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*9,416 volunteer abstracts, 18 symposia abstracts, and 64 teaching of neuroscience abstracts.

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THEMATIC LIST OF SESSIONS

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405.	Chemical senses: central pathways II	Poster			Wed PM		
626.	Chemical senses: central pathways III	Poster					Fri AM
49.	Chemical senses: peripheral mechanisms I	Poster	Mon AM				
251.	Chemical senses: peripheral mechanisms II	Poster		Tue PM			
438.	Chemical senses: peripheral mechanisms III	Slide				Thu AM	

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
481.	Chemical senses: peripheral mechanisms IV	Poster				Thu AM	
480.	Cochlea: nerve responses, transmitters and second messengers	Poster				Thu AM	
440.	Cochlear nerve, micromechanics and receptors	Slide				Thu AM	
253.	Invertebrate sensory systems	Poster		Tue PM			
214.	Nociception	Slide		Tue PM			
622.	Pain modulation: CNS	Poster					Fri AM
45.	Pain modulation: behavior	Poster	Mon AM				
623.	Pain modulation: monoamines	Poster					Fri AM
46.	Pain modulation: opioids I	Poster	Mon AM				
121.	Pain modulation: opioids II	Poster	Mon PM				
478.	Pain modulation: peptides and excitatory amino acids	Poster				Thu AM	
179.	Pain modulation: peripheral	Poster		Tue AM			
402.	Pain modulation: pharmacology I	Poster			Wed PM		
548.	Pain modulation: pharmacology II	Poster				Thu PM	
547.	Pain modulation: spinal and trigeminal	Poster				Thu PM	
120.	Pain pathways I	Poster	Mon PM				
291.	Pain pathways II	Slide			Wed AM		
477.	Pain pathways: central	Poster				Thu AM	
178.	Pain pathways: hyperalgesia	Poster		Tue AM			
401.	Pain pathways: spinal and trigeminal	Poster			Wed PM		
624.	Retina and photoreceptors: chemistry and anatomy	Poster					Fri AM
403.	Retina and photoreceptors: circuits and signals	Poster			Wed PM		
143.	Retina and photoreceptors: ganglion cells	Slide		Tue AM			
549.	Retina and photoreceptors: ganglion cells and centrifugal control	Poster				Thu PM	
122.	Retina and photoreceptors: photoreceptors	Poster	Mon PM				
9.	Retina and photoreceptors: receptors and circuits	Slide	Mon AM				
44.	Somatic and visceral afferents	Poster	Mon AM				
399.	Somatic and visceral afferents: central projections	Poster			Wed PM		
546.	Somatic and visceral afferents: nociception	Poster				Thu PM	
248.	Somatosensory cortex and thalamocortical relations	Poster		Tue PM			
330.	Somatosensory cortex and thalamocortical relations: physiology	Poster			Wed AM		
331.	Somatosensory cortex and thalamocortical relations: plasticity	Poster			Wed AM		
441.	Somatosensory pathways	Slide				Thu AM	
400.	Spinal cord	Poster			Wed PM		
119.	Subcortical somatosensory pathways: brainstem	Poster	Mon PM				
247.	Subcortical somatosensory pathways: thalamus	Poster		Tue PM			
282.	Subcortical visual pathways	Slide			Wed AM		
249.	Subcortical visual pathways: LGN	Poster		Tue PM			
550.	Subcortical visual pathways: cortical inputs and subcortical responses	Poster				Thu PM	
47.	Subcortical visual pathways: midbrain	Poster	Mon AM				
118.	Visceral afferents	Poster	Mon PM				
431.	Visual cortex: architecture and interactions	Slide				Thu AM	
48.	Visual cortex: cortical circuits	Poster	Mon AM				
180.	Visual cortex: extrastriate response properties	Poster		Tue AM			
511.	Visual cortex: far extrastriate cortex	Slide				Thu PM	
208.	Visual cortex: near extrastriate cortex	Slide		Tue PM			
332.	Visual cortex: organization and connections	Poster			Wed AM		
625.	Visual cortex: stimulation and evoked responses	Poster					Fri AM
73.	Visual cortex: striate cortex	Slide	Mon PM				
404.	Visual cortex: striate mechanisms	Poster			Wed PM		
7.	Visual psychophysics and behavior	Slide	Mon AM				

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
333.	Visual psychophysics and behavior: basic processes	Poster			Wed AM		
479.	Visual psychophysics and behavior: higher functions and models	Poster				Thu AM	
Theme G: Motor Systems and Sensorimotor Integration							
429.	**Human Eye Saccades—Sensory and Cortical Factors**	**SYMP.**				**Thu AM**	
138.	**Neural Network Models of Vertebrate Sensorimotor Systems**	**SYMP.**		**Tue AM**			
70.	**The Basal Ganglia in the '90s: Wedding the Neural System to Cellular and Molecular Mechanisms**	**SYMP.**	**Mon PM**				
183.	Basal ganglia and thalamus I	Poster		Tue AM			
184.	Basal ganglia and thalamus II	Poster		Tue AM			
336.	Basal ganglia and thalamus III	Poster			Wed AM		
483.	Basal ganglia and thalamus IV	Poster				Thu AM	
520.	Basal ganglia and thalamus V	Slide				Thu PM	
334.	Basal ganglia and thalamus: electrophysiology	Poster			Wed AM		
335.	Basal ganglia and thalamus: molecular	Poster			Wed AM		
482.	Basal ganglia and thalamus: unit activity	Poster				Thu AM	
368.	Cerebellum I	Slide			Wed PM		
551.	Cerebellum II	Poster				Thu PM	
552.	Cerebellum III	Poster				Thu PM	
628.	Cerebellum IV	Poster					Fri AM
627.	Cerebellum: anatomy	Poster					Fri AM
50.	Circuitry and pattern generation I	Poster	Mon AM				
595.	Circuitry and pattern generation II	Slide					Fri AM
630.	Circuitry and pattern generation III	Poster					Fri AM
51.	Circuitry and pattern generation: models	Poster	Mon AM				
409.	Control of posture and movement I	Poster			Wed PM		
442.	Control of posture and movement II	Slide				Thu AM	
484.	Control of posture and movement III	Poster				Thu AM	
553.	Control of posture and movement IV	Poster				Thu PM	
629.	Control of posture and movement V	Poster					Fri AM
410.	Control of posture and movement in humans	Poster			Wed PM		
125.	Cortex I	Poster	Mon PM				
126.	Cortex II	Poster	Mon PM				
443.	Cortex III	Slide				Thu AM	
406.	Cortex: anatomy	Poster			Wed PM		
407.	Cortex: lesion and stimulation	Poster			Wed PM		
485.	Invertebrate motor function I	Poster				Thu AM	
554.	Invertebrate motor function II	Poster				Thu PM	
185.	Oculomotor system I	Poster		Tue AM			
186.	Oculomotor system II	Poster		Tue AM			
218.	Oculomotor system III	Slide		Tue PM			
337.	Oculomotor system IV	Poster			Wed AM		
338.	Oculomotor system V	Poster			Wed AM		
254.	Reflex function	Poster		Tue PM			
256.	Sensorimotor integration: muscle I	Poster		Tue PM			
555.	Sensorimotor integration: muscle II	Poster				Thu PM	
84.	Spinal cord and brainstem I	Slide	Mon PM				
187.	Spinal cord and brainstem II	Poster		Tue AM			
408.	Spinal cord and brainstem III	Poster			Wed PM		
188.	Spinal cord and brainstem: anatomy	Poster		Tue AM			
255.	Spinal cord and brainstem: motoneurons	Poster		Tue PM			
127.	Vestibular system I	Poster	Mon PM				
128.	Vestibular system II	Poster	Mon PM				

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
Theme H:Other Systems of the CNS							
631.	Association cortex and thalamocortical relations	Poster					Fri AM
16.	Brain metabolism and blood flow I	Slide	Mon AM				
190.	Brain metabolism and blood flow II	Poster		Tue AM			
216.	Brain metabolism and blood flow III	Slide		Tue PM			
339.	Brain metabolism and blood flow IV	Poster			Wed AM		
413.	Brainstem systems	Poster			Wed PM		
129.	Comparative neuroanatomy I	Poster	Mon PM				
257.	Comparative neuroanatomy II	Poster		Tue PM			
412.	Hypothalamus I	Poster			Wed PM		
486.	Hypothalamus II	Poster				Thu AM	
52.	Limbic system I	Poster	Mon AM				
189.	Limbic system II	Poster		Tue AM			
411.	Limbic system III	Poster			Wed PM		
Theme I: Neural Basis of Behavior							
278.	**Dynamical Behavior of Neural Systems**	**SYMP.**			**Wed AM**		
279.	**The Neuron Doctrine 1891 - 1991**	**SYMP.**			**Wed AM**		
269.	Antipsychotics I	Poster		Tue PM			
270.	Antipsychotics II	Poster		Tue PM			
15.	Biological rhythms and sleep I	Slide	Mon AM				
262.	Biological rhythms and sleep II	Poster		Tue PM			
263.	Biological rhythms and sleep III	Poster		Tue PM			
264.	Biological rhythms and sleep IV	Poster		Tue PM			
265.	Biological rhythms and sleep V	Poster		Tue PM			
292.	Biological rhythms and sleep VI	Slide			Wed AM		
345.	Biological rhythms and sleep VII	Poster			Wed AM		
346.	Biological rhythms and sleep VIII	Poster			Wed AM		
490.	Biological rhythms and sleep IX	Poster				Thu AM	
491.	Biological rhythms and sleep X	Poster				Thu AM	
565.	Drugs of abuse—alcohol	Poster				Thu PM	
151.	Drugs of abuse—alcohol, barbiturates and benzodiazepines	Slide		Tue AM			
567.	Drugs of abuse—amphetamine	Poster				Thu PM	
494.	Drugs of abuse—amphetamine and nicotine	Poster				Thu AM	
60.	Drugs of abuse—benzodiazepines	Poster	Mon AM				
636.	Drugs of abuse—cellular effects of ethanol	Poster					Fri AM
349.	Drugs of abuse—cocaine	Poster			Wed AM		
348.	Drugs of abuse—cocaine: antagonists and serotonin	Poster			Wed AM		
61.	Drugs of abuse—cocaine: binding and neurophysiology	Poster	Mon AM				
62.	Drugs of abuse—cocaine: development	Poster	Mon AM				
267.	Drugs of abuse—cocaine: dopamine	Poster		Tue PM			
150.	Drugs of abuse—cocaine: genes, molecules and buprenorphine	Slide		Tue AM			
268.	Drugs of abuse—cocaine: monoamines and brain stimulation	Poster		Tue PM			
566.	Drugs of abuse—cocaine: pharmacology	Poster				Thu PM	
80.	Drugs of abuse—cocaine: transporters and toxins	Slide	Mon PM				
564.	Drugs of abuse—ethanol	Poster				Thu PM	
568.	Drugs of abuse—opioids	Poster				Thu PM	
132.	Drugs of abuse—opioids: dopamine and dependence	Poster	Mon PM				
635.	Drugs of abuse—prenatal ethanol	Poster					Fri AM
197.	Hormonal control of behavior I	Poster		Tue AM			
419.	Hormonal control of behavior II	Poster			Wed PM		
561.	Hormonal control of behavior III	Poster				Thu PM	
562.	Hormonal control of behavior IV	Poster				Thu PM	

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
13.	Human cognition	Slide	Mon AM				
258.	Human cognition: event-related potentials, attention, methods	Poster		Tue PM			
340.	Human cognition: hemispheric lateralization, gender differences	Poster			Wed AM		
487.	Human cognition: language, other	Poster				Thu AM	
191.	Human cognition: learning and memory	Poster		Tue AM			
217.	Ingestive behavior: CCK, bombesin and NPY	Slide		Tue PM			
81.	Ingestive behavior: molecular	Slide	Mon PM				
57.	Ingestive behavior: monoamines	Poster	Mon AM				
196.	Ingestive behavior: neural, hormonal and GI	Poster		Tue AM			
195.	Ingestive behavior: peptides	Poster		Tue AM			
347.	Ingestive behavior: salt and water	Poster			Wed AM		
220.	Invertebrate learning and behavior I	Slide		Tue PM			
418.	Invertebrate learning and behavior II	Poster			Wed PM		
521.	Invertebrate learning and behavior III	Slide				Thu PM	
633.	Invertebrate learning and behavior IV	Poster					Fri AM
53.	Learning and memory—anatomy I	Poster	Mon AM				
54.	Learning and memory—anatomy II	Poster	Mon AM				
192.	Learning and memory—anatomy III	Poster		Tue AM			
259.	Learning and memory—anatomy IV	Poster		Tue PM			
341.	Learning and memory—anatomy V	Poster			Wed AM		
414.	Learning and memory—anatomy VI	Poster			Wed PM		
140.	Learning and memory—anatomy: animal studies	Slide		Tue AM			
5.	Learning and memory—anatomy: human studies	Slide	Mon AM				
55.	Learning and memory—pharmacology: acetylcholine I	Poster	Mon AM				
488.	Learning and memory—pharmacology: acetylcholine II	Poster				Thu AM	
194.	Learning and memory—pharmacology: excitatory amino acids	Poster		Tue AM			
557.	Learning and memory—pharmacology: monoamines	Poster				Thu PM	
342.	Learning and memory—pharmacology: other I	Poster			Wed AM		
632.	Learning and memory—pharmacology: other II	Poster					Fri AM
130.	Learning and memory—physiology I	Poster	Mon PM				
193.	Learning and memory—physiology II	Poster		Tue AM			
260.	Learning and memory—physiology III	Poster		Tue PM			
415.	Learning and memory—physiology IV	Poster			Wed PM		
437.	Learning and memory—physiology V	Slide				Thu AM	
556.	Learning and memory—physiology VI	Poster				Thu PM	
266.	Monoamines and behavior: D_1 and D_2 mechanisms	Poster		Tue PM			
493.	Monoamines and behavior: amphetamine and others	Poster				Thu AM	
131.	Monoamines and behavior: development, ingestion and sex	Poster	Mon PM				
198.	Monoamines and behavior: dopamine	Poster		Tue AM			
293.	Monoamines and behavior: human and animal	Slide			Wed AM		
59.	Monoamines and behavior: norepinephrine and serotonin	Poster	Mon AM				
261.	Motivation and emotion I	Poster		Tue PM			
344.	Motivation and emotion II	Poster			Wed AM		
416.	Motivation and emotion III	Poster			Wed PM		
489.	Motivation and emotion IV	Poster				Thu AM	
56.	Neural plasticity I	Poster	Mon AM				
213.	Neural plasticity II	Slide		Tue PM			
343.	Neural plasticity III	Poster			Wed AM		
558.	Neural plasticity IV	Poster				Thu PM	
492.	Neuroethology: arthropods	Poster				Thu AM	
417.	Neuroethology: bird song	Poster			Wed PM		

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
560.	Neuroethology: fish	Poster				Thu PM	
559.	Neuroethology: molluscs, amphibians, mammals, models	Poster				Thu PM	
420.	Neuropeptides and behavior I	Poster			Wed PM		
563.	Neuropeptides and behavior II	Poster				Thu PM	
634.	Neuropeptides and behavior III	Poster					Fri AM
82.	Psychotherapeutic drugs	Slide	Mon PM				
569.	Psychotherapeutics: affective disorders	Poster				Thu PM	
637.	Psychotrophic agents: anxiety	Poster					Fri AM
133.	Psychotrophic drugs: sigma receptors	Poster	Mon PM				
58.	Stress, hormones and the autonomic nervous system	Poster	Mon AM				
366.	Stress, hormones and the autonomic nervous system: neurotransmitters	Slide			Wed PM		

Theme J: Disorders of the Nervous System

582.	**Neural Grafting and Parkinson's Disease**	**SYMP.**					**Fri AM**
206.	**Therapeutic Potential of Neurotrophic Factors**	**SYMP.**		**Tue PM**			
576.	Affective illness and related disorders	Poster				Thu PM	
364.	Alzheimer's disease: amyloid I	Slide			Wed PM		
439.	Alzheimer's disease: amyloid II	Slide				Thu AM	
517.	Alzheimer's disease: amyloid III	Slide				Thu PM	
572.	Alzheimer's disease: amyloid IV	Poster				Thu PM	
573.	Alzheimer's disease: amyloid V	Poster				Thu PM	
422.	Alzheimer's disease: cytoskeleton	Poster			Wed PM		
421.	Alzheimer's disease: experimental models	Poster			Wed PM		
83.	Alzheimer's disease: genetics and growth factors	Slide	Mon PM				
290.	Alzheimer's disease: neurochemistry I	Slide			Wed AM		
423.	Alzheimer's disease: neurochemistry II	Poster			Wed PM		
498.	Alzheimer's disease: neuroimaging and diagnostic tests	Poster				Thu AM	
147.	Alzheimer's disease: neuropathology I	Slide		Tue AM			
271.	Alzheimer's disease: neuropathology II	Poster		Tue PM			
272.	Alzheimer's disease: neuropathology III	Poster		Tue PM			
273.	Alzheimer's disease: neuropsychology	Poster		Tue PM			
274.	Alzheimer's disease: pharmacology and drug trials	Poster		Tue PM			
580.	Clinical CNS neurophysiology	Poster				Thu PM	
574.	Degenerative disease	Poster				Thu PM	
351.	Developmental disorders of the nervous system I	Poster			Wed AM		
495.	Developmental disorders of the nervous system II	Poster				Thu AM	
63.	Developmental genetic models	Poster	Mon AM				
199.	Developmental genetic models: transmitter systems and second messengers	Poster		Tue AM			
68.	Epilepsy: animal models I	Poster	Mon AM				
200.	Epilepsy: animal models II	Poster		Tue AM			
497.	Epilepsy: anticonvulsant drugs	Poster				Thu AM	
67.	Epilepsy: basic mechanisms I	Poster	Mon AM				
201.	Epilepsy: basic mechanisms II	Poster		Tue AM			
571.	Epilepsy: basic mechanisms III	Poster				Thu PM	
596.	Epilepsy: basic mechanisms IV	Slide					Fri AM
638.	Epilepsy: basic mechanisms V	Poster					Fri AM
369.	Epilepsy: human studies and animal models I	Slide			Wed PM		
496.	Epilepsy: human studies and animal models II	Poster				Thu AM	
570.	Experimental genetic models	Poster				Thu PM	
350.	Genetic models of behavior	Poster			Wed AM		
503.	Infectious diseases	Poster				Thu AM	
77.	Ischemia I	Slide	Mon PM				
425.	Ischemia II	Poster			Wed PM		
426.	Ischemia III	Poster			Wed PM		

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
427.	Ischemia IV	Poster			Wed PM		
433.	Ischemia V	Slide				Thu AM	
500.	Ischemia VI	Poster				Thu AM	
502.	Ischemia VII	Poster				Thu AM	
501.	Ischemia: excitotoxicity	Poster				Thu AM	
586.	Neurobiology of affective illness and related disorders	Slide					Fri AM
148.	Neurobiology of schizophrenia	Slide		Tue AM			
639.	Neuromuscular diseases	Poster					Fri AM
285.	Neurotoxicity I	Slide			Wed AM		
577.	Neurotoxicity II	Poster				Thu PM	
504.	Neurotoxicity: MPTP	Poster				Thu AM	
505.	Neurotoxicity: biogenic amines	Poster				Thu AM	
579.	Neurotoxicity: environmental	Poster				Thu PM	
578.	Neurotoxicity: metals and free radicals	Poster				Thu PM	
515.	Parkinson's disease	Slide				Thu PM	
424.	Parkinson's disease: animal and transplant studies	Poster			Wed PM		
275.	Parkinson's disease: animal studies	Poster		Tue PM			
499.	Parkinson's disease: human studies	Poster				Thu AM	
575.	Schizophrenia	Poster				Thu PM	
65.	Trauma I	Poster	Mon AM				
66.	Trauma II	Poster	Mon AM				
288.	Trauma III	Slide			Wed AM		
64.	Trauma: spinal cord	Poster	Mon AM				

Other:

202.	Teaching of neuroscience: courses and programs	Poster		Tue AM Tue PM			
203.	Teaching of neuroscience: computer-based education	Poster		Tue AM Tue PM			

2

SYMPOSIUM. INSIGHTS INTO BRAIN AND SPINAL COMPONENTS OF THE OPIATE WITHDRAWAL SYNDROME. J.J. Buccafusco, Med. Col. Georgia & DVAMC and D.C. Marshall, Pfizer Internatnl. (chairpersons); H.N. Bhargava*, Univ. Illinois; G.K. Aghajanian, Yale Univ. Sch. Med.; G.F. Koob, Scripps Clinic; E.D. London, NIDA Addiction Res. Ctr.

The purpose of this symposium is to summarize recent advances in the understanding of the central neuronal processes underlying the expression of the opiate withdrawal syndrome. **Dr. Buccafusco** will introduce the concept of spinal *vs.* brain-mediated withdrawal phenomena and discuss the role of cholinergic neurons. **Dr. Bhargava** will discuss the changes in specific brain and spinal opiate, dopamine and serotonin receptor subtypes associated with physical dependence and withdrawal. Different mechanisms operate in μ/k-induced tolerance-dependence and abstinence processes. **Dr. Aghajanian** will discuss the electrophysiological and biochemical changes within the locus coeruleus during opiate withdrawal. The activation of noradrenergic neurons during withdrawal may be related to an hypertrophied cAMP/protein kinase A system. Both excitatory amino acid inputs and intrinsic enhancement of the cAMP system may occur. **Dr. Koob** will present his studies which identify disruptions in operant behavior following microinjections of opiate antagonists into selective brain regions, particularly the nucleus accumbens in the morphine dependent rat. **Dr. London** will provide detailed analysis of brain and spinal regions most active during opiate withdrawal by employing the technique of regional cerebral glucose utilization in rats. Studies using positron emission tomography (PET) scanning to obtain objective measures of withdrawal processes in the human brain will also be discussed. It is expected that this symposium will provide a forum for the latest theories of the opiate withdrawal phenomenon and help underscore consistent trends in research findings for this area.

3

SYMPOSIUM. ACTIVITY-DEPENDENT REGULATION OF SOMATOSENSORY PROCESSING AT THALAMIC AND CORTICAL LEVELS IN ADULT PRIMATES. E.G. Jones, UC,Irvine (Chairperson); M.M. Merzenich, UCSF; J.H. Kaas, Vanderbilt University; T.P. Pons, NIMH.

Research in the 1980s established that topographic maps in the adult brain are susceptible to reorganizational changes following peripheral deafferentation. Emerging research indicates that the areal extent of such changes can be an order of magnitude greater than previously thought. Behavioral manipulations involving enhanced usage rather than deprivation, have now also been demonstrated to influence tuning characteristics and receptive field properties of neurons that probably underlie activity dependent representational changes. In addition, morphological, chemical and molecular correlates have been demonstrated in adult brains. These may be responsible not only for the changes in representational maps that occur under activity-dependent conditions, but also for serious perturbation of sensory perception such as pain of central origin that commonly accompany chronic deafferentation.

The speakers will present evidence on the extent of reorganization that can occur in the somatosensory cortex under activity-dependent conditions. They will show that the character and extent of such reorganization depends to a large extent on the nature of a peripheral perturbations and probably on a variety of cortical and subcortical mechanisms. The remodeling of cortical representations will be considered in relation to different forms of peripheral deafferentation over short and very long periods, to stimulation of nerves, and to behaviorally relevant usage of the limbs. The differential effects of central lesions will be explored. The relative contributions of existing anatomical circuits and of regulation of gene expression for transmitters, receptors and related molecules at cortical and subcortical sites in the activity-dependent plasticity of cortical cell assemblies will be a major feature of the symposium.

LONG-TERM POTENTIATION: PHYSIOLOGY AND PHARMACOLOGY I

4.1

LTP REDUCES K^+ CHANNEL ACTIVITY IN HIPPOCAMPAL SYNAPTOSOMES. J. Farley and A. Routtenberg. Program in Neural Science, Indiana University, Bloomington, IN 47405 and Cresap Neuroscience Laboratory, Northwestern University, Evanston, IL 60201.

Recent quantal analyses suggest that maintenance of LTP at Schaffer collateral synapses is due to enhanced neurotransmitter release from presynaptic terminals. Because K^+ ion channels in presynaptic terminals are involved in regulation of such release at many synapses, a persistent reduction in their activities induced by tetanizing stimulation might be expected to contribute to LTP. We have examined this possibility by incorporating hippocampal synaptosomal membranes from animals that sustained LTP into planar lipid bilayers on patch electrodes. We observed near-complete elimination of the activities of several classes of large-conductance calcium-dependent K^+ channels (Farley & Rudy, 1988, Biophys. J., 53: 919-934) in membranes prepared from perforant path-dentate gyrus synapses, 1 hr following high-frequency stimulation and LTP-induction, as compared to that of sham and low-frequency stimulation controls. The low incidence of K^+ channel activity did not appear to be due to a selective failure of vesicle fusion for LTP membranes, since calcium channel activity was often observed in these same membranes. Potassium channel activity in membranes from LTP-animals could be restored by addition of alkaline phosphatase to the bath, suggesting the involvement of a protein kinase in the persistent reduction of K^+ channels and LTP. Supported by NIH NS 26106 to JF and NIMH 25283 and AFOSR 90-0240 to AR.

4.2

ANGIOTENSIN II BLOCKS LONG-TERM POTENTIATION. J.B. Denny, J. Polan-Curtain, D.L. Armstrong and M.J. Wayner. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78249.

We have found that injection of 1 ul of 4.78 uM angiotensin II in artificial cerebrospinal fluid (ACSF) immediately above the hippocampus in the rat blocks the induction of long-term potentiation (LTP) in perforant path-stimulated dentate granule cells. This effect was completely eliminated if the specific angiotensin antagonist saralasin was also present in the injected solution at a concentration of 239 uM. Saralasin injected alone resulted in enhanced excitability in the dentate gyrus, but as was the case with the inhibitory action of angiotensin II, the effect was only observable after tetanic stimulation. Both angiotensin II and saralasin had no effect on baseline synaptic transmission. Angiotensin III also blocked LTP induction but required concentrations 40-50 fold greater than those using angiotensin II. These results suggest that angiotensin II is released in the hippocampus following tetanic stimulation and that it serves to modulate LTP through an inhibitory action. A ten-fold lower dose of angiotensin II was not effective in blocking LTP, and the ratio of saralasin to angiotensin II was required to be fifty-fold as given above. A lower ratio of saralasin to angiotensin II (five-fold) resulted in partial blockade of LTP.

4.3

LONG TERM POTENTIATION OF AN INHIBITORY PATHWAY IN THE CNS. Y. Oda and H. Korn. INSERM, Institut Pasteur, Paris

The Teleost Mauthner(M-) cell is subjected to a strong Cl^- dependent inhibition carried by commissural interneurons which are excited by primary auditory afferents. Current and voltage clamp experiments have shown that following low intensity classical tetanization of the contralateral eighth nerve subthreshold for LTP of excitation in the other M-cell, IPSPs and IPSCs are enhanced for the remainder of the recording session which could exceed one hour. This potentiation reflected, in part, an increased synaptic efficacy at VIIIth N with inhibitory cells connections since i) there was an associated LTP in the LVN, ii) the presynaptic volley onto the M-cell, recorded extra- and intracellularly, was augmented simultaneously with iii) this neuron's inhibitory conductance (Gipsp, peak enhancement m±SEM = 135±19%, range 63 to 392%, n=18). M-cell input conductance, RP and collateral IPSPs remained unchanged. Several observations suggest that potentiation also occurred at inhibitory synapses themselves. Specifically, i) the slope of the relation between the input volley and Gipsp could be greater after the tetanus, and ii) this effect was not found when BAPTA was injected postsynaptically. Thus LTP is not restricted to excitatory pathways.

***Indicates nonmember of the Society for Neuroscience**

4.4

Synaptic Ca^{2+}-responses restricted to postsynaptic spines of central neurons are NMDA-receptor dependent. W. Müller and J.A. Connor. Dept. of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

Ca^{2+} and NMDA-receptor dependent synaptic plasticity, e.g. long-term-potentiation, has met considerable interest as a possible substrate for associative memory. Independent plasticity of adjacent synapses on the same postsynaptic dendrite would boost the performance of such a memory network. The synaptic independence might depend on NMDA-receptor mediated Ca^{2+}-influx specifically into activated postsynaptic spines. High resolution microfluorometry of fura-2-injected CA3 pyramidal neurons in guinea pig hippocampal slice revealed that synaptic stimulation can indeed elicit very uneven Ca^{2+}-accumulations in postsynaptic spiny dendrites, eventually being clearly restricted to single postsynaptic spines. Stronger stimulation elicits more widespread Ca^{2+} accumulations with significant concentration peaks in activated spines. The NMDA-receptor antagonist AP-5 effectively antagonized synaptically evoked Ca^{2+} responses in spines of distal dendrites. Repeated train stimulation led, facilitated by picrotoxin, eventually to the expression of sustained spine or dendritic Ca^{2+} gradients, indicating long lasting postsynaptic changes. Our results demonstrate a space and time dependent microstructure of postsynaptic Ca^{2+} responses that we surmise is significant for neuronal memory capabilities.

W.M. was supported in part by the DFG.

4.5

AN INHIBITOR OF NITRIC OXIDE SYNTHETASE PREVENTS LONG-TERM POTENTIATION (LTP). E.M. Schuman and D.V. Madison. *Dept. of Molecular and Cellular Physiology, Stanford School of Medicine, Stanford, CA 94305.*

While LTP in the CA1-Schaffer collateral synapse in the hippocampus is induced by postsynaptic factors, recent evidence suggests that LTP may be expressed by presynaptic alterations in neurotransmitter release. If the induction of LTP is postsynaptic and the expression is presynaptic then this necessitates some sort of back-communication from the post to the presynaptic neuron. We have been conducting studies which investigate whether the diffusible molecule nitric oxide, NO, might function in such a capacity. We first tested whether an inhibitor of NO synthetase, N-methyl-l-arginine (L-NMMA) blocks or otherwise attenuates LTP. Extracellular application of L-NMMA (100 uM) effectively prevented tetanus-induced potentiation of the field epsp, whereas an inactive isomer N-methyl-d-arginine, (D-NMMA), was ineffective in preventing LTP. Extracellular application of hemoglobin (100 uM) also blocked LTP of the field epsp. Since hemoglobin is a large protein which is unable to cross cell membranes this result implies that NO must travel extracellularly to exert its effect. To determine the site of NO synthetase activity, we have introduced L-NMMA into the postsynaptic neuron via an intracellular recording electrode. Confirming the above results, we found that we were unable to produce LTP by the pairing technique (postsynaptic depolarization coupled with presynaptic stimulation, 1 Hz). Surprisingly, however, the postsynaptic inhibitor did not prevent LTP induced by tetanus. One interpretation of this finding is that during tetanic stimulation many neurons generate NO which can then travel to the neighboring presynaptic terminal(s) of the inhibited neuron to produce LTP. Further substantiation for this idea is gained from whole cell studies of LTP. It is known that the ability to achieve LTP by the pairing technique is seriously compromised following dialysis of the postsynaptic neuron (20-30 min.). We have confirmed this finding but have found that following a failure to induce LTP by pairing, we can reliably induce LTP by tetanus.

D.V.M. is a Lucille P. Markey Scholar and this work was supported by a grant from the Lucille P. Markey Charitable Trust.

4.6

THE FREQUENCY OF NMDA RECEPTOR ACTIVATION INFLUENCES THE STABILIZATION OF LTP. A. Colino*, Y.-Y. Huang*, and R.C. Malenka. Dept.'s of Psychiatry & Physiology, Univ. of California, San Francisco, CA. 94143.

A defining feature of LTP is the stability of the increase in synaptic efficacy. To determine the interval over which the processes activated by a single EPSP can be integrated and result in stable LTP, NMDA receptor-dependent processes were activated by pairing synaptic stimulation with postsynaptic depolarization and a fixed number of pairings were performed at intervals ranging from 2 seconds to 1 minute. Using field recordings and a brief tetanus to an independent conditioning pathway to evoke postsynaptic depolarization, a single pairing routinely elicited a modest potentiation of synaptic transmission which decayed to baseline within 3-10 minutes (STP). Ten pairings at 1 minute intervals rarely resulted in stable LTP (1 of 7) while ten pairings given at 2-10 second intervals routinely resulted in LTP (13 of 17). A 30 second interval gave mixed results (5 of 9 resulted in stable LTP). Similar experiments were also performed on single cells using whole-cell recording. Two independent inputs were alternatively activated to obtain stable baselines. The cell was then depolarized and one input stimulated every 40-60 seconds while the other input received the same number of stimuli every 5-10 seconds. Because whole-cell recording may result in "wash-out" of important intracellular constituents, the pairing at shorter intervals was started at the end of the longer interval pairings. In 6 of 8 cells, the shorter interval pairings resulted in stable LTP while the longer interval pairings resulted in STP.

These results suggest that following synaptic stimulation of the NMDA receptor sufficient to elicit STP (but not LTP), processes are activated that can be integrated over a period of approximately one minute and result in stable LTP. This "integration period" likely depends on induction conditions (e.g. magnitude of postsynaptic calcium increase) and is another parameter which may prove to be important when considering the mechanisms and role of LTP.

4.7

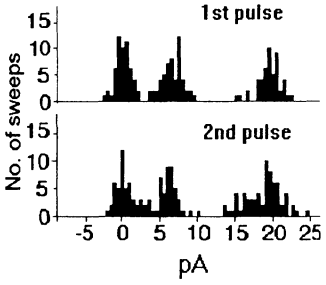
OSCILLATIONS IN SYNAPTIC TRANSMISSION AND LONG-TERM POTENTIATION. Neal Hessler and Roberto Malinow. Neuroscience Program and Dept of Physiology and Biophysics, University of Iowa.

Oscillations in quantal content have been described in the neuromuscular junction (Meiri and Rahamimoff, 1978). Similar oscillations in mean amplitude of elicited synaptic currents are observed at the Shaffer collateral/CA1 synapse in the hippocampal slice, using minimal stimulation and whole-cell recordings (Malinow, 1991). The oscillations in transmission have a period that ranges from 2-15 min for different experiments and they appear to be of presynaptic origin as in-phase oscillations are seen in mean^2/variance, percent synaptic failures, and paired pulse facilitation. Oscillation of one or more of these measures can occur with little change in mean transmission, showing that apparent stationarity in mean transmission does not guarantee stationarity in underlying mechanisms, placing greater restrictions on use of traditional quantal analysis. In most cases, some oscillatory activity is seen in the absence of induced LTP. However, in about 30% of experiments, little oscillatory activity is seen prior to LTP; pairing pre- and postsynaptic activity triggers oscillations in addition to LTP. Quantification of these non-stationarities is being pursued using Fourier analysis and chaotic models. The correlation between different potentiated pathways and relation between pre-existing oscillations and likelihood of obtaining LTP is being examined. Oscillations of transmission may reflect presynaptic calcium oscillations and could functionally associate different inputs potentiated at the same time.

4.8

RESOLUTION OF SYNAPTIC FLUCTUATIONS USING WHOLE CELL PATCH CLAMPING IN THE HIPPOCAMPUS. D.M. Kullmann*, T. Manabe*, P. Renner* & R.A. Nicoll. Depts. Pharmacol. & Physiol., UCSF, CA 94143.

Several studies of synaptic transmission in the hippocampus based on amplitude fluctuations have relied on untested assumptions to estimate the quantal parameters (see Korn et al, Nature, 350, 282). Using minimal stimulation of stratum radiatum, we have obtained whole-cell voltage clamp recordings from guinea-pig CA1 cells which often show clear separation between transmission failures and EPSCs. In some cases the EPSCs are further seen to fluctuate between discrete amplitudes, which are not always separated by a constant increment. Paired-pulse facilitation is consistently accompanied by a reduction in the probability of transmission failure. It is therefore unlikely that the failures are due exclusively to intermittent excitation of the presynaptic fibers. We are currently using the nystatin perforated patch technique to prolong the time during which EPSC fluctuations can be recorded. The figure shows the peak amplitudes of 160 trials (paired pulses, 60 ms delay).



Supported by the NIMH, AES, HFSPO and Smith & Nephew Foundation.

4.9

ROLE OF MEMBRANE POTENTIAL AND CALCIUM IN THE INDUCTION OF LONG-TERM POTENTIATION (LTP). D. J. Perkel, T. Manabe* and R. A. Nicoll Depts. Pharmacol. and Physiol., UCSF, San Francisco, CA 94143.

It is widely accepted that one early step in the induction of LTP of synaptic transmission in hippocampal CA1 neurons is an influx of calcium ions through NMDA receptors. We have studied the effects of manipulating postsynaptic membrane potential, and presumably postsynaptic calcium concentration, on LTP induction.

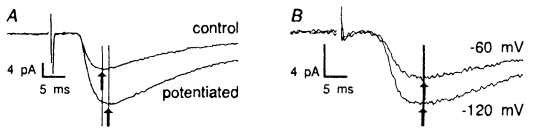
We have recorded excitatory postsynaptic currents from CA1 neurons in slices of guinea pig hippocampus using the "blind" whole-cell voltage-clamp technique and Cs-based intracellular solutions. To induce LTP we have paired low-frequency synaptic stimulation with steady postsynaptic depolarization to different membrane potentials ("pairing"). One synaptic pathway onto each cell was paired at its reversal potential (RP; 0 to +20 mV) and served as an internal calibration for the amount of potentiation generated in another pathway, which was paired at a test potential (-40 mV or +100 mV). Pairing at +100 mV produced approximately 20% of the potentiation induced by pairing near the RP (cf. Malenka et al., *Science* 242:81, 1988), while pairing at -40 mV was also less effective than pairing at the RP. The voltage-dependence of calcium entry induced by synaptic activation of NMDA receptors is not known, but our results suggest that the calcium level achieved by pairing at the RP may exceed that at -40 mV.

Although previous studies have found that postsynaptic calcium-spike activity alone does not affect synaptic efficacy, we have found that prolonged (3 sec, 0.2 Hz, 20 pulses) postsynaptic voltage pulses to 0 mV, chosen to maximize calcium entry through voltage-dependent calcium channels, can enhance synaptic transmission. This phenomenon is transient, lasting approximately 10 - 30 min., and is resistant to D-APV (25 μM). Although additional data linking this enhancement to LTP are required, the effects of membrane potential in enhancing transmission provide further support for a role for calcium in regulating synaptic efficacy.

4.10

THE ONSET OF THE EXCITATORY POSTSYNAPTIC CURRENT IS SLOWED DURING LONG-TERM POTENTIATION **J.A. Kauer, F.E. Schweizer, D.D. Friel & R.W. Tsien. Dept. of Mol. & Cell. Physiology, Stanford University, Stanford CA 94305.**

We have used whole-cell voltage clamp recordings and minimal stimulation to look for changes in the time course of EPSCs in CA1 pyramidal cells during long term potentiation. LTP was induced by pairing postsynaptic depolarization with continued afferent stimulation (2 Hz). EPSC amplitude and total charge increased upon induction of LTP (*A*, averages of 20 EPSCs before and during LTP). Little or no change was detected in EPSC latency or time constant of decay. However, the onset of the EPSC became slower and the time to peak was increased during LTP. Time-to-peak (arrows) increased by 1.7±0.8 ms, or 25±10% (N=11), a change that was maintained during LTP. The difference in time course was still observed when comparing EPSCs of similar amplitude taken before and after LTP. Control experiments showed no such slowing in time course when the EPSC amplitude was increased by postsynaptic hyperpolarization (*B*, averages of 20 EPSCs each). These results might signify recruitment of slow/distant synapses or presynaptic modifications such as (1) spike broadening, (2) decreased calcium buffering, or (3) changes in the release machinery. Thus, it is unlikely that LTP is caused by recruitment of additional synaptic units with invariant properties.



4.11

QUANTAL AMPLITUDE REMAINS CONSTANT WITH LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL SLICES. F.E. Schweizer, J.A. Kauer, D.D. Friel, & R.W. Tsien. **Dept. of Mol. and Cell. Physiology, Stanford University, Stanford, CA 94305.**

Long-term potentiation is characterized by a persistent increase in synaptic current, but it remains controversial whether this arises from enhanced quantal content (number of unit events) or increased quantal size (amplitude of unit event). To address this issue we estimated quantal size from amplitude histograms of EPSCs recorded with whole cell voltage clamp from CA1 pyramidal cells (minimal afferent stimulation at 2 Hz). Fitting each EPSC with the sum of two exponentials aided accurate estimation of EPSC amplitude. A small proportion of records was excluded from the analysis on the basis of excessive baseline noise, spontaneous events, or unusual onset latency. Amplitude histograms were constructed from 500-1000 records and showed peaks of roughly equal spacing. Histograms were judged robust if independent histograms made from every other data point or from consecutive portions of the epoch showed close agreement in peak locations. We used maximum likelihood analysis to estimate the position and width of peaks. Typically, we found a quantal size of 1-2 pA at -70 mV; quantal variance was negligible relative to recording noise, in agreement with Larkman et al. (1991).

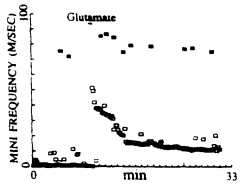
Mean EPSC amplitude increased by 30-50% when the postsynaptic neuron was hyperpolarized from -60 to -110 mV. The corresponding amplitude histograms showed an increase in quantal size with hyperpolarization in proportion to the enhancement of mean EPSC amplitude. This result gives confidence in our ability to estimate quantal size under varying experimental conditions.

LTP was induced by depolarizing the postsynaptic neuron during continued afferent stimulation. In experiments where robust peaks were observed before and during LTP, mean EPSC amplitude increased to at least 150% of control. In contrast, peak spacing did not change with LTP. These results support the idea that LTP is caused by an increase in quantal content rather than an increase in quantal size.

4.12

PRESYNAPTIC EXPRESSION MECHANISM OF LONG-TERM POTENTIATION OF MINIATURE EPSC FREQUENCY IN CULTURED HIPPOCAMPAL NEURONS. A. Malgaroli & R. W. Tsien, Department of Molecular and Cellular Physiology, Stanford CA 94305.

Analysis of miniature excitatory postsynaptic currents offers novel information about mechanisms of LTP expression. We made whole-cell recordings from rat hippocampal pyramidal cells in culture and directly applied glutamate to induce a global synaptic enhancement. Like the amplitude of evoked responses, the frequency of mini epscs was strongly increased (2- to 10-fold in 41/60 experiments). The rise in mini frequency (1 μM TTX present) was (1) persistent up to the end of the recording (up to 80 min), (2) prevented by strong postsynaptic hyperpolarization (Mg^{2+} present), (3) prevented by block of NMDA receptors with MK801 (10 μM). Several observations support the idea that postsynaptic responsivity remained unchanged during LTP of mini frequency. (1) The distribution of mini amplitudes was not significantly changed. (2) Responses to brief puffs of glutamate or AMPA stayed the same. (3) When vesicle release from nerve terminals was evoked by brief challenges with hypertonic solutions, the peak mini frequency response (■) was barely increased by glutamate-induced potentiation (n=6), in contrast to the 4-10 fold increase in spontaneous mini frequency (□). These findings run contrary to expectations for expression of LTP by purely postsynaptic recruitment of latent spines or previously inactive receptor clusters. Instead, the near-occlusion between mini potentiation by glutamate and by hypertonicity suggest actions involving the same rate-limiting step along the presynaptic secretory pathway.



LEARNING AND MEMORY—ANATOMY: HUMAN STUDIES

5.1

PATIENTS WITH ALZHEIMER'S DISEASE SHOW NORMAL PRIMING IN PERCEPTUAL IDENTIFICATION OF PSEUDOWORDS. M.M. Keane, J.D.E. Gabrieli, J.H. Growdon, and S. Corkin. Dept. of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139; Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Previously, we reported that patients with Alzheimer's disease (AD) showed normal priming in perceptual identification of briefly presented words. The aim of the current study was to determine whether normal perceptual priming in AD extended to pseudowords. The subjects of study included 12 AD patients (mild to severe), and 12 normal control subjects (NCS). The stimuli were 128 three-letter pseudowords that conformed to the rules of English orthography; 64 were used in a perceptual priming task and the other 64 were used in a recognition memory task. Prior to a perceptual identification or recognition task, 32 pseudowords were presented in a study list. In the perceptual identification task, subjects were asked to identify 64 briefly presented, masked pseudowords, of which half had appeared in the prior study list and half had not. Priming was reflected in enhanced performance with studied compared to unstudied pseudowords. In the recognition task, 64 pseudowords were presented one at a time; subjects were asked to indicate which ones had appeared in the prior study list. Relative to NCS, AD patients showed impaired recognition memory and normal priming. We conclude that intact perceptual priming in AD extends to novel stimuli; that perceptual priming of words and pseudowords may reflect the operation of a unitary neural mechanism (which is independent of the mechanism supporting recognition memory); and that such priming may depend upon posterior (occipital) circuits that are relatively preserved in AD.

5.2

INTACT REPETITION PRIMING FOR TIME TO NAME PICTURES IN PATIENTS WITH ALZHEIMER'S DISEASE: DISSOCIATIONS FROM RECOGNITION MEMORY AND FROM WORD-COMPLETION PRIMING. J.D.E. Gabrieli, W.S. Francis*, D.A. Grosse*, and R.S. Wilson. Rush Alzheimer's Disease Center and Department of Neurological Sciences, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612.

Patients with Alzheimer's disease (AD) have impaired memory and reduced repetition priming (influence of repeated processing of a stimulus) on word-completion tests. There are reports of intact magnitudes of perceptual priming in AD, but these findings have been complicated by AD patients' baseline performance deficits. In the present study, 12 AD patients and 12 normal control (NC) subjects named 58 pictures of objects and animals; naming latencies were recorded. Next, subjects saw 29 pairs of pictures in a two-choice recognition memory test. Finally, subjects named the remaining 29 drawings named earlier but not included in the recognition test. The measure of priming was the reduction in time to name the repeated pictures. The AD patients were significantly less accurate on the recognition memory test than the NC subjects, but had a normal magnitude of priming without a significant difference in baseline naming latency. The same AD patients exhibited impaired priming on two word-completion tasks (one had the names of the pictures as the stimuli). The unambiguous dissociation between preserved picture-naming priming and impaired word-completion priming reveals that separable memory systems subserve distinct components of repetition priming. This result is consistent with the notion that perceptually-based repetition priming remains intact in early AD, perhaps due to selective sparing of posterior cortical areas that mediate perceptual priming. Supported by Alzheimer's Association and Illinois Department of Public Health.

5.3

INTACT PERCEPTUAL PRIMING OF NOVEL INFORMATION IN AMNESIA AFTER A SINGLE STUDY TRIAL. F. Haist,[1,2] G. Musen[1] & L.R. Squire.[1] [1]VA Med. Ctr. and [1]UCSD Dept. of Psychiatry, La Jolla, CA 92161, and [2]SDSU Dept. of Psychology, San Diego, CA.

Amnesic patients, who are severely impaired on conventional tests that assess explicit (declarative) memory, can exhibit entirely normal performance on implicit (nondeclarative) memory tests that do not require conscious recollection of prior events. For example, amnesic patients exhibit intact priming, i.e., an improved ability to detect or identify stimuli based on recent encounters with them. It has been unclear whether implicit memory can support the acquisition of novel information or whether implicit memory depends on the activation of pre-existing knowledge structures. We examined whether priming can support the acquisition of novel verbal information presented for only a single study trial. At test, amnesic patients (n=10) and normal subjects (n=15) were shown words and pronounceable nonwords for an average of 51 msec and 118 msec, respectively. Half of the items had been studied once prior to test and half had not been encountered previously. The presentation time was sufficient to permit about 50% of the unstudied items to be identified correctly. Amnesic patients and normal subjects identified significantly more studied words than unstudied words (27% and 24% facilitation, respectively) and also identified more studied nonwords than unstudied nonwords (18% and 20% facilitation, respectively). These results support the idea that perceptual priming depends on changes in early-stage perceptual priming systems and that these changes can result in the acquisition of new information, not simply the activation of pre-existing knowledge.

5.4

INTACT, LONG-LASTING FACILITATION OF PICTURE NAMING IN AMNESIC PATIENTS. C.B. Cave and L.R. Squire, VA Med Ctr, and Dept of Psychiatry, UCSD, La Jolla, CA 92093.

Eleven amnesic patients and 9 control subjects were shown 130 pictures, one at a time, and named the pictures as quickly as possible. Then both 2 and 7 days later, 50 new pictures were presented together with 50 old pictures, and subjects again named them as quickly as possible. Control subjects and patients named previously seen pictures significantly faster than pictures not seen before (facilitation = 120 msec for controls, and 129 msec for patients). Similar facilitation was observed at each delay. In contrast, at both delays, amnesic patients were much poorer than control subjects at recognizing items that had been seen previously. Normal picture-naming facilitation in amnesic patients, together with their poor recognition performance, provides a striking dissociation between long-lasting repetition priming and explicit memory.

Facilitation of picture naming could depend on repetition of the same visual percept or on repetition of any visual stimulus that has the same name. In a second study, 14 normal subjects named pictures once and again 3 minutes later. At the second test, the pictures were of 4 types randomly intermixed: 1) pictures identical to those seen earlier; 2) pictures presented earlier, but changed in size or shading; 3) different examples of the objects presented earlier (e.g. a beagle instead of a retriever, but both identified as "dog"); or 4) pictures not presented previously. Facilitation was found to be based both on repetition of object names and on repetition of exact visual percepts. Repeated items (whether unchanged or different in size, shading, or example) were named significantly faster than new items, indicating facilitation due to the repetition of object names (regardless of the specific physical appearance of the stimuli). Identical pictures were named significantly more rapidly than pictures that were changed in size or shading, or pictures that were different examples of previously presented objects. This finding indicates that additional facilitation is also supported by repetition of exact visual percepts.

5.5

NORMAL ACQUISITION OF COLOR-WORD ASSOCIATIONS IN AMNESIC PATIENTS. G. Musen & L.R. Squire VA Med Ctr & UCSD Psychiatry Dept., La Jolla, CA 92093.

Recent research has considered whether implicit (nondeclarative) memory can support the learning of new associations. Acquisition of novel word pairs (such as valley-trophy) occurs in both normal subjects and amnesic patients after multiple study trials (Musen & Squire, Soc. Neurosci. Abstr. 16:287, 1990). We investigated whether the learning of new associations might also occur for stimuli other than word pairs. We used a color naming task in which 7 color words (e.g., red, green, blue) were presented in an incongruent color, i.e., the word *green* was printed in the color *red* as in the familiar Stroop paradigm. Amnesic patients and age-matched control subjects named the color a word was printed in as quickly as possible. Each color word was presented 6 times, always in the same incongruent color. Thus, the word *green* was always printed in the color *red*. Then the color-word associations changed such that the word *green* was always printed in the color *blue*. Amnesic patients and control subjects improved with repetition to the same extent. Specifically, the time required to name each color decreased gradually across the 6 repetitions of the color words (amnesic patients from 712 msec to 580 msec; control subjects from 760 msec to 632 msec). Naming times then increased when the color-word associations changed (amnesic patients 683 msec; control subjects 632 msec). This study provides evidence for gradual acquisition of new associations in implicit (nondeclarative) memory across multiple trials.

5.6

NORMAL ACQUISITION OF AN ARTIFICIAL GRAMMAR BY AMNESIC PATIENTS. B.J. Knowlton, S. Ramus, & L.R. Squire. VA Med. Ctr. and Dept. of Psychiatry, UCSD, La Jolla, CA 92093.

Study of amnesic patients has revealed a distinction between explicit (declarative) memory, and implicit (nondeclarative) memory. Examples of implicit memory include skill learning, priming, and simple forms of conditioning. Normal subjects can acquire an artificial grammar based on rules that determine the order of letters within letter strings. Conscious knowledge of the rules appears inadequate to account for performance, suggesting that this learning is implicit. If so, amnesic patients should be capable of acquiring artificial grammars normally, despite their severely impaired explicit memory. Ten amnesic patients and 13 control subjects were shown 23 letter strings, which were formed according to a finite-state rule system. After 5 min, subjects were told that the items had been formed according to a complex set of rules, and they were instructed to decide for each of 46 new items whether the item obeyed these rules. Control subjects classified 65.0% of these new items correctly, compared to 62.0% for the patients. In contrast, the patients performed much worse than control subjects on a 46-item recognition test for the letter strings that had been presented (73.1% vs. 59.6%, $p < .005$). These results provide strong evidence that artificial grammar acquisition is based on implicit memory, and that the learning of such rules depends on brain structures other than those damaged in amnesia.

5.7

A FUNCTIONAL ANATOMICAL STUDY OF HUMAN MEMORY. L. Squire*, J. Ojemann, F. Miezin, S. Petersen, T. Videen, and M. Raichle. *UCSD Dept. of Psychiat., VA Med. Ctr., San Diego, CA, 92161. Dept. of Neurol. and Neurol. Surg. Washington Univ. Med. Ctr., St. Louis, MO 63110.

We studied regional cerebral blood flow using the [^{15}O] H_2O method while normal subjects performed four closely similar tasks. In each task, subjects saw words and then saw 3-letter word stems. Local blood flow was monitored during a 40-sec period while subjects 1) silently viewed word stems; 2) completed stems to form the first words to come to mind, but the stems could not form study words (baseline); 3) completed stems and half of them could form study words (priming); or 4) tried to recall study words, and half of the stems could form these words (memory). There were three major findings: 1) The memory task engaged the right hippocampal region (but not the amygdala), when the memory task was compared to either baseline or a fixation-point condition. The right hemispheric locus suggests that performance is driven by the visual characteritics of the words rather than by semantic or phonetic analysis. 2) Right prefrontal cortex was also activated in the memory minus baseline condition. 3) In the priming minus baseline comparison, there was reduction in blood flow in the right posterior cortex. The results provide the first evidence for selective activation of the human hippocampal region in association with memory function. The results also provide a specific proposal about the neural basis of priming: following presentation of a stimulus, less neural activity is required to process the same stimulus.

5.8

SUCCESSFUL EYEBLINK CLASSICAL CONDITIONING IN H.M. D. S. Woodruff-Pak[1] & S. Corkin[2], Department of Psychology, Temple University[1], Philadelphia, PA 19126 & Department of Brain and Cognitive Sciences and Clinical Research Center, MIT[2], Cambridge, MA 02142

Amnesic patients with medial temporal-diencephalic lesions show impaired explicit memory (recall and recognition) but normal implicit memory (skill learning and priming), suggesting that the latter capacities do not require hippocampal integrity. Another kind of implicit memory performance, classical conditioning, does not depend upon the hippocampus in rabbits and may not in humans as well. We therefore evaluated classical conditioning in the amnesic patient, H.M. (aged 64). He had undergone bilateral removal of medial temporal-lobe structures (including the anterior 8 cm of the hippocampus) in 1953 for seizure control; long-term dilantin therapy is believed to have damaged his cerebellar vermis and hemispheres. First, in a delay paradigm we used a 500 msec, 1 KHz, 80 dB SPL tone conditioned stimulus (CS), followed 400 msec after its onset by a 5 psi, 100 msec corneal airpuff unconditioned stimulus (US). In a series of 90-trial, 45-minute sessions, H.M. required 473 trials to reach learning criterion, producing 10 consecutive conditioned responses (CRs). Throughout 8 sessions, he never recalled or recognized the conditioning apparatus or procedure. A healthy 66-year-old control subject reach learning criterion in 316 trials. Five weeks after training in the delay paradigm we conditioned H.M. in a trace paradigm (250 msec, 1 KHz, 80 dB SPL tone CS, 500 msec blank "trace" period, 100 msec corneal airpuff US). He reached criterion in 91 trials. We then repeated the initial delay paradigm, and H.M. attained criterion in 277 trials. We conclude that humans (like rabbits) can acquire the eyeblink CR without the hippocampus intact. Cerebellar hemispheric atrophy may have slowed H.M.'s acquisition. *Supported by grants from the Alzheimer's Association and from NIH, RR-00088.*

5.9

NAME DROPPING: RETRIEVAL OF PROPER OR COMMON NOUNS DEPENDS ON DIFFERENT SYSTEMS IN LEFT TEMPORAL CORTEX. A.R. Damasio, J.P. Brandt, D. Tranel, & H. Damasio. Div. of Cognitive Neuroscience, University of Iowa College of Medicine, Iowa City, IA 52242.

Damage to human temporal cortices causes striking dissociations between retrieval of concepts and of the nouns they denote. We present new evidence on the dissociation between retrieval of common and proper nouns. Visual recognition and naming were investigated in (a) 30 controls, and (b) 14 subjects with lesions in left or right inferotemporal (IT) region or temporal pole (area 38). Stimuli were 77 *unique* entities (familiar faces, whose naming requires a proper noun) and 155 *non-unique* entities (which require a common noun). Results: (1) Damage to both left area 38 and IT impairs retrieval of both proper and common nouns, but spares recognition. (2) Damage restricted to left area 38 (sparing IT) impairs retrieval of **proper** nouns but allows normal retrieval of **common** nouns (recognition of unique entities is intact). (3) Damage to right 38/IT does not seem to affect naming. We propose that the lesions disrupt a bidirectional access device which operates as a third party mediator between concept retrieval systems and word reactivation systems. Such devices are based in left temporal cortices, and appear to be placed hierarchically, e.g., the device concerned with proper nouns depends on a cortical station located more anteriorly than the one concerned with common nouns.

5.10

OVERT AND COVERT FACE RECOGNITION: A DOUBLE DISSOCIATION. D. Tranel, H. Damasio, & A.R. Damasio. Div. of Cognitive Neuroscience, University of Iowa College of Medicine, Iowa City, IA 52242.

Familiar faces are normally recognized at both overt and covert levels but one of the two levels may be missing. In the most typical dissociation, patients with face agnosia caused by occipito-temporal damage cannot perform recognition of the identity of familiar faces at *overt* level. However, many such patients can give discriminatory *covert* responses to the familiar faces they fail to identify. For example, in 4 face agnosics we found that the average skin conductance response (SCR) to familiar faces was 0.66 μS, while the average SCR to unfamiliar faces was 0.04 μS. We now report the opposite dissociation: a patient who has entirely normal **overt** identification of familiar faces but who fails to generate discriminatory **covert** responses to those faces. The patient [EVR-318] has bilateral ventromedial frontal lesions. His overt face identity recognition is 100% correct, in both retrograde and anterograde compartments. At covert level, however, he is profoundly impaired: SCR magnitudes generated to familiar retrograde and anterograde faces, respectively, were 0.04 μS and 0.00 μS (despite normal SCRs to "orienting" stimuli). Taken together, these findings indicate that the neural systems which process the somatic-based valence of stimuli, are separate from and parallel to those which process the factual, non-somatic information associated with those same stimuli.

5.11

MEMORY PROFILE OF A BILATERAL DIENCEPHALIC INFARCT PATIENT WITH PRESERVED INTELLIGENCE AND SEVERE AMNESIC DISTURBANCES. H.J. Markowitsch*[1], D.Y. von Cramon*[2] and U. Schuri*[2] (SPON: ENA), [1]Department of Psychology, Ruhr-University Bochum, P.O.B. 102148, D-4630 Bochum and [2]Department of Neuropsychology, City Hospital Munich-Bogenhausen, D-8000 Munich, Germany

The case of a patient with above-average intelligence and an IQ-MQ difference of 40 points is documented. Extensive neuroradiological material of his focal bilateral brain damage in the dorsal diencephalon is available. A wide range of tests on all aspects of intelligence, attention, immediate retention, learning, skill and problem solving abilities, concept formation, cognitive flexibility, priming, constructional ability, and retrograde memory was used. The patient's shortterm memory and attention were well above average. He gave a number of examples of still intact skill and implicit memory abilities, but had no awareness of his severe anterograde and retrograde amnesia. The results from our patient confirm the dichotomy between declarative and non-declarative mnestic functions and demonstrate that disconnecting portions of the medial and basolateral limbic circuits have devastating consequences on memory.

5.12

EFFECT OF SEPARATE VS COMBINED LESIONS OF AMYGDALA, HIPPOCAMPAL FORMATION AND BASAL FOREBRAIN ON HUMAN MEMORY. **P.J. Eslinger, Neurology and Behavioral Science, Penn State University College of Medicine, Hershey, PA 17033.**

Experimental animal studies indicate different effects of separate and combined lesions of medial temporal lobe structures on learning and memory. As a direct test of human brain function and related hypotheses derived from animal studies, standardized methods of anatomic localization and cognitive study were applied to 7 human cases with acquired, focal lesions of the amygdala (A), hippocampal formation (HF), and basal forebrain region (BF). Comparisons were made between lesion location within the left hemisphere and verbal learning and memory abilities. Anatomic study indicated cases of separate lesion to A, posterior HF, BF (n=3), and of combined lesion to A+HF and A+HF+BF. The combined lesions were also associated with anterior temporal cortex damage from herpes simplex encephalitis. The A+HF+BF lesion produced the most severe verbal amnesia, followed by A+HF. BF lesions alone produced moderate impairment and amnesia. Separate lesions to A and posterior HF (sparing rostral hippocampus and entorhinal cortex) produced only minor impairment and no verbal amnesia. Together with other human and animal studies, findings indicate that: (1) Combined lesions result in severe amnesia, (2) damage to A alone and to posterior HF alone produces only minor disturbance, (3) HF lesion probably requires strategic hippocampus or entorhinal cortex damage to produce amnesia, (4) BF lesions alone produce amnesia and have an additive effect on A+HF lesions.

5.13

AMNESIA FOLLOWING ANTERIOR COMMUNICATING ARTERY ANEURYSM: PROPOSED CERBRAL MECHANISMS. J. DeLuca. Kessler Institute for Rehabilitation, West Orange, NJ 07052.

Aneurysm of the anterior communicating artery (ACoA) in humans frequently results in a cluster of neurobehavioral symptoms often referred to as the "ACoA syndrome." These include a "Korsakoff-like" amnesia, confabulation and personality change. Two hypotheses concerning the neuropathologic mechanisms have been proposed: 1) A focal lesion hypothesis involving anterior cerebral structures, and 2) diffuse cerebral involvement. Two studies are presented addressing the issue of neurobehavioral mechanism, examining confabulation and amnesia.

Two groups were examined in both studies: patients with ACoA aneurysm and those with intracranial hemorrhages elsewhere in the brain. In study I, confabulation was observed during two naturally occurring conditions: disoriented and oriented periods. Confabulation was more frequent, severe, and persisted into the oriented phase in the ACoA group. In study II, neuropsychologic testing revealed no differences among groups on tests of immediate memory, but ACoA subjects were significantly more impaired on tests of delayed verbal recall. The ACoA group also performed significantly worse on tests of frontal lobe functioning. Results support a focal lesion hypothesis, and suggests both basal forebrain and frontal lobe involvement.

5.14

DEFICITS IN RECALL, BUT NOT RECOGNITION FOLLOWING POSTERIOR COMMISUROTOMY. E.A. Phelps[1], W. Hirst*[2], M.S. Gazzaniga[3]. [1]Center for Neural Science, New York Univ., NY., N.Y. 10003, [2]New School for Social Research, [3]Dartmouth Medical School.

The role of the hippocampal commissure in memory functioning was examined in three studies. The first compared pre and post-operative performance on the logical memory and visual reproduction subtests of the Weschler Memory Scale for patients receiving either anterior or posterior sectioning of the commissure. Posterior sectioning generally includes the hippocampal commissure while anterior sectioning does not. A deficit in both visual and verbal recall was found in patients with posterior sectioning, but not those receiving anterior sectioning. The second study examined pre and post-operative verbal memory performance in two anterior sectioned patients. Patients were given four lists of 20 words both pre and post-operatively. Each word was presented for 5 seconds followed by a retention interval of either 0 sec., 30 sec., 2 min., or 1 hour. Patients were then tested with free recall followed by forced-choice recognition. There was no deficit in either recall or recognition memory following sectioning of the anterior commissure at any of the retention intervals. The final experiment examined the nature of the memory deficit following complete commissurotomy, including the hippocampal commissure. Two split-brain patients and control subjects were presented 8 lists of 20 words. Each word was presented for 5 seconds followed by a 10 minute retention interval. Subjects were tested with free recall followed by forced-choice recognition. Patients with complete commissurotomies showed a deficit in recall, but not recognition in comparison to normal controls. This finding is consistent with the finding of relatively preserved recognition in amnesic patients and suggests that the mnemonic role of the hippocampal commissure may be more important for recall than recognition memory.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY I

6.1

SYSTEMIC ADMINISTRATION OF L-CYSTEINE (CYS) GREATLY ELEVATES CEREBRAL CYSTEINE SULFINATE (CSA) LEVELS AND PRODUCES HYPOGLYCEMIA-LIKE HIPPOCAMPAL PATHOLOGY. A. Lehmann, O. Orwar* and M. Sandberg*. Inst. of Neurobiol., Univ. of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden.

When administered systemically to infant animals, CYS produces a widespread brain damage mediated by NMDA receptors. It is not known if CYS is directly neurotoxic, or if its oxidation product CSA mediates the toxicity. In the present study, the effects of toxic doses of CYS on cortical CSA levels were determined in 4-days-old rats. Changes in the CYS metabolites alanine and taurine were also followed. Further, the distribution of CYS-induced damage was reinvestigated. S.c. injection of 1 mg/g CYS produced a 19-fold increase in cortical CYS levels after 1 h after which time they declined. There was a delayed increase in CSA and alanine amounting to 18 and 3 times of control levels, respectively, after 6 h. Taurine decreased after CYS injection, while most other amino acids increased. With the exception of CYS, CSA and alanine, CYS-induced alterations in other amino acids were prevented by the NMDA antagonist MK-801, suggesting that MK-801 does not interfere with cerebral uptake and metabolism of CYS, and that changes in other amino acids are caused by CYS neurotoxicity. The concentration of CSA was 0.1 µmol/g protein after 6h. Provided that this value chiefly reflects intracellular levels, it seems unlikely that CSA mediates CYS toxicity. At 0.5 mg/g, CYS inflicted a selective damage to the cingulate and medial parietal cortices. At 1.0 mg/g, CYS caused massive hemorrhages and a severe injury in many forebrain regions, especially in the cortex and hippocampus. In the latter region, damage was confined to neurons exposed to CSF indicating that the major route of uptake of CYS is across the choroid plexus. Although MK-801 (1.0 µg/g), but not the non-NMDA antagonist NBQX (15 µg/g), prevented CYS toxicity, the distribution of hippocampal injury did not correlate with the distribution of NMDA receptors previously reported by others.

6.2

GLUTAMATE MODULATES PHOSPHOLIPASE A_2 (PLA_2) ACTIVTY IN CULTURED CORTICAL NEURONS. G.Rordorf*, W.J.Koroshetz, R.A.Nemenoff* and J.V.Bonventre. Departments of Medicine and Neurology, Massachusetts Genl. Hosp., Harvard Med. Sch., Boston, MA 02114.

We have recently demonstrated that PLA_2 activity is stably enhanced in gerbil brain with ischemia and reperfusion. Using cultured rat cortical neurons we found that glutamate increases PLA_2 enzymatic activity. We have further characterized PLA_2 activity in cultured cortical neurons. PLA_2 activity was assayed in cell free extracts, by directly measuring release of arachidonic acid (AA) from exogenous [^{14}C]AA-phospholipid. Two forms of the enzyme are present in the cell cytosolic extracts, a larger molecular weight form and a smaller form with a Mr of approximately 14 kDa. Glutamate enhances Ca2+ sensitivity and the activity of the smaller form. Both forms are Ca^{2+}- dependent with optimal activities at pH 8.5. Phorbol 12,13-mystrate acetate (PMA) (but not an inactive form of phorbol ester), and a calcium ionophore, stimulate PLA_2 activity. Downregulation of protein kinase C (PKC) activity by chronic exposure to PMA reduces the stable glutamate-induced PLA_2 activation by one-half. These results suggests that PKC plays an important role in the upregulation of PLA_2 activity seen with glutamate. The glutamate-induced stable enhancement of PLA_2 activity in cells occurs prior to cell death as determined by LDH release. These results suggest that PLA_2 may play a central role as a causative factor in glutamate-induced toxicity.

6.3

DOES PROTEIN KINASE C PLAY A ROLE IN THE DEVELOPMENT OF SLOWLY-TRIGERRED EXCITOTOXICITY IN CORTICAL CELL CULTURES? D.M. Hartley and D.W. Choi, Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

The neuroprotective effect of gangliosides against glutamate-induced neurotoxicity has been proposed to be due to interference with protein kinase C (PKC) function (Manev et al., FASEB J. 4:2789, 1990). Murine cortical cell cultures exposed to 15 μM NMDA or 35 μM kainate + 10 μM MK-801 for 24 hr developed submaximal neuronal degeneration. A two hour pretreatment with 200 μM GT1b reduced NMDA-induced injury approximately 80-90%. This neuroprotection was specific to NMDA-induced cell death, as neither GT1b nor GM1 blocked kainate-induced cell death.

Does inhibition of PKC mimick this protective effect of gangliosides? A 24 hr pretreatment with 0.1 to 10 μM phorbol 12-myristate 13-acetate down-regulated PKC activity in our cultures, approx. 90-95%, as measured by phosphorylation of the altered pseudosubstrate peptide, [(ser)25 PKC (19-31)]. However, sister cultures exposed to similar conditions showed little reduction in NMDA- or kainate-induced death. Furthermore, staurosporine at > 1.5 μM blocked more than 90% of PKC activity, but concentrations up to 10 μM did not reduce NMDA- or kainate-induced death. We speculate that the neuroprotective effect of GT1b on cortical neurons may be mediated at least in part by a mechanism other than reduction of PKC function (the alpha, beta, and gamma isoforms).

6.4

RAPID CA1 INJURY INDUCED BY NON-NMDA AGONISTS IN HIPPOCAMPAL SLICES. R.A. Wallis, K.L. Panizzon, J.M. Boring* and C.G. Wasterlain. Dept. of Neurology, Sepulveda VAMC and UCLA Sch. of Medicine, Sepulveda, CA 91343.

In dissociated cortical cultures, NMDA induces rapid neuronal injury while non-NMDA causes slow neuronal injury. We examined the non-NMDA excitotoxicity in the CA1 sector of hippocampal slices, using the CA1 population spike (PS) amplitude as a physiological end point. An 8 min. exposure to AMPA (50 μM) induced 98.4% ± 1.7 (SE) damage of the antidromically evoked CA1 PS. This damage was blocked by 100 μM DNQX, provided DNQX was continued for 60 min. during recovery. Low calcium, dantrolene (20 μM), MK-801 (32 μM) and azelastine (15 μM) offered little protection. Trans-ACPD (up to 200 μM) for 35 min. failed to cause significant CA1 neuronal injury. An 8 min. exposure to kainic acid (65 μM) caused severe CA1 neuronal damage (recovery 19.9% ± 6.6) which was blocked by DNQX (100 μM) but not by MK-801, dantrolene or azelastine. These data suggest that in the hippocampal slice, non-NMDA agonists can produce rapid neuronal injury through activation of AMPA and kainic receptors, while stimulation of "metabotropic" receptors alone is not excitotoxic.

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6.5

TWO MECHANISMS OF KAINATE NEUROTOXICITY IN RAT CORTICAL NEURONS *IN VITRO*. A.G. Knapp, C.J. Kirk* and T.J. Wolcott*. Cambridge NeuroScience, Inc., Cambridge, MA 02139.

To clarify the mechanisms by which excitatory amino acid agonists can cause neuronal damage, we have examined the time course and concentration dependence of kainic acid neurotoxicity in cultured rat cortical neurons. Neurons were enzymatically dissociated from neonatal rats, grown in 96-well tissue culture plates for 15-17 days and then exposed to kainate (1-1000 μM) for varying durations (5 minutes to 24 hours). Cell death was measured by automated colorimetric assay of lactate dehydrogenase released into the culture medium 24 hours after the start of the exposure. In contrast to previous reports, we have found that exposures to kainate as brief as 5 minutes are sufficient to kill more than 90% of neurons. The concentration-response relationship for this rapid component of excitotoxicity was very steep (Hill slopes > 3), with a threshold near 100 μM and half-maximal damage occurring at 200-300 μM. Neuronal death caused by brief (5-30 minutes) exposures to kainate could be almost completely prevented by both competitive (APV) and non-competitive (MK-801) antagonists of NMDA receptors. With longer exposure durations, kainate damaged neurons at lower concentrations (EC_{50} = 40-60 μM at 24 hours) and the concentration-response relationship became less steep (Hill slopes 1-2). This slower form of neurotoxicity was insensitive to NMDA antagonists, but the competitive non-NMDA receptor antagonist DNQX (1-10 μM) shifted the concentration-response curve to the right in a dose-dependent manner. We propose that the rapid form of kainate neurotoxicity results from indirect activation of NMDA receptors by release of an endogenous NMDA agonist (e.g. glutamate) from the cultures or by potentiation of basal amounts of such an agonist. The slower form of toxicity likely reflects kainate's direct actions on non-NMDA receptors.

6.6

CALCIUM ACCUMULATION IN EXCITOTOXIC BRAIN LESIONS: TIME COURSE AND CORRELATION WITH CELL DEATH. J.B.P.Gramsbergen and A.J. van der Sluijs-Gelling*. Erasmus Univ. Rotterdam Sch of Med and TNO Med Biol Lab, Rijswijk, Netherlands.

In order to examine the relationship between calcium overload and excitotoxic cell death, we investigated the time course of regional ^{45}Ca accumulation in the rat brain following intrastriatal quinolinic acid (QUIN) or systemic kainic acid (KA) administration using semi-quantitative $^{45}CaCl_2$-autoradiography and radioactivity counting of dissected tissues. In addition, using different doses of QUIN, the ^{45}Ca contents in the basal ganglia were correlated with biochemical or histological markers of cell dysfunction or death in those areas. One week after QUIN striatal ^{45}Ca accumulation was highly correlated with reductions of striatal glumatic acid decarboxylase (GAD; r=0.9) and with histological damage of the same brain sections. Time course studies of 25 ug QUIN rats showed that, although GAD was maximally reduced (74%) at day 2, striatal ^{45}Ca continued to accumulate up to day 42 (671%), suggesting that ^{45}Ca was also trapped in extracellular calcium deposits. Several hours after KA (12 mg/kg) both CA_3 and CA_1 cells in the hippocampus were labeled, whereas at day 2 ^{45}Ca could not be detected in CA_3 and was diminished in CA_1. However, 1 and 2 weeks following KA ^{45}Ca accumulated again in CA_1, but not in CA_3. Thus, CA_3 pyramidal cells seem to die quickly, whereas CA_1 neurons die more slowly or can accumulate more ^{45}Ca before death.

6.7

SERUM ALBUMIN STRONGLY POTENTIATES GLUTAMATE NEUROTOXICITY IN CULTURED RAT CEREBELLAR GRANULE CELLS. M. SCHRAMM AND S.EIMERL*, Department of Biological Chemistry, Institue of Life Sciences, Hebrew University of jerusalem, Jerusalem 91904, Israel.

Addition of 1% serum to 14 day cultured granule cells in a Locke salt-glucose solution caused acute cell death. The glutamate in the serum, acting on the NMDA receptor, in conjunction with a putative potentiating factor, accounted for the acute toxicity (M. Schramm et al., Proc. Natl. Acad. Sci. USA 87,1193-1197,1990). Serum albumin appears to be the potentiating factor, showing no toxicity on its own, but boosting toxicity of glutamate 3-10 fold. Bovine serum albumin (BSA), regular or fatty acid-free, or human serum albumin, were all about equally effective, with maximal enhancement at 2 mg/ml. Casein was the only substance, among some other polymers tested, which produced enhancement of toxicity. Potentiation by BSA occurred in presence and absence of Mg^{++}, with glutamate or NMDA as agonist. BSA was only effective when present together with the NMDA receptor agonist. Potentiation by BSA required 1 mM Ca^{++}, with a maximum effect at 1.7 mM. It is suggested that serum albumin, by magnifying threshold toxic activation, of the NMDA receptor may play an important role in neuronal degeneration.

6.8

AMANTADINE DERIVATIVES PREVENT NMDA RECEPTOR-MEDIATED NEUROTOXICITY. James W. Pellegrini*, H-S. Vincent Chen, Sizheng Z. Lei, Nikolaus J. Sucher and Stuart A. Lipton. Dept of Neurology, Children's Hospital & Progr. in Neurosci., Harvard Med Sch, Boston, MA.

Increasing evidence supports the hypothesis that escalating levels of excitatory amino acids are responsible for neuronal cell death in a variety of acute and chronic neurological conditions including hypoxia/ischemia, trauma, hypoglycemia, epilepsy, and degenerative diseases such as AIDS dementia. In some areas of the central nervous system, the predominant form of this neuronal injury is mediated by excessive stimulation of NMDA receptors. Recently, the anti-viral and anti-Parkinsonian drug memantine, a derivative of amantadine, has been shown to antagonize NMDA-evoked electrical currents in neurons, probably by a mechanism similar to that of MK-801 (J. Bormann, *Eur. J. Pharm.* 1989;166:591-2). Unlike MK-801, however, memantine and other amantadine derivatives are clinically tested drugs with relatively few side-effects at micromolar levels. Here, we show that memantine (6 μM) blocks NMDA-evoked electrical currents in primary cultures of central neurons, i.e., postnatal rat retinal ganglion cells. Also, we report that amantadine (200 μM) or congener memantine (6 μM) attenuates NMDA-induced increases in intracellular [Ca^{2+}] and prevents NMDA receptor-mediated neurotoxicity.

6.9

PENTAMIDINE, A NOVEL NMDA RECEPTOR ANTAGONIST IS NEUROPROTECTIVE *IN VITRO*. I.J. Reynolds and E. Aizenman. Depts. Pharmacol. and Physiol., U. Pittsburgh, Pittsburgh PA 15261.

Dementia frequently accompanies acquired immunodeficiency syndrome (AIDS). This may arise from excessive stimulation of NMDA receptors by either quinolinate or a similar neurotoxin produced by HIV-infected macrophages (Giulian et al, *Science* 250:1593, 1991). A prediction of this hypothesis is that an NMDA antagonist may delay or prevent AIDS related dementia.

We have found that pentamidine, frequently used to treat *P. carinii* infections in AIDS patients, is an effective and potent NMDA antagonist. Pentamidine inhibits [^{3}H] dizocilpine binding with an IC_{50} of about 2μM. It is non-competitive with glutamate, glycine and spermidine, and also slows the dissociation of [^{3}H] dizocilpine. Pentamidine also inhibits NMDA- and glycine induced increases in $[Ca^{2+}]_i$ in cultured cortical neurons at similar concentrations. The inhibition of whole-cell voltage clamped responses is independent of membrane potential, and, with the protocol used, does not show use dependence.

Approximately 70% of cultured cortical neurons die within 24hr of being exposed to 200μM NMDA for 30min. This excitotoxic action of NMDA was completely reversed by the addition of 5μM pentamidine during the NMDA exposure. However, concentrations of pentamidine above 30μM were directly neurotoxic when present for 24hr.

These findings suggest that pentamidine may be useful in treating AIDS-related dementia in addition to its antimicrobial activity.

6.10

HIV ENVELOPE PROTEIN gp120 AND THE NMDA RECEPTOR-CHANNEL COMPLEX. Stuart A. Lipton, Linda A. Wong, Maureen Oyola, Nikolaus J. Sucher, & Evan B. Dreyer. Dept. of Neurology, Children's Hospital & Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Picomolar concentrations of the HIV coat protein gp120 increase $[Ca^{2+}]_i$ and produce neuronal injury in rat retinal cultures; within 24 hr of exposure to 20 pM gp120, 15-50% of the retinal ganglion cell neurons die compared to controls (Dreyer et al., *Science* 1990;248:364). Previously, we found that calcium channel antagonists ameliorate this form of neurotoxicity as do the NMDA antagonists APV and MK-801 (Lipton et al., *Neuron*, in press). Here we report that in preliminary experiments gp120-induced neurotoxicity was partially attenuated by an antagonist of the glycine co-agonist site, 7-Cl-kynurenate (50 µM). Nevertheless, whole-cell recordings with patch electrodes failed to demonstrate any conclusive action of gp120 (20-600 pM) in evoking NMDA-like electrical responses or in enhancing NMDA- or glutamate-activated currents. To assess effects requiring intracellular messengers, we also recorded from retinal ganglion cells using the 'perforated patch' technique (n = 30). However, gp120 still produced no consistent NMDA-like effects. Thus, at 20-600 pM, gp120 apparently lacks a direct excitatory effect on the NMDA receptor-channel complex, and other actions must be sought either upstream or downstream from this site.

6.11

IMPAIRMENT OF TRANSMITTER GLUTAMATE SYNTHESIS AND KCL-INDUCED RELEASE BY PHENYL SUCCINATE:
A MICRODIALYSIS STUDY.
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Excessive activation of the glutamate (glu) transmission seems to be involved in the neuron damage after cerebral ischemia and kainate induced seizures. Besides blockade of postsynaptic glutamate receptors another possibility for reduction of glutamatergic transmission would be to decrease the pool of transmitter glutamate. Studies on cultured cerebellar granule cells (Paleiologos, Hertz, Schousboe 1988) have shown that phenylsuccinate (PS) is able to reduce KCl stimulated glutamate release by interfering with a ketodicarboxylate carrier in the mitochondrial membrane. We studied this in anaesthetized rats by means of stereotactically placed microdialysis fibers in dorsal hippocampi. Both fibers were perfused with 5 µl/min Krebs buffer; to the right fiber was added 50 mM PS. Fifty mmol KCl was periodically added to the perfusion fluid on both sides (10 min on, 20 min off). Sampling time was 5 min and the dialysates were analysed for aminoacids after OPA derivatization on a HPLC system with fluorescense detector. During the unstimulated period of sampling no side difference in glu concentration was observed. KCl induced distinct elevations of glu on the control side whereas the PS-treated side showed no release of glu. Thus also in vivo PS is able to reduce glu release; the significance of this is currently investigated in ischemia and kainate induced neurone damage.

6.12

NMDA RECEPTORS ON BRAIN CAPILLARIES MEDIATE COLD INJURY-INDUCED ACTIVATION OF POLYAMINE SYNTHESIS AND BLOOD-BRAIN BARRIER BREAKDOWN. H. Koenig*, A. Goldstone*, J.J. Trout*, C.Y. Lu* & Z. Iqbal. Neurol. Serv., VA Lakeside Med. Ctr. & Dept. of Neurol., Northwestern U. Med. Sch., Chicago, IL 60611

Focal cold injury (CI) induces blood-brain barrier breakdown (BBBB) & brain edema linked to a biphasic increase in ornithine decarboxylase activity (ODC-A) & polyamine (PA) levels in rat brain capillaries (BC). A rapid increase appears at 1-2 min coincident with BBBB, & lasts > 72 h (Koenig et al., J. Neurochem. 52:622-631, 1989). The ODC inhibitor DFMO, & verapamil, dexamethasone & aspirin block the CI-induced activation of the ODC/PA cascade & BBBB, & putrescine (PUT) reverses these effects (Koenig et al., 1983, 1989). The NMDA receptor antagonist MK-801 (1-10 mg/kg) also blocks the early (2 min) increase in ODC/PA & BBBB, monitored by fluorescein, [^{14}C]AIB, & HRP transport. NMDA evokes a conc.-dep. increase in ODC-A, PUT & transport of $^{45}Ca^{2+}$, [^{3}H]deoxyglucose & HRP in isolated BC. This response is Ca^{2+}-dep., blocked by AP5, MK-801 & DFMO, & DFMO inhibition is reversed by PUT. BC membranes show NMDA-displaceable L-[^{3}H]glutamate binding (K_d 50 nM, B_{max} 176 fmol/mg) & GLU + GLY stimulatable [^{3}H]MK-801 binding sensitive to AP5 & DFMO. These data suggest that NMDA-R are present on BC, are coupled to the ODC/PA cascade, upmodulate BC transport, & mediate BBB breakdown induced by CI.

VISUAL PSYCHOPHYSICS AND BEHAVIOR

7.1

WHEN POPULATION CODING SURPASSES SINGLE NEURON PERFORMANCE E. Zohary[1*], A. C. Sittig[2*], and Shaul Hochstein[1], Neurobiology Dept., Life Sciences Inst., Hebrew University, Jerusalem, Israel[1], Delft University of Technology, The Netherlands[2]

We recorded the activity of V1 neurons in the awake macaque monkey. Neural responses to gratings of different orientations or spatial or temporal frequencies indicate that the reliability of the most selective neurons in signalling a difference between two stimuli differing in one dimension may be comparable with psychophysical performance. Also, the channel capacity of some neurons reached the perceptual limits reported in one-dimensional absolute judgement tasks (around 2.5 bits). Thus, there are cases where behavior may be governed by the performance of single units. However, neurons in the visual cortex are typically selective to a number of stimulus dimensions, resulting in an ambiguity in relating the response level of a single neuron to the stimulus values. We show that a multi-dimensional stimulus may be coded reliably by an ensemble of neurons, using a weighted average population coding model. We simulated neurons with physiological parameters, modelling a two-dimensional case of orientation and spatial frequency tuning, and found that 10^3-10^4 neurons are needed to reach psychophysical discrimination levels. Introduction of each additional dimension requires 1.7 times the number of neurons in the ensemble to reach the same level of accuracy. Since the number of neurons in the hypercolumn is less than 10^5, only 3 to 5 dimensions could influence neuronal response. This provides another rationale for the existence of parallel processing pathways in vision.

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7.2

DIRECTION DISCRIMINATION THRESHOLDS AND RESPONSE VARIANCE IN AREA V1 AND MT OF THE MONKEY
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The ability of a direction-tuned neuron to discriminate two super-threshold patterns moving in different directions depends not only on the neuron's tuning curve but also on the variability of it's firing. We determined the tuning curves and the response variance of neurons in visual areas V1 and MT of the awake behaving monkey.

Response variance was about equal to the mean response rate in both areas. This suggests that the response variance of a MT cell arises from mechanisms inherent in the cell itself, rather than being inherited from its inputs since MT cells should otherwise achieve a lower variance by pooling the signals from many V1 cells.

From the above measurements we were able to derive a neuron's neurometric function for any firing level ('criterion rate'). This function plots the probability that the neuron fires above the criterion rate against the stimulus direction. From the neurometric functions derived for a range of criterion rates we calculated the cell's direction discrimination thresholds.

Discrimination thresholds followed a U-shape when plotted as a function of criterion rate. The best discriminations are achieved along the flanks of the neuron's tuning curve. This is in agreement with psychophysical adaptation studies which suggest that the channels involved in direction discrimination of super-threshold stimuli (rather than stimulus detection at threshold) are those with a preferred direction off the test directions. Our experiments further show that some MT neurons can discriminate differences as low as 1.5° similar to the ability of humans performing the same task.

7.3

LESIONS OF MT IMPAIR SPEED DISCRIMINATION PERFORMANCE IN THE JAPANESE MONKEYS (MACACA FUSCATA). E. Vandenbussche, R.C. Saunders and G.A. Orban. Laboratorium voor neuro- en psychofysiologie, K.U.Leuven, Belgium and NIMH, Washington D.C., U.S.A.

Monkeys (n=4) were trained to discriminate between two speeds (different or the same) of horizontally moving random dots presented in succession. By pressing on one of the two levers, the monkeys decided if they saw two bars with the same or a different speed. Although two stimuli were presented in each trial, control experiments showed that the monkeys based their discrimination solely on the second stimulus and thus used an absolute identification strategy.

The just noticeable differences (JNDs) in speed were measured using a staircase procedure over a range of reference speeds (2 to 128 deg/sec). JNDs in speed were minimal for intermediate reference speeds (of 16 to 64 deg/sec). Using the same stimulus set-up JNDs in speed were also measured in humans (n=2) and found to be very similar to those of the monkeys.

In three monkeys, area MT of both hemispheres was ablated by a combination of subpial aspiration (posterior bank of ST sulcus) and injections of ibotenic acid (floor of the sulcus). Animals were retested postoperatively for three months at the reference speeds of 8 or 32 deg/sec. In one animal JNDs increased from a Weber fraction of 15% obtained preoperatively to a Weber fraction of 40%. In two other animals the MT lesions had a more devastating effect. For these two animals no stimulus control was obtained for a difference in speed equal to 120%. Six months after the lesion stimulus control was obtained for one of the two animals, but not the other. The third monkey remained at the same level (40% Weber fraction) after six months. In control experiments these three monkeys were shown to perform postoperatively at a 73.5% level of correct responses in a Vernier acuity task for an offset of 1.5 minutes of arcs. In a second control experiment, area TE was ablated bilaterally in two monkeys without effect on speed discrimination.

7.4

PERMANENT DEFICITS IN SPEED DISCRIMINATION AFTER MT/MST LESIONS IN MACAQUE MONKEYS. William H. Merigan, Tatiana Pasternak, Vincent Ferrera, John H.R. Maunsell, University of Rochester Medical Center, Rochester, NY 14642.

Anatomical and physiological evidence suggests a central role for cortical areas MT and MST in motion processing, and behavioral lesion results are generally consistent with such a role. This study examined motion related psychophysical thresholds in two macaque monkeys, before and after large bilateral lesions were made of cortical areas MT and MST. Lesions were placed by making a grid of ibotenic acid injections in the anterior and posterior banks of the dorsal 7 mm of the superior temporal sulci. Histological reconstruction, completed in one monkey, showed the damage was centered on areas MT and MST, which were severely damaged. Some cortex in the region of V3 in the lunate sulcus was also damaged. Contrast sensitivity for detecting 1 c/deg drifting gratings over a wide range of drift rates was unaffected by the lesion. Moreover, the monkeys discriminated the direction of motion at contrasts near or just below detection threshold (.5-1.3%). On the other hand, the lesion produced deficits in the discrimination of stimulus speed. Prelesion, thresholds were about 10-13% of the speed of a 1 c/deg grating. Postlesion, the speed threshold was elevated about 2-4 fold at all grating contrasts. This elevation was still present at the time of the last testing, about five months after the lesions were made, and therefore can be considered essentially permanent. These results show that the ability to discriminate speed is reduced, but not abolished, by extensive lesions of areas MT/MST. Thus, extrastriate areas MT and MST do not appear essential to either detection or opposite direction discrimination measured as contrast sensitivity with drifting gratings. On the other hand, these areas appear important for the computation of the speed of stimulus motion, although some discrimination ability survives their removal.

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7.5

GLOBAL MOTION PERCEPTION AFTER MT/MST LESIONS IN A MACAQUE. Tatiana Pasternak, John H. R. Maunsell, Vincent Ferrera, William H. Merigan, University of Rochester Medical School, Rochester NY 14642.

Dynamic random-dot stimuli were used to study the cortical mechanisms that underlie the integration of local direction information into a global motion percept. In such stimuli, each dot is displaced with a constant step size in a direction chosen at random from a distribution, and the stimulus appears to move in the direction of the mean of the distribution. We examined the perception of global motion in two monkeys with ibotenic acid lesions of areas MT and MST (see Merigan et al, Soc. Neurosci. 1991). We measured range thresholds for the **discrimination of opposite directions** as a function of the fraction of dots that moved in random directions. When no dots moved randomly, the postoperative decrease in range threshold was modest (from 330° to 250-280°). This deficit increased when the proportion of randomly moving dots increased, and beyond 50% the monkeys were unable to perform the task. This compares to preoperative thresholds of 96% of the dots in the target moving randomly. Thus, the discrimination of opposite directions of global motion in the presence of directional noise was severely disrupted. The lesions also produced a 2-4 fold loss in **direction difference thresholds** that was independent of both stimulus speed and the range of directions in the global motion stimulus. These deficits, like those described above, persisted throughout the 5 months of postlesion testing.

Our studies demonstrate two effects of MT/MST lesions on direction sensitivity in the absence of directional noise: direction difference thresholds were elevated, and the range of directions that could be integrated to judge opposite direction was decreased. The relatively modest size of these effects suggests some sparing of direction integration and direction difference sensitivity after MT/MST lesions. On the other hand, the addition of even small amounts of directional noise severely compromised direction sensitivity, suggesting that these extrastriate areas may be most critical for preserving motion visibility in the presence of motion noise.

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7.6

SPEED DISCRIMINATION AND GLOBAL MOTION PERCEPTION IN PATIENTS WITH UNILATERAL POSTERIOR BRAIN LESIONS. L. M. Vaina,[1,2,3] M.LeMay,[4] N. Stratton,[1] and T. Kemper[2]. [1]Intelligent Systems Laboratory and [2]Neurology Department, Boston University, Boston, MA 02215; [3]Division of Health Sciences and Technology, MIT, Cambridge, MA 02139; [4]Harvard Medical School, Boston, MA 02115

Recent studies show that MT/MST lesions in the macaque produce severe deficits of speed discrimination and global motion perceptions. We have reported (1) that bilateral lesions involving the occipital-parietal-temporal junction in humans produce similar deficits. Here we show further examples of such deficits from patients with unilateral brain lesions. We will also show possible dissociations between impaired speed discrimination and global motion perception similar to that of our patient AMG reported at ARVO in 1991 (2). We will contrast both these results with the anatomical description of the lesions and with the performance on other motion psychophysical tasks.

(1) L.M. Vaina, et al. Visual Neuroscience, 1990, (V. 5)
(2) L.M. Vaina, et al. Inv. Oph. Vis. Sci., 1991, p. 824.

This research was supported by a grant from the National Institutes of Health, #EY07861-02.

7.7

REDUCTION IN DIRECTIONALLY SELECTIVE NEURONS EXTENDS SPATIAL LIMIT FOR GLOBAL MOTION Kirsten K. Rudolph, Tatiana Pasternak, and Vincent Ferrera, Center for Visual Science, University of Rochester, Rochester, NY 14627.

Global motion, the perception of coherent flow, requires integration of many localized direction signals. We used dynamic random-dots to study the neural mechanisms underlying this process. In such displays, each dot is displaced with a constant step size in a direction chosen at random from a specified range. We've shown that cats with severely reduced proportions of directionally selective cortical neurons (reduced DS) discriminate opposite directions of global motion at normal levels, but are unable to make judgments of direction difference more refined than 20-30 deg. In this study, we manipulated step size to determine whether the same pattern of results would hold over a range of spatial displacements. Opposite direction discrimination was tested in humans, normal, and reduced DS cats. As step size increased, range thresholds decreased for all observers, but reduced DS animals were less affected than normals and could do the task at step sizes beyond those which limited performance for other observers. Discrimination of small direction differences revealed the same pattern of results. Whereas the accuracy of normal observers decreased sharply as a function of step size, at intermediate and especially large step sizes (2.4-3.8 deg), animals lacking the majority of their directionally responsive cells did as well, and often better than normals. The spatial displacement limit for these animals was extended relative to that of normals.

We used autocorrelation to compute the signal-to-noise in the stimuli, and used this ratio as input to a modified line-element model. By varying spatial weights used in computation of the signal, good fits could be achieved for all observers. This result, together with the finding of improved performance with reduction in dot density suggest that spatial scale change in residual directional mechanisms, along with a reogranization of sampling in the visual systems of reduced DS cats, may account for the superiority of their performance at large step sizes.

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7.8

INVARIANCE IN APPARENT MOTION STRENGTH WITH VIEWING DISTANCE IS DUE TO CANCELING OF SEPARATE 2-D SIZE AND PROXIMITY EFFECTS. Michael E. Rudd and Paola Bressan*, Psychology Department, Johns Hopkins University, Baltimore, MD 21218 and Dipartimento di Psicologia Generale, Universita di Padova, 35139 Padova, Italy

The separate modulating effects of stimulus element luminance and size on the variation of apparent motion strength with 2-D proximity were studied using the stimulus display and competition paradigm of Shechter and Hochstein (Vis. Res. 29:579, 1989). Luminance-proximity and size-proximity interactions were analyzed on the basis of trials run in blocks in which luminance and size were held constant while proximity varied. This method allowed us to quantitatively model the interaction effects in the absence of confounds introduced in earlier studies.

Our results reveal a significant size-proximity interaction, consistent with earlier findings. Furthermore, we show that this interaction obeys a quantitative law which accounts for the previously reported "scale invariance" of motion strength with viewing distance (Burt and Sperling, Psych. Rev. 88:171, 1981): Z-scores for motion in the reported direction vary in proportion to the element diameter. This finding cannot be explained as an artifact of the covariation of element size and element interedge distance. No effects of either luminance or luminance flux were observed. The results are related to a recent model of motion computation by the cortex (Rudd and Grossberg, Neurosci. Abst. 16, 962, 1990).

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7.9

PERCEIVED VELOCITY OF GRATINGS VARIES WITH TARGET AND MOTION PATH TEMPORAL FREQUENCY. E. Katz, J. Victor and M. S. Gizzi. Department of Neurology and Neuroscience, Cornell University - Medical College, New York, and Department of Neurology, Mount Sinai School of Medicine, New York.

Last year we reported that the perceived velocity of an object is inversely related to the length of the motion path and thus positively related to the temporal frequency (TF) of the motion path (Katz et al. *Neurosci. Abstr.* 46.3, 1990). In the present study we measured perceived velocity of grating patches as a function of their true velocity, the TF of the grating and the TF of the motion profile. Vertical grating patches (2.2°x2.2°) moving at constant speed, back and forth for 1 cycle of a triangular motion profile were produced on a 608 Tektronix oscilloscope and viewed at 57 cm. Each trial consisted of randomly ordered sequential presentation of standard and test stimuli. Velocities were judged in a two-alternative forced-choice paradigm. The standard stimulus moved at 8.3°/s or 10.2°/s, along 4.0° or 4.8° motion path. The velocity of the test stimulus was 9.28°/s and its motion path varied from 0.55° to 4.4°. In addition, we manipulated the TF of both test and standard stimuli by presenting them at spatial frequencies of 0.91 cyc/° and 3.6 cyc/° in matched and mismatched conditions.

The data indicate that when standard and test stimuli were identical in grating TF and motion path TF, the 10% velocity difference was barely discriminable. When standard and test stimuli differed in grating TF but not in motion path TF, the stimulus with the higher grating TF was perceived as faster, independent of true velocity. Finally, when the stimuli differed in motion path TF, the stimulus with higher motion path TF was perceived as faster regardless of grating TF and true velocity.

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7.10

CROSS DIRECTIONAL INHIBITION IN HUMAN MOTION PROCESSING.

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Selective adaptation experiments have led to the notion of 'visual channels'. For instance adaptation to a pattern moving upward increases the threshold for seeing patterns moving upward, the effect decreasing as the angle between the adapting and test pattern increases until no effect is seen (typically when the patterns differ by more than 120 deg). This suggests that there are independent directional specific channels in human vision.

To test this notion I have used a 'double adaptation' procedure. Subjects adapt to a random dot pattern which alternates between two directions (A & B). If directions A & B activate the same channel threshold elevation to direction A should be greater than adapting to A alone; if A & B activate independent channels then elevation should be the same as A alone; whereas if there is inhibition between channels A and B elevation will be less than A alone. The results show that a clear inhibitory effect between patterns which differ by more than 100 deg, with the greatest inhibition when the patterns differ by around 120 deg.

Patterns which differ by 120 deg do not affect each others threshold for detection, yet appear to be inhibitory in the 'double adaptation' procedure. This suggests a 'divisive' inhibition. This notion was further tested by measuring threshold elevation as a function of adapting contrast either in the presence of absence of such an inhibitory pattern. Again the results clearly show that the inhibitory effect is divisive in nature. These results bear great resemblance to those reported for individual cells of the extrastriate area MT (Snowden et al, Soc. Neurosci. Abs. 16: 7.5, 1990).

7.11

COLOR SATURATION CONSTANCY

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In color constancy, the perceived color of an area depends on the relationship between the light from that spot and the light from its surround. These effects have been widely studied for the color dimensions of lightness and hue. We report such surround effects occur also for color saturation.

Colors appeared more saturated in a low-saturation surround than in a high-saturation surround, even when the mean lightness and chromaticity of the two surrounds were identical. Similarly, achromatic colors appeared blacker or whiter in surrounds with low contrast variance than in high contrast surrounds.

These saturation effects may be analogous to the well-known phenomena of induction and constancy for lightness and hue, but they are neither predicted nor explained by the usual accounts and models of color constancy. Saturation constancy implies compensatory expansion or compression of the range of colors present in a scene, without necessarily shifting the centroid (or neutral grey point) of those colors.

Color constancy is commonly considered as a matter of "discounting the illuminant". Most changes in the illuminant primarily affect lightness and chromaticity, with relatively minor effects on saturation. Color constancy, including constancy for saturation, may be important for additional visual functions, such as vision through haze, transparency, depth, etc.

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7.12

Surface representation vs features in visual search.

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Often implicit in the interpretation of visual search tasks is the assumption that the detection of targets is determined by the properties of early feature processing. In this report, we cast doubt on this view by manipulating binocular disparity so that supposed "features" can become parts of surfaces which render them less clearly distinguishable as targets and distractors. For example, an L next to a square (see figure beside) can appear to be part of an occluded surface particularly if its disparity is uncrossed with respect to the square. Similarly, an L in a slightly more complex display can appear as part of a transparent surface in front if the sign of binocular disparity is crossed and the luminance conditions are correct (Nakayama, et al., Perception, 1990). In a series of experiments manipulating binocular disparity, distractor number, and luminance ratios, we were able to show that the detection of a reversed L among other Ls was significantly impaired when the L formed part of a more complete surface. These results raise the possibility that visual search has little or no access to the level of feature extraction but must have as an input, a later process of surface representation.

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PATTERN FORMATION, COMPARTMENTS AND BOUNDARIES I

8.1

Eye morphogenesis in the zebrafish. E.A. Schmitt*, C. Fulwiler, L.V. Goodrich* & J.E. Dowling. Biological Laboratories, Harvard University, Cambridge, MA 02138.

As a foundation for genetic analysis of retinal development in zebrafish, we examined eye morphogenesis in wild type and haploid embryos by scanning EM and light microscopy. Here we describe novel features of eye development observed in the course of these studies.

After evagination of the optic vesicles between the 6 and 10 somite (s) stages, the vesicles of the zebrafish flatten as they extend laterally. Then upon contact with ectoderm, they rotate downward along the long axis of the embryo. A central crevice appears separating the distal and proximal neuroepithelia and will later form the optic lumenae. Shortly afterwards, an antero-posterior furrow becomes visible on the surface of the vesicle (15s). Invagination commences with a central depression in the furrow, spreading symmetrically across the vesicles. At the anterior end of the furrow a shallow groove marks the first appearance of the choroid fissure (16s) which will increase in depth along with the optic cup between 16 and 20s.

Between 1 and 1.5 days, ventral flexure of the head results in rotation of the eye by 90°. This brings the choroid fissure into a ventral position. In haploids, the fissure is abnormally wide and fails to close. Subsequently, as the surrounding ventral retina differentiates, the organization of cellular layers in this region is disrupted.

Our observations indicate that the epithelium which becomes neural retina originates in the dorsal half of the vesicle prior to its downward rotation.

8.2

Pattern regulation in the retina of the zebrafish mutant *cyclops*. C. Fulwiler, E.A. Schmitt*, & J. E. Dowling. Prog. in Neuroscience, Harvard Medical School and Biological Laboratories, Harvard Univ.

We examined retinal development in the γ-ray induced zygotic lethal mutation *cyc*-1(*b*16), obtained from Dr. C. Kimmel, U. of Oregon (Hotta, et.al., Nature 350:339, 1991) in order to investigate how synophthalmia affects retinal pattern formation. Plastic sections from mutants between 1 & 3 d. were compared with sections from wild type siblings.

In the *cyclops* eye at 1.5-2d, a midline inner retinal pattern develops between two laterally placed inner retinas. Two eye fields appear to have fused and induced a third field. The cellular and plexiform layers in the midline are continuous with the layers on either side.

By 3d., three outer retinal layers are apparent, one midline and one on each side. The photoreceptor, outer plexiform and horizontal cell layers are continuous across the midline, there being a gradual transition in cell polarities between them. Abnormal cell death is seen, primarily medially where the two eye fields overlap. We have tentatively identified new germinal cells moving into this region, perhaps to contribute to the emerging midline pattern. The result is a ventral to dorsal gradient of retinal cells with the oldest ganglion cells across the ventral margin and the youngest photoreceptors across the dorsal margin.

The effect of the *cyclops* mutation on the retina is probably indirect, reflecting a regulative response of cells to abnormal positional relationships created by the fusion of two eye fields. Our observations suggest that in the retina, interactions between neighbors may be more important to the generation and maintenance of the laminar pattern than information about a cell's position within the eye field.

8.3

CORRESPONDING ORTHOGONAL GRADIENTS OF TOP MOLECULES IN THE DEVELOPING RETINA AND OPTIC TECTUM. D. Trisler, S. Gill*† and J. Joshi*. Lab. of Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20892 and †George Washington Univ. School of Medicine, Washington, DC 20037.

The topographic map of cell position in the avian retina is conserved and inverted when retinal ganglion neurons synapse with neurons in the optic tectum. Developmental mechanisms based on molecular gradients that specify positional information have been postulated in the establishment of these topographic maps of cells in the retina and tectum. Two cell surface proteins in chicken retina, TOP_{DV} and TOP_{AP}, are distributed in dorsoventral and anteroposterior topographic gradients, respectively (Trisler, 1990, J. Exp. Biol. *153*: 11-23). Corresponding gradients of TOP molecules, present in the tectum, are inverted with respect to the retinal gradients; TOP_{DV} is more abundant in dorsal retina and ventral tectum and TOP_{AP} is more abundant in posterior retina and anterior tectum. Both TOP_{DV} and TOP_{AP} are present at embryonic day 2 and persist throughout development and in the hatched chick. Regulation of TOP expression was studied *in vivo* and *in vitro* using the quail neuroretina cell line, QNR/D (Pessac, *et al.*, 1983, Nature *302*: 616-617). Eye rotation and retinal sector ablation studies show that TOP_{DV} expression is determined before embryonic day 3. The level of TOP_{DV} expression throughout the retina is established by cell lineage. These orthogonal gradients of TOP_{DV} and TOP_{AP} molecules provide a possible Cartesian coordinate system for designation of cell position at all points in the retinotectal map.

8.4

MOLECULAR CLONING AND EXPRESSION OF TOP_{AP}; A GRADIENT MOLECULE IN THE CHICK RETINOTECTAL SYSTEM. J.M. Savitt*, D. Trisler and D.C. Hilt. Dept. of Biochemistry and Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201. Laboratory of Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20892.

TOP_{AP} is a 40 kD protein found in an exponential gradient in chick retina and tectum. Studies involving monoclonal antibodies have shown that the TOP_{AP} epitope is found preferentially in posterior retinal cells and their major projection area, anterior tectum. These studies and additional *in vivo* experiments suggest that TOP_{AP} may be involved in position-specific neural connections in the developing chick visual system. In order to better understand the structure and function of this molecule we constructed a cDNA expression library from posterior quadrant chick retina mRNA and screened 10^6 clones using an anti-TOP_{AP} monoclonal antibody. A single positive clone was identified, and subsequently expressed as a 45 kD Lac Z fusion protein which was recognized by the monoclonal antibody on Western blots. Analysis of the cloned sequence demonstrated a near full-length cDNA of approximately 3.1kb with an open reading frame coding for 371 amino acids. The translated sequence showed limited similarity to known protein-coding regions found in the NBRF database. Further analysis has shown that a single mRNA species of approximately 3.5kb codes for TOP_{AP} and appears to be preferentially expressed in posterior quadrant chick retina.

8.5

A ROSTROCAUDAL GRADIENT OF TRANSGENE EXPRESSION IN ADULT MOUSE SKELETAL MUSCLE. Maria J. Donoghue*, John P. Merlie, and Joshua R. Sanes. Departments of Anatomy and Neurobiology and of Molecular Biology and Pharmacology, Washington University Medical School, St. Louis, MO 63110.

We previously generated transgenic mice in which a promoter and enhancer from a myosin light chain (MLC) 1/3 gene are linked to a chloramphenicol acetyltransferase (CAT) gene. CAT expression is muscle-specific and developmentally regulated in these mice. Analysis of 35 individual muscles now reveals that CAT and CAT mRNA expression are positionally graded along the rostrocaudal axis. The gradient spans the entire body, and has a >100-fold range from lowest levels in muscles innervated by cranial nerves to highest levels in muscles innervated by lumbar and sacral nerves. The relationship between rostrocaudal position and level of transgene expression is not due to the site of transgene integration into the chromosome or to sequences in CAT. A novel histochemical stain for CAT reveals that transgene expression is fiber type-dependent (Type IIB>IIX>IIA>I), but that levels of CAT are positionally graded among fibers of each type. However, there is no gradation in the expression of the endogenous MLC 1/3 gene. Our interpretation of these results is that MLC sequences, when taken out of their genomic context, respond to an endogenous regulator of transcription whose activity is positionally graded. The stability of this positional memory is demonstratable in vitro: myoblasts from individual muscles divide in culture and form myotubes that express levels of CAT characteristic of their muscle of origin. This pattern of transgene expression provides a molecular correlate of positional gradients of synaptic preference demonstrated electrophysiologically (e.g., Wigston and Sanes, J. Neurosci. 5:1208, 1985), and the transgene may be useful for investigating the endogenous determinants of this positional information. (Supported by NIH and MDA.)

8.6

FETAL INJECTIONS OF NERVE GROWTH FACTOR INTERRUPTS WHISKER-RELATED PATTERN FORMATION IN BRAINSTEM. T.A. Henderson, P.A. Osborne*, R. Srisumrid*, T.A. Woolsey, E.M. Johnson, & M.F. Jacquin. Dept. Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104; Dept. Molec. Biol. & Pharm., Div. Exp. Neurol. & Neurosurg., Washington Univ. Sch. Med., St. Louis, MO 63110.

Mechanisms normally controlling pattern formation in the developing trigeminal (V) system are unknown. The only direct manipulation known to alter development of whisker-related CNS patterns is peripheral nerve injury. To test the role of naturally occurring ganglion cell death in this process, rat embryos were given transuterine, sub-Q injections of exogenous NGF (20 or 30 ug) on either embryonic day 15 (E15), E16, E18, or both E15 + E18. Control embryos received injections of matching volumes of antibodies to the NGF receptor or vehicle. Fetuses were brought to term (E21.5 $\pm$ .5; conception = E0), weighed, and perfused. Brainstems were processed for cytochrome oxidase (CO) histochemistry. Experimental and control pups had equivalent body weights that were on average 20% below normal. All controls, and pups given NGF on E16 or E18, exhibited distinct whisker-related CO patterns that did not differ from those in untreated pups at birth. E15 NGF cases had a less distinct pattern. Pups given NGF on E15 + E18 did not display whisker-related CO patterns in any portion of the V brainstem complex, despite dense CO staining where patterns normally appear. Other E15 + E18 NGF pups sustained for 24 or 72 hours postnatally, and given daily injections of 5 mg/kg NGF, also did not develop CO patterns. Here too, dense CO staining was seen in the V complex. Other than lacking a whisker pattern, V nuclei looked normal. Why patterns do not form is under study. NGF may alter projections to the face or rescue "interpatch-projecting" ganglion cells from a natural death. DE07734, DE07662, NS24679.

8.7

NERVE GROWTH FACTOR INJECTIONS RESCUE CELLS, BUT NOT CNS WHISKER PATTERNS, AFTER INFRAORBITAL NERVE INJURY AT BIRTH. P.A. Osborne*, T.A. Henderson, E.M. Johnson, C.A. Bennett-Clarke, P.A. Young, R.W. Rhoades, & M.F. Jacquin. Dept. Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104; Dept. Molec. Biol. & Pharm., Washington Univ. Sch. Med., St. Louis, MO 63110; Dept. Anat., Med. Coll. Ohio, Toledo, OH 43699.

Neonatal infraorbital nerve section rapidly kills large numbers of trigeminal first- and second-order cells, and interrupts pattern formation in brainstem, thalamus, and cortex. To determine whether rescue of axotomized ganglion cells by NGF prevents second-order cell death and permits development of whisker-related patterns, rats were given sub-Q injections of 5 mg/kg NGF prior to, and every day after, left infraorbital nerve section on the day of birth until sacrifice on postnatal day (P) 1, 3, 5, or 7. Unlike pups not given NGF (Henderson & Jacquin, Neurosci. Abstr. 16, '90), NGF-treated animals displayed no significant cell loss in the ganglion (P1: 97$\pm$4, P3: 103$\pm$12, P5: 103$\pm$15, P7: 95$\pm$11; % $\pm$ SD vs. control right side, N=5 each) or brainstem nucleus principalis (P1: 102$\pm$5, P3: 102$\pm$7, P5: 100$\pm$7, P7: 99$\pm$3; N=5 each). 5 other NGF-treated nerve cut pups from each age were processed to show whisker-related cytochrome patches in the brainstem and thalamus, and 5-HT patches in the cortex. Deterioration of brainstem patterns, and failure to develop higher-order patterns, proceeded as in cases not given NGF. These data are consistent with prior proposals that communication with the periphery is necessary for the normal development of CNS whisker patterns. They also suggest that rescuing primary afferents prevents second-order cell death, but this conclusion must be tempered by known direct effects of NGF on CNS cells. DE07734, DE07662, NS24679, NS28888.

8.8

ACTIVITY-DEPENDENT COMPETITIVE INTERACTIONS IN THE DEVELOPING WHISKER-BARREL NEURAXIS. P.H. Lee*, W.R. Weaver*, T.A. Henderson, R.V. Sonty, T.A. Woolsey, & M.F. Jacquin. Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104; Exp. Neurol. & Neurosurg., Washington Univ. Sch. Med., St. Louis, MO 63110.

We (Henderson et al., Neurosci. Abstr. 15, '89) have shown that postnatal blockade of infraorbital nerve impulses does not alter pattern formation or cytochrome oxidase (CO) staining in developing whisker-barrel pathways. Yet, others have documented reduced CO staining following neonatal deafferentation/deprivation or chronic whisker-trimming in adult rats (eg. Land & Simons, Brain Res. 341, '85). In an attempt to reconcile these data, additional whisker deprivation paradigms were studied. 5 rats had all of their left (L) whiskers trimmed daily for 2 wks from birth and CO staining patterns and intensities were evaluated. L and right (R) trigeminal nuclei, thalami, and cortices were indistinguishable. Identical methods were used in 6 littermates to determine whether CO patches stain differentially when all of the whiskers, except those in the C-row, were trimmed daily. Again, L and R staining patterns and densities, as well as patch sizes, were equivalent in each station. In another litter, we found that cortical B+D row patch sizes were not altered by L C-row cautery at birth (vs control: 97$\pm$11%, N=4). In contrast, cortical B+D row patches were larger than normal when littermates were subjected to both C-row cautery at birth and daily whisker trimming (vs control: 117$\pm$17, N=4, $p < .01$). Taken together, the above data suggest that under normal conditions, postnatal ganglion cell activity does not control pattern formation or metabolic activity in the developing barrel neuraxis. However, subordinate activity-dependent competitive interactions can be revealed when a competitive advantage is conferred upon B+D row neuropil by C-row cautery at birth. NIH DE07734, DE07662, NS17763.

8.9

CORTICAL FIELD TRANSITIONS DO NOT FORM CELL LINEAGE BOUNDARIES IN RAT HIPPOCAMPUS. E.A. Grove and J. Price*, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

We employed the retroviral method for tracing cell lineage to ask whether clonal restriction boundaries define the borders between areas in a portion of the cerebral cortex: the hippocampus. A replication-incompetent retrovirus, carrying the *lac-Z* gene, was injected into the cerebral vesicles of embryonic rat brains at the peak of cortical neurogenesis, embryonic day 16. Pups were allowed to survive until the third postnatal week, when cortical area boundaries appear mature. Clones of neurons derived from infected progenitor cells were histochemically identified and their dispersion examined relative to area boundaries determined in the same tissue sections.

The spread of individual neuronal clones in the hippocampus appears limited compared with that seen in contiguous neocortex; pairs of clonally related neurons rarely lay more than 100-200um apart. Nonetheless, clones freely crossed field boundaries among the CA fields of Ammon's horn and between CA1 and the subiculum. Statistical analysis of data from our sample of 49 hippocampi indicated that the dispersion of neuronal clones is not constrained by structural boundaries at all; rather clones distribute in an apparently random manner over the surface of the hippocampus. Cortical fields, at least in the hippocampus, therefore appear to differ from structural subdivisions in the hindbrain and diencephalon, whose borders form clonal restriction boundaries during neurogenesis.

8.10

RHOMBOMERIC ORGANIZATION IN THE EMBRYONIC VERTEBRATE HINDBRAIN. R. Baker, E. Gilland*, and D. Noden*, Dept. of Physiol. and Biophys. NYU Med. Ctr., New York, NY 10016 and Dept. of Anat., NY Coll. of Vet. Med., Cornell Univ., Ithaca, NY 14583.

Rhombomeres are metameric units in the hindbrain of vertebrates that may delineate compartments in which neurons develop at different times and project to specific peripheral and central targets. To better understand the phylogeny and ontogeny of rhombomeric organization we have examined embryonic motor nuclei in segmenting hindbrain neuroepithelium utilizing cationic lipophilic dyes applied to formaldehyde preserved and living skate, shark, turtle, bird, mouse, and ferret embryos. All species exhibited seven clear hindbrain rhombomeres, but boundaries of an eighth were less evident, especially in shark and skate. An alternating wedge-like segmental shape was prominent in r2-5, especially in the bird and ferret. Motoneurons of nerves V, VII, IX, and X originated in adjacent pairs of rhombomeres (r2-3, r4-5, r6-7, r7-8) and the sensory/motor fibers entered and exited at rhombomeres 2, 4/5 junction, 7 and 8, respectively. In the shark and skate, most Vth motoneurons originated from r3 and the nerve rootlet exited from that rhombomere. In mouse and ferret, V and VII motoneurons largely originated from r2 and r4, but in turtle and bird equal numbers were observed in r2-3 and r4-5. The VIth nerve emerged from the floor plate of r6 (skate and shark), r5 and r6 (turtle and bird), but only from r5 in ferret and mouse. Motoneurons of the VIth nerve were medial to those of VII and IX in r5 and r6. We conclude that the branchio-motor origins of V, VII, IX and X are similar in all species; however there is considerable interspecific disparity in the VIth nerve, some in V and less in VII. These data suggest that rhombomeric compartmentalization is a highly conserved template upon which species-typical developmental modifications are executed.

8.11

EVOLUTION OF INSECT SEGMENTATION AND HOMEOTIC GENES AND THEIR FUNCTION DURING NEUROGENESIS. E.E. Ball[1], E.J. Rehm[2*] and N.H. Patel[2*], and C.S. Goodman[2].
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In *Drosophila*, segmentation and homeotic genes are expressed in the blastoderm and control the pattern and identity of segments. Many of these same genes are also expressed later during the development of the nervous system and several have been shown to control the differentiation of particular neurons. Previous studies on *engrailed (en)* indicate that this *Drosophila* segment polarity gene is utilized for both segmentation and neurogenesis in arthropods, but its only conserved function in chordates appears to be in neural development. To expand our knowledge of the evolution of genes involved in segmentation and neurogenesis, we have cloned the grasshopper homologs of the *Drosophila* pair-rule gene *even-skipped (eve)* and the homeotic gene *Antennapedia (Antp)*, both of which encode homeodomain-containing proteins. In both insects, *eve* is expressed in a very similar subset of neurons and by muscles that are innervated by the motoneurons that express *eve*, suggesting a potential role for this gene in regulating neuromuscular connectivity. The grasshopper *eve* gene, however, is neither expressed in a pair-rule nor any other pattern during segmentation. In contrast, the grasshopper *Antp* gene is expressed during both segmentation and neurogenesis in a manner very similar to its *Drosophila* counterpart. These results suggest that the early pattern formation and neurogenic functions of homeotic (*Antp*) and segment polarity (*en*) genes have been largely conserved between grasshopper (a more primitive insect) and *Drosophila*, whereas only the neurogenic function of a pair-rule gene (*eve*) has been conserved between these two species.

8.12

RETINOIC ACID DISRUPTS ANTEROPOSTERIOR PATTERNING AND NEURAL GENE EXPRESSION INSIDE AND OUTSIDE EMBRYONIC FROG BRAIN: EVIDENCE FOR POSTERIOR TRANSFORMATION? W.P. Hayes and Y.P. Loh. Lab. Dev. Neurobiol., NICHD, Bethesda, MD 20892.

Increasing evidence suggests all-trans retinoic acid (RA) is a morphogen during embryogenesis. A strong case exists for RA in anteroposterior (A–P) patterning of chick limb bud (Smith et al. 1989, Development **Supplement**). More recently, RA has been implicated in A–P patterning of the embryonic CNS in *Xenopus*. Like Sive et al. (1990, Genes & Development 4), we found a progressive and dose-dependent A–P deletion of the CNS by RA. Since the RA-sensitive period precedes neural induction, RA may be toxic to more 'anterior' gastrulating mesodermal cells and thus block anterior neural induction. But since RA is present in gastrulae and neurulae, and since an apparent enlargement of the remaining posterior CNS was observed, it has been proposed that RA normally acts to *transform* anterior-specified neural tissue to more posterior CNS (Durston et al. 1989, Nature **340**). Regional homeobox (HB) gene expression also supports this, because RA only acts in concert with growth factors that have differentially induced anterior or posterior HB genes (Cho and De Robertis 1990, Genes & Development 4).

To test this hypothesis we are using neuropeptide genes to map regional neural differentiation after progressive A–P 'ablation' by RA. Our previous *in situ* hybridization studies showed that proopiomelanocortin (POMC) is the anteriormost embryonic marker of neural gene transcription (Hayes and Loh 1990, Development **110**), whereas thyrotropin-releasing hormone (TRH) and proenkephalin label more posterior CNS. Interestingly, 10-7 and 5x10-8 but not 10-8 RA eliminated POMC expression in pituitary and CNS, although at 5x10-8 RA, forebrain areas which normally express POMC were still present. Nevertheless, at 10-7 RA, anterior brain did, as previously reported, resemble enlarged hindbrain tissue. We are now using proenkephalin and TRH probes to determine if as predicted this 'transformed' region exhibits a pattern of neural differentiation characteristic of hindbrain and spinal cord.

8.13

REGULATION OF EARLY DORSAL ROOT GANGLION (DRG) SIZE DEPENDS UPON THE TYPE OF MESODERM ENCOUNTERED BY MIGRATING NEURAL CREST CELLS. C. Kalcheim, R.S. Goldstein and G. Gvirtzman. Dept. Anat. & Embryol. Hebrew Univ.-Hadassah Med. School, Jerusalem 91010, Israel.

The environment created by grafting rostral somitic halves in place of normal somites, leads to the formation of non segmented peripheral ganglia (Kalcheim & Teillet, Dev. 106, 85-103, 1989; Goldstein & Kalcheim, Dev., 1991, in press) and is mitogenic for neural crest (NC) cells that become DRG (Goldstein et al., PNA'S., 87, 4476-80, 1990). We have now studied the effect of additional mesodermal tissues on DRG growth in chick embryos: a. unilateral deletion of epithelial somites (SD), similar deletions followed by grafting: b. a 3 - dimensional collagen matrix (CM), or c. fragments of quail lateral plate mesoderm (LPM). Neural structures were identified with the HNK-1 antibody and the graft of quail cells with hematoxylin counterstaining. NC cells migrated into the grafted LPM, and unsegmented DRG formed whose volume was increased by 68%(n=3,E4) compared to the contralateral ganglia. In contrast, NC cells did not penetrate the intermediate mesoderm that was apposed to the neural tube in SD embryos, or the CM invaded by loose mesenchyme. In SD and CM embryos, unsegmented ganglia formed whose volumes were decreased by 19% (n=5,E4) and 13% (n=5,E4), respectively, compared to controls. These results suggests that although DRG precursors do not require the sclerotome to begin migration and condensation processes, DRG size is largely regulated by the properties of the mesoderm. Permissiveness to migration is then correlated with an increase in DRG volume. This increase observed in LPM grafts presumably results from contribution of sympathetic precursors remaining in dorsal positions and/or by increased proliferation of NC cells as previously demonstrated for rostral somitic grafts.

9.1

AN EYE- SPECIFIC PROTEIN KINASE C IS REQUIRED FOR MEDIATING EXTRACELLULAR CALCIUM DEPENDENT LIGHT ADAPTATION IN DROSOPHILA PHOTORECEPTORS. R. Ranganathan*, D. P. Smith*, C. F. Stevens, and C. S. Zuker. Howard Hughes Medical Institute, Depts. of Biology and Neuroscience, UC San Diego, La Jolla, CA 92093, and the Salk Institute, La Jolla, CA 92037.

With the goal of examining the role of calcium ions in invertebrate phototransduction, we have developed a novel preparation of isolated *Drosophila* photoreceptors suitable for an electrophysiological characterization of the light response using patch clamp techniques. Our general characterization of the *Drosophila* light activated conductance indicated that it is permeable to many monovalent cations, and is highly permeable to calcium ions. The responses to both brief flashes and longer pulses of light show a marked asymmetry about the reversal potential in which the kinetics of adaptation and inactivation are much faster during inward current flow. Our results indicate that this behavior is not directly voltage dependent, but is instead dependent on the direction of ion flow. Experiments in which we recorded from photoreceptors under varying $[Ca^{2+}]_{out}$ showed that extracellular calcium is both sufficient and necessary for mediating a rapid stimulus-dependent inactivation of the phototransduction cascade that underlies the observed asymmetries.

In order to understand the biological significance of this calcium-dependent inactivation process, we screened putative phototransduction mutants for those defective in this process. We show that mutations in a gene that encodes a photoreceptor-specific isoform of a protein kinase C(PKC) are specifically defective in this calcium-dependent inactivation mechanism and that the expression of the mutant phenotype completely requires extracellular calcium. These data suggest a model in which calcium mobilization as a result of channel activation modulates the phototransduction cascade largely through the activation of this protein kinase C, and that this mechanism may represent a molecular basis for light adaptation in Drosophila.

9.2

LIGHT ADAPTATION OF HUMAN PHOTORECEPTORS. D. C. Hood and D. G. Birch* Columbia Univ., NY 10027 and Retina Foundation of the SW, Dallas, TX 75231

The role our photoreceptors play in our ability to adjust to steady ambient lights is incompletely understood. In this study, we measured human photoreceptor activity by examining the leading edge of the a-wave of the ERG.

We have recently shown that the leading edge of the a-wave provides a measure of the activity of human photoreceptors[1]. In particular, the a-wave varies with time and flash energy in ways consistent with known computational models[2] of receptor responses. In separate experiments, we isolated the cone and rod a-wave by procedures previously described[1]. The 600 µs flashes were red (W26) or blue (W47B) and were presented upon 'white' adapting fields. Amplitude-intensity data for full-field ERGs were obtained in the dark and upon steady adapting fields ranging in intensity from 10 to 10,000 trolands for the cones and from 1 to 250 scot td for the rods. Plots of response amplitude at fixed times after the flash confirm that the data can be described by a class of models with a linear filter followed by a static nonlinearity[1,2]. A computational model of adaptation was fitted to the data.

Both receptor types show adaptation but not until the adapting field is relatively intense, greater than 1.0 log scot td for the rods and 3.0 log td for the cones. Adaptation includes a gain change and some change in the time course. A similar computational model of adaptation fits both receptor types.

1. Hood & Birch (1990) *IVOS* **31**; Hood & Birch (1990) *Vis. Neurosci* **5**; Hood & Birch (1991) *IVOS* **32/4**. 2. Baylor et al (1974) *J. Physiol.* **242**; Baylor et al (1984) *J. Physiol.* **357**; Penn & Hagins (1972) *Biophys J.* **12**. Schnapf et al (1990) *J. Physiol.* **427**.

9.3

STRUCTURE OF THE HUMAN SAMPLING MOSAIC IN PERIPHERAL RETINA. J. Hirsch and M. Bianchi*. Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CT 06510.

The structure and packing of retinal photoreceptors determines the spatial detail available to the visual system for subsequent neural processing. Although the sampling mosaic plays a fundamental role in vision, sampling strategies have not been explored beyond the foveal region. We compare the foveal mosaic[1,2] with a region of the same retina between 9 and 11 degrees of eccentricity, and find that the cone center-to-center spacings exceed those in the fovea (factors of 4 to 5), rods are more numerous than cones (factor of 16), and the cone spacings exceed cone aperture diameters (factor of 1.8) all resulting in a substantial loss of sampled spatial detail. Further, measures of highest visual acuity in this region (8.6 to 11 c/deg)[3] are below the resolution capacity (as estimated from cone spacings) of this peripheral cone mosaic (14 to 15 c/deg) suggesting that peripheral postreceptoral processes that mediate resolution may be compromised in comparison to the more central systems where the visual acuity coincides with the anatomical resolution.[2]

1. Curcio, et al, 1987, Science, 236, 579-582.
2. Hirsch and Curcio, 1989, Vision Res., 29,1095-1101.
3. Westheimer, 1982, Vision Res., 22, 157-162.

9.4

PHOTORECEPTOR TRANSPLANTATION: ANATOMIC, ELECTROPHYSIOLOGIC AND BEHAVIORAL EVIDENCE FOR THE FUNCTIONAL RECONSTRUCTION OF RETINAS LACKING PHOTORECEPTORS M.S. Silverman, S.E. Hughes, and T.L. Valentino[1]. Sensory Neuroscience Laboratory, Central Institute for the Deaf, and [1]Dept. of Biology, St. Louis University, St. Louis, MO 63110.

We have shown that sheets of photoreceptors can be transplanted to retina lacking photoreceptors (Silverman and Hughes, 1989, IOVS 30:1684). However we wished to determine whether these transplanted photoreceptors functionally integrate with the dystrophic retina and if so, whether such integration results in any degree of visual function. Sheets of neonatal photoreceptors were transplanted to the subretinal space of one eye of hosts (albino rats with severe photoreceptor loss induced by constant light exposure) using the photoreceptor isolation and transplantation procedures previously described. Two to 10 weeks post-transplantation cortical evoked potentials (VEP) were recorded from skull screws located over the visual cortex. Flash stimulation of the reconstructed eye resulted in a cortical potential comparable in waveform and up to 50% the amplitude of VEP recorded from normal rats. Little or no response was recorded by such stimulation of the fellow unreconstructed eye or sham operated eyes. The reconstructed eyes also show a conventional pupillary reflex to light, while fellow unreconstructed or sham-operated eyes show a much attenuated response that was aberrant in form (a small pupillary dilation to continuous light). Ultrastructural examination of the reconstructed retinas revealed a new outer plexiform-like layer (OPL) at the interface of the transplanted outer nuclear layer and the host inner nuclear layer (Hughes et al., 1990, IOVS 31:594). Ribbon synapses are evident within this OPL. These synapses are characteristic of those formed by rod photoreceptors, displaying an electron-dense ribbon surrounded by a cluster of vesicles. Ribbon synapses are found only rarely in control light-damaged retina. These results show that transplanted photoreceptors can functionally integrate with the dystrophic retina to reestablish evoked activity and behavioral responsivity to light.

9.5

ROD OUTER SEGMENT LENGTH: A CASE OF STRUCTURE-FUNCTION RELATIONSHIPS. *K. N. Leibovic* and *R. Moreno-Diaz Jr.** Biophysics Department, Sch. of Med. SUNY/Buffalo, N.Y. 14214 and Computer Science, Universidad de Las Palmas, E-35016 Las Palmas, Spain.

There are numerous examples of structure subserving function in the literature, a case in point being diameter and spacing of photoreceptors with respect to acuity and the limits of diffraction. Photoreceptor length, on the other hand, has not received such attention. Vertebrate rods are exquisitely sensitive, being able to detect single photons in spite of the presence of noise due to the transduction machinery. Efficient absorption of photons favors a long outer segment (OS), while noise control requires a short one. These competing demands lead to an optimality criterion for rods operating in dim illumination. We have shown that this criterion predicts the correct OS length in Bufo marinus. Here we extend the optimality criterion to several other species including Macaca fascicularis. We show that our computed OS lengths are in close agreement with the experimental values. It supports the notion that these rods are designed to act as efficient photon detectors in the presence of noise. On the other hand, there is no such agreement for the rods of the skate. This is consistent with the fact that the latter rods operate over a range of light intensities covered by rods and cones in most other vertebrate eyes. Our results provide a rationale for rod OS length in a variety of vertebrate species and can be used as an aid in classifying functionally different photoreceptors.

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9.6

VISUALIZING SYNAPSES WITH CONFOCAL MICROSCOPY IN A LIVE RETINA. R.F. Miller, R.M. Wolfe, J.L. Eiesland, R.W. Fuller and C.B. Toris. Department of Physiology and Graduate Program in Neuroscience, University of Minnesota Medical School, Minneapolis, MN 55455.

We have labeled and visualized synaptic boutons in the amphibian and rabbit retina, using activity-dependent dyes (ADD) and optical, 3D reconstruction methods based on confocal microscopy. This approach was combined with electrophysiological experiments in which single neurons were identified and intracellularly labeled with a dye, such as lucifer yellow (LY). The combination of the two labelling techniques permitted visualization of presumed synaptic contacts between ADD filled terminals and the dendrites of single neurons. Several different types of dyes proved useful for labelling terminals, including highly polar dyes which are taken up endocytotically, dyes which label "active" mitochondria and voltage-sensitive dyes. LY filled processes could be separated from ADD filled profiles by switching barrier filters in the light pathway to the PMT. Localization of ADD dyes to nerve terminals was established by dissociating retinas which had been exposed to the dye and using a cooled CCD camera to obtain image data of the cell together with dye localization information. ADD fluorescence was detected in the terminals of photoreceptors, bipolar cell-like neurons and presumed amacrine cells. No ADD dye was observed in ganglion cells which had been back labeled. Single LY filled dendrites, surrounded by ADD filled terminals were optically reconstructed (Voxel View, Vital Images) to evaluate the proximity and density of contacts between these structures. Results with this approach suggest that only a small number of ADD profiles are sufficiently close to any single dendrite to be considered as a likely synaptic connection. These observations demonstrate the feasibility of labelling and studying synaptic terminals in the intact retina. Supported by NEI grants EY03014 and EY07376.

9.7

HORIZONTAL CELLS IN THE CONE-DOMINATED RETINA OF THE TREE SHREW. L. Peichl and B. Müller*. Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, W-6000 Frankfurt/M.71 Germany.

We have studied the morphology, distribution, and synaptic connections of tree shrew horizontal cells (first described by Mariani, J.C.N. 233:553, 1985) by intracellular injection and photoconversion of Lucifer Yellow, and by staining with a neurofilament antibody.

The larger (A-type) cells have sparsely branched dendrites; up to eight dendritic processes end in extensive bushy arborisations (not found in any other mammal). Along the dendrites and on the bushy arborisations, photoreceptor contacts are made through single spiny terminals and small terminal aggregates. Electron microscopy shows that all contacts are with cones, like in the A-type cells of other mammals. A-type densities decrease from 1220/mm² in central retina to 450/mm² in the periphery. Conversely, dendritic fields increase from 400 µm to 540 µm diameter.

The smaller (B-type) cells have a densely branched dendritic tree with 40-80 terminal aggregates, and a single axon with a few terminals that presumably contact rods. The terminal aggregates synapse with practically all cone pedicles in the dendritic field, i.e. there is no colour specificity. Dendritic tree sizes increase from central to peripheral retina (40-70 µm diameter).

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9.8

COMPARISON OF THE EFFECTS OF STEADY AND FLICKERING LIGHT ON DOPAMINE-MEDIATED CHANGES IN HORIZONTAL CELL COUPLING IN MUDPUPPY RETINA. C.-J. Dong* and J.S. McReynolds, Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

We recently reported that in mudpuppy retina steady light (SL) causes a dopamine (DA)-mediated uncoupling of horizontal cells (HC), probably by an action through ON-center bipolar cells. However, reports that flickering light (FL) is a more effective stimulus for DA release in other species raises the possibility that FL may release additional DA via the OFF pathway. The effects of SL and FL on HC coupling in dark-adapted retinas were compared by measuring changes in HC responses to illumination of the center and surround portions of the receptive field. For a given period of adaptation, SL was more effective than FL of equal intensity with 1/10 the total on-time and 10 times the number of light-dark transitions. For SL and FL of equal on-time, FL had about the same effect as SL when the duty cycle of the FL was 1:10 (light on : light off), and was slightly more effective than SL when the duty cycle of the FL was increased to 1:1. The uncoupling effects of both SL and FL were blocked by DA antagonists and by APB. These results suggest that both FL and SL affect DA release and HC coupling via the ON pathway. The greater effectiveness of more rapidly changing illumination may be due to the initial transients of bipolar cell responses at the onset of each light flash.

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9.9

GABAERGIC TRANSMISSION IN DISTAL MAMMALIAN RETINA. T. E. Frumkes and R. Nelson. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, Md. 20892

Histological studies have provided conflicting findings regarding the role of GABA in mammalian outer plexiform layer. We studied the influence of GABAergic substances applied by means of ophthalmic artery perfusion upon intracellular responses of retinal horizontal cells (HCs) of the cat. GABA agonists depolarized HCs, decreased the amplitude and slowed the time course of the light response as reported for many cold-blooded vertebrates. However, such effects required long application of high drug concentrations (>20 mM GABA, >0.2 mM Muscimol). 1 mM Baclofen had negligible effects. Other GABAergic drugs produced more striking results. 1 minute exposure to 4 mM dAVA (delta amino valeric acid, which is reported to be a $GABA_A$ agonist and a $GABA_B$ antagonist) totally abolished the photic response and depolarized HCs by >20 mV; a smaller, similar effect could be obtained with 1 mM concentration. Nipecotic acid, a presumed GABA uptake blocker, produced effects similar to dAVA. Application of Bicuculline (BCC) greatly speeded up HC responses to light offset. The influences of dAVA and BCC did not antagonize each other and, hence, appear to act by independent mechanisms. Collectively, these results suggest that GABA plays some role in synaptic transmission in the outer plexiform layer of mammalian retina, a role not adhering to "conventional" $GABA_A$ or $GABA_B$ pharmacology.

9.10

DAPI LABELS THREE TYPES OF BIPOLAR CELL IN RABBIT RETINA. Stephen L. Mills*, M.L.J. Crawford, and Stephen C. Massey*. Sensory Sciences, Graduate School of Biomedical Science, University of Texas Health Science Center, Houston, TX.

The fluorescent dye DAPI has been shown to label starburst amacrine cells in rabbit retina a day or two after intraocular injection (Vaney, 1984, Proc. R. Soc. Lond. B, **220**, 501; Tauchi and Masland 1984, Proc. R. Soc. Lond. B, **223**, 101) and AII amacrine cells following in vitro incubation (Mills and Massey 1991, J. Comp. Neurol., **304**, 491). We now show that 3 types of cone bipolar cell can be labeled by brief (< 15 min) exposures to DAPI in vitro. All 3 types ramify in sublamina a, suggesting they are "OFF" bipolar cells. The type most brightly labeled was characterized by a small field in both the OPL and IPL, with the axon branching rather narrowly in layer 1. A second type, appearing darker under DAPI fluorescence, branched more diffusely throughout the whole of sublamina a. The third and largest type branched very narrowly in layer 1 of sublamina a and was most notable for the large spatial extent of its processes in the IPL, which generally exceeded the area of the OPL processes by a factor of about 3.

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9.11

Synaptic Connections of the Axon Terminal of a Cone Bipolar Cell in the Rabbit Retina. E. Strettoi*, R.E. Dacheux, and E. Raviola. Istituto di Neurofisiologia del CNR, Pisa, Italy and Dept. Anatomy and Cell. Biology, Harvard Medical School, Boston, MA 02115.

The synaptic connections of the axonal arborization of one cone bipolar cell of the rabbit were reconstructed at the electron microscope from continuous series of thin, radial sections. Its processes branched in the vitreal sublamina of the inner plexiform layer (S4) and established gap junctions with the AII amacrine cell; thus, this bipolar was probably involved in the transfer of scotopic signals to ganglion cells. The processes of this cone bipolar were presynaptic at ribbon synapses and usually contacted a dyad of postsynaptic dendrites that belonged to amacrine and ganglion cells. The pattern of connections at the dyad synapse was not constant: about one third of the postsynaptic amacrine cell dendrites returned a reciprocal synapse onto the cone bipolar and the other two thirds did not; a small number of these nonreciprocal amacrine cells were in turn presynaptic to nearby rod bipolar axon terminals. The reciprocal synapses represented only a minor portion of the input to this cone bipolar terminal, for the majority of the presynaptic processes originated from a heterogeneous population of nonreciprocal amacrines. An important characteristic of this cone bipolar was the large number of synapses onto ganglion cell dendrites (30%) when compared to rod bipolars (.5%) and AII amacrine cells (3%). This indicates that this cone bipolar mediates the access to ganglion cells of both rod and cone signals. EY01344 & EY03011.

9.12

PEPTIDERGIC MODULATION OF CALCIUM CURRENTS IN ISOLATED RETINAL BIPOLAR CELLS. George S Ayoub & Gary Matthews. Dept of Neurobiology, SUNY, Stony Brook, NY 11794

Retinal bipolar cells are non-spiking interneurons that relay information from photoreceptors to amacrine and ganglion cells. We have recorded the calcium currents of enzymatically isolated, rod-dominated depolarizing bipolar cells of the goldfish (type Mb1) using patch pipette/whole cell recording. These cells have a prominent synaptic Ca influx in response to depolarization which is carried by L-type Ca channels (Matthews & Heidelberger, 1990 Neurosci Abst). Application of peptides known to be present in amacrine cells presynaptic to the Mb1 cells modulated this calcium current. Specifically, application of nanomolar concentrations of substance P, somatostatin, or met-enkephalin increase the activation potential of the Ca current to more positive potentials, with the maximal Ca current suppression at membrane potentials of -30 to -20 mV. While 1 nM substance P suppresses approximately 20% of the Ca current, 10 nM suppresses about 40%. Higher concentrations are no more effective than 10 nM. The Ca current modulation by substance P requires GTP in the patch pipette, implicating G-protein mediation. Additionally, though the peptide effects sum with low drug concentrations, there is no summation with high concentrations, indicating a common mechanism of operation. The modulation we observe with these peptides appears similar to the effect of GABA on the Ca current of these cells (Matthews, Ayoub & Heidelberger, 1991 Neurosci Abst).

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10.1

RAPID LAMININ-INDUCED CHANGES IN SYMPATHETIC GROWTH CONES D.W. Burmeister, R. I. Rivas*, A.M. Coriano*, D.I. Goldberg Dept Pharm. & Cntr for Neurobiol. & Behavior, Columbia Univ., NY NY 10032

Adding laminin (ln) to cultures of sympathetic neurons growing on polylysine substrates induces a rapid change in growth cone (gc) morphology as a prelude to enhancing the rate of neurite elongation. Using video microscopy of living gcs, and subsequent immunocytochemistry, we find that the earliest and most pronounced effect of ln addition is an acceleration of the net movement of membranous organelles and microtubules into peripheral lamellipodia (Rivas & Goldberg, JCB 111: 490a). This movement is termed engorgement, and appears to be a necessary stage in the growth of neurites (Goldberg & Burmeister, TINS 12: 503). The advance of the leading edge of the gc and the rate of engorgement on polylysine treated substrates was about 5 μm/h. After the addition of 25μg/ml ln the rate of engorgement increased more than 4-fold within 20 min, while advance of the leading edge continued at a slow pace. This initial, rapid effect on engorgement, but not other stages of neurite advancement, indicates 1. engorgement, in some circumstances, may be a rate limiting step in neurite elongation, 2. engorgement is particularly sensitive to ln, and 3. stimulation of engorgement could be a mechanism by which ln stimulates neurite growth. Ln-induced engorgement was blocked by antibodies to the β_1 subunit of integrin, but not by pre-immune serum, indicating that integrins probably mediate the engorgement response. However, interfering with the binding of ln to cell surface β 1,4-galactosyltransferase with α-lactalbumin (which inhibits neurite outgrowth from PC12 cells), also blocked ln-induced engorgement in sympathetic gcs. These results are consistent with the idea that multiple surface receptors mediate the ln-induced changes in gcs.

10.2

EXPRESSION OF A CHIMERIC HUMAN/RAT $\alpha 5/\beta 1$ INTEGRIN HETERODIMER IN A RAT NEURONAL CELL LINE (PC12): EFFECTS ON ATTACHMENT AND NEURITE OUTGROWTH IN RESPONSE TO FIBRONECTIN. K. J. Tomaselli*, S. C. Bodary*, L. L. Rubin and L. F. Reichardt. Athena Neurosciences Inc., So. S.F., CA 94080; Dept of Physiology and Howard Hughes Med. Inst., UCSF, S.F., CA, 94135.

Previous studies showed that rat PC12 cells attach well to laminin (LN) and several collagens (COL), but attach to fibronectin (FN) at levels only 10-20% of those achieved on LN or COL. The comparatively weak attachment to FN is inhibited by β1 integrin antibodies or RGD peptides, indicating that attachment is mediated by either the $\alpha 5\beta 1$ or $\alpha 3\beta 1$ integrins. Immunoprecipitation studies demonstrated, however, that while PC12 cells express both $\alpha 3\beta 1$ and $\alpha 1\beta 1$, they do not express detectable amounts of $\alpha 5\beta 1$ (Tomaselli *et. al.*, J. Cell Biol., 107:1241-1252). We have attempted to enhance the responsiveness of PC12 cells to FN by transfection of the integrin α5 subunit cDNA. The human α5 cDNA (gift of L. Fitzgerald) was subcloned into the pHβAPr-1-*neo* expression vector and was transfected into PC12 cells. Of about 20 stably transfected clones that attached at high levels to FN, four were characterized in more detail. Each expressed the human α5 subunit in association with the endogenous rat β1 subunit as assayed by immunoprecipitation of radiolabelled cells using a rat MAb (B2G2) that recognizes human, but not rat, $\alpha 5\beta 1$ dimers. Attachment to FN was inhibited by the B2G2 MAb and also by an RGD peptide. In addition, α5 transfectants showed an increased ability to regenerate neurites on FN after priming with NGF. These results demonstrate the feasability of manipulating the responses of neuronal cells to components of the ECM by expression of exogenous integrin subunits.

10.3

THE FIBRONECTIN RECEPTOR ($\alpha 5\beta 1$ INTEGRIN HETERODIMER) AND FIBRONECTIN ARE REGULATED DURING PERIPHERAL NERVE DEVELOPMENT AND REGENERATION. Frances Lefcort and Louis F. Reichardt, Dept. of Physiology and HHMI, University of Califronia, San Francisco, San Francisco, CA. 94143-0724

Fibronectin, a major glycoprotein constituent of extracellular matrix (ECM), has been demonstrated to potently promote peripheral neurite outgrowth in vitro. Immunohistochemical studies have shown that fibronectin is present in the environment of the developing and regenerating peripheral nerve and that regenerating neurites are in close contact with the ECM. The Integrin family of ECM receptors has been identified as the primary class of ECM receptors for peripheral neurons in vitro, with the $\alpha 5\beta 1$ subunit serving as a major fibronectin receptor. To understand the significance of $\alpha 5\beta 1$ and fibronectin for actively growing neurites in vivo, we have examined the protein expression levels and distribution of the $\alpha 5\beta 1$ integrin receptor and its ligand, fibronectin, in developing and regenerating chick peripheral nerve by quantitative analysis of western blots and immunocytochemistry. We have found that in vivo, 1) peripheral neurites express $\alpha 5\beta 1$, 2) $\alpha 5\beta 1$ is dramatically down regulated with peripheral nerve maturation on both axons and Schwann cells, 3) $\alpha 5\beta 1$ is upregulated in adult nerve following nerve transection on both neurites and non-neuronal cells. Similarly, fibronectin is also down regulated in adult nerve and strongly upregulated during nerve regeneration. The expression patterns of $\alpha 5\beta 1$ and fibronectin are overlapping and interestingly in regenerating peripheral nerve, fibronectin is most strongly expressed immediately in advance of the distal front of regenerating growth cones (a region where laminin expression is extremely low). Our results implicate a major role for the $\alpha 5\beta 1$ integrin receptor and fibronectin in nerve regeneration and development and functional tests of this prediction are now in progress. (We thank Drs. J.A.MacDonald, R. Horwitz, J. Muschler for the anti-$\alpha 5\beta 1$ antibodies and K. Venstrom for the anti-fibronectin).

10.4

SINGLE CELL CHROMOPHORE ASSISTED LASER INACTIVATION (CALI) SHOWS THE SITE OF FASCICLIN I ACTIVITY IS AT THE GROWTH CONE. Anne M. Sydor, Joel Bard*, Aneil Mallavarapu*, Timothy P. O'Connor, and Daniel G. Jay Dept. of Cell. and Dev. Biology, Harvard Univ., Cambridge, MA and Dept. of Molecular and Cell Biology, Univ. of CA, Berkeley, CA.

CALI inactivates specific protein function with laser light directed to the protein through a dye-labeled antibody (PNAS 85:5454, 1988). The dye moiety absorbs light at 620 nm, a wavelength which is not absorbed by cellular components. We have developed single cell CALI in which a laser beam is focused through a microscope to a 10 μm diameter such that protein function can be inactivated in single cells.

We tested single cell CALI by inactivating the enzyme HRP bound to the surface of a single S2 *Drosophila* cell. Single cell CALI was done on S2 cells expressing fasciclin I, a neuronal membrane glycoprotein that causes these cells to aggregate. Inactivation of fasciclin I on a single cell caused that cell to separate from a cluster. This was not observed when laser light was directed at clusters incubated with dye-labeled BSA.

We have now used single cell CALI to localize the function of fasciclin I to the neuronal growth cone. The Til neurons are a fasciclin I-expressing cell pair in the grasshopper limb bud which fasciculate together as they project to the CNS. Previously we have shown that fasciclin I functions in adhesion between these sister axons (Nature 338:548, 1990). To visualize the growth cones, Til neurons were labeled with dil in an open limb bud preparation (J. Neurosci. 10:3935, 1990). Embryos were incubated with dye-labeled anti-fasciclin I. Laser light directed to the growth cones resulted in defasciculation in 3 out of 3 limbs while a control limb that was not subjected to laser light appeared normal.

Our results show that the adhesive function of fasciclin I is initiated at the growth cone. Single cell CALI is the molecular analog of cellular laser ablation and provides an unprecedented level of spatial and temporal resolution for studying neurodevelopment.

10.5

LASER INACTIVATION OF FASCICLIN II INHIBITS AXONOGENESIS OF THE PIONEER NEURONS OF THE GRASSHOPPER LIMB BUD. James W. Booth*, Lisa Park* and Daniel G. Jay. Dept of Cellular and Developmental Biology, Harvard University, Cambridge, MA.

Previously, we showed that fasciclin I plays a role in axon adhesion using chromophore assisted laser inactivation (CALI) (Nature 348:548, 1990). We now show that CALI directed against fasciclin II, the insect NCAM homolog, results in a perturbation of axonogenesis but not axon adhesion in the grasshopper PNS. Laser irradiation of 31% embryos injected with Malachite green-labeled anti-fasciclin II resulted in an inability of the Til pioneer neurons of the metathoracic limb buds to extend axons even though their cell bodies appeared to differentiate (8 out of 10). This was visualized by anti-HRP immunostaining. Control embryos injected with dye-labeled BSA or not subjected to laser light showed normal axonogenesis (6 out of 6) and axon extension up to 100 μm. CALI against fasciclin II in later embryos (33%) showed no significant inhibition of axon growth nor defasciculation, a result of CALI using anti-fasciclin I.

These results suggest that fasciclin II functions in the initiation of axon outgrowth and that its role in neurodevelopment is distinct from fasciclin I. Its concurrent expression on the neighboring epithelial cells and its homology to NCAM suggest that it functions in the initial adhesive contacts of the growth cone to the epithelium.

The use of CALI to determine the functions of fasciclin I and II in the development of Til neurons suggests that this technique will be useful in understanding molecular mechanisms in neurodevelopment.

10.6

AN ENDOGENOUS GLIAL RECEPTOR FOR THE NEURONAL GLYCOPROTEIN THY-1: FURTHER BIOCHEMICAL CHARACTERIZATION. Evan B. Dreyer†, Paul Lucek*, Kristen Upchurch*, Dana Leifer, & Stuart A. Lipton. Program in Neuroscience, Harvard Medical School, Children's Hospital, & †Massachusetts Eye and Ear Infirmary, Boston, MA.

Previous work in our laboratory (Leifer, et al., Science,224:303,1984) has demonstrated a role for Thy-1 in neurite outgrowth by central mammalian neurons. Thy-1 antibodies promote neurite outgrowth of cultured retinal ganglion cells. Using two monoclonal anti-idiotypic antibodies as probes, we have identified endogenous binding sites for Thy-1 that may represent part or all of an endogenous Thy-1 receptor. We have shown that this putative receptor has a similar effect in modulating neurite outgrowth to that seen with the Thy-1 antibodies. The proposed Thy-1 receptor has been identified in cultured astrocyte and whole brain preparations. Anti-idiotypic binding to the proposed receptor is competitively blocked by purified Thy-1.

Several Thy-1 binding proteins (MWs 90,175,180kD) have been identified in Western blots of whole rat brain and astrocyte preparations. Two dimensional blots have now been analyzed by similar techniques, and a partial protein sequence determined. This provides further information on the biochemistry of the proposed Thy-1 receptor. These data suggest that a receptor for the neuronal glycoprotein Thy-1 exists on glia, and that this receptor plays a role in modulating retinal ganglion cell neurite outgrowth.

10.7

IDENTIFICATION AND ANALYSIS OF THY-1 RECEPTOR cDNA CLONES FROM BRAIN AND MUSCLE.

Dana Leifer, Evan B. Dreyer, Kelly S. Rothe*, John Heng*, Rachael L. Neve† and Stuart A. Lipton. Dept. of Neurology, Children's Hospital, Massachusetts General Hospital and Harvard Medical School, Boston, MA, and †Dept. of Psychobiology, Univ. of California, Irvine, CA.

We have previously used anti-idiotypic monoclonal antibodies against Thy-1 antibodies to identify putative Thy-1 receptor proteins that appear to modulate neurite outgrowth in culture. We screened a human fetal brain cDNA library with the anti-idiotypic antibodies and identified candidate receptor clones. An antiserum against a fusion protein derived from one of the clones binds to the same bands on Western blots of brain and astrocyte preparations that the anti-idiotypic antibodies identify. Moreover, purified Thy-1 blocks binding of the anti-idiotypic antibodies to these bands. The clones contain an open reading frame at least 1.34 kb in length. Searches of the GenBank and NBRF databases indicate that the sequence has not been reported previously. The sequence has several potential glycosylation sites. RNA blot analysis demonstrates that the putative receptor cDNA identifies specific bands in brain and muscle, but not in a variety of other tissues. The RNA blots and the sequence data indicate that the clones initially isolated were partial clones. We have therefore used one of them to re-screen brain and muscle libraries. We have isolated potentially full-length clones from both brain and muscle. Taken together, our results suggest that we have now identified Thy-1 receptor proteins and corresponding cDNA clones.

10.8

NCAM AND N-CADHERIN DIRECTLY PROMOTE MORPHOLOGICAL AND BIOCHEMICAL DIFFERENTIATION OF PC12 CELLS VIA A PERTUSSIS TOXIN SENSITIVE PATHWAY.

P. Doherty, S.V. Ashton, F.S. Walsh. Dept. Experimental Pathology, UMDS, Guy's Hospital, London SE1 9RT, U.K.

Neurite length and specific cell surface glycoprotein expression have been measured for PC12 cells cultured on confluent monolayers of control 3T3 cells or 3T3 cells expressing either transfected human NCAM or chick N-cadherin. NCAM and N-cadherin in the monolayer directly induced a change in the morphology of PC12 cells from an adrenal to neuronal phenotype. This was accompanied by a transient increase in Thy-1 immunoreactivity, but not L1 or NGF-receptor immunreactivity. The morphological responses could be specifically inhibited by antibodies to NCAM or N-cadherin but not by antibodies to NGF or FGF. The response differed from NGF induced morphological differentation in that it did not depend on transcription. The NCAM and N-cadherin responses could also be fully inhibited by pertussis toxin and partially inhibited by the calcium channel antagonists verapamil and diltiazem. These data suggest that NCAM and N-cadherin can directly modulate cell phenotype and that they may do so via activation of similar second messenger pathways.

10.9

ULTRASTRUCTURE OF AN IDENTIFIED GROWTH CONE ARRAY IN EMBRYONIC LEECH REVEALS SPATIALLY DIVERSE CELL AND MATRIX INTERACTIONS DURING DIRECTED MIGRATION.

D.M. Kopp, D. McCarthy*, and J.Jellies. Neurobiology Research Center and Dept. of Physiol. and Biophysics, Univ. of Alabama at Birmingham, Birmingham, AL, 35294.

Unique, transient muscle-organizing cells (Comb or C-cells) in the embryonic medicinal leech, *Hirudo medicinalis*, each extend about 70 parallel growth cones and establish a framework of processes used to collect myocytes into an oblique array sandwiched between circular and longitudinal muscle layers. It has been suggested that an interplay of spatio-temporally regulated cell-cell and cell-matrix events mediate the parallel alignment of growth cones followed by their rapid, directed extension through the body wall. A simple alternative is that direction is intrinsically limited by the cytoskeleton and that C-cell growth cones are mechanically constrained to grow between existing layers of circular and longitudinal muscle, perhaps even within pre-existing channels. Our initial ultrastructural examination of HRP-filled C-cells makes this simple scheme seem unlikely. C-cell growth cones in 11 day old embryos appear neuron-like with microfilaments, microtubules, and large and small vesicular elements. Filopodia and growth cones associate not only with the orthogonal grid of circular and longitudinal muscle, but also with basolateral epidermal surfaces, undifferentiated mesenchyme, the basement membranes associated with all of these developing cells, and diffuse matrix. C-cell growth cones ocassionally enwrap and are themselves enveloped by cells in these layers, and filopodial insertions into muscle were observed. Close (<10 nm) growth cone-cell contacts are common, but not restricted to a particular cell type in the environment. Processes, in contrast to leading growth cones, appear to be more restricted in position, often being closely apposed to mesenchymal cells. Thus, at the developmental stage examined here, C-cell growth cones are capable of sampling a variety of cell surfaces and matrices, potentially extracting guidance cues from any, or all, of them. Overall, these observations are consistent with the idea that a range of cell/matrix interactions may mediate the emergence of order in C-cell growth cone navigation and the subsequent assembly of the oblique muscles, rather than a simple scheme of pre-existing anatomical pathways guiding C-cell growth cones inescapeably along particular oblique trajectories. (Supported by NIH NS28603 to JJ).

10.10

A CYTOPLASMIC GRADIENT OF CYCLIC AMP ACROSS THE *XENOPUS* GROWTH CONE IS SUFFICIENT TO INDUCE TURNING.

A.M. Lohof, M. Quillan, Y. Dan, and M. Poo. Dept. of Biological Sciences, Columbia Univ. N.Y., N.Y. 10027.

We have studied the role of cAMP in growth cone guidance in cultured embryonic *Xenopus* spinal neurons. Stable microscopic gradients of membrane-permeable drugs known to alter intracellular cAMP activity were established extracellularly by repetitive pulsatile application of the drug from a micropipette. The behavior of the growth cone in the applied gradient was determined by measuring the angle of growth cone orientation and neurite extension from video records. We found that when a gradient of dibutyryl cAMP (20 mM pipette solution) was produced at a 45^{o} angle across the growth cone, significant growth cone turning (average angle 29.1 ± 4.9^{o}, N = 20) toward the pipette was observed within 2 hr. Application of similar concentrations of sucrose, underivatized cAMP, or dibutyryl cGMP produced no significant turning. Gradients of drugs which perturb the endogenous level of cAMP were also tested. The phosphodiesterase inhibitor IBMX (1.5 mM) produced orientation similar to dibutyryl cAMP. The adenylate cyclase activator forskolin (5 mM) produced a mean turning angle of 19.9 ± 5.4^{o} (N = 34) within 30 min. A cytoplasmic gradient of cAMP thus appears to be sufficient for inducing the turning response; whether such a gradient is necessary for growth cone turning induced by extracellular guidance cues remains to be determined. The consistency of the orientation response in the chemical gradient offers the opportunity to explore the sequence of cellular events leading to the reorientation response of the growth cone.

10.11

DIFFERENTIAL REGULATION OF NEURITE GROWTH IN RAT SYMPATHETIC NEURONS BY PHOSPHORYLATION INHIBITORS AND NGF.

R. B. Campenot and D. D. Draker*. Dept. of Anatomy and Cell Biology, Faculty of Medicine, Univ. of Alberta, Edmonton, Alta., Canada T6G 2H7.

Long-term application of phorbol 12-myristate 13-acetate (PMA) locally to distal neurites of NGF-supplied (200 ng 2.5S NGF/ml) rat sympathetic neurons in compartmented cultures caused a concentration-dependent reduction in neurite extension rate over a broad range (10 mM-10 μM), but was without effect when applied to cell bodies and proximal neurites. PMA is a short-term activator, but long-term downregulator of protein kinase C (PKC). Similar results were obtained with staurosporine (range 100 nM - 1μM), a general inhibitor of phosphorylations specific for PKC in its low nM range. No other agents have been observed to modulate the rate of neurite extension in compartmented cultures, and NGF over a broad range (5-200 ng/ml 2.5S NGF) modulates neurite density without effect upon extension rate. Also, substantial neurite growth was observed with 2 μM PMA, and 10 nM staurosporine had little or no effect on neurite growth. Thus, protein kinase C activity appears to be unnecessary for neurite growth or NGF signaling. Neurite growth in 2 μM PMA was blocked by anti-NGF IgG. Thus, it is unlikely that long-term PMA treatment depressed neurite growth by interfering with NGF action. These results suggest the presence of phosphorylation-mediated signalling systems in sympathetic neurites that regulate their growth independently of NGF. What phosphorylations or ligands may be involved are presently unknown, but such systems may play major roles in regulating nerve growth and regeneration. Supported by the AHFMR, MRC, and NCE.

10.12

AXON RETRACTION IS MEDIATED BY ENHANCEMENT OF PROTEIN PHOSPHORYLATION.

S. Finnegan Sloan, E. Koenig and V. Lemmon. Dept. of Physiology, Univ. at Buffalo, Buffalo, NY 14214, and Center for Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106.

Videomicroscopic studies reveal that mAb 8A2 induces retraction in regenerating retinal ganglion cell axons of goldfish explants *in vitro* characterized by: 1) growth cone collapse, 2) collection of axoplasm into a mass, and 3) retrograde translocation of the mass leaving multiple strands distally (*Soc. Neurosci. Abstr.* Vol. 15, p. 1027, 1989). The mAb 8A2 retraction response may serve as a model for retraction associated with axon elimination during development. The response is arrested by cytochalasin D, but continues in the presence of nocodazole or taxol suggesting that microtubules are probably passive components and that the actin filament network plays an active role. (*Soc. Neurosci. Absrt.* Vol. 16, p. 314, 1990). Microtubules are translocated proximally out of the distal strands where only components of the membrane cytoskeleton such as spectrin and talin remain. Exposure of axons to phorbol 12-myristate 13-acetate (10 nM) triggers a similar retraction response while inactive phorbol ester analogs do not. In addition, okadaic acid, a phosphatase inhibitor, triggers axon retraction with very short latency. Both mAb 8A2 and phorbol ester stimulated retraction are blocked by kinase inhibitor staurosporine. Genistein, another kinase inhibitor, blocks mAb 8A2 induced retraction but was not tested with phorbol ester. These results indicate that the mAb 8A2 response may involve kinase activation and/or phosphatase inhibition. This strongly suggests that axon retraction is associated with an enhanced phosphorylation state of one or more axonal proteins.

10.13

GAP-43 IS A UNIQUE TYPE OF GUANINE NUCLEOTIDE RELEASE PROTEIN FOR G_o. S.M. Strittmatter*, D. Valenzuela*, Y. Sudo*, D. Platika and M.C. Fishman. Depts. of Neurology and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, MA

We previously showed that G_o is a major protein in the growth cone membrane, suggesting that it may transduce some extracellular signals into second messanger changes which lead to directed neurite outgrowth. In addition, G_o activity is susceptible to GAP-43. This is the first intracellular protein reported to regulate G proteins, an activity previously thought to be restricted to ligand-receptor complexes. Now we have studied the mechanism of G_o and GAP-43 interaction, comparing it to that of receptors and G proteins. Like receptors, GAP-43 increases the rate of GDP release, the initial rate of GTPγS binding and the steady state GTPase activity of G_o, but does not affect the intrinsic turnover number for hydrolysis of bound GTP. GAP-43 can be distinguished from receptors in that its stimulation of G_o is not blocked by pertussis toxin, is not dependent on the presence of $\beta\gamma$ subunits, and occurs equally effectively in detergent solution and in phospholipid vesicles. These data demonstrate that GAP-43 is a guanine nucleotide release protein (GNRP) for G_o, but that it has some unique properties compared to other G protein stimulators. The GNRP activity of GAP-43 argues that it is an upstream positive regulator of G_o in vivo, and that therefore G_o may integrate extracellular and intracellular signals in the growth cone.

10.14

G-PROTEINS MODULATE GROWTH CONE FUNCTION IN SYMPATHETIC GANGLION CELLS. T. Vartanian*, S. M. Strittmatter*, J. Vanselow* and M. C. Fishman, Depts. of Neurology and Medicine, Mass. General Hospital and Harvard Medical School, Boston, MA 02114.

Previous work has demonstrated that G_o is both a major component of the growth cone membrane and is regulated by the growth associated protein, GAP-43, thus leading to the speculation that growth cone G-proteins regulate neuronal morphogenesis. To examine this hypothesis we have perturbed G-protein function in cultured embryonic chick sympathetic neurons. We find that pertussis toxin-mediated uncoupling of G_o and G_i from receptors causes an increase in both the length and the total number of neurites, and that this effect is modulated by the nature of the substratum. In contrast, direct stimulation of G-proteins with mastoparan or aluminum flouride nearly abolishes neurite extension, in a reversible manner. These data provide support for the notion that G-proteins play a critical role in growth cone function.

SECOND MESSENGERS I

11.1

TYROSINE PHOSPHORYLATION OF MAP KINASE INDUCED BY STIMULATION OF PI-LINKED GLUTAMATE RECEPTOR. J.M. Baraban, R.S. Fiore, and T.H. Murphy. Depts. of Neuroscience, Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Recent studies have identified a novel serine-threonine phosphorylating enzyme, referred to as MAP kinase, that is activated by tyrosine phosphorylation. In previous studies, we have demonstrated activation of this kinase in hippocampus by electroconvulsive treatment (*J. Neurochem.* 56, 147 (1991)). To study the regulation of this kinase by neurotransmitters, we have employed primary cortical cultures. We have found that phorbol 12,13 diacetate, an activator of PKC, produces a marked increase in tyrosine phosphorylation of a 42 kD protein that co-migrates with MAP kinase. Furthermore, trans-ACPD, a selective agonist of the PI-coupled glutamate receptor, produces a similar effect. These findings indicate that the tyrosine kinase signalling pathway can be activated by neurotransmitter stimulation of the PI-PKC system and that MAP kinase is regulated via this cascade.

11.2

SYNERGISTIC ACTIVATION OF PROTEIN KINASE C BY ARACHIDONIC ACID AND DIACYLGLYCEROLS IN HIPPOCAMPAL NEURONS. D.O. KEYSER AND B.E. ALGER. University of Maryland School of Medicine, Dept. of Physiology, Baltimore, MD 21201.

Activation of protein kinase C (PKC) influences neuronal activity through regulation of Ca^{2+} and other ion channels, enhancement of spontaneous synaptic activity and induction of LTP. Diacylglycerols (DAG) and arachidonic acid (AA) each can activate PKC but the high concentrations tested have raised questions about their actual role.

We tested the hypothesis that AA and DAG can synergistically activate PKC at low concentrations using two PKC-mediated responses as electrophysiological bioassays in hippocampal neurons: 1) We found a marked synergistic interaction between AA and DAG on PKC-mediated depression of whole-cell Ca^{2+} current. EC_{50}'s of the dose-response curves for AA or DAG alone shifted from $\geq$25 μM to ~2 μM when both were present. Enhanced responses were blocked by PKC inhibitors and were not seen with inactive compounds. Interestingly, synergy occurred over a limited dose range, suggesting a novel specificity, perhaps related to different PKC isoforms. 2) Spontaneous synaptic activity was increased by AA-plus-DAG in a PKC-dependent way, providing the first physiological evidence that the α and β PKC isoforms are susceptible to the synergistic interactions of AA and DAG and are, indeed, involved in synaptic transmission. In view of these data, a role of AA in PKC activation assumes an even more likely physiological significance.

11.3

Characterization of [3H]Phorbol 12,13-dibutyrate binding in the circumesophageal ganglion of *Hermissenda crassicornis*. Donna L. McPhie, David S. Lester, James L. Olds and Daniel L. Alkon. Neural Systems Section, NINDS, NIH, Bethesda MD 20892.

Hermissenda crassicornis exhibits a learning specific increase in [3H] Phorbol 12,13-dibutyrate binding that is localized to individual types of nerve cells as assessed by emulsion autoradiography of the circumesophageal ganglion(McPhie et al, 1990). In the present study saturation binding conditions, subcellular distribution and competition with binding of diacylglycerol were further characterized to demonstrate the specificity of [3H]PDBU binding to Protein Kinase C in *Hermissenda*. [3H]PDBU binding was shown to be saturable at 15-25nM. Kd was estimated (Bmax/2) at 9±2nM, similar to that found in vertebrates and other invertebrates. Incubation of cytosol and detergent extracted membrane fractions at a saturating concentration of [3H]PDBU (25nM) showed *Hermissenda* CNS to bind 4.7±0.4 fmol [3H]PDBU/mg protein and 3.9±0.5 fmol [3H] PDBU/mg protein, giving a cytosol/membrane distribution of 55% and 45% respectively. *Hermissenda* CNS incubated in 2.5nM [3H]PDBU specifically bound 80 fmol [3H]PDBU/mg protein. In the prescence of OAG, a cell permeable diacylglycerol analog, and (1 x10-5 M) PDBU there was 17.7 fmol [3H] PDBU/mg protein bound, indicating that OAG displaced 78% of specific [3H] PDBU binding. The above results support the proposal that [3H] PDBU binds to PKC in *Hermissenda* CNS. Also to our knowledge this is the first demonstration of competition of OAG in an intact tissue system.

11.4

EFFECT OF ALCOHOL DEPENDENCE AND WITHDRAWAL ON PHOSPHO-INOSITIDE TURNOVER IN RAT BRAIN. S.C. Pandey*, M. Plano*, P.L. Reddy*, D. Schwertz*, J.M. Davis and G.N. Pandey. College of Medicine, University of Illinois at Chicago.

In order to examine if alcohol dependence or withdrawal are related to changes in receptor-mediated phosphoinositide signalling systems, we studied the effects of long-and short-term ethanol treatment and withdrawal on agonist- stimulated inositol phosphate formation in rat brain. Rats were maintained on either lieber-decarli liquid diet containing ethanol (9% v/v) or control liquid diet for 2 month (chronic) or 15 days (subchronic). Rats were sacrificed after 0 and 24 hrs of withdrawal, and $5HT_2$, $alpha_1$ adrenergic receptors, NE and 5HT stimulated inositol phosphates formation was determined in cerebral cortex. We observed that 5HT-stimulated [^{3}H]-IP_1 formation was significantly decreased in chronically treated rats as compared to control rats. $5HT_2$ receptors number, as measured by [^{3}H]-Ketanserin binding tended to decrease but was not significantly changed in cortex of chronic ethanol treated rats compared to control rats. 5HT-stimulated [^{3}H]-IP_1 formation was also decreased in subchronically treated rats before ethanol withdrawal and after 24 hours of ethanol withdrawal. Neither NE-stimulated [^{3}H]-IP_1 formation nor B_{max} and K_d of [^{3}H]-prazosin binding were significantly changed in chronic or subchronic ethanol treated rats. Our results thus indicate that chronic or subchronic treatment with ethanol decreases 5HT-induced but not NE-induced PI turnover. This decrease in 5HT-induced PI turnover may be due to decrease in coupling of $5HT_2$ receptors to effector pathway of PI system after chronic exposure to ethanol.

11.5

MODULATION BY LITHIUM OF BASAL AND CHOLINERGIC-INDUCED PHOSPHOINOSITIDE SIGNALING IN THE BRAIN. K.M.Savolainen and M.-R.Hirvonen. Natl. Publ. Hlth Inst., Dept. Env. Hyg. & Toxicol., P.O.B. 95, SF-70701 Kuopio, Finland.

Effects of LiCl on basal and cholinergic-induced cerebral regional phosphoinositide (PI) signaling were studied in rats after a single (2.5-16 mEq/kg) and 14 repeated i.p. doses (2.5 mEq/kg) of LiCl. To study the effects of LiCl on cholinergic-induced PI signaling rats were pretreated with saline or LiCl (10 Meq/kg) 24 h prior to a convulsive dose of an indirect cholinergic agonist malaoxon (MO; 39.2 mg/kg i.p.). Changes in mass amounts of inositol and inositol-1-phosphate (Ins1P) were used as indices of changes in PI signaling. A single dose of LiCl decreased cerebral inositol dose-dependently with a threshold maximum at 10 mEq/kg and caused a reduction of inositol from about 20 to 12 mmol/kg (dry brain weight). Similarly, the threshold maximum of LiCl-induced increase of Ins1P was at 10 mEq/kg, but the increase of Ins1P was only 5 % of the decrease of inositol at this dose of LiCl. Inositol-4-phosphate levels remained stable. Repeated doses of LiCl caused a slight but transient increase of Ins1P, but inositol levels were unaffected. When rats were given 39.2 mg/kg of MO, 60 % of the rats had tonic-clonic convulsions, and a stimulation of PI signaling occurred in most brain regions during the 72 h observation period. Increased PI signaling correlated well with the occurrence of convulsions. LiCl pretreatment decreased the dose of MO which produced convulsions (26.2 mg/kg) but, at the same time, attenuated cholinergic-induced PI signaling. These results provide evidence that lithium may not work through the PI signaling system in cyclic affective disorders. Also, although LiCl increases the potential of a cholinergic agonist to produce convulsions it at the same time attenuates cholinergic-induced PI signaling. Supported by the Research Council of Environmental Sciences, The Academy of Finland.

11.6

A NOVEL INOSITOL PHOSPHATE ACTS AS A SELECTIVE NEUROPEPTIDE Y (NPY) ANTAGONIST. C. Wahlestedt, D.J. Reis and L. Edvinsson*. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021; Dept. of Internal Medicine, Lund Univ., S-221 85 Lund, Sweden.

The novel compound D-myo-inositol 1,2,6-triphosphate (Ins[1,2,6]P_3, PP56) is a synthetic isomer of the Ca^{2+} mobilizing second messenger, Ins[1,4,5]P_3. While the potent sympathetic vasoconstrictor, NPY, indeed is a Ca^{2+} mobilizing agonist in vascular smooth muscle cells (VSMC), the peptide does not elicit appreciable changes in Ins[1,4,5]P_3 levels. Since there exists an array of inositol phosphates, we hypothesized that (at least) one might be involved in and/or modulate the signal transduction of NPY. In several blood vessel segments, including guinea-pig basilar and human subcutaneous arteries, extracellularly applied Ins[1,2,6]P_3, in sub- to low micromolar concentrations, inhibited (by >80%) vasoconstriction elicited by NPY, but not by norepinephrine or a range of other vasoconstrictors. Parallel experiments in cultured VSMC from rat vena cava (RVC) revealed the ability of Ins[1,2,6]P_3 to selectively inhibit NPY-induced elevations of intracellular Ca^{2+}. Ins[1,2,6]P_3 did not appear to act as a dihydropyridine Ca^{2+} channel antagonist nor did it show high affinity to previously characterized inositol phosphate binding sites. Moreover, binding studies using intact or homogenized VSMC from RVC (or rat brain homogenates) indicated that Ins[1,2,6]P_3 does not affect the binding characteristics of ^{125}I-NPY at its specific binding sites. We therefore conclude that the novel inositol phosphate acts as a selective (functional) NPY antagonist, presumably by inhibiting the pathway involved in NPY-induced Ca^{2+} mobilization.

11.7

TREATMENT OF CEREBELLAR GRANULE CELLS WITH CHOLERA TOXIN REDUCES CARBACHOL-INDUCED PHOSPHOINOSITIDE HYDROLYSIS. R. Raulli, E. Costa, and J.T. Wroblewski. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Sch. of Med., Washington, DC 20007.

Primary cultures of cerebellar granule cells express muscarinic receptors coupled to phosphoinositide (PI) hydrolysis. In these cells, the cholinergic muscarinic agonist carbachol induced a concentration-dependent increase in PI hydrolysis (EC_{50}= 10 μM). Overnight treatment of granule cells with cholera toxin (CTX), a potent endotoxin that causes ADP-ribosylation of G proteins, decreased the response to carbachol. The effect CTX was dose-dependent reaching a plateau at toxin concentration of 1.5 μg/ml. At maximal concentrations CTX inhibited the carbachol-induced PI hydrolysis by about 50%. The carbachol dose-response curves performed with and without CTX pretreatment indicated a noncompetitive character of CTX-induced inhibition. In order to investigate the ability of CTX to cause ADP-ribosylation of protein substrates in cerebellar granule cells the cell homogenates were incubated with increasing concentrations of CTX in the presence of [^{32}P]NAD. The results indicated a dose-dependent increase in ^{32}P incorporated into a 45 kDa protein band. The incorporation of label was reduced in the presence of snake venom phosphodiesterase, or when the labelled proteins were treated with NaOH or neutral hydroxylamine, indicating that the label is incorporated via covalent ADP-ribosylation. These data indicate that the G protein involved in the coupling of muscarinic receptors to phospholipase C can be inhibited by the ADP-ribosyltransferase activity of CTX.

11.8

IMAGING OF Ca^{2+} LIBERATED IN XENOPUS OOCYTES BY AGONISTS AND PHOTO-RELEASED INOSITOL TRISPHOSPHATE Y. Yao and J. Parker. Lab. Cellular & Molecular Neurobiology, Dept. Psychobiology, University of California Irvine, CA 92717.

By the use of long-wavelength fluorescent indicator dyes we had recorded Ca^{2+} transients in Xenopus oocytes evoked by photo-released inositol 1,4,5-trisphosphate ($InsP_3$) (Parker & Ivorra, Science 250, 977-979; 1990). We have now extended these studies by using an intensified CCD camera to image intracellular Ca^{2+} transients evoked by agonists and by spatially defined photorelease of $InsP_3$.

Experiments were done on isolated, defolliculated oocytes of Xenopus laevis, obtained from albino frogs and injected 1 hr before recording with 1-5 nl of a solution containing 5 mM fluo-3 and 0.5 mM caged $InsP_3$. Following bath application of serum to activate the $InsP_3$ pathway, Ca^{2+}-fluorescence signals began after a few tens of seconds, and appeared as discrete foci. Over several seconds these enlarged, and additional foci appeared, until the Ca^{2+} level was diffusely elevated over the whole oocyte. The fluorescence was then greater in the animal hemisphere as compared to the vegetal, and this polarity was maintained as the fluorescence subsided after washing out the serum. Flash-photolysis of caged $InsP_3$ by a slit of UV light oriented along the animal/vegetal axis evoked a fluorescence signal that began following a short (a few hundred ms) latency and was brighter near the animal pole. However, unlike the spreading wave of Ca^{2+} seen with agonist application, Ca^{2+} liberation remained confined to that region stimulated by the light. Supported by grant GM39831.

11.9

INOSITOLTRISPHOSPHATE RECEPTOR: IDENTIFICATION OF PHOSPHORYLATION SITES AND CHARACTERIZATION OF THE RECONSTITUTED ION CHANNEL COMPLEX Christopher D. Ferris, Andrew M. Cameron, David S. Bredt, Sonye K. Danoff, Christopher A. Ross, Richard L. Huganir and Solomon H. Snyder. Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205.

The inositoltrisphosphate receptor has been biochemically isolated, molecularly cloned, and functionally reconstituted in lipid vesicles. In the published cDNA sequences of both the rat and mouse receptors two consensus sequences for PKA phosphorylation are observed. Recently we have isolated and sequenced tryptic fragments from the rat cerebellar receptor following PKA phosphorylation and directly demonstrated phosphoserine at amino acids 1589 and 1755. S-1755 is much more readily phosphorylated in the cerebellar form of the receptor. Also, we have found a 120 nucleotide insert between S-1589 and S-1755 which is removed by alternative splicing in non-neuronal forms of the receptor. We have used the purified rat vas deferens receptor to characterize this shorter form. In the shorter form the Km for PKA phosphorylation is five fold higher (3nM) and serine 1589 is preferentially labeled. We have recently cloned the human cerebellar receptor and found that the phosphorylation sites are conserved. PKC and Cam kinase II have also been found to phosphorylate the receptor in reconstituted vesicles on serine. The sites which are labeled are being investigated. We are also investigating the functional regulation of the reconstituted receptor by calcium, nucleotides and phosphorylation.

11.10

DISTINCT IP_3R FORMS DERIVED BY ALTERNATIVE SPLICING. S.K. Danoff, S.H. Snyder and C.A. Ross. Dept of Neuroscience, Johns Hopkins University Sch. of Med., Baltimore, MD 21205.

The inositol 1,4,5-trisphosphate receptor (IP_3R) mediates the release of calcium from intracellular stores. The IP_3R has been purified from rat cerebellum and is regulated by calcium concentration, ATP concentration and protein kinase A (PKA) phosphorylation. The receptor cDNA has recently been cloned from mouse, rat and human. We have identified a 120 nucleotide alternative splice site with tissue specific expression. The splice site is located between two PKA phosphorylation consensus sequences. IP_3R isolated from tissues expressing the short or long forms of the message display distinct phosphorylation patterns and kinetics with PKA. This splice site may represent a regulatory mechanism conferring different phosphorylation characteristics to receptors differing in the expression of the splice site. We have developed anti-peptide antibodies directed at the two splice forms which recognize the distinct proteins derived from the short and long messages. We are carrying out genomic cloning to determine the intron/exon structure.

11.11

INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS (IP_3R): CLONING OF THE HUMAN cDNA AND AN IP_3R-RELATED MOUSE cDNA INDICATING A FAMILY OF IP_3R-RELATED GENES. C.A. Ross, S.K. Danoff, C.D. Ferris, C. Donath, G.A. Fischer, S. Munemitsu, S.H. Snyder and A. Ullrich. Johns Hopkins University School of Medicine, Department of Neuroscience, Baltimore, MD 21205; Max Planck Institut für Biochemie, Department of Molecular Biology, 8033 Martinsried bei München, Germany.

IP_3 stimulation of the IP_3R mediates the mobilization of intracellular calcium in response to hormones or neurotransmitters. We are in the process of cloning a cDNA for the human IP_3R. In the 5 kb we have sequenced, there is over 90% identity with the mouse cDNA sequence. Northern blot analysis of human cerebellar RNA showed a single 10 kb band, and in situ hybridization showed dense label over cerebellar Purkinje neurons.

We have also identified cDNAs with sequences related to the IP_3R, using PCR and low stringency hybridization. One clone, M1, is homologous to a portion of the mouse IP_3R mRNA coding for part of the membrane spanning regions. It has 85% nucleotide identity with the IP_3R and about 60% identity with the ryanodine receptor. This is consistent with the hypothesis that the IP_3R is a member of a gene family.

11.12

INOSITOL TETRAKISPHOSPHATE AND HEXAKISPHOSPHATE RECEPTORS: ISOLATION AND PHOTOAFFINITY LABELING IN RAT BRAIN. A. Theibert, V. Estevez*, R. Mourey, C. Ferris, R. Barrow*, G. Prestwich*, and S. Snyder. Dept. Neuroscience, Johns Hopkins School of Med, Baltimore, Md. 21205 and Dept. Chemistry, SUNY at Stony Brook, Stony Brook, N.Y.

Using an Inositol tetrakisphosphate (IP4) affinity column (V.Estevez and G. Prestwich, *Tetrahedron Lett.* 32, 1623-1626, 1991) we have isolated high affinity IP4 and inositol hexakisphosphate (IP6) binding proteins from detergent solubilized rat cerebellar membranes. Three binding peaks can be eluted from the IP4 column. An IP6-selective site elutes first with a Kd for IP6 of 10-20 nM. IP4 and IP5 bind with 2-3 fold lower affinity. By SDS-PAGE, three proteins of 115, 105, and 50 kDa coelute with the binding peak. Under native conditions, binding migrates as a 300-350 kDa complex. The 105 and 115 kDa subunits, but not the 50 kDa subunit is specifically photoaffinity labeled with [125I]1-O-[N-(4azido-salicyloxy)-3-aminopropyl-1-phospho myo-inositol 3,4,5 trisphosphate.

Two distinct IP4-selective binding peaks can be eluted off the IP4 column with high salt that have high affinity and selectivity for IP4 (Kd = 2-6nM) with IP5, IP6 and IP3 only 10,5, and 1 %, respectively, as potent as IP4. By SDS-PAGE, binding corresponds to proteins of 182 kDa in the first peak and 174 and 84 kDa in the second peak. These proteins are specifically photolabeled with the [125I]ASA-IP4. Photolabeled receptors have distinct pH optima, sensitivity to divalent cations, high affinity and selectivity for IP4, and are enriched in the brain. The IP4 receptor proteins are phosphorylated in vitro by protein kinases C and A. We are determining whether these are part of a single receptor if they are distinct receptors and examining their physiological function.

NERVE GROWTH FACTOR I

12.1

NERVE GROWTH FACTOR REGULATES MUSCARINIC RECEPTORS mRNA IN TELENCEPHALIC NEURONAL CULTURES FROM NEONATAL RATS. [1]Carola Eva, [2]Mariella Fusco, [1]Rossella Brusa*, [1]Alessandra Oberto*, [2]Guido Vantini and [1]Enrico Genazzani*. Istituto di Farmacologia e Terapia Sperimentale, University of Torino (1),and Fidia Research Laboratories, Abano Terme(2), Italy.

The effect of nerve growth factor (NGF) on muscarinic cholinergic receptors (mAChR) was investigated in a primary culture of telencephalic neurons prepared from newborn rats. The treatment with NGF (100 ng/ml), that elevates both choline acetyltransferase activity and intracellular ACh content, differentially regulates the expression of mAChR during cultivation. NGF induces a significant increase of mAChR number at the 3rd and the 5th day of in vitro (DIV), as measured by [^{3}H]-quinuclidinyl benzylate binding to cell homogenate, that is followed by a dramatic decrease of the receptor density from the 9th day of culture. The decrease of mAChR induced by prolonged exposure of telencephalic neurons to NGF is associated with a comparable reduction of the relative content of the mRNA encoding m1 and m3 receptors at the 10th DIV, while the m4 mRNA is increased by the treatment. We suggest that the stimulation of cholinergic neurons by NGF induces a downregulation of postsynaptic muscarinic receptors (m1 and m3) in the cerebral cortex and the hippocampus, while it increases the synthesis of the m4 receptor that may be mainly presynaptically localized on striatal interneurons. The transient increase of the receptor number that occurs at the first days of culture, is not paralleled by changes in the relative content of mRNA encoding mAChR subtypes and might be associated with the trophic activity of NGF on cholinergic synapses during early development.

12.2

TRANSYNAPTIC REGULATION OF THE NGF RECEPTOR (LNGFR) GENE AND OF VGF, AN NGF-INDUCIBLE GENE, FOLLOWING PARTIAL NEURAL LESIONS. G.A. Kuchel[1,2], S. Salton[1], R. Hellendall[1] and M. Blum[1], Fishberg Research Center in Neurobiology[1] and Dept. of Geriatrics[2], Mount Sinai School of Medicine, New York, NY 10029.

The rat pineal gland receives bilateral innervation from the two sympathetic superior cervical ganglia (SCG) through internal carotid nerves (ICN). Cutting the ICN on one side has been shown to remove one-half of the pineal innervation and to be followed by the development of collateral sprouting from the other SCG which can be detected by 3 days. We used a quantitative ribonuclease protection assay to measure NGF receptor (LNGFR) mRNA levels in individual SCG. LNGFR mRNA levels (pg/μg total RNA) were increased 25-30%, with no change in cyclophilin mRNA, in the "intact" SCG, contralateral to the lesion performed 1 or 3 days earlier, as compared to "intact" SCG in sham-operated animals (2-way Anova; $p < .05$). These results, combined with *in-situ* hybridization studies, suggest that there is increased expression of the LNGFR gene in the SCG from which collateral sprouting takes place. We also measured levels of NGF mRNA in pooled pineal glands and VGF mRNA in SCG after this lesion. The latter gene is known to be rapidly and quite selectively induced by NGF in PC12 cells. A finding of elevated VGF mRNA levels in the SCG involved in collateral sprouting would support the hypothesis that LNGFR gene induction in collateral sprouting may be mediated through greater stimulation by target tissue-derived NGF. (supported by MRC of Canada, the Brookdale Foundation and the MacArthur Foundation Program on Successful Aging)

12.3

REGULATION OF GENE EXPRESSION IN MATURE SYMPATHETIC NEURONS BY TERMINALLY-DERIVED NGF. F.D. Miller, T.C. Mathew,and A. Speelman.* Dept. of Anat. and Cell Biol., Univ. of Alberta, Edmonton, Alberta, CANADA.

We have previously demonstrated that systemic administration of NGF increases neuronal expression of mRNAs encoding the low-affinity NGF receptor (LNGFR), tyrosine hydroxylase (TH), and Tα1 α-tubulin (Tα1) in neonatal rats (Miller et al., JCB, 112, 303, 1991). To determine whether terminally-derived NGF is capable of eliciting similar changes in mature neurons, we retrogradely labelled neurons that project from the SCG to the iris with fast blue, and labelled those that project from the SCG to the ear with fluorogold. For 7 days following labelling, 2.5S NGF was injected daily into the anterior chamber of one eye, and PBS or cytochrome C into the other. We subsequently performed in situ hybridization to compare levels of LNGFR, TH, and Tα1-tubulin mRNAs in fast blue-labelled neurons innervating the iris versus fluorogold-labelled neurons of the same ganglia innervating the ear. This analysis demonstrated that NGF delivered to nerve terminals in the iris caused hypertrophy of mature sympathetic neurons, increasing cross-sectional areas 25-35% relative to control neurons innervating the PBS injected eye. Grain-counting of fast blue and fluorogold-labelled neurons within the same ganglion (done to minimize intersection variability) indicated that LNGFR and TH mRNAs were at least two-fold higher in neurons whose terminals were treated with NGF. Preliminary results indicate a somewhat smaller increase in levels of Tα1 mRNA. These results suggest that the availability of target-derived neurotrophins may regulate the synthesis of proteins important to neuronal growth and transmitter function in the mature nervous system.

12.4

GENETICALLY ENGINEERED CELLS PRODUCING NGF REDUCE THE NEUROTOXICITY OF QUINOLINATE AND QUISQUALATE *IN VIVO* J.M. Schumacher, W.S Rosenberg, M.P. Short, S.R. Bossi*, X.O. Breakefield and O. Isacson. Dept. Neurology and Neurosurgery, Harvard Med. Sch., McLean Hospital, Belmont MA 02178 and Neuroscience Center, Massachusetts General Hospital.

Trophic factors, such as NGF, may elevate the neuronal threshold towards excitotoxicity in vivo. In order to test this hypothesis (1) a genetically engineered NGF-producing rat fibroblast cell line (NGF+) was produced and then stereotaxically implanted into the brain, providing a continuous supply of NGF prior to, during, and after the neuronal insults. A second group of rat received the same number of fibroblasts without the NGF gene inserted; (NGF-) (2) One week later, quinolinic acid (120 nmoles) or quisqualic acid (105 nmoles), was infused as a neurotoxin into the neostriatum in both groups . The animals with prior graft placement of NGF(-) fibroblasts, had quinolinate and quisqualate lesions of the neostriatum with sizes similar to those in animals without any implants. In contrast, the lesion area in the NGF(+) transplanted group was between 50-70% smaller than those in animals containing NGF(-) grafts. Relative sparing of cholinergic neurons was significantly increased within lesion zones in the NGF(+) groups, while NADPH diaphorase neuronal counts were not affected by this treatment. These results show that NGF released by genetically modified cells can reduce neurotoxicity produced by glutamate-receptor agonists in vivo .

12.5

7S-NGF PENETRATES FROM THE BLOOD TO THE CNS IN THE NEONATAL RAT. R.H. Fabian and C.E. Hulsebosch. Dept. of Neurol. and of Anat. and Neurosci. and the Marine Biomed. Inst., Univ. Tex. Med. Branch, Galveston, TX 77550.

Recent evidence indicates that NGF is present in significant amounts in the serum of neonatal rats. We postulate that blood borne NGF has a role in the development of the CNS as a humoral trophic factor. We began to investigate this possibility by determining the extent to which NGF can penetrate from the blood to the brain in neonatal rats.

Radioiodinated NGF was injected intraperitoneally into 1 or 2 day old rats. Following a survival of 2, 4, 6, 12, 24, and 48 hours, the rats were deeply anesthetized and perfused with saline and paraformaldehyde following sampling of blood. Blocks of brain tissue were prepared and gamma activity and weights of all tissue samples determined. Some blocks were prepared for autoradiography.

The results indicate that NGF penetrates from the blood to the CSF to bind to NGF receptors in nervous tissue. Levels of NGF in the brain reach levels of 3 to 5% of blood activity within 4 hours. This supports a role for blood-borne NGF as a humoral trophic factor for CNS development.

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12.6

NGF RECEPTORS, FGF RECEPTORS, AND CHOLINERGIC NEURONS IN THE DEVELOPING AVIAN CERULEUS COMPLEX. C.S. von Bartheld and M. Bothwell. Dept. of Physiology & Biophysics (SJ-40), University of Washington, Seattle, WA 98195.

The mesencephalic tegmentum and ceruleus complex contain neuronal populations with heterogeneous phenotypes. We used in situ hybridization in adjacent sections to determine FGF- and NGF receptor mRNA expression, and immunocytochemistry to visualize choline acetyltransferase in the developing chick brain (E6-P14).

As early as E6, about 2000 cholinergic neurons extend from the nucleus mesencephalicus profundus, pars ventralis (MPv) to the locus ceruleus. FGF-R expression begins at E13 in the same population of at least 1600 neurons and appears codistributed with the cholinergic phenotype. While FGF-R expression remains at high levels, NGF-R expression generally decreases during development, but not in a distinct population of about 700 neurons in the caudal ceruleus complex which retains high levels of NGF-R expression and may coincide with the noradrenergic population of the ceruleus complex.

Our results indicate that the MPv and part of the avian ceruleus complex are homologous to the cholinergic pontomesencephalotegmental complex of mammals (Woolf, N.J. & Butcher, L.L.: *Brain Res. Bull.* 16:603, 1986). Transmitter heterogeneity of the avian ceruleus neurons is reflected by a heterogeneity of growth factor receptors, with FGF-R expressed in the cholinergic subpopulation, and NGF-R in the presumptive noradrenergic population. These results are consistent with the hypothesis that transmitter phenotypes and target specificity may be regulated by neurotrophic factors.

Supported by NIH grants NS08990 and NS23343.

12.7

POTENTIAL INVOLVEMENT OF NERVE GROWTH FACTOR (NGF) IN SPATIAL MEMORY FORMATION. J.E. Springer, B.J. Gwag, G. Woertwein, R. Stackman, R. Rogers, K. Opello, and T.J. Walsh, Dept of Neurology, Hahnemann Univ, Phila, PA 19102 (JES and BJG), and Dept of Psychology, Rutgers Univ, New Brunswick, NJ 08903.

Molecular, biochemical and morphological data support the role of NGF as a neurotrophic molecule for basal forebrain cholinergic neurons. This population of cholinergic neurons appears to be critical in spatial memory formation in rats. Due to the close association of NGF with the basal forebrain cholinergic system, we have suggested that NGF may play a more dynamic role in memory formation. To test this, we determined the effects of NGF treatment on acquisition of a well-defined spatial task, the Morris water maze. Animals received daily infusions of NGF (3.0 µg) prior to exposure to the spatial task. We found that daily infusions of NGF, but not basic fibroblast growth factor, or control infusions disrupted the animals ability to learn the location of the escape platform. In addition, NGF treatment resulted in increased NGF receptor gene expression in cells of the medial septum and striatum, areas known to contain NGF-responsive neurons. In a second study, we analyzed levels of NGF messenger RNA (mRNA) in the hippocampus of animals following 20 trials in the spatial task. Using this single day testing paradigm, one population of animals showed excellent learning of the spatial task, while another group of animals exhibited difficulty in acquisition. A third group of animals was run as a yoked control group, which received exposure to the task but were not allowed escape the maze. Using the RNA protection assay procedure, we determined that animals exhibiting rapid learning in the spatial task had lower levels of NGF mRNA in the hippocampus when compared to animals exhibiting slow learning, or control animals. We suggest that in addition to its role as a neurotrophic factor, NGF may function as a modifier of memory formation in the hippocampal formation, and "flooding" the central nervous system with NGF may mask or disrupt stimulus-specific signals necessary for normal memory formation. Supported by PHS grants RR 07058 (TJW) and AG-08969 and the Alzheimer's Disease Research Foundation (JES).

12.8

NGF-LIKE AND NGFR IMMUNOREACTIVITY IN PITUITARY OF DEVELOPING MACAQUES. S. Borson*, G. Schatteman, P. Claude, M. Dubach, and M. Bothwell. Depts of Psychiatry and Physiology & Biophysics, Univ Wash Sch Med, Seattle WA 98195.

NGFR immunostaining of fibers in the median eminence has been observed in rat and primate, but neither NGF nor NGFR immunoreactivity (IR) has been well characterized in pituitary. We examined the pituitary and median eminence of macaques for the presence of NGF-like and NGFR IR. Nine animals, including fetal (≥ 100d), newborn, and adult specimens, were examined using NGFR5 (a monoclonal antibody to human NGFR) and an affinity-purified polyclonal antibody to mouse submandibular NGF. At all ages, NGF IR was associated with what appear to be adenohypophyseal secretory cells. Stained cells were most numerous in anterior pars distalis and in pars tuberalis, where they represented up to 50% of the cells. NGF-IR cells were arrayed singly, in groups along capillaries, or in duct-like or pseudoacinar structures. Sequential staining of adult glands with Abs to NGF and ACTH, GH, or PRL (gifts of the National Hormone and Pituitary Program) revealed few double-stained cells. The distribution of NGFR-IR contrasted markedly with that of NGF-IR. In pars distalis, NGFR-IR was limited to cell bodies and processes of folliculostellate cells. In fetal animals these processes formed an extensive network throughout the gland, becoming less prominent in the newborn and adult. In pars nervosa, intense NGFR-IR was seen adjacent to NGF-IR cells of pars tuberalis in fetal specimens, and on fibers and in the capillary network of the hypophyseal portal system at all ages. In the median eminence and proximal infundibulum, tanycyte processes, capillary walls and the contractile loops of the portal circulation were NGFR-IR. These anatomic findings suggest that NGF may be a secretory product of a subset of adenohypophyseal cells present by midgestation. NGFR's are also well developed by day 100 of fetal life, but the pattern of NGFR-IR varies during development, perhaps reflecting differing functions of pituitary NGF.

12.9

PERINATAL CHOLINE TREATMENT INCREASES BASAL FOREBRAIN CELL SIZE AND HIPPOCAMPAL NGF. R. Loy, and Y.S. Choe*. Canandaigua VAMC and Department of Neurology, University of Rochester, 435 E. Henrietta Road, Rochester, NY 14620.

Perinatal choline treatment results in long-lasting facilitation of spatial memory (Meck *et al.*, 1988, *Devel. Psychobiol. 21*, 339). In rats treated *in utero* from ED12 (300 mg/kg/day, *p.o.* to the dam) and postnatally until day 30 (150 mg/kg/day, s.c.) with choline chloride the average area of diagonal band somata immunoreactive for NGF receptor (NGFRir) or ChAT is up to 30% larger than controls (Loy *et al.*, 1991, in:*The Basal Forebrain: Anatomy to Function*). To determine if choline treatment causes cells to grow larger throughout development or prevents the naturally occuring regression of cell size after PD15 (Koh and Loy, 1989, *J. Neurosci. 9*, 2999), we compared the area of NGFRir cells in the diagonal band on the day of birth in rat pups treated *in utero* from ED12-17 and found that choline-treated cells have nearly twice the area of controls. In addition, hippocampi were dissected from PD20 rats following choline treatment given between ED12 and PD19, tissues frozen, then homogenized and assayed by ELISA. NGF levels in choline-treated hippocampus were approximately 3-fold higher than controls. We conclude that the organizational effects of perinatal choline treatment on spatial memory may be based in part on an early increase in the size and presumably enhanced cholinergic function in the septohippocampal system, which in turn may be regulated by altered levels of NGF in the developing hippocampus.

We thank Dr. W. Mobley for assistance with the NGF ELISA, D. Heyer and J. Miller for technical assistance, and W. Meck and C. Williams for helpful discussions. Supported by AG09525.

12.10

BASAL FOREBRAIN NEURONS IMMUNOREACTIVE TO NERVE GROWTH FACTOR RECEPTOR INNERVATE THE RETICULAR THALAMIC NUCLEUS IN THE RAT.

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Institute of Anatomy, University of Verona, Italy

Recent observations have shown that the reticular thalamic nucleus (RTN) is richly innervated by nerve growth factor receptor (NGF-R) immunopositive fibers, whose staining is considerably enhanced by intracerebroventricular administration of NGF. We searched in the present study for the origin of this RTN innervation in the basal forebrain (BF), using retrograde tracing with immunohistochemistry. Preliminary findings based on fluorescent tracer injection in the anterior pole of RTN indicate that some (5-10%) retrogradely labeled BF cells were also immunostained with NGF-R. Immunohistochemistry performed on alternate sections revealed that 5-10% of the BF labeled neurons were choline acetyl transferase-positive, and 10-15% were parvalbumin-positive. These data indicate that RTN is one of the targets of BF neurons expressing NGF-R. Moreover, in agreement with previous findings, the present results suggest that BF neurons projecting to RTN are chemically heterogeneous. The study of the relationships among NGF-R immunopositive cells, GABAergic cell bodies, and neurons projecting to RTN in the BF, as well as triple labeling experiments aimed at investigating the co-localization of NGF-R with other neuroactive compounds in the BF cells innervating RTN, are in progress.

12.11

EFFECTS OF FIMBRIA-FORNIX AND ANGULAR BUNDLE TRANSECTION ON NGFR mRNA-EXPRESSING CELLS LOCATED IN THE MEDIAL SEPTUM AND DIAGONAL BAND OF BROCA. R.B. Gibbs[1], M.V. Chao*[2], & D.W. Pfaff[1]. [1]Laboratory of Neurobiology & Behavior, Rockefeller University, and [2]Department of Cell Biology & Anatomy, Cornell University, N.Y., N.Y. 10021

Quantitative in situ hybridization techniques were used to examine the effects of lesions which sever hippocampal cholinergic and cortical afferents on nerve growth factor receptor (NGFR) mRNA-expressing cells located in the medial septum (MS), and the vertical (VDB) and horizontal (HDB) limbs of the diagonal band. Animals received either bilateral transection of the fimbria-fornix (FFX), unilateral transection of the angular bundle (ABX), or sham surgery (4 animals/group). Four days later, animals were sacrificed and sections through the MS, VDB, and HDB were processed for detection of NGFR mRNA using a ^{3}H-labeled riboprobe and in situ hybridization techniques previously described (Gibbs et al., Mol. Brain Res. 6: 275-287, 1989).

In the MS and VDB, FFX resulted in a significant decrease in the size of NGFR-expressing cells (25.9% and 15.1% respectively) which was accompanied by a significant reduction (37.9% and 12.7% fewer grains/cell) in relative levels of NGFR mRNA. In the HDB, FFX had no significant effect on the average size of NGFR-expressing cells; however, a significant increase (49%) in the mean relative level of NGFR mRNA was observed. ABX resulted in small but significant increases (9.4% and 10.9%) in relative levels of NGFR mRNA in the MS and VDB, as well as an increase (19.6%) in the number of NGFR mRNA-expressing cells in the HDB, on the injured side. No increase in NGFR expression in the MS, VDB, or HDB contralateral to the lesion was observed; however, a decrease in the size (8.0%) and message content (19.4%) of NGFR-expressing cells was detected in the MS contralateral to the lesion.

These data are consistent with the effects of FFX and ABX on the subsequent survival and growth of cholinergic neurons located in the MS, VDB and HDB, and therefore provide evidence for a link between NGF-related effects and lesion-induced changes in the organization and sprouting of cholinergic projections in the adult CNS.

12.12

NERVE GROWTH FACTOR ENHANCES NEURONAL PLASTICITY IN THE AGING RAT NEOCORTEX: A QUANTITATIVE GOLGI STUDY. R.F. Mervis[1,2], R. Lewis[1,2], R.M. Dvorak[1,2], D. Pope[2], and L.W. Williams[3]. [1]Neurocognitive Research Labs, Inc., Columbus, OH. 43212, [2]Div. Neuropathol., Ohio State Univ. Med. Ctr., Columbus, OH., [3] The Upjohn Co., Kalamazoo, MI.

The role of NGF on cortical neurons and circuitry is unknown. Here. we evaluated effects of chronic exogenous NGF treatment on extent of dendritic branching and dendritic spine density from the basilar tree of layer V frontal cortex pyramids in 3 groups of Fischer 344 rats: Young Controls (4 mo) [YC]; Old Controls (24 mo) [OC] and Old-NGF-Treated (24 mo) [O-NGF]. Old Ss were administered NGF (or control vehicle) unilaterally via osmotic minipump (1.2 ug/day) i.c.v. unilaterally for four weeks. Sholl Analysis of basilar dendritic branching showed that in 24 mo rats, the dendritic domain was **larger** than in 4 mo controls (compensatory hypertrophy). NGF treatment resulted in **regression** of dendritic branching. Dendritic spines were sampled on terminal tip and internal branch segments. Aging resulted in significant spine loss in both regions. However, NGF treatment caused a significant plastic response on the terminal tip segments: there was plumping of the segment and **formation of new dendritic spines in old rats**. Internal (non-plastic) segments showed no response. [Supported by Sigma Kappa Foundation, Roessler Foundation and the UpJohn Company]

HUMAN COGNITION

13.1

MODULATION OF EARLY SENSORY PROCESSING IN HUMAN AUDITORY CORTEX DURING AUDITORY SELECTIVE ATTENTION. M.G.Woldorff*(1), C.C.Gallen(2), S.R.Hampson*(2), S.A.Hillyard(1), C.Pantev*(2), and F.E.Bloom(2). Dept. of Neurosciences, Univ. of Calif. at San Diego, La Jolla, CA 92093-0608 (1), Dept. of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA, 92037 (2).

A dichotic listening experiment was conducted in which the stimulus and task conditions were optimized for the selective focusing of attention. Subjects (N=7) listened selectively to sequences of rapidly presented tone pips in one ear while ignoring tone pips of a different pitch in the opposite ear. Thirty-seven channels of event-related magnetic fields (ERFs) and three channels of event-related potentials (ERPs) were simultaneously recorded from over the left hemisphere. Previous ERP studies using this paradigm had revealed a small enhanced positivity between 20-50 msec (the "P20-50") in the ERPs to attended-channel tone pips, followed by a relative enhancement of several of the exogenous ERPs, including subcomponents of the central N1 and P2 waves and the temporal T-complex. The ERPs recorded in the present study confirmed the basic results of the previous studies. The magnetic counterpart to the electrical N1 wave, the "M1", also showed a strong effect of attention which, similar to the electrical N1 effect, closely overlapped the exogenous M1 in time. Moreover, the field patterns of the magnetic M1 and the attention effect on it were highly dipolar, inverting in polarity at anterior relative to posterior sensor locations. MRI scans were obtained from several subjects, and the anatomical locations of the neural generators of the attention-sensitive activity were determined by dipole source localization techniques. These calculations placed the sources of both the M1 and its enhancement with attention to be within Heschl's gyri on the supratemporal plane (primary and secondary auditory cortex). In addition, the earlier P20-50 attention effect also demonstrated a dipolar magnetic field distribution indicative of a source in Heschl's gyri.

13.2

WITHDRAWN

13.3

PHYSIOLOGICAL AND ANATOMICAL EVIDENCE FOR A MAGNOCELLULAR DEFECT IN DEVELOPMENTAL DYSLEXIA — A.M. Galaburda, Depts. of Neurology and Neurobiology, Harvard Medical School, G.D. Rosen and F.W. Drislane*, Dept. of Neurology, and M.S. Livingstone, Dept. of Neurobiology, Boston, MA 02215.

Developmental dyslexics do poorly in tests of rapid visual information processing[1]. In the primate, fast visual information is carried by the magnocellular subdivision of the visual pathway. We recorded visually evoked scalp potentials in dyslexics and found that they showed diminished potentials for rapid, low contrast stimuli but normal responses to slow or high contrast stimuli. The differences in the dyslexics' evoked responses were consistent with an abnormality in the magnocellular division at the level of Visual Area 1 or earlier. We therefore compared the lateral geniculate nuclei in 5 dyslexic and 5 non-dyslexic brains and found that the magnocellular neurons of the dyslexic brains were significantly smaller, on average 27% smaller, but the parvocellular neurons were the same size as in normals. Dyslexics often do poorly on tests of rapid auditory transitions as well,[2] and we hypothesize that auditory, and possibly other sensory and motor systems, are similarly divided into a fast and a slow subdivision and that dyslexics are specifically affected in the fast subdivisions.

[1] Martin F and Lovegrove W, *Perception 16*:215-221, 1987.
[2] Tallal P, *Brain and Language 9*:182-198, 1980.

13.4

Asymmetries of Probe Evoked Potentials and Intellectual performance in cognitively impaired Children. D.W.Shucard and J.L. Shucard*, Department of Neurology, SUNY at Buffalo School of Medicine and Biomedical Sciences, 100 High Street (D-6), Buffalo, NY 14203.

In a series of investigations, we examined the relationship between electrocortical activity and specific patterns of cognitive abilities as measured by the Wechsler Intelligence Scale for Children-Revised (WISC-R). The subject groups included males and females with no known cognitive deficits, and two groups with cognitive deficits: males with reading disability (RD) and females with Turner Syndrome (TS). Subjects ranged in age from 8-15 years. Auditory evoked potentials (AEPs) to task-irrelevant tone stimuli (Probes) were obtained while subjects performed specific Verbal or Spatial tasks. These tasks were presented in both auditory and visual modalities. The probe tones were superimposed on the on-going visual or auditory information. AEPs to the probe tones were obtained from temporal scalp sites of the left and right cerebral hemispheres.

The findings indicated that significant differences were present in the patterns of AEP amplitude asymmetries among the groups studied. Further, significant relationships were found between AEP amplitude asymmetry and WISC-R verbal- performance scores, regardless of the group. For example, the pattern of right - left temporal site amplitude asymmetry seen in TS girls paralleled the visual spatial deficits seen in this group. In addition, within the TS group, AEP amplitude asymmetry correlated significantly with the degree of discrepancy between verbal and performance WISC-R scores.

These findings, obtained across different populations, suggest that AEPs to probe stimuli may offer insight into the relationship between the functional organization of the brain and human abilities. Supported in part by NICHD grant HD11681 and Genentech, Inc.

13.5

ANATOMICAL SUBSTRATES OF EVENT-RELATED POTENTIALS ELICITED DURING MEMORY CHALLENGE: CLINICAL OBSERVATIONS.
L. deToledo-Morrell, D. Charletta*, F. Morrell and T. McNally*. Dept. of Neurological Sci. and Dept. Diagnostic Radiology, Rush Med. Coll., Chicago, IL 60612.

The P300 component of long-latency evoked potentials generated during a mnemonic task considered to engage hippocampal circuits may provide an index of the anatomical integrity of the hippocampal formation. We studied four patients electrophysiologically and with a special high resolution MRI protocol. Two patients had low-grade glioma; in one, the tumor infiltrated the entorhinal cortex, perforant path and dentate gyrus while in the other it involved the amygdala and uncus, sparing the hippocampus entirely. Two patients with vascular disease had primarily neocortical atrophy, one with and one without associated hippocampal destruction. P300 potentials were recorded referentially during a modified Sternberg memory scanning task having different degrees of memory load. P300 amplitude was strikingly diminished with increasing memory load only over the involved hemisphere and only in the patients with hippocampal destruction. Neocortical involvement alone did not diminish P300 amplitude or render it sensitive to memory load. We conclude that the P300 component of event related potentials recorded in the context of mnemonic demand may provide a sensitive marker of hippocampal dysfunction.

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13.6

REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING OBJECT AND WORD NAMING.
S.Y.Bookheimer, T.A.Zeffiro, C.Pelizzari, W.Theodore, W.Gaillard, P.Fedio and M.Hallett Medical Neurology Branch, 10-5D49, NINDS, National Institutes of Health, Bethesda, MD, 20892.

Although lesions of the inferior frontal gyrus (IFG) can produce expressive aphasia, positron emission tomography (PET) studies have not consistently demonstrated rCBF changes associated with language in that region. In the present study, rCBF was measured using H_2O^{15} in normal volunteers while naming objects or words, viewing objects or words, or viewing meaningless designs. After global normalization, image pairs were subtracted to obtain task-specific changes in rCBF. The resulting difference images were aligned with corresponding MRI using a surface registration algorithm. rCBF values were then obtained for cortical regions identified on MRI and analyzed using a 2x2x2 ANOVA design (hemisphere x naming condition x stimulus).

We found a significant increase in rCBF in the left IFG during viewing of both pictures and words ($p<0.001$), and decreased rCBF in the IFG during naming compared to viewing ($p<0.01$). In contrast, orofacial regions in pre- and post-central gyri were bilaterally active during naming but not viewing ($p<0.001$). In the medial superior frontal gyrus (supplementary speech area), rCBF was greater during naming than during viewing and greater on the left than on the right ($p<0.01$). The location of task-related changes in the IFG varied markedly across subjects.

These results indicate a more complex set of region-task interactions in the IFG than previously assumed. Specifically, the higher IFG activation with viewing rather than naming suggests a role in receptive language processing. Our results may differ from previous studies that found no activation in the IFG after subject averaging of PET data, which may reduce apparent activity due to anatomical variation in functional localization.

13.7

PRACTICE-RELATED CHANGES IN HUMAN BRAIN FUNCTIONAL ANATOMY.
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A previous PET study (Nature 331:585, 1988) led to the hypothesis that two distinct pathways can be used for verbal response selection, and the use of these pathways reflects the degree to which a given response is automatic. As a test of this hypothesis, a PET study, using measurements of local blood flow in 11 normal humans, was conducted to determine the functional effects of practice on a verb generation task. For the generation task, subjects were instructed to say aloud an appropriate verb for each of 40 visually presented nouns. As a control state subjects were asked to repeat the seen noun. Subjects were scanned during performance of the generation task in three different states of practice: 1) NAIVE -- initial (unpracticed) performance of the verb generation task, 2) PRACTICED -- following 9 blocks of practice with the same set of nouns, and 3) NOVEL -- following practice with the task, but with a novel set of nouns as stimuli. Six regions of interest were selected for analysis. An ANOVA was performed for each region across the 3 conditions followed by post hoc t-tests. All 6 regions varied significantly. Regions most active during NAIVE performance (left prefrontal, left temporal, anterior cingulate cortices and right cerebellar hemisphere) were all less active during PRACTICED performance. These changes were accompanied by reciprocal changes in sylvian-insular cortex bilaterally. The functional changes were correlated behaviorally with a significant reduction in response times and the occurrence of stereotyped responses across practice blocks. The practice-related changes appear to be largely item-specific, since analysis of the NAIVE vs. NOVEL subtraction images only reveals a significant difference in left prefrontal cortex. Thus, subjects appear to change areas used during the verb generation task following less than 15 minutes of practice.

13.8

CHANGES IN LOCATION OF BLOOD FLOW ACTIVATION IN EXTRASTRIATE CORTEX CORRELATE WITH PERFORMANCE DURING OBJECT AND SPATIAL VISUAL PROCESSING.
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We measured cerebral blood flow (CBF) in 11 young subjects using [15O]-water and positron emission tomography during 2 runs of 3 visual tasks- face and dot-location matching and a control task. Normalized difference images were computed by subtracting the first and second sets of control task images from the first and second sets of images obtained during the matching tasks, respectively. Areas of extrastriate cortex were identified based on our previous finding of occipitotemporal (OT) activation during face matching and superior parietal (SP) activation during dot-location matching. The centers of gravity (COG) of the CBF increases in OT and SP were determined from the difference images and compared between the 2 runs. The mean absolute change in the anterior/posterior location of the COG was 4.6 ± 1.1 mm (mean $\pm$ s.e.) in OT during face matching and 5.9 ± 1.5 mm in SP during dot-location matching. Some subjects showed COG changes of 8 mm or more, which were twice as likely to occur in the left hemisphere; 85% were in the anterior direction. There were significant correlations between the size of the left hemisphere COG shift and reaction time in the first run during both face ($r=0.71$, $p<0.01$) and dot-location matching ($r=0.74$, $p<0.01$). Although the location of rCBF activation in extrastriate cortex is relatively stable during repeated visual testing, some large changes are found, suggesting that more anterior cortical areas may be recruited during subsequent performance of the task. The left hemisphere COG shift that follows slower performance may reflect a change in strategy.

13.9

VARIETIES OF PROSOPAGNOSIA: NEUROBIOLOGICAL EVIDENCE.
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Studies of brain-damaged patients have revealed the existence of a selective impairment of face processing, prosopagnosia. Although the functional deficit is the same in all patients - an inability to experience a feeling of familiarity at the view of faces of known persons - the qualitative nature of the impairment varies considerably across patients. In this study, the patterns of successes and failures on various tasks of face and object processing by 4 prosopagnosic patients are considered in relation with radiological (MRI) and physiological (PET) data, in an attempt to determine what cortical areas are critically involved in the processing of faces, what functional aspects of face processing are served by these areas, and whether these areas are uniquely devoted to the processing of faces. This examination indicates that the locus of the lesion determines the functional locus of the cognitive impairment, and that damage in one of three cortical regions within the ventral occipito-temporal cortex of the right hemisphere may result in a break-down of facial information processing. The data from the patients are further compared to the results from a combined MRI and PET study in normal subjects.

13.10

CORTICAL STIMULATION EVIDENCE FOR TOPOGRAPHIC REORGANIZATION OF SPEECH IN BILINGUAL PATIENTS.
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During surgery for medically intractable epilepsy, performed in awake patients under local anesthesia, we have mapped speech cortex related to naming of line-drawn objects in 2 languages for each of 7 bilingual patients, based on errors characterized as naming arrest, substitution, or delay. A multinomial distribution and median absolute deviation methods were used in error analysis. Topographic analysis for 1st and 2nd languages in these patients usually showed partial overlap. We divided our patients into 2 groups, one more fluent in the 1st language and the other in the 2nd. Comparison of areas for 1st and 2nd languages within each group showed that the 2nd language territory on average was larger than the 1st for both groups, but with predominant fluency in the 2nd language, territories for both languages were smaller.

For pooled independent (single language) sites, the 1st language followed a diagonal from midfrontal to posterior temporal cortex, while the 2nd language appeared in parallel diagonals on either side of the 1st language. Independent 2nd language sites of lesser fluency could fall on the usual 1st language diagonal, though still independent from their 1st language sites in those patients.

(Supported by the Horbach Fellowship and by NIH Grants NS21724, NS17111, and NS20482).

13.11

MENTALLY ROTATING THREE-DIMENSIONAL FIGURES AND LISTENING TO TEXT: AN EEG MAPPING STUDY ON COGNITIVE PROCESSES IN HEALTHY PERSONS AND SCHIZOPHRENICS

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Recordings were obtained from 23 healthy and 11 schizophrenic males with eyes open and eyes closed and during two separate tasks: trying to determine if figures differently oriented in space were identical and listening to text with eyes closed. EEG was recorded with 19 electrodes placed on the scalp according to the international 10/20 system and against averaged signals picked up from both ear lobes (TC 0.3s, Filter 35 Hz). Topographic mapping was used to illustrate differences in spectral parameters such as amplitude (AM), local coherence (LC) and interhemispheric coherence (IHC).

In healthy males, during the rotation task, LC was increased mostly in parietal and central regions presumably related to spatial analysis and most pronounced in the beta3 range. LC was decreased mostly in frontal regions and in posterior areas known to process visual informations and was most pronounced in the alpha range. IHC was increased in posterior and decreased in frontal areas. When listening to text, main changes were present in local coherence (LC) between left temporal, central and parietal electrodes. In both tasks, regional changes involved both hemispheres and interhemispheric cognitive processing was increased.

In schizophrenics, important differences in LC and IHC were present suggesting altered language and visuo-spatial processing. When listening to text, changes in the left hemisphere were noticeably reduced although comprehension of the text was not altered. IHC results suggest significantly modified cognitive processing between both hemispheres.

13.12

ASSOCIATION OF ELECTROPHYSIOLOGIC BRAIN FUNCTION AND NUTRITIONAL STATUS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) INFECTION.

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There is evidence to suggest that abnormalities of vitamin B_{12} (cobalamin) and vitamin B_6 (pyridoxine) may affect neurologic function. This is of particular interest in light of recent findings demonstrating that alterations in these specific nutrients occur early in HIV-1 infection, and may be related to cognitive changes. The association between electrophysiologic brain function, as measured by quantified cognitive evoked potentials (CEP), and nutritional vitamin B_6 and B_{12} status was evaluated in 22 HIV-1 seropositive homosexual individuals (CDC Stages III, IV) over an eighteen month period. Our findings indicate that the overall level of vitamin B_{12} was related to electrophysiologic function ($r = -0.56$ $p<0.05$) and that a decrease in vitamin B_6 status (measured as an activity coefficient) was associated with more deviant cognitive evoked potentials ($r = 0.58$ $p<0.01$). These results suggest that an indirect mechanism (i.e. alteration of CNS metabolism via vitamin B_6 and/or vitamin B_{12} deficiency) may be one method by which an HIV-1 infection produces cerebral dysfunction and changes in the CEP.

ACETYLCHOLINE RECEPTORS: NICOTINIC

14.1

THYMOPOIETIN PREVENTS NICOTINE INDUCED ALTERATIONS IN MORPHOLOGY AND NICOTINIC RECEPTORS IN NEONATAL RAT MUSCLE CULTURES.

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The present results show that thymopoietin (TPO), a 49 amino acid polypeptide isolated from thymus, potently inhibits α-BGT binding (IC50 = 4 nM) to cultured neonatal muscle cells. Studies were done to evaluate the potential functional consequences of this interaction of TPO at the receptor. Long term exposure (3 days) of muscle cells in culture to nicotine ($3x10^{-6}$ M) or carbachol (10^{-6} M) inhibited muscle cell branching and cell size. TPO (3-6 nM), as well as α-BGT, prevented the effects of the nicotinic agonists on muscle cell morphology; furthermore, the decline in α-BGT binding which occurred after exposure to nicotine or carbachol was prevented by TPO. Thus, the long term effects of TPO on cellular morphology and on the nicotinic receptors correlate well with its interaction at the α-BGT site. TPO also inhibited nicotinic receptor mediated ^{22}Na uptake in cultured muscle cells, but at higher concentrations (IC50 = 20 nM). These studies suggest that TPO may modulate muscle cells characteristics during development through an interaction at the nicotinic receptor.

14.2

THE δ-SUBUNIT DETERMINES VOLTAGE DEPENDENT CLOSING OF TORPEDO AChR CHANNELS.

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Acetylcholine receptor (AChR) channels of *Torpedo californica* were expressed in *Xenopus laevis* oocytes by injecting different combinations of subunit-specific mRNA transcripts of the individual AChR subunits α, β, γ, δ. The order of efficacy in producing ACh induced oocyte depolarization for different subunit combinations was $\alpha_2\beta\gamma\delta > \alpha_2\gamma\delta > \alpha_2\beta\delta > \alpha_2\beta\gamma > \alpha_2\delta > \alpha_2\gamma > \alpha_2\beta$. These results indicate the efficacy of non-α subunits in substituting for one another is $\delta > \gamma > \beta$. Oocytes injected with $\alpha_2\beta\gamma\delta$ displayed brief current events with a channel conductance of 100 pS (measured under symmetrical 100 mM K^+ at 16°C) and an average burst duration of 0.35 ms at -100 mV which increased with hyperpolarization (e-fold per 120-140 mV). Single AChR channels formed from the subunit deficient combinations $\alpha_2\beta\gamma$, $\alpha_2\beta\delta$ and $\alpha_2\gamma\delta$ also displayed a channel conductance of around 100 pS and a similar voltage-dependent burst duration kinetics, with the exception that the δ-less combination expressed channels with burst kinetics that were relatively voltage insensitive (i.e. e-fold charge per 600 mV). We hypothesize that a specific structural domain resides in the δ-subunit which influences the voltage dependent closing step of AChR channels.

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14.3

A 90 KD PROTEIN RECOGNIZED BY ANTI-SRC ANTIBODIES COLOCALIZES WITH THE nAChR.

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The nicotinic acetylcholine receptor (nAChR) is a ligand gated ion channel that mediates signal transduction at the postsynaptic membrane of synapses including the neuromuscular junction and the electric organ of electric fish. The nAChR is known to be modulated by both serine and tyrosine phosphorylation. Although the kinases responsible for serine phosphorylation have been identified, the kinase responsible for tyrosine phosphorylation has been elusive. In an effort to identify this protein tyrosine kinase (PTK), antibodies were raised to a synthetic peptide based on the sequence of the autophosphorylation site of the *src* PTK. On Western blots of *Torpedo* electric organ, this antibody primarily recognizes a 90 kD protein which is most abundant in postsynaptic membranes rich in nAChR. Anti-phosphotyrosine antibodies also recognize a 90 kD protein with the identical subcellular distribution. Immunofluorescent double labeling with the anti-*src* antibody and rhodamine conjugated α-bungarotoxin suggest that the 90 kD protein colocalizes with the nAChR in the electric organ. These results suggest that the 90 kD protein may be a PTK that phosphorylates the nAChR or a substrate for a PTK. The anti-*src* antibodies are currently being used to screen expression libraries of the electric organ to isolate a cDNA clone encoding the 90 kD protein.

14.4

Agonist-sensitive Photolabeling of the M2 Region of the Nicotinic Acetylcholine Receptor (AChR) by a Hydrophobic Noncompetitive Antagonist (NCA).

Benjamin H. White and Jonathan B. Cohen. Dept. of Anatomy and Neurobiology, Washington University Sch. of Med., St. Louis, MO 63110

To characterize agonist-induced changes of AChR structure, we have mapped the site of photoincorporation of a novel photoaffinity NCA, 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine ([^{125}I]TID). Although [^{125}I]TID binds to the AChR with similar affinity in the presence and absence of agonist, agonist inhibits labeling of all subunits by >75%. Sites on the β- and δ-subunits labeled in the absence of agonist were identified by N-terminal sequencing of tryptic fragments purified by SDS-PAGE followed by reverse phase HPLC. [^{125}I]TID specifically labels homologous aliphatic residues (βL-257, δL-265, βV-261, and δV-269) in the M2 region of both subunits. In the presence of agonist labeling of these residues is reduced ~90% and the distribution of labeled residues is broadened to include a homologous set of serine residues at the N-terminus of M2. In the β-subunit residues βS-250, βS-254, βL-257 and βV-261 are all labeled in the presence of carbamylcholine. This pattern of labeling supports an α-helical model for M2 with the labeled face forming the ion channel lumen. The observed redistribution of label provides the first evidence for an agonist-dependent rearrangement of the M2 helices.

14.5

THE ROLE OF ASPARTATE-200 IN LIGAND ACTIVATION OF *TORPEDO* ACETYLCHOLINE RECEPTORS. Michael E. O'Leary* and Michael M. White, Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

The requirement for a positive charge on nicotinic agonists and antagonists has led to the assumption that electrostatic interactions are important for ligand binding.Chemical modification studies have localized the binding site to a region containing residues 180-210 on the α subunit. Within this region is a conserved aspartate (αD200) that could function as the anionic subsite thought to be part of the binding site. We have examined the role of this residue in agonist-mediated activation of the *Torpedo* AChR by means of site-directed mutagenesis and expression in *Xenopus* oocytes. Wild-type (WT) receptors are half-maximally activated (K_{act}) by 20 μM ACh with a Hill coefficient n=1.8. Replacement of aspartate-200 by asparagine (αD200>N) shifts K_{act} for ACh to 75 μM with no change in n, suggesting that αD200 is not absolutely essential for ACh binding. To further investigate the role of αD200, we examined receptor activation by two partial agonists, phenyltrimethylammonium (PTMA) and tetramethylammonium (TMA), which interact with the alkylammonium binding subsite of AChRs. WT receptors are activated by PTMA and TMA with K_{act} of 75 μM and 2 mM, respectively. In contrast, αD200>N receptors do not respond to either of these compounds; however, they do act as competitive inhibitors of the mutant receptors with K_i of 30 μM and 1.5 mM for PTMA and TMA, respectively. The close agreement for the WT K_{act} and mutant K_i for the partial agonists suggests that the affinity for these two compounds is not markedly affected by the mutation, but it does abolish the ability to couple binding to activation. We suggest that αD200 is not involved in agonist binding *per se*, but rather serves as a structural element involved in coupling ligand binding to activation. Supported by NIH grants R01-NS23885 and F32-NS08880 and the Lucille P. Markey Charitable Trust.

14.6

MAPPING THE NEGATIVE SUBSITE OF THE NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST BINDING SITE. C. Czajkowski and A. Karlin. Center for Molecular Recognition, Columbia University, New York, NY 10032.

Our model of the acetylcholine (ACh) binding site places a negative subsite about 1 nm from a readily reducible disulfide bond. Previously, α Cys192 and Cys193 were shown to form the binding site disulfide. We have now identified a region that may contribute to the negative subsite. Starting with mildly reduced ACh receptor from Torpedo, we attached the 0.9 nm long ^{3}H-N-glycylcysteamine (^{3}H-GC) to either α Cys192 or Cys193 via a disulfide bond. We added a carbodiimide to form an amide bond between the ^{3}H-GC glycyl amino group and nearby receptor carboxyl group(s). We then reduced the disulfide between ^{3}H-GC and α Cys 192/193 so that all ^{3}H-GC retained by the receptor was linked via an amide bond. The major site of amide bond formation was on the δ subunit. This crosslinking reaction from α to δ was blocked in the presence of ACh. By CNBr cleavage, HPLC, SDS-PAGE, and N-terminal sequencing, we identified δ164-257 as the region specifically and uniquely crosslinked to α Cys192/193. This region therefore may contain all or part of the negative subsite of one of the two ACh binding sites. Supported in part by NIH grants NS07065 and NS07258 and by MDA.

14.7

EHRLICH'S MAGIC BULLET REVISITED: AROMATIC ARSENOXIDES AS SELECTIVE REAGENTS FOR NICOTINIC RECEPTORS. R.H. Loring[1], W. Lane[1*], Y. Dou[1*], and K. Stevensen[2*]. [1]Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA 02115 and [2]Dept. of Biol. Sci., Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4

An unusual feature of nicotinic receptors is the presence of a disulfide between adjacent cysteine residues near the agonist binding site. Aromatic arsenoxides selectively form stable ring structures with vicinal (spatially close) sulfhydryls. We investigated the ability of aromatic arsenoxides to covalently bond to reduced nicotinic receptors in the intact chick retina. Reduction with dithiothreitol (DTT; 2 mM, 20 min) increases the concentration of agonist needed to activate nicotinic receptors while decreasing the concentration of NMDA needed to activate NMDA receptors. These effects are reversed by reoxidation with dithiobis(nitrobenzoic acid) (DTNB; 1mM, 5 min). Neither 4-aminophenylarsenoxide (APA) nor bromoacetyl-4-aminophenylarsenoxide (BAPA) change nicotinic responses by themselves. When either compound is applied (20 μM, 20 min @) after reduction with DTT, nicotinic responses remain diminished even after attempted reoxidation with DTNB. Furthermore, concentrations of nicotinic agonists that would normally activate DTT-treated receptors are ineffective after APA or BAPA application. However, treatment with the anti-arsenical, 2,3-dimercaptopropane-sulfonic acid (2 mM, 20 min), allows reoxidation of nicotinic receptors (recovery 60-90%). APA and BAPA have no effect on reduced or oxidized NMDA receptors, suggesting no functionally important vicinal cysteines in those receptors. Preliminary binding assays suggest that APA protects DTT-reduced Torpedo receptors against alkylation by bromoacetylcholine (as detected by inhibition of ^{125}I-α-bungarotoxin binding) with an EC_{50} of 1-10 nM. These results suggest that aromatic arsenoxides may be the basis for novel covalent, yet reversible, probes for nicotinic receptors. Supported by NS22472, STRC, and NSERC (Canada).

14.8

NEREISTOXIN: REDOX EFFECTS ON NEURONAL NICOTINIC RECEPTORS IN CHICK RETINA. Y.Xie and R.H. Loring Dept. of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115

Nereistoxin (NTX; 4-N,N-dimethylamino-1,2-dithiolane) is reported to block nicotinic acetylcholine receptors in both muscle and neurons, as well as reversibly inhibit the binding of [^{3}H]-ACh and [^{125}I]-α-bungarotoxin to Torpedo nicotinic receptors. We studied the effects of NTX in intact chick retina by electrophysiological recordings, and by the binding of [^{125}I]-neuronal bungarotoxin (^{125}I-NBT) to retinal homogenates. NTX (100 μM, 10 min) substantially and irreversibly blocked the retinal responses to the nicotinic agonist, dimethylphenylpiperazium (DMPP). The oxidizing compound, dithiobis-(nitro-benzoic acid) (DTNB, 1 mM), transiently and repetitively reversed the action of NTX in chick retina (85%±6, N=7 experiments). However after the application of the alkylating agent bromoacetylcholine (BAC, 100 μM, in the presence of 1-2 μM neostigmine), DTNB can no longer restore the function of nicotinic receptors. Both the agonist DMPP (10-300 μM) and the noncompetitive antagonist hexamethonium (1 mM) partially protected, 74%±16 (N=3) and 78%±15 (N=4), respectively, against the irreversible effects of NTX in chick retina preparations. NTX reversibly displaced the binding of ^{125}I-NBT to chick retina homogenates (IC_{50}=10 μM). ^{125}I-NBT binding was recovered by washing away NTX, and BAC treatment, followed by washing, did not inhibit ^{125}I-NBT binding to the chick retina homogenates preincubated with NTX. However, preliminary binding assays indicate that treatment of living retinas with NTX followed by BAC treatment irreversibly inhibits ^{125}I-NBT binding. We hypothesize that NTX (or a metabolite) covalently attacks neuronal nicotinic receptors, possibly in the channel or near the agonist binding site, and alters the conformation in such a way that the disulfide bond in the agonist binding site becomes unstable and susceptible to alkylation by BAC. Supported by NS22472 and the Smokeless Tobacco Research Council (STRC).

14.9

ACHR GENE PRODUCTS IN CHICK CILIARY GANGLIA: TRANSCRIPTS, SUBUNITS, AND RECEPTOR SUBTYPES. A.B. Vernallis, W.G. Conroy, R.A. Corriveau, S.W. Halvorsen, and D.K. Berg. Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093.

Chick ciliary ganglion neurons have at least two classes of nicotinic acetylcholine receptors (AChRs). One class binds the monoclonal antibody mAb 35, occurs predominantly in synaptic membrane, and generates nicotinic responses mediating synaptic transmission through the ganglion (mAb 35-AChRs). The other class binds α-bungarotoxin, occurs predominantly in non-synaptic membrane, and only recently has been shown to function as a receptor (αBgt-AChRs). RNAse protection experiments demonstrated that ciliary ganglia from 18 day chick embryos contain substantial amounts of AChR gene α7 mRNA, moderate amounts of α3, α5, β2, and β4 mRNAs, and traces of α4 mRNA. The subunit composition of ciliary ganglion AChRs was examined with mAbs raised against fusion proteins containing relatively unique portions of the α3, α7, and β4 gene products. Anti-α3 and anti-β4 mAbs immunoprecipitated mAb 35-AChRs solubilized from embryonic ciliary ganglia. When immunoblots of affinity purified mAb 35-AChRs were probed with the anti-α3 mAb, bands of about 58 and 55 kD were obtained, as previously seen with an anti-α3 antiserum. The smaller band is likely to be a degradation product of the larger. Probing the immunoblots with an anti-β4 mAb revealed a band of about 54 kD. mAb 268, raised against AChRs purified from brain, detected a component in the vicinity of the β4 band that has yet to be correlated with a known gene. αBgt-AChRs were immunoprecipitated with an anti-α7 mAb but not with anti-α3 or anti-β4 mAbs. Most if not all ciliary ganglion neurons express α3 mRNA and have mAb 35-AChRs and αBgt-AChRs. The results suggest that α3 and β4 gene products co-assemble with each other but not with the α7 gene product, though all are likely to be present in the same cell. The multiplicity of AChR transcripts also implies that either neuronal AChRs can have more than two types of subunits or ciliary ganglia contain more than two species of AChRs. (Supported by R01 NS12601 & PO1 NS25916)

14.10

NICOTINIC RECEPTORS THAT BIND α-BUNGAROTOXIN ON NEURONS RAISE INTRACELLULAR FREE CA^{++}. S. Vijayaraghavan, M.M. Rathouz, P.C. Pugh*, and D.K. Berg. Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093.

Many vertebrate cholinoceptive neurons have a membrane component that binds α-bungarotoxin (αBgt) with high affinity and has physical properties characteristic of nicotinic receptors. Gene cloning studies indicate that a presumed subunit of the component can function as a ligand-gated ion channel, but no function has yet been demonstrated for the native component *in situ*. We find that the component (αBgt-AChRs) serves as a nicotinic receptor and increases intracellular free Ca^{++}.

Chick ciliary ganglion neurons have high levels of αBgt-AChRs in addition to a lower level of receptors previously shown to generate nicotinic responses and to bind the monoclonal antibody mAb 35 (mAb 35-AChRs). A fluorescence assay for nicotinic responses was devised by loading cells in culture with fluo-3, a Ca^{++}-sensitive fluorescent dye, and relying on AChRs to permit Ca^{++} flux. Nicotine at 0.1-1.0 μM produced an increase in fluorescence that was blocked by 20 μM d-tubocurarine and by EGTA, but not by 0.1 μM atropine or 10 μM tetrodotoxin. αBgt blocked the signal with an EC_{50} of 1-2 nM which agreed well with its K_i of 0.7 nM for competition with ^{125}I-αBgt for binding to the neurons. Similarly, methyllycaconitine (MLA) blocked the signal with an EC_{50} of 1-2 nM and had a K_i of 2.8 nM against ^{125}I-αBgt for binding. Neither αBgt nor MLA acted on mAb 35-AChRs at these concentrations, and neither blocked K^+-induced fluoresence. Whole cell voltage clamp analysis confirmed that currents induced by low concentrations of nicotine could be blocked in part by αBgt and that the blockade did not represent αBgt acting on mAb 35-AChRs. Previous failures to detect αBgt-AChR function *in situ* probably resulted from assay conditions that allowed other nicotinic receptors to dominate the response. The increase in intracellular free Ca^{++} caused by activation of αBgt-AChRs may involve more than one mechanism. (Supported by RO1 NS12601 & PO1 NS25916)

14.11

TISSUE SPECIFIC ALTERNATIVE SPLICING OF THE NICOTINIC RECEPTOR β_4 SUBUNIT GENE. K. E. Isenberg, S. G. Holstad*. Dept. of Psychiatry, Washington University School of Medicine, St. Louis College of Pharmacy, St. Louis, MO 63110.

The neuronal nicotinic acetylcholine receptor (nAChR), a multi-subunit ligand-gated ion channel, is thought to be composed of two types of subunits, an α or acetylcholine binding subunit, and a β or structural subunit. Alternative splicing is a means of regulating gene expression, generating protein products whose functional characteristics may contribute to the specific cellular phenotype. β_4 is a recently described neuronal nAChR structural subunit whose primary transcript may be spliced to generate two different transcripts. These transcripts result in β_4 subunits with significantly different amino termini reinforcing the possibility that selective expression of a transcript product would be tissue specific. We utilized a PCR assay employing primers that would generate a PCR product from mRNA for each of the possible transcripts to measure expression of each transcript. PCR products specific for both β_4 transcripts were synthesized and judged to be the correct products by size and hybridization criteria. Rat brain RNA predominantly generates a larger (356 bp) transcript in the PCR assay while PC-12 cells express both transcripts, a smaller (287 bp) product predominating. The PCR products observed in our assay confirms the proposed complexity of β_4 subunit expression, presumably with functional consequences for the cells expressing the β_4 subunit.

14.12

THE PREDOMINANT HIGH AFFINITY NICOTINIC RECEPTOR IN RAT BRAIN IS COMPRISED OF α4 AND β2 SUBUNITS AND IS UP-REGULATED BY NICOTINE. C.M. Flores[1], S.W. Rogers[2], L.A. Pabreza[1]*, B.B. Wolfe[1] and K.J. Kellar[1]. Department of Pharmacology, Georgetown University, Washington, DC 20007[1] and The Salk Institute for Biological Studies, San Diego, CA 92138[2].

Several different α and β subunit components of neuronal nicotinic receptors have been cloned from rat brain. Although the transcripts for these subunits are expressed throughout the rat CNS, at the protein level neither the precise subunit composition and stoichiometry nor the regional distribution are known. It has also yet to be determined which subtype(s) is up-regulated in response to chronic administration of nicotine. Polyclonal antisera directed against non-homologous regions of the α2, 3, 4, 5 and β 2, 3,4 subunits were used in an immunoprecipitation (IMP) protocol to address these issues. Washed membrane homogenates from rat brain were bound with ^{3}H-cytisine, solubilized with Triton X-100 and immunoprecipitated by sequential incubation with antibody and stripped Pansorbin. Non-specific IMP was determined with either pre-immune or normal rabbit sera. Specific IMP was obtained only with sera directed against the α4 and β2 subunits. Moreover, preclearing solubilized receptor with any of the other antisera did not decrease specifically immunoprecipitable α4 and β2. However, specific IMP of both subunits was almost completely abolished by preclearing with either anti-α4 or anti-β2 sera. These data indicate that in rat brain the predominant if not only subtype of nicotinic receptor with high affinity for agonists is comprised of α4 and β2 subunits in association with each other. Furthermore, these subunits exhibit the same regional distribution profile generated by conventional nicotinic agonist binding assays in rat brain homogenates. Additional experiments demonstrated that this α4/β2 subtype is up-regulated in the cortex of rats injected for 10 days with nicotine bitartrate dihydrate (2.0 mg/kg).

This work was supported by NIDA grant DA06486 and NIH grant NS11549.

BIOLOGICAL RHYTHMS AND SLEEP I

15.1

EXPRESSION OF AP-1 PROTEINS IN THE RAT SUPRACHIASMATIC NUCLEI DURING THE LIGHT PHASE. WJ Schwartz, E Davis,* K Chase,* & N Aronin. Depts Neurology & Medicine, U. Massachusetts Med School, Worcester, MA 01655

We (PNAS 87:5959) and others (Exp Neurol 109:353; Neurosci Lett 120:105) have reported a daily rhythm of immunoreactive Fos protein(s) in the rat SCN, with high levels during the light phase of the light-dark (LD) cycle and low levels during the dark phase. This rhythm is not endogenous; both the number of cell nuclei and the intensity of their labeling are reduced to dark phase levels if lights are not turned on at expected dawn and the rats remain in darkness. We now show that expression of c-Fos protein *per se* and AP-1 DNA binding activity both occur in the rat SCN during the light phase of the LD cycle.

In male albino rats entrained to a 12 hr:12 hr LD cycle, SCN c-*fos* mRNA levels peak 30 min after lights-on (by *in situ* hybridization using a ^{32}P-labeled rat cDNA probe, gift of T Curran) whereas levels of Fos protein(s) are greatest 2 hr after lights-on (by immunohistochemistry using an affinity-purified antibody to c-Fos(132-154), gift of SM Sagar & FR Sharp). Protein from nuclear-enriched SCN cell extracts was obtained during the light phase; Western blot analysis identified the c-Fos protein (≈60 kD) as the predominant immunoreactive species, and gel retardation assay demonstrated specific binding to a ^{32}P-labeled oligonucleotide probe corresponding to the AP-1 consensus sequence. Since Fos binds to the AP-1 site only when complexed as a heterodimer to Jun, the latter results also imply that Jun protein(s) are present in the SCN during the light phase. In rats free-running in constant darkness, high levels of c-*fos* mRNA and Fos immunoreactivity are expressed after light pulses during subjective night but not subjective day.

Altogether, these data suggest that the immunostaining we see during the light phase probably reflects the occurrence of light at dawn; for animals with $\tau > 24$ hr, a daily phase advance at dawn is required for entrainment to the 24-hr LD cycle.

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15.2

LIGHT AND CIRCADIAN PHASE REGULATE JUN-B AND AP-1 EXPRESSION IN THE HAMSTER SUPRACHIASMATIC NUCLEUS. J.M. Kornhauser[1], D.E. Nelson[2], K.E. Mayo[1]*, and J.S. Takahashi[2]. [1]Dept. of Biochemistry, Molecular Biology, and Cell Biology, and [2]Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

The suprachiasmatic nucleus (SCN) of the mammalian hypothalamus contains a circadian pacemaker that regulates many physiological and behavioral rhythms. Photic information entrains this pacemaker to the environmental light-dark cycle. A molecular correlate of circadian entrainment has been identified in the regulation of the immediate-early gene c-*fos* in the rodent SCN by light. Photic induction of c-*fos* mRNA expression exhibits a circadian phase-dependence and an irradiance threshold matching those of the behavioral effects of light. To determine if another AP-1 transcription factor component is regulated by light in the same manner, we examined the effects of light upon *jun*-B mRNA in the golden hamster using *in situ* hybridization techniques. Light pulses of 5 minute duration induce a dramatic increase in *jun*-B mRNA in the SCN; peak *jun*-B mRNA levels occur 30 minutes following light onset, corresponding to the time-course of c-*fos* mRNA induction by light. In addition, the ability of light to induce *jun*-B mRNA in the SCN is gated by the circadian pacemaker. Light pulses during the subjective night that cause phase shifting of activity also induce *jun*-B mRNA expression; light at circadian times when no behavioral phase shift occurs does not affect *jun*-B mRNA levels. Since light-induced *jun*-B and c-*fos* expression exhibit the same anatomical and temporal specificity, we investigated if light regulates levels of the dimeric AP-1 protein complex. A gel mobility-shift assay was performed on SCN tissue extracts to determine levels of specific binding to an AP-1 oligonucleotide. Light increases levels of AP-1 binding activity in the hamster SCN, and this induction is dependent upon circadian phase. Thus, light may be coupled to coordinated changes in gene transcription mediating circadian entrainment in the SCN.

15.3

LIGHT INDUCES PROTO-ONCOGENE EXPRESSION IN GASTRIN RELEASING PEPTIDE NEURONS WITHIN THE SUPRACHIASMATIC NUCLEUS. D.J. Earnest, S.M DiGiorgio, L.A. Trojanczyk and J.A. Olschowka. Dept. of Neurobiology & Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642

During photostimulation at specific circadian times, the induction of c-*fos* proto-oncogene expression occurs within the ventrolateral subdivision of the suprachiasmatic nucleus (SCN), coinciding with the terminal fields of fibers that provide the photoentrainable pacemaker in the SCN with light information. Close associations between photic inputs to the ventrolateral SCN and a number of neuronal perikarya containing gastrin releasing peptide (GRP) and vasoactive intestinal polypeptide (VIP) suggest that these SCN neurons may be involved in processing visual information. Consequently, the present study was conducted to determine whether the photoinduction of c-*fos* expression in the ventrolateral SCN occurs within these populations of peptidergic neurons.

Ten male rats were transferred from LD 12:12 to constant darkness for ≈24h, exposed to light for 1h near the mid-subjective night and then perfused for immunocytochemistry. Analysis was conducted to double label Fos protein(s) along with either GRP or VIP in the SCN in brain sections (4μm).

In all light-treated animals, SCN cells with Fos-positive nuclei were mainly segregated within the ventrolateral subfield and approximately 25% of these cells also exhibited cytoplasmic immunoreactivity for GRP. In the converse comparison, not all of the GRP neurons in the SCN expressed Fos-immunoreactivity in response to light. No sign of light-induced Fos expression was observed in VIP-positive neurons in the SCN. These results demonstrate that light induces c-*fos* expression in at least a subpopulation of the GRP-containing neurons in the SCN, suggesting that these cells may be involved in processing entraining light signals. Analysis of the effect of light on c-*jun* expression in SCN neurons containing GRP or VIP is in progress to complement these observations. Supported by AFSC Grant #90-0182 (D.E.).

15.4

THE PUTATIVE SUPRACHIASMATIC NUCLEUS OF BIRDS RESPONDS TO VISUAL MOTION. Josh Wallman, Elizabeth Teakle*, and Rae Silver§. Dept. of Biology, City College, CUNY, New York, NY 10031 and §Barnard College, Columbia Univ., New York, NY 10027

The mammalian suprachiasmatic nucleus (SCN) is a necessary pacemaker for various physiological and behavioral daily rhythms, and its own circadian rhythms of neural and metabolic activity can be entrained by daily light cycles.

In birds, a nucleus in the lateral hypothalamus has been identified as the avian SCN because it receives retinal afferents and contains neurotransmitters similar to the mammalian SCN, although a non-retinorecipient nucleus more medial and rostral is also a candidate SCN. Recently Rusak, et al. (1990) has shown that the SCN of hamsters and rats expresses the gene c-fos when exposed to light during subjective night, but not during subjective day. To help identify the avian SCN, we have repeated this experiment in chickens.

Chicks with one eye covered by a translucent occluder from hatching were put in darkness. During either subjective night or day the occluder was switched to the other eye and they were exposed either to brightly illuminated surroundings moving vertically at 15 deg/sec or to 10 Hz strobe light for 90 min. Brain sections were reacted with polyclonal antibodies to the protein products of the fos family (provided by R. Bravo, Bristol-Myers Squibb Inst.).

Surprisingly, the lateral nucleus was strongly labeled contralateral to the eye seeing visual motion, but not to the other eye, which saw diffuse light. In stroboscopic illumination, neither lateral nucleus was labeled. The labeling seen was similar to c-fos label in nuclei of the accessory optic system and pretectum known to signal whole-field visual motion (Rojas, et al Soc. Neurosci, 1990). The sensitivity to motion, but not to light, suggests that this nucleus is not the SCN, but may be part of an undiscovered motion pathway.

15.5

IN VIVO ASSESSMENT OF 5-HT AND AMINO ACID METABOLISM IN THE SCN: CORRELATION WITH OVERT CIRCADIAN RHYTHMS. J.D. Glass, U.E. Hauser, J.L. Blank[1] and M.A. Rea[1]. Dept. Biological Sciences, Kent State Univ., Kent, OH 44242 [1]U.S.A.F. School of Aerospace Med., Brooks A.F.B., TX 78235.

In vivo microdialysis was used to characterize serotonergic (5-HT) and amino acid activity in the SCN region of freely-behaving golden hamsters under L:D 14:10. Animals with a microdialysis probe in or near the SCN (n=5) exhibited an apparent circadian rhythm in release of 5-hydroxyindoleacetic acid (5-HIAA), with the peak (127±1.5% of the daily mean) occurring 2 h after lights-off. This peak occurred with the large initial bout of wheel-running activity and 1.2°C rise in core temperature. The increase in 5-HIAA may reflect enhanced 5-HT synaptic activity, since the suppressive effect of the 5-HT reuptake blocker, citalopram, on 5-HIAA was greater during the dark phase than light phase (52.9±2.1% vs 80.0±9.0% of baseline; $p<0.05$). Amino acid neurotransmitters glutamate (GLU) and aspartate (ASP) in SCN dialysates also exhibited apparent circadian fluctuations with peaks during the dark phase. The GLU peak (239±68% of the daily mean) occurred 5 h after lights-off and gradually declined to daytime levels by morning. This peak was not associated with motor activity. Release of ASP was elevated throughout the dark phase, and glutamine was linear throughout the experiment. The differences in activities of 5-HT, GLU and ASP is evidence for a diversity of function of these substances in the SCN. AFOSR 440785 (J.D.G.) & AFOSR 2312W6 (M.A.R.).

15.6

ENCAPSULATED FETAL SCN GRAFTS SURVIVE TRANSPLANTATION TO THE VENTRICLE. R. Silver[1], J. Le Sauter[1], M-T Romero[1], P. Aebischer[3] and M.N. Lehman[2]. [1]Barnard College of Columbia Univ., New York, NY 10027, [2]Univ. Cincinnati Coll. Med., Cincinnati, OH 45267, [3]Brown Univ., Providence, RI 02912.

In mammals, a circadian pacemaker has been identified in the suprachiasmatic nucleus (SCN) of the hypothalamus. Ablation of this nucleus results in the loss of circadian rhythms of locomotor activity, and this response is restored following placement of intraventricular grafts of fetal hypothalamic tissue containing the SCN. In order for transplanted tissue to restore activity rhythms, the grafted SCN presumably sends a signal to appropriate target brain regions. Tract-tracing for neural efferents from intraventricular SCN grafts reveals scant neural connections with host brain (Canbeyli et. al., Br.Res, in press). To set the stage for analysis of a diffusible signal(s) from the SCN, we encapsulated fetal SCN tissue within permselective acrylic copolymer tubes with a nominal molecular weight cut-off of 50,000 daltons, allowing nutrients, growth factors and transmitters to diffuse freely. The tubes were sealed with bone wax and compatible polymer glue and implanted stereotaxically in the third ventri cle of SCN-X hamsters. Encapsulated E15 whole tissue grafts or cell suspension grafts of fetal SCN transplanted into the 3rd ventricle of adult hamsters survive and develop VIP-immunoreactivity. Immunostaining for 5HT reveals fibers in host brain that do not cross the capsule wall. In the next series of experiments, the capacity of encapsulated grafts to restore locomotor rhythms in SCN-lesioned hamsters will be examined. (Support: NIH NS 24292).

15.7

PROPERTIES OF CULTURED SUPRACHIASMATIC NEURONS. D. K. Welsh and S. M. Reppert. Laboratory of Developmental Chronobiology, Massachusetts General Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

The suprachiasmatic nucleus (SCN), site of a circadian clock, contains a heterogeneous population of neurons and glia. Which cell types are essential for clock function is not known. Also unknown is whether the clock emerges from specific patterns of connections in a neuronal network, or whether it consists of a coupled population of independent cellular oscillators. As an initial approach to these questions, we are examining the properties of dissociated SCN neurons in long-term culture. Neurons were dissociated from newborn rat SCN using papain, and plated on poly-D-lysine and laminin in medium containing 5% rat serum. Approximately 2/3 of dissociated neurons attached to the substrate, 1/2 of these were viable at 2 days, and 90% of the remaining neurons were still alive after 3 weeks in culture. Survival was clearly inferior in serum-free medium. Insulin-like growth factors (IGF-I, IGF-II) were no more effective than insulin in promoting cell survival in serum-free medium. IGF-I (10 nM) was less effective than insulin (1 μM; p=.01), whereas IGF-II (10 nM) was just as effective. Addition of nerve growth factor (NGF) promoted neurite outgrowth and fasciculation but did not improve survival. Preliminary morphological and immunocytochemical findings are consistent with known *in vivo* properties of SCN neurons. Whole cell patch recordings indicate that some SCN neurons fire spontaneous action potentials in culture. We are therefore testing the feasibility of long-term, multi-electrode recording of spontaneous activity in cultured SCN neurons.

15.8

ULTRADIAN CALCIUM OSCILLATIONS IN SUPRACHIASMATIC NUCLEUS CELLS. A.N.van den Pol, A.Cornell-Bell, S.Finkbeiner. Sect.Neurosurgery, Cell Biol., Molec.Neurobiol., Yale University, New Haven, Ct. 06510.

To study cellular ultradian oscillators, which have been postulated to underlie circadian clocks, we used digital video microscopy to examine calcium fluctuations in cultured suprachiasmatic (SCN) cells with the Ca^{2+} indicator dye fluo-3. SCN neurons and astrocytes showed an intracellular Ca^{2+} increase in response to glutamate and serotonin. Expression of regular neuronal oscillations (periods about 15 sec) could be blocked by the inhibitory transmitter GABA. Astrocytes showed very regular rhythms of cytoplasmic Ca^{2+} concentrations with periods ranging from 7 to 20 seconds. These periodic Ca^{2+} oscillations could be initiated by in vitro application of glutamate, a putative retinohypothalamic transmitter. Cellular Ca^{2+} oscillations arose from intracellular Ca^{2+} stores, and could be induced by neurotransmitters in the absence of extracellular Ca^{2+}. Communication between glial cells, capable of increasing the period length of cellular Ca^{2+} oscillations to 45 to 70 sec, mediated via gap junctions, could be induced by SCN neurotransmitters. These results show that both neurons and glia in the SCN exhibit regular ultradian oscillations which can be influenced by SCN transmitters. The interaction of these ultradian oscillators may play a role in SCN function.

15.9

NEURONAL CIRCUITRY INVOLVED IN CIRCADIAN RHYTHM REGULATION CHARACTERIZED BY TRANSNEURONAL PASSAGE OF PSEUDORABIES VIRUS. R.Y.Moore, J.C.Speh, L.W.Enquist* and J.P.Card, Univ. of Pittsburgh, Pittsburgh, PA 15261 and DuPont Merck Pharmaceutical Co.,Wilmington, DL 19880.

Intraocular injection of an attenuated pseudorabies virus strain (Bartha, PRV-Ba) results in infection of a distinct class of ganglion cells with subsequent transneuronal passage of virus to centers of the circadian timing system, the suprachiasmatic nucleus (SCN) and the intergeniculate leaflet (IGL) of the lateral geniculate complex (Card et al, Neuron, in press). PRV-Ba is not transported by ganglion cells projecting to the dorsal lateral geniculate nucleus or superior colliculus. In the present study we have extended the post-injection interval to further define the neuronal circuits labeled by transneuronal passage of virus through the SCN and IGL. Labeled neurons are found in a series of basal telencephalic, diencephalic and brainstem cell groups including the septal nuclei, organum vasculosum lamina terminalis, medial preoptic area, anterior hypothalamic area, lateral a area, retrochiasmatic area, ventromedial and dorsomedial nuclei, perifornical and dorsal hypothalamic areas, periacqueductal gray, reticular formation, raphe nuclei and laterodorsal and pedunculopontine tegmental nuclei. The pattern of labeling is complex but its continuing analysis should provide further insight into pathways by which the SCN regulates physiological and behavioral rhythms. (Supported in part by NIH grant NS-16304 and by DuPont Merck Pharmaceutical Co.)

15.10

DOES THE FETAL CIRCADIAN CLOCK PLAY A ROLE IN THE TIMING OF BIRTH? N.Viswanathan and F.C.Davis. Department of Biology, Northeastern University., Boston, MA 02115.

In rodents, a circadian clock begins to oscillate prenatally and is entrained by maternal rhythm(s). A hypothesis for the function of such prenatal maternal entrainment is that the fetal clock plays a role in the circadian timing of birth; i.e., the entrainment by the mother makes the fetus aware of the time of day. Prenatal injections of melatonin to the mother hamster can predictably set the phases of her offsprings' circadian rhythms. It is possible that the prenatal injections of melatonin to the mother would affect the timing of birth. We examined this possibility in two groups of SCN-lesioned, pregnant syrian hamsters which received daily subcutaneous injections of melatonin on days 9-15 of gestation. One group received melatonin injections in the morning and the other in the evening. Following the last set of injections, animals were observed every hour until birth. The timing of birth was *not* significantly different between the two groups although the pups' activity rhythms at weaning were found to be related to the timing of melatonin injection. The results indicate that the fetal clock entrained by the exogenous melatonin is unable to influence the timing of birth. Preliminary studies using mutant *tau* hamsters born to wild-type mother also suggest that the fetal clock has no role in the timing of birth. Supported by NIH grant HD 18686 to FCD.

15.11

USE OF SILICON-BASED MULTICHANNEL MICRO-ELECTRODES TO CHARACTERIZE SINGLE-UNIT ACTIVITY OF NEURONS IN THE RAT SUPRACHIASMATIC NUCLEUS. M.N. Ghazzi*, K.D. Wise*, D.J. Anderson, S.W. Newman and A.R. Midgley Jr*, Rep. Sci.& Bioeng. Prog. Univ. Michigan, Ann Arbor, MI 48109.

The nature of neuronal interactions that generate oscillatory rhythms and light responsiveness of suprachiasmatic nucleus (SCN) neurons are not understood. For example, although some SCN neurons have been previously classified as light-inhibited, the bursting pattern of these neurons has not been characterized. This study attempts to characterize neuronal interactions and light response properties of monitored SCN neurons. Miniature solid state electrodes (15 μm by 35 μm by 15 mm) with five 36 μm^2 recording sites spaced 100 μm apart were used for recordings of up to 8h. Probes were inserted stereotaxically into the SCN of urethane-anesthetized, mature, male, Sprague Dawley rats (12h light:12h dark). The recorded signal was buffered, amplified, filtered, and connected to a custom-designed, computerized, data acquisition system for analysis. In response to 10 min of light exposure at 1500 lux, some SCN neurons showed a reduction in spike rate. Closer examination, however, revealed that the decrease in overall spike rate for some of these "light inhibited" SCN neurons was accompanied by an increase in average number of spikes per burst. Analysis of spontaneous firing of neurons not exposed to light indicated that both inhibitory and excitatory interactions exist between SCN neurons. In some of these neurons the latency period between the firing of the leading and the lagging neuron was observed to shift intermittently from 4.5 ms to 5.5 ms to 4.5 ms. This suggests that a multi-path interactive mechanism may be involved in oscillatory SCN behavior. Thus, multichannel single-unit recording electrodes coupled with computerized data analysis revealed neuronal interactions otherwise indecipherable by conventional techniques. (5K11 HD00828-04)

BRAIN METABOLISM AND BLOOD FLOW I

16.1

THE PHASIC INCREASE IN CBF BY ENDOTHELIN IS MEDIATED BY THE RELEASE OF NITRIC OXIDE THROUGH AN ACTIVATION OF NMDA RECEPTORS. K.Nakai, M.Nakai*, K.Kubo*, T.Okuno, T.Itakura*, S.Hayashi*, N.Kornai* Dept. Neurol.Surg., Wakayama Med.Col., Wakayama Japan

We have demonstrated a several minutes-long phasic increase in the regional cerebral blood flow (rCBF) with remarkably dilated parenchymal blood vessels by the intracortical injection of endothelin (ET), in spite of its strong vasoconstrictive activity (Nakai et al., Neurosci. Abst. 1989). Similar study using NMDA instead of ET also showed the phasic increase in the rCBF and dilated parenchymal blood vessels as well (Nakai et al., Neurosci. Abst.1990). Present study tried to elucidate the underlying mechanism to cause such phasic increase in the blood flow caused by these two particular chemicals. After the intraperitoneal injection of MK801 (2mg/kg) or an inhibitor of the nitric oxide (NO) formation (NO_2arginine, 5mg/kg), parietal cortices of S-D rats were injected with either ET ($1x10^{-4,-6,-8}$M) or NMDA ($1x10^{-3,-4,-6}$M) at a constant rate(1μl/10 min) through a glass capillary. Throughout the course of experiment rCBF was monitored by laser flowmetry. Higher doses of each chemicals (ET $1x10^{-4,-6}$M,NMDA $1x10^{-3,-4}$ M) injected intracortically caused the phasic increase in the rCBF for a few minutes. Pretreatmemnt of MK801 suppressed the phasic increase caused by NMDA($1x10^{-3,-4}$M). Interestingly, the phasic increase by ET was significantly suppressed by the pretreatment of MK801, when the blood vessels in the vicinity of injection site were not remarkably dilated any more. There was no phasic increase in rCBF in animals pretreated with NO_2arginine under all doses of ET and NMDA examined in this study. Above results suggest that the temporary vasodilatatory action of ET on the brain blood vessels is primarily mediated by NMDA receptor activation leading to a release of NO either from the neurons, as was reported by Garthwaite (Nature 1988), or from the vascular endothelium in the brain parenchyma .

16.2

CEREBRAL HYPEREMIC RESPONSES TO HYPOGLYCEMIA (HG) VS HYPOXIA (HX): ROLE OF ADENOSINE (ADO). D.A. Pelligrino and A. Sharp, Dept. of Anesthesiol., Univ. of Ill./Humana-Michael Reese Hosp., Chicago, IL 60616.

In the present study, we sought to determine whether ADO receptor (R) blockade, via 8-phenyltheophylline (8-PT), could attenuate the regional cerebral blood flow (rCBF) increases that accompany HG in the rat. Previous work has established a significant role for ADO in the rCBF increases associated with HX. Thus, to confirm the efficacy of 8-PT in blocking ADO-R mediated rCBF responses in our experimental system, the influence of 8-PT on the cerebral hyperemic response to HX also was evaluated. For study, the rats were anesthetized (fentanyl/70% N_2O), paralyzed and mechanically ventilated. rCBF was measured in the cortex, subcortex, brainstem, and cerebellum using radiolabeled microspheres. One μl 8-PT (10 μg/kg) or vehicle (VEH) was administered into a lateral cerebral ventricle (icv). In HX, injections were given following an initial rCBF measurement. rCBF was then measured at 10 min post-injection (normoxia) and at 10 min HX (PaO_2=30-35 mmHg). In HG, rCBF was measured prior to iv insulin and when plasma glucose (G) reached 1.5 mM. 8-PT or VEH was given and rCBF evaluated after 15 min (with G clamped at 1.5 mM) and 30 min (G permitted to fall to 1 mM). During HX, 8-PT produced a 50-60% attenuation of the normal 3-5 fold rCBF increase in all regions, indicating the efficacy of our protocol to induce ADO-R blockade. On the other hand, in HG, 8-PT had no effect on the 2-4 fold rCBF increases seen in VEH-injected rats at G≈1.5 mM, but was associated with a 40-60% attenuation of the 4-6 fold rCBF increase normally observed at G≈1 mM. Thus ADO appears to play a role in mediating rCBF increases only during severe HG, when G approaches or exceeds coma threshold.

16.3

COUPLING OF REGIONAL CEREBRAL METABOLISM AND BLOOD FLOW IN THE CONSCIOUS RAT IS MAINTAINED AFTER TREATMENT WITH THE MUSCARINIC AGONIST ARECOLINE. K. Maiese, H.W. Holloway*, D.M. Larson*, D.J. Reis, and T.T. Soncrant, Div. of Neurobiol., Cornell Univ. Med. Coll., NY, NY 10021 and Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

The muscarinic cholinergic agonist arecoline (AREC) selectively increases local cerebral glucose utilization (LCGU) in the conscious rat (Soncrant, T.T. et. al., Brain Research, 347:205-216, 1985). We sought to determine whether these focal increases in metabolism were coupled to, or independent of, local cerebral blood flow (LCBF). In groups of young Fisher-344 rats, LCGU and LCBF were determined in 60 brain regions by the ^{14}C 2-deoxyglucose and the ^{14}C iodoantipyrine autoradiographic methods at three minutes following administration of normal saline, AREC 1mg/kg, and AREC 15mg/kg i.p. Each rat was pretreated with methylatropine 4mg/kg s.c. to reduce systemic parasympathetic discharge. In saline treated rats, LCBF was correlated with LCGU ($r=0.838$, $p<0.01$). AREC 1mg/kg produced only small changes in blood flow and metabolism, but the two parameters remained closely related ($r=0.891$, $p<0.01$). In contrast, AREC 15mg/kg yielded widespread (mean 242%) but regionally heterogeneous (range 130%-361%) increases in LCBF, and LCBF remained coupled to LCGU ($r=0.803$, $p<0.01$). Maintenance of coupling between LCGU and LCBF indicates that changes in LCBF following arecoline administration reflect regional drug effects on brain function.

16.4

BASAL FOREBRAIN(BF) REGULATION OF CORTICAL CEREBRAL BLOOD FLOW (CBF): EFFECTS OF AGING AND CHRONIC NICOTINE TREATMENT. D.G. Linville, S. Williams*, M.J. Malchrzak, J.L. Raszkiewicz* and S.P. Arneric, Neuroscience, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064-3500, and Dept. of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62702.

Microstimulation of BF elicits profound increases in cortical CBF (Excerpta Int. Cong. Series 869:381, 1989). This response is enhanced by acute (-)nicotine (NIC) (Soc. for Neurosci. 16:129.9, 1990). Conversely, it shows age-related impairments, but is largely preserved in the frontal cortex (Neurobiol. Aging 11:73, 1990). This study sought to determine: is the enhancement in cortical CBF elicited by NIC preserved following subacute administration? If so, is it effective in aged rats? Sprague-Dawley (3 or 18 mo.) rats were implanted with pellets delivering either placebo or 1.2 mg NIC/day (Innovative Research). After 14 days rats were anesthetized (urethane), paralyzed, artificially ventilated and arterial blood gases controlled. Resting and BF-elicited increases in cortical CBF were assessed using laser-doppler flowmetry on the dural surface (1.5 mm rost.& 3.3 mm lat. to bregma). Unilateral electrical stimulation (100 uA; 10 sec.; 6.25-50 Hz) of BF resulted in graded increases in CBF (up to 240% of resting). Acute infusion of NIC (12.5-200 ug/kg, iv) to 3 mo. rats further enhanced the responses by 100-500%; greatest enhancement was at lower Hz ($N=7$; $p < 0.05$). Subacute NIC showed a remarkable, although diminished effect to acute NIC administration (50-350% enhancement). In aged rats the enhancement by acute NIC was similar following placebo or subacute NIC treatment ($N=5$), although diminished with respect to 3 mo. rats (50-350 % enhancement). CONCLUSION: Cortical CBF governed by the BF is facilitated by NIC following acute and, although somewhat diminished, subacute administration. Aged rats have a diminished response to acute NIC, but are not further diminished by subacute NIC. (Support: ABBOTT & NIA Grant P-30 AG0 8014-01A1)

16.5

BASAL FOREBRAIN (BF) REGULATION OF CORTICAL CEREBRAL BLOOD FLOW (CBF): MODULATION BY SUBTYPE-SELECTIVE NEURONAL NICOTINE RECEPTOR AGONISTS. S.P. Arneric, D.G. Linville, S. Williams*, and J. Raszkiewicz*. Neuroscience, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064-3500, and Dept. of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62702.

Chemical and electrical microstimulation of the BF elicits profound increases in cortical CBF (Arneric, Excerpta Int. Cong. Series 869:381, 1989), an effect that is enhanced by nicotinic receptor activation (Linville & Arneric, Soc. for Neurosci. 16:129.9, 1990). The existence of multiple neuronal nicotinic receptors (nAChRs) in brain is supported by molecular biological and pharmacological evidence. Recently, Luetje & Patrick (J. Neurosci. 11:837,1991) have shown that nAChRs containing beta4 subunits are remarkably more sensitive to (-)cytisine (CYT) than (-)nicotine (NIC). This study sought to determine: Is the enhancement in cortical CBF elicited by nicotinic agonists linked to beta2 or beta4 containing nAChRs? Resting and BF-elicited increases in cortical CBF were assessed using laser-doppler flowmetry. Sprague-Dawley rats (3-5 months) were anesthetized (urethane), paralyzed, artificially ventilated and arterial blood gases controlled. Flow probes were stereotaxically positioned on the dural surface (2.5 mm rostral & 2.6 mm lateral to Bregma). Unilateral electrical stimulation (100 uA; 10 sec.; 6.25-50 Hz) of BF resulted in graded, stimulus-locked increases in CBF (up to 271% above resting control). Infusion of NIC (50-200 ug/kg, iv) further enhanced the responses by another 100-300%; greatest enhancement was at lower Hz. CYT had a biphasic effect, with modest enhancement (30-40 %) at low doses (0.2-3 ug/kg), while the response was inhibited by 50% at 200 ug/kg. These data indicate that control of the cortical cerebral circulation by the BF is facilitated by nicotinic receptor agonists similar to NIC, not CYT. These data also suggest this response is mediated predominantly by nAChRs containing beta2, not beta4, subunits. (Support: ABBOTT & NIA Grant P-30 AG0 8014-01A1)

16.6

CLOMIPRAMINE, A TRYCICLIC ANTIDEPRESSANT, DOSE-DEPENDENTLY REDUCES LOCAL CEREBRAL GLUCOSE METABOLISM IN AWAKE RATS. U. Freo, M. Dam*, G. Pizzolato*, G. Chinaglia*, S. Ruggero*, P. Pietrini#, and L. Battistin*. Neurology Department of Padova University, 35100 Padova, and #Psychiatric Department of Pisa University, 56100 Pisa, ITALY.

The relation of dose to regional cerebral metabolic rates for glucose (rCMRglc) were measured in awake male 3-month-old Fisher-344 rats after administration of clomipramine (CMI), a 5-HT uptake inhibitor used as an antidepressant and antiobsessive drug. Using the quantitative autoradiographic [^{14}C]2-deoxyglucose technique, rCMRglc was determined in 47 brain regions 2 hours after intraperitoneal administration of 2, 10 or 50 mg/kg of CMI.

At the lower doses (2 and 10 mg/kg), CMI reduced rCMRglc in 12 (29%) and 14 (32%) brain regions, respectively. These areas belong to limbic (hippocampus, amygdala basolateral) and visual (superficial layer of superior colliculus, lateral geniculate, visual cortex) regions, locus coeruleus and raphe (dorsal and median) nuclei. Limbic regions as well as raphe nuclei and locus coeruleus are involved in mood regulation and possess high concentrations of 5-HT uptake sites. High dose CMI (50 mg/kg) produced a more widespread rCMRglc reduction (28 brain regions; 59%). Most of these latter regions were diencephalic and telencephalic projection areas of serotonin fibers arising from raphe nuclei system. Therefore, CMI affects areas associated with regulation of mood and control of behavior.

16.7

THREE-DIMENSIONAL RECONSTRUCTION OF DORSAL RAPHE NUCLEUS CEREBROVASCULAR REGULATORY SUBREGIONS IN RAT. *M.D. Underwood, M.J. Bakalian, V. Arango, R.W. Smith and J.J. Mann.* Labs. of Neuropharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15213.

We have previously reported that electrical stimulation of the dorsal raphe nucleus (DRN) in rat elicits either decreased or increased local cortical blood flow (CrtBF) as measured by laser Doppler flowmeter (Soc. Neurosci. Abstr. *16*: 290, 1990). We sought to determine whether the different effects on CrtBF could be explained by activation of different subregions of the DRN.

Animals were anesthetized (chloralose), paralyzed and ventilated. Arterial pressure (AP), heart rate and arterial blood gases were continuously monitored and controlled. The DRN was stimulated electrically (10 min; 1 sec on/1 sec off; 100 μA; 200 Hz) and CrtBF was measured with a laser-Doppler flowmeter. Marker lesions (200 μA, 10 sec) were made in all animals. A composite of electrode placements in DRN was reconstructed in 3-dimensions using a PC-based system based on data from Nissl-stained serial sections (25 μm).

A marked difference in cerebrovascular response was observed depending on the rostrocaudal placement within the DRN. Stimulation within rostroventral portions of DRN elicited a slow onset sustained decrease in CrtBF (85 ± 4% of baseline; $p < 0.05$; $n = 9$). In contrast, stimulation in caudal DRN with identical stimulus parameters resulted in increased CrtBF (126 ± 6% of baseline; $p < 0.05$; $n = 5$). The different cerebrovascular responses could not be attributed to differences in arterial pressure, blood gases or hematocrit between groups.

We conclude that electrical stimulation of the DRN can evoke either increases or decreases in CrtBF depending on the anatomical sublocalization and is evidence for a functional topographical organization of the DRN. (Supported by PHS grant MH46745)

16.8

EXCITATION OF NEURONS OF DISCRETE REGION OF VENTROLATERAL MEDULLA MODIFY CEREBRAL BLOOD FLOW, BUT NOT METABOLISM, DEPENDENT UPON PROSTAGLANDIN(S) AND NO. E.V. Golanov*, M. Springston, S.B. Berger, D.A. Ruggiero, R. Blasberg, M. Itkis and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Stimulation within areas of ventrolateral medulla (VLM) modifies arterial pressure, respiration and, conceivably, cerebral blood flow (Underwood et al., 1990). To assess the topographic organization of the cerebrovascular response, we examined the effects of excitation of the VLM on regional cerebral blood flow (rCBF) in frontal cortex of anesthetized, spinalized rats. Site-specific electrical simulation of VLM increased rCBF, measured with a laser doppler flowmeter, up to 260±40% of control (n=12). With a 10 sec stimulus (60Hz, 3x threshold current), the onset was between 5 and 10 sec and maximum responses were reached at 30-40 sec, persisting for >5 min. rCBF was comparably elevated by microinjection of glutamate (20nl, 15μg) or kainic acid (20nl, 0.4μg). The active zone was caudal to and distinct from the cardiovascular zone of rostral VLM. The evoked rCBF response was reduced by 57% with indomethacin (10mg/kg, i.v.; n=7) and 70% by blockade of NO synthesis with NMMA (30mg/kg, i.v.; n=5). Autoradiographic analysis of rCBF in cortex and glucose utilization by ^{14}C-2-deoxyglucose (n=6) demonstrated a significant ($p<0.01$) increase in rCBF unassociated with changes in glucose utilization. Neurons in VLM distinct from those regulating AP may regulate rCBF independently from metabolism by mechanisms largely dependent upon release of prostaglandins and NO.

16.9

EDRF PARTICIPATES IN THE NEOCORTICAL VASODILATION ELICITED BY STIMULATION OF THE FASTIGIAL NUCLEUS. C. ladecola, Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Electrical stimulation of the cerebellar fastigial nucleus (FN) increases cerebral blood flow (CBF) globally and maximally in neocortex, wherein the vasodilation is dependent on local release of acetylcholine (ACh). In brain as in other organs ACh probably mediates vasodilation through an endothelium-derived relaxing factor (EDRF), a substance, thought to be nitric oxide (NO), which induces smooth muscle relaxation by stimulating guanylyl cyclase (GC). We used the specific inhibitor of EDRF/NO synthesis N^G-nitro-L-arginine (NA) and the GC inhibitor methylene blue (MB) to determine whether EDRF/NO participates in the cortical vasodilation elicited by FN stimulation. Rats were anesthetized (halothane: 1-3%) and ventilated. FN or pontine reticular formation (PRF) were stimulated through microelectrodes. Hypertension was prevented by spinal cord transection with arterial pressure maintained by i.v. phenylephrine. Aerated Ringer (37C°) with and without MB or NA was superfused on the parietal cortex wherein CBF was monitored by laser-doppler flowmetry. With normal Ringer superfusion FN stimulation (100μA; 50Hz) increased CBF by 86±7% (n=26; $p<0.01$, ANOVA) and PRF stimulation (100μA;100Hz) by 128±18% (n=9; $p<0.01$). Superfusion with MB (10^{-3} M) did not change resting CBF but attenuated the CBF increase elicited by FN stimulation by 80±4% (n=15; $p<0.01$). MB did not affect the CBF increase elicited by PRF stimulation (+98±18%; n=9; $p>0.05$) or hypercapnia (+97±16%; pCO_2 54±4; n=11). Similarly, NA attenuated the CBF increase elicited by FN stimulation (-67±5%; n=11; $p<0.01$ from Ringer) but not PRF stimulation (+104±14%; n=9; $p>0.05$). Thus, the neocortical vasodilation elicited by FN stimulation is substantially attenuated by inhibition of NO synthesis or action. Such attenuation is not a consequence of impaired vascular reactivity as the vasodilation evoked from the PRF or hypercapnia is not affected. The results strongly suggest that the vasodilation elicited by FN stimulation is mediated by EDRF/NO and raise the possibility that EDRF/NO may also be important in the regulation of the cerebral microcirculation.

16.10

BLOOD FLOW INCREASES IN RAT BARREL CORTEX DURING WHISKER STIMULATION. S.B. Cox*, T.A.Woolsey and C.M. Rovainen. Depts. Neurosurgery and Cell Biology, Washington Univ. Sch. of Med.,St. Louis, MO 63110

We used videomicroscopy of the rat barrel cortex and natural stimulation of the whiskers to test the hypothesis that functional groups of neurons are linked to discrete groups of cortical vessels. These experiments extend previous studies by Winn *et al.* (1991). Our model is that one or a few penetrating pial arterioles supply each barrel, a group of cells that is activated by a vibrissa. Adult female rats were fitted with a closed cranial window over the barrel cortex. Vessels were imaged using a fluorescence microscope with a SIT or ICCD camera, and analyzed by computer. This provided an *in vivo*, instantaneous and continuous, record of vascular changes during stimulation. Following intravascular injection of fluorescent dextrans and 3μm latex beads, changes in vessel diameter, arterial-venous transit time, and bead velocity were used to measure changes in blood flow. The row C whiskers were stimulated at 5 Hz for 1 min by stroking . All measured parameters for blood flow gave similar results: blood flow increases with whisker stimulation. The vessels in which flow increased supplied the appropriate barrels which were identified histologically. Increases in blood flow were not observed in vessels that supplied other cortex. Apnea increased blood flow more dramatically than whisker stimulation. The increases occurred throughout the arterial tree and independent of barrel location. Increases in local cerebral blood flow with whisker stimulation demonstrate a functional link between cerebral vascular units and corresponding cortical modules. This provides a basis for focal regulation of cerebral blood flow.

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16.11

CUTANEOUS STIMULATION REGULATES REGIONAL CEREBRAL BLOOD FLOW IN ANESTHETIZED RATS.

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In previous study[1], the effects of pinching of a forepaw on cerebral blood flow (CBF) in the cortex were examined using laser Doppler flowmetry in halothane anesthetized, spinalized rats (transection at the first thoracic (T1) level) after eliminating pressor responses to pinching. It was demonstrated that the pinching of the forepaw produced a significant increase in CBF in the cortex irrespective of responses of systemic blood pressure (BP). The present experiment further evaluated whether pinching of the forepaw for one min influences regional CBF (rCBF) in various other brain regions in the anesthetized, spinalized rats. Bilateral rCBFs in a total of 27 brain regions on the one side were examined with autoradiography using [^{14}C]iodoantipyrine. There were remarkable increased responses of rCBF in the bilateral cerebral cortex, caudate nucleus, hippocampus, thalamus and midbrain, but there were no regions to show the decrease. These results indicate that pinching stimulation of the forepaw produces widespread increases in rCBF bilaterally, but not decreases, irrespective of BP.

[1]Adachi,T. *et al. NeuroReport* 1, 41-44 (1990)

16.12

SPINAL CORD BLOOD FLOW FOLLOWING A COMPLETE LOW THORACIC TRANSECTION. O.U.Scremin, D.Heuser*, E.Romero* and E.Duran*. V.A.Medical Center and University of New Mexico School of Medicine, Albuquerque, NM 87108.

Profound alterations in functional activity of the spinal cord are observed below a complete transection (SCT). All reflexes are depressed within 24 hrs after SCT while enhanced reflex activity and new reflex modalities are present 4 weeks later. The status of spinal cord blood flow (SCBF) was investigated with the autoradiographic Iodo-^{14}C-antipyrine technique, above and below a Th9 level SCT in 3 groups of 6 rats: non-transected controls, 24 hrs and 4 weeks after SCT. SCBF was measured in ventral (VC), lateral (LC) and dorsal (DC) column, ventral horn (VH) and dorsal horn (DH) at cervical (C), thoracic above SCT (Th) and lumbar (L) levels. SCBF of untreated controls were (ml*g^{-1}*min^{-1}, Mean±S.E.) CVC= .35±.07, CLC= .31±.03, CDC=.22±.04, CVH= 1.14±.18, CDH= 1.06±.19, ThVC= .29±.06, ThLC= .22±.05, ThDC= .19±.04, ThVH=.83±.15, ThDH=.84±.14, LVC=.41±.08, LLC=.44±.11, LDC=.33±.09, LVH= 1.38±.21, LDH= 1.25±.20. Statistical differences were assessed by ANOVA followed by Tukey's protected t. No significant differences with regard to controls were observed for any of the regions 24 hrs after SCT. At 4 weeks post-SCT, white matter regions of cervical (CLC= .64±.1, $p<.025$) and thoracic cord (ThLC= .58±.08, $p<.01$; ThDC= .52±.07, $p<.01$) showed higher SCBF than untreated controls. In conclusion, no variations in SCBF of white or gray matter were observed below SCT at 24 hrs or 4 weeks post-SCT in spite of large differences in reflex patterns. White matter columns above the level of SCT demonstrated about 100% increase in SCBF. It is hypothesized that this phenomenon may be related to degeneration/regeneration processes in white matter columns.

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AUDITORY AND VESTIBULAR HAIR CELLS AND EPITHELIA

17.1

MISMATCH OF ELECTRICAL AND ACOUSTIC TUNING IN COCHLEAR HAIR CELLS OF THE ALLIGATOR LIZARD. R.A. Eatock and M. Saeki. Department of Physiology, University of Rochester, Rochester, NY 14642-8642.

In the "free-standing" region of the alligator lizard's cochlea, hair cells are tuned to sound frequencies between 1 and 4 kHz. It has been proposed that the acoustic tuning of these cells derives largely from micromechanical resonances of their hair bundles (Weiss and Leong, Hearing Res. **20**:157,1985). In cochlear hair cells of turtles and some other species, acoustic tuning may instead arise from electrically resonant properties of the cell membrane (e.g., Fettiplace, Trends Neurosci. **10**:421, 1987). Such electrical resonance has been documented only in hair cells tuned to frequencies below 500 Hz. To probe a higher frequency range and to look for potential interactions between micromechanical and electrical tuning mechanisms, we have examined electrical resonance in hair cells isolated from the free-standing region of the lizard's cochlea. Using conventional and perforated-patch whole-cell recording, we find that depolarizing current steps evoke lightly damped voltage oscillations at frequencies well below the acoustic characteristic frequencies of the free-standing region. Unlike turtle cochlear hair cells, the lizard's cells do not exhibit voltage oscillations spontaneously but only in response to injected currents $\geq +50$ pA. For 8 free-standing hair cells the mean electrical quality factor (Q) was 8±2.4 (SEM) and the mean electrical resonant frequency was 134±19.2 Hz (range 36-205). (For all cochlear hair cells, some of which may not be free-standing, the mean Q was 14±4.8 (n=21), and the mean resonant frequency was 141±11.3 (range 27-224).) Thus the electrical resonance of isolated free-standing hair cells is not congruent with their *in vivo* acoustic tuning, indirectly supporting the proposal that micromechanical mechanisms determine acoustic tuning in these cells. *Supported by NIH.*

17.2

MEMBRANE PROPERTIES OF SOLITARY TALL COCHLEAR HAIR CELLS ISOLATED FROM THE ADULT PIGEON. M. J. Correia, D. G. Lang* and P. A. Fuchs, Departments of Otolaryngology and Physiology and Biophysics, UTMB, Galveston TX

Using dissociation and recording procedures detailed elsewhere (Correia et al., *J. Neurophysiol.* **62** 924 1989), tall hair cells (17-20μm) were isolated from basal, central and apical regions of the auditory papillae and studied using whole cell patch clamp techniques. Recordings were made in 20° C pigeon Ringers solution containing 10mM HEPES and buffered to a pH of 7.4. The recording electrodes contained potassium or cesium as the major cation and 11 mM EGTA. The mean (±SEM) zero current potential (during voltage clamp) of 35 hair cells was -47.7 ± 1.8 mV. Mean input resistances, at rest, for apical, central and basal hair cells were 1.30 ± 0.13 GΩ (n=13), 2.39 ± 0.27 GΩ (n=7) and 0.97 ± 0.84 GΩ (n=3), respectively Ten hair cells, (studied during voltage clamp), produced at least three putative types of ionic currents: an inward, calcium current; an outward calcium-dependent current (suggested by N-shaped I(V) curve) and an outward calcium-insensitive current (suggested by linear I(V) curves). Electrical resonance was observed in some cells. In general, the resonant frequency increased as the cells were depolarized. The resonant frequencies and the voltage dependency of the resonant frequencies of 9 apical, 6 central and 4 basal hair cells were compared and were the same, suggesting that electrical resonance in adult pigeon tall hair cells may not be tonotopically arranged. This work was supported in part by grants, NAG-2-293 and N00014-91-J-1027.

17.3

A STOCHASTIC MOTOR THEORY OF COCHLEAR OUTER HAIR CELL MOTILITY. B.N. Evans, R. Hallworth and P. Dallos. Auditory Physiology Lab. (The Hugh Knowles Center) and Dept. Neurobiol. & Physiol., Northwestern Univ., Evanston, IL 60208.

The nature of the mechanism that generates electrically-induced length changes in isolated cochlear hair cells is unknown. Using the microchamber method, we have previously described the relationship between the applied voltage command and the length change (Evans et al., Hear. Res, 52, 288-304, 1991). The function is nonlinear over its entire range and saturates in both the contraction and extension directions. The length change function may be described by a second-order Boltzmann equation, although in many cells a first-order equation is a good approximation.

By varying the partitioning of the cell in the microchamber, we have demonstrated that the cell segments contract or extend independently as a function of the local membrane potential (Hallworth et al., Soc. Neuro. Mtg., 1990). This suggests the existence of a large number of independently-acting motor elements. Considering this concept, together with the saturation property of the mechanism and its characterization, we propose a stochastic model of outer hair cell motility. By analogy with voltage-dependent ion channels, we suggest that the mechanism consists of a large number of molecular motors which fluctuate between two states, "long" and "short". The constants governing the rates of transition between the states are modulated by the membrane potential. The observed length change is therefore the statistical sum of the motor states.

Our previous studies also suggested a motor mechanism that generates the length change by axial deformation rather than radial constriction. Nevertheless, diameter changes, in opposite phase to the length change of the corresponding segment, are also observed. The diameter change amplitudes as a function of cell segment extrusion indicate that they are actively driven. They also exhibit the same nonlinearities as the length changes, demonstrating that the nonlinearity is the property of the molecular motor itself. We therefore propose that axial and radial changes are vector components driven by motors aligned at an angle with the cell's axis.

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17.4

THE DISTRIBUTION OF VIBRATION-SENSITIVE FIBERS IN THE FROG INNER EAR. J. Christensen-Dalsgaard, D.D. Simmons and P.M. Narins, Dept. of Biology, UCLA, Los Angeles, CA 90024-1606.

We studied the responses of inner ear afferents in Rana pipiens to substrate-borne vibrations. We also injected fibers in the saccular nerve with biocytin and traced the course of the fibers in VIIIth nerve and brainstem. Recordings were made from the VIIIth nerve using a dorsal approach and from the anterior branch of the VIIIth nerve and the saccular nerve using a ventral approach. Nearly all fibers in the saccular nerve were vibration-sensitive with BF's ranging from 10 to 40 Hz, and clear responses were seen to levels of 0.01 cm/s^2. Vibration-sensitive fibers were also found in the anterior branch of the VIIIth nerve (lateral to its mergence with the saccular branchlet) and in the dorsal part of the VIIIth nerve. Therefore, vibrational sensitivity is distributed among several inner ear organs. The saccular fibers form a homogeneous population with low-pass frequency characteristics.

Comparative data on the distribution of vibration-sensitive VIIIth nerve fibers in the Puerto Rican frog Leptodactylus albilabris will also be presented. DNSRC 11-7765 (JCD), Sloan Foundation (DDS) and NIH #DC00222 (PMN).

17.5

CENTRAL AND PERIPHERAL GENERATORS OF SHORT LATENCY VESTIBULAR RESPONSES. A. M. Nazareth* and T. A. Jones. Department of Oral Biology, College of Dentistry, Univ. of Nebraska Med. Ctr., Lincoln, NE 68583-0740.

Vestibular compound action potentials are elicited by linear acceleration pulses and appear as a series of four to seven waves in far-field recordings in the bird (*Gallus domesticus*). These responses occur within 8msec following the stimulus, exhibit peak-to-peak amplitudes ranging from 0.3 to 20 μv, and have been shown to be dependent upon the activation of vestibular neurons bilaterally (Jones and Pederson 1989; Jones 1991). The exact neuroanatomical origins of vestibular responses have yet to be determined. The purpose of the current study was to distinguish response components generated by the vestibular periphery from those arising from central neuronal relays.

To accomplish this, responses were recorded before and after unilateral surgical interruption of the vestibulocochlear nerve near its exit at the internal acoustic meatus. Prior to sectioning, a contralateral intralabyrinthine injection of tetrodotoxin (TTX) was used to abolish all activity from that side. The effectiveness of the contralateral TTX blockade was confirmed ultimately by a second ipsilateral TTX application. Each animal (n=18) was anesthetized and tracheotomized. The cranium was embedded in plaster for delivery of vertical acceleration stimuli. Responses were recorded using traditional computer averaging techniques. Electrophysiological activity from the skull (vertex-mastoid) was amplified (gain=100,000), filtered (LF=300Hz, HF=3KHz), and directed to an analog to digital converter for processing. Following each study, heads were fixed, decalcified and blocked in preparation for histological sectioning. Histological sections were used to characterize the precise limits of surgical manipulations.

Sectioning of the eighth nerve abolished or substantially reduced response peaks beyond P2 (vertex positive, peaks numbered sequentially). P1 and N1 were consistently the least affected by sections. In two animals sections produced little or no effect indicating that vestibular relays were spared. These findings are consistent with the hypothesis that P1 and N1 represent components of the eighth nerve compound action potential and therefore reflect peripheral neural activity. We conclude that peaks arising later than P2 are generated by vestibular brainstem relays. This research was supported by NASA NAGW 1275, (TAJ) NIH IRI5DC00474-01, (TAJ) NIH, Grad Res. Asst. COD (AMN).

17.6

COMPUTER-AIDED ANATOMICAL RECONSTRUCTION OF THE SHAPE AND THE ORIENTATION OF HUMAN UTRICULAR AND SACCULAR MACULAE. U.J. Bucher*, M.A. Maier*, C.F. Ramos, R.K. Dismukes. Department of Psychology, Biomathematical Section, University of Zurich, CH-8032 Zurich, Switzerland.

To understand functional aspects of the vestibular organs in spatial orientation, one is critically dependent on information about their shape and orientation in the head. Most of the available material up to date allows only poor visualization or measurement of the three-dimensional properties.

The present study presents a technique to reconstruct the macular epithelia in a three-dimensional virtual space, based on histological sections of one human left and right temporal bone. In this approach it is not necessary to previously mark the sections. Once the sections are digitized each can be aligned to its neighbor section by translation and rotation using the semicircular canal system as a frame of reference.

As a result, the maculae utriculi and sacculi cannot only be displayed graphically as stereopicture, but, furthermore, their shape and position relative to the Reid stereotactic system can be analytically defined with a polynomial function. Such data may conveniently be used in computer simulations as shown e.g. in a functional simulation of the otoliths' response to varying gravitational forces.

17.7

CYTOCHEMICAL LOCALIZATION OF ADENYLATE CYCLASE IN THE TROUT SACCULE. R.C. Kern[1*], M.J. Drescher[1,2*], K.M. Khan[1,2], S.F. Myers[1], and D.G. Drescher[1,2]. [1]Dept. of Otolaryngology, [2]Lab. of Biotology, Wayne State University, Detroit, MI 48201.

The trout saccular sensory epithelium contains a large population of receptor cells (hair cells) and serves as a model system for octavolateralis end organ function in higher vertebrates. Adenylate cyclase, the enzyme of synthesis of cAMP, an important second-messenger regulator of cell function, has been cytochemically localized in the trout saccular epithelium by a modification of the histochemical method of Mees (1984). Pyrophosphate released from the artificial substrate AMP-PNP was precipitated by strontium ion and converted to a lead reaction product, visible in the electron microscope. Dithiothreitol and theophylline were added to inhibit nonspecific activity. Parallel control incubations were performed with substrate deleted.

In tissues incubated with the complete reaction mixture, precipitate was found to be associated with the stereocilia and apical membranes of the hair cells. Reaction product was also observed on lateral membrane specializations of mitochondria-rich cells in the region of transitional epithelium and noted in vesiculated, presumed efferent, nerve terminals on receptor cells. Little nonspecific precipitate was observed for control incubations.

Adenylate cyclase activity in the apical portion of hair cells could play a role in modulation of mechanotransduction processes. Activity on lateral membrane specializations in presumed ion-transporting cells may have a correlate in the stria vascularis and dark cells of higher vertebrates. Adenylate cyclase activity in presynaptic efferent neurons may subserve regulation of the synthesis and release of efferent neurotransmitter(s).

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17.8

ONTOGENESIS OF F-ACTIN IN HAIR CELLS OF RAT'S COCHLEA. A. Zine, A. Hafidi, and R. Romand*. Lab. de Neurobiologie, Université Blaise Pascal, 63177 Aubière Cedex, France.

Actin is the main componant of stereocilia of the stato-acoustic receptors. Phalloidin is a specific marker for the polymerized form of actin, F-actin. This property was used with rhodamine-labelled phalloidin in order to follow the ontogenesis of this cytosqueletal protein in hair cells of the cochlea.

This study was performed in the rat from the 16th day of gestation (16 DG) up to adulthood on surface preparations and cryostat cross-sections of the organ of Corti. At 16 DG, no specific phalloidin labelling is visible on the apex of hair cells, except close to the plasmic membrane outlining the shape of cells. At 18 DG, specific labelling is present on the cuticular plate and very faint labelling on stereocilia of the inner hair cells, while in the outer hair cells (OHCs) the labelling on the cuticular plate is only visible at 20 DG and on stereocilia at birth. At this stage of development, the cuticular plate and stereocilia of both types of hair cells are clearly visible, although the W shaped stereocilia of OHCs appear later during the first postnatal week.

From these observations it can be concluded that F-actin follows the known pattern of lateral gradient of development between both types of hair cells and shows an adult distribution well before the onset of cochlear function in the rat.

(Supported by INSERM grant 896013).

NEUROGENESIS

18.1

CELL OUTPUT OF THE VENTRICULAR ZONE OF THE E14 MOUSE NEOCORTEX T. Takahashi[1] R.S. Nowakowski[2] M. Jacobson*[1] and V.S. Caviness, Jr.[1] [1]Dept. of Neurology, Mass. General Hospital, Boston MA 02114 and [2]Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

The fraction of cells quitting (Q) and persisting (P, where P+Q = 1) in the proliferative cycle following cell division was determined by a ^{3}H-thymidine (^{3}H-TdR) and bromodeoxyuridine (BUdR) double labeling strategy for the E14 murine neocortical ventricular zone (VZ). In principle, the Q fraction should increase from 0 at E10, before neuronogenesis begins, to 1.0 by E17, when the VZ becomes exhausted (Nowakowski, *Soc. Neurosci. Abstr.* 17: 1991). E14 is a time when the length of the overall cell cycle and S-phase are changing dramatically (Caviness et al., *Soc. Neurosci. Abstr.* 17: 1991)

A pulse of ^{3}H-TdR was given 2 hr before the initiation of the cumulative labeling schedule with BUdR. This sequence labels those cells which leave the S-phase during the two hour period after the ^{3}H-TdR pulse only with ^{3}H-TdR; cells which leave the S-phase subsequent to the initiation of the BUdR labeling will be labeled with both markers or with BUdR alone. From a prior analysis of cytokinetic parameters at E14 (Takahashi et al., *Soc. Neurosci. Abstr.* 16:1147,1990) it is known that 5.5 hr after ^{3}H-TdR injection, cells singly labeled with ^{3}H-TdR will have completed mitosis. Thus at 5.5 hr the number of singly labeled cells corresponds to the total number of daughter cells produced within the two hour period (N_P+N_Q). At 14.5 hr after the ^{3}H-TdR pulse the singly labeled cells will correspond to the Q fraction, that is cells which complete the full cycle without reentering S-phase. The ratio of the numbers of cells in these two populations, i.e. $N_Q/(N_P+N_Q)$, determined at E14, is significantly less than 0.5. This means that at E14, the proliferating population is not in a "steady-state" and that most daughter cells return to S phase following M-phase. Therefore, at E14 the size of the proliferative population of the VZ is increasing.

Supported by NIH 2 R01 NS 12005

18.2

CYTOKINETIC PARAMETERS OF THE VENTRICULAR ZONE IN DEVELOPING MOUSE NEOCORTEX V.S. Caviness, Jr.[1] T. Takahashi[1] M. Jacobson*[1] and R.S. Nowakowski[2] [1]Dept. of Neurology, Mass. General Hospital, Boston MA 02114 and [2]Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

Cytokinetic parameters of the ventricular zone (VZ) in the neocortex of mice of the CD1 strain were determined by an analysis based upon cumulative S-phase labeling with bromodeoxyuridine (BUdR, Nowakowski et al., *J. Neurocytol.*, 18:311-318, 1989, Takahashi et al., *Soc. Neurosci. Abstr.*,16:1147, 1990). The analysis spanned embryonic days 12 (E12) through E17 (E0 = day of conception), when the majority of infragranular and granular and all the supragranular neurons are generated (Caviness, *Dev. Brain Res.* 4:293-302,1982).

The growth fraction (GF, the proportion of the total population that is proliferating) of the VZ is maintained at 1.0 between E12 and E15 and is only slightly less than 1.0 at E16. On E17, because the VZ is rapidly disappearing, the lengths of the cell cycle (Tc) and S-phase (Ts) and GF could not be reliably estimated. At every age considered here, the overall VZ population behaves as a homogeneous population with respect to Tc and Ts (single population model). Tc lengthens from 10.4 to 19.1 hr between E12 and E16. Ts declines from values above 5 hr before E14 to values less than 5 hr afterwards. The ratio Ts/Tc falls sharply between E13 and E14.

These changes in the cytokinetic parameters of the VZ occurring around E14 are coincident with the appearance of the cortical plate within the primitive plexiform zone and of the subventricular zone. They may accompany an augmentation in the fraction of cells which leave the VZ (Takahashi et al., *Soc. Neurosci. Abstr.*, 17: 1991). Supported by NIH 2 R01 NS 12005

	Ts(hr)	Tc(hr)	Ts/Tc	GF
E12	5.1	10.4	0.49	1.0
E13	6.3	14.7	0.43	1.0
E14	3.0	13.6	0.22	1.0
E15	4.0	18.3	0.22	1.0
E16	4.5	19.1	0.24	0.94

18.3

THE CONTRIBUTION OF COMPETITION DURING G1 AND REENTRY INTO THE PROLIFERATIVE POPULATION TO VARIATION IN CLONE SIZE. R.S. Nowakowski, Dept. of Neuroscience and Cell Biology, UMDNJ-RWJMS, Piscataway, NJ 08854.

The behavior of the cells in proliferative populations can be studied in a variety of ways. Using S-phase markers (e.g., ^{3}H-TdR or BUdR), the average behavior of the cells comprising the population can be analyzed. Using retroviral or other single cell marking techniques, the behavior of the progeny of a single cell can be determined. For this study, data from both approaches have been analyzed in order to determine when during the cell cycle the decision to reenter S-phase is made by the two daughter cells which result from a single mitotic division. At each pass through the cell cycle, a proportion of the cells "decide" to continue to proliferate. The remainder of the cells leave the cell cycle to become permanently post-mitotic or quiescent. If the decision for each daughter cell to reenter the S-phase is made by competitive interaction with constituents of its environment, then the average behavior of the population can be described probabilistically. The range in variation in size of single clones as reported by retroviral experiments can be accounted for only if those competitive interactions are independent for each of the two daughter cells. Since the only time that independent decisions can be made by two daughter cells is after mitosis and during the early part of G1 (i.e., before the restriction checkpoint), it is concluded that the decision to reenter the proliferative population or to leave the proliferative population is made during G1.

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18.4

REGULATION OF NEURON NUMBER BY THE PROLIFERATIVE POPULATION IN THE DENTATE GYRUS OF MICE. N.L. Hayes and R.S. Nowakowski, Dept. of Neuroscience and Cell Biology, UMDNJ-RWJMS, Piscataway, NJ 08854.

During development of the mouse the neurons of the dentate gyrus are produced over a period of time extending from approximately embryonic day 10 (E10) through the third postnatal week. We have compared the characteristics of the proliferative population of mice of the C58/J inbred strain, which have approximately 250,000 granule cells in the dentate gyrus, to that of the LG/J inbred strain, which have approximately 400,000 granule cells. Our data indicate that on the day of birth (P0) the length of the cell cycle (T_C) and of the S-phase (T_S) in the two inbred strains are approximately equal. However, the number of cells which comprise the proliferative population (i.e., the growth fraction GF) differs by a factor of approximately 2. These data indicate that the difference in the size of the adult population of neurons in the dentate gyrus of these two inbred strains of mice arises as a consequence of events which occur during the embryonic period at a time which precedes the generation of most of the granule cells. These events affect specifically the number of cells in the proliferative population but not other major aspects of the cell cycle.

Supported by NSF Grant BNS 8921020.

18.5

STABILITY OF TIMING OF NEUROGENESIS IN RODENTS, MARSUPIALS, CARNIVORES AND PRIMATES AND ITS CONSEQUENCES FOR CELL PROLIFERATION. K.A. Jordan*, A.Baernstein*, L. Hinds* and B.L.Finlay Dept. of Psychol. Cornell Univ., Ithaca NY 14853

How schedules of neurogenesis in mammals are adjusted to conform to maturational periods that may differ in length by an order of magnitude, and the consequences of these neurogenetic schedules for cell proliferation is the subject of this study. Tritiated thymidine birthdating studies from five rodents (rat, mouse, gerbil, hamster and spiny mouse), two marsupials (possum and quokka), and in the cat and rhesus monkey were reviewed to determine the duration and peak day of neurogenesis for as many neural structures or cell classes as possible. All pairs of species were compared to determine the regression equation best relating the set of common neurogenetic events in one species to the other. In every case, the relationship was fit well by a linear equation, with correlation coefficients ranging from 0.66 to 0.93.

A test case of of the consequences of extended cell proliferation for cell number was examined. The gerbil retina has twice the retinal ganglion cells of the hamster, and its retinal neurogenesis is about twice as long. Total retinal neuron number in early development was quantified to determine if extended duration of neurogenesis alone accounts for the greater number of retinal neurons in the gerbil, or if rate of neuron production also differed. The rates of cell proliferation were close to identical. Comparison of the slopes of increase of cell number over days showed no significant difference ($t=1.785$, $df=16$, $p<.20$) with the trend favoring faster proliferation in the hamster. Thus, duration of neurogenesis accounts for the species difference in neuron number. Supported by NIH RO1 NS19245.

18.6

CELL PROLIFERATION IN THE VENTRICULAR ZONE OF RING DOVES. C. Ling & M.-F. Cheng, Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

Recent studies in adult canaries have identified areas within the VZ of the lateral ventricle (hot spots) in which new cells proliferate. In this study, we examined patterns of cell proliferation in the VZ of the ring dove, a non-song learner. Sixteen young doves (about three months old, adult body weight) received a single intramuscular injection of [^{3}H] thymidine (4 uCi/g), a marker for DNA synthesis and therefore for cell replication. Birds were sacrificed under deep anesthesia after various survival times: 1h, 8h, 24h, and 48h. Brains were removed, sectioned, processed and scanned for silver grain as described by Alvarez-Buylla et al. (1990). There are 3 salient findings: (1) Most of labeled cells were found along the lateral wall of the VZ of the lateral ventricle, (2) concentration of labeled cells in the VZ increased gradually from anterior sections, reaching a peak at the level where the lobus parolfactory (LPO, avian basal ganglian) reaches its caudal edge and declining sharply in sections thereafter, (3) there are consistent sex differences in the pattern of cell proliferation in the VZ. **Supported by Johnson and Johnson Discovery Award.**

18.7

IN VITRO NEUROGENESIS BY VENTRICULAR ZONE EXPLANTS OF THE ADULT SONGBIRD FOREBRAIN S. A. Goldman and A. Zaremba*, Dept. of Neurology, Cornell Univ. Medical College., N.Y., N.Y. 10021.

The vocal control nucleus, HVc, of the songbird forebrain exhibits neurogenesis in adulthood, with the production of new neurons from local ventricular zone precursor cells. Ventricular zone explants derived from the adult canary HVc can be maintained in vitro, with the continued migration and differentiation of newborn neurons (J. Neurosci.10:2931-39, 1990). Neuronal mitogenesis, however, has only rarely been observed in these cultures when raised in high-serum media (25% total). In the present study, we tested the possibility that serum-borne factors might suppress the mitosis of adult-derived neuronal precursor cells in vitro. When HVc ventricular zone explants derived from the adult zebra finch were raised in high concentrations of charcoal-pretreated fetal bovine serum (FBS, 25%), neuronal DNA replication, and inferentially precursor cell mitosis, was minimal: Only 4.4% of those neurons counted after 8 days in vitro (n=1418) were labelled by a sustained exposure to ^{3}H-thymidine begun at 24 hrs in vitro. In contrast, 19.3% of neurons (n=1972) raised in low-serum medium (5% FBS) incorporated ^{3}H-thymidine by the eighth day in vitro. Neuroblastic ^{3}H-thymidine uptake continued through the first 96 hours in culture, with a decline thereafter in the neuronal labelling index. Immunolocalization of MAP-2 followed by autoradiography confirmed the neuronal identity of the ^{3}H-thymidine labelled cells. This facilitation of neurogenesis by serum depletion suggests that serum-borne factors may inhibit the in vitro division of adult-derived neuronal precursors, either directly or by agents released by serum-stimulated glial or ependymal cells. (Supported by the Mathers Charitable Foundation, Mr. and Mrs. F. Merle-Smith, and NIH NS01316.)

18.8

DEVELOPMENTAL PATTERNS OF NEUROGENESIS IN THE CNS OF THE CANARY. Alvarez-Buylla,A.,and Ling,C-Y Rockefeller University, New York, N.Y. 10021.

Many new neurons are produced in the brain of adult canaries (*serinus canaria*). These cells are born in the lateral ventricle and differentiate only within the telencephalon. Other regions of the brain receive no new neurons in adulthood. We have now investigated the time of neuronal birth in different canary brain structures. [^{3}H]-thymidine was injected at the following ages: before hatching: E5,E9,E10,E12; and after hatching: P1-5,P6-10,P11-20,P21-30,P31-40,P41-50,P91-100,P120-129. All birds were killed at 10-13 months of age. Labeled neurons were identified in sagittal sections stained with cresyl violet. The positions of labeled neurons were mapped in the telencephalon, diencephalon and hind brain (except cerebellum) with the aid of a computer-microscope. With the exception of neurons of the dorsomedial thalamus, most of the neurons in regions caudal to the telencephalon were born before E9. Neurons in the dorsomedial thalamus were produced through E12 with very few if any born after hatching. The addition of new neurons throughout the brain decreased drastically after hatching, but continued in the telencephalon, where it was particularly high in lobus parolfactorius. Very few neurons outside the telencephalon were labeled after hatching and non after P50. Neurogenesis restricted to the telencephalon is therefore established very early, suggesting that both developmental and adult neurogenesis, may allow epigenetic and environmental factors to constantly sculpt telencephalic circuits.

18.9

ACCURATE DETERMINATION OF TIME OF NEURONAL BIRTH USING SEQUENTIAL LABELING WITH ^{3}H-THYMIDINE AND BUdR.

A. Repka*, E. Adler-Graschinsky and R. Adler. Wilmer Inst., Hopkins Univer. Sch. of Med., Baltimore, MD.

Accurate data about the "birthdate" of neuronal precursors is of crucial importance for understanding its relationship with phenotypic commitment. Pulse and cumulative labeling methods using either ^{3}H-Thymidine (^{3}H TdR) or bromodeoxyuridine (BUdR) are useful, but only allow an approximate determination of the day of neuronal birth. We have developed a "window-labeling" technique to precisely determine the time of cell birth by labeling retinal precursor cell cultures first with ^{3}H TdR and, after a specified time interval, with BUdR. Precursors that are already postmitotic prior to ^{3}H TdR labeling are ^{3}H TdR(-)/BUdR(-), while those that continue to divide after BUdR labeling are ^{3}H TdR(+)/BUdR(+). Window-labeled cells, ^{3}H TdR(+)/BUdR(-), are those that undergo the last round of DNA synthesis during the brief time interval between ^{3}H TdR and BUdR treatments and can be easily and accurately identified in preparations processed for autoradiography and BUdR immunocytochemistry. This technique has no detectable deleterious effects upon precursor proliferation, survival or differentiation, and allows accurate determination of the time of terminal mitosis. Supported by USPHS EY04859 and EY07047-13.

CELL LINEAGE I

19.1

CG-4, A NEW BIPOTENTIAL GLIAL CELL LINE FROM RAT BRAIN, CAPABLE OF DIFFERENTIATING INTO EITHER OLIGODENDROCYTES OR TYPE-2 ASTROCYTES.

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Primary cultures of oligodendrocyte-type-2 astrocyte (O-2 A) progenitor cells from brain can be induced to differentiate into either oligodendrocytes or type-2 astrocytes under defined conditions. The O-2A cells are characterized by the presence of the A2B5 surface marker and the absence of potential differentiation markers for oligodendrocytes (galactocerebroside and myelin basic protein) or type-2 astrocytes (GFAP). From these cultures, we have established a permanent cell line, designated CG-4 (Central Glia-4). In contrast to primary O-2A cells, which proliferate for only a limited number of passages, the CG-4 cells can be propagated in serum-free culture medium (DME, with N1 supplement and biotin) supplemented with medium conditioned by the neuronal cell line B104 (B104-CM) for unrestricted periods of time as undifferentiated precursor cells. The CG-4 cells have a normal karyotype and display the same phenotype and differentiation potentials as normal O-2A cells. The proliferating CG-4 cells can display two interconvertible morphologies, bipolar or multipolar, depending on cell density. The multipolar morphology (perhaps a step towards oligodendrocyte differentiation) is favored in high density cultures and has a slower proliferation rate. Similar to normal O-2A cells, the CG-4 cell line can be induced to differentiate into either oligodendrocytes or type-2 astrocytes. Differentiation into oligodendrocytes is observed when proliferating CG-4 cells are transfered to serum-free medium in the absence of the B104-CM. Alternatively, CG-4 cells are induced to differentiate into type-2 astrocytes by medium supplemented with 20% fetal calf serum. Following differentiation, pure cultures of oligodendrocytes or type-2 astrocytes can be obtained in virtually unlimited numbers and maintained for several weeks in medium containing 5% fetal calf serum. In summary, we have established a line that shares all the characteristics with normal brain O-2A cells, except the limitation in proliferative capacity. Support: NINCDS NS16349; NSF BSN-88-08285.

19.2

EXPRESSION OF OLIGODENDROCYTE AND O2A PROGENITOR CELL CHARACTERISTICS IN A HUMAN-HUMAN NEURAL HYBRID CELL LINE

G.C. Trudel, I. Shaw*, J.P. Antel, N.R. Cashman. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

One of the barriers to understanding the cell biology of oligodendrocytes (OL) and OL precursors is the difficulty in obtaining primary neural cells in sufficient yield and purity. We have developed a series of immortal human-human hybrid cell lines which express many phenotypic characteristics of OLs and O2A progenitors. A thioguanine-resistant mutant of the human rhabdomyosarcoma (TE671-TR6) was fused with primary human OLs (cultured from surgical specimens) by a lectin-enhanced polyethylene-glycol procedure, and hybrids were selected in aminopterin-containing medium. In contrast to TE671-TR6, the hybrid line MO3-13 expressed surface immunoreactivity for A2B5, galactocerebroside (GalC), and myelin associated glycoprotein (MAG), and intracellular immunoreactivity for myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP). By fluorescence cytometry, MBP, GalC, and MAG were upregulated by treatment of MO3-13 with phorbol esters, which was corroborated by northern analysis for MBP mRNA. Preliminary data suggest that serum-starved MO3-13 proliferate on exposure to PDGF, and that expression of MBP, GFAP, and A2B5 are regulated by serum concentration in a similar fashion to that described in cultured O2A progenitor cells. MO3-13 cells may provide a model to investigate OL differentiation in an immortalized clonal system.

19.3

DEVELOPMENTAL EXPRESSION AND MODULATION OF INTERMEDIATE FILAMENTS (IF) IN HUMAN FETAL ASTROGLIAL (HFA) STRAINS.

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During astroglial development, expression of IF switches from vimentin, a pre-astrocytic form, to glial fibrillary acidic protein (GFAP), the specific marker of mature astrocytes. All mammalian systems studied thus far demonstrate this developmentally linked expression of IF suggesting a fundamental event in astrocyte differentiation. Little is known, however, about, the mechanism governing differential expression of the IF gene family. Previous work in this lab has demonstrated the ability to grow purified human fetal astrocytes for prolonged periods in vitro and multiply passage them without obvious changes in differentiated cell characteristics. We now report that these HFA cultures have differential expression of GFAP and vimentin and that their IF expression can be modulated with certain cytokines. As previously described, human cell strains consisting predominantly of GFAP-positive astrocytes were established from abortuses of 8 - 18 weeks gestation. Growth requirements and characteristics were established over a 24 month period and greater than 45 passages. Two (2) HFA strains (HFA-1 and HFA-2), derived from the same fetal tissue, were further characterized. IF and cell surface and cytoplasmic markers were examined by immunocytochemistry, western blot analysis, and mRNA analysis by northern blotting. HFA-1 strains uniformly (>93% of cells) expressed high levels of GFAP and demonstrated minimal degrees of positivity for vimentin, MHC class II antigens, and A2B5. Interestingly, HFA-2 cells expressed GFAP at low or undetectable levels while vimentin and A2B5 are expressed at high levels, suggesting an astrocytic progenitor cell type. Neurofilament and galactose cerebroside were not detectable in either strains. In addition, HFA-1 and HFA-2 cultures were exposed to various cytokines (i.e. PDGF, TNF, FGF) to determine whether IF expression could be modulated or switched from one type to the other. The data suggests that HFA-1 represent a population of cells that are typical of Type I Astrocytes, while HFA-2 represents an progenitor cell type similar to the previously described O2A bipotential cell.

19.4

RT4: NEW INSIGHTS INTO IN VIVO COUNTERPARTS.

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The RT4 cell line family was derived from an ENU-induced rat peripheral neurotumor and consists of a stem cell line which spontaneously and stably converts in culture to three derivative cell types in a process called cell type conversion. The stem cell expresses properties of both neurons and glia (Schwann cells) and upon cell type conversion the neuronal and glial properties segregate appropriately. We are studying transcriptional regulation of Na^+-channel gene expression in the RT4 system with the idea that understanding cell type-specific gene regulation may lead to an understanding of how a cell becomes committed to a particular fate. We have shown that (1) upon cell type conversion, Na^+-channel mRNA expression segregates primarily with the RT4 neuronal derivatives and (2) the SkM2 Na^+-channel gene, which was originally isolated from rat muscle cDNA libraries, is the predominant Na^+-channel gene expressed by the RT4 neuronal derivatives. Since the RT4 cell lines were derived from a peripheral neurotumor, our results present the possibility that the SkM2 Na^+-channel gene may be important in vivo in the rat PNS. In addition our findings suggest that the RT4 system may derive from dorsal root ganglia (DRG) since neurons from rat DRG have tetrodotoxin (TTX) resistant Na^+-current and the only known Na^+-channel gene thought to encode a TTX-resistant channel is the SkM2 gene.

19.5

A CENTRAL NEURONAL-LIKE CELL LINE IMMORTALIZED WITH A RETROVIRUS ENCODING THE TEMPERATURE-SENSITIVE SV40 LARGE T ANTIGEN. K. Hisanaga, D.E. Bredesen and F.R. Sharp. Dept. of Neurology, UCSF and VA Medical Center, San Francisco, CA 94121 and Dept. of Neurology, UCLA, Los Angeles, CA 90024-1769.

A temperature-sensitive immortalized neuron-like cell line has been obtained from a primary fetal rat (E17) hippocampal cell culture using a retroviral vector encoding SV 40 large T antigen and neomycin phosphotranspherase (generously provided by K. Frederiksen and R. McKay). The clonal cells, selected by the neomycin analogue G418 resistance, exhibit flat shape, and SV 40 large T antigen and vimentin-like immunoreactivity (IR) at the permissive temperature (33ºC). At the non-permissive temperature (39ºC), the cells exhibit fibrous morphology, and the rate of cell division is significantly reduced. The removal of fetal calf serum (FCS) from DME medium induces neuronal morphological changes in the cells at 39ºC, but not 33ºC, although most of cells die within 3 days. The addition of dibutyryl-cyclic AMP to 10 % FCS containing DME induces marked morphological changes in most of cells to bipolar- or tripolar-shaped neuron-like cells at 39ºC, but not 33ºC, and most of cells can survive for at least 5 days at 37ºC. The cells exhibit NFP, VIP, and GAD-like IR, while GFAP is negative. These cells should be useful to investigate the neuronal differentiation in vitro and possibly in vivo (transplantation to the CNS).

19.6

EARLY AND LATE TRANSDIFFERENTIATING EFFECTS OF NGF ON CULTURED ADRENAL CHROMAFFIN CELLS MAY BE MEDIATED BY TWO DIFFERENT MECHANISMS. *M.A. Herman*[†], *C.A. Schulz*[*] *and P. Claude*[*]. Wisconsin Regional Primate Research Center, [†]Department of Physiology and [*]Neuroscience Training Program, University of Wisconsin, Madison, WI 53715.

During the first week in nerve growth factor (NGF), cultured rat adrenal chromaffin cells increase their rate of mitosis and extend neurites. Following the first week, as cells transdifferentiate into sympathetic neurons, a dense neuritic network forms, cell bodies enlarge, and cells become post-mitotic and dependent upon NGF for survival.

Recently we found that early effects of NGF may be mediated by protein kinase C (PKC), since chronic exposure to the PKC activator, phorbol myristate acetate (PMA; 30 ng/ml) closely mimics the early proliferative and differentiating effects of NGF (*Devel. Biol.*, in press). However, cells grown in PMA remain in an arrested state of development and fail to transdifferentiate into sympathetic neurons. Even after 4 weeks in PMA, cells continue to incorporate ^{3}H-thymidine and fail to develop the dense neuritic network and somatic hypertrophy of sympathetic neurons. This suggests that modulation of PKC is insufficient to complete transdifferentiation and that another biochemical pathway might be involved, perhaps one that uses cAMP.

Forskolin (FS; 50 μM) an activator of adenylate cyclase (AC), inhibits both the proliferative and differentiating effects of either NGF or PMA during the first week in culture. However, at later times, FS does not inhibit the effects of either NGF or PMA. If FS is added to cultures grown for 2 weeks in PMA, a dense neuritic network develops and cells appear similar to those in sister cultures grown in NGF. FS also supports the neurites of NGF-dependent cells. Thus, the later phases of transdifferentiation may involve the activation of AC. [Supported by NSF BNS-8616958, NICDH HD-07118 and NIH RR00167]

19.7

EXPRESSION OF BASIC FIBROBLAST GROWTH FACTOR IN HUMAN NEUROBLASTOMA CELLS REGULATES IN A NEGATIVE MANNER AROMATIC L-AMINO ACID DECARBOXYLASE GENE TRANSCRIPTION. M.J. Weber, P. Barraillé*, A. LeVanThai*. Lab. Pharmacologie Toxicologie Fondamentales, CNRS, 31077 Toulouse France, B. Couderc*, F. Amalric*, H. Prats*. Centre Recherche Biol. Génét. Cell., CNRS, Toulouse.

Human bFGF mRNA is translated from 3 alternative initiation codons, the usage of which determines the cellular localization of the protein products. Iniatiation at 2 CUG generates the 22.5 and 21 kD nuclear forms, whereas the initiation at a AUG generates the 18 kD cytoplasmic form (1,2).

To study the effects of intraneuronal bFGF's, human neuroblastoma cells SK-N-BE and SK-N-BE K2 have been transfected with a retroviral vector expressing the 3 forms of bFGF. The clonogenic capability of the resulting clones in soft agar was reduced compared to the parental cell lines. Moreover, neurofilaments immunoreactivity and aromatic L-amino acid decarboxylase (AADC) activity were suppressed. The supression of AADC activity was also observed in the rat pancreatic exocrine cells AR4-2J. Exogenous bFGF had no effect on AADC activity in SK-N-BE cells.

A construct containing 9 kb of the presumptive promoter of human AADC gene linked to LacZ gene was transiently expressed in SK-N-BE and in its retrovirally transfected derivative. Whereas a strong β-gal activity was measured in the parental line, the corresponding promoter activity was strinkglingly reduced by the expresison of the bFGF's, in agreement with the suppression of AADC activity. Therefore intraneuronal bFGF might regulate the transcription of AADC gene in a negative manner.

Experiments are in progress to determine the effects of the expression of individual forms of bFGF. These experiments might help understanding how intraneuronal bFGF affects the neurotransmitter phenotype of neurons.

1-PRATS H. *et al.*(1989) Proc. Natl. Acad. Sci. U.S.A. 86 1836-1840
2-BUGLER B., AMALRIC F. and PRATS H. (1991) Mol. Cell. Biol. 11 573-577

19.8

REGULATION OF THE TYROSINE HYDROXYLASE IN QUAIL IMMORTALIZED NEURAL CREST CELLS. M. FAUQUET*, E. DUPIN* and A. PROCHIANTZ. CNRS URA 1414, Ecole Normale Supérieure 75005 Paris and Institut d'Embryologie, CNRS UMR 0009,94130 Nogent sur Marne France

The catecholaminergic differentiation of avian sympathoadrenal precursors,cultured *in vitro*, is highly dependant of the presence of chick embryonic extract (CEE) in the culture medium. We isolated a 1.5Kb 5' flanking region of the quail tyrosine hydroxylase (TH) gene . Constructs in which the 5' flanking sequences of the quail gene directed the expression of the bacterial chloramphenicol acetyltransferase (CAT) were transfected into cell lines and assayed for transient CAT expression. The CAT activity was 2% in the mouse fibroblast line LTK- but reached 15% in the TH-positive rat pheochromocytoma line PC12 and 20% in the quail immortalized neural crest cells (Fauquet et al., 1990). To test the possibility that CEE acts on the TH promotor we added CEE to the culture medium of the immortalized crest cells and proceeded to the transient expression assay. We found a 60% increase of the promotor expression in the presence of 10 % CEE. We showed that a region of 70 nucleotides including the TATA box and the cyclic AMP responsive element (CRE) is sufficient for the response to the CEE. Furthermore, forsfolin was able to stimulate the adrenergic expression of the primary neural crest cells *in vitro*. These findings strongly suggest that CEE could act on the catecholaminergic metabolism via the cAMP pathway.

19.9

ROLE OF ADRENERGIC AUTORECEPTORS IN THE DESIPRAMINE (DMI)-MEDIATED INHIBITION OF ADRENERGIC EXPRESSION BY NEURAL CREST CELLS. J.-M. Zhang[1)], A. D. Strosberg[2)], and M. Sieber-Blum[1)]. [1)]Dept. of Cellular Biology and Anatomy, Med. Col. of Wisconsin, Milwaukee, WI 53226, and [2)]Institut Cochin de Génétique Moléculaire, 75014 Paris, France.

Norepinephrine (NE)-uptake inhibitors, such as DMI, block expression of the adrenergic phenotype in clonal neural crest cell cultures (Sieber-Blum, Dev. Biol. **136**, 372, 1989). We have investigated the possibility that this inhibition is caused by a down-regulation of adrenergic autoreceptors. Phentolamine (10 μM), which blocks α-receptors (α-R; major type of adrenergic autoreceptors in the peripheral sympathetic nervous system), did not affect adrenergic expression by neural crest cells in clonal culture. By contrast, propranolol (10 μM; β1- and β2- blocker; also known to directly inactivate tyrosine hydroxylase), caused a 59% decrease. Indirect immunofluorescence using antibodies against dopamine-β-hydroxylase and Mab against either β1-R (B120; Chapot et al., Hybridoma **8**, 535, 1987) or β2-R (BRK-1; Kaveri et al., Eur. J. Biochem., **167**,449, 1987)) indicated that some adrenergic neural crest cells express β-1 and β-2 autoreceptors. DMI caused a >6-fold decrease in the proportion of $\beta1\text{-R}^{+}/\text{DBH}^{+}$ cells, while the number of adrenergic cells expressing β2 autoreceptors did not seem to be affected. We have shown previously that while DMI causes a decrease, added NE causes an increase in adrenergic expression. If DMI acts via adrenergic autoreceptors, NE-agonists would be expected to also cause an increase in adrenergic expression. However, this was observed neither with clonidine (10 μM; α1-, α2-agonist), nor with isoproterenol (10 μM; β1-, β2-agonist). In summary, the data indicate that DMI-treatment is accompanied by a decrease of adrenergic β1-, but not α- or β2-, autoreceptors. Whether this effect has a causative relationship to the observed inhibition of adrenergic differentiation remains to be determined. However, the fact that isoproterenol did not increase phenotypic expression supports the notion that by increasing adrenergic expression NE acts upon an intracellular target that remains to be identified. Supported by USPHS grant HD21423 (M. S-B.) and by CNRS (A.D.S.).

19.10

A CATECHOLAMINERGIC SENSORY PHENOTYPE IN CRANIAL DERIVATIVES OF THE NEURAL CREST: EVIDENCE FOR REGULATION BY CELL AGGREGATION. **D.M. Katz and M.J. Erb.* Depts. of Neuroscience and Medicine, CWRU Sch. of Med., Cleveland, OH 44106.**

Tyrosine hydroxylase (TH) is transiently detectable in cells of the root (crest-derived) sensory ganglia of most cranial nerves between embryonic day (E) 13.5-15.5; TH cells are only rarely found, however, in older ganglia (Jonakait, et al. 1983; Katz & Erb, 1990). To examine the possibility that the loss of TH after E16.5 was due to modulation by the ganglionic microenvironment, the jugular-superior ganglion (JSG) of nerves IX-X was used as a model system of TH development. E16.5 and older ganglia were grown for 24 h in explant and dissociate culture and monitored for TH by immunocytochemical staining. In contrast to explant cultures and the ganglion in situ, 20% of neurons in dissociate cultures of E16.5 JSG were TH+, suggesting that TH expression might be regulated by the state of cell aggregation. In support of this hypothesis, we found that a 4-fold increase in cell density, accompanied by an increase in aggregation, resulted in a 30% decrease in the percentage of TH neurons in dissociate cultures. This decrease was accompanied by changes in neuronal morphology characteristic of ganglion cell maturation in vivo. Our data indicate that factors associated with cell aggregation may play a role in regulating neuronal differentiation and transmitter phenotype during cranial sensory gangliogenesis. HL-42131 and Dysautonomia Fndtn.

19.11

MULTIPLE bFGF FORMS EXPRESSED IN AVIAN EMBRYOS PROMOTE NEURAL CREST CELL COMMITMENT. L. Sherman*, K. Stocker*, R. Morrison, and G. Ciment. Dept. Cell Biology & Anatomy, Oregon Health Sciences University, Portland, OR 97201.

The neural crest (NC) is a transient embryonic structure whose cells differentiate into a variety of phenotypes including sympathetic and sensory neurons, Schwann cells, and melanocytes. In previous studies, we demonstrated that cultured embryonic avian dorsal root ganglia (DRG) and peripheral nerves (PN), which do not normally give rise to melanocytes, become committed to the melanocyte lineage following treatment with basic fibroblast growth factor (bFGF). We also showed that the cells becoming melanocytes probably belonged to the Schwann cell lineage, and that bFGF appeared to be necessary for melanocyte commitment to occur *in vitro*.

In this study, we assayed avian embryo tissue extracts from NC migratory pathways and NC derivatives for their patterns of bFGF expression using protein immunoblots. Embryo extracts were shown to contain 17, 20, 23, 28 and 32 kDa forms of bFGF, each with unique patterns of expression dependent on embryonic age. We have partially purified these forms and compared their abilities to cause melanogenesis in avian embryonic DRG and PN cultures. Preliminary data suggest that the 17 and 23 kDa forms have similar activities. In addition, antibodies which neutralize these bFGF forms have been introduced into the wing buds of quail embryos. Embryos treated with these antibodies appear to demonstrate localized hypopigmentation in treated wings. These data suggest that bFGF may play an important role in melanocyte commitment, and that different forms for bFGF may have different activities during NC development.

CELL SHAPE AND DIFFERENTIATION I

20.1

DISTRIBUTION OF SECRETORY PROTEINS IN CULTURED NEURONAL CELLS. G.Terenghi, A.M.Suburo, S.C.Wheatley*, D.Horn*, D.S.Latchman*, J.M.Polak. Dept. Histochemistry, RPMS, Hammersmith Hospital and * Medical Molecular Biology Unit, University College & Middlesex School of Medicine, London, UK.

To obtain morphological information concerning cellular mechanisms regulating both the shape of nerve processes, and compartmentalisation and release of neurosubstances, we have used the mouse neuroblastoma N18Tg2 cell line and several clones of hybrid ND cells (ND7, ND9 and ND21) derived from the fusion of rat sensory neurones with neuroblastoma. These cell lines can be induced to differentiate *in vitro* and were studied using immunocytochemical methods to detect various neuronal markers, neuropeptides and proteins associated with secretion mechanisms. In all cell lines there was immunostaining for protein gene product 9.5, neuropeptide Y, C-flanking peptide of NPY (CPON), tyrosine hydroxylase and chromogranin. Synaptophysin could only be detected in ND cells. Immunoreactivities to substance P, calcitonin gene-related peptide, galanin and somatostatin were not detected in any of these cell lines. *In vitro* differentiation of N18Tg2 and ND7 cells induced appearance of cell processes of various length. With differentiation, immunostaining for NPY, CPON and chromogranin was found in the tips of the processes, whereas synaptophysin immunoreactivity was seen mainly in cell bodies. Thus the regulated secretory pathway associated with chromogranin was segregated into nerve processes at an early stage of differentiation, when synaptophysin-associated pathway in not yet completely expressed. As NPY, CPON and chromogranin are found in secretory granules, and synaptophysin is a marker for small synaptic-like vesicles, the results of this study are consistent with the view that these markers are associated with different subcellular structures. ND7 cells thus provide a useful model system to study changes in the distribution of neuropeptides, cytoskeletal elements and proteins associated with cell secretion during neuronal differentiation.

20.2

GLYCOCONJUGATE CHANGES DURING SYNAPSE FORMATION. V.V.T.S. Prasad*, S. Fitzgerald*, P. Nelson, and J.R. Moskal. Chicago Institute for Neurosurgery and Neuroresearch, Chicago, IL 60614 and Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20814.

The role that cell-surface glycoconjugates play in the formation of appropriate, stable synapses remains to be determined. We have begun to address this question using 2 model systems; 1) the murine neuroblastoma x rat glioma hybrid cell line, NG108-15, in co-culture with rat myotubes under synapse forming conditions and 2) primary cultures of murine spinal cord (SC) in co-culture with dorsal root ganglia (DRG) also under synapse forming conditions. Gangliosides were purified from cultured cells and subjected to high-performance thin layer chromatography. Immunoautoradiography experiments were then performed using A2B5, a monoclonal antibody that recognizes a family of gangliosides present in both neuronal and non-neuronal tissues. It was found that a ganglioside was expressed in the NG108-15 co-cultures that was not expressed in controls (i.e., N18TG-2 x muscle co-cultures or muscle cultured alone). This ganglioside migrates between GM1 and GM2 and may be the O-acetylated form of GD3. The ganglioside patterns observed with A2B5 in DRG x SC co-cultures were distinct from either NG108-15 x muscle co-cultures or DRG or SC cultured alone. These results demonstrate that cell-cell interactions between synapse forming cells and their targets induces significant changes in ganglioside expression; some of which may be important for appropriate synapse formation. This work was supported in part by NINDS grant NS 26186.

20.3

ETHANOL PROMOTES NEURITE ELONGATION IN RAT CEREBELLAR MACRONEURONS IN VITRO. JY Zou,* RA Rabin,* RJ Pentney. Depts. of Anatomical Sciences and Pharmacology and Therapeutics, State University of NY at Buffalo, Buffalo, NY 14214.

Ethanol reportedly inhibits neurite growth in chick DRG (Dow and Riopelle, Science 228:951, 1985) but accelerates neurite extension in PC12 cells (White and Wooten, FASEB J 5:A457, 1991). In the study reported here, cerebellar macroneurons were grown in vitro on poly-L-lysine-coated coverslips (1000-1500 cells/mm^2) from E17. The cells were stained with antibody against MAP2 for morphometric analysis. Ethanol (75mM), added to the cultures immediately after plating, did not affect neuronal survival or attachment to the substrate. Between 8 and 24 hrs in vitro, neurites in ethanol-treated neurons grew by 56% compared with 31% in the control cells. Between 24 and 48 hrs, they grew by 16% compared with 6% in the control cells. The ethanol-induced increases in total length resulted from linear extension of neurites and from increased branching of neurites. The observed changes were independent of the tissue culture medium employed. (Supported by NIAAA Grants AA05592 and AA06207)

20.4

DIFFERENTIAL CONTROL OF MOTILITY, SHAPE AND DIFFERENTIATION OF OLIGODENDROCYTE PROGENITOR CELLS BY bFGF AND PDGF. R.D. McKinnon*[1], C. L. Smith[2], T. N. Behar[3], T. G. Smith[3], and M. Dubois-Dalcq[1]. Lab. of Viral and Molecular Pathogenesis[1], Neural Control[2] and Neurophysiology[3], NINDS, NIH, Bethesda, MD 20892.

We examined the role of two CNS-derived mitogens, basic fibroblast (bFGF) and platelet-derived (PDGF) growth factors, on the proliferation, migration, and differentiation of perinatal rat cerebral oligodendrocyte progenitor cells in vitro. In the continued presence of bFGF, progenitors were stellate, non-migratory, divided, and remained undifferentiated expressing high levels of PDGF α-receptor and a POU-homeodomain protein (Oct-6/Scip) transcripts in absence of myelin gene expression. On addition of PDGF, FGF-treated progenitors quickly became bipolar and actively migratory while dividing. The complexity of the cells borders was quantitated by fractal analysis. When PDGF was removed, the cells reverted to their characteristic bFGF stellate shape and stopped migrating, demonstrating the plasticity of the cells response to PDGF. Finally, when bFGF was removed the progenitor cells quickly differentiated into highly branched, non-motile and non-dividing oligodendrocytes, acquired myelin gene transcripts (MBP, PLP, MAG), and down-regulated PDGF α-receptor and Oct-6 transcripts. In the presence of PDGF alone, in contrast, progenitor cells slowly differentiated into oligodendrocytes. Thus, bFGF and PDGF have distinct influences on shape, motility, and differentiation of oligodendrocyte progenitor cells.

20.5

TERMINAL DIFFERENTIATION OF A HUMAN NEUROBLASTOMA CELL LINE. P. LoPresti*, W. Poluha*, D. Poluha*, H. Piwnica-Worms* and A.H. Ross. Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545.

Human neuroblastoma cell line SHSY5Y expresses both the 75 kDa low-affinity nerve growth factor (NGF) receptor and the *trk* protooncogene and is responsive to NGF. Treatment of these cells with NGF resulted in neurite extension, but not in cessation of cell growth. SHSY5Y cells were treated with NGF and the DNA polymerase inhibitor aphidicolin. The concentration of aphidicolin was varied to optimize differentiation and to minimize the cell death associated with aphidicolin treatment. There were few differentiated cells immediately following the aphidicolin-NGF treatment, but treatment of the cells with NGF in the ensuing week, resulted in extension of long neurites (>400 μm). If maintained in NGF, the differentiated cells were stable for at least a month and were mitotically inactive. The protooncogene *c-myc* was downregulated. The mRNA for MAP 1B, a microtubule-associated protein associated with neurite extension, was upregulated in the differentiated cells, but there was little or no induction of synaptic vesicle antigens. To elucidate the relation between neuronal differentation and cell cycle regulation, we are analyzing the expression of cell cycle-related proteins. The $p34^{cdc2}$ kinase is expressed by the differentiated cells, and we are testing whether it is active. Supported by NIH grant NS21716.

20.6

AGE-RELATED EFFECTS OF NERVE GROWTH FACTOR ON THE MORPHOLOGY OF DEVELOPING SENSORY NEURONS. S.A.Scott and A. M. Davies. Dept of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794 and Dept. of Anatomy, St. George's Hospital Medical School, London UK SW17 ORE.

Studies on neonatal mammals have shown that neuronal morphology is regulated by the availability of target-derived neurotropic factor (Purves *et al.*, 1988. Nature **336**:123). We tested whether the same is true for embryonic neurons, which are critically dependent on target-derived neurotrophic factors for survival. We grew trigeminal sensory neurons from avian embryos of different ages (E6-E16) *in vitro* in different concentrations of nerve growth factor (NGF) (0.025-20 ng/ml) for 15 or 48 hr, and measured the size and complexity of the resulting arborizations; these levels of NGF supported 4-70% of neurons for at least 72 hr. As expected, the size and complexity of arborizations increased with embryonic age (up to E14), regardless of NGF concentration. Surprisingly, however, for embryos younger than E14 neuronal morphology at a given age was similar in all levels of NGF. By contrast, at E14 and E16, when trigeminal neurons begin to lose their absolute dependence on NGF, neurons established significantly larger and more complex arborizations in higher concentrations of NGF. Thus, in young embryos the extent of neurite outgrowth appears to be independent of neurotrophic factor levels; apparently all neurons that receive enough NGF to survive elaborate full-size arbors. As neurons mature and lose their absolute requirement for NGF, neurite growth becomes dependent on the availability of neurotrophic factor, as in neonates.

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20.7

NGF AND SATELLITE CELLS EFFECT ON DEVELOPMENT OF DENDRITES BY RAT NODOSE SENSORY NEURONS IN CULTURE. P.De Koninck*, S.Carbonnetto + & E.Cooper', Dept. of *Biol.,+Neurol. & 'Physiol., McGill Univ., Montréal, Québec, H3G 1Y6.

Nodose neurons are pseudo-unipolar sensory neurons whose somas are devoid of synaptic contacts and lack conventional dendrites. Previously, it was shown that nodose neurons were capable of expressing high density nAchRs and forming cholinergic synapses among each other when they develop in culture in the absence of other cell types and with NGF. Therefore, we have asked whether these sensory neurons develop dendrites under these conditions. To distinguish dendrites from axons, we have used immunostaining with an antibody to MAP2, together with an antibody to phosphorylated neurofilaments (SMI31). Our results indicate that after 3 weeks in culture with NGF and without satellite cells, 24% (350/1450) of the neurons had MAP2 positive (SMI31 negative) processes which we have classified as dendrites. Of these neurons, 65% had 2 to 5 primary dendrites. In contrast, less than 5% (47/999) of neurons had dendrites in sister cultures without NGF. In addition, dendrites were two fold longer (155 μm $\pm$9) after 3 weeks in cultures with NGF than without NGF (75 μm $\pm$8). These results indicate that some neonatal sensory neurons are capable of developing dendrites and that NGF plays an important role in this process.

It was previously shown that nodose neurons do not form functional synapses when co-cultured with their ganglionic satellite cells and NGF. Our preliminary results suggest that few nodose neurons develop dendrites under these conditions. Our results, taken together, suggest that the expression of dendrites correlates with synapse formation in this system. Funded by NSERC, FRSQ and MRC.

20.8

TARGET INTERACTIONS INFLUENCE MORPHOLOGY OF SV-2 POSITIVE CONTACTS IN CO-CULTURES OF CHICK EDINGER WESTPHAL NEURONS WITH APPROPRIATE AND INAPPROPRIATE TARGETS. J.T. Fujii. Dept. of Anatomy and Cell Biology, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

In vivo a subset of Edinger Westphal neurons forms large hand-like synaptic terminals known as calyces or calyciform terminals on a subset of ciliary ganglion neurons. Previous work (Fujii and Berg, 1987) has shown that Edinger Westphal neurons in culture, identified by their enkephalin immunoreactivity, form calyx-like contacts preferentially on ciliary ganglion neurons. These contacts have now been examined for the presence of synaptic structures using a monoclonal antibody raised against the synaptic vesicle protein SV-2.

EW nuclei were specifically dissected from 400 μm thick embryonic brainstem slices, dissociated, and cultured with ciliary ganglion or sympathetic ganglion neurons. Double label immunohistochemistry experiments found that greater than 90% of the enkephalin-positive calyx-like contacts in these co-cultures were also positive for SV-2 immunoreactivity. Furthermore, the proportion of SV-2 positive contacts with calyx-like morphology was three times greater in co-cultures with ciliary ganglion neurons than in co-cultures with sympathetic neurons. The finding that calyx-like contacts are immunoreactive for SV-2 and that interaction with an appropriate target, ciliary ganglion neurons, favors their formation *in vitro* suggests that direct surface interactions with the target neuron may be important in determining the morphology of synaptic terminals *in vivo*.

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20.9

THE RELATIONSHIP OF GLIAL CELLS TO SYNAPTIC REMODELLING VIEWED IN LIVING MICE. S.L. Pomeroy, Department of Neurology, Children's Hospital, Harvard Medical School, Boston MA 02115.

Synaptic boutons and satellite cells associated with autonomic ganglion neurons have been observed to change in position and number when viewed over time in living mice (Purves et al. 1987, Science 238:1122-6; Pomeroy and Purves 1988, J Cell Biol 103:1167-75). Since synapses are made preferentially near satellite nuclei, I have assessed whether synaptic remodelling is related to concurrent changes of these glia.

Salivary ganglion neurons were viewed in anesthetized adult male CF-1 mice using in vivo video microscopy. The positions of synaptic boutons (stained with the styryl pyridinium dye 4-di-1-ASP) and satellite cell nuclei were recorded initially and again 2-3 months later.

My results show that synaptic remodelling may occur without coincident change of glial cell nuclei, although a strong preference is retained for synaptic boutons to remain associated with nuclei during remodelling. Although changes of satellite nuclei are not necessary for synaptic remodelling, the persistence of their association suggests an important role of glial cells in the maintenance of these synaptic contacts.

20.10

LIMITED EFFECT OF AFFERENT INPUT ON OLFACTORY BULB DEVELOPMENT. J.H. McLean and A. Darby-King.* Basic Medical Sci., Memorial University of Nfld, St. John's, Newfoundland, Canada A1B 3V6

Cholinergic and noradrenergic (NE) axons have been suggested to be essential for providing synaptic plasticity during critical periods of cortical development (Bear & Singer '86). Recently, cholinergic inputs to the cortex have been shown to produce cytoarchitectural changes suggesting that the input influences cell migration (Hohmann *et al.* '88). In this study, Nissl, immunocytochemistry (ICC) of intrinsic dopaminergic neurons and glia, acetylcholinesterase histochemistry, and Golgi studies have been used to elucidate the pretransmission role of cholinergic inputs to the developing rat olfactory bulb (OB).

On the day of birth, an electrolytic lesion was made in the diagonal band (source of cholinergic input to the OB) and 6-OHDA was injected subcutaneously to deplete the locus coeruleus (NE) input to the OB. Following survival of 10 or 35 days the animals were sacrificed by perfusion. At PND 11, the main effect of the depletions was that the OB was smaller and fewer cells appeared to be present in the proliferative region (subependymal zone). In some cases there was also disruption of cytoarchitecture in the OB. ICC staining of intrinsic dopaminergic cells indicated that some of these cells had migration problems on the side ipsilateral to the diagonal band depletion. The distribution of glia appeared normal. Results at 35 day survival was similar to the 11 day survival. Golgi impregnation of the OB at different ages revealed normal cellular differentiation of intrinsic mitral, granule and periglomerular cells. Thus, the major change in the OB following depletions was fewer migrating cells. We interpret the results to indicate that the cholinergic/NE input has a role in cell migration or the electrolytic lesions directly interrupted migration of cells destined to reach the OB. Supported by MRC of Canada MT-10931.

20.11

CELL-CELL INFLUENCES ON THE MORPHOLOGICAL DEVELOPMENT OF AN IDENTIFIED MOTOR NEURON IN THE MOTH MANDUCA SEXTA. Karla S. Kent, Dept. Anat., Sch. Dent., Oregon Health Sciences University, Portland, OR 97201

During metamorphosis of the moth Manduca sexta, thoracic leg motor neurons (MNs) undergo substantial morphological changes, including dendritic loss and dendritic growth. In order to determine if these dendritic changes are influenced by cell-cell interactions, sensory and interneuronal inputs to one identified leg MN, the metathoracic femoral extensor MN, have been manipulated using surgical and chemical ablation techniques. Although ablation of one or both metathoracic legs does not substantially alter the course or magnitude of dendritic loss and growth, the overall morphology of the MN dendritic arbor is affected such that a characteristic dorsomedial process is much more extensive in preparations deprived of metathoracic legs during adult development. The extensive growth of this process occurs even when all three pairs of thoracic legs are absent suggesting that its elaboration is not induced by sensory inputs from other legs. In addition, elimination of postembryonically generated interneurons by use of an inhibitor of DNA synthesis (hydroxyurea) does not alter the overall extent of MN dendritic reorganization nor does it affect growth of the dorsomedial process. Current studies are aimed at identifying specific cell-cell interactions that influence MN dendritic growth and distinguishing between influences of specific leg sensory inputs and contact with specific leg muscles.

20.12

DEVELOPMENTAL REMODELLING OF IDENTIFIED LEECH MOTOR NEURON PERIPHERAL ARBORIZATIONS. J. Jellies and D.M. Kopp, Neurobiology Research Center and Dept. Physiol. and Biophysics, Univ. of Alabama at Birmingham, Birmingham, AL, 35294.

Many neurons employ a developmental strategy whereby their final form and target innervation is sculpted by selective modification of "appropriate" versus "inappropriate" projections (Macagno *et al.*, J. Neurobiol., 21:107, 1990), yet the mechanisms driving this process *in vivo* are somewhat obscure. We have recently discovered a particularly robust example of such plasticity in the medicinal leech, *Hirudo medicinalis*. The muscular heart tubes in this animal are peripheral targets for a pair of excitatory motor neurons, the HE's (Maranto & Calabrese, J. Comp. Physiol., 154:367, 1984). We used intracellular dye-filling and immunostaining to compare embryonic to mature HE's. During early embryogenesis, all HE's project into multiple territories, including the primordial heart tubes and the ventro-lateral body wall. The body wall projections are extensive, often showing tertiary and quaternary branches. However, they are transient - being absent in the mature leech. Also, while there are no physiologically identified HE's in the first 2 or last 2-3 midbody segments in adults (Thompson & Stent, J. Comp. Physiol., 111:261, 1976), we propose that there are, in fact, HE homologs in these segments. Our identification of these homologs is based upon comparisons of cell position and size, ipsilateral projection out the anterior nerve root, early dendritic morphology, and peripheral arborization (in early embryos these cells also project to the nascent heart tubes and ventro-lateral body wall). Additionally, the definitive HE's (segs. 3-18) and these homologs (segs. 1,2 & 19-21) have another feature in common, all being labelled by a mAb directed against human fibronectin. We further discovered that these anterior and posterior homologs probably persist, but as predicted, they are not driven by the central circuitry that generates the rhythmic heartbeat. Instead they exhibit primarily tonic discharge. We are examining the possibility that the HE homologs also remodel their peripheral arborizations, but perhaps do so in a fashion opposite to the definitive HE's, losing heart tube projections while elaborating the body wall arborization, ultimately being incorporated into different circuits. Whether HE remodelling is causally related to target contact or initial assembly of the heartbeat central pattern generator remains to be determined. (Supported by NIH NS28603 to JJ).

20.13

THE UNIQUE AND BIZARRE MORPHOLOGY OF LATE-APPEARING, PERIPHERALLY INDUCED CENTRAL NEURONS OF THE LEECH. Eduardo R. Macagno and Timothy Robin Gershon*, Dept. of Biological Sciences, Fairchild Center, Columbia University, NY, NY 10027

The peripherally induced central (PIC) neurons are found only in the segmental ganglia (SG) of the 5th and 6th body segments, the only segmental ganglia that innervate the reproductive organs. These neurons, of which there are approximately 350 per SG, are born later in embryogenesis than the other central neurons, in response to a signal generated by the male genitalia and conveyed along nerves to the sex SG (Baptista, C.A., et al, *Nature* **6287** 855-858; 1990). PIC cells do not express their neuronal phenotype, however, until several months after the end of embryogenesis. After their delayed differentiation, the PIC neurons must incorporate themselves into the already formed and functioning CNS. We are endeavoring to shed light on the mechanisms controling the timing of PIC neuron differentiation and the growth of PIC neuronal projections through existing circuitry.

We have found that the number of mature PIC neurons increases gradually during the animal's first year of life, but that it increases dramatically with body weight following episodic feeding. The arborization of PIC cells was studied using intracellular dye injection. Each PIC neuron was found to have a single long axon with short secondary branches. Indidual axons followed different courses; some travelled in nerve roots to the periphery; others ran in the interganglionic connective nerve to adjacent SG. Most completed at least one, and often several 360° turns. The wide variation in PIC neuron morphology and the presence of multiple loops in their axons suggests that there may be a stochastic element in the guidance of their growing processes. We have proposed that the PIC neurons may be neurosecretory cells (Baptista, C.A. & Macagno, E.R. *J. Neurobiol.* **19** 707-726,1988), and if so, precise pathways and connections within the neuropil might not be required.

20.14

DEVELOPMENT OF ROSTROCAUDAL DENDRITES OF RAT PREGANGLIONIC SYMPATHETIC NEURONS E.B. Ezerman and C.J.Forehand, Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405

The longitudinal organization of preganglionic sympathetic neurons in the adult mammalian spinal cord takes the general form of a ladder (Barber et al. J. Comp. Neurol. 229: 329, '84). Dispersed clusters of neurons in the intermediolateral cell column (IML) and intercalated area form the rungs of the ladder. In the rat, preganglionic neurons of the IML have extensive dendritic arborizations in both rostrocaudal and mediolateral directions (Forehand, J. Comp. Neurol. 298:334, '90). We have investigated the development of the dendritic arborization of preganglionic sympathetic neurons. By embryonic day (E) 15, an extensive mediolateral arborization is present; however, development of the rostrocaudal arborization is largely postnatal and occurs in two spurts. Rostrocaudally oriented dendrites were first seen at E20 when the average rostocaudal dendritic extension was 35µ. At postnatal days (P) 1, 3, and 5, the average rostrocaudal extension was 130µ; at P7 and 9, it was 250µ. Growth spurts occurred between E20 and P1 and between P5 and P7. These correlate with similar spurts in the distances between the developing ladder rungs. However, by P3, the rate of rostrocaudal dendritic extension exceeds the development of the spacing between the rungs of the ladder. By P7 the extent of the rostrocaudal arborization is almost double the distance between the rungs. The signal for this postnatal growth of a subpopulation of the dendrites of preganglionic neurons is unknown.

20.15

MOSSY FIBER OUTGROWTH PRECEDES DENDRITIC MATURATION IN DEVELOPING DENTATE GRANULE CELLS. B.J. Claiborne, M.P. O'Boyle* and S.P. Jones*. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78249.

Previous studies of early granule cell development in the dentate gyrus have not addressed the relationship between axonal outgrowth and dendritic maturation. In this study, DiI was used to retrogradely label developing granule cells whose axons had reached the CA3 region of the hippocampus. Crystals of DiI were placed in field CA3c of fixed hippocampal slices from rat pups between the ages of 2 and 9 days and the dye was allowed to diffuse for 18 to 36 hours.

Labeled granule cells at various stages of maturity were visible in all slices, reflecting the long period of granule cell neurogenesis in the rat. In general, labeled neurons from the youngest animals had very immature dendritic trees with thick primary dendrites and short stubby branches. Dendrites exhibited growth cones and numerous large varicosities, but had few or no spines. Basal dendrites were present on most cells. As the animals matured, dendritic branches elongated and spines began to appear. Varicosities, growth cones and basal dendrites decreased in number, but were still present on many labeled cells. In the adult rat, granule cells do not display dendritic varicosities, nor do they have basal dendrites. The present results indicate that the mossy fibers of at least some granule cells reach the CA3c region of the hippocampus long before the adult form of the dendritic tree has been established. (Supported by NSF)

20.16

ELEVATED GM2 GANGLIOSIDE IS ASSOCIATED WITH THE PROLIFERATION OF DENDRITES ON PYRAMIDAL NEURONS IN DEVELOPING NEOCORTEX. L.A. Goodman, P.O. Livingston* and S.U. Walkley. Dept. Neuroscience, Rose F. Kennedy Center, Albert Einstein College of Medicine, Bronx, NY. 10461.

Recent studies of cats with the neuronal storage disease, α-mannosidosis, established that elevated GM2 ganglioside in mature cortical pyramidal neurons is associated with proliferation of new dendrites, suggesting that GM2 may stimulate dendritic growth (Goodman et al., Soc. Neurosci. Abstr. *16*:315). To determine whether the association between GM2 ganglioside and dendritic growth occurs under normal conditions, we used a monoclonal antibody to localize GM2 in cerebral cortex of developing cats. The present study shows that neurons contain GM2-like immunoreactivity (GM2-LIR) throughout the period when pyramidal cells elaborate their normal dendritic arbors. Whereas neurons in adult cats contained no GM2-LIR, pyramidal-like neurons in cats at late gestation through 2.5 weeks of age contained diffuse GM2-LIR that filled the soma and proximal portions of basilar and apical dendrites. Densely-stained cells were located in cortical layer V and in undifferentiated cortical plate above layer V. Some of the older (i.e., deep) neurons contained dark dots of GM2-LIR; in the electron microscope, the dots looked like lysosomes, suggesting that these cells were degrading excess GM2 ganglioside. At 3 and 4 weeks of age, only younger (i.e., superficial) cells contained GM2-LIR. By 6 weeks of age, when pyramidal neurons were morphologically mature, reactivity was no longer evident. Studies of developing rat cortex produced similar results; viz., neurons contained GM2-LIR from postnatal day 5 through day 21, once again coinciding with normal dendritogenesis. Thus, GM2 ganglioside appears to regulate dendritic growth on pyramidal neurons during normal development as well as on mature pyramidal neurons in a disease state. (NS18804 and NS07098)

20.17

A DEVELOPMENTAL GOLGI STUDY OF PURKINJE CELL DENDRITIC MORPHOLOGY IN LURCHER CHIMERIC MICE. J. M. Soha and K. Herrup. E.K. Shriver Center, Waltham, MA 02254.

Previous studies have shown that wild type cerebellar Purkinje cells (PCs) in mature lurcher mouse chimeras (+/Lc↔+/+) exhibit aberrant and atrophic morphologies that may reflect developmental deafferentation and an altered trophic environment. The present study analyzes PC morphology in lurcher chimeras and wild type chimeras (+/+↔+/+) at postnatal day 20 to determine if the aberrant phenotype arises through regression after normal growth, or instead via a failure in development.

Mutant (+/Lc) PCs that remained in P20 lurcher chimeras were easily recognized by their immature morphology (typical of P7), indicating that the stunted morphological phenotype of lurcher PCs is a cell intrinsic effect of the lurcher gene. Several features of wild type PC dendrites, including height, width, and areal extent, were measured in sagittal sections of Golgi-Cox material. At P20, preliminary analysis suggests that wild type PCs in lurcher chimeras and wild type chimeras are similar with respect to these measures. Comparison to the same features in adult chimeras suggests the phenomenon is regressive; e.g., dendritic extent (area in the sagittal plane) in both lurcher and wild type chimeras at P20 closely resembled average extent in adult wild type chimeras, but was roughly twice that of adult lurcher chimeras. The same comparison also suggests that the phenotype is not widely expressed at P20. Supporting this conclusion, 1 µm plastic sections of a P20 lurcher chimera stained with toluidine blue do not exhibit the distinctive abnormal staining of primary dendrites previously reported in adult lurcher chimeras. Supported by NIH grants NS18381 and NS20591 (KH), NS08896 (JS), and by March of Dimes 1-1175 (KH).

20.18

EARLY DENDRITE DEVELOPMENT DESCRIBED BY FRACTAL DIMENSION. E.A. Neale, L.M. Bowers*, and T. J. Smith, Jr. Lab. Develop. Neurobiol., NICHD and Lab. Neurophysiol., NINDS, NIH, Bethesda, MD 20892

The early development of neurons in cell culture was studied using fractal dimension (D) as a quantitative descriptor of the morphologic complexity of cellular borders. Dissociated fetal murine spinal cord cells, plated at low density onto a glial substrate, were stained using tetanus toxin Fragment C immunohistochemistry at intervals from shortly after plating until one week in vitro. Fluorescent images were captured and processed to obtain estimates of the value of D for individual neurons (Smith et al., J. Neurosci. Meth. 27: 173, 1989). D was consistent with perceived morphologic complexity and, after one week in culture, ranged from 1.28 to 1.46. The sample of neurons was subdivided into four groups on the basis of the number of primary processes emerging from the soma. Differences were significant among the groups in the plateau value and time-course of development of D. Neurons with 3 or 4 primary processes were analyzed additionally, over 7 days in vitro, for total dendrite length, area occupied by the arbor, number of interbranch segments, and number of intersections with a superimposed pattern of concentric circles. The mean value of D increased from 1.0, the value for a circle, to 1.34. Half of this increase occurred during the first 9 hr in culture. Increases in the other measures occurred over a different time-course, with half-maximum values attained after 4 days. The early rise in D occurred before the formation of interneuronal contacts, suggesting intrinsic control. The observation that growth of the dendrite arbor, during the first week in culture, was associated with a continued increase in D indicates that, over this interval, the arbor does not exhibit "fractal growth"; i.e., increase in size with constant D. Fractal analysis provides an objective quantitative measure of branching complexity for studies of the effects of extrinsic developmental factors, including physiologic activity, on dendrite morphology.

20.19

DEVELOPMENT OF COMMISSURAL NEURONS IN MAMMALIAN SPINAL CORD. I. Silos-Santiago and W.D. Snider. Department of Neurology, Washington University, School of Medicine, St. Louis, MO 63110.

Little is known about the development of interneurons in the mamalian spinal cord. We have studied the migration and dendritic arborization of spinal neurons with an axon in the ventral commissure in embryonic rats. Crystals of the lipid soluble tracer, DiI, were placed in various locations in the thoracic the spinal cord in order to label commissural neurons within the intermediate zone and ventral horn at E13.5, E15, E17, and E19.

DiI revealed the morphology of these neurons with the detail of intracellular staining. At E13.5 we observed seven groups of neurons sending their axons to the ventral commissure. These groups appear to have originated in different places along the neuroepithelium adjacent to their position within the gray matter. By E15, a surprisingly early stage, commissural cells are near their final locations and exhibit characteristic morphologies. On the basis of dendritic arborization and position we can identify at least 15 different groups. Groups in the most dorsal location within the intermediate zone are widely separated and have the most characteristic morphologies with dendrites primarily orientated in the dorso-ventral axis. In more ventral regions cells with oblique, radial or horizontal dendritic trees are intermingled. Near the midline two types of cells are present, one with short radially distributed dendrites, and another with a long straight dendritic stem projecting toward the dorsal horn. How the differing dendritic arbors of commissural neurons relate to their target projection and the innervation they receive is currently under investigation.

We conclude that, in mammals, a large number of neuronal classes send an axon to the ventral commissure. Some of these can be classified on the basis of dendritic morphology and location. Characterization of the morphology of intrinsic spinal neurons is the first step in understanding how the complex circuitry of the spinal cord is generated.

CELL SHAPE AND DIFFERENTIATION II

21.1

DELIVERY OF DOCOSAHEXAENOIC ACID (22:6) TO MEMBRANE PHOSPHOLIPIDS DURING SYNAPTOGENESIS. R.E. Martin and N.G. Bazan. LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.

***In vivo* experiments were designed to investigate 22:6 delivery to the central nervous system and to follow its incorporation into growth cones and synaptosomal membranes. Instead of using [^{14}C]18:3 (*PNAS* 86:2903;1989), in this study mice were injected with [^{3}H]22:6 at 5 days postnatal and were later killed to follow the metabolism of 22:6 and its trafficking in the plasma from liver to brain and retina. Free 22:6 was rapidly esterified into phospholipids, triacylglycerols (TG), and cholesterol esters for transport in the plasma. Peak labeling of liver occurred 2 h after injection and decreased biphasically over 72 h. Enrichment of label in plasma was highest 2 h post-injection and decreased steadily over the next 70 h. Uptake of [^{3}H]22-6 in retina and brain occurred later than incorporation in liver and increased with time. Label acquired by retina and brain paralleled loss of label from liver. When synaptosomes and less mature nerve endings (growth cones) were assayed for [^{3}H]22-6 content 5 days after injection, growth cones were more enriched with [^{3}H]22-6 than synaptosomes or the crude homogenate. The distribution of [^{3}H]22-6 in growth cone and synaptosomes was similar. Phospholipids were most heavily labeled, followed by TG and free fatty acid. The data indicate that during development, 22:6 is transported from the liver in the plasma to the retina and brain as a fatty acid esterified in phospholipids, cholesterol ester, and TG. Upon arrival to the brain, [^{3}H]22:6 is preferentially transferred to growth cones rather than to more mature synaptic endings. Supported by NEI EY04428.**

21.2

HISTOCHEMICAL INCREASE OF CHICK CREATINE KINASE ACTIVITY DURING MATURATION OF INTERNEURONAL CONNECTIONS IN VITRO. O. Ramírez, V. Alemán, E. Jiménez* and B. Osorio*. Departamentos de Bioquímica y de Fisiología, Biofísica & Neurociencias, Centro de Investigación y de Estudios Avanzados del I.P.N. México, D. F. 07000.

Formation of synapses have high energy requirements not completely covered by anaerobic and aerobic glycolisis. ATP must be produced by transphosphorylation. To study neuronal maturation, chick embryo brain neurons from stage 30 were cultured for 11 days on polylysine coated dishes. At 2 day intervals, representative samples were utilized for phase contrast observation, electron microscopy, and histochemical formazan-localization of creatine kinase (CK). An increase in the number of long processes between nerve cell clusters was followed by a 123% increase of cytosolic BB-CK specific activity from the 5th to the 7th day of culture. The intensity of catalytically active CK staining paraleled the cytosolic BB-CK specific activity increase in developing processes and nerve cells clusters. Formazan granules along neurites and in clusters clearly appeared from 5 day. Coinciding with the CK burst, synapses ($\geq$ 200 nm long) showed, in the electron micrographs, a progressive increase in complexity as they matured from the fifth day. These findings support the view that, as a molecular marker, CK activity can be utilized during the establishment of interneuronal connections and synapse maturation.

21.3

PC12 CELL DIFFERENTIATION IS ALTERED BY ADENOVIRUS-5 E1A PRODUCTS. Kim E. Boulukos* and Edward B. Ziff. Howard Hughes Medical Institute, Dept of Biochemistry, NYU Medical Center, N.Y., N.Y., 10016.

The rat pheochromocytoma-derived PC12 cell line is a useful system for studying growth arrest and neuronal differentiation in the presence of neurotrophic factors such as nerve growth factor (NGF) or fibroblast growth factor (FGF). We have used viral transforming proteins to disrupt neuronal differentiation of PC12 cells to understand how regulation of PC12 cell growth contributes to neuronal differentiation. Since the human adenovirus E1a proteins are capable of altering cell growth properties, genomic E1a sequences were stably introduced into PC12 cells by electroporation and neomycin selection. The E1a transformed PC12 cells (PC-E1a cells) are flattened, irregular in shape and divide rapidly compared to parental PC12 cells. Gene expression is also modified such that mRNAs encoding the intermediate filament protein peripherin as well as other neuronal markers abundant in non-treated or NGF-treated PC12 cells are not expressed in PC-E1a cells. Effects of NGF and FGF treatment of PC-E1a cells were investigated by measuring DNA synthesis, morphological differences and changes in gene expression. We find that PC-E1a cells fail to respond to both NGF and FGF. Stimulation of parental PC12 cells with these ligands via their respective receptors transduces signals which eventually result in PC12 cells exiting the cell cycle and extending neurites. It appears that E1a products disrupt the expression of the receptor for NGF and possibly FGF, thereby abolishing the potential for signal transduction at the beginning of the differentiation pathway.

Since PC-E1a cells proliferate rapidly, we next investigated whether epidermal growth factor (EGF), which induces a proliferative response in PC12 cells, could still elicit such a response in PC-E1a cells. Thymidine labelling experiments indicate that EGF does not augment the proliferation of PC-E1a cells suggesting that some other mechanism(s) is responsible for inducing growth. Since the effects of E1a proteins are pleiotropic, we are currently investigating how E1a products alter gene expression and what role these genes play in growth arrest and neuronal outgrowth.

21.4

REGULATION OF PROTEIN KINASE C IN HUMAN AND MURINE NEUROBLASTOMA CELL DIFFERENTIATION. F. Battaini, S. Bergamaschi*, S. Govoni*#, L. M. Vicentini^, M. Parenti^ and M. Trabucchi*. Dept. Exptl. Med. Bioch. Sci. IInd Univ. Rome, ^Dept. Med. Pharmacol. Toxicol. and Chemother. Univ. Milan, #Pharmacobiol. Dept., Univ. Bari, Italy.

The induction of neurite outgrowth in neuroblastoma cells is a useful model for the analysis of the neurochemical parameters related to neuronal differentiation. Protein kinase C (PKC) is one of the key enzymes involved in cell growth and differentiation; its inhibition, in fact, induces neuroblastoma cells to differentiate. In this study we have investigated PKC in human (SK-N-BE) and murine (NG108-15) neuroblastoma cells. In both cell lines the differentiation is associated with neurite formation and with a decrease in PKC. In SK-N-BE differentiated (D) cells (Retinoic acid 2 μM for 10 days) there is almost a 40% decrease in 3H-Phorbol 12,13 dibutyrate total binding. In NG108-15 D cells (dibutyryl cAMP 1 mM for 4 days) phorbol ester binding is 50% of that in non D cells. The analysis of PKC function (histone-directed) indicates that the differentiation elicits a decrement in soluble (sol) and membrane-bound (mem) activity in both SK-N-BE (sol -41%,. mem -22%) and in NG108-15 cells (sol -50%, mem -62%). The phosphorylating activity in both cell lines appears to be due to the α isoform of PKC. In fact Northern blot analysis reveals the presence of α, but not β or γ isozymes mRNA. The data indicate that PKC is modified when processes controlling neurite (SK-N-BE) and synapse formation (NG108-15 cells) are activated.

21.5

INDUCTION OF RAPID DIFFERENTIATION IN IMMORTALIZED RAT SEPTAL CELL LINES. E.M.Eves[1], J. Kwon*[1], and B.H.Wainer[1,2,3]. Depts. [1]Pharm. & Phys. Sci., [2]Path. and [3]Comm. on Neurobiol., The University of Chicago, Chicago, IL 60637.

Temperature sensitive alleles of the SV40 large T antigen were employed to immortalize rat embryonic (E14-15) septal cells. The resultant cell lines proliferate at 33° and cease or greatly slow proliferation at 39°. Seven of thirteen lines tested demonstrated a dramatic response to a cocktail of differentiation agents composed of 0.5mM 1-isobutyl-3-methyl xanthine, 0.5mM dibutyryl cAMP, and 25ng/ml NGF (XAN) (Ronnett et al., 1990, Science 248, 603). The undifferentiated cells had a flat, epithelioid appearance. Within one hour following the addition of XAN the cells became refractile and had begun to produce long, multibranched processes. This morphological differentiation reached a maximum at approximately 4 hours. Neurofilament protein was detectable by immunocytochemistry in the differentiated cells; glial fibrillary acidic protein was not detectable. The effect of XAN on proliferating cells (33°, 10% FCS) was transient with many of the cells regaining epithelioid morphology within 24 hours. In non-proliferating or slowly proliferating cells (39°, 1% FCS) XAN treatment was lethal for the majority of the cells. (Supported by NS 25787 and The Alzheimer's Disease and Related Diseases Assn.)

21.6

INTERACTION OF ACIDIC FIBROBLAST GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR BETA IN FETAL RAT ASTROCYTES *IN VITRO*. T.C. Ryken*, R.K. Osenbach, V.C. Traynelis* and R. Lim. Divisions of Neurosurgery and Neurochemistry, University of Iowa College of Medicine, Iowa City, Iowa 52242.

The mitogenic and morphologic effects of acidic fibroblast growth factor (aFGF) and transforming growth factor beta (TGF-β) were assayed on fetal rat astrocytes in the presence and absence of serum using growth curves, phase contrast, and scanning electron microscopy. Astrocytes incubated with aFGF underwent a characteristic morphological change involving extensive process formation and rounding of cell bodies, accompanied by an increase in mitotic activity. Astrocytes incubated with TGF-β underwent a slight decrease in mitotic activity and remained morphologically unchanged. The cells incubated in a combination of aFGF and TGF-β demonstrated an attenuation of both the mitogenic and morphological changes observed in the presence of aFGF alone. These results suggest that in normal fetal rat astrocytes, TGF-β is capable of attenuating the mitogenic and morphological changes induced by aFGF *in vitro* and may have a regulatory role in astrocyte growth and differentiation.

21.7

MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL DE-DIFFERENTIATION OF N1E-115 CELLS BY SERUM BORNE FACTOR(S). L.M.Ireland*, C.Marr*, R.H.Rech, & P.Cobbett. Dept. Pharmacol./Toxicol. & Neuroscience Program, Michigan State University, East Lansing, MI 48824-1317.

Murine neuroblastoma derived N1E-115 cells may be morphologically and electrophysiologically differentiated by various stimuli including removal of serum from the culture medium (Cobbett et al., Soc.Neurosci.Abstr. 16, 990, 1990). We have examined whether this differentiated state is maintained when the stimulus for differentiation is removed. N1E-115 cells were first maintained in serum free medium (SFM) for 12 days: cells were process bearing and generated large amplitude, short duration, Na^+-dependent action potentials. SFM was then replaced with medium containing 10% new born calf serum: cells rapidly altered morphologically and electrophysiologically. Process withdrawal occurred with a variable latency but was rapid once initiated: after 24 hours, mean process length was not changed but the occurrence of process bearing cells decreased significantly (from 100.4±0.4% to 37.0±6.6%). Within 48 hours, <20% of cells were process bearing. Morphologically de-differentiated cells had significantly decreased resting membrane potentials, and action potential amplitudes but had increased input resistances and action potential durations. The data clearly show that differentiation of N1E-115 cells may easily be reversed by as yet unknown factor(s) in the serum. Morphological and electrophysiological de-differentiation are closely associated temporally suggesting the possible dependence of one on the other. (Supported by the Pharmaceutical Manufacturers Association Foundation.)

21.8

NEURONAL CELLS DERIVED FROM THE CELL LINE N-TERA 2 EXHIBIT A CHOLINERGIC PHENOTYPE S.R.Kleppner, S.J.Pleasure, V.M.-Y. Lee*. Inst. of Neurosci., Dept. of Path., Univ. of PA Sch. of Med., Phila., PA 19104.

We have previously developed a culture system which yields nearly pure post-mitotic neuronal cultures from N-Tera 2 (NT2) cells treated with retinoic acid (RA). In this study, we sought to determine the neurotransmitter phenotype of these cells. Our initial work focussed on cholinergic properties as measured by choline acetyl transferase (ChAT), an enzyme responsible for acetyl choline production. ChAT levels were assayed using a modification of the radiochemical method described by Fonnum (J. Neurochem. 24: 1975). Measurements of ChAT using this method established that neuronal cells derived from the NT2 cell line with RA treatment exhibited cholinergic activity. Antibodies directed against ChAT confirmed its presence in these pure cultures. We also characterized the onset and time course of cholinergic activity following exposure of the cells to RA as well as the effects of various concentrations of RA on ChAT activity. ChAT first appeared at low levels in mixed cultures one week after initial exposure to RA, and activity increased dramatically over the following two weeks. To determine whether various concentrations of RA yielded cells committed to different phenotypes, pure neuronal cultures induced with RA concentrations between 10^{-5} and 10^{-8} M were examined. These experiments yielded pure cultures with cells exhibiting ChAT activity. Finally, we attempted to correlate the onset of ChAT activity to other cellular events occurring during the first three weeks following RA treatment. Preliminary results indicate that ChAT activity accompanies the terminal commitment to a neuronal phenotype. Thus, RA treatment of NT2 cells leads to a cholinergic phenotype of resulting neuronal cells. Expression of this activity follows a specific time course which seems to match the appearance of other neuronal markers. We are now working to define more precisely the relative levels and timing of ChAT expression in these cells.

21.9

IN VITRO CYTOSKELETAL DIFFERENTIATION OF NEURONAL CELLS INDUCED BY RETINOIC ACID IN NTERA 2 CELLS. S.J. Pleasure and V.M.-Y. Lee*. Dept. of Path., Univ. of PA Sch. of Med., Phila., PA 19104.

NTera 2 (NT2) cells are a human teratocarcinoma cell line which differentiates into neuronal cells in response to retinoic acid (RA). We have shown previously that it is possible to isolate large numbers of neuronal cells (NT2-N cells) in pure cultures from RA treated cells. We have now examined the changes in cytoskeletal phenotype from undifferentiated stem cells to nearly pure neuronal cultures. Before treatment, NT2 cells are flat, epithelioid cells that express nestin, keratins 8 and 18, vimentin and small amounts of NF-L, NF-M and NF66. P+++ NF-M begins to appear in clusters 3 days following the first RA treatment and by 1 week there is extensive expression of P+++ NF-M. At 2 weeks, the cultures contain two distinct populations of cells; the first resembles the original stem cells and the second elaborates rudimentary processes and no longer expresses nestin or keratins. The latter also express P+++ NF-M, MAP2 and tau. By 3 weeks, the second group of cells have extended long processes. Following purification of the NT2-N cells there is a period of extensive neurite outgrowth during which MAP2 and P+++ NF-M are ubiquitously expressed throughout the cell. At 3-4 weeks, as the neuritic network stratifies, segregation of these antigens begins to appear. By 5 weeks, the NT2-N cells have established a more differentiated neuronal phenotype with readily recognizable dendrites and axons. The dendritic processes and cell bodies of these cells contain MAP2 and P- NF-M while the axonal processes have P+++ NF-M.

In summary, RA induces a complete conversion of NT2 cells from a cell line with neuroepithelial stem cell characteristics into post-mitotic neurons that eventually develop the polarized morphology of neurons. Thus, NT2 cells are an excellent system for studying both the regulation of polarity in neurons and factors controlling the commitment of neuroepithelial stem cells to neurogenesis.

21.10

INDUCTION OF PROTEIN KINASE C-β AND PHOSPHORYLATED NEUROFILAMENT-IMMUNOREACTIVITY IN CULTURED ADRENAL CHROMAFFIN CELLS AND PINEALOCYTES. R. Roivainen and J. Koistinaho. Department of Public Health, University of Tampere, P.O. Box 607, SF-33101 Tampere, Finland.

Adrenal chromaffin (AC) cells of the newborn rat and human fetuses were cultured with and without nerve growth factor (NGF) for 3-7 days. Pinealocytes of the newborn rat were cultured without NGF for 14 days. The cultures were immunostained with antibodies to phosphorylated M- and H-subunits of neurofilaments (PNF) and protein kinase C-β (PKC-β). Noncultured human and rat AC cells as wells as AC cells maintained in culture without NGF showed no immunoreactivity, whereas strong PKC-β and PNF-staining was seen when the cells were supported with NGF for 3-7 days. Likewise intact rat pinealocytes contained no PKC-β or PNF-LI, but when they were cultured for two weeks a proportion of them started to express PKC-β and PNF-immunoreactivity. Both in the pinealocyte and AC cell cultures the immunopositive cells had developed neuron-like characteristics and they grew out processes of varying length. The results indicate that PKC-β may contribute to the phosphorylation of neurofilaments, which is induced in certain endocrine cells cultured in conditions favouring the transformation to a neuronal phenotype.

21.11

PHENYLETHANOLAMINE N-METHYLTRANSFERASE-EXPRESSING ADRENAL CHROMAFFIN CELL LINES DERIVED FROM PNMT-SV40 TRANSGENIC MICE. A. Messing, E.E. Baetge and J.P. Hammang. School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706; Bristol-Myers Squibb Co., Wallingford, CT 06402.

Clonal lines of phenylethanolamine N-methyltransferase (PNMT)-expressing (adrenergic) chromaffin cells have been derived from adrenal medullary tumors in PNMT-SV40 transgenic mice. When cultured in the presence of glucocorticoid, two immortalized lines (designated AT-1 and AT-2) express the nuclear SV40 large T antigen (Tag), maintain a characteristic chromaffin cell morphology, are catecholaminergic, and continue to divide. The AT cell lines are unique in that the cells express both TH (tyrosine hydroxylase) and PNMT protein and PNMT mRNA, and consequently produce both norepinephrine and epinephrine. When the AT cell lines are grown in the absence of glucocorticoid, Tag levels fall and most of the cells slow or stop dividing. After 1-2 weeks in the absence of glucocorticoid a number of the cells then increase their expression of the neurofilament proteins and extend neurite processes. Unlike PC12 cells, the AT cells do not express cytokeratin, the epithelial cell-specific intermediate filament. These AT cell lines represent an improved system for studying the biochemistry and development of the PNMT-expressing adrenal chromaffin cell.

21.12

IN VITRO DEVELOPMENT OF TYROSINE HYDROXYLASE-IMMUNOREACTIVE NEURONS FROM THE EMBRYONIC CHICK HYPOTHALAMUS. A.A. Romero*, J.K. Lobner*, A.M. Gabaldon*, R.L. Lopez* and J.A. Wallace. Dept. of Anatomy, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

Unlike dopaminergic (DA) neurons in the mammalian hypothalamus, the majority of tyrosine hydroxylase-immunoreactive (TH+) neurons in the chick diencephalon do not synthesize dopamine (Romero et al., SON abs 16:646, '90). Here we have examined whether chick hypothalamic TH+ neurons develop similarly *in vitro* as they do *in vivo* with respect to the expression of the DA phenotype. Dissociated cell cultures of the diencephalon were developed from embryos at ages 5-7 or 10-12 days of incubation, times at which the TH+ cell groups first appear embryologically or are already well established. From either embryonic age, the number of TH+ neurons observed in the cultures was less than that anticipated from the incidence of these cells *in vivo*. Nonetheless, as in the intact animal, only a small proportion of the hypothalamic TH+ neurons appear to synthesize DA, detected immunocytochemically. This contrasts markedly with the large ratio of DA to TH-immunoreactive cells found in sister cultures of the chick midbrain. Therefore, the characteristics expressed in culture by developing hypothalamic TH+ neurons is consistent with that observed *in vivo* by these diencephalic cells in the chick embryo. Supported by NIH grants GM08222 and RR08139.

21.13

CEREBELLAR GRANULE NEURONS PROMOTE PURKINJE CELL SURVIVAL AND DIFFERENTIATION *IN VITRO*. C.A. Baptista, D.H. Baird, W.-Q. Gao, M.E. Hatten and C.A. Mason, Dept. Pathology, Columbia Univ., Coll. Physicians and Surgeons, New York, N.Y. 10032.

To analyze afferent-target cell interactions *in vitro*, we have been co-culturing purified target cells with specific afferents, taken from the developing rodent cerebellum. We have improved our purification method for Purkinje cells (Baptista et al., Soc. Neurosci.16:1150,1990) to obtain more than 80% purity, by a combination of density gradients and immunoadsorption (panning). At this level of purity, Purkinje cells plated alone did not survive for more than 3 days. When plated on monolayers of purified cerebellar astroglia, Purkinje cells survived for longer periods but their morphological differentiation was impaired.

To test the influence of one of the principal Purkinje cell afferents, the parallel fibers, on Purkinje cell survival and morphogenesis, highly purified Purkinje cells were co-cultured with small reaggregates of purified granule cells that extended numerous neurites. Purkinje cells contacting these neurites survived, extended dendrites, and developed profuse spines by 2-3 weeks *in vitro*. Moreover, the extent of dendritic development appeared to depend on the density of granule cell axons contacting Purkinje cells. Thus, interactions with one appropriate set of afferents are necessary for the long term survival and differentiation of characteristic morphology by Purkinje cells. We will next determine the effect of contact with the other Purkinje cell afferents, the climbing fibers, as well as inappropriate afferents, on Purkinje cell survival and differentiation. These experiments suggest that specific afferent connections promote CNS target cell survival and differentiation.

21.14

PATTERNS OF ANTIGEN EXPRESSION IN THE DEVELOPING RAT THALAMIC RETICULAR NUCLEUS. J. Mitrofanis* and R.W. Guillery. Department of Human Anatomy, South Parks Road, University of Oxford, OX1 3QX, UK.

The thalamic reticular nucleus (TRN) is a sheet of cells covering most of the dorsal thalamus (dT). Its major afferents are collaterals from passing corticothalamic and thalamocortical fibres and the majority of its efferents go to the dT. We have examined the postnatal development of the TRN in rats with antibodies to gamma-aminobutyric acid (GABA), parvalbumen (PV) and pro-alpha-thryrotropin-releasing hormone (TRH). Each of these antibodies labels most, if not all TRN cells in adults. PV- and TRH-immunoreactive (IR) cells during early development (P0 to P10) were apparent along the lateral border and rostral pole of the TRN only. Labelled cells extended lateral to the TRN into the internal capsule (IC) and into the globus pallidus (GP) and entopeduncular nucleus (EPN). Later in development (P10 to P24), some PV-IR cells were still in the GP and EPN but all the PV- and TRH-IR cells in the IC and rostral TRN had disappeared. The fate of these cells is unclear: they could either have been migratory, died, or transiently expressed these antigens. During this period (P10 to P24), a second wave, or pattern of PV- and TRH-IR expression was apparent: labelled cells emerged first in central TRN and with increasing maturity, labelled cells appeared in caudal and then in rostral TRN. GABA-immunoreactivity in the developing TRN was similar to the second pattern of PV- and TRH-immunoreactivity, but it began much earlier (P0). For example. GABA-IR cells appeared first in central TRN and then subsequently in caudal and rostral TRN. Thus, different immunocytochemical markers of TRN cells show distinct patterns of development. It remains to be determined how these patterns of antigen expression relate to, firstly, patterns of TRN cell birth and secondly, to the maturation of the connections of different sensory modalities related to distinct TRN sectors.

21.15

THE NUMBER AND BIRTHDATE OF NEUROPEPTIDE NEURONS IN DEVELOPING CHICK GUT. Miles L. Epstein and Kristian T. Poulsen*. Dept of Anatomy, University of Wisconsin, Madison, WI 53706.

A diverse array of neurotransmitters is found within enteric ganglia. We have investigated the number, coexistence, and birthdate of myenteric neurons expressing vasoactive intestinal peptide (VIP), somatostatin (SOM) and substance P (SP) in the developing proventriculus of the chick gut. The number of neurons expressing a particular peptide was counted in immunostained whole mounts obtained from E4-E21 embryos. Coexistence was examined by double labeling techniques. Birthdate was determined by applying ^{3}H-thymidine to eggs and preparing sections of immunostained gut for radioautography. We found that VIP appeared first at E5.5, SOM at E6.5, and SP at E10.5. At hatching the largest number of neurons contained VIP and about one-half that number each expressed SOM and SP. After double-staining we found a large percentage of cells showed the coexistence of VIP and SOM at E8.5, but a very small percentage at E15.5 and E21. No coexistence was found for VIP and SOM or for SP and SOM. The majority of the myenteric neurons were born between E4 and E11, although a small number were born even at E16.5. Our results suggest that decisions about transmitter phenotype occur concurrently in different cells. Supported by NSF BNS 8820658.

21.16

BRAIN EXPANSION IN THE CHICK EMBRYO REQUIRES NA+/K+-ATPASE PUMPS FOR FLUID TRANSPORT. X.Y. Li* and M. E. Desmond. Department of Biology, Villanova University, Villanova, PA. 19085.

The brain region of the embryonic central nervous system expands markedly once the tube is sealed at both ends as a result of closure of the rostral neuropore and occlusion of the spinal cord. This growth is rapid and directly controlled by intra-luminal pressure (ILP) generated by neural tube fluid. How does the fluid cross the neuroepithelium into the brain cavity during this period of rapid enlargement? This study reports on experiments designed to test the hypothesis that fluid transport via the Na+/K+- ATPase pump is essential for normal brain expansion. Over 25 dozen chick embryos (stages 11-25) were treated with ouabain, a specific inhibitor of the Na+/K+-ATPase pump, at various concentrations (5-1000 µM) for 24 hrs while in shell-less culture at 38° C . Embryos were critically staged, photographed atop the yolk and fixed in Carnoy's for serial sectioning and volume measurements. Preliminary results show that stage 18 embryos treated 24 hrs with 50 µM ouabain exhibited extensive folding of the neuroepithelium similar to previously reported studies in which the ILP was decreased, thus suggesting a significant decrease in the cavity size after ouabain treatment. Volume measurements are in progress to determine the actual amount of reduction. The younger embryos (stage 11-14) were less sensitive to ouabain than the older embryos (14-25) suggesting that either Na+/K+-ATPase pumps were not present in the younger embryos or, if present, were inactive. Autoradiography is being done in order to confirm the first appearance of the pumps. Supported by NIH RO1 HD24710-01A2.

21.17

CLONED CEREBRAL ENDOTHELIAL CELLS: AN *IN VITRO* MODEL FOR BRAIN VASCULARIZATION?

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We have recently shown that cloned cerebral endothelial cells (cEC) of porcine and murine origin differentiated reversibly into two distinct phenotypes depending on the presence of endothelial cell growth factor and heparin in the culture medium. Factor enriched medium produced cobblestone like cEC (type I) which responded to glial and neuronal stimmulation of blood brain barrier (bbb) related enzyme activities (Tontsch U. and Bauer H.C., 1991, Brain Res. 539, 247). Depletion of factors resulted in a phenotypic switch producing spindle shaped cells (type II) which did not respond to neural membrane stimulation. This phenotype expressed different proteins, such as smooth muscle α-actin as shown by immunofluorescence, Immunoblots and Northernblots. In addition, the cells showed enhanced migratory behaviour and different protease activities depending on the cell substrate. The data support the hypohesis that type II cEC are involved in neovascularization and that type I cEC are related to bbb function.

AXON GUIDANCE MECHANISMS AND PATHWAYS I

22.1

THE RELATIONSHIP OF OPTIC GROWTH CONES TO THE TRACT OF THE POSTOPTIC COMMISSURE IN THE DEVELOPING ZEBRAFISH BRAIN. J. D. Burrill and S. S. Easter, Jr., Department of Biology, University of Michigan, Ann Arbor MI, 48109.

Previous work has suggested that the pial basal lamina and/or the axons in the tract of the postoptic commissure (TPOC) may provide local guidance cuesfor optic axons to reach the tectum. We have examined what strucures are contacted by optic growth cones en route to the tectum.

The optic axons of fixed embryos (34-48h) were labelled by intraocular injection of DiI, and then photoconverted. The embryos were then embedded and sectioned for electron microscopy.

The first retinal ganglion cell axons leave the eye between 32-34h, and reach the tectum about 46-48. In most cases 1-3 axons had advanced farther up the diencephalon then a larger group of axons (5-20). The leading optic growth cones are separated from the basal lamina 1-3 um by neuroepithelial endfeet. The leading optic growth cones do not extensively contact TPOC axons; in fact, only occasional filopodial contact with other axons was observed. At the midline of 33-36h embryos the optic axons are separated from the TPOC by neuroepithelial processes. These processes are smaller at 48h, causing the optic axons to appear adjacent to the TPOC axons.

Our work indicates that the neuroepithelial endfeet rather than either the basal lamina or other axons probably provide the local guidance cues for the optic axons. Supported by RO1-EY-00168 and T32-EY-007022.

22.2

THE MIDLINE OF THE MOUSE OPTIC CHIASM: CELLULAR COMPOSITION AND ANTIGEN EXPRESSION DURING RETINAL AXON GROWTH. C.A. Mason, R. Blazeski, J. Dodd, and P. Godement. Depts. of Pathology, and Physiology and Cellular Biophysics, Columbia Univ., Coll. of Physicians and Surgeons, New York, N.Y. 10032, and Inst. Neurosciences, CNRS, Univ. P. M. Curie, Paris 75005.

Our previous studies on retinal axon guidance suggest that a region spanning the midline of the optic chiasm inhibits the advance of uncrossed retinal fibers, while it is permissive for the growth of crossed fibers. The inhibitory zone on either side of the midline is occupied by a palisade of radial glia, recognized in the mouse by the MAb RC2 (Mason et al., Soc. Neurosci. 16:1125, 1990). The palisade is present from the time the earliest retinal axons arrive in the diencephalon at E13, and loses RC2 immunoreactivity in late fetal periods. The midline glial cells were further characterized by immunostaining for the presence of antigens expressed by the floor plate of the spinal cord, another midline structure important for axon guidance. Among the antigens expressed by both structures is SSEA I, a stage-specific embryonic antigen expressed on embryonic stem cells. This antigen is expressed on some fibers of the glial palisade, and at the midline seam of the chiasm. In addition, with the Pan-Macrophage monoclonal (Serotec), we have identified cells of the macrophage/monocyte lineage. These cells form a carpet of cells subpially, where recently arrived axons grow and turn. Immunopositive cells are also found in the subventricular zone of the third ventricle.

Characterization of the resident cells of the chiasm should reveal properties of the chiasm midline important for guidance of retinal axons, and will allow the cells used *in vitro* assays of retinal axon growth (Guillaume et al., this volume) to be identified unambiguously.

22.3

RETINAL AXON-OPTIC CHIASM CELLULAR INTERACTIONS *IN VITRO*. P. Guillaume*, L.-C. Wang, C.A. Mason and P. Godement, Inst. Neurosciences, CNRS, Univ. P. M. Curie, Paris 75005 and Dept. Pathology, Coll. Physicians and Surgeons, Columbia Univ., New York, N.Y., 10032.

Our previous studies suggest that the midline of the optic chiasm inhibits the advance of uncrossed retinal fibers, while it is permissive for the growth of crossed fibers. To investigate the cellular interactions that underly retinal axon guidance to both sides of the brain, we are developing an *in vitro* model system, in which retina is co-cultured with dissociated cells from the optic chiasm, both taken from mouse embryos at E15-16.

The majority of cells in these cultures are glial, showing both RC2 immunoreactivity, as does the midline glial palisade, and GFAP immunoreactivity. After 2-3 days in culture, RC2 immunoreactivity diminishes, and GFAP immunoreactivity is retained. In these cultures, other cells, in greater numbers in serum-free conditions, appear to be neurons, based on reactivity with antibodies against neurofilaments and alpha internexin, a new neuronal intermediate filament expressed early in the CNS (gift of Dr Liem, Columbia University). A striking cellular interaction is the formation of "islands" of such neuronal cells settled around "spokes" of RC-2 positive elongated processes, partially mimicking the glial palisade in vivo.

Retinal fibers from regions of retina giving rise to crossed fibers readily grow on optic chiasm cells as unfasciculated axons, suggesting that these cells are very supportive to their elongation. Fibers from inferior temporal retina grow as fascicles among optic chiasm cells, or stop next to the fields of chiasm cells. Some growth cones appear to turn back, in a fashion that is reminiscent of what is observed for uncrossed retinal fibers in vivo.

These observations suggest that divergence of crossed and uncrossed retinal fibers involves contact interactions with optic chiasm cells.

22.4

THE ORIGIN AND COURSE OF UNCROSSED RETINAL GANGLION CELL AXONS IN XENOPUS. Jeremy S.H. Taylor*. (SPON: Brain Research Association).
Department of Human Anatomy, University of Oxford, South Parks Road, Oxford. OX1 3QX. U.K.

To investigate the location of possible cues which guide ipsilaterally projecting axons, HRP was injected into the optic tracts post-metamorphic frogs. Labelled axons were then traced through the optic tract and nerve to their cells of origin in the ipsilateral retina.

The largest ipsilateral retinal ganglion cells form a mosaic array. They have somata approximately 20μm diameter, and dendritic arbors which are sparsely branched but extend over a wide area (radius 250μm). Smaller cells cannot be designated to any particular class.

In the optic nerve ipsilateral axons are intermixed with the contralateral axons from adjacent retinal regions as they pass through the zone of reorganisation[1] and become distributed around the periphery of the nerve. As the nerve nears the diencephalon the ipsilateral axons gather at the posterior nerve margin, with axons lying in the anterior periphery of the nerve abruptly traversing the width of the nerve. The turning of axons into the ipsilateral optic tract coincides with the alteration in glial environment at the nerve/tract junction[2]. The fibres turn directly into the optic tract as they enter the diencephalon and occupy the rostral-most edge of the tract. The decision to project ipsilaterally is made before the fibres reach the mid-line and encounter axons from the other eye suggesting that the ipsilateral decision is independent of the other eye.

In animals with embryonic monocular enucleation, the course and distribution of the ipsilateral projection in the post-metamorphic frog is shown to be unaffected.

It is concluded that the largest ipsilateral cells are of a specific sub-class. All ipsilateral axons have a predilection for the anterior edge of the ipsilateral optic tract. This behaviour is manifest as the fibres reach the cellular environment of the diencephalon. The ipsilateral projection is independent of inter-action with contralateral projection of the other eye.

Taylor, J.S.H. (1987). Development **99** 393.
Wilson, M.A. et al. (1988) Development **102** 537.

22.5

EVIDENCE OF A TRANSFORMATION OF AXONAL ORDER AT THE OPTIC NERVE HEAD IN THE DEVELOPING CHICK. Cheri V. Williams and S.C. McLoon, Department of Cell Biology, University of Minnesota, Minneapolis, MN 55455.

The retina projects to the tectum in a topographic manner such that neighboring retinal ganglion cells project to neighboring regions of the tectum. However, there is a mirror-reversal in this projection between retinal ganglion cells and their terminals in the tectum. This reversal requires a transformation of axon order at some point along the pathway. There is conflicting evidence as to where such a transformation might occur. Previous work with monoclonal antibodies that recognize axons from specific retinal regions suggested that there is a decussation of axons from the two sides of the retina at the optic nerve head. The present study analyzed the course of axons from various retinal regions through the optic nerve head. DiI was used to label groups of axons in the retina. Labeled axons were then followed from the retina into the optic nerve. Axons from dorsal retina entered directly into the optic nerve with no detectable crossing of axons. Many axons from both the nasal and temporal sides of the retina appeared to cross within the nerve head to the opposite side of the nerve. However, some axons from both sides entered the nerve without decussating. These results suggest that there is a partial decussation of axons in the optic nerve head of the chick. Other sites of axonal reorganization have been reported in the visual pathway. It seems likely that the mirror-reversal of axons between the retina and the visual centers is the result of several reorganizations along the pathway beginning at the optic nerve head.

22.6

OUTGROWTH OF TRANSPLANT-DERIVED RETINAL AXONS IN CEREBRAL CORTEX OF NEONATAL RATS. A.J. Sefton*, K. Rao, M.H. Hankin and R.D. Lund. Univ. of Pittsburgh, Pittsburgh, PA 15261 and *Univ. of Sydney, Sydney, NSW 2006, Australia.

Embryonic (E13) mouse retinae were transplanted to cortical locations in newborn (P1) rats to determine if optic axons could grow in regions of the brain not normally encountered during development. Transplant-derived retinal axon outgrowth was examined immunohistochemically using mouse-specific monoclonal antibodies, and peroxidase-coupled secondary antibodies.

Retinal transplants were located at various depths in the cortex, ranging from superficial (subpial) to deep (in cortical plate and/or white matter). Two projection patterns could be detected during the first week post-transplantation (PT): (1) locally along the edges of the implantation lesion created by the introduction of the grafted tissue with a glass pipette, and (2) for distances of several mm along cortical layer 1 (not associated with obvious surgical damage). Layer 1 projections were usually confined to one or two directions along the cortical margin (they did not extend radially as they might in a culture dish), although there was no preference outgrowth in any one particular direction or cortical area. Such projections were not seen after the first week PT, but they could be sustained for long periods if they contacted co-grafted target tissue, even if the co-graft was located several mm from the retina.

These results suggest that retinal axons can use outgrowth-promoting substrates present along the subpial margin of the developing cortex, and are consistent with the idea of target-derived maintenance. In addition, lesions in early postnatal animals appear not to represent an impediment to axonal outgrowth, and may, in fact, produce outgrowth-promoting substances.

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22.7

RETINOTOPIC REFINEMENT OF INDIVIDUAL AXONAL ARBORS DURING OPTIC NERVE REGENERATION IN GOLDFISH WITH AND WITHOUT OPTIC ACTIVITY. Z.R. Wang* and R.L. Meyer, Developmental Biology Center, Univ. California, Irvine CA 92717 and Biology Dept., Langzhou Univ., China.

The retinotopy of axon arbors was analyzed by making small injections of DiI into retina to label a small number of optic fibers. These fibers were visualized in tectal wholemounts using conventional and confocal fluorescence microscopy. In normals, small largely overlapping arbors were observed in the appropriate retinotopic position of tectum. Contrary to previous HRP studies, regenerating fibers at early times after nerve crush had few branches, and these were small and distributed near the distal segment. With time, fibers formed small arbors which initially had little overlap with each other but became progressively coextensive. Elimination of impulse activity by repeated intraocular injections of TTX did not prevent the formation of restricted terminal arbors but did prevent them from becoming overlapped.

The results suggest that the development of arborization is an activity independent process while retinotopic localization of the arbor requires activity. It is also noteworthy that arborization develops in a progressive fashion during regeneration like that reported for development even though the target cells are already differentiated.

Supported by NIH grant RO1-EY06746.

22.8

A SPECIFIC BRAIN TRACT GUIDES FOLLOWER GROWTH CONES IN TWO REGIONS OF THE ZEBRAFISH BRAIN. A.B. CHITNIS, C.K. PATEL, and J.Y. KUWADA, Neuroscience Prog., Dept. of Biology, University of Michigan, Ann Arbor, MI 48109.

Neurons of the nucPC, an identifiable cluster of neurons in the embryonic zebrafish brain, project growth cones ventrally along the posterior commissure (PC) to an intersection in the anterior tegmentum where the commissure crosses two longitudinal tracts, the TPOC and the MLF. Once at the intersection nucPC growth cones turn posteriorly onto the TPOC in the dorsolateral tegmentum and follow it to the hindbrain. Previously we showed that in the absence of the TPOC, nucPC growth cones often extend along aberrant paths suggesting that fasciculation with axons in the TPOC helps to guide the growth cones along their normal pathway. However, many nucPC growth cones also continue to extend along the dorsolateral tegmentum in the absence of the TPOC suggesting that cues associated with the dorsolateral tegmentum, independent of the TPOC, also help to guide the nucPC growth cones.

We have now confirmed using electron microscopy, that nucPC growth cones normally first fasciculate with axons in the PC and then with axons in the TPOC. In the absence of the TPOC the growth cones extend along the dorsolateral tegmentum in contact with the endfeet of dorsolateral neuroepithelial cells suggesting that cues associated with these cells guide the nucPC growth cones in the absence of the TPOC axons. We also show that in the absence of the TPOC, significantly more nucPC growth cones turn aberrantly at the midbrain/hindbrain border. This suggests that fasciculation with TPOC axons may also help the nucPC growth cones to cross this border in the brain. Supported by grants from N.I.H. and M.O.D.

22.9

Subplate cells in medial cortex send the first axons across the corpus callosum.

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Subplate (SP) cells extend the first axons out of cortex through the internal capsule (McConnell et al Science 245 '89; DeCarlos & O'Leary Soc NS Abs '90). Here, we report that in rats SP cells extend the first axons through another major cortical pathway, the corpus callosum (CC). Anterograde DiI labeling in fixed tissue shows that the first growth cones reach the midline of the nascent CC at E17 -- 1.5 days earlier than previously reported. By E18, many axons cross the midline and extend dorsolaterally in the intermediate zone (IZ). To determine the origin of these earliest crossing axons, we injected DiI into the medial IZ of fetuses fixed at E18. In the cortex contralateral to the injection, retrogradely labeled neurons are seen only in presumptive cingulate cortex, an area adjacent to the midline. Axons emerge from the labeled cells, descend into the IZ, turn abruptly, and can be followed into the nascent CC. Where the SP layer is distinct from the emerging cortical plate (CP), the labeled cells are clearly in the SP. Nearest the midline, where cortex is less differentiated, the labeled cells have morphologies characteristic of SP cells, and distinct from CP neurons, at this stage of development. In utero injections of Rhodamine beads label a similarly arrayed population at E18.5. Further evidence that these neurons are SP cells comes from birthdating them. ^{3}H-Thy was injected on E12 to label SP or on E15 to label CP; pups were fixed at E18, and DiI was injected into the IZ of medial cortex. Most DiI labeled cells in contralateral cortex are co-labeled with ^{3}H-Thy injected at E12, but are rarely co-labeled with E15 injections, as would be predicted for SP cells. Neurons in more dorsal and lateral parts of cortex can be first labeled from contralateral cortex on E19 and are found in both CP and SP. We conclude that in the rat a population of SP cells in medial (cingulate) cortex send the first axons across the corpus callosum. This population of SP cells is distinct from that which extends the first cortical axons through the internal capsule, which may indicate that these are specialized populations of cells with unique roles in cortical development. Support: NEI grant EY07025, NSF predoctoral fellowship.

22.10

MANIPULATION OF CEREBRAL CORTICAL PHENOTYPE IN TRANSPLANTS SPECIFIES THALAMIC AFFERENT PROJECTION PATTERNS.

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Previous studies using a molecular marker of limbic phenotype (limbic system associated membrane protein, LAMP), in combination with brain tissue transplants, have shown that there is an early critical period for the commitment of the fate of developing cortical neurons (Barbe and Levitt, J. Neurosci., 11: 519-533). The present study has taken advantage of the ability to manipulate cortical phenotype of the grafts to determine whether LAMP expression by the transplanted tissue correlates with the organization of limbic and nonlimbic thalamic afferent projections to the grafts. Slabs of presumptive perirhinal and sensorimotor cortex were obtained from fetal rats at 12 and 14 days of gestation, times before and after the committment to the limbic molecular phenotype, respectively, and were transplanted into perirhinal (PR) or sensorimotor (SM) cortex of newborn rats. Fixed brains from hosts were harvested 2 weeks after grafting and DiI-coated glass micropipettes were inserted into the transplant for retrograde labeling. Control homotopic transplants at both ages received appropriate SM or limbic thalamic projections. E12 heterotopic transplants of both presumptive limbic and SM cortex, in which the molecular phenotype is regulated by the host environment, receives thalamic afferents that reflect the grafts' new identities. Thus, LAMP-positive presumptive SM tissue placed in PR cortex of the host receives limbic projections from the thalamus, while LAMP-negative presumptive PR tissue placed in SM cortex of the host receives ventrobasal nuclear projections. E14 heterotopic transplants, in which the molecular phenotype is specified irrespective of the environment, receive thalamic afferents that correspond to the grafts' original fate. The data suggest that developing thalamic afferents respond to alteration of target phenotype by specifying their limbic and sensorimotor projections in correspondence to LAMP expression. (Supported by NIMH Grant MH45507).

22.11

HIPPOCAMPAL CA3 AFFINITY IS MAINTAINED BY NEONATAL GRANULE CELL MOSSY FIBERS IN VITRO.

A. Hussein*, S. Trikha*, N. Ortiz*, S. Springfield and P. Sajovic.

In the developing hippocampus, granule cells of the dentate gyrus project mossy fiber axons to the CA3 subfield of the hippocampus proper. Mossy fiber growth cones form synaptic contacts with the apical dendrites of pyramidal cells in this region. In the present study we sought to determine if neonatal granule cell projections maintain CA3 affinity in coculture with hippocampal explants varying in age.

Under sterile conditions the granule cells from P1-3 Sprague-Dawley rats were trypsinized and plated out at low density on poly-d-lysine treated coverslips in DMEM. Hippocampal explants obtained from: 1) adult, 2) 1 month old, and 3) P1-3 rats were cocultured with granule cells in a sandwich arrangement.

The addition of the explants significantly increased the degree of arborization and projection lengths of granule cells. Granule cells grown in the presence of explants exhibited mean projection lengths of 470 u whereas the control cells had mean projection lengths of 75 u. The viability of granule cells was increased in the presence of an explant. In all three types of cocultures CA3 affinity was maintained, however, granule cells cocultured with 1 month old rats demonstrated the greatest affinity for the CA3 region. Possible explanations for this affinity are that the pyramidal cells of the CA3 region may be involved in the secretion of a diffusible factor that may serve as a target for mossy fiber growth cones or that the cell surfaces of the granule cells may interact with surface molecules along the pathway to the target cells.

22.12

GUIDANCE IN CULTURE OF NEURITIC PROCESSES OF IDENTIFIED RAT BRAIN STEM MOTONEURONS.

J.P. Ternaux, P. Portalier*, P. Clark* and A. Curtis* - Unité de Neurocybernétique Cellulaire - UPR 418 CNRS, Marseille, France. Department of Cell Biology. University of Glasgow, Scotland.

Dissociated cells from the hypoglossal nucleus of 18 day old rat foetuses or 1 to 16 day old new born rats were cultured on poly-L-Lysine coated petri dishes for several weeks in a DMEM/F12 medium supplemented with additives and 3% FCS. Neurons, motoneurons and glial cells were identified using immunohistochemical staining with specific antibodies respectively raised against enolase, choline acetyl transferase and glial fibrillary acidic protein. Motoneurons were also identified by using retrograde transport of both fluorescent latex microspheres and carbocyanines (DiI and DiO) previously injected in the tongue. Cells were cultured on topographical microstrustures (glass and quartz) with engraved grooves of various width and deepth. Guidance of the neuritic tree was observed with grooves of 3 μm deep and wide, and was related to the size of cell bodies and to the angular position of the neurite outgrowth in function of the orientation of the groove. Microphotolithographic method combined with silanes chemical treatment allows to obtain controlled routes of adhesive proteins, such as laminine, on which dissociated cells can be cultured. In these conditions, we can arranged that the cells have 1, 2, 3 or 5 final neurites, 0,1 or 2 levels of bifurcation in each neurite and simple architecture of neuritic tree can be drawn. This method would be helpful to study the processing of information and its relationship with the shape of the dendritic tree.

22.13

ONTOGENESIS OF DESCENDING PATHWAYS FROM VARIOUS BRAINSTEM NUCLEI TO THE SPINAL CORD IN THE RAT.

F. Auclair*, M.-C. Bélanger and R. Marchand. Centre de recherche en neurobiologie, Hôpital de l'Enfant-Jésus, Québec, Qc, Canada, G1J 1Z4

In order to gain better understanding of the development of the descending pathways from the brainstem to the spinal cord in the rat, we implanted a crystal of DiI, a retrograde and anterograde plasma membrane fluorescent tracer, into the neural tube of young embryos. The tracer was allowed to diffuse in plasma membranes for a period of 2 to 4 months. After implantation in both the ventral and lateral funiculi in the cervical spinal cord of 14 and 15 day embryos, we encountered somas in the bulbar reticular formation which were retrogradely labeled by the marker. This group of neurons extended along the whole length of the ventral medulla oblongata. Their axons coursed into the ventral marginal zone at bulbar levels and entered the ventral funiculus when reaching the cervical spinal cord. In another set of experiments, we placed a crystal of DiI in the region of the interstitial nucleus of Cajal in embryos of 12,0 to 14,5 days (E12,0 to E14,5) to determine when the projections from that nucleus reached the cervical spinal cord. Fibers from that nucleus reached the rostral pons by E12,0 and arrived to the first segments of the cervical spinal cord by E13,5. As expected, these fibers coursed into the primordium of the medial longitudinal fasciculus. Finally, we placed a crystal of the same tracer into the vestibular complex of E14,5 and E19,0 embryos. Fibers from these nuclei in the E14,5 embryo could be followed as far as lower cervical segments while at E19,0 they reached at least the lower thoracic segments and in both cases, these fibers coursed into the ventral and ventrolateral funiculi. At E19,0, some fibers entered the ventral horn of the spinal cord at all levels mentioned above. These results among others suggest that the supraspinal control on spinal cord neurons is initiated early during the development of the rat.
Supported by MRC of Canada, FRSQ and FCAR.

22.14

COMMISURAL PROJECTIONS OF THE HOFMANN KÖHLICHER NUCLEI CELLS IN EMBRYONIC CHICKEN SPINAL CORD.

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The Hofmann-Köhlicher (HK) nuclei are segmentally iterated clusters of large cells found on the ventrolateral surface of the avian spinal cord at lumbosacral levels. Little is known about the anatomy and function of the cells in these nuclei.

The purpose of this study was to follow the embryonic development of the axonal projections of the HKcells. Chicken embryos were fixed at successive developmental stages and the lipophilic tracer DiI was applied in the HKnuclei for anterograde tracing and in the ventral funiculus or the ventral midline for retrograde tracing. The typical HKcell extends an axon that crosses the ventral midline in the segment of origin without branching on the ipsilateral side. The axon bifurcates in the contralateral ventral funiculus near the midline, one branch extends rostrally and the other extends caudally. By embryonic day (E)9 collaterals that extend towards the medial MNpool are established in the transverse plane. At E10 tertiary branching appears. By E16 the longitudinally running axons from the HKnucleus lying between lumbar segments 4 and 5 project 4 segments rostrally and 4 segments caudally, giving off collaterals all along their length. The HKcells at one segmental level may innervate the ventral horn of the entire lumbosacral extent of the spinal cord.

The HKnuclei may provide an accessible model system for studying the development of commissural axon projections in the spinal cord.

22.15

CYCLOPAMINE INDUCES A SPECIFIC PATTERN OF NEURAL DEFECTS IN EMBRYONIC ZEBRAFISH. Scott E. Stachel and Paul Z. Myers. Dept. of Human Genetics & Dept. of Biology, U. of Utah, Salt Lake City, UT.

Cyclopamine is a teratogenic alkaloid produced by several Western range grasses. Zebrafish embryos exposed to cyclopamine exhibit abnormalities that mimic many of the defects caused by the *cyc-1* mutation in zebrafish. The most prominent defects caused by both the drug and the mutation are cyclopia and a failure of the trunk myotomes to properly differentiate. However, unlike *cyc-1* animals which lack a floorplate, cyclopamine-treated animals show no disorganization in the neural midline and have a normal floorplate. Our analysis suggests that one effect of cyclopamine is to suppress formation of the cartilaginous branchial arches, allowing the eyes to slump together ventrally. Cyclopia can therefore develop in the absence of any defect in the neural midline.

The earliest trunk defect in cyclopamine-treated animals can be traced to the failure to differentiate of a specific longitudinal array of early muscle fibers, termed muscle pioneers, that express the *engrailed* protein. We thereafter see that a longitudinal furrow called the horizontal septum does not form and that the myotomes retain an immature organization. These myotomal defects lead to aberrant pathfinding by a peripheral sensory structure, the lateral line. In normal animals, axons of the lateral line extend longitudinally, following a migrating primordium. In animals exposed to cyclopamine at high concentrations, the primordium fails to migrate and axons accumulate in a tangled knot just caudal to their somata. At lower concentrations, the primordium migrates, but fails to find its normal cues and instead wanders aberrantly. The lateral line nerve follows the path of the errant primordium, and neuromast organs form in abnormal locations. We suggest that the aberrant migration of the lateral line primordium is a consequence of the disruption of the horizontal septum by the cyclopamine treatment.

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22.16

BRACHIAL PLEXUS FORMATION IN CHICK EMBRYOS WITH DISTURBED SEGMENTATION OF THE VENTRAL ROOTS AND SENSORY GANGLIA. R.S. Goldstein and C. Kalcheim, Dept. Anat. and Embryol. Hebrew Univ.-Hadassah Medical School, Jerusalem, Israel.

A possible role for the segmented pattern of the peripheral nervous system (PNS) is the carrying of positional information necessary for the sorting out and establishment of axonal bundles and plexuses. To test this possibility, we have studied embryos with unilateral disruption of normal segmentation of paraxial PNS structures. Replacing somites at the prospective brachial level of the neural tube in chick embryos with rostral half-somites from quail donors, prevents the segmentation of the sensory ganglia, ventral roots and sympathetic trunk. Three-dimensional reconstructions were made of 6 and 9 day embryos, in which neural tissues were distinguished with an antibody to HNK-1 and grafted quail cells with hematoxylin.

In normal embryos, three major (14,15,16) and one smaller (13,17) spinal nerves participate in the formation of the brachial plexus. These nerves merge and then separate into two trunks, as well as giving off smaller nerves. In spite of the lack of individual ventral roots and spinal nerves corresponding to each segment, the axons on the operated side of chimeric embryos converge and then diverge to produce trunks and nerves very similar to their counterparts in normal embryos and in the control plexuses of chimeric embryos.

The zone proximal to the plexus contains environmental cues that cause axons to selectively gather into groups that become individual peripheral nerves. Our results demonstrate that segmentally-repeating cues present in this proximal zone are distinct from the cues responsible for 1) the convergence of axons into a plexus and 2) the normal formation of trunks and nerves arising from the plexus.

22.17

THE ORGANIZATION OF PRECOCIOUS NEURONS IN THE RAT EMBRYO REPRESENTS A BLUEPRINT FOR THE ORDERLY GROWTH OF AXONS. M.-C. Bélanger, F. Auclair*, L. Bertrand* and R. Marchand. C.R. Neurobiologie, Hôpital de l'Enfant-Jésus, 1401, 18e rue, Québec (Qc), Canada, G1J 1Z4

During embryogenesis, the fiber tracts grow in a highly stereotyped pattern. Katz and Lasek (1980) have elaborated the concept of substrate pathways, defined as *(...)a set of similar guidance cues which are aligned in a continuous discrete pathway (...)*. How are such guidance cues settled in pathways? In other words, what is the *origin* of substrate pathways? We had previously observed that the precocious neurons displayed a very peculiar arrangement that predicted the pattern of some CNS axonal tracts. To determine if the first neurons play a role in the guidance of early nerve fibers, they were mapped in the brain of an $E12_{15}$ embryo (embryonic day 12 and 15 hours) after injection of tritiated thymidine to gestating rats at $E11_{15}$. In the embryo, early-generated neurons were organized in longitudinal columns throughout the entire length of the brain. In the prosencephalon, they formed a complex and ramified basal prosencephalic column. In the brainstem, two columns were seen: a medial one, including members of the somatomotor column, and a more lateral one, in the reticular formation. All these columns were associated with well-differentiated regions in the marginal zone, characterized by open spaces, progressively filled by growing fibers. These regions represented the prospective accessory olfactory tract, medial forebrain bundle, optic tract, medial longitudinal fasciculus and central tegmental tract. To further verify the relation between the columns and the growth of axons, nerve fibers were traced with DiI in the brain of young embryos. Fasciculi were seen to travel in close proximity to regions of early-generated neuronal columns. It is proposed that the primitive pattern of precocious neurons is responsible for the orderly growth of longitudinal axonal tracts.

Supported by MRC of Canada, FRSQ, FCAR and Fondation de l'U. Laval.

22.18

DOES PERIPHERAL NERVE REPAIR INCREASE THE ACCURACY OF AXONAL REGENERATION? R.D. Madison[1,2,3], C. Harsh[1*], and S.J. Archibald[1]. 1Department of Surgery (Neurosurgery) and 2 Department of Neurobiology, Duke University Medical, and 3 Research Service, VA Hospital, Durham, NC 27710.

We have recently refined a model of nerve regeneration to allow the anatomical accuracy of nerve regeneration at the single neuron level (Madison et al., Soc. Neurosci. Absts. 16: 336.2, 1990). We applied this model to answer the question: Does direct suture repair of the rat femoral nerve increase the accuracy of regeneration? Control experiments showed that the primary sensory neurons which project into the saphenous nerve (a purely sensory terminal branch of the femoral nerve) can be reliably prelabeled by exposure of the saphenous nerve to DiI, and two weeks later approximately 99% of the same population of neurons can be labeled with Fluorogold application to the same nerve. Following prelabeling of the sensory projection to the saphenous nerve in 20 adult rats, the parent femoral nerve was cut and either simply reopposed (N=10) or repaired by direct suture (N=10). Two weeks later the percentage of returning sensory neurons from the L4 DRG was quantified by Fluorogold application to the saphenous nerve; $27\pm$ 12 % and $8\pm$11% (respectively) of the neurons correctly regenerated an axon into the saphenous branch ($p<.05$, t-test). Thus although the rodent PNS demonstrates spontaneous regeneration, accuracy can be significantly improved by nerve repair techniques. This double labeling technique provides a powerful and accurate mechanism to evaluate the effectiveness of various nerve repair techniques and prostheses. Supported by NS 22404-06 (RDM).

GROWTH FACTORS AND TROPHIC AGENTS I

23.1

THE EFFECTS OF bFGF ON THE DEVELOPMENT OF DOPAMINE NEURONS FOLLOWING EXPOSURE TO MPP^+: COMPARISON WITH THE EFFECTS OF EGF. T. Bak and C. Mytilineou. Dept. of Neurology, Mt. Sinai Sch. of Med. New York, N.Y. 10029

bFGF has been shown to increase dopamine (DA) neuron survival and stimulate ^{3}H-DA uptake in mesencephalic cultures (Ferrari et al., 1988) and to induce regrowth of damaged DA neurons in vivo (Otto and Unsicker, 1990). We examined whether bFGF could protect cultured DA neurons from the neurotoxin MPP^+, or affect the recovery of MPP^+-damaged neurons. We also examined whether bFGF effects on DA neurons are direct or mediated by other cell populations. Mesencephalic cultures were established from E16 rats and maintained in defined medium. bFGF was added to the appropriate groups and was present throughout the experiment. Cultures were exposed to 10uM MPP^+ for 24hrs on 4 DIV and analyzed at various times for ^{3}H-DA uptake and TH immunocytochemistry. Following removal of MPP^+, a similar reduction of DA uptake (~60%) was observed in control and bFGF treated cultures, suggesting a lack of a protective effect of bFGF against MPP^+-induced damage. However bFGF promoted long term survival of DA neurons and increased DA uptake in both control and MPP^+-treated cultures. Similar effects were observed with EGF on parallel cultures. Treatement with FUDR along with EGF resulted in a reduction of >90% in DA uptake as well as a decrease in TH+ neurons, suggesting that the EGF effects are mediated by proliferating glia. In cultures treated with FUDR and bFGF, there was a reduction of ~50% in DA uptake, and no loss of TH+ neurons, suggesting that the bFGF effects are only partially dependent on the presence of glial cells. Our results indicate that EGF and bFGF do not prevent MPP^+ neurotoxicity but support the survival and neurite outgrowth of remaining DA neurons in a similar manner, but by different mechanisms.

23.2

MULTIPLE FORMS OF BFGF ARE DEVELOPMENTALLY EXPRESSED IN THE RAT CNS. Suzanne Giordano, Larry Sherman*, and Richard Morrison. R.S. Dow Neurological Sciences Institute and Department of Cell Biology & Anatomy Oregon Health Sciences University, Portland, OR.

Recently multiple molecular weight forms of the bFGF protein have been identified. Some of these molecular weight forms appear to be amino terminal extensions of the 18Kd bFGF protein that are translated from a single mRNA species.

Here we demonstrate changes in the expression of different bFGF protein forms during the development of the rat CNS. In the adult rat brain western blots demonstrated the presence of four bFGF protein forms with approximate molecular weights of 18, 21, 22 and 34Kd using a previously characterized monoclonal antibody. All four bFGF protein forms bound to heparin and their staining on blots was eliminated in the presence of the 18 Kd human recombinant bFGF.

The expression pattern of these protein forms changed during CNS development. Proteins were extracted from whole rat brains at age E15, E18, P1, P7, P14, and adult. Embryonic brain contained three bFGF forms at 18, 21 and 34Kd, but lacked the 22Kd bFGF form present in the adult. The expression of the 22Kd form was first observed in brain following birth and then steadily increased to adult levels. bFGF protein forms appeared to be regulated differently in different regions of the developing rat CNS. Although the spinal cord and cortex showed the same pattern of bFGF expression as observed in whole brain, the adult cerebellum lacked the 21 and 22Kd forms.

These alterations in the expression of bFGF during CNS development suggest that individual forms may have discrete functions and that these changes may underlie specific developmental events. Research support (to RM) from NIH Grant NS 26125-01 and a grant from the M.J. Murdock Charitable Trust.

23.3

PROLIFERATION OF NEURAL PRECURSORS REGULATED BY ENDOGENOUS PRODUCTION OF FIBROBLAST AND INSULIN-LIKE GROWTH FACTORS. P.F. Bartlett, J. Drago* and M. Murphy*. The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia 3054.

The first stage of CNS formation is the rapid expansion of neuroepithelial cells within the neural tube. It has been shown in vitro that fibroblast growth factor (FGF; both acidic and basic) is capable of stimulating this proliferation. (Murphy et. al. J. Neurosci. Res. 25:463, 1990). This response has been found recently to be dependent on the presence of insulin-like growth factors (IGF), especially the truncated form of IGF-1.(Drago et. al. Proc. Nat. Acad. Sci.,USA. 88:2199, 1991).

As the requirement for these exogenous factors can be overcome in vitro by plating neuroepithelial cells at high cell density, we investigated whether this resulted from the production of endogenous FGF and IGF. This was examined by using antibodies that neutralised either IGF-1 or bFGF activity. Pure populations of neuroepithelial cells obtained from embryonic day 10 CBA mouse mesencephalon and telencephalon were plated at 10^4 cells/well (Terasaki wells containing a final volume of 15µl) in insulin- and serum-free medium in the presence of varying dilutions of a monoclonal anti-IGF-1 (SM-1.2) or a polyclonal anti-bFGF (Gift of D. Gospadarowicz). The anti-IGF-1 completely inhibited growth whereas the anti-bFGF only partially inhibited this response.

This finding coupled with our demonstration that neuroepithelium also contains mRNA for both IGF-1 and bFGF supports the concept that early neuroepithelial growth may be regulated in an autocrine/paracrine manner and indicates that both IGF and FGF may be important in this regulation.

23.4

ß-AMYLOID (1-42) ENHANCES THE RELEASE OF FIBROBLAST GROWTH FACTOR (FGF) AND INTERLEUKIN-1 (IL-1) FROM GLIAL CELLS. A. J. Walencewicz, D. M. Araujo, and C. W. Cotman, Dept. Psychobiol., Univ. California, Irvine, CA, 92717 USA.

Microglia and astrocytes surrounding plaques in Alzheimer's disease (AD) appear to play a significant role in the neurodegenerative changes observed in the disease. However, it is not yet clear whether alterations in the function of these cells contribute to the ß-amyloid-induced neurodegenerative changes, nor is it evident whether the proliferation of these cells around neuritic plaques is caused by the peptide. Therefore, the main objective of the present study was to determine the effects of ß-amyloid (1-42) on glial cells *in vitro*. The results show that ß-amyloid (1-42) (10 and 100 µg/ml) enhances ^{3}H-thymidine uptake by microglia (by 49 and 75%, respectively) and astrocytes (by 10 and 20%, respectively) *in vitro*. Moreover, ß-amyloid (1-42) appears to increase glial cell activity. First, the release of basic FGF-like immunoreactive material from astrocytes and microglia was augmented by the peptide (by 58-98%). Second, the release of IL-1 from microglia and astrocytes was also increased (by 51-97%) by ß-amyloid (1-42). In addition, exogenous application of either basic FGF or IL-1 (10-100 ng/ml) appears to enhance the neurotoxic effects of ß-amyloid (1-42) on hippocampal neurons *in vitro*, an effect that is apparent by 24h in culture. Thus, it is possible that ß-amyloid stimulates the release of various GFs and lymphokines from glial cells that may contribute to the neurotoxic properties of the peptide observed in AD.

23.5

EXPRESSION OF BIOLOGICALLY ACTIVE BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN PRIMARY FIBROBLASTS. Jasodhara Ray*, Mitsuru Kakihana*, Malcolm Schinstine*, Andrew Baird# and Fred H. Gage*. * Dept. of Neurosci., Univ. of Calif. San Diego, La Jolla, CA 92093, # Dept. of Mol. & Cell Growth Biology, Whittier Institute, La Jolla, CA 92037

Basic fibroblast growth factor (bFGF) is a potent growth and mitogenic factor and possesses both trophic and neurite-promoting activity for a variety of central (CNS) and peripheral nervous system (PNS) neurons. Expression and characterization of bFGF producing primary fibroblasts will be reported.

The cDNA encoding for the entire 18 kd coding region for bFGF was cloned into an expression vector under the control of Rous sarcoma virus long terminal repeat (RSV-LTR) promoter. The recombinant plasmid was introduced into primary fibroblasts and stable transformants were selected by growing cells in medium containing G418.

The success of expression of bFGF was confirmed by measurement of bFGF in the cell lysate by radioimmunoassay. Fibroblasts (FF) endogenously express bFGF at a low level (0.1 µg/mg protein). Genetically modified fibroblasts (FF/FGF) produced bFGF 18 times higher than control fibroblasts (1.8 µg/mg protein). Basic FGF was not secreted into the medium but was expressed on the cell membrane. Treatment with 0.1% Triton X-100 removed the immunoreactivity. Soft agar assay showed that the cells were not transformed. To determine if these cells have any of the biological effects of purified soluble bFGF, hippocampal (HC) neurons were co-cultured with either control FF or FF/FGF cells and the neurite lengths and survival of neurons were measured. Results showed that 93% of HC neurons survived in the presence of FF/FGF cells after 7 days in culture compared to 73% in the presence of FF cells. At day 2, the number of neurons bearing neurites (that were >50 µm) was significantly higher in presence of FF/FGF (61%) than control cells (39%). Additional studies, including the growth and expression of bFGF during different stages of cell growth, will be presented.

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23.6

NGF, FGF AND EGF RAPIDLY STIMULATE p21^{ras}.GTP BINDING IN PC12 CELLS BY SEPARATE, CONVERGENT MECHANISMS. M.-S. Qiu, and S.H. Green. Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242

NGF and basic FGF (bFGF) are trophic factors for PC12 cells and induce neuronal differentiation. Treatment with EGF, by contrast, does not produce these effects. Since the receptors for these factors all appear to be tyrosine kinases, the differences in the responses presumably derive from differences in intracellular signal mechanisms activated by these receptors. By immunoblotting with antiphosphotyrosine, we have observed only small differences between substrates of EGF- and NGF-activated tyrosine kinases but bFGF-induced tyr-phosphorylations have a very different pattern. p21^{ras} has been implicated as a signal transducer for NGF induction of neuronal differentiation in PC12 cells. Here we show directly that NGF, bFGF and EGF all transiently activate p21^{ras} to a similar extent. p21^{ras} was immunoprecipitated from [^{32}P]-labeled-PC12 cells and p21^{ras}-associated nucleotide phosphates were chromatographically separated. Activation of p21^{ras} by growth factor was detected as a ≈20× increase in p21^{ras}-associated GTP — maximal within 3 m but decaying nearly to prestimulus levels within 30 m. The timecourse was similar for the three factors, although somewhat prolonged in bFGF-treated cells. p21^{ras} activation appears to require tyr-phosphorylation as it is inhibited by genistein, although low genistein concentrations which block most tyrosine phosphorylation inhibit only EGF and not NGF or FGF responses. PC12 mutants that lack *trk* respond to EGF but not to NGF. Methylation inhibitors block p21^{ras} activation by NGF and bFGF but not by EGF. K-252a (a general kinase inhibitor) blocks only p21^{ras} activation by NGF; other kinase inhibitors (H-7, sphingosine, 6-thioguanine, 2-aminopurine) do not block at all. Effects of NGF and EGF are not additive, implying a common pathway downstream of these receptors. These results imply that activation of p21^{ras} is not sufficient for neuronal differentiation but reveal differences in the mechanism of p21^{ras} activation by the three factors that may be exploited for the identification of signals specific to neurotrophic effects.

23.7

ACIDIC FGF PROMOTION OF NEURITE OUTGROWTH IN SYMPATHETIC NEURONS IS MEDIATED BY NON-NEURONAL CELLS. M. Deschuyteneer, K.W. Roche*, W.D. Matthew and J.N. Davis, Neurology Research, Veterans Adm. Med. Ctr., Durham NC 27705 and Dept. of Neurobiology, Duke Univ. Med. Ctr., Durham NC 27710.

Fibroblast growth factors (FGF) appear to influence the kinetics and morphology of neuronal outgrowth in a variety of *in vitro* models. We studied the ability of human recombinant acidic FGF and basic FGF to elicit neurite outgrowth from whole explants and dissociated neurons of the neonatal mouse superior cervical ganglion. In explants, aFGF consistently promoted neurite outgrowth and was 25 times more potent than bFGF (ED_{50} = 0.06 nM and 1.5 nM, respectively). In a pure population of dissociated sympathetic neurons, aFGF and bFGF elicited little neurite outgrowth. By contrast, in mixed populations of dissociated ganglia cells that combined both the neurons and non-neuronal cells, aFGF and bFGF promoted significant neurite outgrowth in individual neurons and aFGF was more potent than bFGF. In addition, aFGF significantly enhanced neurite outgrowth in dissociated neurons co-cultured with a line of immortalized mouse Schwann cells or with Balb/c 3T3 mouse fibroblasts. The Schwann cell line was more efficient in eliciting neurite outgrowth than the fibroblast one. These results show that non-neuronal cells mediate the potent aFGF neurite outgrowth promotion in sympathetic neurons and suggest that Schwann cells are the primary intermediate in this process. (Supported by NS 06233 and the Dept. of Veterans Affairs).

23.8

THE ROLE OF BASIC FIBROBLAST GROWTH FACTOR IN THE HYPOGLOSSAL SYSTEM. C. Grothe and K. Unsicker. Dept. of Anatomy and Cell Biology, Univ. of Marburg, D-3550 Marburg, F.R.G.

We found bFGF-like immunoreactivity (bFGF-IR) in the hypoglossal nucleus (HN), localized in cell bodies and fibers of a neuronal subpopulation. Transection of the hypoglossal nerve led to a nearly complete reduction of bFGF-IR in the ipsilateral HN after 48 h. Cresyl violett-stained consecutive sections revealed no signs of chromatolysis. Injection of ^{125}J bFGF into the tongue resulted in a prominent labeling of neuronal perikarya in the HN. No labeling was found in animals that received heat-denatured ^{125}J-bFGF or an excess of unlabeled bFGF in addition to ^{125}J bFGF. These results suggest a specific retrograde axonal transport of bFGF in the hypoglossal system.

In vitro bFGF stimulated the survival of hypoglossal neurons of 7 day old rats in a dose-dependent manner (ED_{50} 2 ng/ml). In vivo administration of bFGF did not prevent lesion-induced (i) neuron death in the HN of 7 day old rats and (ii) decrease of choline acetyltransferase in the HN of adult rats. We suggest that, in vivo, bFGF may have a non-neurotrophic role in the hypoglossal system or act as a co-neurotrophic factor with a hitherto unknown protein.

23.9

TRANSIENT LESION-INDUCED EXPRESSION OF bFGF AND ITS RECEPTOR IN LAYER VIb (SUBPLATE CELLS) OF THE ADULT RAT CEREBRAL CORTEX. F. Gómez-Pinilla, J. Won-Kyun Lee* and C. W. Cotman. Dept. of Psychobiology, University of California, Irvine, CA 92717.

bFGF is a potent trophic factor with a wide spectrum of activity which appears to play a role in various stages of neuronal development. Cortical layer VIb of adult rats seems to be homologous with the transient subplate cell population of embryonic cerebral cortex of cats and primates. Since cells of cortical layer VIb survive into adulthood in rats, it is possible that these cells could possess special trophic capabilities. Accordingly, we examined the effect of brain injury on the expression of bFGF and its receptor (bFGFR) by the subplate cell population of the adult rat. Male Sprague-Dawley rats three to six months old, were deeply anesthetized and received unilateral electrolytic entorhinal cortex ablation, fimbria-fornix transection or partial aspiration of the dorsal cortex (2 x 2 mm). Rats were sacrificed 2, 7 or 14 days after lesion and processed for bFGF immunohistochemistry by the ABC method. We used an affinity purified monoclonal antibody that recognizes the configuration of bFGF related to its biological activity. In normal rats, layer VIb did not show bFGF/bFGFR immunoreactivity. After each of the lesions, there was a transient lesion-induced expression of bFGF/bFGFR immunoreactivity in the layer VIb of the cerebral cortex. This induction of bFGF appeared by postlesion day 2 and had already disappeared by day 7. Our findings suggest that, endogenous bFGF may have a neuroprotective role after insult/trauma and thus may help to maintain subplate cells throughout life.

23.10

LIMBIC SEIZURES INCREASE BASIC FIBROBLAST GROWTH FACTOR (bFGF) GENE EXPRESSION IN HIPPOCAMPUS AND ENTORHINAL CORTEX. M.A. Riva[1], K. Gale[2], J. Graham[2], A. Pazos[2] and I. Mocchetti[1]. Departments of [1]Anatomy and Cell Biology and [2]Pharmacology, Georgetown University Medical Center, Washington, D.C. 20007.

bFGF, an important growth factor for maintenance of neurons, has been postulated to serve a protective role in degenerative processess. Using an RNase protection assay, we investigated the regulation of bFGF gene expression in selected limbic regions of rats following convulsive seizures evoked focally from area tempestas (AT), an epileptogenic site in the deep prepiriform cortex.

Following infusion of bicuculline methiodide (118 pmol in 120 nl) into the AT, limbic motor seizures, characterized by rearing with facial and forelimb clonus, recurred over a 45 min period. At 5 and 10 hr following initiation of seizures from AT, the levels of bFGF mRNA in entorhinal cortex and hippocampus were significantly increased (by more than twofold). An increase in bFGF mRNA was also detected in substantia nigra, but no increases occurred in striatum or hypothalamus. Infusions of saline into AT, or infusions of bicuculline adjacent to AT (which did not induce seizures) were without effect on bFGF mRNA levels. Moreover, infusions of bicuculline into the inferior colliculus, which evoked characteristic brain stem seizures (explosive running and bouncing convulsions), did not increase bFGF mRNA in any of the forebrain regions investigated.

These data suggest that the regional expression of bFGF mRNA selectively changes in response to the propagation of seizure discharge through limbic circuits and raises the possibility that bFGF may participate in adaptive changes occurring following limbic seizure activity.

Supported by HHS grant #NS20576 and a grant from Farmitalia Carlo Erba Pharmaceuticals.

23.11

ENDOGENOUS EXPRESSION OF ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS IN 1-METHYL-4-PHENYL-1,2,3,5-TETRAHYDROPYRIDINE (MPTP) LESIONED MOUSE BRAIN. J. Logel, D. Luthman*, J. Luthman, M. Hall*, B. Hoffer and S. Leonard. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, Colorado, 80262.

Substantial evidence suggests that fibroblast growth factor may influence the survival of dopaminergic neurons both in vitro and in vivo, but endogenous response of the FGFs to chemical lesion in dopaminergic systems has notbeen measured. The fibroblast growth factor family comprises at least five unique gene sequences. To study endogenous gene expression in dopaminergic neurons, we have treated Swiss-Webster mice with 3 X 30 mg/kg of the neurotoxin 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine (MPTD) on successive days. After one week and five weeks, animals were sacrificed and the brains were removed for dissection of the substantia nigra and striatum. RNA was isolated from a pool of each of these tissues for each time point and mRNA quantitated by multiplex PCR for acidic and basic fibroblast growth factors. Results suggest that the endogenous expression of both acidic and basic fibroblast growth factor increases at one week post-MPTP-lesion.

23.12

INJURY-INDUCED INCREASE IN BASIC FIBROBLAST GROWTH FACTOR IN THE RAT BRAIN. C. Wolak*, S. Webb*, L. Hjelmeland* and V. Vijayan. Dept. of Cell Biol. & Human Anat. and Dept. of Med. Ophthalmol., University of California, Davis, CA 95616.

Basic fibroblast growth factor (bFGF) has been localized to neurons and astrocytes in the C. N. S. bFGF level increases following brain injury and this may contribute to repair and neurotrophic support of injured neurons. We undertook immunohistochemical studies to examine the loci of bFGF increase following injury in a previously characterized model of neural trauma in the rat (Topp et al., 1989; Vijayan et al., 1990).

We observed bFGF immunoreactivity in many neurons, in ependymal cells and in subpopulations of gray and white matter astrocytes throughout the rat brain in the absence of injury. Two to 15 days following injury, the wound area demonstrated acid phosphatase- and OX 42 -positive macrophages in the cavity and adjacent neuropil, and intensely GFAP-reactive hypertrophied astrocytes in the border zone. bFGF staining appeared in the extracellular space near the wound and in the nuclei and cytoplasm of macrophages. In addition, reactive astrocytes demonstrated an increase in nuclear bFGF immunoreactivity.

Our observations suggest that bFGF released into the extracellular space and produced by brain macrophages and reactive astrocytes are potential sources for the growth factor following a traumatic injury to the rat brain.

Supported by NIH grant AG06159.

23.13

IMMUNOHISTOCHEMICAL LOCALIZATION OF bFGF IN THE CNS. W.R. Woodward, C.K. Meshul, R. Nishi, T.E. Williams* and F.P. Eckenstein. Depts. of Neurology and Cell Biology and Anatomy, Oregon Health Sciences Univ. and VA Medical Center, Portland, OR 97201.

Basic fibroblast growth factor (bFGF) is present in relatively high levels in the brain where it may play an important role in the development and maintenance of the tissue. We used a mouse monoclonal anti-bFGF to examine the immunocytochemical distribution of bFGF in frozen sections of rat brain. We found bFGF-immunoreactivity to be predominantly associated with GFAP-positive astrocytes throughout all regions of the CNS. Only a few neuronal populations were found to contain bFGF-immunoreactivity: prominent among them were neurons in the CA2 area of hippocampus and small neurons in the retrosplenial cortex. The bFGF-immunoreactivity in these cells appeared to be present in both the nucleus and the cytoplasm. The nuclear localization of bFGF-immunoreactivity was confirmed by EM analysis for both astrocytes and CA2 neurons. In CA2 bFGF-immunoreactivity was only observed in a subpopulation of pyramidal neurons with irregular-shaped nuclei. The only other cells in the CNS showing bFGF-immunoreactivity were ventricular ependymal cells. These data suggest that bFGF might be involved in mediating astrocytic influences on late postnatal maturation and plasticity in the CNS. The nuclear localization suggests that bFGF may be important for the differentiation of astrocytes and specific neuronal populations.

This work was supported by an Oregon Medical Research Foundation grant (WRW), a VA Merit Review grant (CKM), NIH grants [AG07424,NS17493 (FPE); NS25767 (RN)] and a March of Dimes Basil O'Connor grant (FPE).

23.14

BASIC FIBROBLAST GROWTH FACTOR (bFGF) SUPPRESSES AN INITIAL PHASE OF NEURONAL DIFFERENTIATION IN CULTURE IN CHICK NEURAL TUBE DEVELOPMENT. Y. Kinoshita, C. Kinoshita*, and M. Bothwell. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

Neurotrophic activities of bFGF have been demonstrated for a variety of developing neuronal populations. Here we report that bFGF instead suppresses neuronal differentiation in culture when it is applied to cells at a very early stage of neuronal differentiation. Dissociated neural tube cells obtained from a thoracic region of ~E2.5 (26-30 somites) chick embryos were cultured on polyornithine-coated substrate in Keratinocyte Serum-Free Medium (Gibco) + 1 % FBS with or without bFGF (10 ng/mL), in which little cell proliferation occurs. bFGF reduces the number of neurons, as identified after 2 days of culture using an anti-neurofilament antibody, down to ~40% for well-differentiated neurons (neurite>2 cell body size) and to ~55% for the total neuronal count, with a half-maximal effective concentration below 0.1 ng/mL. This adverse effect of bFGF is seen only when cells are exposed to bFGF within 12 hrs following cell plating (roughly corresponding to E3), and later exposures to bFGF result in a weak neurotrophic action. During development *in ovo*, the suppressive effect of bFGF becomes largely unnoticeable by E3.5 for well-differentiated neurons that are thought to be born earlier, although poorly-differentiated neurons are still susceptible to bFGF. bFGF also suppresses neuronal differentiation of cells cultured on collagen substrate in F12 + 10% FBS medium.

These results indicate that in an initial phase of neuronal development there is a critical period during which bFGF can act suppressively on neuronal differentiation and support the hypothesis that FGFs may exert different functions at two discrete phases of neuronal differentiation (Heuer *et al.*, Neuron 5:283, 1990).

23.15

CHARACTERIZATION OF A PC12 VARIANT UNRESPONSIVE TO FIBROBLAST GROWTH FACTOR. M. E. Zuber, D. J. Marshall* and B. B. Olwin*. Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

The rat pheochromocytoma cell line PC12 has been used as a model system in the study of neurite outgrowth. PC12 cells differentiate reversibly when treated with nerve growth factor (NGF), acidic or basic fibroblast growth factor (aFGF and bFGF respectively). We have isolated a PC12 variant (PC12–) that is not responsive to either aFGF or bFGF yet retains the ability to extend neurites in the presence of NGF. We have examined FGF receptors in PC12– cells to determine if an alteration in FGF receptors is responsible for the loss of FGF-dependent neurite outgrowth in these cells. ^{125}I-aFGF cell surface binding studies indicated that PC12– cells bind less than 25% of the aFGF bound to control PC12+ (FGF-responsive) cells. Chemical cross-linking of ^{125}I-aFGF to intact PC12– and PC12+ cells revealed that PC12+ cells express a ~160 kDa cell surface FGF binding protein not present in PC12– cells. Northern blot (mRNA) analysis demonstrated that PC12+ cells express a transcript(s) that hybridizes to a member of the tyrosine kinase FGF receptor (TK FGFR) family. PC12– cells lack detectable levels of this transcipt. The simplest interpretation of these results is that PC12– cells lack a TK FGFR and therefore cannot respond to FGF as a factor that stimulates neurite outgrowth. Experiments are in progress to test this hypothesis.

23.16

EVIDENCE FOR A PERTUSSIS TOXIN-SENSITIVE G-BINDING PROTEIN COUPLED TO THE CLONED HUMAN ACIDIC FIBROBLAST GROWTH FACTOR (aFGF) RECEPTOR. Michael F. Jarvis and George Gessner, Rhône-Poulenc Rorer Central Research, King of Prussia, PA 19406.

Acidic fibroblast growth factor (aFGF) is a neurotrophic agent that mediates neuronal differentiation via a specific cell surface receptor. The cloned human aFGF receptor possesses a single membrane spanning region and is functionally linked to the activation of tyrosine kinase. [^{125}I]aFGF binds to NIH 3T3 murine fibroblast cell membranes containing the recombinant and overexpressed human aFGF receptor with high affinity (Kd = 300 pM) and limited capacity (Bmax = 19 pmol/mg protein). Preincubation of the membranes with pertussis toxin resulted in a concentration-dependent decrease in ligand affinity (Kd = 800 pM) with no significant change in apparent Bmax. Experiments with cholera toxin failed to produce binding parameters different from control values. Under appropriate experimental conditions, the binding of [^{125}I]aFGF was also reduced by GTP, GTP-γ-S, and Gpp(NH)p. These results provide direct evidence that the aFGF receptor is coupled to a G (possibly G_i)-binding protein and that functional receptor activation involves the formation of a ternary (agonist/receptor/G-protein) complex.

23.17

EFFECTS OF bFGF ANTISENSE OLIGONUCLEOTIDES ON ASTROCYTES AND HIPPOCAMPAL CELL CULTURES. W.Gerdes, W.Brysch, K.H.Schlingensiepen, W.Seifert Dept.Neurobiology, Max-Planck-Inst.biophys.Chem. 3400 Göttingen, Germany

Basic fibroblast growth factor (bFGF) has been recognized as a neurotrophic growth factor for CNS neurons in recent years. We have used a stable synthetic bFGF antisense oligodeoxynucleotide in order to block the bFGF gene expression in astrocyte cultures from rat cortex and in neuronal cultures from rat hippocampus (E18).

Astrocytes from 8-10 days old cultures of newborn rats grown in DMEM plus 10% calf serum were trypsinized and replated in 24- or 96-multiwell plates, either in serumfree DMEM or in hormone-supplemented DMEM. After addition of the antisense oligo, growth factors (10% serum / FGF / EGF) were added and cell proliferation was tested at 24 hrs and 48 hrs by ^{3}H-thymidine incorporation into DNA and by the FDA assay in a cytofluorimeter. In cultures stimulated by these factors a significant inhibition of DNA synthesis and cell proliferation was observed. In hippocampal neuronal cultures (E18, 3 days in serumfree cult) the antisense oligodeoxynucleotide had no effect on survival after 24 hrs.

23.18

DEVELOPMENTAL CHANGES IN PDGF AND FGF mRNA LEVELS IN CAT CORTEX AND CEREBELLUM. M.A McCormack, P. Tolentino, K.M. Rosen, G.D. Mower and L. Villa-Komaroff. Department of Neurology and Program in Neuroscience, Children's Hospital and Harvard Medical School, Boston, MA 02115

Northern/slot blot analysis was used to describe the postnatal developmental expression of mRNAs encoding the two chains of Platelet-Derived Growth Factor (PDGF) and the two forms of Fibroblast Growth Factor (FGF). For the PDGF family, the developmental pattern of expression was different in different structures. In visual cortex, RNA encoding PDGF-B showed a peak of expression at 5 weeks of age, mirroring the postnatal critical period in this structure. By contrast, levels of this RNA were highest at 1 week and showed a steady 2-3 fold decline to adulthood in frontal cortex and cerebellum. Levels of PDGF-A mRNA declined 6-8 fold in frontal and visual cortex but remained constant in cerebellum. RNA encoding bFGF rose sharply over the first 10 weeks, then declined 2-3 fold to adult levels in all three structures, correlating with the time course of synaptogenesis and gliogenesis. Levels of aFGF mRNA were essentially constant in all three structures. To investigate the effect of environmental input on the expression of these RNAs, we are examining the effect of dark rearing and exposure to light after dark rearing in young (5 week) and older (20+ week) cats. Preliminary results suggest that there is no striking effect of these conditions on mRNAs encoding these growth factors in either visual or frontal cortex. These results indicate that the two members of each of these growth factor families have different patterns of expression during postnatal life. The consequence of these patterns is that the relative amounts of the two forms of FGF and the homodimers and heterodimers of PDGF change over the course of normal brain development.

23.19

Increase in immunoreactivity and mRNA level of basic fibroblast growth factor in rat brain after transient forebrain ischemia. K. Takami, Y. Kiyota, M. Iwane[1]*, M. Miyamoto, A. Nagaoka*, A. Shino* and R. Tsukuda*. Biology Res. Lab. & Biotechnology Res. Lab.[1], Res. and Dev. Div., Takeda Chemical Ind. Ltd., Osaka 532, Japan.

Using immunohistochemistry and *in situ* hybridization, we examined whether basic fibroblast growth factor (bFGF) is generated as one of the 'self-repair' responses in rat brain following 20 min of forebrain ischemia. We used monoclonal anti-human bFGF antibody for immunostaining and synthesized oligonucleotide probe complementary to bFGF mRNA. In ischemic rats, remarkable neuronal degeneration and necrosis were observed in the CA1 subfield of the hippocampus and the caudate putamen. In these area, intense bFGF immunoreactivity was observed while slight in normal rats. Marked immunoreactivity was also evident in the temporal cortex, corpus callosum and CA4 subfield of the hippocampus. In addition, transient forebrain ischemia markedly increased the levels of mRNA for bFGF in the hippocampus, especially in the CA1 subfield. These results suggest that induction of bFGF may be related to the healing which follows brain ischemia.

23.20

CYTOKINE GENE EXPRESSION IN THE DEVELOPING BRAIN T.M. Burns*, J.A. Clough*, M. De *, N.E.J. Berman, R.M. Klein, G.W. Wood*. Departments of Pathology and Oncology and Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS, 66103.

Cytokines have been shown to have profound effects on the proliferation and differentiation of numerous cell types, but little is known about their potential role in brain development. To determine whether cytokines are expressed in the developing brain, we extracted total RNA from fetal and early postnatal mouse brains (E12-P39) and performed Northern blots using formaldehyde gel electrophoresis. IL-1α, a potent mitogen, was expressed at all time periods examined, and the mRNA was present as more than one transcript. TNF-α, which is known to be cytostatic and cytotoxic, increased during fetal and neonatal development, but decreased dramatically after postnatal day 36. IL-6, which is known to be induced by IL-1 and TNF-α, was also present at all time periods. TGF-β_2, which regulates extracellular matrix deposition, increased from E12 to P6, and increased again between P12 and P39. These results show that cytokine genes are expressed at different times during CNS development. The differential expression of cytokine genes may reflect regional differences in brain development and/or involvement of cytokines in distinct developmental processes. Supported by MH38399, BNS881997, and HD17678.

23.21

REGIONAL DIFFERENCES IN OPIATE-DEPENDENT ASTROCYTE GROWTH IN ORGANOTYPIC CULTURES OF THE MOUSE CEREBELLUM, HIPPOCAMPUS AND CEREBRAL CORTEX. K.F. Hauser. Dept. of Anat. and Neurobiol., Univ. Kentucky Sch. Med., Lexington, KY 40536

To determine whether opiates directly modulate neural growth, opiate-dependent growth was examined in organotypic explant cultures derived from rat cerebellum, hippocampus, and cerebral cortex. Each of these brain regions was taken from a total of 36 male, Swiss (ICR) mice on postnatal days 1 or 7, and dissected into 6 symmetrical (right vs left) explant pairs. One explant from each pair was continuously exposed to 10^{-5} M morphine, 10^{-5} M morphine plus 3 x 10^{-5} M naloxone, or 3 x 10^{-5} M naloxone alone for 7-10 days *in vitro*, while the other explant served as a control. In general, maximum growth occurred in hippocampal and cerebral cortical explants derived from PD 1 mice, and in cerebellar tissue from PD 7 mice. Moreover, initial observations of opiate-dependent growth in living explants demonstrated that cells with astrocytic morphology were highly responsive. To better explore differences in astrocyte growth, glial fibrillary acidic protein (GFAP) immunoreactivity was assessed in whole-mount explants derived from tissue at the above ages. In cerebellar cultures, the outgrowth area of GFAP-immunoreactive cells was significantly reduced by morphine treatment (prevented by concomitant naloxone treatment), while the total number of GFAP-immunoreactive cells per μm^2 of outgrowth was unaffected. The growth of hippocampal explants was unaffected by opiates, whereas cerebral cortical cultures displayed variable growth responsiveness - perhaps due to differing responsiveness within the cortex itself. Collectively, the results indicate that opiates can inhibit the growth of astrocytes in organotypic neural cultures, but that intrinsic regional differences in the astrocytes themselves or associated neural cells mediate the responsiveness of astrocytes to opiates. Supported by NIDA grant DA 06204.

23.22

DEVELOPMENTAL APPEARANCE OF PROENKEPHALIN IMMUNOREACTIVITY IN PRIMARY EXPLANT CULTURES OF RAT CEREBELLUM. J.G. Osborne[1], B.A. Spruce[2], and K.F. Hauser[1]. [1]Dept. of Anat. and Neurobiol., Univ. of Kentucky Sch. of Med., Lexington, KY 40536 and [2]Dept. of Biochem., Med. Sci. Inst., The University, Dundee DD1 4HN, UK.

Enkephalin immunoreactivity is reported to transiently appear within the germinative cells of the cerebellar external granule layer (EGL) *in vivo* and *in vitro*. Moreover, endogenous opioids are reported to modify the rate of EGL cell proliferation *in vivo*. To better understand the regulation of opioid expression in the neuronal progenitor cells of the EGL, proenkephalin immunoreactivity (PE-IR) was examined in primary explant cultures using a novel monoclonal antibody (PE-25) specific for the intact/partially processed proenkephalin peptide. Cerebella from 8 male, neonatal Sprague-Dawley rats were dissected into 6 symmetrical explant pairs (i.e., comparable right vs left portions were compared) per cerebellum, and maintained as organotypic cultures for 7, 14, or 21 days. In whole-mount explants, PE-IR was detected in a portion of the EGL cells and their granule cell progeny, as well as in Golgi cells and some immature glia. When EGL cells and their derivatives were quantitatively compared in explant pair homologues at different times *in vitro*, the number of cells displaying PE-IR increased with progressive development - an interesting observation in light of previous reports of decreasing numbers of EGL cells possessing enkephalin immunoreactivity (ENK-IR) with increased maturation. Collectively, the increases in the number of cells with PE-IR appear to be developmentally regulated by local factors intrinsic to the cerebellum. Furthermore, differences between the patterns of PE-IR and those previously reported for ENK-IR suggest significant modulation of proenkephalin posttranslational processing by cerebellar neurons and their progenitors during maturation. Supported by NIDA grant DA 06204 and NIH grant RR-05374.

23.23

OLIGODENDROCYTE SURVIVAL SELECTIVELY DETERMINED BY A SOLUBLE ASTROCYTE-DERIVED FACTOR. A.L. Gard and S.E. Pfeiffer. Dept. of Microbiology, Univ. of Connecticut Med. Sch., Farmington, CT 06032 and Dept. of Structural & Cellular Biology, Univ. of South Alabama Med. Sch., Mobile, AL 36688.

Oligodendrocyte (OL) survival and its trophic regulation have received considerably less attention experimentally than neuronal growth in this regard. However, when OL progenitors bearing the $O4^+GalC^-$ antigenic surface phenotype are purified directly from their normal germinal environment in the postnatal rat telencephalon by immunoselection, >95% of the cells are viable initially but die thereafter in a basal defined culture medium (BDM) which otherwise promotes differentiation of the remaining population (Gard and Pfeiffer, Development 106:119, 1989). Death of ~ 75% of the cells occurs between days 1 and 3 in culture just before, or soon after, their maturation into OL expressing galatocerebroside (GalC). Hypothesizing that a specific trophic factor is deprived from the system, supplemental BDM conditioned individually by meningeal cells, astroglia, cerebellar interneurons, macrophages were screened for survival-enhancing activity in low-density progenitor cultures. Only astrocyte-conditioned medium (ACM) was effective. Acting in a dose-dependent manner, 33% (v/v) ACM maximally increased the OL progenitor plating efficiency 9-10 fold. The size and purity (>96% $O4^+$cells) of the population was sustained through 21 days of study. ACM was neither mitogenic for progenitors nor did it impair differentiation as determined by the ordered expression of galactocerebroside and myelin basic protein into myelin-like membrane. These data support the concept of an interval during OL development when survival is regulated independently of proliferation and differentiation by a soluble astroglial factor. Supported by NIH grant NS10861.

23.24

THE EFFECTS OF EPIDERMAL GROWTH FACTOR (EGF) ON SEPTAL CHOLINERGIC NEURONAL DIFFERENTIATION. I.E.Mazzoni* and R.L.Kenigsberg. Centre de Recherche Pediatrique, Hopital Ste-Justine, Montreal, Quebec, Canada, H3T 1C5.

We found that 7 days of exposure to EGF decreases choline acetyltransferase (ChAT) activity in primary dissociated fetal rat medial septal cultures indirectly via proliferating glia. The decrease in ChAT enzymatic activity was accompanied by a comparable decrease in the number of acetylcholinesterase (AChE) positive neurons. In order to clearly determine whether EGF was affecting the survival of a subset of the cholinergic neurons in our cultures, 7 days of EGF treatment was followed by 2 days of exposure to nerve growth factor (NGF). In these cultures, ChAT activity levels returned to or surpassed control values, while the number of AChE positive cells returned to control levels. This suggests that EGF is not affecting cholinergic cell survival but acting as a de-differentiating factor on a subset of cholinergic neurons in our cultures which are responsive to NGF. In addition, we found that the proliferative response of the glia in our cultures to EGF as assessed by ^{3}H-thymidine incorporation, preceded the decrease in ChAT enzymatic activity by 48 h. Furthermore, the EGF-induced increases in thymidine uptake, like the decreases in ChAT were dose-dependent. At the moment the response of potential proliferating glial cells in our culture (i.e. astroglia, oligodendrocytes and microglia) to EGF are being assessed in order to identify which glial cell types and in turn which glial-derived molecules are implicated in the cholinergic cell response to EGF.

23.25

THE ROLE OF bFGF IN NEURAL CONTROL OF ADRENOCORTICAL CELL PROLIFERATION. D.P.Basile* and M.A.Holzwarth. Department Physiology & Biophysics, Univ. Illinois, Urbana, IL 61801

We are investigating the role of bFGF as an autocrine mitogen in compensatory adrenocortical growth (CAG), a neurally mediated cell proliferation which occurs in the remaining adrenal in response to unilateral adrenalectomy. Isolated rat capsule-glomerulosa (CG) preps incubated in vitro 24 hrs after unilateral adrenal-ectomy demonstrate a 33±13% increase in ^{3}H-thymidine uptake. Basic FGF (3ng/ml) stimulates a 68±14% increase in DNA synthesis rate in rat CG preps in vitro and has been localized immunocytochemically (anti-$_{N1-24}$bFGF) in glomerulosa and outer fasciculata cells and in some medullary cells, but not in adrenocortical nerves. Molecular forms of rat adrenal bFGF are being identified by SDS-PAGE and Western blot. Suramin (1mM), which blocks bFGF-receptor interaction, inhibited the effect of bFGF (3ng/ml) on CG preps in vitro. Suramin was then used to test the role of bFGF in CAG in vivo; a high dose (5mg/100gBW) enhanced the baseline DNA synthesis rate within 24 hrs. A low dose (0.2mg/100gBW, 1 wk prior) did not affect basal DNA synthesis but surprisingly enhanced rather than blocked the CAG response. Presumably this is the result of bFGF receptor up-regulation because CG preps from suramin-pretreated rats showed an enhanced response to exogenous bFGF in vitro. These data suggest that bFGF participates in the mediation of CAG.

24.1

REGENERATION CONTRIBUTES TO THE FUNCTIONAL REPAIR OF SPINAL CORD INJURIES IN EMBRYONIC CHICKEN. S.J. Hasan, H.S. Keirstead* and J.D. Steeves. Departments of Anatomy and Zoology, University of British Columbia, Vancouver, B.C., V6T 1Z4.

Previous results have demonstrated complete anatomical and functional repair of descending brainstem-spinal projections in chicken embryos that underwent thoracic cord transection prior to embryonic day (E)13. To determine to what extent regeneration is contributing to this repair process, we have conducted experiments using a double retrograde tract-tracing protocol. On E8-12, the upper lumbar spinal cord was injected with the first fluorescent tracing dye to label the existing brainstem-spinal neurons. One to two days later (on E10-14), the mid-thoracic spinal cord was completely transected. After an additional 5-7 days, the second fluorescent tracing dye was injected into the lumbar cord, caudal to the site of transection. Two days later (on E17-20), the CNS was fixed, sectioned, and the brainstem and spinal cord tissue sections were then viewed with epifluorescence microscopy. Our findings indicate that there are double labelled brainstem-spinal neurons after a transection prior to E13 and the number of double labelled brainstem-spinal neurons decreases after an E13-14 transection. In addition, at each subsequent stage of development (between E9-13), a higher ratio of double labelled brainstem-spinal neurons (indicating regeneration of previously severed axons) to the number of cell bodies labelled with the second fluorescent tracer alone (indicating possible subsequent development) was observed. This would suggest that regeneration of previously axotomized fibers increasingly contributes to the observed anatomical and functional recovery after thoracic cord transections prior to E13. (Supported by the MRC and NSERC of Canada.)

24.2

DEVELOPMENTAL TRANSITION IN THE EMBRYONIC CHICK SPINAL CORD FROM PERMISSIVE TO NONPERMISSIVE SUBSTRATES FOR NEURONAL DIFFERENTIATION. K.W. Cheng, D.W. Ethell[1], L.M. Jordan and J.D. Steeves[1], Dept. of Physiology, Univ. of Manitoba, Winnipeg, MB, Canada, and [1]Dept. of Zoology, Univ. of British Columbia, Vancouver, BC, Canada.

Recent studies of spinal cord development and plasticity, in embryonic chick, have demonstrated a loss in regenerative ability correlated with a developmental stage. This report describes effects of plasma membranes from embryonic chick spinal cords on cultures of neuroblastoma X glioma hybrid NG108-15 cells, as an *in vitro* neurite outgrowth assay for permissive and nonpermissive substrates. Thoracic spinal cord segments were isolated from embryonic chicks at different stages of development (E10-E21), homogenized in isotonic buffer, and fractionated by differential centrifugation to obtain plasma membranes for coating dishes as substrate for cultures of NG108-15 cells. Upon coatings of E10 or E12 membranes, NG108-15 cells adhered strongly to the substrate, began to differentiate after 1-2 h, and became highly differentiated with neurites longer than 2-3 times cell diameter after 24 h in culture. The permissive effect of E10 membranes was observed to be slightly higher than that of the E12 cords. In contrast, pre-coating with E18 membranes prevented adhesion and differentiation of NG108-15 cells, which remained undifferentiated as clumps with round morphology even after 24 h in culture. These results indicate that the embryonic chick spinal cord undergoes a developmental transition from permissive to nonpermissive substrates for neuritogenesis, correlating with our previous observation that, upon transection of the thoracic spinal cord prior to, but not after, embryonic day 13, chicks can repair to a complete functional recovery.

24.3

PROTEIN CHANGES ASSOCIATED WITH THE PERMISSIVE AND RESTRICTIVE PERIODS FOR EMBRYONIC SPINAL CORD REPAIR IN THE CHICK. D.W. Ethell and J.D. Steeves. Departments of Anatomy and Zoology, University of British Columbia (UBC), Vancouver, B.C., V6T 1Z4

Recent experiments in our lab have established both a permissive and restrictive period for axonal repair/regeneration, following an upper thoracic spinal cord transection. The transition from permissive to restrictive environments occurs around embryonic day (E) 13 of chick development. Major protein changes accompanying the transition in regenerative capacity have been investigated. Upper thoracic spinal cord segments were micro-dissected from chick embryos aged E6 to E20. Proteins were purified from the spinal cords and run on 2D gels. Protein spots were visualized using either a high resolution silver stain protocol or autoradiography.

Two sets of proteins have been sought in comparing these 2D gels: 1) proteins present exclusively after E13 (possibly contributing to inhibition of repair), termed late neural proteins (LNPs) and, 2) proteins present exclusively before E13 (possibly supporting axonal repair), termed early neural proteins (ENPs). At least seven potential LNPs and eight ENPs have been identified as conforming to these criteria. Since the functions of these proteins cannot be determined from 2D gels alone, LNPs and ENPs are currently being purified for micro-sequencing. Subsequent cloning of cDNAs for LNPs and ENPs is a long term strategy. (Supported by MRC).

24.4

DEVELOPMENTAL EXPRESSION AND THE EFFECT OF SPINAL CORD TRANSECTION ON BRAINSTEM GAP 43 AND α-TUBULIN mRNA LEVELS IN THE CHICK EMBRYO. D.M. Pataky, S.J. Hasan and J.D. Steeves, Dept. of Zoology, UBC, Vancouver, Canada V6T 1Z4.

Previous work in our lab has determined there is a permissive (before E13) and restrictive (E13 or older) period for functional repair after a complete thoracic spinal cord transection. This study examines GAP 43 and α-tubulin mRNA levels during development, and after spinal cord transection during both the permissive and restrictive periods for repair. Cytoplasmic RNA was isolated from the pons/medulla of E4-E21 chick embryos, some of which received complete spinal cord transections at E11 (permissive) or E14 (restrictive), with 1,3,5 and 7 days survival post-transection (PT). Northern blotting was performed using cRNA probes generated from a chicken GAP 43 cDNA (kind gift of Dr. L. Baizer) and a rat α-tubulin cDNA (kind gift of Dr. F. Miller).

Developmentally, α-tubulin mRNA levels are very low at E4, with a large increase at E6, and a smaller increase to peak at E8. Levels then decrease gradually to E17-E19, and drop to just above E4 levels by E21. Transection on E11 results in little change, until 7 days PT when there is a decrease below control α-tubulin mRNA levels. Transection on E14 results in increased α-tubulin mRNA levels 1 day PT, followed by a subsequent decrease below control levels by 5 days PT, returning to control levels by 7 days PT. GAP 43 mRNA is almost undetectable at E4, with a considerable increase at E7.5, and a smaller increase to peak at E10. Levels decrease at E14, then drop to almost undetectable amounts through E17-21. Transection on E11 results in decreased levels 1 day PT, rising above control levels at 3 days PT, then decreasing below control levels by 7 days PT. Transection on E14 results in increased levels peaking 5 days PT, followed by a decrease, though still above control levels, by 7 days PT. Future experiments will include *in situ* hybridization histochemistry. (Supported by Rick Hansen Man in Motion Legacy Fund and British Columbia Health Research Foundation)

24.5

ACUTE SPINAL CORD COMPRESSION INJURY RESULTS IN REGENERATION-ASSOCIATED GENE EXPRESSION IN ADULT RAT RUBROSPINAL NEURONS. E. Theriault[1], K.M. Mearow[2], W. Tetzlaff[3] and C.H. Tator[1]. Univ. of Toronto[1], McMaster Univ.[2], and Univ. of Calgary[3], Canada.

The recent application of in situ hyridization techniques to experimental spinal cord injury has allowed the study of the expression of specific genes in lesioned CNS neurons. Since the mechanism of injury in the majority of human traumatic spinal cord injuries involves rapid cord *compression* due to bone displacement, accompanying fracture-dislocation, or burst fracture (Tator, 1983), we have used the rat model of clip compression injury to examine this question in identified populations of adult rubrospinal neurons. We have used in situ hybridization techniques with both radioactive and non-radioactive labelled probes to examine the mRNA levels for GAP-43, tubulin, actin, ChAT, GAD and glutaminase (GLU). Our preliminary results reveal a population of magnocellular red nucleus (RN) neurons with elevated expression of GAP-43 mRNA at 1-2 but not at 4 weeks post-lesion. Changes in neurotransmitter-related mRNA for GAD and ChAT were not detected, however transcription of GLU mRNA appeared to decrease in large magnocellular RN neurons. We are currently using fluorescent retrograde labelling techniques to more clearly identify the responding populations of RN neurons. These and other findings suggest that contusion injuries of the spinal cord can result in regeneration-associated gene expression in intrinsic CNS neurons and raise the question of how to sustain and manipulate this 'new growth' mode of gene expression in the adult nervous system.

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24.6

CHANGES IN mRNA LEVELS FOR GAP43, TUBULIN AND NEUROFILAMENT-M IN RAT SPINAL MOTONEURONS AFTER PROXIMAL VERSUS DISTAL AXOTOMY. B.J. Tsui, S.L. Cassar and W. Tetzlaff, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

We have examined whether spinal motor neurons display distance-dependence in their expression of mRNA for GAP43, α-tubulin and the medium neurofilament subunit, NFM, following axotomy. For a proximal lesion the L5 spinal nerve was transected 5 mm distal to the DRG. For a distal lesion the common peroneal nerve was transected at the entry into the muscle. The cells were retrogradely filled with either Fast Blue or Fluorogold to restrict the analysis to axotomized (labelled) neurons only. *In situ* hybridization was performed on frozen sections of the spinal cords. At 7, 14 and 21 days post lesion, there was an increase in both GAP43 and α-tubulin mRNA expression. The increase in GAP43 mRNA expression following distal lesion was not different than that observed after proximal lesion. The increases in α-tubulin mRNA were greater after proximal lesion than after distal lesion. The mRNA levels for NFM decreased following both proximal and distal axotomy, and were not different after 14 days. The differing extent of the changes in mRNA expression in response to distal versus proximal lesions suggests that different molecular signals are involved in the regulation of these regeneration associated genes.

Supported by Medical Research Council of Canada.

24.7

RAP-14: A PROTEIN ASSOCIATED WITH THE TIME COURSE OF THE ABILITY OF RAT SPINAL CORD TO RECOVER FUNCTION FOLLOWING INJURY W. E. Edmonston, Jr., Colgate University, Hamilton, NY 13346

Electrophoretic analysis of the polypeptide composition of fetal, neonatal and adult rat spinal cord tissue reveals a 14kD protein which is present in the younger tissue (Fetal 19 through Neonatal 15 to 18 days), but absent in older tissue. The disappearance of the protein (RAP-14 -- Regeneration Associated Protein) coincides with the cessation of the ability of rat spinal cord to reorganize and re-establish function following injury. Adult frog CNS also contains RAP-14. Immunoblot analysis established that RAP-14 is neither Myelin Basic Protein nor Nerve Growth Factor. Differential centrifugation of 3-6-, 10-, 12-, 16-, and 23-day old rat tissue revealed a progressive disappearance from specific cell fractions with age in accord with expectations regarding its manufacture and storage within the cell.

24.8

THE GROWTH-ASSOCIATED PROTEIN GAP43 IS EXPRESSED IN DEGENERATING PERIPHERAL NERVE AFTER AXOTOMY. M.A. Bisby[1], K.C. Harrington[2]* and W. Tetzlaff[2]. [1]Dept. of Physiology, Queen's University, Kingston, Ontario, Canada and [2]Depts. of Anatomy and Pathology, University of Calgary, Calgary, Alberta, T2N 4N1, Canada.

We reported that GAP43 immunoreactivity was associated not only with regenerating axons in rat sciatic nerve, but also with Schwann cells (Tetzlaff et al., J. Neurosci., 9:1303, 1989). Further, GAP43 immunoreactivity was detected surrounding unmyelinated axon bundles in normal nerve. These findings raise the possibility that GAP43 is synthesized by cells within peripheral nerve. Northern blot analysis showed that an appropriate size signal (1.4 kB) was recognized by a GAP43 cDNA probe (Basi et al., Cell; 49:785, 1987) in degenerating rat facial nerve samples. No signal was detectable in normal nerve. Now, using the more sensitive technique of reverse transcription followed by polymerise chain reaction amplification with primers spanning the entire coding region, we have detected amplified products of the expected size in normal samples, and higher levels in degenerating samples. GAP43, once thought to be exclusively neuronal, may also be expressed by cells within peripheral nerve. Increased expression in degenerating peripheral nerve suggests a site of action for GAP43 additional to its implied role within the growing axon.

24.9

LOCALIZATION OF THROMBOSPONDIN FOLLOWING OPTIC NERVE CRUSH IN THE MOUSE AND GOLDFISH.
J.R. Hoffman, K.S. O'Shea, and V.M. Dixit*
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Thrombospondin (TSP) is a 420 kDa component of the extracellular matrix present at high levels during development and in regeneration of the peripheral nervous system. In the current study, adult male CD-1 mice or 5-10 cm goldfish were anesthetized and the optic nerve was crushed within 2-3 mm of exiting the eye. After 1-60 d, frozen sections were cut and processed to localize TSP. Control mouse optic nerve showed low levels of TSP associated with glial cells, while the goldfish optic nerve had much higher levels of TSP in a fasiculated pattern. Following nerve crush in the mouse, TSP levels increased in regions of gliosis and cellular infiltration near the crush site. In the goldfish optic nerve, TSP levels rapidly increased throughout the nerve forming distinct tracts of TSP running from the retina past the crush site. Following nerve crush, TSP levels in mouse optic nerve increased somewhat randomly in association with gliosis. TSP levels in the goldfish optic nerve were much higher and very organized.

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24.10

MATRIX REMODELLING AND INTERSTITIAL CELLS AT DENERVATED FROG NEUROMUSCULAR JUNCTIONS. E. A. Connor and H. A. Martin.* Univ. of Massachusetts. Amherst, MA.

When a motor nerve is damaged, the connective tissue in junctional regions of skeletal muscle undergoes striking changes. Interstitial cells and several matrix molecules including fibronectin and tenascin are concentrated near denervated neuromuscular junctions. These interstitial cells may be responsible for remodelling the extracellular matrix and thereby may influence synapse regeneration. Previously, we identified two monoclonal antibodies (mAbs), 2G3 and 7B11, that stain muscle connective tissue and whose staining patterns are altered by denervation; both antigens are concentrated in junctional regions of denervated muscle. To determine if the mAb 2G3 and 7B11 antigens were produced by interstitial cells, muscles were dissociated enzymatically and the freed interstitial cells were maintained *in vitro*. By 1 week in culture, interstitial cells produced mAb 2G3 and 7B11 antigens as well as tenascin and fibronectin. The mAb 2G3 and 7B11 antigens were found to be extracellular and bound to glass coverslips. MAb 2G3 stain was distributed in a filamentous pattern coincident with that of fibronectin. In Western blots, mAb 2G3 recognized a protein of 235kD molecular weight. MAb 7B11 stain was distributed in a punctate pattern and in young cultures was excluded from substrate areas apposed to stress fibers. In older cultures, the substrate was uniformly stained. These data suggest that the interstitial cells remodel the extracellular matrix in junctional regions of muscle after denervation and are consistent with findings in rat muscle (Gatchalian et al., 1989). The mAb 2G3 and 7B11 antigens may be previously uncharacterized matrix molecules whose distribution is altered by denervation.

24.11

ROLE OF EXTRACELLULAR MATRIX MOLECULES AND MICROGLIAL CELLS IN REGENERATION OF LEECH CNS
L. Masuda-Nakagawa*, C. Wiedemann*, A. Walz*, D. Neely* and J.G. Nicholls Dept. Pharmacology, Biocenter, 4056 Basel, Switzerland

The aim of these experiments is to analyze how protein molecules in extracellular matrix (ECM) of leech CNS influence the shapes of neurons and of microglial cells that accumulate at sites of injury. An interesting paradox is that microglial cells in situ become closely associated with laminin and regenerating axons as they grow through a lesion; by contrast in co-cultures of microglia and identified neurons, they produce contact inhibition of neurite outgrowth. The pattern of neuronal outgrowth depends critically upon the molecular composition of the substrate. For example on laminin the processes of a Retzius cell are slender, straight and unbranched; on the plant lectin Con A they are thick, curved and branched. Another molecule, tenascin, has now been purified by gradient centrifugation of high pH extracts of ECM surrounding leech CNS. On highly enriched tenascin fractions the pattern of neurite outgrowth resembles that on Con A. Substrate molecules also influence the shapes of cultured microglial cells. On Con A they are rounded and resemble fried eggs; on ECM they are thin and spindle-shaped. Tests are now being made to determine whether microglial cells secrete growth promoting molecules and whether their functions are influenced by the substrates that they come into contact with.

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24.12

EFFECTS OF AXOTOMY ON PROTEIN SYNTHESIS AND DEGRADATION IN THE RAT HYPOGLOSSAL NUCLEUS Y. Sun*, L. Sokoloff and C. Beebe Smith, Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20892

In previous studies we found that unilateral XII[th] n. axotomy results in an apparent increase in the rate of protein synthesis in the ipsilateral hypoglossal nucleus (Smith et al. *J.Neurosci* 4:2489, 1984). This effect is seen as early as 48 h postaxotomy and returns to normal by postaxotomy Day 35. Local rates of protein synthesis were determined with the quantitative autoradiographic [1-^{14}C]leucine method with the assumption that there was no recycling of unlabeled leucine derived from protein degradation back into the precursor amino acid pool for protein synthesis. We recently determined that there is such recycling and that in the brain as a whole 42% of leucine in the precursor pool is derived from protein degradation (Smith et al. *PNAS* 85:9341, 1988). The rates of protein synthesis determined in our previous study were, therefore, underestimated. In the present study we have examined the degree of recycling in the regenerating nucleus. In order to determine the degree of recycling in whole brain we measured the ratio of specific activity of leucine in the precursor pool (tRNA-bound leucine) to that in plasma in rats with the brain in a steady state for both labeled and unlabeled leucine. The concentration of leucyl-tRNA in brain is too low to measure in individual brain regions, however; instead we measured the steady state specific activity of leucine in the total acid-soluble pool as an index of the tRNA-bound pool in the ipsilateral and contralateral hypoglossal nuclei of nine adult, male rats five days after unilateral hypoglossal axotomy. The steady state ratios of specific activities were significantly (6%) lower in the axotomized as compared with the control side ($P<.05$, paired t-Test). These results confirm our earlier finding that protein synthesis is increased in the regenerating hypoglossal nucleus, but indicate that protein degradation is also increased under these conditions.

24.13

PURIFICATION AND CHARACTERIZATION OF p68/70 FROM GOLDFISH BRAIN. M.L. Leski*, M. Moos*, K. Seamon* and B.W. Agranoff. Dept. of Biological Chemistry and Neuroscience Lab, Univ. of Michigan, Ann Arbor, MI 48104-1687; Div. of Biochemistry and Biophysics, Center for Biological Evaluation and Research, FDA, Bethesda, MD 20892.

Comparison of autoradiographs of two-dimensional polyacrylamide gels following incorporation of radioactivity from labeled amino acids into goldfish (*Carassius auratus*) retinal proteins in controls and in retinas in which the optic nerve had been previously lesioned, reveals a number of differences. One such difference is a dramatic increase of labeling of two acidic proteins of apparent molecular weights 68 and 70 kDa (p68/70). We have purified this protein doublet to homogeneity from goldfish brain homogenates. This was accomplished by sequential chromatography of the 100,000 x g supernatant on DEAE-Sephacel, copper (II)-charged iminodiacetic acid agarose and TSK-3000. Initial attempts to sequence p68/70 indicated that it is N-terminal blocked. Chemical and enzymatic treatment of p68/70 has yielded several sequenceable stretches. One thirteen amino acid segment has identity with a synaptic vesicle protein from the *Torpedo*, VAT-1 (Linial, M., Miller, K. and Scheller, R.H., *Neuron* 2:1265-73, 1989). However, a polyclonal antibody against p68/70 does not recognize VAT-1. While p68/70 is not associated with synaptic vesicles, much of it appears to be membrane-bound. In extraction studies neither 1 M NaCl, 1 M neutral hydroxylamine, 5 M urea nor 200 mM sodium carbonate buffer, pH 11 completely released p68/70 from the membrane, while 1% Triton X-100 did, a result that suggests that the doublet is integral to the membrane. In phase partitioning experiments using Triton X-114, both cytosolic and membrane-bound p68/70 partitioned into the aqueous phase, indicating that the membrane association is not likely attributable to covalently-bound lipid. (Supported by NIH grant EY 05947.)

24.14

EXPRESSION OF p68/70 IN REGENERATING GOLDFISH VISUAL SYSTEM. G.R. Wilmot, P.R. Raymond and B.W. Agranoff. Mental Health Research Inst. and Dept. Anat. and Cell Biol., University of Michigan, Ann Arbor, MI 48104.

We previously described increased amino acid incorporation into a nominally 68-70 kDa protein doublet in the goldfish retina following optic nerve crush. This doublet (p68/70) is slowly transported via the optic nerve to the tectum, indicating that it is present in retinal ganglion cells (RGC's). We have developed an affinity-purified polyclonal antibody (α–p68/70) which specifically recognizes p68/70, and have utilized α–p68/70 to characterize the expression of p68/70 in normal and regenerating goldfish visual system. The cellular distribution of p68/70 was examined in cryostat sections of fixed tissue using avidin-fluorescein or avidin-biotin-peroxidase. In the central retina, immunoreactivity was highest in RGC dendrites, somata and axons. During regeneration the immunoreactivity of RGC somata increased markedly. Occasional immunolabeled cells were also observed throughout the retina. Most of these scattered cells were also recognized by the monoclonal antibody NN1, which recognizes phagocytic cells of presumed monocytic origin. The remaining α–p68/70 positive cells that were NN1 negative were located in the outer nuclear layer and appear to be rod precursors and/or recently born rods. In peripheral retina adjacent to the germinal epithelium, which contains recently differentiated retinal neurons, α–p68/70 immunoreactivity was observed in all layers. Immunostaining was also observed in the germinal epithelial cells, which give rise to new retinal neurons. In tectum, young neuronal cell bodies adjacent to the tectal germinal zone were prominently labeled. Immunolabeling of the remainder of the tectum was restricted to fiber layers and was most prominent in the *s. marginale*, which receives input from the *torus longitudinalis*. RGC fibers terminating within the *s. fibrosum et griseum superficiale* were lightly labeled in normal tectum but were intensely immunoreactive during regeneration. Immunofluorescent labeling of retinal explants revealed that p68/70 is present in growth cones, filopodia and intracellular membranous organelles of regenerating RGC neurites. The expression of p68/70 thus suggests that this protein plays a role in axonal growth and possibly in cellular differentiation as well. (Supported by NIH grant EY 05947.)

24.15

IN SITU HYBRIDIZATION STUDIES OF J1/TENASCIN REAPPEARANCE FOLLOWING TRAUMATIC BRAIN INJURY IN MOUSE AND HUMAN. E.D. Laywell*, T.F. O'Brien, K. Harrington*, D.A. Steindler, M. Schachner, and U. Dorries*. Univ. of Tenn., Memphis and ETH, Zurich

J1/tenascin is an extracellular matrix (ECM) molecule with a prominent distribution during development. In the brain, J1/tenascin is associated during late embryonic and early postnatal development with boundaries that outline many functional units such as cortical barrels, neostriatal striosomes, and various nuclei. After functional brain patterns have been stabilized, J1/tenascin expression is greatly reduced, or disappears altogether as seen immunocytochemically or biochemically.

Recently, our labs have presented immunocytochemical evidence for enhanced J1/tenascin expression following lesions of the adult mouse cerebellum and cerebral cortex. In immunocytochemical studies of developing and lesioned adult brains, astrocytes appear to be the primary source of tenascin; however, because other cell types have been identified as sources of J1/tenascin (eg. fibroblasts), a more precise approach was needed to unequivocally assign enhanced J1/tenascin expression to a particular cell type. Here, we use a J1/tenascin riboprobe to identify and localize the cell type responsible for this reappearance. *In situ* hybridization, using a digoxygenin labeled riboprobe to J1/tenascin, was performed on sections of lesioned adult mouse brain and postmortem human brain tissue following traumatic head injury. In all cases the J1/tenascin probe proved to be an extremely sensitive marker of some of the earliest cellular changes following brain lesion. In double labeling studies, the J1/tenascin probe identifies a population of glial fibrillary acidic protein positive astrocytes. In particular, Golgi epithelial cells of the cerebellum and astrocytes of the cortex exhibit a discretely localized distribution around lesion sites.

In conclusion, *in situ* hybridization with this newly developed riboprobe to J1/tenascin appears to be a very reliable indicator of early astrocyte response to brain injury. The functional relevance of injury-induced, enhanced expression of such ECM molecules is not yet known. However, it is possible that these molecules may adversely affect neuritic regeneration in and around brain wounds. Supported by NSF and NIH grants BNS-8911514 and NS 20856.

24.16

IMMUNOCYTOCHEMICAL DETECTION OF TENASCIN IN THE TRANSECTED ADULT RAT OPTIC NERVE. A. Ajemian* and S. David, Centre for Research in Neuroscience, Montreal Gen.Hosp.Res.Inst. and McGill Univ., 1650 Cedar Ave. Montreal, Quebec, Canada.

Growth promoting and growth inhibitory molecules are both likely to play a role in controlling axonal regeneration in the adult mammalian CNS. The presence of an inhibitory molecule associated with mammalian oligodendrocytes and CNS myelin has been reported (Schwab, TINS 13: 452). The ECM molecule tenascin (TEN) which is produced by astrocytes in the CNS, was also recently reported to be inhibitory for cell attachment and neurite growth of CNS neurons (Faissner & Kruse, Neuron 5: 627).

In this study we have examined the distribution of TEN in the lesioned adult rat optic nerve. Longitudinal cryostat sections of fixed rat optic nerve, obtained 5 days to several weeks after transection were double labeled by indirect immunofluorescence with anti-TEN and anti-GFAP antibodies. Intense tenascin-like immunoreactivity was seen in a narrow region at the site of transection, at 5 and 10 days after transection, but not after 3 weeks. In some areas the TEN^+ labeling showed correspondence with $GFAP^+$ astrocytes. It is possible that tenascin which delineates the edge of the lesion, might serve to limit the growth of axons across the lesion site. (*Supported by the Canadian MRC*).

24.17

STUDY OF GLYCOSAMINOGLYCANS DERIVED FROM AXONALLY TRANSPORTED PROTEOGLYCANS IN REGENERATING GOLDFISH OPTIC NERVE, Jean F. Challacombe* and J. S. Elam, Department of Biological Science, Florida State University, Tallahassee, Florida 32306

Sulfated proteoglycans (PGs) present in brain contain heparan sulfate (HS) and chondroitin sulfate (CS) GAGs, both of which have been implicated in control of neuronal growth. Previous work in our lab has shown that regenerating goldfish optic nerves axonally transport 60% of $^{35}SO_4$-labelled GAG in CS and 40% in HS, while unoperated control nerves transport 26% in CS and 74% in HS (Coughlin and Elam, Br. Res. 493, 326(89)). To see if there are additional regeneration-related differences in the structure or distribution of transported GAGs, fish were administered unilateral optic nerve crushes and were stored for 21 days at 21 C. Eight hours following intraocular injection of $^{35}SO_4$, optic tracts were removed and processed for isolation of axonally transported GAGs. Results of gel filtration on Sepharose CL-6B show that CS and HS from regenerating tracts are of similar size to those from unoperated control tracts, while paper chromatographic analysis shows that sulfation of CS in regenerating tract remains in the C-4 position of GalNAc. In contrast, a regeneration-correlated increase in ionic strength required for elution is seen during DEAE ion exchange chromatography of CS. Sequential homogenization of tracts with neutral buffer (to extract soluble proteoglycans) followed by buffer containing 1M NaCl-1% Triton X-100 (to extract membranous proteoglycans) showed that regeneration is correlated with a shift in transported $^{35}S04$ (from 49% to 72%) affiliated with the membranous compartment. Taken together, these results indicate that the regeneration-related shift in the proportion of GAGs is accompanied by changes in charge density and in subcellular localization. (Supported by NS 20502)

24.18

ACCUMULATION OF NEUROFILAMENT PROTEINS IN THE REGENERATING FACIAL NERVE. L.T. Wang-Bennett, A. de Jong* and D.J. Liebl*. Dept. of Otorhinolaryngology and Communicative Sci., Baylor College of Med., Houston, TX 77030.

The accumulation of the neurofilament (NF) proteins in the regenerating facial nerve of adult New Zealand rabbits was compared in two surgical models. The animals were operated on bilaterally, with a chamber model placed on one side and a cable graft model inserted on the contralateral side. Normal nerve from unoperated animals or nerve removed during nerve repair surgery served as controls. Using immunoblot techniques and densitometric measurement, we examined specific changes in the individual NF [High (H), Medium (M), and Low (L) molecular weight] at regeneration time of 3 and 7 wk. Linearity of the densitometric system was established by separation and immunostaining of serial dilutions of known NF.

The amount of all three NF's decreased during the regeneration, but did not behave similarly. The NFH in the distal segment of the chamber repaired nerve at 7 wk was 60% - 70% of the preoperative state, which correlated with a previous morphological study of axonal caliber during regeneration. The NFH contents showed a regeneration time-dependent change and an interaction effect from time x repair models. Accumulation of NFL displayed a site-dependent change. The result was discussed in relation to the progress of axonal sprouting and the differential appearance of NF proteins in the developmental stage. (Supported by Texas Advanced Technology Program #1165 and Coker Memorial Research Foundation.)

24.19

CHANGE IN EXPRESSION OF ACETYLCHOLINESTERASE IN TERMINAL FIELDS OF GUSTATORY PRIMARY AFFERENTS FOLLOWING PERIPHERAL NERVE DAMAGE, REGENERATION, AND REINNERVATION OF TASTE BUDS. M.A. Barry, R.G. Wehby*, L.D. Savoy*, and M.E. Frank, Dept. of Biostructure and Function, Univ. Conn. Health Ctr., Farmington, CT 06030.

Damage to the peripheral processes of primary sensory afferents generally has subtle morphological but often profound physiological effects in adult mammals. Little is known about these effects for the gustatory system. Previous studies (Barry et al. 1991, J. Elect. Micros. Tech., In Press) have shown that the terminal fields of the chorda tympani branch of the facial nerve and lingual branch of the glossopharyngeal nerve in the nucleus of the solitary tract (NST) are characterized by intense staining with acetylcholinesterase (AChE) in hamsters. We examined AChE staining in the NST after cutting or crushing these nerves unilaterally in adult male golden hamsters (*Mesocricetus auratus*). At 1 week following nerve damage there was a decrease in AChE staining limited to the area corresponding to the terminal fields. By 2 weeks, the terminal fields could not be easily distinguished from surrounding regions. There was a concomitant loss of foliate taste buds on the affected side of the tongue. At 4-5 weeks, the nerves had apparently regenerated since foliate taste buds reappeared, but there was little or no recovery of AChE staining in the NST. The recovery of AChE activity may correlate with the complete return of gustatory function. NIH-DC00168-10.

24.20

APOLIPOPROTEIN-E ACCUMULATION IN THE RAT LIMBIC SYSTEM FOLLOWING SYSTEMIC ADMINISTRATION OF KAINIC ACID. G.E. Handelmann and D.H. Lowenstein. Gladstone Foundation Labs. and Dept. of Neurology, Univ. of California, San Francisco, CA 94140.

Apolipoprotein (apo)-E, a lipid transport protein, is synthesized by astrocytes in the rat brain. One of the responses of astrocytes to brain injury is the increased secretion of factors which may aid processes of tissue repair. We therefore examined the distribution of apo-E in the brain by immunocytochemistry at various times after systemic administration of kainic acid (KA; 10 mg/kg). We also examined the production of apo-E mRNA in the hippocampus by Northern blots. The KA produced extensive tissue damage in the hippocampus, amygdala, and entorhinal cortex. Within 7 days, astrocytes in these regions demonstrated increased apo-E immunoreactivity and there was considerable extracellular accumulation of apo-E. Apo-E positive macrophages were also present within the meninges and the third ventricle. After 28 days, all three brain regions still contained large amounts of extracellular apo-E, although the immunoreactivity within the astrocytes was decreased. At this time, apo-E-positive macrophages were present in the neuropil of the amygdala and entorhinal cortex, but not the hippocampus. The Northern blots indicated that apo-E mRNA increased in the hippocampus around 14 days after KA treatment, although there was considerable variability among rats in the timing of the increase. In summary, apo-E accumulates in injured portions of the limbic system following KA treatment. At first, apo-E is probably contributed by astrocytes. Later, apo-E may also be derived from invading macrophages. The production of apo-E mRNA does not appear to be associated with the immediate effects of KA, but instead may be related to the repair or remodeling of nervous tissue.

24.21

POSTTRANSLATIONAL ARGINYLATION OF PROTEINS IN EXPLANTS OF CRUSHED SCIATIC NERVES OF RATS. N-S Xu, *G. Chakraborty* and N. Ingoglia. Dep't of Physiology, N. J. Med. Sch, Newark, N.J. 07103.

Posttranslational arginylation of proteins has been demonstrated in fractions of high speed supernatants of sciatic nerves but not in intact tissue. In the present experiments we have utilized the finding that Arg is added at the N-terminus of the target protein to demonstrate posttranslational modification of endogenous proteins in sciatic nerve explants. Sciatic nerves were crushed in vivo and 2 hrs later segments of nerve, including the site of the crush, were removed and incubated in media containing ^{3}H-Arg. Incorporation of ^{3}H-Arg into proteins was analyzed by acid precipitation and the presence of label at the N-terminus by micro Edman degradation. 20% of the protein bound ^{3}H-Arg was released by the Edman reaction indicating that it was added posttranslationally rather than through protein synthesis. Nerves not crushed prior to explant and incubation showed no evidence of N-terminal labeling. These results support previous in vitro evidence for increases in N-terminal arginylation of proteins following injury to rat peripheral nerves. (Supported by the Foundation of UMDNJ)

24.22

A LOW MOLECULAR WEIGHT FACTOR CAN INHIBIT THE ARGINYLATION OF tRNA AND THE POSTTRANSLATIONAL ARGINYLATION OF PROTEINS IN RAT BRAIN. M. Yu and N. Ingoglia. Dep't of Physiology, New Jersey Medical School, Newark, NJ 07103.

The tRNA mediated N-end arginylation of proteins can only be demonstrated in vitro if low molecular weight molecules are removed by gel filtration chromatography. We have proposed that an endogenous peptide is responsible for the regulation of these reactions. In the present experiments we have isolated and attempted to purify this peptide. Rat brains were extracted with acidic methanol and the 20k xg supernatant was used as the source of the putative regulator. Fractionation on a Sephadex G-50 column demonstrated inhibitory activity (I.A.) eluting at < ~4kDa. When purified by Sep-Pak C18 cartridges (Waters), I.A. was found in a 30% but not an 80% acetonitrile/TFA fraction and eluted at approx. 34 ml when purified further by reverse phase HPLC. This fraction was able to block the arginylation of rabbit liver tRNA suggesting that the regulator prevents protein arginylation by inhibiting the charging of tRNA. We suggest that this factor is an endogenous regulator of the arginylation of RNA and proteins.

TRANSPLANTATION: CORTEX

25.1

SURVIVAL AND DIFFERENTIATION OF FETAL RAT CORTICAL CELL SUSPENSION AFTER TRANSPLANTATION INTO AN ADULT RAT CEREBRAL CORTICAL LESION CAVITY. B.W. Chopko, R.L. Berry, T.J. Voneida. Dept. of Neurobiology, N.E. Ohio Univ. College of Medicine, Rootstown, Ohio 44272, U.S.A.

The anatomical characteristics of a fetal cell suspension graft transplanted into an adult cerebral cortical lesion cavity are largely unknown. A suspension of frontal cerebral cortical tissue, obtained from rats on embryonic day 15, was prepared by sequential trypsinization and centrifugation. Immediately before transplantation, a sample of suspension was used to establish cell cultures. Cortical suspensions were grafted into 3 adult rat hosts; the transplantation site consisted of a transcortical cavity in the rostral motor cortex, which was produced by aspiration 45 minutes before grafting. After survival periods of 2, 12 and 12 weeks, grafts were studied using Nissl, myelin and axonal stains. All grafts were viable and attached to the lesion cavity, but only 1 completely filled the cavity. Grafts consisted of a dense, nonlaminar mixture of neuronal, glial, inflammatory and spindle cells. Axons were short and sparse at 2 weeks. At 12 weeks, axons were abundant and arranged into a dense tangle of single axons and fascicles; focally, graft axons entered the host brain for distances of greater than 500 μm. Myelin was present only at 12 weeks. Cultures contained cells arranged in both monolayers and multicellular spherical aggregates; filamentous cellular processes were also present. Suspension grafts of fetal cortical tissue survived and differentiated when transplanted into cerebral cortical lesion cavities. Graft success in this paradigm is further testimony to the resilience and plasticity of fetal brain tissue.

25.2

MIGRATION OF TRANSPLANTED EMBRYONIC NEOCORTICAL NEURONS INTO SELECTIVELY NEURON-DEFICIENT CORTEX OF EARLY POSTNATAL MICE. J.D. Macklis. Dept. of Neurology, Prog. in Neuroscience, Harvard Medical School, Children's Hospital, Boston, MA, 02115

The central purpose of this work is the study of neuronal migration and cellular integration following transplantation of immature neocortex into a "custom-generated" pyramidal neuron-deficient host, as both a developmental and transplantation model. Unfocused, noninvasive laser illumination, at long wavelengths that penetrate through nervous system tissue without major absorption, can cause extremely selective, noninvasive, cell-specific damage to desired subpopulations of neurons, targeted by retrograde incorporation of latex nanospheres containing the cytolytic, photoactive chromophore chlorin e_6.

Postnatal day 1-5 mouse pups (n=54) served as hosts for transplants into control (n=49) and experimental lesioned cortices (n=5). Lesions were effected to pups following retrograde neuronal labeling in vivo with photoactive nanospheres. Embryonic day 17 neurons were labeled in vitro with nanoparticles that allowed identification of host and graft cells at both the fluorescence and EM levels. After survival times of 6 days to 12 weeks, serial sections were cut and processed for nanosphere intracellular fluorescence and routine histology; 6 were studied by EM. Camera lucida drawings and higher magnification fluorescence microscopy were used to identify and assess donor neuron migration and cellular integration; EM was used to confirm identities of migrated neurons. Neurons placed near host zones of neuron deficiency within lamina II/III migrated to and integrated within them; control grafts revealed only local spread without laminar preference. Donor neurons within the lesioned zones largely assumed a pyramidal phenotype. These results suggest that transplanted neocortex may seek to restore normal cytoarchitecture, using developmentally age-specific cues to guide migration and integration. Supported by HD00859, Alzheimer's Association, and Hearst Fund.

25.3

HETEROTOPIC AND HOMOTOPIC FETAL CORTICAL TRANSPLANTS TO THE DEVELOPING ROSTRAL CORTEX OF RATS PRODUCE DIFFERENT BEHAVIORAL EFFECTS. T. M. Barth and B. B. Stanfield, Dept. of Psychology, Texas Christian University, Fort Worth, Texas 76129 and Lab. Neurophysiol., NIMH, NIH Animal Center, Poolesville, MD 20837.

Fetal occipital cortical neurons transplanted to the rostral cortex of newborn rat pups (i.e. heterotopic transplants), behave like the host neurons of the recipient cortical region in terms of their eventual projections. Specifically, the transplanted occipital cortical neurons maintain a corticospinal projection even though this projection is eliminated when these neurons develop in their original occipital locale. As expected, homotopic transplants (i.e. fetal rostral transplant to rostral cortex) also maintain a corticospinal projection. These anatomical findings suggest that the behavioral effects of homotopic and heterotopic transplants should be similar. The present experiment was designed to compare the functional capacity of heterotopic and homotopic transplants.

Newborn (P0) rats received small unilateral lesions of the rostral cortex and pieces of 5-bromo-2'-deoxyuridine labeled fetal cortex (E17) from either the rostral or occipital region was placed in the cavity. The rats were tested for somatosensory asymmetries (as measured by bilateral-tactile stimulation tests) and forelimb placing on P45. Previous behavioral experiments have shown that rats with large unilateral neonatal cortical lesions exhibit an enduring ipsilateral somatosensory asymmetry, but no impairments in forelimb placing. The principal finding of the present experiment is that rats with heterotopic transplants showed a marked somatosensory asymmetry and contralateral impairments on forelimb placing tasks while rats with homotopic transplants failed to show significant impairments on any of the behavioral tests used. Bromodeoxyuridine-immunohistochemistry was utilized for positive identification of the transplants in both groups. These data suggest that while homotopic fetal cortical transplants may help ameliorate behavioral deficits following neonatal lesions of the rostral cortex, heterotopic transplants do not, and may indeed exacerbate the deficits, despite their seemingly appropriate projections.

25.4

NOREPINEPHRINE (NE) AND DOPAMINE (DA) INNERVATION OF EMBRYONIC CORTEX GRAFTS IN ADULT RATS WITH CATECHOLAMINE (CA) LESIONS OF THE MEDIAL FOREBRAIN BUNDLE (MFB).
A. Dunn-Meynell & B.E. Levin, Neurol.Svc., DVA Med. Ctr., E.Orange, NJ 07019, Depart. Neurosci., NJ Med.Sch., Newark NJ 07103.

The study was performed to determine the nature of CA innervation of grafts placed in the adult rat mfb. 6-hydroxydopamine was injected into the mfb to lesion CA fibers followed after 2 wk by E15-16 frontal cortex grafts, or sham grafts, to the lesion site. NE and DA innervation were distinguished using dopamine-β-hydroxylase and tyrosine hydroxylase immunoreactivity (DBH-IR and TH-IR) or high affinity ^{3}H desmethylimipramine (DMI) or mazindol uptake. Lesions produced massive depletion of these parameters in distal host structures, regardless of graft presence. However, DA fibers grew into grafts as reflected by patches of high mazindol binding (up to 803 fM/mg) and TH-IR. NE graft innervation was much more sparse with few DBH-IR fibers and no significant DMI binding. Conversely, in additional rats with grafts and no mfb lesions DBH-IR fiber ingrowth was much more extensive. Thus, following CA lesions of the mfb which, in the absence of grafts, allow little or no regenerative sprouting, grafts promote mainly DA fiber sprouting. Supported by DVA Med. Res. Svc. and Foundation of UMDNJ.

25.5

FETAL NEOCORTICAL TRANSPLANT PROJECTIONS DEMONSTRATED BY PHA-L AXONAL TRACING. J.C.Sørensen*, B. Klausen*, J.Zimmer, and A.J.Castro. Instit. of Neurobiology, Aarhus Univ., DK-8000 Aarhus C, Denmark and Dept. of Cell Biol., Neurobiol. and Anatomy, Loyola Sch. Med., Maywood, IL 60153.

Fetal neocortical grafts placed into newborn recipients receive extensive inputs from the host brain. In the present study, PHA-L was used to examine graft *efferents* from fetal (E14-16) neocortical transplants placed homotopically into frontal cortical lesions made immediately prior to grafting in newborn rats. At maturity, PHA-L was injected iontophoretically into the grafts and the animals were sacrificed two weeks later.

In four animals showing injections confined to the transplants. efferent projections were traced into the cortex adjacent to the graft, and other fibers traversed the subcortical white matter to the ipsilateral parietal cortex or traversed the callosum to the contralateral forelimb area. Descending fibers projected through the striatum and internal capsule giving terminal branches to the caudate-putamen, the thalamic ventrobasal and posterior nuclei, claustrum and pontine gray. In one animal, fibers were seen approaching the red nucleus. These projections resembled normal connectivity.

(Supported by the Danish Research Academy, the Danish State Biotechnology Program, the NOVO and Lundbeck Foundations and NIH Grant 13230.)

25.6

PHARMACOLOGIC SPECIFICITY OF REDUCED IMMOBILITY IN A RODENT DEPRESSION MODEL BY MONOAMINERGIC NEURAL TRANSPLANTS D. Dougherty, C.E. Sortwell, and J. Sagen. Dept. Anat. and Cell Biol., Univ. IL at Chicago, Chicago, IL 60612.

Previous findings in our laboratory have indicated that the transplantation of monoaminergic tissue into the frontal neocortex of rats can reduce behavioral deficits in rodent depression models. The purpose of the present study was to pharmacologically characterize the effects of these transplants using specific antagonists. After transplantation of serotonin (5-HT) containing pineal gland tissue, norepinephrine (NE) containing adrenal medullary tissue, a combination of both pineal and adrenal medullary tissue, or sciatic nerve (control) into the rat frontal neocortex, the animals' behavior was assessed using the behavioral despair model. The behavioral despair model measures immobility time while swimming in a clear cylinder as a correlate to antidepressant activity. As in previous studies, monoaminergic transplants but not control transplants produced marked decreases in immobility time. After establishing these baselines, the transplants were assessed pharmacologically with norepinephrine antagonists phentolamine (α-adrenergic) and propranolol (β-adrenergic) and serotonin antagonists metergoline (5-HT1) and pirenperone (5-HT2). If the reduction in behavioral deficits by the transplants is due to the release of monoamines, it should be blocked by specific monoaminergic antagonists. Both serotonergic antagonists blocked the decreased immobility produced by the pineal transplants and the pineal-adrenal cografts, but had no effect on either adrenal or control transplanted animals. Interestingly, α- and β-adrenergic antagonists not only blocked, but overcompensated for the adrenal transplants, producing a large increase in immobility and blocked decreased immobility produced in cografted animals. The α- and β- antagonists had no effect on pineal or control transplanted animals. Immunocytochemical analysis of the transplants revealed that both the adrenal medullary and pineal transplants survived well and continued to produce high levels of monoamines. The results of this study suggest that the transplantation of monoaminergic tissue into the CNS may reduce depressive symptoms by increasing CNS levels of NE or 5-HT.

25.7

BIOCHEMICAL AND BEHAVIORAL ANALYSIS OF NEUROTOXIN LESIONS AND MONOAMINERGIC NEURAL TRANSPLANTS IN THE RAT FRONTAL NEOCORTEX C.E. Sortwell and J. Sagen. Dept. Anat. and Cell Biol., Univ. IL at Chicago, Chicago, IL 60612.

Monoaminergic neural transplants into the rat frontal cortex have been demonstrated in our laboratory to reduce immobility in the Forced Swimming Test (FST), an animal model where reduced immobility time is regarded to reflect increased antidepressant activity. Our past findings suggest that neural transplants may provide a long-term source of monoamines that may correct the central imbalance of noradrenergic and serotonergic function believed to cause depression. This study investigates the effect of injections of monoaminergic neurotoxins to the frontal neocortex of rats on FST immobility time and the ability of monoaminergic neural grafts to reverse this depletion. Either serotonergic neurotoxin 5,6-DHT, adrenergic neurotoxin 6-OHDA or an equal volume of saline vehicle were stereotaxically injected into the frontal neocortex of rats. One week after injecting, 5,6-DHT lesions were found to produce a marked increase in immobility times when compared to saline injected and naive controls. In contrast, 6-OHDA lesions produced a reduction in immobility time when compared to saline lesioned and naive controls. Most 5,6-DHT lesioned rats were then transplanted with either serotonin (5-HT) containing pineal gland tissue or equal volumes of sciatic nerve tissue to the lesion site. Six weeks following transplantation FST immobility times were assessed once again. While all three 5,6-DHT lesioned groups displayed reduced immobility times, the pineal grafted group displayed significantly further reduced immobility times when compared to the 5,6- DHT sciatic group and 5,6-DHT nontransplanted group. Biochemical analysis using HPLC revealed that 5,6-DHT lesioned frontal neocortex contained lower levels of 5-HT than vehicle injected and control frontal cortex. Pineal grafted neocortex contained higher levels of 5-HT than sciatic nerve grafted or nongrafted controls. There were decreased levels of both norepinephrine and dopamine in the 6-OHDA lesioned frontal neocortex. These results provide further evidence to suggest an important role for neocortical 5-HT transmission in antidepressant activity and that transplants of 5-HT containing tissue may eliminate biochemical deficits in depression.

25.8

Expression of amyloid in cortical transplants of trisomy 16 mouse. C.F. Hohmann, G.T. Capone, A.M. Diggs and J.T. Coyle. Dept. of Psychiatry, Johns Hopkins University and The Kennedy Institute, Baltimore, Maryland.

Trisomy of murine chromosome 16 [Ts16] shares phenotypic and genotypic similarities with human trisomy 21, Down Syndrome [DS], thus providing a suitable model to study the effects of triplication of these genes on brain development. Since Ts16 does not survive to birth, we devised a transplantation paradigm to examine cortical development. We here investigate the expression of APP protein and mRNA in grafts of late embryonic Ts16 and euploid litter mate cortex transplanted to the neocortex of neonatal euploid hosts.

Immunocytochemistry was performed using an antiserum (F5) to amino acid 687-695 of the APP molecule and in *situ* hybridization using a RNA probe (AB1) coding for the carboxyterminus and A4 region (both courtesy of Dr. R. L.Neve). Compared to euploid (N=4), immunoreactivity [IR] to APP was elevated diffusely in the neuropil of Ts16 grafts (N=4) and the surrounding host tissue at 2 weeks following transplantation. Furthermore, IR was seen in neurons and other, to be identified, cellular elements, predominantly in the border regions between host and transplants. Such stained cells were not found as frequently in euploid grafts and the immediate host environment. Transplants of both euploid (N=4) and Ts16 (N=4) tissue, 4-6 weeks following grafting, did not display similar elevations of diffuse or cell associated APP IR. *In situ* hybridization displayed a pattern similar to IR. Experiments are currently underway to study in greater detail the time course of APP expression between 1 week and 6 month post-grafting. In agreement with previous studies on GAP43 and SOM, these data indicate that host and graft tissue interact with each other in the regulation of the expression of genes located on chromosome 16.

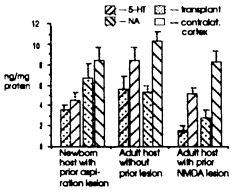
25.9

INTRACEREBRAL NEOCORTICAL TRANSPLANTS CONTAIN MEASURABLE AMOUNTS OF NEUROTRANSMITTERS NOREPINEPHRINE AND SEROTONIN. M.K. Schulz, J.A. McNulty, T.P. Hogan*, J. Zimmer and A.J. Castro. Dept. of Cell Biol., Neurobiol. and Anatomy, Loyola Sch. Med., Maywood, IL 60153 and Instit. of Anatomy, Aarhus Univ., DK-8000 Aarhus C, Denmark.

Fetal neocortical grafts placed homotopically into newborn recipients receive extensive input from the host brain including afferents from the locus coeruleus and midline raphe. To measure these latter inputs, the present study examined noradrenaline (NA) and serotonin (5HT) content in fetal (E14–15) neocortical grafts placed into cortical aspiration cavities made immediately before grafting in newborn rats or into NMDA-induced cortical lesions made one week earlier in adult rats. Unablated adults were also used.

At three months postgrafting and using high performance liquid chromatography with electrochemical detection, tissue levels of NA and 5-HT were measured in dissected samples of the transplants and the contralateral cortex.

As indicated in the accompanying figure, NA and 5HT levels reached control, i.e., contralateral cortex, levels in newborn recipients whereas grafts placed into adult hosts were significantly below control values.



(Supported by NIH Grant 13230, NSF Grant BNS 8801726 and the Danish MRC.)

25.10

FETAL NEOCORTICAL TRANSPLANTS INTO FOCAL ISCHEMIC LESIONS IN ADULT RATS. G.L. Tillotson, M.K. Schulz, T.P. Hogan*, P.L. Shaw* and A.J. Castro. Dept. of Neurology and Dept. of Cell Biol., Neurobiol. and Anatomy, Loyola Sch. of Med., Maywood, IL 60153 USA.

The present study was undertaken to investigate the viability of embryonic cortical tissue transplanted into adult ischemic cortical lesions.

Adult rats received focal ischemic lesions by permanent occlusion of the left middle cerebral artery at the level of the rhinal fissure. Seven days later, rat embryonic neocortical graft tissue (E14-15) was transplanted into the cortical ischemic zone. After a 20 day survival period, animals were sacrificed and brains processed for acetylcholinesterase and routine Nissl stain. Upon histological inspection, ischemic infarctions were localized to the left cerebral cortex, primarily involving the sensorimotor area, with relative sparing of subcortical structures. Surviving transplants were found integrated into host tissue in cortical areas surrounding the infarct as well as deep to the infarct in the striatum. Acetylcholinesterase positive fibers were found within the transplant; although less dense than in the surrounding host tissue, these fibers appeared to be derived from the host brain.

(Supported by NIH Grant 13230).

25.11

SUPERIOR CERVICAL GANGLION REGENERATING AXONS BRIDGED BY AUTOLOGOUS NERVE GRAFTS INNERVATE DISCRETE BRAIN REGIONS. E. Fernandez, R. Pallini *, L. Lauretti *, V. Bozzini * and E. Dell'Anna * (#). Institutes of Neurosurgery and Neurology (#), Catholic University, Rome, Italy.

Transplantation studies in animal models have shown that intracerebral superior cervical ganglion (SCG) grafts may survive and sprout cathecolaminergic (CA) fibers. However, the number of surviving CA cells rapidly decreases after grafting. The present work explored the possibility to innervate selected brain areas by SCG derived axons circumventing the ganglion cell loss seen after the direct SCG grafting procedure. In adult rats the SCG was microsurgically exposed. The post-ganglionic branch was cut and a segment of autologous sciatic nerve 35 mm long was grafted between the SCG and a surgically created cortical cavity in the parietal region. After 15-20 weeks of survival, axonal regeneration was evaluated by horseradish peroxidase (Sigma VI, 8-10% solution) injected into the graft and tyrosine hydroxylase immunocytochemistry. SCG derived axons do regenerate into the graft, penetrate the glial scar, and extend into the cortex.

AGING PROCESSES I

26.1

DIFFERENTIAL EXPRESSION OF βA4 PRECURSOR PROTEIN mRNA TRANSCRIPTS DURING HIPPOCAMPAL NEURON DEVELOPMENT *IN VITRO*. M.J. Strong and D.M. Jakowec*. Dept. of Clin. Neurological Sciences, UWO, London, Ont., Canada, N6A 5A5.

We have confirmed and extended our previous qualitative observations of a developmentally-specific pattern of βA4 precursor protein (APP) mRNA hybridization during neuronal maturation *in vitro* (Exp Neurol 1990;109:171). using synthetic junctional oligonucleotide probes (Kitaguchi et al., Nature 1988;331:530). Hippocampal neurons from fetal New Zealand white rabbits were grown in chemically-defined medium and at intervals of 6, 12, 18, 24 and 30 days postplating, the total cellular RNA isolated using guanidine isothiocyanate isolation on CsCl gradients. Prior to hybridization, RNA samples were normalized by slot-blot analysis with a [^{32}P]-labelled TTP probe synthesized on poly A$^+$ oligo dt$_{12-18}$ with M-MLV reverse transcriptase. The oligonucleotide probes were 3'-labelled with terminal deoxynucleotide transferase and [^{32}P]-dCTP (10 μCi/μmole) and the hybridization carried out at 52^{0}C (42^{0}C for APP$_{770}$ probe) in 5x SCC, 1x Denhardts, 1% SDS, 100 μg/ml ssDNA on Nytran membrane following RNA separation on 1.0% formaldehyde-agarose gels. Hybridization patterns to a β-tubulin oligoprobe (Duponf) and a cDNA probe encoding the NF-L protein served as controls. The following patterns of APP mRNA transcript expression were observed: peak levels of APP$_{695}$ mRNA at d18-24 and thereafter declining; progressively increasing levels of APP$_{751}$ mRNA to d30; peak levels of APP$_{770}$ mRNA at d18-24 with a marked decline thereafter. Current experiments are attempting to determine if an intrinsic neuronal signal linked to the acquisition of synaptic polarity mediates this temporal profile of APP mRNA transcript expression.

26.2

ISOLATION AND CHARACTERIZATION OF THE NEURONAL PHOSPHOPROTEINS B-50/GAP-43 AND BICKS/NEUROGRANIN FROM HUMAN BRAIN. M. R. Martzen[*1], A. Nagy[*2], P. D. Coleman[1] and H. Zwiers[2]. Dept. of Neurobiol. and Anatomy[1], Univ. of Rochester Med. Center, Rochester, NY 14642 and Depts. of Med. Physiol. and Med. Biochem.[2], Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1.

The neuronal phosphoproteins B-50 and BICKS share a highly conserved amino acid sequence which includes a PKC phosphorylation site and calmodulin binding domain. The B-50 molecule has at least one proteolysis product (B-60) whose cleavage is inhibited by thiol protease inhibitors. B-50 has been associated with neuronal development, axonal regeneration, polyphosphoinositol metabolism and synaptic plasticity. The function of BICKS, immunohistologically localized to perikaria and dendrites, is less clear but may ultimately share some functional homology with B-50 through their common domains. In normally aging brain neurons mount a plastic compensatory response resulting in a net increase in dendritic extent. This response may be defective in certain pathological states. Neuronal proteins associated with plasticity, therefore, may provide some insight into these events. B-50 and BICKS were isolated from normal and Alzheimer's disease post-mortem human brains and separated into membrane and cytoplasmic fractions, followed by C18 reverse phase HPLC in TFA and CH_3CN. Isolated proteins from superior temporal gyrus, visual cortex and cerebral spinal fluid were then analyzed by gel scanning, Western blotting and analytical C4 reverse phase HPLC. B-50 and BICKS were found present in both cytoplasmic and membrane fractions. In addition, a series of immunoreactive bands were found concentrated in the cytoplasmic fractions with apparent molecular weights ranging from 43-15 kDa. A similar pattern was not seen with BICKS. These results suggest a series of B-50 immunoreactive molecules potentially resulting from proteolytic processing of native B-50 in its PEST regions. Implications of these results and their correlation with aging and Alzheimer's disease will be discussed. Supported by grants AG 01121 and by a LEAD award (PDC) and by the MRC of Canada (HZ).

26.3

CALCIUM BINDING PROTEINS IN THE SEPTO-HIPPOCAMPAL SYSTEM OF YOUNG AND AGED RATS. M.L. Smith and R.M. Booze
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Altered calcium homeostasis has been proposed as a mechanism of neuronal cell death in aging and Alzheimer's disease. In this study we examined the localization of calcium binding proteins within the septo-hippocampal system, a brain region affected by age-related cell loss and atrophy. We investigated 1) the distribution of calmodulin and calbindin D_{28k} within the septal nucleus and the hippocampus and 2) the age-related differences in calmodulin and calbindin D_{28k} immunoreactivity.

Pairs of young (4-5 month) and aged (24-25 month) Fischer-344 male rats were perfused and the brains sectioned on a Vibratome (40 μm). Serial sections from each pair were collected through the septal nucleus and dorsal hippocampal formation. Adjacent sections were processed simultaneously for calmodulin (1:1000; Chemicon), calbindin D_{28k} (1:500; Sigma), ChAT (1:1000; Chemicon) and GAD (1:300; Chemicon) using standard ABC immunocytochemical techniques.

Both young and aged rats showed extensive calmodulin immunoreactivity which was not confined to a specific region or cell population. No clear age-related differences were detected in the intensity of calmodulin binding. Calbindin D_{28k} immunoreactivity was highly localized in the neocortex, striatum, septal nucleus and hippocampus. Within the hippocampus, CA1 and CA2 pyramidal cells were immunoreactive. Lower reactivity was present in dentate gyrus granule cells. No calbindin D_{28k} was observed in the CA3 pyramidal cell layer. There was an age-related decrease in calbindin D_{28k} staining intensity within the hippocampus; however, there were no detectable decreases in the cortical and striatal areas. Within the septum the calbindin D_{28k} was localized to the medial septal nucleus, as well as in the lateral septum. The localization of calbindin D_{28k} within the septum and CA1/CA2 pyramidal cells suggests that calbindin D_{28k} may be an important factor in calcium-dependent mechanisms of age-related cell loss.

26.4

INCREASED GFAP EXPRESSION IN THE AGED RAT BRAIN DOES NOT RESULT FROM INCREASED ASTROCYTE DENSITY. M.N. Gordon and D.G. Morgan. Division of Neurogerontology, Univ. of Southern California, Los Angeles, CA 90089-0191.

Brain sections from Wistar or Fisher 344/Brown-Norway F1 hybrids were stained for GFAP or S100 using a direct coupling technique in which the secondary antibody is conjugated to peroxidase. We previously demonstrated that such sections may be analyzed by image analysis to yield quantitative measurements of area occupied by reaction product (Gordon et al, Soc. for Neuroscience Abst. 16:347, 1990). Rats from 5 to 28 mo. of age were examined. Area occupied by GFAP or S100 reaction product was measured in two brain regions, the caudate nucleus containing a low astrocyte density, and the hilus of the dentate gyrus containing a high astrocyte density. Sections stained for S100 were also used to measure astrocyte density (cells / mm^2) and the cross-sectional area of S100 positive cells containing visible nuclei.

Astrocytes stained for GFAP possess reaction product restricted to cellular processes; in contrast, S100 staining is largely confined to the cell soma. Area occupied by GFAP reaction product increased with age by 100% in caudate nucleus and 40% in the hilus. With aging, S100 staining revealed no increase in the area occupied by reaction product, astrocyte cell soma area or astrocyte density (cells/mm^2). Similar results were observed in both brain regions of both rat strains. These findings demonstrate that the age-related increase in GFAP expression is specific for this protein, and does not result from increased astrocyte cell density accompanying aging. Technical assistance was provided by D.G. Berg, X. Ou, C.M. Flores and C.S. Young.

Supported by AG-7892, AHA-GIA 891079, and AHA-EIA 890173.

26.5

AGE-RELATED CHANGES IN ASTROCYTE mRNAs IN FIVE REGIONS OF MOUSE BRAIN. J.R. Goss & D.G. Morgan. Gerontology Center & Dept. of Biol. Sci., Univ. of Southern California; Los Angeles, CA 90089-0191.

In previous studies we found a 40-80% increase in the message level for glial fibrillary acidic protein (GFAP) in aged mouse cortex, cerebellum, and hippocampus. In these same studies we found no change in glutamine synthetase (GS), another astrocyte message, in the cortex (Goss, Finch and Morgan; *Neurobiol. Aging* **12**:165-170). In this study we have expanded on these findings by examining GFAP, GS, and three other astrocyte messages: sulfated glycoprotein-2 (SGP-2), apolipoprotein-E (APO-E), and amyloid precursor protein (APP; also found in neurons), in five brain regions from four ages of mice.

Male C57BL/6Nia mice of ages 4, 12, 20, and 28 months were used. Brains were removed from six animals per age group and dissected into five regions: cortex; cerebellum; brainstem (medulla, pons, and midbrain); a region containing hippocampus, and diencephalon; and a region containing basal ganglia and basal forebrain. Northern blot hybridization was performed using 3μg of total RNA per lane and single strand [^{32}P]labelled cRNA probes.

Results confirm a 20-100% increase in GFAP RNA between 4 and 28 months over the five brain regions. All other RNA markers changed less than 20% except for a 60% increase in the cerebellum for GS, a 45% increase in the cerebellum for APO-E, and a 30% decrease for APP in the cortex. These results suggest that the increase in GFAP RNA with age may be specific for GFAP, and not due to general astrocyte hypertrophy or hyperplasia in most areas of the brain; although the increases in APO-E and GS found in the cerebellum may indicate some increase in astrocyte size or number. S-100 RNA is presently being investigated with these same samples. Supported by AG-07892, AG-00093, AHA-GIA 891079, and AHA-EIA 890173.

26.6

SPECTRIN BREAKDOWN PRODUCT AND CHANGES IN N-CAM INCREASE WITH AGE IN SPECIFIC BRAIN AREAS. L.T. Ha*, B.A. Bahr, P. Vanderklish*, M.T. Tin*, B. Murray[1] & G. Lynch. Ctr. Neurobio. Learning/Memory and Dept. Devmntl. & Cell Biol.[1], Univ. of Calif., Irvine, CA 92717

It has been suggested that many neurodegenerative conditions are, at least in part, a consequence of the normal aging process. It is of interest, therefore, to ask whether components of cytoskeletal and adhesive structures which contribute to neuronal shapes and properties are modified as part of normal aging. The membrane cytoskeletal protein spectrin is present throughout neurons and is a preferred substrate for the calcium dependent protease calpain. Proteolysis of spectrin produces characteristic breakdown products (BDPs of 155 and 150 kDa) together which are a biochemical marker that correlates with the onset of many instances of brain pathology. This study indicates that a highly significant linear correlation (r) exists between age (3 to 30 months) and the level of spectrin BDPs (5 to 16% of the anti-spectrin immunoreactivity) in the telencephalon (slope= 0.29, r= 0.91, $p< 0.01$) but not in the hindbrain (slope= -0.10, r= 0.53, $p> 0.1$) of Balb/c mice. BDPs in the mesencephalon also exhibited a linear increase across life-span, but was weakly correlated with age (slope= 0.12, r= 0.76, $p> 0.05$). These data suggest that the spectrin breakdown process associated with pathogenesis increases with age in some but not all brain regions. Second, N-CAM adhesion molecules whose presence in brain membranes has recently been shown to be sensitive to calpain (Sheppard, Wu, Bahr, Lynch, *Synapse* 1991, in press) were tested across mouse age. A highly significant linear correlation between age and percent loss of immunoreactivity toward NCAM-140 was found in the mesencephalon (slope= 2.1, r= 0.90, $p< 0.01$) but not in other brain areas. NCAM-180, however, was unchanged across life-span in all brain areas tested eventhough it was similarly concentrated as NCAM-140 in synaptic plasma membranes vs. homogenates in the mesencephalon as well as the telencephalon. Note, the N-CAMs in hind brain material did not exhibit such localization. This suggests that normal aging involves the loss of distinct adhesion molecules from specific brain areas. (Supported by NIA grant #AG00538 and the Pew Foundation.)

26.7

SENESCENCE OF VIRALLY STIMULATED NEURORETINAL CELLS INVOLVES ALTERED EXPRESSION OF c-Fos AND v-Src ONCOPROTEINS G.M. Seigel and M.F.D. Notter Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

Embryonic day 7 chick neuroretinal cell cultures were mitotically stimulated *in vitro* with the transforming v-Src oncogene of Rous Sarcoma virus. The result was a transformed cell population (LA29NR) with an extended mitotic lifespan in culture. Interestingly, over the course of 5 months in culture, these cells reached a state of senescence which we have characterized with regard to cell growth, morphology, as well as c-Fos and v-Src protein expression.

At one month in culture, "early" LA29 NR cells were 85% to 95% mitotic, as shown by tritiated thymidine incorporation. These "early" cells expressed significant levels of c-Fos and v-Src oncogene proteins as seen by Western blot analysis. As LA29NR cells aged in culture, a precipitous decline in mitotic index was accompanied by unusual morphological changes and decreases in oncogene expression. By 5 months in culture, "late" LA29NR cells exhibited a three-fold decrease in v-Src expression and a six-fold decrease in c-Fos expression compared with their "early" counterparts. In addition, the level of tyrosine phosphorylation of a 41 kilodalton phosphoprotein was significantly decreased by 5 months in culture. Therefore, specific modulations in gene expression were associated with senescence of LA29NR cells and their ability to overcome the mitogenic effects of Rous Sarcoma virus. These studies may prove useful in the characterization of cellular events accompanying senescence, as well as tumor regression. This work was supported, in part, by training grant T32AG00107 (G.M.S.) and EY 05262 from the National Eye Institute.

26.8

LOSS OF MUSCARINIC RESPONSIVENESS IN SENESCENCE MAY BE THE RESULT OF DECREASED MEMBRANE FLUIDITY. J.A. Joseph, K. Yamagami, and G.S. Roth*. Gerontology Res. Ctr./ NIA, Baltimore, MD 21224.

Previous research has indicated that reductions in muscarinic (m) agonist enhancement of K$^+$-evoked dopamine release (K$^+$ERDA) and decreased IP_3 release upon m receptor (mAChR) agonist stimulation are partially the result of deficits in signal transduction (ST). The present experiments were carried out in order to test the hypothesis that these putative ST deficits may occur as a result of alterations in the ligand-mAChR-G protein complex produced by age-related decreases in membrane fluidity. Oxotremorine (oxo) enhancement of K$^+$ERDA was examined in perifused striatal slices from mature (6 mo) and old (24 mo) Wistar rats incubated (30 min, 37° C) with S-adenosyl-L-methionine (SAM, 0, 0.2, 0.5, 1.0 mM) in a modified Krebs medium containing 2.5 mM KCl. Tissue was then aliquoted into a perifusion apparatus and washed (10 min) with the low KCl medium, followed by a switch to a hi KCl (30 mM) medium containing 0, 0.1, or 0.5 mM oxo and 5 min samples collected for HPLC analysis. Results indicated that SAM treatment significantly reduced the age deficit in oxo enhancement of K$^+$ERDA (e.g. no SAM treatment, 0.5 mM oxo peak DA release diff. from 0.0 mM oxo, Mature 69.22 $\pm$ 5.00 Old, 44.71 $\pm$ 9.59 p moles/mg protein; $t(6) = 3.74$; $p < 0.02$; 0.5 mM SAM treatment Mature 122.73 $\pm$ 24.62, Old 126.18 $\pm$ 17.11 pmoles/mg protein; $t(8) < 1$). Direct measurements of fluidity are being carried out, but these preliminary results suggest that loss of m-agonist responsiveness in senescence may be explained partially by decreases in membrane fluidity.

26.9

THE EFFECT OF AGING AND DIETARY RESTRICTION ON INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) BINDING SITES IN THE BRAIN. A. D'Costa, C.R. Breese, and W.E. Sonntag, Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

IGF-1 is a peptide hormone which demonstrates mitogenic activity in many tissues and has been reported to show decreased plasma levels with age and in animals that are dietary restricted. This study was undertaken to examine IGF-1 receptor binding sites in the brain and examine the modification of these receptors with aging (7, 17 and 28 months) and dietary restriction. Autoradiography was performed on slide mounted sections, incubated with ^{126}I-IGF-1, and apposed to Hyperfilm-^{3}H. Non-specific binding was estimated by the addition of 2μg of IGF-1. Receptor binding was observed in the s. oriens and s. lucidum of CA3 and the dentate gyrus of the hippocampus. Laminar binding was seen in the cortex with high levels of binding in layer VI of the frontal cortex and layer II of the parietal and temporal cortex. Moderate binding was seen in the cerebellum (molecular layer). Quantitative autoradiographic analysis revealed an increase in IGF-1 binding in layer II and VI of the cortex with increasing age ($p<0.02$). CA3, dentate gyrus, and cerebellum showed no changes in binding with age. Receptor binding assays were performed by incubation of isolated membranes (50μg) from dissected cortex, cerebellum, and hippocampus of rats with varying concentrations of cold IGF-1. Scatchard analysis showed no significant changes in K_D values in all the tissues examined. Receptor number increased in the cortex ($p<0.05$) with age and in response to dietary restriction. B_{max} values for the cerebellum and the hippocampus were not significantly different. These results suggest that changes observed in IGF-1 receptor density with age may have an important role in maintenance of cortical function in the aging animal (supported by NIH grant AG07752 to WES)

26.10

AGE RELATED DECREASES IN NEOSTRIATAL HIGH AFFINITY [^{125}I] RECOMBINANT HUMAN NERVE GROWTH FACTOR (NGF) BINDING AND CHOLINE ACETYLTRANSFERASE (ChAT).
R. H. Soriano, M. Dugich-Djordjevic, C. A. Altar. Endocrine Research Department, Genentech, Inc., South San Francisco, CA 94080.

Numerous reports have shown decreases in rat cholinergic neuronal cell number, density, size, biosynthetic capacity and acetylcholine release during aging. We examined aging effects on the high affinity binding of NGF to rat neostriatum using quantitative receptor autoradiography (Altar, C.A. *et al.*, *PNAS* **88**, 281, 1991). ChAT activity was measured in the contralateral neostriatum, olfactory tubercle, hippocampus and neocortex from 1-24 month old male Sprague Dawley rats (6 per group) using a radioenzymatic assay.

Saturable neostriatal binding of NGF was decreased in 6, 7, 8, and 12 month compared to 1 and 2 month rats ($p < 0.05$). The B_{max} decreased 42% from 9.52 ± 0.96 fmol/mg in 1 month to 5.54 ± 0.49 fmol/mg in 24 month rats ($p < 0.001$). ChAT activity decreased from 1 to 24 months in the neostriatum, olfactory tubercle and neocortex by 58, 33, and 42%, respectively ($p < 0.05$). ChAT did not change in the hippocampus at any age. This loss of NGF receptor number and reduction in cholinergic activity may be related to the recognized cell loss in these forebrain regions during aging.

26.11

MODULATION OF BRAIN TRANSDUCING MECHANISMS BY L-ACETYLCARNITINE (LAC) IN YOUNG AND AGED RATS.
T.Florio, Q.Meucci, M.Grimaldi, A.Scorziello *Q. Ghirardi A.Marino G.Schettini, Dept. Pharmacology, II School of Medicine, University of Naples, and *Inst. for Research on Senescence, Rome ITALY .

In the present study, we evaluated the effect of LAC on frontal cortex adenylate cyclase (AC) activity, inositol phosphate (IP) production and free intrasynaptosomal calcium levels ([Ca++]i), in young and aged rats. Both acute and subchronic treatments with LAC were carried on and we observed that: 1) Acute administration of LAC did not significantly modify basal and stimulated AC, IP production, [Ca++]i; 2) The subchronic treatment with LAC (250 mg/kg, 30 days) potentiated AC activity under carbamilcholine (CCh), norepinephrine (NE) and dopamine (DA)-stimulated conditions, in young as well as in aged animals, whereas it did not affect basal enzyme activity. 3) In synaptoneurosomes from old rat cortex, LAC increased CCh-stimulated inositol phosphate production while in young rats also basal IP production was enhanced. 4) The subchronic administration of LAC neither affected basal [Ca++]i levels in hyppocampal synaptosomes from young and aged rats, nor modified [Ca++]i rise induced by K+- depolarization or maitotoxin.

26.12

Free Radicals, glutamate and 5-HT after reperfusion in aging gerbil brain.
G. Delbarre, B. Delbarre and F. Calinon*.
Faculté de Médecine, 37032, Tours, France.

Levels of neuromediators are low at birth, reach a plateau, then decrease with aging. In gerbil brain, there is no indication of stroke index (SI) and levels of these amines and OH° in aging after ischemia reperfusion insult (IRI). We have determined the SI and the levels of glutamate (Xu, X., J. Liquid Chromatog., 9, 2253, 1986), 5-HT (Rips, R., Progress in HPLC, Vol. 2 : 375-94, 1986) and OH° (Floyd R.A., J. Free Radic. Biol. & Med., 2 : 13, 1986) in the gerbil brain (3, 9 and 15 months old) after IRI. Carotid artery was occluded during 60 min ; the SI (Delbarre G., Stroke, 19 : 26, 1988) was determined 4, 24, 48 and 72 h after release of the clip and for levels determination of amines and OH°, the brain was dissected 30 min after release of the clip (Delbarre G., Stroke, 19 : 26, 1988). After IRI, the SI and glutamate, 5-HT and OH° were significantly increased in 9 and 15 months old gerbil compared with 3 months old one. These results demonstrate that after IRI, the SI, glutamate, 5-HT and OH° are more important in old brain gerbils.

Percentage of increase versus 3 months old

	OH°	Glutamate	5-HT
9 months	146.37***	216.55***	407.60**
15 months	418.10***	478.05***	487.56*

Unpaired Student t test, $p < 0.05$*, $p < 0.001$***

NEUROGLIA AND MYELIN I

27.1

REGULATION OF CARBOXYPEPTIDASE E SECRETION AND SYNTHESIS IN CULTURED ASTROCYTES.
R. S. Klein, B. Das, L. D. Fricker. Depts. Molecular Pharmacology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Cultured astrocytes have been shown to produce neuropeptides and neuropeptide processing enzymes. In this study, we used the neuropeptide processing enzyme carboxypeptidase E (CPE) as a marker for neuropeptide biosynthesis and to examine the secretory pathway in cultured astrocytes. In pulse/chase and metabolic labeling experiments cultured astrocytes were labeled with ^{35}S-methionine and CPE was isolated from media and cells using substrate affinity columns. Results from the pulse/chase studies showed that the secretion of labeled CPE from the astrocytes occurred after a 30 minute lag time and was complete by 120 minutes, indicating that all newly synthesized CPE is rapidly secreted. In metabolic labeling experiments, the secretion of CPE protein from cultured astrocytes was increased 2-3 fold by prolonged treatment (3-48 hours) with the phorbol ester 12-O-tetradecanoyl phorbol 13-acetate (TPA). This increase occurred after 3 hours of TPA treatment in hypothalamic and frontal cortical astrocytes, with no further increase upon longer TPA exposure, and after 24 hours of treatment in astrocytes cultured from the striatum and cerebellum. In addition, Northern blot analysis of expression of CPE mRNA by cultured cerebellar astrocytes showed a slight (1.3-fold) increase after 24 hours of exposure to TPA but not after 3 hours of exposure. These results indicate that the neuropeptide secretory pathway in astrocytes does not include a storage step, and that the secretion of neuropeptides and the synthesis of CPE may be regulated through a TPA-inducible second messenger system.

27.2

HYPEROSMOTIC EXPOSURE ALTERS GLUTAMATE TRANSPORT AND GLUTAMINE SYNTHETASE ACTIVITY OF CEREBRAL ASTROCYTES.
J.E. Olson. Dept Emerg Med, Wright State Univ, Dayton, OH 45401

The content of glutamine, glutamate, and other amino acids increases in astrocytes following prolonged exposure to hyperosmotic conditions *in vitro* (J Neurosci Res 27: 241, 1990). To understand the mechanisms which produce these changes in amino acid content, we measured glutamate transport and the activity of glutamine synthetase (GS) in hyperosmotic-treated and normal astrocyte cultures.

Cells obtained from 2-4 day-old rat pups were grown for 2 weeks in normal medium. Thereafter, the osmolality of the culture medium was increased 50 mOsm at each feeding (3 time/week) by adding NaCl. Astrocytes were used after 4 weeks *in vitro* when the culture medium was 600 mOsm. Transport was determined in intact cells using glutamate concentrations ranging from 10-1000 μM and osmolalities matched to that of the final culture medium. GS was determined after lysing the cells in a hypoosmotic solution.

GS activity was 4.30±0.44 μMole/(mg protein hr) in hyperosmotic-treated astrocytes compared with 3.06±0.49 μMole/(mg protein hr) in control cells (N=8, $p<0.02$). Hyperosmotic treatment decreased the V_{max} for glutamate transport by approximately 50% while K_m was unchanged.

The increased glutamine content of hyperosmotic-treated cells may be due in part to increased GS activity. The increase in glutamate content is not due to an increase in inward glutamate transport. Supported by NINDS (NS23218).

27.3

EICOSANOID-INDUCED INHIBITION OF GLUTAMATE UPTAKE INTO CORTICAL ASTROCYTES. D.M. Vaughn, R.C. Rossmanith* and N.R. Cox*. Scott-Ritchey Research Program, College of Veterinary Medicine, Auburn University, Auburn, AL 36849.

Elevated concentrations of arachidonic acid (AA) metabolites in brain are known to occur in association with trauma, ischemia & enhanced neuroimmune reactiveness. We investigated the capabilities of AA, the cyclooxygenase metabolites PGE2 & carbacyclin-TxA_2, & the lipoxygenase metabolites LTB_4 & LTD_4 to inhibit [14C]-glutamate uptake into cultured rat cortical astrocytes. All eicosanoids were incubated at 50, 25, 12.5 uM for 90 min prior to a 5 min glutamate uptake. All cpds, except LTD_4, induced a 95-45% dose-dependent inhibition of glutamate uptake with the following rank order of potency PGE2> AA> TXA_2> LTB_4. Both arachidic acid (20:0) & palmitic acid (16:0) stimulated glutamate uptake into astrocytes by approximately 100% at 50 uM. Therefore, both polyunsaturated lipooxygenase and cyclooxygenase metabolites may contribute to the neurotoxic effects of glutamate by increasing extracellular glutamate concentrations.

27.4

SERUM EFFECTS AND REGIONAL DIFFERENCES IN ASTROCYTIC SEROTONIN AND GLUTAMATE UPTAKE

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Na^+-dependent, fluoxetine-sensitive high-affinity uptake of serotonin and Na^+-dependent uptake of glutamate were studied in primary astrocyte cultures from 1-day-old rat neocortex. High-affinity serotonin uptake was decreased when cells were grown in horse serum as compared to fetal bovine serum and was almost absent when cells were grown in chemically defined medium. In contrast glutamate uptake was unaffected by the composition of the medium in which the cultures were grown. For cultures prepared from different brain regions grown in fetal bovine serum, Na^+-dependent [^{3}H] L-glutamate and fluoxetine-sensitive [^{3}H] 5HT uptake varied markedly. For astrocytes the relative order of uptake for [^{3}H] L-glutamate was HP (hippocampus) > CC (cerebral cortex) > B G + DI (basal ganglia + diencephelon) $\geq$ MS (mesencephalon) $\geq$ BS (brain stem) > CB (cerebellum) and for [^{3}H] 5HT was MS > CS $\geq$ HP > BS > CB. For [^{3}H] 5HT this essentially corresponds to the order of binding of the [^{3}H] 5HT specific uptake ligand [^{3}H] citalopram in situ. For [^{3}H] glutamate regional variation of the uptake from the different cultures exactly corresponds to the regional uptake by rat brain. Uptake was also analyzed at the single cell level by simultaneous autoradiography (ARG) and GFAP staining and for different morphological, GFAP(+) cells. Flat cells, with or without processes and comprising 65-98% of the cultures, represented most of the uptake. These differences in transmitter uptake by GFAP(+) astrocytes in primary culture which are dependent on the region of origin or morphological type, suggests that such uptake in vitro is an expression of differences shown by astrocytes in situ. (Supported by grant NS 19491 to H.K.K.)

27.5

IONOTROPIC GLUTAMATE RECEPTOR mRNA EXPRESSION IN CULTURED RAT BRAIN ASTROCYTES AND RAT OPTIC NERVE. Abbie Jensen* and S.Y.Chiu. Neuroscience Training Program & Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Northern blot analysis of poly (A)$^+$ RNA obtained from cultures of neonatal cortical astrocytes was performed to test for the presence of ionotropic glutamate receptors, GluR1 -GluR5. The cultures were ~2 months old, and contained ~ 60% type-1 astrocytes, 30% type-2 astrocytes, as determined by double GFAP and A2B5 antibody labeling of a sister culture, and the remaining percentage consisted of O-2A progenitor cells, oligodendrocytes and meningeal cells. Partial-length probes (~1.4 kb, 5' end through first membrane spanning region) were generated using the random prime method. Prehybridization, hybridization, and washes were done using high stringency conditions. After overnight exposure substantial signals were observed in the GluR1 and GluR2 lanes (3 µg mRNA/lane). Even after several days of exposure no signal was observed in the GluR3, GluR4, and GluR5 lanes. The GluR1 probe recognized a transcript of 5.5 kb; GluR2 probe recognized two major transcripts of 6 kb and 3.8 kb, and a minor transcript of 4.8 kb.

In Situ hybridization of postnatal day 7 rat optic nerve was performed using full length sense and antisense ^{35}S-riboprobes generated from GluR2 cDNA. Prehybridization, hybridization (approximately same probe cpm of sense and antisense added) and stringency conditions were identical for both probes. Slides were dipped in emulsion and exposed for 14 days. Silver grains were counted overlying optic nerve sections in both the sense and antisense cases. The grain density was 4-10 times higher in the antisense hybridized sections as compared to sense.

Supported by NS-23375 (NIH) and RG-1839 (National MS Society)

27.6

GLUTAMATE-INDUCED INCREASE IN ASTROCYTE GLYCOGEN IS MEDIATED BY CELL VOLUME CHANGES. **R.S. Dombro*, A.S. Bender, L. Machado*, J. Blicharska*, D.G. Hutson* and M.D. Norenberg. VAMC, Miami, FL 33125 and Depts of Surgery and Pathol., Univ. Miami School of Medicine, Miami, FL 33101.**

Elevated glycogen content is a common response of astroglial cells following exposure to a variety of agents associated with conditions leading to astroglial swelling. Glutamate increases the glycogen content in cultured astrocytes (Swanson et al., J. Neurochem. 54, 490, 1990), and also induces astrocytic swelling (Chan et al., J. Neurosci. Res. 25, 87, 1990). We tested the possibility that the glutamate-induced increase in astrocyte glycogen levels is mediated by changes in cell volume. Glycogen levels were measured using the method of Lust et al., (Anal. Biochem. 68, 328, 1975), and cell volume was determined as described by Kletzien et al., (Anal. Biochem. 86, 537, 1975). When astrocytes were incubated with 1 mM glutamate in isotonic Krebs-Ringer bicarbonate medium for 90 min, glycogen levels increased by 60% and cell volume increased by 123%. Treatment of these cells with hypertonic medium (216 mM NaCl) blocked the swelling and also inhibited the glutamate-induced increase in glycogen. These results suggest that glutamate-induced glycogen accumulation in astrocytes is mediated by a signal that is generated by an increase in cell volume. **(Supported by VA Medical Research Service and NIH grant AM 38153).**

27.7

Potential second messengers mediating proliferation of rat glial cells in culture. L. H. Fossom and G. H. DeVries, Medical College of VA, Richmond, VA 23298.

In the peripheral nervous system, Schwann cells (SC) proliferate and subsequently differentiate in response to neurons. Although a signal for proliferation apparently resides in the neuronal plasma membrane (since axolemma (AEF) can induce rat SC to divide in cell culture), the identity of the axolemmal mitogen(s) remains unresolved and the associated signal transduction system(s) remains to be elucidated. Although several agents that elevate cAMP levels in cultured SC also stimulate SC to divide (Raff et al., 1978), cAMP levels in rat primary SC are unaffected by 3-9 hr treatment with mitogenic concentrations of AEF (Meador-Woodruff et al., 1984). In the current study we measured protein kinase A (PKA), the unique mediator of cAMP responses in eukaryotes, as an index of cAMP involvement in the interaction of AEF with SC. Transformed SC (TSC)(Tennekoon et al., 1987) were maintained in serum-free defined medium; PKA was measured in cell supernatant as the transfer of 32P from ATP to histone IIA substrate. PKA in TSC was maximally activated *in vitro* by 1 uM 8-Br-cAMP (EC50=0.1 uM) and maximally inhibited by 10 uM synthetic peptide of the Walsh inhibitor (IC50=0.3 uM). Under control culture conditions approximately 20% of PKA in TSC was activated; 30 min treatment of TSC with AEF did not alter this activity, although treatment with 0.5 mM 8-Br-cAMP completely activated the kinase. This data corroborates the earlier data on cAMP levels suggesting that the axolemmal mitogen does not work directly through cAMP.

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27.8

ROLE OF CALCIUM IN ATP-STIMULATED EICOSANOID PRODUCTION IN ASTROCYTES G. Bruner and S. Murphy Dept. of Pharmacology, College of Medicine, Univ. of Iowa, Iowa City, IA 52242

Stimulation of rat cortical astrocytes with ATP can elicit eicosanoid production via activation of a P_{2Y}-purinergic receptor. ATP does not alter the sensitivity of phospholipase A_2 (PLA_2) to Ca^{2+}. Activation of PLA_2 could occur secondary to a rise in $[Ca^{2+}]_i$. Alternatively, physiologic increases in $[Ca^{2+}]_i$ alone may not be sufficient to elicit production of eicosanoids. In fura-loaded cells, $[Ca^{2+}]_i$ rose from 106±18 nM to 321±42 nM (n=8) within 6 sec upon stimulation with 500µM ATP. In Ca^{2+}-free buffer (plus 0.5mM EGTA) basal $[Ca^{2+}]_i$ was decreased (50±8 nM), as was peak $[Ca^{2+}]_i$ (105±25 nM) (n=7) and the time to peak was slower (20 sec), suggesting that much of the initial rapid increase in $[Ca^{2+}]_i$ is due to influx.

Previously we have demonstrated that pertussis toxin (PTx) pretreatment inhibits ATP-stimulated eicosanoid production in a concentration-dependent manner. However, PTx did not alter ATP-stimulated increases in $[Ca^{2+}]_i$ either in the absence or presence of extracellular Ca^{2+}. Furthermore, PTx did not completely inhibit inositol trisphosphate formation, suggesting that mobilization of Ca^{2+} from intracellular stores is not the stimulus for PLA_2 activation. These data indicate that an increase in $[Ca^{2+}]_i$ alone is an insufficient stimulus for PLA_2 activation.

27.9

ENHANCEMENT OF Ca^{2+} SIGNALS IN ACUTELY ISOLATED ASTROCYTES BY β-ADRENERGIC AGONISTS AND cAMP

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Neurotransmitters acting through specific membrane receptors have been shown to modulate ionic channels in cultured glial cells. For example, β-adrenergic agonists and cAMP analogues increase Ca currents in cultured astrocytes (MacVicar and Tse, Glia 1: 359, 1988). However, it is not known if astrocyte Ca channel modulation occurs *in vivo*. We have previously shown that astrocytes acutely isolated from mature rat hippocampus possess functional high-threshold voltage-gated Ca channels (Duffy et al Soc Neursosci Abstr 16: 279.12, 1990). To investigate the possibility that these channels are modulated by neurotransmitters, acutely isolated astrocytes were loaded with the Ca-sensitive dye Indo-1, and changes in $[Ca^{2+}]_{in}$ monitored in response to application of neurotransmitters and second messenger analogues. It was found that the β-adrenergic agonist isoproterenol and the cell permeant cAMP analogues dibutyryl-cAMP and 8-bromo-cAMP can increase both resting $[Ca^{2+}]$ and depolarization-evoked $[Ca^{2+}]_{in}$ signals by enhancing the activity of these channels. Cell autofluorescence at 485 nm, which is correlated with intracellular NADH levels, decreased upon Ca^{2+} influx. This is indicative of an increase in cell metabolic turnover. It is proposed that increases in $[K^+]_{ex}$ associated with neuronal activity triggers Ca^{2+} influx into astrocytes and that this can be regulated by neurotransmitters. One possible function of astrocyte Ca^{2+} signals is to trigger the release of metabolic substrates during brain activity. The control of astrocyte Ca^{2+} signals by K^+ and transmitters released from neurons may constitute an important form of neuron-glia signalling.

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27.10

HETEROGENEITY OF RECEPTOR MEDIATED RESPONSES WITHIN CLONES OF TYPE 1 ASTROGLIA

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Previous studies from this laboratory indicated that type 1 astroglia are pharmacologically heterogeneous. Experiments were designed to determine if astroglia within a given clone were pharmacologically identical and if the ability of individual astroglia to respond to a neuroligand was stable over time in culture. Astroglia derived from neonatal rat cerebral cortical tissue were plated at low density such that the development of clones from individual cells could be monitored via time lapse microscopy. A video-based imaging system and the Ca^{2+} indicator dye fura-2 were used to measure changes in Ca_i^{2+}. Interestingly, only a fraction of the cells within a given clone responded to carbachol or histamine with an increase in Ca_i^{2+}, whereas treatment with a P_{2Y} purinergic receptor agonist generally increased Ca_i^{2+} in 100% of the cells within the clone. To examine the stability of the receptor signalling, individual astroglia within a number of clones were tested on different days for their ability to respond to neuroligands. The results of these experiments indicated that the ability of an astroglial cell to respond to ligands changes with time in culture. For example, certain cells responded to carbachol but not NE in the first week, lost the carbachol response during the second week and became responsive to NE. These results suggest that intrinsic mechanisms must operate in these cells. Whether the pharmacological heterogeneity reflects a change in receptor expression or other subcellular events remains to be determined. (NS20212)

27.11

EFFECT OF HYPOTONIC STRESS ON Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE ACTIVITY IN ASTROCYTES.

A.S. Bender, J.T. Neary, J. Blicharska* and M.D. Norenberg. Laboratory of Neuropathology, University of Miami School of Medicine & Veterans Administration Medical Center, Miami, FL 33101.

When astrocytes are exposed to hypotonic stress, they immediately swell and then subsequently return to almost their normal volume. These events are associated with an increased Ca^{2+} uptake (Bender et al., 1991, J. Neurochem., in press). This effect on Ca^{2+} uptake could affect the enzymatic activity of Ca^{2+}-dependent protein kinases. Because calmodulin antagonists were shown to block volume regulation, we investigated the effect of hypotonic stress on Ca^{2+}/calmodulin (CaM) protein kinase. Cells were exposed to hypotonic stress (105 mOsm) for various time periods ranging from 0.5 to 30 min, and CaM kinase activity was assayed as described by Babcock-Atkinson et al. (Glia 2: 112-118, 1989). CaM kinase activity was increased three-fold in the supernatant fraction, reaching maximal activity at 5 min, whereas it was concomitantly decreased in particulate fraction by 65%, reaching its lowest activity at 2 min. Thus, hypotonic stress activates CaM kinase, and appears to induce its translocation from the particulate to the supernatant fraction. These changes in CaM kinase may mediate the ionic changes which are involved in volume regulation. (Supported by M.R.C. of Canada and the Veterans Administration).

27.12

EFFECT OF CALCIUM ON ASTROCYTE VOLUME REGULATION, ION AND AMINO-ACID RELEASE.

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Swelling of astrocytes in hypotonic media causes regulatory volume decrease (RVD) and release of ions and amino-acids. Using a dynamic method to measure cell volume in attached cells, which measures extracellular electrical impedance, volume regulation in astrocytes exposed to hypotonic media without calcium is abolished. The addition of 1 mM $CaCl_2$ to swollen astrocytes in hypotonic media without calcium causes an almost immediate initiation of volume regulation. The removal of extracellular calcium also abolishes swelling-induced K^+ (^{86}Rb) and $^{36}Cl^-$ efflux, but does not affect swelling-induced aspartate or taurine release. The addition of 1 mM quinine·HCl, which is known to block Ca^{2+}-activated K^+ channels, abolishes volume regulation and K^+ efflux in hypotonic-induced swollen astrocytes in the presence of calcium. Measuring intracellular free calcium with Fura-2, swollen astrocytes show a rapid increase in $[Ca^{2+}]_i$, followed by a decrease to an elevated plateau that mirrors the time course of volume regulation. These results suggest that an increase in free $[Ca^{2+}]_i$ triggers activation of Ca^{2+}-dependent ion channels, releasing intracellular ions and osmotically obligated water, leading to a return to near normal cell volume (RVD). (Supported by grant NS 23750 to H.K.K.).

27.13

NEURONAL ACTIVITY ELICITS ASTROCYTE CA^{2+} WAVES AND OSCILLATIONS WITHIN HIPPOCAMPAL SLICES.

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Using laser confocal microscopy and the Ca^{2+} indicator dye fluo-3, we have imaged intracellular Ca^{2+} concentrations in organotypic hippocampal slices following electrical stimulation of the dentate gyrus. Specific cells within the slice were identified as astrocytes on the basis of correlative GFAP immunocytochemistry and electron microscopy. Both neurons and astrocytes located within area CA3 exhibited elevated intracellular Ca^{2+} levels with electrical stimulation. These responses were mediated through a TTX-sensitive fiber pathway. Astrocyte Ca^{2+} responses were delayed by 1-2 seconds when compared with the neuronal Ca^{2+} response, which appeared within milliseconds following the onset of stimulation. In addition, the astrocytes displayed both propagating waves of intracellular Ca^{2+} and Ca^{2+} oscillations, whereas the neurons exhibited only sustained Ca^{2+} rises. Kynurenic acid, a glutamate antagonist, completely blocked astrocytic responses to electrical stimulation, suggesting that neuronal glutamate release may mediate this aspect of the response. Astrocyte Ca^{2+} waves and oscillations were observed with stimulus frequencies as low as 2 Hz. At room temperature, wave velocities ranged from 7 to 15 um/s, and the periods for oscillations averaged 20 seconds. These findings indicate that a prompt form of neuronal-to-glial communication and a long-distance glial signalling system exists within the brain. If one supposes that glial Ca^{2+} signals in some way feed-back to influence neuronal excitability or synaptic transmission, these findings suggest that astrocytes may be partners with neurons in the brain's information processing functions. *Supported by the G. Harold and Leila Y. Mathers Charitable Foundation.*

27.14

CALCIUM-MEDIATED RESPONSES IN CULTURED CORTICAL ASTROCYTES: COMPARISONS BETWEEN cAMP INDUCED AND NON-INDUCED CELLS.

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Previous studies have shown that astrocytes become more rounded, express a larger voltage-dependent calcium current and produce increased GFAP mRNA when treated with cAMP. Calcium and cAMP can act together to produce changes in mRNA abundance. Many stimuli may converge upon the calcium messenger system to regulate astrocyte function and the calcium response may depend upon the state of the astrocyte. Here we investigate the effects of various stimuli on calcium levels in cultured cortical astrocytes.

This astrocyte culture system has been developed to minimize contamination by cell types, such as microglia. We have observed changes in calcium levels induced by a variety of stimuli in cultured astrocytes using fura-2 imaging. Depolarization causes large calcium elevations only in forskolin treated astrocytes. In contrast, quisqualate induced elevations are unaffected by treatment. These results suggest that some aspects of calcium regulation depend upon the state of the astrocyte.

Stimulation with any of several cytokines, including Il-1 β, IFNγ, and TNFα, produce small elevations similar to those observed with quisqualate stimulation. Responses to cytokines, such as Il-1 β, are unaltered in forskolin differentiated cells while some differences may be observed in response to cytokines such as IFN. Few interactive effects have been observed with combined cytokine stimulation.

The effects of cytokines on the abundance of a variety of transcripts is being investigated using northern blotting techniques. The transcripts include those coding for GFAP, cytokines, and their receptors. Preliminary results indicate that changes in the amount of some transcripts can be detected in response to cytokines and that forskolin treatment may further alter some mRNA abundancies. The role of calcium in receptor mediated changes is being determined.

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27.15

Na/Ca EXCHANGE: A MAJOR DETERMINANT OF ASTROCYTE RESPONSIVENESS TO NEUROTRANSMITTERS AND EXCITOTOXINS. M.P. Blaustein, B.K. Krueger, P.J. Yarowsky, & W.F. Goldman*. Depts. of Physiol. & Pharmacol., U. MD Sch. Med., Baltimore, MD 21201

The influence of the Na^+ gradient on resting intracellular Ca^{2+}, $[Ca^{2+}]_i$, and on evoked increases in $[Ca^{2+}]_i$ was measured in cultured rat brain astrocytes. The cells were loaded with fura-2, introduced as the acetoxymethyl ester; the apparent free intracellular Ca^{2+}, $[Ca^{2+}]_{App}$, was determined by digital imaging. In unstimulated cells, $[Ca^{2+}]_{App}$ was heterogeneously distributed; in most cytoplasmic areas $[Ca^{2+}]_{App}$ was 50-100 nM, but much higher levels were seen in some areas. Glutamate, 100 μM, or 40-100 mM K^+ evoked transient, non-uniform increases in $[Ca^{2+}]_{App}$ as high as 1 μM. Replacement of 135 mM Na^+ by isosmolar N-methylglucamine, or 15 min exposure to ouabain, induced a small increase in $[Ca^{2+}]_{App}$ in most cells, but substantially potentiated the amplitude and duration of glutamate- and K^+-evoked Ca^{2+} increases. After exposure to ouabain, reduction of $[Na^+]_o$ induced a dramatic, external Ca^{2+}-dependent rise in $[Ca^{2+}]_{App}$. Thus, reduction of the plasmalemmal Na^+ gradient not only increases $[Ca^{2+}]_i$ in resting astrocytes, but also augments the Ca^{2+} transients evoked by depolarizing agents. The latter may be due to increased intracellular Ca^{2+} storage as well as to inhibition of Ca^{2+} extrusion via Na/Ca exchange: the exchanger may not only play an important role in regulating $[Ca^{2+}]_i$ in astrocytes, but it may also be a major determinant of the responsiveness of these cells to neurotransmitters and excitotoxins by regulating Ca^{2+} in intracellular stores.

27.16

THE EXPRESSION OF SODIUM CHANNELS IN ASTROCYTES FROM NEONATAL RAT SPINAL CORD *IN VITRO*. J.A. Black, H. Sontheimer, B.R. Ransom and S.G. Waxman. Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06510 and Neuroscience Research Center, VA Medical Center, West Haven, CT 06516.

Astrocytes derived from optic nerve, hippocampus, and cerebral cortex have been shown to express voltage-sensitive sodium channels *in vitro*. However, it has become clear that neither the pattern of expression nor the physiological properties of sodium channels are invariable for astrocytes derived from differing CNS regions. Thus, we have begun to examine the expression of sodium channels in astrocytes derived from neonatal rat spinal cords.

Cells from P-0 rat spinal cords were dissociated and plated at a density of 10^5/ml on poly-ornithine/laminin-coated glass slides; cells were maintained in Earle's MEM containing 10% fetal calf serum. At 1 day *in vitro* (DIV), many cells exhibited GFAP and/or vimentin staining; in addition, A2B5 and O4 immunoreactivity was present on some cells. Sodium channel immunostaining with antibody 7493 was present but weak at 1 DIV. At 7 DIV, sodium channel staining was robust on $GFAP^+$ cells. Many of the $GFAP^+$ cells exhibited A2B5 or O4 staining. By 14 DIV, sodium channel immunostaining was reduced, and by 28 days in culture it was not detectable; A2B5 staining was greatly attenuated at 14 DIV and completely absent by 28 DIV. In contrast, $GFAP^+/O4^+$ cells continued to be present at 28 DIV.

These observations demonstrate that astrocytes derived from neonatal rat spinal cord exhibit a different pattern of sodium channel expression than astrocytes derived from several other CNS regions, and suggest a regional difference of sodium channel expression in astrocytes.

[Supported in part by VA, NMSS and NIH]

27.17

TRANSIENT PERIOD OF ELECTROGENESIS DURING THE DEVELOPMENT OF RAT SPINAL CORD ASTROCYTES *IN VITRO*: H. Sontheimer, J.A. Black, B.R. Ransom, and S.G. Waxman. Dept. Neurology, Yale University, New Haven, CT 06510 and VA Hospital, West Haven, CT 06516.

The expression of voltage-activated Na+ and K+ currents was studied in astrocytes cultured from P0 rat spinal cord using whole-cell patch-clamp recordings. As with optic nerve cultures, two antigenically distinct astrocyte populations ($GFAP^+/A2B5^-$ and $GFAP^+/A2B5^+$) can be identified. Both astrocyte types express 4-AP-sensitive, voltage-activated outward K+ currents at high densities (100-200 pA/pF) immediately after dissociation. However, at such early times *in vitro*, voltage-activated Na+ currents are only expressed in $A2B5^+$ astrocytes. $A2B5^-$ astrocytes begin to show robust Na+ currents after 5-6 DIV; in fact, Na+ currents are so large (up to 18 nA; I-dens. 100-300 pA/pF) that channel densities are 10-50fold higher (~ $3/\mu m^2$) than in optic nerve or hippocampal astrocytes. Outward K+ currents are almost lost in $A2B5^-$astrocytes at the time that these large Na+ currents are expressed. At 5-10 DIV $A2B5^-$ astrocytes exhibit action-potential-like responses in response to hyperpolarizing current injections ("anode break spikes"), without pharmacological blockage of outward currents. These responses overshoot 0 mV and return to resting potential with a time-constant of ~ 6 msec. Trains of such responses can be elicited by repetitive stimulation. Hyperpolarization in excess of -150 mV is required to overcome Na+ channel inactivation since even at potentials as negative as -130 mV Na+ current inactivation is not always removed (h_∞-curves of $A2B5^-$ astrocytes show midpoints around -90 mV whereas $A2B5^+$ astrocytes typically have midpoints around -60 mV). Action-potential-like responses could not be recorded in $A2B5^+$ astrocytes since these cells continue to express large outward K+ currents that exceed Na+ current amplitudes by 2-10fold and stabilize the cells resting-potential. Since ion channel densities appear to be modulated by extraglial factors *in situ* and by *in vitro* culture conditions, it is not clear whether astrocytes *in situ* display regenerative responses. (Supported by: NIH, VA, EPVA)

27.18

POTASSIUM AND ANION CHANNELS OPENED BY OSMOTIC GRADIENTS ACROSS ASTROCYTE PLASMA MEMBRANES *T. Jalonen, A.J. Popp and H.K. Kimelberg
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Astrocytic swelling can be induced in vitro by osmotic gradients and high extracellular K^+. After a fast initial volume increase in hypoosmotic solutions astrocytes undergo a regulatory volume decrease (RVD), but usually remain swollen in high K^+. Activation of "stretch-sensitive" channels has been proposed to contribute to this RVD. We have found increased occurrence of large-conductance, potential-dependent anion (A^-) channels in isolated astrocyte membrane patches when hypoosmotic solutions were present in the bath and isoosmotic solutions were present in the pipette. Currently about half (12 of 26) of such recordings have clearly shown a large-conductance Cl^- channel, as determined by the reversal potential for the current. High K^+ in bath, both in inside-out and cell attached recording configurations, induced K^+ channel activity while A^- channels were only rarely detected. With gluconate present on the extracellular side only K^+ channels have been detected, suggesting that gluconate is inhibiting the A^- channel. This anion channel is also blocked by L-644,711, an anion transport inhibitor that has been shown to be useful in recovery from experimental stroke and head injury. Supported by NS 23750 (H.K.K.) and the H. Schaffer Foundation (A.J.P.).

27.19

L 644,711 INHIBITS K^+ - STIMULATED CHLORIDE ION UPTAKE AND CELLULAR INJURY IN CORTICAL ASTROCYTES. P. Thakran, M.P. Leuschen, A. Chatterjee*. Div. Newborn Med., Univ. Nebraska Med. Ctr., Omaha NE 68198.

Cytotoxic edema of astrocytes is the major hypoxic-ischemic lesion in the brain of premature infants and has been attributed to a high extracellular K^+ - stimulated, chloride mediated process. The effect of the anion transport inhibitor L 644,711(5,6,-Dichloro-2,3,9,9a-Tetrahydro-3-oxo-9a-propyl-1H-fluoren-7yl) oxyacetic acid was investigated in an in vitro model consisting of primary cultures dissociated with dispase from cerebral cortices of neonatal guinea pigs, plated on Vitrogen™, maintained in modified DMEM with 10% FCS and characterized as >90% astrocytes by immunohistochemistry with antiserum to GFAP. The extracellular milieu of 18-day, confluent astrocytes was manipulated by incubation in either a basal buffer (BB) with an ionic composition similar to DMEM or one with 60 mM K^+ (HiK). Cellular injury was quantitated by three parameters, uptake of ^{36}Cl, efflux of lactate dehydrogenase (LDH) and the MTT viability assay. Incubation in HiK (vs. BB) resulted in an increase in ^{36}Cl uptake within 10 minutes, a time-dependent increase in LDH efflux (30 min. - 4 hours), and significantly reduced cell viability ($p<0.05$). L 644,711 significantly inhibited HiK-stimulated ^{36}Cl uptake and reduced LDH efflux in a dose dependent manner. The MTT assay revealed 10^{-11} M L 644,711 as the peak effective dose for preventing injury when administered simultaneously with the HiK paradigm in a 1-hour incubation. The same dose was effective in reversing injury when administered 10 min. after exposing the cells to HiK. These findings indicate the potential usefulness of agents which modify ion transport processes in hypoxic-ischemic cerebral injury.

27.20

APPLICATION OF POTASSIUM CHLORIDE TO THE CORTICAL SURFACE DRAMATICALLY UPREGULATES LEVELS OF MESSENGER RNA FOR GLIAL FIBRILLARY ACIDIC PROTEIN IN CORTEX AND HIPPOCAMPUS. D.J. Bonthius, J.L. Stringer, E.W. Lothman and O. Steward. Departments of Neuroscience, Pediatrics and Neurology, University of Virginia, Charlottesville, Virginia 22908.

It has previously been shown that electrically-induced seizures lead to increased levels of mRNA for GFAP in the hippocampus (Steward et al., P.N.A.S., in press). The present study investigates whether the signal for this increased expression might include elevated extracellular potassium.

Adult male rats underwent unilateral parietal craniotomy, and pledgets soaked in KCl (3M) were applied to the exposed parietal cortex for ten minutes. Previous studies have shown that this treatment can induce cortical spreading depression. K^+-sensitive microelectrode recording revealed a negetive shift in DC potential and a sharp increase in extracellular K^+, conditions characteristic of spreading depression. The animals were killed 24 hours following KCl application and levels of GFAP mRNA were assessed by *in situ* and dot blot hybridization.

In situ hybridization revealed increased labelling throughout the ipsilateral cortex and throughout the hippocampus bilaterally. Dot blot hybridization revealed a greater than 10-fold increase in GFAP mRNA in the hippocampus, relative to saline-treated controls. In a time-course study, animals were killed 1.5, 3, 6, 12 and 24 hours post KCl application, and *in situ* hybridization was used to evaluate levels of GFAP mRNA. Increases in GFAP mRNA first became detectable at 6 hours, and levels continued to increase up to 24 hours. These results suggest that elevated extracellular K^+ can upregulate GFAP gene expression and that this effect can be seen over widespread areas, possibly as a result of spreading depression. (Supported by NIH Grant NS12333 to O.S.)

28.1

DIFFUSIONAL TRANSPORT OF MACROMOLECULES IN DEVELOPING NERVE PROCESSES. S. Popov and M-m. Poo, Dept. of Biological Sciences, Columbia Univ. N.Y., N.Y. 10027.

Passive transport of macromolecules in growing nerve processes was analyzed quantitatively by measuring the rate of diffusional spread of fluorescently-labeled molecules injected into the soma of cultured *Xenopus* neurons, using digital fluorescence imaging techniques. The diffusion coefficient (D) of a typical globular protein bovine serum albumin (BSA) was found to be 12.5 + 2.3 x 10^{-8} cm^2/s (s.e., N=6), a value 10-fold higher than the previously reported values for BSA diffusion in fibroblast cytoplasm. The relatively high D value of soluble macromolecules in developing neurons suggests that passive diffusion could serve as an effective transport mechanism. When the diffusion of dextrans of various sizes was examined, we found that the dependence of D on the molecular size deviates considerably from that expected for diffusion in a viscous aqueous medium. Treatment of the neuron with microfilament-disrupting agent cytochalasin B, or pre-loading the neuron with dephospho-synapsin I, a molecule that induces bundling of actin filaments, significantly increased the diffusion rate for large dextrans, but had little effect on the diffusion of small dextrans. This finding suggests that the microfilament meshwork imposes a selective constraint on the diffusion of large macromolecular components within the neuronal cytoplasm.

28.2

UNC-116 ENCODES C.ELEGANS KINESIN. N. Patel, G. de Feo*, J.R. Mancillas. UCLA, Los Angeles , CA. 90024

During a screen for genes involved in axonal guidance, a single hypomorphic allele, e2281, representing a new locus, unc-116, was identified. (J.R. Mancillas, Neuroscience 22:S236, 1987). We have cloned unc-116 using a combination of the transposon tagged allele and the physical map. Analysis of putative full length cDNA and predicted amino acid (aa) sequence reveals that unc-116 encodes the heavy chain of kinesin. At the aa level, unc-116 is 55% identical to Drosophila kinesin (Yang et. al. , Cell 1989) and to squid kinesin (Kosik et. al. , Journal of Biological Chemistry, 1990). The three major domains of kinesin (Yang et. al.) are present in unc-116 : the motor domain is 71% identical to Drosophila and contains the consensus ATP binding sequence; the stalk region is 150 aa shorter than in Drosophila but has the heptad repeat characteristic of coiled-coil α-helices; and the tail is 60% identical. The transposon insertion in e2281 occurred near the stalk-tail junction; the resulting alteration in kinesin may affect transport of specific membrane-bound organelles and thus account for the specific axon misplacements observed in this allele (de Feo et. al, SNS abstract 1991). Embryonic lethality in a second, independently isolated allele (J. Plenefisch and E. Hedgecock, unpublished data), suggests a function for kinesin in early development, in addition to its neuronal function.

28.3

UNC-116, A MUTANT IN THE C. ELEGANS KINESIN HOMOLOG HAS SPECIFIC NERVOUS SYSTEM DEFECTS G. de Feo*, N. Patel, and J.R. Mancillas. UCLA, Los Angeles, CA. 90024

Unc-116, the *C. elegans* kinesin homolog (N. Patel et. al., Society for Neuroscience abstract,1991), was identified as a new genetic locus in a screen for mutants in abnormal backward locomotion (J. R. Mancillas, Neuroscience 22:S236, 1987). We are characterizing the neuroanatomical defects of two alleles of this gene. The first, e2281, is a weak hypomorph which has a restricted set of defects in the nervous system. By using a variety of antibody stains that mark specific subsets of neurons, we have detected neuronal defects such as abnormal varicosities in the Alm(anterior lateral microtubule cell, a touch receptor neuron), an abnormal posterior projection in the Alm, and dorsally misplaced Alm axons. These defects seem to be variable from individual to individual which may explain the variable behavioral phenotype of these animals. The second allele we are investigating is rh24 (provided by J. Plenefisch and E. Hedgecock), a much stronger allele which is 80% embryonic lethal(J. Plenefisch, personal communication). This allele has more severe neuroanatomical defects, which correlates with more severe locomotor defects in these animals. These studies indicate that a disruption in kinesin has consequences for both early development, and nervous system development.

28.4

Distribution of kinesin in squid axoplasm by rapid freeze immunocytochemistry. J. E. Moreira*, A. Kladakis* and T. S. Reese. Laboratory of Neurobiology, NIH, Bethesda MD 20892.

Axoplasmic kinesin was originally thought to be the source of the transport motor for moving organelles, but recent work shows that the anterograde organelle motor appears to be very tightly attached to the organelle surface (Schnapp *et al.*). It now becomes essential to distinguish cytoplasmic kinesin that might bind to the organelle surfaces during fixation from kinesin originally on organelle surfaces. In order to prevent displacement of kinesin during preparation, squid axoplasm was extruded and immediately frozen on a liquid helium cooled copper block, freeze-substituted in 0.1% uranyl acetate in acetone at -80°C, infiltrated with Lowicryl K11M at -60°C, and warmed up from -70°C during ten days of UV polymerization. This protocol avoids contact with water, cross-linking fixatives, and stabilizes the tissue prior to cutting. Electron microscopic immunolabeling of thin sections with rabbit polyclonal anti-kinesin and protein A-gold showed gold grains on both organelles and cytosol. Specific labeling by a polyclonal antibody against squid neurofilament was used along with the anti-kinesin to compare the localization of both proteins. Backgrounds were very low and controls, incubated with rabbit non-immune IgG or whole serum, showed only a few gold particles. Our results suggest that the antigens reacting with the kinesin polyclonal antibody are widely distributed in cytoplasm. Though organelles showed labeling density slightly above cytoplasmic levels it remains unclear whether this organelle staining corresponds to the motors that move the organelles.

28.5

SUPPRESSION OF KINESIN EXPRESSION USING ANTISENSE OLIGONUCLEOTIDES IN CULTURED HIPPOCAMPAL NEURONS. A. Ferreira*, J. Niclas*, K. Kosik, R. Vale*, and G. Banker, Univ. of Virginia, Charlottesville, Virginia; Harvard Medical School, Boston, MA; UCSF, San Francisco, CA.

As one approach to examine the functional role of kinesin in living nerve cells, we used antisense oligonucleotides to inhibit kinesin expression in dissociated-cell cultures prepared from embryonic rat hippocampus. Based upon the sequence of the human kinesin gene, a 25-mer oligonucleotide in the antisense orientation was prepared against a region of the heavy chain that spanned the initiator methionine. Subsequent analysis has revealed only two discrepant nucleotides in the corresponding region of the rat gene. Antisense oligonucleotide was added to cultures every 12 hours, beginning 4 hours after plating. After 48 hours kinesin levels were assessed using a monoclonal antibody directed against the heavy chain (SUK-4, J. Scholey). In comparison to untreated or sense-treated controls, kinesin immunostaining was abolished under antisense conditions and the content of immunoreactive kinesin was reduced by more than 90% based on dot immunobinding. Analysis of neurite length revealed that elongation was inhibited by almost 50% in the presence of the antisense oligonucleotide, axons and minor processes being equally affected. As one measure of the transport of vesicle-associated proteins, we examined the distribution of synapsin I and GAP-43. Under antisense conditions, immunostaining for both proteins was largely confined to the cell body, whereas in control cultures immunoreactivity extended into the neurites and was concentrated at their distal tips. These results are consistent with the suggestion that kinesin mediates vesicle transport toward the plus ends of microtubules in vivo, and that interference with this transport interferes with process outgrowth.

28.6

AXONAL TRANSPORT IN TRANSECTED NERVES: FURTHER EVIDENCE FOR BIDIRECTIONAL TRANSPORT OF NEUROFILAMENTS. J.D. Glass, P.N. Hoffman and J.W. Griffin. Departments of Pathology, Neurology, and Ophthalmology and the Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Peripheral nerves of C57BL/6/Ola mice degenerate very slowly following separation from their nerve cell bodies. Previously, we have described the massive accumulation of neurofilaments (NF) at both proximal-and distal-cut sites of doubly transected sciatic nerves, suggesting the bidirectional transport of NF. In the present study, the axonal transport of pulse-labeled NF proteins was analyzed in axons isolated from their cell bodies. At either 7 or 14 days after [^{35}S] methionine was injected into the L5 dorsal root ganglion (DRG) of C57BL/6/Ola mice, DRG were removed (ganglionectomized) and the distribution of labeled proteins in the isolated sciatic nerve was analyzed 7 or 14 days later, respectively (i.e., 21 days after labeling). Comparison of these transport profiles with those from corresponding intact nerves demonstrated that NF proteins were located more proximally in ganglionectomized nerves. These findings support the hypothesis that NF proteins are transported in a retrograde direction in isolated nerve segments.

28.7

BLOCKAGE OF FAST AXOPLASMIC TRANSPORT BY MECHANICAL COMPRESSION. Paul E. Gallant, Laboratory of Neurobiology, NINDS, NIH, Bethesda MD 20892

Fast axoplasmic transport is often studied by collecting material that accumulates focally after crushing or tying off an axon. These procedures might block fast transport indirectly by cutting off the blood supply or more directly by damaging axonal structures necessary for fast transport. The direct effects of mechanical compression on fast axoplasmic transport were studied by video-enhanced differential interference microscopy of isolated squid giant axons that were compressed with 0.5, 5.0, 20, or 100 g weights over a 1 mm length of axon. Compressions with 0.5 g/mm momentarily deformed the axon but produced no change in fast axoplasmic transport. Compressing the axon with 5-20 g/mm broke and separated the axoplasm at the site of the crush. Though the axoplasm usually returned to the crush site after the weight was removed, organelles failed to cross this rejoined crush site, instead they accumulated over time at the crush site. This block was not due to an influx of harmful extracellular ions into the axon at the crush site, since replacing the normal external ions with an internal-type media had no effect on the blockage or accumulation of organelles produced by a 5-20 g/mm crush. Compression with 100 g/mm led to a rapid ion dependent liquefaction of axoplasm at the site of the crush. Replacing the harmful external media with internal-type media prevented the axoplasmic liquefaction, but the internal media did not prevent the breakage of the axoplasm or the blockage of transport in the axons compressed with 100 g/mm. Moderate pressures thus appear to block transport mostly by breaking the axoplasm, while higher pressures break both the axoplasm and the plasma membrane.

28.8

BREFELDIN A INHIBITS FAST AXONAL PROTEIN TRANSPORT AND DISASSEMBLES GOLGI APPARATUS BUT DOES NOT DIMINISH ANTEROGRADE AXONAL VESICLE TRANSPORT R.S. Smith, H. Chan* and R. E. Snyder, Department of Anatomy and Cell Biology and Department of Applied Sciences in Medicine, University of Alberta, Edmonton, Canada T6G 2H7

Newly synthesized proteins that undergo rapid axonal transport are carried by vesicles and tubulovesicular organelles, but whether the transport of these organelles is dependent on the recent synthesis of protein is not known. To investigate this relationship we have prevented the export of newly synthesized protein from the cell body using brefeldin A, a drug that has been shown to disassemble the cis and medial Golgi in non-neuronal cells and have determined the effect of this agent on the rapid axonal transport of proteins and vesicles.

The sciatic nerves and dorsal root ganglia (DRG) with or without the spinal cord were isolated from the amphibian Xenopus laevis and maintained for periods up to 27 h in a physiological saline. Newly synthesized protein was metabolically labeled in DRG with ^{35}S-methionine or ^{3}H-leucine. The DRG were exposed to brefeldin A (10 µg/ml), and for comparison to cycloheximide (125 µg/ml), or puromycin (500 µg/ml). Rapidly transported protein was assayed in the sciatic axons by liquid scintillation analysis. The status of the Golgi apparatus was determined by transmission electron microscopy. Organelle transport was studied in isolated sciatic axons using video microscopy.

Brefeldin A did not affect the synthesis of proteins in the DRG but rapidly (<1 h) inhibited the amount of axonal transport of protein undergoing axonal transport to less than 1 % of control values. The inhibition of axonal transport was concomitant with the ultrastructural disappearance of Golgi stacks, and was similar in time course and in magnitude to the inhibition achieved with cycloheximide or puromycin. In contrast, the anterograde flux of vesicles as determined by video microscopy remained at control values for times up to 24 h in the presence of each of the inhibitors. We conclude that newly synthesized proteins are not required for the formation or transport of vesicles from the neuronal cell body. (Supported by MRC, Canada)

28.9

TRANSPORT AND TURNOVER OF AXOPLASMIC PROTEINS IN CONTROL AND ANUCLEATE MAUTHNER AXONS OF GOLDFISH J. W. Moehlenbruck, J. A. Cummings*, and G. D. Bittner
Dept. of Zoology, Univ. of Texas, Austin, Tx. 78712

After severance, the distal segments of giant Mauthner axons (anucleate M-axons) in goldfish remain morphologically and physiologically intact for months at 10-30°C, but eventually degenerate in a proximal-to-distal (P-D) direction at a temperature-dependent rate (Blundon et al., 1990). Long term survival (LTS) of anucleate M-axons could be due to a very slow turnover of axoplasmic proteins. In goldfish maintained at 15°C, the turnover of 80 kD and 130 kD neurofilament proteins in single anucleate M-axons was analyzed using 1D SDS/PAGE, silver staining for total protein, and/or Bodian's silver stain for neurofilaments. The neurofilament bands, and other axoplasmic protein bands, were intensely stained in gels from anucleate M-axons severed 70 days previously (or from intact M-axons), but not in samples from anucleate M-axons severed for over 80 days.

We have also analyzed the transport of neurofilament proteins within control M-axons. In goldfish maintained at 15°C and 25°C, axoplasmic proteins were radiolabelled at the Mauthner cell body in the medulla. At postinjection intervals of 10-60 days, consecutive 3 mm segments of single M-axons were isolated and labelled M-axon proteins visualized using SDS-PAGE and fluorography. Slow transport of neurofilaments within intact M-axons occurred at rates of 0.1-0.2 mm/day at 15°C and 0.4-1.3 mm/day at 25°C. These rates are similar to observed rates of P-D degeneration in anucleate M-axons. We are now measuring slow transport rates in anucleate M-axons. Our results are consistent with a very slow turnover of axoplasmic proteins during LTS of anucleate M-axons and during slow transport in intact M-axons. Supported by NSF and TATP grants to GDB.

28.10

CYCLIC AMP INCREASES THE QUANTITY OF ELH-CONTAINING PROTEINS IN THE NEURITES OF *APLYSIA* BAG CELL NEURONS. E. Azhderian*, C.-H. Lin, D. Hefner*, P. Forscher and L. K. Kaczmarek, Yale University, New Haven, CT. 06510

Brief synaptic stimulation of the bag cell neurons triggers an afterdischarge during which cAMP levels are elevated. During the discharge, the neuropeptide ELH is released from the terminals of the bag cell processes. Previous work has shown elevations in cAMP increase the synthesis of ELH and accelerate processing of the initial ELH prohormone. We now report that cAMP increases the transport of ELH-immunoreactive proteins from the bag cell somata towards the terminals. Bag cell clusters in intact abdominal ganglia were labeled with ^{3}H leucine and then incubated with TTX to inhibit afterdischarges. Experimental ganglia were exposed to a cAMP analog CPTcAMP(500 uM) after which the bag cell somata and the neurite-containing sheath were dissected away from the rest of the ganglion. After a 10 min treatment with CPTcAMP we found that the amount of ELH-containing proteins in the neurite-containing sheath increased several fold over control. We also analyzed the distribution of ELH immunofluoresence in the processes of cultured bag cell neurons incubated with the adenylate cyclase activator forskolin in the presence of 10 uM anisomycin and 50 uM TTX. ELH immunoreactivity was found to extend relatively uniformly along the length of the neurites in control cells while forskolin treatment resulted in an increased concentration of ELH immunoreactivity toward the distal end of the neurites. These findings are consistent with the hypothesis that an elevation of cAMP increases the transport of ELH-containing secretory granules into and along the neurites. We are presently using video-enhanced microscopy to determine whether the rates of granule movement and the density of granules on microtubule tracks are modulated by cAMP.

28.11

RETROGRADE AXONAL TRANSPORT OF PHOSPHATIDYLCHOLINE AFTER INTRANEURAL INJECTION OF [^{3}H]CHOLINE INTO THE RAT SCIATIC NERVE. S.Padilla*, E.H.Bennett*[1], P.Tandon[2]. Neurotox. Div. (MD-74B), US E.P.A. and [1]METI, Res.Tri.Pk., NC 27711; and [2]Center for Environmental Medicine, Univ.of NC, Chapel Hill, NC 27514.

Retrograde axonal transport of phosphatidylcholine (PC) in the sciatic nerve has only been demonstrated after injection of lipid precursor into the cell body regions. When [^{3}H]choline was injected locally into the sciatic nerve the majority of the label was incorporated into myelin phospholipids (Gould et al. 1987 J.Neurochem. 48; 1121). We now report, however, that after microinjection (1 µl) of [methyl-^{3}H]choline chloride (87.6 Ci/mmol;15 µCi) into the rat sciatic nerve (45-50 mm distal to L_4 and L_5 dorsal root ganglia; DRGs), time-dependent accumulation of [^{3}H]labeled lipid occurred only in DRGs ipsilateral to the injection site. The dpm present in the contralateral DRGs remained constant at 140-193 dpm between 2 and 72 hrs after injection; the labeling in the ipsilateral ganglia was minimal at 2 hrs (96 dpm) and increased thereafter: 7 hrs=272 dpm (median); 24 hrs=1590 dpm; 48 hrs=748 dpm and 72 hrs= 1055 dpm (n=3 to 5 per time point). One dimensional TLC separation of the ipsilateral DRG lipid extract revealed that ≥90% of the label was present in PC at all time points. Because colchicine injection into the sciatic nerve prevented the accumulation of radiolabel in the ipsilateral ganglia, the presence of this delayed radiolabeling of the ipsilateral DRGs is probably due to retrograde axonal transport of PC.

28.12

THE THIOL-PROTEASE INHIBITOR E64 INHIBITS THE REVERSAL OF ACETYLCHOLINESTERASE AT A NERVE SECTION. G. Filliatreau*, B. Tavitian, R. Hässig*, L. Di Giamberardino. INSERM U.334, Service Hospitalier Frédéric Joliot, CEA, F-91406 Orsay, France.

Axonal acetylcholinesterase (AChE) carried by rapid anterograde axonal transport "turns around" at a nerve section and is carried back to the cell body. This mechanism is probably identical to the "anterograde to retrograde (A-R) conversion mechanism" occurring at the nerve endings, which was reported to be blocked by protease inhibitors (Sahenk and Lasek, 1988).

We analyzed the effect of E64 and leupeptin, two thiol-protease inhibitors, on the turnaround of AChE in transected sciatic nerves of young Leghorn chickens.

When the tip of the cut nerve was dipped *in vivo* for 30 min to 5 hours into a solution of E64 (10 mg/ml) immediately after the section, the turnaround of AChE was inhibited up to 50%. However, no inhibition was observed if the application of E64 was delayed for one hour or more after the section. On the contrary, leupeptine had no effect on the turnaround of AChE.

These observations suggest that cystein proteases could be implicated in the events leading to the establishment of the A-R conversion mechanism at the tip of a cut nerve.

Sahenk Z, Lasek R (1988) Brain Res., 460: 199-203.

28.13

TISSUE IMMUNOLOCALIZATION OF A 60KD PROTEIN ORIGINALLY ISOLATED FROM CNS PRESYNAPTIC TERMINALS. J.A. Garner and M. J. Cullen, Dept. of Anatomy and Cell Biology, USC School of Medicine, Los Angeles, CA 90033.

Previously, a 60KD protein was shown by axonal transport studies to be a major constituent of guinea pig retinal ganglion cell (RGC) collicular terminals. It is a major slow component b protein (6.9% of total cpm), the group of proteins thought to convey the cytoplasmic matrix. The unusual solubility characteristics of this 60KD presynaptic protein (when exposed to micromolar levels of calcium) has allowed its ultimate purification from whole guinea pig cortex. A polyclonal antibody was generated in chickens against the purified protein and has been used in the present study to investigate the tissue immunolocalization of the antigen. Immunoblots of a number of central nervous system and other guinea pig tissues (including superior colliculus, spinal cord, cerebellum, cerebrum, retina, lung, liver, adrenal gland, kidney, small intestine, striated muscle, and cardiac muscle) revealed the presence of a 60KD antigen within all tissues except lung and liver, with some enrichment in CNS tissues, and substantial enrichment in retina, striated muscle, and cardiac muscle. Tissues stained with primary and fluorescently-derivatized secondary antibody reveal cytoplasmic staining specifically in pyramidal type neurons in cerebral cortex, in alpha motoneurone and radial glia in spinal cord, punctate staining in outer plexiform layer and generalized RGC staining in retina, punctate cytoplasmic staining in adrenal medulla, and a distinct striated pattern of staining in striated and cardiac muscles. (Supported by NIH, NINDS, NS22402.)

28.14

IS THE SMALL GTP-BINDING PROTEIN RAB6 INVOLVED IN THE VECTORIAL DELIVERY OF SYNAPTIC PROTEINS ? B.J. Jasmin, B. Goud*, G. Camus* and J. Cartaud*. Institut Jacques Monod and Institut Pasteur, Paris, France.

Recently, the Rab genes have been cloned and sequenced in mammals, and their products represent good candidates for small GTP-binding proteins involved in the intracellular transport of vesicles in higher eukaryotes. Remarkably, each of the Rab proteins appears associated with a distinct step of either the exocytic or endocytic pathway. In particular, Rab6p has been localized to the outermost Golgi cisternae in NRK cells where its function remains unclear (Goud et al., Nature 345: 125, 1990). In this work, we carried out immunocytochemical analyses of the distribution of Rab6p in a polarized cell, the electrocyte of Torpedo marmorata, to gain insights into the role of this small GTP-binding protein in exocytic events. We report that the bulk of Rab6p associates selectively with clusters of distinctive post-Golgi vesicles: only those located at the cytoplasmic face of the innervated membrane of the electrocyte. Thus, Rab6p presents a polarized distribution in this cell. Also, we show that this distribution is dependent on the integrity of the microtubule network of the electrocyte. These data are coherent with the notion that Rab6p is involved in the regulation of membrane traffic from the trans-Golgi network to specialized domains of the plasma membrane. As such, Rab6p may represent a key element involved in the sorting and targeting of synaptic proteins.

28.15

TRANSYNAPTIC AND NON-TRANSYNAPTIC FORMS OF WGA-HRP. Michael J. Russell, Hong Liu* Richard J. Nunes*, and Vijaya Vijayan. Dept. Anesthesiology, Univ. of Calif. Davis, Med. Sch., Sacramento, CA 95817.

The mechanisms of intraneuronal transport are reasonably well understood. However, why some materials cross a synaptic barrier while others do not is still largely a unknown. It is essential for the study of transynaptic carriers to develop the ability to control when a material will cross the synapse, and when it will not. In collaboration with E-Y Labs of San Mateo, CA we have developed two closely related forms of WGA-HRP. Both of these were tested by placing them into the right naris of rats to determine if they would be taken up and carried through the olfactory system into the brain. We found that one form WGA-aminocaproic-HRP ($H_2N(CH_2)_2COOH$) was taken up by the olfactory receptor neurons, but stopped at the first synapse. The other form WGA-HRP (coupled directly) was taken up and also carried across the synapse into the olfactory bulb. The coupling of HRP to WGA with aminocaproic acid results in a charge difference in the molecule as well as some conformational change. This modification is apparently not enough to prevent the material from being taken up by olfactory receptor neurons, but it does prevent the material from being transported to the next cell. This work is an important step in developing tools for the study of transynaptic transport mechanisms.

28.16

THE ALZHEIMER'S AMYLOID PRECURSOR PROTEIN (APP) IS CLEAVED INTRACELLULARLY IN A POST-GOLGI COMPARTMENT. K. Sambamurti*, J. Shioi, and N. K. Robakis. Dept. of Psychiatry, Box # 1229, The Mount Sinai Medical Center, New York, N.Y. 10029.

APP is secreted from many different cell lines as a part of its normal metabolism. The cleavage site resulting in the secreted form of APP (nexin II) is located within the amyloid peptide sequence and is inconsistent with amyloid formation. To identify the enzyme responsible for this cleavage, we have characterized the subcellular location of the cleavage event. Using PC12 cells as a model, we found that nexin II was also present in freshly prepared cell extracts. Furthermore, nexin II was detected in sedimentable vesicles and was released after permeabilization by digitonin. Pulse-chase analysis demonstrated that APP had a short half-life and was cleaved intracellularly at a late stage in the secretory pathway. Inhibition of protein-transport at the trans-golgi network (TGN) by conducting the chase reaction at 20°C resulted in the inhibition of APP cleavage suggesting that the cleavage event occurs after the APP molecule crosses the TGN. Nexin II secretion was not significantly affected at 23°C and therefore, it is unlikely that the cleavage inhibition was due to the lack of activity of the secretase at this temperature. Cell-surface labelling did not detect any APP on the surface of PC12 cells. These results indicate that APP is cleaved intracellularly in a post-golgi compartment, presumably the constitutive transport vesicles. Such a cleavage reaction has been reported earlier for certain viral proteins such as the Semilike forest virus surface glycoprotein P62.

28.17

ALTERATIONS IN THE PATTERN OF NUCLEAR PROTEINS IN *AEDES ALBOPICTUS* CELLS INFECTED WITH *MAYARO VIRUS*. Andrade, A. and Carvalho, M.G.C.. Dept. de Biofisica Molecular, IBCCF, UFRJ, Rio de Janeiro, RJ 21941, Brazil.

Comparative studies on the replication of *Sindbis* virus (Alphavirus) in cultured vertebrate and invertebrate cells revealed that enucleated mosquito cells, unlike enucleated vertebrate cells, are incapable of producing significant amounts of viral particules. This suggested that some host function was required for efficient virus replication in invertebrate cells. In the present work, we isolated *Aedes albopictus* nucleus in an attempt to characterize the pattern of nuclear protein of infected cells. We compared isolated nucleus obtained from infected cells maintained at 28°C (normal temperature of growth) and at 37°C (induces inhibition of celular growth and viral replication). We observed the presence of a viral structural protein (34 Kd) and a high molecular weight group of proteins in nucleus from infected cells incubated at 28°C. In contrast, at 37°C, a modification in the pattern of this group could be detected. The molecular weight of these proteins corresponded to that of the nonstructural proteins of *Sindbis*. The above results were reinforced by electron microscopy studies. Our data suggested that viral infection can alterate the pattern of nuclear proteins through the transport of viral proteins to nucleus and/or induction of celular proteins. The viral proteins translocation could be associated to the dependence of the virus upon the nuclear function of invertebrate cells. Support: FINEP, FAPERJ and CNPq.

29.1

NOREPINEPHRINE-INDUCED CALCIUM CURRENT INHIBITION IN ADULT RAT SYMPATHETIC NEURONS DOES NOT REQUIRE PROTEIN KINASE C ACTIVATION. G.G. Schofield and T.P. Abrahams. Dept. Physiology, Tulane University Medical School, New Orleans, LA 70112.

The α_2-adrenoceptor-mediated inhibition the Ca^{2+} current of adult rat superior cervical ganglion (SCG) neurons is transduced via a pertussis toxin sensitive G-protein. In dorsal root ganglion neurons norepinephrine-induced inhibition of the Ca^{2+} current is also transduced via a G-protein but requires activation of protein kinase C. Experiments were performed to investigate if protein kinase C is involved in the norepinephrine-induced inhibition of the Ca^{2+} current in adult rat SCG neurons. Ca^{2+} currents were recorded from dispersed SCG neurons, acutely isolated from adult rats using the whole-cell patch-clamp technique. The Ca^{2+} current induced by step depolarizations to +10 mV from a holding potential of -80 mV was decreased by both norepinephrine and by the protein kinase C activator 1,2-dioctanoyl-sn-glycerol (diC_8). The Ca^{2+} current rising phase became double exponential in the presence of norepinephrine whereas in the presence of diC8 the rising phase remained mono exponential and the current displayed a prominent decay. Intracellular application of three protein kinase C inhibitors; PKC 19-36, staurosporine and H7, from the patch pipette, had no effect on the norepinephrine-induced Ca^{2+} current inhibition. Moreover, these inhibitors did not decrease the Ca^{2+} current inhibition induced by diC8. Thus, it appears that the α_2-adrenoceptor-mediated inhibition the Ca^{2+} current of adult rat superior cervical ganglion neurons is not transduced by protein kinase C activation, and that diC8 can inhibit Ca^{2+} currents by a protein kinase C independent mechanism. Supported by PHS grant HL 43656.

29.2

MUSCARINIC MODULATION OF N- AND L-TYPE CALCIUM CHANNELS IN RAT SYMPATHETIC NEURONS. L. Bernheim, A. Mathie and B. Hille. University of Washington, School of Medicine, Seattle WA 98195.

Our previous work on freshly isolated neurons from adult rat superior cervical ganglion showed that muscarinic receptor activation suppresses more than 80% of the whole-cell calcium current. This occurs through parallel pathways, one of which uses a diffusible second messenger. To study modulation at the single channel level, we recorded barium currents from cell-attached patches with pipettes containing 110 mM $BaCl_2$. The membrane potential of the rest of the cell was zeroed with a bath solution containing 140 mM K-aspartate and 5 mM EGTA. L-type calcium channels were recorded in the presence of 10 μM ω-conotoxin in the pipette; their activity was greatly enhanced by the dihydropyridine agonist (+)202791 and was not inactivated by holding the patch at -30 mV for several minutes. N-type calcium channel activity recorded without ω-conotoxin was not enhanced by (+)202791 and could be inactivated by holding the patch at -30 mV. Application of 10 μM oxotremorine-M (a specific muscarinic agonist) by bath perfusion reduced the averaged current through L-type channels by 67±19% (n=4) and the averaged current through N-type channels by 81±15% (n=4). The suppression of L-type activity was confirmed using whole-cell voltage-clamp. (+)202791-enhanced calcium tail-currents could be reduced by the muscarinic agonist. Interestingly, noradrenaline (10 μM in presence of 1 μM propranolol), which reduces calcium current by about 50% without using the second messenger pathway, failed to act on the (+)202791-enhanced calcium tail-current. This work shows that neuronal L-type calcium channel activity can be decreased by activation of muscarinic receptors but not by activation of α-adrenergic receptors whereas both agonists decrease N-type activity. (NIH NS08174, McKnight Research Award, Fondation Suisse de Bourses en Médecine et Biologie, Fogarty Fellowship F05 TW04457)

29.3

NEUROPEPTIDE Y DECREASES THE CALCIUM CURRENT IN NEURONS ACUTELY DISSOCIATED FROM THE RAT SUPERIOR CERVICAL GANGLION. S. Foucart, D. Bleakman and R.J. Miller. Dept. of Pharmacological and Physiological Sciences. University of Chicago, Chicago, IL 60637.

The calcium current (I_{Ca}) and the intracellular calcium signal $[Ca^{2+}]_i$ were measured in acutely dissociated neurons from the adult rat superior cervical ganglion (SCG). The neurons were prepared according to the method of Schofield and Ikeda (Pflüger Arch. 411:481, 1988). In these neurons, simultaneous whole cell patch clamp and $[Ca^{2+}]_i$ measurements showed that somatostatin (SMS) and noradrenaline (NA) reduced the I_{Ca} and the $[Ca^{2+}]_i$. In the whole cell patch clamp configuration, neuropeptide Y (NPY) also inhibited the I_{Ca} in these cells. While the effects of NA and SMS were observed in most neurons, it was observed that NPY inhibited I_{Ca} mainly in cells with smaller diameter. Overall (n=50), 75% of neurons smaller than 22 μm responded to NPY whereas only 23% of the neurons larger than 22 μm were affected by NPY. The maximum current inhibition was 32.4% at a concentration of 1 μM NPY (n=8) and the IC_{50} value was 143nM. Pretreatment of cells with pertussis toxin (200 ng/ml, 16 hrs) abolished the effect of NPY, NA and SMS. It is concluded that NPY inhibits Ca^{2+} current in a specific population of SCG neurons.

29.4

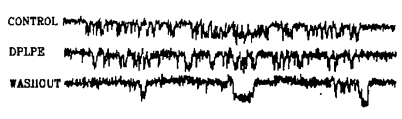
CHARACTERIZATION OF INTRACELLULAR CALCIUM INCREASE INDUCED BY M1 AND M3 RECEPTOR SUBTYPE STIMULATION IN LAN-1 HUMAN NEUROBLASTOMA CELLS. **A. Fatatis, A. Bassi*, L.M.T. Canzoniero, G.F. Di Renzo* and L. Annunziato. Section of Pharmacology, Department of Human Communication Sciences II School of Medicine - University of Naples "Federico II" Via Pansini 5 80131 Naples, ITALY.**

In LAN-1 human neuroblastoma cells, Carbachol (CCh) in a dose dependent manner (1-1000 nM) caused an increase of [Ca++]i, detected on single cell using Fura-2. This increase was characterized by a "peak phase" (10'') and a "plateau phase" that lasted 5-10 minutes in presence of CCh. If the extracellular Ca++ was removed, the "peak phase" was still present even if reduced of about 40%, whereas the "plateau phase" was completely abolished, suggesting an involvement of an extracellular calcium influx. This Ca++ entrance was Nimodipine insensitive and Verapamil and Gadolinium sensitive. Furthermore, Tetrodotoxin did not prevent CCh-induced [Ca++]i increase. Pirenzepine and 4-DAMP, two M1 and M3 receptor antagonists respectively, reduced of about 80% the [Ca++]i increase after CCh treatment, whereas Atropine completely abolished CCh induced calcium influx. Moreover, Pertussis Toxin and Staurosporine did not show any inhibitory activity on CCh induced [Ca++]i increase, whereas the two intracellular Ca++ blockers Phytic acid and TMB-8 did interfere with CCh induced Ca++ increase.(Supported by C.N.R. 883416 grant to L.A.)

29.5

OPIOID INCREASES MEAN OPEN TIME OF SINGLE L-TYPE CALCIUM CHANNELS IN N1E-115 NEUROBLASTOMA CELLS. E. Reuveny and T. Narahashi. Dept. of Pharmacol. Northwestern Univ. Med. School, Chicago, IL 60611.

The activation of δ-opioid receptors specifically reduces L-type calcium channel currents in NG108-15 neuroblastoma cells (Tsunoo *et al.* PNAS, 83 9832, 1986). We have previously found that during prolonged application of δ receptor agonist, Ca^{2+} channel currents are gradually restored (desensitization). Upon washout of the agonist, currents increase in amplitude by ≃40% above the control levels (rebound). We have now studied this modulation at the single-channel level. Cell-attached patches were held at -30 mV and step depolarized to 0 mV for 160 msec. The δ-opioid receptor agonist, DPLPE (1 μM, applied to the bath) did not change the peak amplitude. In control, during DPLPE application and after washout, the peak amplitudes were 0.96 ± 0.078, 0.92 ± 0.014 and 0.91 ± 0.12 pA (n=2), respectively. Mean open time histograms for control and in DPLPE were best fitted by single exponential curve to τ = 166 ± 14 and τ = 159 ± 1 μsec, respectively. In contrast, upon washout, the mean open time histogram was best fitted by double exponential curve with τ_f = 93 ± 33 and τ_s = 880 ± 263 μsec. The inability of DPLPE to inhibit single Ca^{2+} channel currents suggests direct G-protein interaction. The increase in open time upon washout suggests the involvement of second messengers. Supported by NIH grant NS14144.



29.6

NICOTINE ACTIVATES A WHOLE-CELL CALCIUM CURRENT IN CULTURED RAT NEOCORTICAL NEURONS. Sam A. Deadwyler, Robert E. Hampson, Jian Mu*, Barbara A. Bennett, Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Pat Lippiello, and Eric Fluhler, Div. of Pharmacology, R.J. Reynolds Tobacco Co., Winston-Salem, NC.

Previous studies from these laboratories have demonstrated the presence of nicotinic cholinergic receptors on neurons cultured from fetal rat neocortex (Lippiello *et al.*, JPET 246:409-416, 1988). Experiments utilizing Fura-2 probe demonstrated increases in intracellular calcium levels following nicotine application (Fluhler *et al.*, SN Abstr. 15:679, 1989). These techniques, however, did not demonstrate how nicotine effected calcium entry into these neurons.

The studies to be presented include whole-cell patch clamp recordings of alterations in voltage-clamp currents in cultured cortical neurons following application of nicotine to the bath (20-100 μM) or via pressure pipette (1mM). At membrane potentials between -50 and -90 mV, a voltage-sensitive potassium current was evoked by depolarizing voltage steps. Following nicotine application, the magnitude and duration of the potassium current was enhanced. The potassium current was also enhanced by barium and blocked by cadmium, indicative of a calcium-mediated potassium (I_{Kc}) current. The nicotine enhancement of I_{Kc} was blocked by d-tubocurarine (60 μM). Brief application of nicotine to cortical neurons in culture produced a depolarizing current, which reversed at +20 mV. Substitution of choline for sodium in the bath resulted in a 50-75% decrease in nicotine-gated current. This residual current was blocked by cadmium, and by nicotinic receptor blockers d-tubocurarine and hexamethonium.

Nicotinic receptor activation in cortical neurons resulted in calcium entry simultaneous with sodium entry into cells. Similar experiments in cultured hippocampal neurons did not result in nicotine-gated sodium and calcium currents. *[Supported by NIDA grant DA05073 (B.A.B.) and a gift from the R.J.Reynolds Tobacco Co. (S.A.D.)]*

29.7

MULTIPLE WAYS TO REGULATE HIGH THRESHOLD Ca^{++} CURRENTS BY OPIOIDS AND PRIOR ELECTRICAL ACTIVITY. M.D. Womack and E.W. McCleskey. Dept. of Cell Biology and Physiology, Washington Univ. Med. Sch., St. Louis, MO 63110.

What is the physical change in neuronal Ca^{++} channels when they are inhibited by μ opioids? As reported for other neurotransmitters (Bean, 1989; Elmslie et al., 1990), we find that μ opioids often cause Ca^{++} currents in rat dorsal root ganglion neurons to shift their range of gating to more positive voltages. Depolarizing prepulses to potentials greater than + 10 mV recruit Ca^{++} channels both in the presence and absence of opioids, but the recruited current is consistently larger in the presence of opioids. These results suggest that μ opioids suppress activity of a population of Ca^{++} channels by shifting their activation range to more depolarized potentials, while electrical activity enhances activity of the same population of channels by shifting the activation curve to more hyperpolarized potentials. Consistent with this hypothesis is the finding that the current recruited by positive prepulses has the same properties as the opioid-sensitive current: it activates at around -40 mV in 3 mM Ba^{++}, it is transient, it inactivates at negative voltages (-90 to -60 mV), and it is blocked by both ω-conotoxin and nifedipine. Opioids also suppress Ca^{++} currents in a voltage-independent manner. In some neurons current amplitude is decreased without a shift of the activation curve. Furthermore, extremely positive prepulses rarely recover all the opioid-sensitive current. Thus, μ opioids regulate Ca^{++} currents through both voltage-dependent and voltage-independent pathways.

Bean, B.P. Nature 340, 153-156 (1989).
Elmslie, K.S., Zhou, W., and Jones, S.W. Neuron 5, 75-80 (1990).

29.8

PHARMACOLOGICAL CHARACTERIZATION OF HIGH VOLTAGE ACTIVATED (HVA) CALCIUM CURRENTS IN DORSAL ROOT GANGLION NEURONS AND THEIR MODULATION BY NOREPINEPHRINE AND GABA. D.H. Cox* and K. Dunlap. Physiology Dept., Tufts Med. Sch., Boston, MA 02111

Norepinephrine (NE) and GABA reversibly inhibit HVA calcium channels in embryonic chick dorsal root ganglion neurons via pharmacologically distinct receptors. Since the HVA calcium current is likely composed of more than a single component, we have first characterized these currents using specific pharmacological agents--omega-conotoxin (ω-CgTx) and a dihydropyridine (DHP) agonist, (+)-(s)-202-791, and antagonist, nimodipine. ω-CgTx (9 μM) irreversibly blocked ca. 85% of the HVA current. The ω-CgTx-resistant component was reversibly augmented by (+)-(s)-202-791 and inhibited completely by nimodipine. The slow tail current produced by (+)-(s)-202-791 was not inhibited by ω-CgTx, indicating that the DHP-sensitive (L-type) calcium current was not blocked by the toxin. GABA and NE inhibited the HVA calcium current by 30-50%. Pretreatment of the neurons with ω-CgTx eliminated the transmitter-induced inhibition suggesting that NE and GABA target the ω-CgTx-sensitive (N-type) component of the HVA current. This was further demonstrated in experiments with (+)-(s)-202-791. The current during the test pulse was inhibited by GABA while the (+)-(s)-202-791-prolonged tail was unaffected.

Work supported by PHS grant NS-16483 and the McKnight Foundation.

29.9

SELECTIVE ADENOSINE RECEPTOR ACTIVATION POTENTIATES Ca CURRENT IN ACUTELY ISOLATED HIPPOCAMPAL CA3 PYRAMIDAL NEURONS. D. J. Mogul and A. P. Fox*. Dept. of Pharmacol./Physiol., Univ. of Chicago, Chicago, IL 60614.

Previously published reports have shown that the exposure of cardiac muscle cells, DRG neurons, and hippocampal pyramidal neurons to adenosine or its analogues elicit a decrement in Ca current (I_{Ca}), possibly by blocking the N-type channel. We have found that the effect of adenosine receptor agonists on I_{Ca} in acutely isolated guinea pig hippocampal pyramidal neurons from the CA3 region depended upon which type of adenosine receptor was activated. We have previously shown that these neurons contain T-, N-, and L-type Ca currents and a whole cell current component not blocked by typical N- and L-type channel blockers. Exposure to the adenosine analogue 2-chloroadenosine (2-CA; 5 μM) decreased I_{Ca} in most neurons studied. However, in some cells, 2-CA caused an increase in I_{Ca}. If the adenosine A_1 receptor was blocked by the A_1 antagonist, cyclopentyl-theophylline (CPT; 500 μM), from a holding potential of -100 mV adenosine (50 μM) potentiated I_{Ca} at test potentials more negative than -30 mV but blocked I_{Ca} at more positive test potentials in the same cell. This potentiation was not blocked by the N-type channel blocker ω-conotoxin but was blocked by Ni^{2+} (100 μM). Potentiation of I_{Ca} was also observed with the A_2 agonist DPMA (50 nM) but was not seen with the selective A_{2a} agonist CGS 21680 (50 nM - 1000 nM) suggesting that potentiation of I_{Ca} with adenosine receptor activation does not occur through A_1 or A_{2a} receptors. In addition, when the holding potential was -60 mV, neither CPT and adenosine nor DPMA produced potentiation of I_{Ca} at any test potential suggesting that the L-type Ca channel was not responsible for the potentiation of I_{Ca}.

29.10

SINGLE CALCIUM CHANNEL CURRENTS IN INSULIN-SECRETING HIT-T15 CELLS AND THEIR MODULATION BY GLUCOSE AND BETHANECHOL. J.A. Love, N. Richards*, C. Owyang*, and D. Dawson*. Depts. of Internal Medicine and Physiology, Univ. of Michigan Med. Center, Ann Arbor, MI 48109

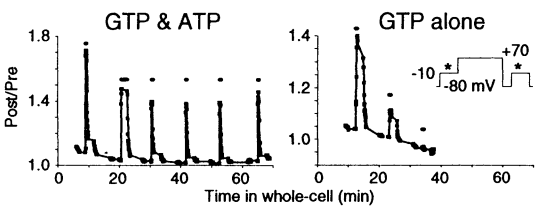
The influx of extracellular Ca^{++} through voltage-dependent channels secondary to membrane depolarization is essential for glucose-stimulated insulin release and its potentiation by cholinergic agonists. To determine whether these secretagogues also modulate Ca^{++} channels independent of changes in membrane potential single-channel (Ba^{++}) currents were recorded from cell-attached patches of HIT-T15 cells. Cells were depolarized to near 0mV by a high K^+ (143 mM) bath solution and depolarizing steps (45-65mV) applied from a holding potential of -70mV. Channel openings with a mean conductance of 30 pS were evoked at patch potentials positive to -40mV and showed little inactivation during prolonged depolarizing steps. BAY K 8644 (1μM) prolonged channel openings and patch excision rapidly abolished activity. Increasing bath glucose concentration from 0mM to 20mM (10-30 minutes) resulted in a 257% increase in the frequency of channel opening (n=5). Mean open times were increased 159% (0.39 vs 0.62 msec) in 3 of 5 cells and unchanged in 2 others. Exposure (5-30 min) of cells (n=6) to glucose-free bath solutions containing bethanechol (100 μM) increased the opening frequency by 218%. Mean open time increased from 0.53 to 0.95 msec (179%) in all 6 cells. Thus, L-type Ca^{++} channels predominate in HIT-T15 cells and channel activity is increased by both nutrient and neural stimulants of insulin secretion independent of effects on membrane potential. Supported by DK32838, DK29786 and DK34933.

29.11

WHOLE-CELL DIALYSIS WITH ATP AND GTP PRESERVES MODULATION OF CALCIUM CURRENT BY NOREPINEPHRINE. Keith S. Elmslie, Jeffrey L. Overholt,* and Stephen W. Jones. Dept. of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106.

Norepinephrine (NE) inhibits whole-cell calcium current in bullfrog sympathetic neurons via a pertussis-sensitive G protein, but it is not clear whether second messengers or protein phosphorylation are also involved. We have examined the effects of internally applied ATP (5 mM) and GTP (0.3 mM) on the NE response. Initially, 10 μM NE inhibited 25 - 60% of the current regardless of added nucleotides, but over tens of minutes the effect disappeared unless *both* ATP and GTP were added. The NE response decreased at a similar rate with GTP only, ATP only, and with no added nucleotide. Thus, both ATP and GTP appear to be necessary for maintenance of receptor-channel coupling.

Figure: NE selectively reduces the current during a prepulse to -10 mV (protocol shown at the *right*), increasing the postpulse/prepulse ratio. This measurement compensates for rundown, which is generally faster without ATP. The short horizontal bars mark applications of 10 μM NE.



29.12

CALCIUM CHANNEL CURRENTS IN CULTURED CEREBELLAR GRANULE NEURONES: EFFECTS OF PERTUSSIS TOXIN. H A Pearson*, E Huston*, A C Dolphin, Department of Pharmacology, Royal Free Hospital, London NW3 2PF, UK.

Studies of Ca^{2+}-dependent glutamate release from cultured rat cerebellar granule neurones have shown that the $GABA_B$ agonist (-)baclofen inhibits K^+-evoked release of glutamate, this action being blocked by 16h pretreatment with pertussis toxin (PTX)(1). 16h PTX also increased the amount of glutamate released following K^+-depolarisation, suggesting a tonic G-protein regulation of Ca^{2+} channels. We have therefore investigated the whole cell Ca^{2+} channel currents (I_{Ba}) in these cells with 10mM Ba^{2+} as the charge carrier.

I_{Ba} recorded from the cell body was found to be small and sustained. The threshold for activation of I_{Ba} was typically -40mV from a holding potential of -80mV and the maximal current, 61·0±4·8 pA occurred at -6·9±1·4mV (n=23). The reversal potential for I_{Ba} was 43·7±2·6mV (n=23). The dihydropyridine agonist (+)-202-791 (1μM) significantly ($p<0·05$) increased the current by 36·1±11·5% (n=4) whereas ω-conotoxin GIVA (1μM) perfused into cells in 10mM Ba^{2+} solution had no effect (n=3). In agreement with this 1μM ω-conotoxin did not inhibit K^+-stimulated glutamate release.

I_{Ba} recorded in cells pretreated with PTX (16h, 150ng/ml) was not significantly different from controls. 16h PTX did, however, attenuate the effect of 100μM (-)baclofen on I_{Ba} in these cells (52·8±6·6% inhibition in controls, n=7, 6·2±1·8% inhibition in treated cells, n=3, $p<0·005$).

These data suggest that I_{Ba} in these cells is carried predominantly by 'L-type' calcium channels which are coupled to $GABA_B$ receptors. The lack of action of PTX on I_{Ba} indicates that there is no tonic G-protein regulation of I_{Ba} at the cell body.

REF: (1) Huston, E., et al (1990) Neuroscience 38, 721-729

29.13

NEUROSTEROIDS BLOCK CALCIUM CURRENT IN FRESHLY ISOLATED GUINEA-PIG HIPPOCAMPAL CA1 NEURONS. K. T. Spence and J. M. H. ffrench-Mullen, Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

Neurosteroid block of Ca^{2+} channel current in enzymatically dissociated CA1 neurons from adult guinea-pig hippocampi was examined using the whole-cell patch clamp technique in solutions containing 3 mM external Ba^{2+} and internal NMG-Cl, Cs^+-BAPTA and Mg^+-ATP. I_{Ba} was examined with 5 ms to 1 s depolarizing command steps from a holding potential of -80 mV to a test potential of -10 mV. The steroids Allotetrahydrocorticosterone (THCC), Allotetrahydrodeoxycorticosterone (DHOC), Dehydroepiandrosterone sulfate (DHEAS), Pregnanolone, Allopregnanolone (3α-OH-DHP), Alfaxalone and Progesterone were applied by rapid superfusion. THDOC, 3α-OH-DHP, THCC, DHEAS and Pregnanolone depressed peak Ca^{2+} current current (0.01-100 μM) with maximal block at 100 μM of 40, 42, 61, 74 and 78% respectively. The Ca^{2+} current was reversibly blocked through 10 μM with partial (60-85%) >100 μM. Alfaxalone showed a 29% block at 100 μM; progesterone had no effect. Picrotoxin (10 μM), had no effect on the Ca^{2+} current and/or the blocking action of these steroids. THCC and Pregnanolone showed no change in the peak I-V relationship or deactivation kinetics. THCC, 0.1-10 μM, selectively depressed the inactivating (N-type) portion of the current. In the presence of 10 μM omega-conotoxin (fraction GVIA; CTX), THCC (10 & 100 μM) had no effect. Conversely, in the presence of THCC (10 & 100 μM), CTX had no effect. In contrast, pregnanolone continued to block. These results demonstrate a novel action of certain neurosteroids: blockade of voltage-gated calcium channels in mammalian hippocampal neurons. Secondly, an endogenous compound, THCC, appears to act at a separate site to selectively depress the CTX sensitive, inactivating or (N-type) channel. Thus, in addition to the GABA-gated Cl^- current, the data suggest an additional site at which the steroids exert their pharmacological actions to modulate brain excitability.

29.14

GLUCOCORTICOIDS ENHANCE DIHYDROPYRIDINE BIINDING TO SYNAPTOSOMAL PLASMA MEMBRANE FROM RAT BRAIN. Paul Y. Sze and Zafar Iqbal. Dept. of Pharmacol. & Mol. Biol., The Chicago Medical School, North Chicago, IL 60064, and Dept. of Neurology, Northwestern Univ. Medical School, Chicago, IL 60611.

The effects of glucocorticoids on the uptake of $^{45}Ca^{2+}$ were determined in intact synaptosomes from rat cerebral cortex following depolarization of the membrane by high K^+ (70 mM). Preincubation of the synaptosomes with corticosterone (50 nM-1 uM) at 37 °C for 15 min. resulted in an increase of the depolarization stimulated uptake of $^{45}Ca^{2+}$ (70-80% above the control at 1 uM). Cortisol, dexamethasone and triamcinolone were similarly effective, whereas 17B-estradiol and progesterone produced only small and insignificant increases.

To examine the effects of glucocorticoids on Ca^{2+} channels, specific binding of [^{3}H]PN-200-110 was determined following the disruption of the synaptosomes by sonication. Preincubation of the membrane preparation with corticosterone (1 uM) had no effect on the radiolabeled dihydropyridine binding. However, when intact synaptosomes were preincubated with the steroid and then disrupted, the membrane binding of [^{3}H]PN-200-110 was markedly increased. Scatchard analysis indicates that the increase of radioligand binding was due to an increase of the binding sites. It appears that a cytosolic factor(s) was required in the steroid action which led to an increase of the active dihydropyridine binding sites on the synaptosomal membrane.

Our results support the notion that glucocorticoids act on synaptosomal plasma membrane and one of such membrane actions is the modulation of L-subtype Ca^{2+} channels.

29.15

USE-DEPENDENT BLOCK OF CALCIUM CURRENT BY PHENCYCLIDINE IN ACUTELY DISSOCIATED HIPPOCAMPAL NEURONS. J.M.H. ffrench-Mullen[1] and M.A. Rogawski[2], [1]ICI Americas, Wilmington, DE 19897 and [2]Neuronal Excitability Section, Bethesda, MD 20892.

Phencyclidine (PCP) is well known to block a variety of ion channels in excitable cells. However, voltage-dependent Ca^{2+} channels in most tissues are relatively resistant to the drug. One exception is the Ca^{2+} channel in acutely dissociated adult guinea-pig CA1 hippocampal neurons activated by step depolarization from −80 to −10 mV which is reversibly depressed by PCP in a voltage-dependent manner by low concentrations of PCP (IC_{50}, 7 μM; *Soc. Neurosci. Abst.* **16**: 510, 1990). We now report that the PCP block occurs in a use-dependent fashion with trapping of the drug by the resting channel. Ca^{2+} channel currents were recorded with patch electrodes containing an ATP regenerating system to minimize rundown and with 3 mM Ba^{2+} in the extracellular solution as charge carrier. PCP (5 μM) was equilibrated with the cells and trains of repetitive 10 ms duration depolarizing stimuli were applied at various rates. Negligible block of the peak current was observed with the first stimulus in the trains. Steady-state block was achieved with subsequent steps according to an exponential course. The use-dependent development of block was analyzed according to the guarded receptor model of Starmer *et al.* (*J. theor. Biol.* **124**: 335, 1987). The uptake rate (λ^*) was dependent upon stimulation interval (0.5 − 15 s) with an apparent unbinding rate (λ_r) of 0.1 s^{-1}. However, there was no rate-dependent increase in steady state block and upon termination of the stimulus trains, no recovery of the current occurred even with recovery intervals of up to 5 min. Upon perfusion with drug-free solution, complete recovery was always obtained upon the presentation of 3 to 6 stimuli. We conclude that PCP block of Ca^{2+} current in CA1 neurons occurs in a strongly use-dependent fashion with an apparent unbinding rate that is distinct from the negligible true unbinding rate suggesting a novel blocking mechanism.

29.16

EFFECTS OF ANESTHETICS ON INTRACELLULAR CA^{2+} LEVELS OF MAMMALIAN NEURONS. M.E. Morris, S.K. Nsonwah* and D. Shnier*. Department of Pharmacology, University of Ottawa, Ottawa, Canada K1H 8M5.

Neurons were isolated from brains of 11-13 day-old mouse embryos, incubated with fura-2 AM, and prepared in a 4 x 10^6 cells/ml suspension for fluorimetric cuvette analysis of Ca_i (*Morris et al. 1987 Exp. Brain Res. 65: 520*). Effects of ketamine (5-200 μM), hexanol (1-800 mM) and ethanol (10-800 mM) were observed on resting Ca_i levels in normal, Ca-free and low-Na Ringer solutions and on responses evoked by 10-100 mM KCl. Ketamine produced only small and variable change in resting Ca_i and had no effect on voltage-dependent Ca^{2+} entry. Hexanol evoked dose-dependent ↑'s in Ca_i in the presence of Ca_o and decreases in its absence; ≥ 30 mM hexanol caused progressively greater Ca^{2+} membrane permeability. In contrast, single/sequential additions of ethanol evoked graded ↑'s in Ca_i in normal, Ca-free and low-Na Ringer. Increasing concentrations of ethanol progressively attenuated and reversed the Ca_i ▲'s evoked by ↑ K_o. It is concluded that an intracellular release of Ca^{2+} significantly contributes to the accumulation of Ca_i evoked by ethanol, and that with the presence/production of high Ca_i/depolarization mechanisms of uptake/outward transport may also be enhanced/activated.

(Supported by the Medical Research Council of Canada).

29.17

DM-9384, A NEW COGNITION-ENHANCING AGENT, IS A POTENT FACILITATOR OF NEURONAL Ca CHANNEL ACTIVITY AS COMPARED WITH OTHER PYRROLIDONE DERIVATIVES. S. Watabe[1], M. Yoshii[2], H. Yamaguchi[1]* and S. Ashida[1]*. [1]Explor. Res. Lab.II, Daiichi Pharmaceutical Co.,Ltd.,Tokyo 134, Japan and [2]Dept. Neurophysiol., Psychiatric Res. Inst. Tokyo, Tokyo 156, Japan.

Recent neurochemical studies have reported that DM-9384 (N-(2,6 -dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)acetamide) increases transmitter release from GABAergic and cholinergic neurons in the rat cortex (Watabe et al., Soc. Neurosci. Abstr. 15, 601, 1989; 16, 137, 1990). One possible way to explain this effect is that the drug may potentiate Ca channel opening that regulates transmitter release. This possibility has been supported by our recent whole-cell patch-clamp study in neuroblastoma x glioma hybrid (NG108-15) cells, in which long-lasting (type II) Ca channel currents were markedly increased by DM-9384 (Yoshii and Watabe, Biophys. J. 59, 82a, 1991). In the present study, we have compared the potency of four different pyrrolidone derivatives in the Ca channel facilitation at a constant concentration of 1μM. Type II channel currents were increased in the following sequence: DM-9384 (by more than 100%) > aniracetam (by 50-70%) > oxiracetam (by 10-25%) >> piracetam (no significant increase). The present results were consistent with the previous reports that GABA and ACh releases were both potentiated by DM-9384 whereas they were not increased remarkably by either aniracetam or oxiracetam.

29.18

ROLE OF GTP-BINDING PROTEINS IN ETHANOL-INDUCED SUPPRESSION OF L-TYPE CALCIUM CHANNELS IN NEURONS OF LONG- AND SHORT-SLEEP MICE. G.-J. Huang & J.J. McArdle, Dept. Pharmacology & Toxicology, New Jersey Medical School (UMDNJ), Newark, NJ 07103-2714.

In previous reports, we noted that ethanol produces a dose-dependent biphasic alteration of the calcium currents (I_{Ca}) mediated by L-type calcium channels of neurons in cultured dorsal root ganglia; i.e., 25 mg% enhances while 200 mg% depresses I_{Ca}. While single channel recording indicates that these effects of ethanol are secondary to an increase and decrease, respectively, of the probability of single channel opening the underlying mechanism remains unknown. In order to explore this mechanism, we have used the whole cell recording technique to dialyze cells of long (LS) and short (SS) sleep mice with intracellular solutions containing 100 μM GTP-γ-S or GDP-β-S. The latter compound did not alter the effect of 25 and 200 mg% ethanol on I_{Ca} for both strains of mice. In contrast, GTP-γ-S produced an interesting change in the influence of ethanol on I_{Ca}. Specifically, the voltage-sensitivity of I_{Ca} for LS neurons was shifted to the right in a time dependent manner. That is, control I_{Ca} was maximal in response to a voltage-clamp command to 8 mV while the same amplitude of I_{Ca} was elicited in response to a step to 35 mV at 10 min after exposure to 200 mg% ethanol. Our findings extend Bean's (Nature 340:152) suggestion that chemicals can suppress I_{Ca} by changing its voltage dependence. This shift of the voltage sensitivity was less for the neurons of SS mice. Supported by NIAAA grant R01 AA08025.

29.19

CHRONIC ETHANOL FEEDING INCREASES RAT CARDIAC BUT NOT NEURONAL CALCIUM CHANNEL DENSITY. M.H. Hawthorn and R. Bangalore*. Dept. of Biochemical Pharmacology, School of Pharmacy, SUNY at Buffalo, Buffalo, NY14260.

The recent observation that tolerance to ethanol is associated with an increase in L-type calcium channel density in the cortex led us to examine if this occurred in other brain areas and with other VDCC subtypes. Rats (220-260g) were chronically fed an alcohol containing diet (4.5%) according to the method of Miller (1980). This diet induced tolerance to ethanol as indicated by the length of time animals could stay on a rotor rod at 60 rpm. An i.p. injection of ethanol (1g/kg) caused a fall of 71.5±5.9% in the time control animals stayed on the rotor rod but only a 26.8±11% fall in the alcohol treated animals. However, unlike previously reported observations, L-type calcium channel density as assessed by [^{3}H]PN200-110 binding was not altered in the cortex of tolerant rats with B_{max} values being 398±29.2 and 313±76.3 fmol/mg in control and treated animals respectively. There was also no change in density in the striatum (200±16.8 fmol/mg control and 194+29 fmol/mg treated) or hippocampus (304±23 fmol/mg control and 308±33 fmol/mg treated). Ethanol had no effect on the K_D in any of the brain regions studied. Ethanol treatment was also without effect on N channel density as assessed by [^{125}I]ω-conotoxin in the brain areas examined. B_{max} values in control animals were 2180±260 fmol/mg, 1109±40 fmol/mg and 937±95 fmol/mg for the cortex, striatum and hippocampus respectively and 2940±330 fmol/mg, 942±103 fmol/mg and 989±117 fmol/mg in treated animals.

In contrast to neuronal tissue, density of L-type calcium channels in the heart was increased 30% after chronic ethanol treatment, with the B_{max} for [^{3}H]PN200-110 increasing from 402±38.6 fmol/mg in control animals to 520±31.2 fmol/mg in treated animals with no change in K_D.

Supported by NIAAA grant AA08182 to MHH.

29.20

ELECTROPHYSIOLOGICAL EFFECTS OF LOW DOSE ETHANOL ON CALCIUM CURRENTS IN DENTATE GRANULE NEURONS AT PHYSIOLOGICAL TEMPERATURE.

S.S. Jahromi and P.L. Carlen. Playfair Neuroscience Unit, Addiction Research Foundation, Departments of Physiology and Medicine (Neurology), Toronto Western Hospital, University of Toronto, Toronto, Ontario, M5T 2S8.

Ethanol has been shown to affect several Ca^{2+}-related processes in different neuronal preparations (Carlen and Wu, Int. Rev. Neurobiol., 29, 161-189, 1988). The purpose of this study is to examine the effects of low dose (20 mM) ethanol on Ca^{2+} currents at physiological temperature (36 C) in a central mammalian neuron. Adult Wistar rat hippocampal dentate granule neurons were perfused with a medium containing K^+ and Na^+ channel blockers as per Blaxter et al. (J. Physiol., 412, 93-112, 1989). Single electrode voltage clamp recordings of Ca^{2+} currents were obtained with 3M CsCl electrodes. Voltage clamp pulses of over 500 ms were delivered at different holding potentials. At hyperpolarized holding potentials, there was little effect on the early peak (unclamped) current, but some depression of the later slowly inactivating inward current was observed. The most consistent effect of ethanol was partial suppression of the L-type current elicited from depolarized holding potentials. In the majority of cells, this effect was reversible after wash.

It is hypothesized that ethanol enhances Ca^{2+}-mediated inactivation of Ca^{2+} currents at physiological temperature.

Supported by the MRC and ABMRF.

POTASSIUM CHANNELS: PHYSIOLOGY AND REGULATION I

30.1

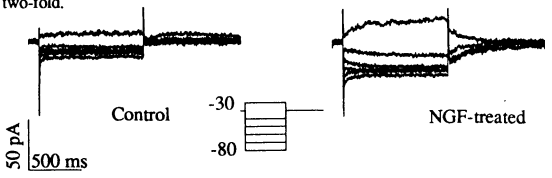
INHIBITION BY CARBACHOL OF M-CURRENT AND POTASSIUM LEAK CONDUCTANCE IN NEURONS OF THE BASOLATERAL AMYGDALA. M.D. Womble and H.C. Moises. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

Stimulation of cholinergic pathways or application of muscarinic agonists depolarizes neurons of the basolateral amygdala (BLA) and increases their input resistance (Washburn & Moises, Neurosci. Abstr. 15:193, 1989). These effects have been examined in rat BLA cells *in vitro* with the single-electrode voltage-clamp. Hyperpolarizing commands from a holding potential (V_H) of -40 mV identify the muscarinic-sensitive M-current (I_M) as a slow, inward current relaxation. Total M-conductance rises steeply with depolarization from -70 mV, the lower limit for I_M activation. At V_H = -40 mV, the application of carbachol produces an inward current shift and decreases membrane chord conductance. These effects represent inhibition of both the voltage-dependent M-current, and a voltage-independent K^+ leak current (I_{leak}). At potentials negative to -70 mV, only inhibition of I_{leak} is seen. Both currents are inhibited by carbachol in a dose-dependent and atropine-sensitive manner, indicating activation of muscarinic receptors. In control saline (3.5 mM K^+), I_M and I_{leak} show reversal potentials of -84 and -108 mV, respectively, and these are shifted in the positive direction by elevation of $[K^+]_{out}$. In a minority of cells, the instantaneous I-V curves at V_H = -70 mV (± carbachol) do not cross, possibly indicating the activation by carbachol of a non-specific dendritic conductance. We conclude that muscarinic activation produces inhibition of both M-current and a nonvoltage-dependent potassium leak conductance. (Supported by NIDA grant DA03365).

30.2

NGF Induced Expression of M-Current in PC 12 Cells. N.V. Marrion+*, N.E. Kremer, S. Halegoua* & P.R. Adams+, Dept. of Neurobiology & Behavior & HHMI*, SUNY at Stony Brook, NY 11794.

NGF induces PC 12 cells to develop a sympathetic neuron-like phenotype. Sympathetic neurons characteristically show a non-inactivating potassium current called the M-current. The presence of M-current in PC 12 cells before and after treatment with NGF was determined. PC 12 cells were whole-cell voltage clamped at -30 mV and the presence of M-current was tested by stepping the voltage depolarized or hyperpolarized for 1 second. M-current was absent from cells not treated with NGF. However, time- and voltage-dependent current relaxations characteristic of M-current were apparent within 24 hours after addition of NGF (50 ng/ml). NGF treatment increased cell capacity less than two-fold.



A channel density of 0.3 μm^{-2} and a unitary current of 0.2 pA (γ~4 pS) were estimated using nonstationary noise analysis.

To determine the signal transduction pathway for NGF's action we have initiated study of M-current expression in a PC 12 subline expressing a temperature sensitive v-src tyrosine kinase. When these cells were grown at the non-permissive temperature (41 °C) the M-current was absent. A shift to the permissive temperature (35 °C) caused appearance of M-current at a channel density similar to that seen with NGF. These results suggest that the expression of M-channels is induced by NGF, an effect which may be mediated via src activation.

30.3

MULTIPLE TYPES OF GLUCOSE-SENSITIVE K^+ CHANNELS IN HIPPOCAMPAL AND CORTICAL NEURONS C.Tromba*, A.Salvaggio*, P.Cassutti*†, G.Racagni & A.Volterra Ctr. Neuropharmacology & Inst. Pharmacol. Sci., Univ. Milan, †Farmitalia Carlo Erba, Milan, Italy.

Glucose-sensitive K^+ channels (K_{ATP}) have been described in central neurons. They may participate in the control of cell excitability and neurotransmitter release and their activity may be altered in hypoglycemic\ischemic conditions. Using the cell-attached mode of the patch-clamp technique we are trying to identify K_{ATP} channels on rat hippocampal and cortical neurons in primary culture. Cells are patched with pipettes containing in mM: KCl 130, $CaCl_2$ 1, $MgCl_2$ 1, Hepes-KOH 10, and superfused with Tyrode's solution with or without 20 mM glucose. Not all the recorded channels show sensitivity to changes in glucose concentration. However, at least 3 different types have their activity reversibly decreased by glucose: a "big" channel of 5 pA, an "intermediate" one of 2 pA, both open at rest (V_p= 0 mV), and a "small" channel of 0.8 pA, seen on hyperpolarization (V_p= +40 mV). Different glucose-sensitive channels sometimes coexist in the same patch. In conclusion, glucose-sensitivity is not a property of a specific type of K^+ channels in brain, but of a discrete "family".

30.4

MAJOR DIFFERENCES IN CNS K_{ATP} CHANNEL DISTRIBUTION BETWEEN RAT (NEWBORN, ADULT) AND TURTLE. Y. Xia and G. G. Haddad, Dept. of Pediatrics, Section of Respiratory Medicine, Yale University School of Medicine, CT 06510.

Using electrophysiological techniques, we have previously shown that 1) K_{ATP} channels are key regulators of K^+ homeostasis and neuronal responsiveness in the rat brain during hypoxia and 2) their role is quantitatively more important in the adult than in the newborn. The purpose of this present study was to examine the distribution of K_{ATP} channels using labelled glibenclamide, a potent sulfonylurea ligand which targets K_{ATP} channels, in the adult and newborn rat CNS. Since the adult turtle is resistant to anoxia, we also compared rat to turtle brain K_{ATP} channel distribution. In all animals, specific glibenclamide binding was saturable. Scatchard plots suggested that glibenclamide was bound to two types of sites in the rat and one type in the turtle. Autoradiographic images shows that distribution of glibenclamide sites was heterogeneous in the adult rat CNS with a higher density in rostral than in caudal regions. The highest binding densities were seen in the cortex, hippocampus, cerebellum, substantia nigra and a few thalamic nuclei. There were intermediate densities in the basal ganglia, septum, and hypoglossal nucleus and low density in most nuclei of the hypothalamus, midbrain, brainstem and spinal cord. The newborn rat (5 day) had a homogeneous distribution throughout the CNS but 5-10 X lower density than the adult CNS. The binding density was even lower in the turtle than in the newborn rat. Our results suggest that the mature mammalian brain is rich in glibenclamide sites and the immature mammalian or reptile brain is not. We suggest that K_{ATP} channels are expressed postnatally with maturation and speculate that they may be new phylogenetic receptors in the CNS.

30.5

GLIBENCLAMIDE ANTAGONIZES PREJUNCTIONAL EFFECTS OF DIAZOXIDE AT FROG NEUROMUSCULAR JUNCTION. Delanthi Salgado and Karim A. Alkadhi, Department of Pharmacology, University of Houston, Houston, TX 77204-5515

The K^+ channel openers cromakalim and diazoxide have been shown to decrease evoked (endplate potentials, EPPs) without affecting spontaneous transmitter release (miniature EPP, MEPPs) at the frog neuromuscular junction (Salgado et. al., *FASEB J.* 5:A1598, 1991). In order to determine whether the observed action is related to opening of the ATP-regulated K^+ channels (K_{ATP}) the effects of diazoxide in the absence and presence of a selective channel blocker were investigated. Glibenclemide (10 μM), a selective K_{ATP} blocker, caused an immediate and significant depolarization of the muscle membrane (control RMP: -82±1 mV; 20 min after glibenclemide: -73±4 mV; $p > 0.05$, n=3). However, the resting potential of the membrane gradually recovered. Other than this depolarization of the membrane potential, glibenclemide by itself had no significant effect on the parameters of transmitter release. Furthermore, glibenclemide had no significant effect on MEPP frequency. In the presence of glibenclemide, diazoxide failed to decrease the EPP amplitude and there was no significant effect on the quantal content. These results indicate that the inhibition of evoked release by diazoxide involves K_{ATP} channels that exist prejunctionally.

30.6

CHARACTERIZATION OF [^{3}H]GLIBENCLAMIDE BINDING TO RAT CEREBRAL CORTEX AND HEART MEMBRANE PREPA-RATIONS. C.E. Stidsen. Dept. of Molecular Pharmacology, Bioscience, Novo Nordisk A/S, DK-2880 Bagsvaerd, Denmark.

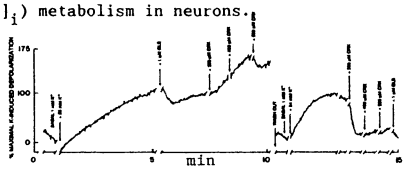
Binding of [^{3}H]glibenclamide, a sulfonylurea, to rat cerebral cortex, heart and pancreatic β-cells is highly specific with only small differences in binding constants. The sulfonylureas are supposed to label ATP-dependent potassium channels due to their ability to inhibit potassium currents in ATP-depleted preparations measured electrophysiologically or using ^{86}Rb. It was recently shown that a radioiodinated analogue of glibenclamide labeled three distinct proteins, the one (140 kDa) with high affinity, the others (65 kDa and 43 kDa) with low affinity. It is still uncertain whether the action of glibenclamide on ATP-dependent potassium channels is due to a direct interaction with the channel protein or is exerted via a modulatory subunit.

Exercising strict control of buffer ionic composition, the biochemical and pharmacological characteristics of [^{3}H]glibenclamide binding to rat cerebral cortex and heart *in vitro* will be discussed, especially evaluating the question of direct or indirect potassium channel modulation.

30.7

CROMAKALIM-INDUCED HYPERPOLARIZATION IMAGED IN SINGLE RAT CULTURED NEURONS IS GLYBURIDE-INHIBITABLE AND CORRELATED WITH INHIBITION OF ELEVATED $[Ca^{2+}]_i$ DURING DEPOLARIZATION. J.Chisholm, J.Davis* and E.Hunnicutt, Miles Inst. Preclin. Pharm., West Haven, CT 06516

The localization of a specific subset of K^+ channels (K_{ATP}), defined pharmacologically by sulfonylurea inhibitors such as glyburide (GLB), and by K-channel agonists including cromakalim (CRK), has recently been extended to the mammalian CNS. We now report that CRK dose-dependently inhibits elevated $[Ca^{2+}]_i$ during sustained depolarization, measured in single fura2-loaded neurons, and dose-dependently hyperpolarizes single neurons during sustained depolarization measured with bisoxonol. Hyperpolarizing responses to CRK are completely inhibited by 1 μM GLB (block by GLB pretreatment is not mimicked by GLB treatment after CRK.) These data are consistent with a physiological relevance of these channels to intracellular calcium ($[Ca^{2+}]_i$) metabolism in neurons.



30.8

EFFECTS OF CALCIUM AND ADRENALINE ON POTASSIUM CURRENT IN TONIC SKELETAL MUSCLE FIBERS OF THE FROG. J. Lomelí*+, C. Vásquez*+, X. Trujillo*+, M. Huerta and J. Muñiz*. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Apdo. Postal 199, 28000 Colima, Col., México.

We investigated the presence of a calcium-activated potassium (K+) current in tonic skeletal muscle fibers. Experiments were done on cruralis muscle of Rana pipiens, using three-microelectrode voltage-clamp technique (Adrian, Chandler & Hodgkin, 1970). The normal solution contained (mM): $Na\text{-}CH_3SO_3$, 117.5; $K\text{-}CH_3SO_3$, 2.5; $Ca(CH_3SO_3)_2$, 1.8 and sucrose 350. Muscle fibers were identified according to their passive electrical properties. Control outward K+ currents were recorded in the normal solution. When the external calcium was omitted from the solution, the amplitude of the K+ currents were reduced c.a. 250% (n=3). In contrast, if external calcium was increased to 11.8 mM, the K+ current was incremented 200% from the previous control currents (n=5). In this same experimental condition, with high calcium, we added adrenaline (5 uM), which augmented the $[Ca^{2+}]i$, and a new increase of potassium current was observed until 50% (n=5). These results suggest the presence of calcium-activated K+ channels in the membranes of tonic skeletal muscle fibers of the frog.

+ CONACyT fellowship.
Supported by SEP C89-01-0150 and CONACyT D111-904366.

30.9

DEPRESSION BY VOLTAGE-DEPENDENT INACTIVATION OR BY BARIUM OF THE DELAYED RECTIFIER OF RAT HIPPOCAMPAL NEURONES. A. Nistri[1] and E. Cherubini[2]. INSERM U29, 123 Bd de Port-Royal, 75014 Paris, France.

Responses from CA3 pyramidal neurones of the adult rat hippocampal slice preparation were recorded under single electrode voltage clamp at 33°C. A TEA (20 mM) sensitive slow outward K^+ current was identified as the delayed rectifier (I_K) after suppression of other voltage-dependent conductances with tetrodotoxin (1 μM), 4-aminopyridine (2mM), Co^{2+} (2 mM), Cs^+ (2 mM), and carbachol (50 μM) in a Ca^{2+}-free superfusing solution. Voltage-dependent inactivation of I_K was shown with hyperpolarizing prepulses (from -55 to -100 mV) which enhanced the steady state amplitude of I_K elicited by steps positive to -50mV. Half inactivation was reached at -80 mV, and was dependent on the interval between prepulse and test pulse (tau 211 ms). Low concentrations of barium (0.1-0.3 mM) reduced, in a voltage independent fashion, the amplitude of I_K by up to 50% without affecting its inactivation properties. These data suggest that membrane hyper-polarization enhances the ability of I_K to inhibit neuronal excitability. Furthermore, the barium-induced depression of I_K may contribute to the bursting behaviour elicited by this cation.

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30.10

VOLTAGE-GATED POTASSIUM CURRENTS IN ACUTELY-DISSOCIATED RAT NEOCORTICAL NEURONS. R.C. Foehring and D.J. Surmeier. Department of Anatomy and Neurobiology, University of Tennessee, Memphis, Memphis, TN 38103-4901.

There is controversy in the literature concerning the number, kinetics and pharmacology of voltage-gated K+ currents in neocortical neurons. We tested the hypothesis that three such currents were present in acutely-dissociated neocortical neurons: a traditional A current (I_{AF}), a transient current with slower kinetics (I_{AS}), and a delayed rectifier (I_K). Cells were dissociated using pronase E treatment followed by mechanical trituration. An attempt was made to select for cells with pyramidal-shaped somas and small processes. TTX (1μM) and CdCl2 (400 μM) were present in the bath to block Na- and Ca-dependent currents.

We found evidence for all three voltage-gated (non-Ca-gated) K+ currents in adult neocortical neurons. All cells displayed a large outward current at voltages above -40 to -30 mV which inactivated relatively slowly. Kinetic analysis of deactivation tails suggest that at least two conductances contribute to this current: I_{AS} and I_K. Some neurons displayed a distinct early current which peaked in 6-10 ms (as expected for I_{AF}), while others did not. The currents were differentiated in several other ways including their sensitivity to the pharmacological blockers 4AP, TEA, and dendrotoxin. Supported by NINDS grant NS27180.

30.11

POTASSIUM CHANNELS OPEN AT RESTING MEMBRANE POTENTIAL IN CULTURED GUINEA PIG ADRENAL GLOMERULOSA CELLS. M.N. Satyanarayan & D.P. Lotshaw, Depts. of Physiol. and Biol. Sci., Univ. of Kentucky, Lexington, KY 40506

Angiotensin II (AII)-induced depolarization of the adrenal glomerulosa cell contributes to the mechanism of AII stimulation of aldosterone secretion. AII-induced depolarization is thought to be mediated by a decrease in resting K^+ conductance (Quinn et al., Endocrinology 120:1581). In order to elucidate AII regulation of plasma membrane ion channels and mechanisms regulating steroidogenesis, we initiated a characterization of K^+ channels involved in the glomerulosa cell resting membrane potential. Guinea pig adrenal capsules were dissociated by collagenase treatment and dispersed glomerulosa cells maintained in primary cell culture. Cultured glomerulosa cells were identified by a nearly circular shape and diameter less than 15 microns. For cell-attached patch clamp recordings, cells were bathed in Kreb's Ringer and patch pipettes were filled with a K^+-rich solution (in mM: 150-KCl, 1.25-CaCl2, 1.2-MgCl2) to select for K^+ channel currents. At the resting membrane potential, multiple current amplitudes and open time kinetics were observed for both inward and outward currents. Outward currents appeared to represent anion channels, usually reversing direction of current flow in response to positive polarization of the pipette by 10 to 20 mV (hyperpolarization of the patch membrane potential). Inward currents behaved like K^+ channels, increasing in amplitude with patch membrane hyperpolarization and decreasing with depolarization. Inward currents were also observed to reverse with depolarization of the patch membrane by 70 to 80 mV, consistant with the estimated reversal potential for K^+ in these cells. These results indicated that multiple species of K^+ channels open at the resting potential and may be involved in determination of membrane potential. This work was supported by BRSG S07 RR07114-21.

30.12

Dendrotoxin and mast cell degranulating peptide block a voltage-activated potassium current in acutely isolated chick ciliary ganglion neurons. M.E. Wisgirda and S.E. Dryer. Program in Psychobiology and Neuroscience, Dept. of Biological Science, Florida State University, Tallahassee, FL 32306.

Voltage-activated K^+ currents were studied using whole-cell recordings from ciliary ganglion neurons obtained from E10-E14 chick embryos. Recordings were made in Ca^{2+}-free salines to minimize activation of Ca^{2+}-dependent K^+ currents. Ciliary neurons express two types of voltage-activated K^+ currents based on differences in kinetics and sensitivity to blockade by TEA. The delayed rectifier current (I_{DR}) is blocked by TEA, activates slowly, and shows a subsequent slow inactivation. The inactivation time constant shows considerable variation from cell to cell, but is always greater than 180ms at +30mV. I_{DR} shows half-maximal activation close to 0mV, and half-maximal steady-state inactivation at -40mV. In contrast, I_A is resistant to TEA, activates rapidly, and becomes inactivated rapidly (time constant of 14-25ms at +30mV). Half-maximal activation of I_A is at -20mV, while steady-state inactivation is half maximal at -80mV. Application of 2mM 4-AP partially blocks both currents. Dendrotoxin (280nM) and mast cell degranulating peptide (200nM) had very similar effects, each producing a partial but selective blockade of I_{DR}. Blockade produced by both peptide neurotoxins was best seen when I_{DR} was evoked by depolarizing steps positive to 0 mV. Neither toxin produced detectable blockade of I_A.

Supported by NIH grant NS-27013.

30.13

TETRANDRINE BLOCKS Ca^{2+}-ACTIVATED K^+ CHANNELS BESIDES INHIBITING A Ca^{2+}-CURRENT IN RAT NEUROHYPOPHYSIAL NERVE TERMINALS. G. Wang and J.R. Lemos. Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545.

(+)-Tetrandrine (Tet), an alkaloid with a bis-benzylisoquinoline structure isolated from the Chinese medical plant *Stephania tetrandra*, has been widely used in China for the treatment of hypertension. The mechanism for its hypotensive action is considered to be related to its negative inotropic effect by blocking L-type Ca channels in myocardium and arteries.

In the present study, the effects of Tet on the Ca^{2+}-current (I_{Ca}) and the Ca^{2+}-activated K^+ current ($I_{K\text{-}Ca}$) of isolated rat neurohypophysial nerve terminals (containing either vasopressin or oxytocin) were investigated using whole-cell and outside-out patch-clamp techniques. I_{Ca} was elicited with either 10 mM Ca^{2+} or Ba^{2+} in the bath solution. There are two components to the neurohypophysial I_{Ca}. Low concentrations of Tet (3-15 μM) inhibit the long-lasting component in a dose-dependent manner. The IC_{50} is 13.5 μM. However, no effect by low doses of Tet on the transient component was observed. Higher doses (100 μM) of Tet significantly inhibit both I_{Ca} components. Furthermore, Tet (7-30 μM) also markedly inhibits, in a voltage-dependent manner, the $I_{K\text{-}Ca}$ of the terminals which is activated by 10 μM Ca^{2+} in the pipette solution. At the single channel level, in the presence of 7 μM Tet, the open time duration of the large-conductance K^+ channel was greatly decreased when depolarizing from -50 mV to +40 mV. Tet, however, did not affect the I_{Na} or I_A of the terminals at such a low concentration.

The results that Tet, at nearly the same doses, blocked only I_{Ca} and $I_{K\text{-}Ca}$ in the isolated nerve terminals suggests the possibility that in these two channels there are receptor sites with similar structure for binding Tet. In addition, by inhibiting I_{Ca}, Tet possibly interferes with the release of vasopressin, which would contribute to its hypotensive action.

30.14

LARGE CONDUCTANCE, CALCIUM ACTIVATED K^+ CHANNEL IN CEREBELLAR GRANULE CELLS. S.-P. Olesen*, E.Ø. Nielsen and J. Drejer*. NeuroSearch A/S, Smedeland 26, DK-2600 Glostrup, Denmark.

Cerebellar granule cells represent the only excitatory neurons in the cerebellar cortex. Neuronal cultures obtained from 7-day-old mouse cerebellum are well characterized and consist of around 95% cerebellar granule cells. Patch-clamp studies on excitatory amino acid activated cation channels and on GABA activated chloride channels have been performed on cerebellar granule cells. Here we report single channel studies demonstrating the presence of a large conductance, calcium activated (BK_{Ca}) channel in cerebellar granule cells.

Single BK_{Ca} channels were found in about 10% of all inside-out patches. The single channel conductance is 154 pS (SD = 17 pS, n = 9) in symmetric 146 mM K^+ and 76 pS (SD = 5 pS, n = 12) in 4 mM external K^+. The channel is highly K^+ selective, i.e. reducing the internal K^+ concentration from 146 to 73 mM shifts the reversal potential by +16 mV.

The open probability is significantly increased by depolarizations, and the channel does not inactivate. Furthermore, rising the free calcium concentration on the cytosolic side from 100 to 1000 nM increases the open probability by 8-60x at V_m = 0 mV. Pinacidil (10 μM) does not activate the channel. In outside-out patches charybdotoxin (20 nM) blocks the channel by inducing long silent periods, and TEA (0.5 mM) inhibits channel activity by a very fast block.

In conclusion, the large K^+ selective channel identified in the cerebellar granule cells exhibits all the characteristics of a BK_{Ca} channel.

30.15

CALCIUM-ACTIVATED POTASSIUM CHANNELS IN SMOOTH MUSCLE CELLS OF RAT CEREBRAL ARTERY. Y.Wang and D.A.Mathers. Department of Physiology, University of British Columbia, Vancouver B.C., Canada V6T 1W5.

This experiments were performed to study the nature of calcium-dependent potassium, K(Ca) channels in smooth muscle cells (SMCs) derived from cerebral arteries of adult Wistar rats. Enzyme dissociated SMCs were maintained in a solution containing (mM): NaCl 130, KCl 5, $CaCl_2$ 0.8, $MgCl_2$ 1.3, glucose 5 and HEPES 10 at 4°C for 1-3 days prior to use. Inside-out patch clamp recordings were made at 21-23°C using a List EPC-5 amplifier(bandwidth DC-2 KHz).

Two populations of K(Ca) channels were identified, these having single-channel conductances of about 200pS and 90pS respectively in symmetrical 140 mM K^+ solutions. Both channel types were activated by membrane depolarization when the cytoplasmic membrane face was bathed in low calcium solutions. The probability of either channel type being open increased when the concentration of calcium ions bathing the cytoplasmic membrane face was raised, especially over the range of 0.1-10 uM. Both channel types showed negligible permeability to Na^+ or Cs^+ and both were blocked by application of tetraethylammonium (TEA, 5 mM) to the cytoplasmic membrane face.

30.16

TRANSIENT POTASSIUM CURRENTS IN NEURONS ACUTELY DISSOCIATED FROM THE ADULT RAT SUPRAOPTIC NUCLEUS: ARE THEY CALCIUM DEPENDENT? M.H.O'Regan & P.Cobbett. Dept. Pharmacol./Toxicol. & Neuroscience Program, Michigan State University, East Lansing, MI 48824-1317.

Magnocellular neurosecretory cells of the mammalian supraoptic nucleus (SON) release the hormones vasopressin and oxytocin into the systemic circulation. In these SON neurons, transient and/or Ca^{2+} dependent K^+ currents may determine interspike intervals and have been implicated in generation of the intrinsic firing patterns exhibited by the two types of cell. Voltage gated K^+ currents were examined in acutely dissociated neurosecretory SON cells using the whole cell configuration of the patch clamp technique. Three separate K^+ currents were distinguished by their voltage dependence, kinetics and pharmacology. A sustained current, present in all neurons, corresponded to the delayed rectifier current (I_K). In addition, two Ca^{2+} independent transient currents, $I_{A(fast)}$ and $I_{A(slow)}$ having significantly different kinetics of inactivation, were recorded in separate populations of SON neurons. I_K was selectively blocked by tetraethylammonium whereas $I_{A(fast)}$ and $I_{A(slow)}$ were blocked by 4-aminopyridine. The kinetics and Ca^{2+} independence of K^+ currents in magnocellular supraoptic neurons may reflect the role of these currents in modulating firing activity of the neurons and thus hypothalamo-neurohypophyseal function. (Supported by NIH NS28206.)

30.17

WHY USE INTRACELLULAR CITRATE FOR RECORDING CALCIUM ACTIVATED POTASSIUM CURRENTS: A POSSIBLE ROLE OF MAGNESIUM. R. Cloues, J. Robbins*, and D.A. Brown. Dept. of Pharmacology, University College London, Gower Street, London WC1E 6BT, England.

Inositol 1,4,5 trisphosphate (IP3) raises intracellular calcium levels and activates an outward Ca-dependent potassium current in NG108-15 cells. This current, evoked by intracellular ionophoresis of IP3, has previously been studied in cells impaled with sharp electrodes (Brown and Higashida, J. Physiol. 397:185-207; 1988). In experiments using the whole cell patch clamp technique we have found the ability to record this current depends on the anion used in the pipette solution. Recordings with acetate, aspartate, gluconate, or methylsulphate showed no currents; with fluoride or chloride, a low percentage of cells showed the response: currents were small and ran down. However, with citrate as the dominant anion, the IP3-induced current could be evoked in over 75% of the cells. In addition, bradykinin, which causes IP3 release through a G protein linked receptor, evoked an outward current only when citrate was used in the recording solution. Since citrate buffers magnesium (Mg2+) to a greater degree than acetate (0.1 mM vs 1.9 mM free Mg2+), we lowered the Mg2+ concentration in the acetate solution. This now enabled us to record the IP3 activated current. As intracellular injections of Ca2+ also activated the outward current only in low Mg2+ solutions, it is suggested that intracellular Mg2+ blocks Ca-activated K channels in these cells. Supported by Wellcome Trust and MRC.

30.18

THE ROLE OF CALCIUM INFLUX AND CALCIUM INDUCED CALCIUM RELEASE IN ACTIVATING POTASSIUM CURRENTS IN GUINEA PIG AND RAT VAGAL NEURONS. P. Sah and E. M. McLachlan. Dept of Physiology and Pharmacology, University of Queensland, Qld 4072. Australia.

In neurons, influx of calcium via voltage gated channels which open during an action potential can activate several types of potassium currents. We have studied the kinetics of potassium currents underlying the afterhyperpolarization (ahp) in vagal preganglionic neurones. Intracellular recordings were made from cells in the dorsal motor nucleus of the vagus(dmv) in transverse slices of brainstem maintained in vitro. Neurons had resting potentials of -60 to -70mV and input resistances of 100 to 600MΩ. In both rats and guinea pigs single action potentials were folowed by prolonged ahps. Under voltage clamp, the tail current underlying the ahp in rat dmv cells was maximal after the action potential, decayed exponentially with a time constant of 150ms and was blocked by apamin. In guinea pigs the tail current had two phases: an early fast phase ($Gk_{Ca,1}$) which decayed exponentially and a slower phase ($Gk_{Ca,2}$)which had a slow rising phase and then decayed exponentially with a time constant of 1.4s . $Gk_{Ca,1}$ had properties identical to the tail current in rat dmv neurons. $Gk_{Ca,2}$ was unaffected by apamin but was selectively blocked by noradrenaline. $Gk_{Ca,2}$ was selectively blocked by compounds which interfere with release of intracellular calcium stores. We conclude that in guinea pig dmv neurons influx of calcium (i) directly activates one class of potassium channels and (ii) leads to release of intracellular calcium which activates a second class of potassium channel. In rat dmv neurons this second type of channel is not expressed.

ION CHANNELS: MODULATION AND REGULATION I

31.1

EFFECTS OF MUSCARINIC RECEPTOR SUBTYPES ON AN INWARD POTASSIUM CONDUCTANCE AND ON EXOCYTOSIS. S.V.P. Jones. Neuroscience Research Unit, Department of Psychiatry, University of Vermont, Burlington, VT 05405.

The effects of activation of two of the five muscarinic receptor subtypes were investigated in RBL 2H3 cells. These cells were transfected with the m1 and m2 muscarinic receptor genes. Electrophysiological effects were monitored using the whole-cell patch clamp technique. RBL 2H3 cells express an inwardly rectifying potassium conductance which is active at potentials more hyperpolarized than -60 mV. The current was sensitive to 5 mM barium and 1 mM cesium. The RBL cell showed little evidence of other conductances. Application of 10 μM acetylcholine (ACh) to m2-transformed RBL cells increased the amplitude of the inward rectifier current. Whole-cell conductance was increased from ~2.5 nS in control to ~5 nS on application of ACh. This action was completely inhibited by 1 μM atropine. Application of 10 μM ACh to m1-transformed RBL cells did not appear to affect the inward rectifier, but resulted in a time-dependent change in morphology, indicative of exocytosis. This effect was abolished on application of 1 μM atropine. An accompanying activation of calcium-dependent conductances was noted on application of ACh to the m1-transformed cells, similar to those observed on antigen-induced exocytosis in these cells.

31.2

EFFECT OF ARACHIDONIC ACID PATHWAY INHIBITORS ON CALCIUM REGULATED M CURRENT ENHANCEMENT S.P.Yu*, P.R.Adams and A.D.Rosen# Howard Hughes Med. Inst., Department of Neurobiol. & Behavior, *Department of Neurology, SUNY at Stony Brook, Stony Brook, NY, 11794

We previously reported (Biophysical J. 59:78a,1991; Neuron 6:533-545, 1991) that intracellular Ca^{2+} can modulate the M current (I_M) in bullfrog sympathetic ganglion neurons. Increasing free $[Ca^{2+}]_i$ from zero to 80 nM by intracellular perfusion doubled the size of M current. The effect was reversible. High free $[Ca^{2+}]_i$ (>300 nM) reduced normal I_M.

Possible Ca^{2+}-dependent transduction pathways involved in M channel regulation were tested in dissociated bullfrog ganglion B cells using whole cell voltage clamp and intracellular perfusion. External solutions contained 0.5 mM EGTA, 2.0 mM $MnCl_2$ and no Ca^{2+}. Internal solutions had, inter alia, 20 mM BAPTA and 1.5 mM ATP. I_M was measured by 1 sec, -20 mV jumps from a holding potential of -30 mV.

The lipoxygenase inhibitor NDGA (1 μM) blocked 80% of I_M at 80 nM free $[Ca^{2+}]_i$ within 15 min, while it had smaller effect when free $[Ca^{2+}]_i$ was zero. The current remaining in 1μM NDGA was still normally sensitive to 1 - 10 μM muscarine but the overrecovery, which usually occurs at 80 nM free $[Ca^{2+}]_i$ with 20 mM BAPTA when washing out muscarine, disappeared. Overrecovery also disappeared when muscarine was tested at zero $[Ca^{2+}]_i$ even if NDGA was not present. The IC_{50} of NDGA for I_M inhibition at 80 nM free $[Ca^{2+}]_i$ was 0.3 μM. NDGA (1 μM), applied either in the bath or intracellularly, completely blocked the I_M enhancement normally seen upon raising intracellular free Ca^{2+} from zero to 80 nM. The cyclooxygenase inhibitor indomethacin (10 μM) had no effect on such I_M augmentation. Two phospholipase A_2 inhibitors 4-BPB (4 μM) and quinacrine (10 μM) partially blocked the Ca^{2+}-induced I_M enhancement. In preliminary tests, arachidonic acid (AA) itself increased I_M by 30-50% and this effect could be blocked by NDGA. The data indicate that AA metabolite(s), together with Ca^{2+}, may act to modulate M channels. The involvement of any particular AA metabolite(s) or other unsaturated fatty acids remains to be probed.

31.3

ROLE OF TARGET ORGAN IN THE MAINTENANCE OF CALCIUM CURRENTS IN BULLFROG SYMPATHETIC NEURONES. B.S. Jassar and P.A. Smith. Dept. of Pharmacology, University of Alberta, Edmonton, Canada.

Axotomy of bullfrog sympathetic ganglion neurones results in an increase in spike width and a decrease in the amplitude and duration of the action potential afterhyperpolarization (Gordon *et al.*, J. Physiol., **392**, 213, 1987). These changes, which are partially reversed following re-innervation of the target organ (Kelly *et al.*, J. Neurobiol., **19**, 357, 1988), have been attributed to functional loss of Ca^{2+}-sensitive K^+ conductances ($G_{K,Ca}$; Kelly *et al.*, Neurosci. Letts., **67**, 163, 1986). Since Ca^{2+} influx is required for the activation of $G_{K,Ca}$ during the action potential (Lancaster & Pennefather, J. Physiol., **387**, 519, 1987), it is possible that the changes in the afterhyperpolarization and action potential which accompany axotomy and re-innervation result from changes in Ca^{2+} currents. We therefore used the whole-cell patch-clamp technique to compare control Ba^{2+} currents with those recorded from neurones 14-15days and 28-30days after *in vivo* axotomy. Axotomy caused peak Ba^{2+} current to decrease to about 53% of control whilst the voltage-dependence of activation was unaffected. τ for activation/deactivation was reduced after axotomy and this was especially apparent for activation at -20mV and for deactivation at -30mV ($P<0.005$). Although the extent and rate of development of inactivation was increased, τ for recovery from inactivation was unchanged. These changes were partially reversed 28-30days after axotomy (when some reinnervation had probably occurred). These results show that contact of neurones with target organs is somehow responsible for the maintenance of Ca^{2+} currents in the cell body and that the properties of the individual channels expressed in axotomized cells differ from those seen in normal cells. (Supported by the MRC of Canada).

31.4

SEROTONIN MODULATION OF A TRANSIENT OUTWARD POTASSIUM CURRENT IN CEREBELLAR PURKINJE CELLS. Y. Wang*, J.C. Strahlendorf and H.K. Strahlendorf. Neurology and Physiology, Texas Tech Univ. Hlth. Sci. Ctr. Lubbock, TX. 79430

Under voltage clamp Purkinje cells (PCs) demonstrate a rapidly inactivating transient outward current (I_{to}) similar to the A-current found in many neurons. A-current may participate in frequency encoding of firing rate in response to graded depolarizations, may modulate excitatory synaptic input and in some cases contribute to spike repolarization. Current and voltage clamp recordings of rat PCs were made from slices. In voltage clamp I_{to} was elicited with depolarizing steps following a hyperpolarizing prepulse. 5-HT (10nM - 10μM) caused a dose-dependent decrease in I_{to} amplitude ranging from 13 to 89%. In current clamp 5-HT (5μM) shortened the delay to the first action potential and increased the slope of the electrotonic membrane response elicited by depolarizing current injection. Suprathreshold depolarizations elicited a higher spike firing frequency compared to control conditions. Resting membrane potential and input resistance were unaffected by these doses of 5-HT. Our results suggest that one mechanism by which 5-HT can enhance PC excitability is via a reduction in I_{to}. Supported by the Tx. Adv. Res. Prog., 010674-020 and NS 19296.

31.5

INTRACELLULAR GUANOSINE-5'-O-(2-THIODIPHOSPHATE) ALTERS THE KINETICS OF THE RESPONSES TO AGONISTS IN BULLFROG SYMPATHETIC NEURONS. Mark A. Simmons and Robert J. Mather*. The Neuropharmacology Lab., Dept. Pharmacol., Marshall University, Huntington, WV, 25755-9310.

Chicken II-luteinizing hormone releasing hormone (cII-LHRH), muscarine, and substance P (SP) inhibit a K^+ current, the M current, in bullfrog sympathetic neurons. The effects of intracellularly applied guanosine-5'-O-(2-thiodiphosphate) (GDPβS) on the responses to these agonists were examined. Whole cell recordings were made from single neurons dissociated from bullfrog sympathetic ganglia. GDPβS was added to the pipette solution with fixed amounts of GTP. At maximal concentrations of agonist, neither the response to the first application of agonist nor the proportion of cells which responded to any of the agonists was altered by GDPβS. With repeated agonist applications, successive responses were decreased in amplitude and increased in time course in the presence of GDPβS. Intracellular GDPβS did not accelerate the rate or magnitude of desensitization to SP. A model of receptor-G protein coupling predicts that a decrease in the available G protein pool should decrease both the magnitude and the time course of the build up of active G proteins. The results are consistent with the hypothesis that GDPβS binds tightly to G proteins in competition with GTP, thereby effectively decreasing the available G protein pool with repeated agonist applications.

31.6

ANALYSIS OF G PROTEIN-GATED K^+ CURRENTS ACTIVATED BY 7-HELIX RECEPTORS. A. Karschin, B.Y. Ho*, N. Davidson, and H.A. Lester. Caltech, Division of Biology 156-29, Pasadena, CA 91125.

We have described vaccinia virus-mediated high-efficiency expression of human 5-HT_{1A} receptors in primary cultures of neonatal rat atrial cells. The expressed receptors coupled to endogenous G protein-gated K^+ channels normally activated by mACh receptors (Karschin et al., Soc. Neurosci. Abstr. 16:462, 1990). Here we report about the kinetic analysis of the currents activated by ACh and 5-HT and suggest a model for direct K^+ channel activation by G protein subunits.

The inwardly rectifying K^+ current $I_{K[ACh]}$ evoked by ACh (5 μM) activated with a time constant of τ_{act}=317±12 ms (mean±SEM; n=32) and deactivated with τ_{deact}=965±75 ms (n=23) upon removal of the agonist. Responses mediated by the expressed 5-HT_{1A}Rs (5 μM 5-HT) showed similar deactivation with τ_{deact}=924±39 ms (n=16), but slower activation time constants τ_{act}=1682±180 ms (n=12) and smaller current amplitudes. Internal perfusion with GTPγS, performed to investigate this difference, caused the persistent activation of $I_{K[ACh]}$ within several minutes. In addition, GTPγS stimulated an increase in the rate of current activation and a decrease in deactivation rate, and especially for 5-HT, an increase in current amplitude. Thus, irreversible G protein activation by GTPγS enhances the efficiency of transduction by a subsequent pulse of agonist, possibly changing the number or nature of rate-limiting steps for activation/deactivation. These effects could be interpreted in a straightforward fashion if the channel was much more likely to be activated by several (n) G protein α-subunits than by a single one. Support: Alexander von Humboldt Foundation and American Heart Association, GM-29836, GM-10991 (NIH).

31.7

MODULATION OF VOLTAGE-DEPENDENT IONIC CURRENTS BY DOPAMINE : EVIDENCE FOR THE INVOLVEMENT OF DIFFERENT G PROTEIN TYPES.

Lledo P-M*, Homburger V*, Israel J M*, Bockaert J* and Vincent J-D. INSERM, unité 176, rue C. St Saëns, 33077 Bordeaux and (H. V. and B. J.) CCIPE, rue de la Cardonille, 34094 Montpellier Cedex 2, France.

Dopamine (DA), acting on D2 receptors, reversibly reduces two voltage-dependent Ca^{2+} currents (the T- and the L-type), and increases two voltage-dependent K^+ currents (IA and IK) in rat pituitary lactotroph cells and thereby decreases prolactin secretion. These effects were potentialized by intracellular application of the nonhydrolizable GTP analog, GTP-γ-S, and blocked by the GDP analog, GDP-β-S. Pretreatment of the cells with pertussis toxin (PTX) abolishes the DA effect, suggesting that PTX-sensitive GTP-binding regulatory proteins (G-proteins) are involved in the signal transduction mechanism which links D2 receptor activation to the modulation of the four types of ionic channel.

To identify the G-proteins involved, we made intracellular injections of affinity-purified polyclonal antibodies raised against the carboxy-terminal sequence of the α-subunit of either Go, Gi3 or Gi1 and Gi2. Ionic currents were recorded using the whole-cell configuration of the patch-clamp technique. Currents were measured 10 min after patch membrane had been broken in the absence or presence of 10 nM-dopamine. The pipette solution contained 180 μg/ml of antibody, heat-inactivated antibody (as control) or antiserum against Go, Gi3 or Gi1 and Gi2. Loading of cells with antibody was demonstrated by immunohistochemistry.

Only cells dialysed with antiserum Go significantly reduced the inhibition of Ca^{2+} currents induced by DA application whereas the response to DA on K^+ currents was markedly attenuated by the injection of antiserum Gi3. We therefore conclude that in lactotroph cells, Ca^{2+} and K^+ currents are both modulated by G proteins with a different type of G protein for channels specific for each ion.

31.8

BIOCHEMICAL EVIDENCE THAT DIHYDROPYRIDINE AND $GABA_B$ RECEPTORS INTERACT WITH G_o AND G_i RESPECTIVELY: M.I. Sweeney, A.C. Dolphin, Department of Pharmacology, Royal Free Hospital School of Medicine, London, NW3 2PF, England.

Recent studies have suggested that the interaction of dihydropyridines (DHPs) with Ca^{2+}-channels is modulated by G-proteins. Here, we have examined a direct interaction by studying the effect of DHPs on GTPase activity. Membranes were prepared from the frontal cortex of male Wistar rats and GTPase was quantitated by liberation of $^{32}P_i$ following hydrolysis of γ-^{32}P-GTP at 37°C for 10 minutes. The $GABA_B$ receptor agonist (-)-baclofen (1-30μM), and the DHP agonist (+)-202-791 and antagonist (-)-202-791 (0·33-10μM) stimulated a high affinity GTPase in a dose-dependent manner. (-)-202-791 was one order of magnitude less potent than the (+)-enantiomer. When data were expressed as a Lineweaver-Burk relationship, 10μM baclofen increased the maximal rate of GTP hydrolysis (V_{max}) from 227±32 to 322±57 pmol/mg protein/min, but had no effect on the affinity of the enzyme (K_M). 1μM (+)-202-791 increased V_{max} by 48±6% also with no effect on K_M (control, 0·65±0·16μM; DHP, 0·71±0·15μM, n=12). The effects of sub-maximal concentrations of baclofen and DHPs were additive. In membranes pretreated at 30°C for 60 minutes with antiserum (OC1, 1:50) against G_o, the effect of DHP on GTPase was completely attenuated while that of baclofen was reduced by 20%. In contrast, pretreatment with the antisera AS7 and SG1 (1:50), which interact with G_i, reduced the effect of baclofen by 83-88% while not affecting stimulation by either DHP. These data indicate that DHP binding produces an increase in GTP hydrolysis, possibly representing a direct coupling between DHP-sensitive Ca^{2+}-channels and G_o. $GABA_B$ receptors may be coupled to a proportion of these G_o proteins, although they also interact with G_i in cortical membranes.

Supported by the MRC (UK and Canada) and Wellcome Trust. We thank Dr G Milligan, Glasgow University for anti-G protein antibodies.

31.9

CLONING OF G PROTEIN cDNAS FROM HELISOMA CENTRAL GANGLIA. S. Durgerian*, P. G. Haydon and D. D. Larson*. Dept. of Zoology and Genetics, Iowa State Univ., Ames, IA 50011.

The tetrapeptide neuromodulator FMRFamide regulates synaptic transmission by inhibiting the release of acetylcholine (ACh) from nerve terminals in the pond snail *Helisoma* . FMRFamide acts through pertussis toxin-sensitive G proteins to reduce calcium influx into the nerve terminal and to reduce the sensitivity of the secretory machinery to calcium. Some effects of FMRFamide are mimicked by microinjection of GTPγS-activated α subunit of purified bovine G_{o2} (Man-Son-Hing et al. '90). In order to study the endogenous G proteins responsible for these effects, we have isolated two unique sets of clones from a *Helisoma* cDNA library which are similar to other invertebrate $G_{\alpha o}$ sequences.

31.10

GLUCOCORTICOID INCREASES THE EXPRESSION OF VOLTAGE-DEPENDENT CA^{2+} CHANNELS OF HUMAN MUSCLE IN CULTURE. S. Braun*, V. Askanas, W.K. Engel. USC Neuromuscular Center, Univ. of Southern Calif. Sch. of Med., Los Angeles, CA 90017.

Glucocorticoid increases the accumulation of non-junctional acetylcholine receptors (AChRs) on aneurally cultured human muscle (Askanas et al., 1986) and of junctional-AChRs on cultured human muscle, that has been innervated by fetal rat spinal cord (Braun et al., 1991). The increased accumulation of AChRs is due to both increased synthesis and decreased degradation. Since activation of Ca^{2+} channels was shown to slow degradation of AChRs in rat muscle culture (Rotzler et al., 1991), we investigated whether glucocorticoid modulates the Ca^{2+} channels in cultured human muscle.

Hydrocortisone (HC) treatment was initiated on 8-day-old aneurally cultured human muscle and continued for up to 14 days. The Ca^{2+} channels were measured by binding of the dihydropyridine derivative [^{3}H]PN200-110, a potent Ca^{2+} channel antagonist. HC significantly increased the specific PN200-110 binding 2.4 times, $p < 0.05$ (18 cultures). The effect was time-related (maximum after 10 to 14 days of treatment) and concentration-dependent. There was a correlation between HC-promoted increase of PN binding and increase of AChR accumulation.

Our study provides the first demonstration that glucocorticoid influences Ca^{2+} channels in cultured human muscle and raises the possibility that its influence on AChR metabolism may be through a Ca^{2+} channel effect.

31.11

ARACHIDONIC ACID HAS DOSE-DEPENDENT EFFECTS ON THE MEMBRANE POTENTIAL OF ACUTELY DISSOCIATED EMBRYONIC CHICK SPINAL CORD CELLS K.S. Madden, A. Prasad, S.V. Smith, G.D. Lange and J.L. Barker, LNP and ICS, NINDS, NIH, Bethesda, MD 20892

Arachidonic acid (AA) metabolism has been implicated in the events of signal transduction in response to various electrical, chemical and mechanical stimuli. These responses are superimposed on the basal metabolic state of cells which is commonly modified by exposure to cold or radiolabeled AA. To assess the effects of this treatment, alone, acutely suspended embryonic chick spinal cord cells (E5-8) were exposed to increasing doses of AA (3 nM to 3 mM). Flow cytometry was used to measure effects on individual cells (20,000/trial) in preparations that were untreated or stained by a fluorescent indicator dye such as oxonol (voltage-sensitive) or fluo-3 (calcium-sensitive). The data revealed four dose-dependent effects on the oxonol signal intensity. 1) Low doses (3 nM-6 μM) decreased the signal intensity of most, if not all, cells without affecting either the autofluorescent (AF) or fluo-3 signals. 2) Transition doses (9 μM-45 μM) decreased, did not change, or increased the signal intensity. 3) Higher doses (ca. 45-90 μM) increased the intensities of oxonol, AF and fluo-3 signals without increasing cell mortality. 4) High doses (>100 μM) killed cells. The low dose effects (1) are consistent with a hyperpolarization caused by the activation of potassium channels. Higher doses (3) probably depolarize cells owing to increases in the intracellular concentrations of calcium ions. Transition effects (2) are likely to result from simultaneous, dual antagonistic effects. This could help to explain some diversity in the reported effects of these concentrations in other preparations.

31.12

INVOLVEMENT OF ATP-SENSITIVE K+ CHANNELS IN SOMATOSTATIN (SRIF)-INDUCED CYTOSOLIC CALCIUM REDUCTION, MEMBRANE HYPERPOLARIZATION BUT NOT INHIBITION OF ADENYLATE CYCLASE ACTIVITY IN MMQ CELLS. Q.Meucci,A.Scorziello*,E.Landolfi*T.Florio, C.Ventra,G.Schettini Dept.Pharmacology,II School of Medicine, University of Naples ITALY.

In MMQ cells, a PRL-secreting rat cell line, which naturally expresses functional SRIF receptor, SRIF dose-dependently reduced intracellular calcium levels ([Ca++]i) and induced a membrane hyperpolarization in basal conditions. SRIF also inhibited the membrane depolarization caused by high extracellular K+, whereas it did not affect the K+ depolarization-induced [Ca++]i rise. The sulfonylurea glibenclamide (Glib), a blocker of K+(ATP)-channels, depolarized the cells, increased [Ca++]i, and markedly reduced SRIF inhibition of basal [Ca++]i. Furthermore, Glib inhibited SRIF reduction of K+-induced depolarization. SRIF inhibited forskolin-stimulated PRL secretion from single cell without affecting basal hormone release, an effect modulated by Glib pretreatment. SRIF reduced adenylate cyclase (AC) activity in basal and forskolin-stimulated conditions in a dose dependent manner (100nm-100uM). Glib did not modify SRIF inhibition of the AC enzyme.

31.13

EFFECTS OF ADENOSINE ON VOLTAGE-DEPENDENT CALCIUM CURRENTS IN IDENTIFIED MOUSE MOTONEURONS. M. Mynlieff and K.G. Beam. Dept. of Physiology, Colorado State University, Fort Collins, CO 80523

Endogenous and exogenous adenosine have been shown to reduce both electrically-evoked and potassium-evoked release of acetylcholine from the neuromuscular junction. This effect is calcium-dependent, blocked by xanthines and exhibits an A1 adenosine receptor antagonist profile. Unlike most A1 receptor mediated effects, the inhibition of neuromuscular transmission by adenosine is not due to the inhibition of adenylate cyclase. The inhibitory effect of adenosine on mammalian motor nerve endings is abolished by pertussis toxin treatment suggesting that activation of the A1 adenosine receptor produces second messengers which alter the conductance of the voltage-dependent calcium channels.

We have used voltage clamp whole-cell patch recording from embryonic mouse motoneurons to study the effects of adenosine on voltage-dependent calcium channels. The motoneurons from 14 day old embryos were identified by retrograde labeling with the carbocyanine dye "dil" prior to dissociation of the cells. Adenosine (40 μM) decreased the transient high-voltage activated (HVA) calcium current (10 mM external Ca^{2+}, 29.1±4.3%, n=10) without affecting the sustained HVA calcium current (8.2±7.2%) and T-current (6.3±9.5%). This decrease in transient HVA current might explain the depressant effect of adenosine on release of acetylcholine at the neuromuscular junction.

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31.14

ATP-ACTIVATED ION CHANNELS IN RAT NODOSE GANGLION NEURONS. Chaoying Li*, Sergio Visentin* and Forrest F. Weight, Section of Electrophysiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The ion currents induced by externally applied ATP were investigated by means of whole-cell patch-clamp method in single neurons freshly isolated from adult rat nodose ganglion. At a holding potential of -60 mV, rapid application of ATP (0.5-250 μM) induced a dose dependent inward current with a K_d=9.3 μM. The current exhibited fast activation and deactivation kinetics. Under normal external ionic conditions, the reversal potential was near 0 mV. In Na^+-free or Na^+-free/Ca^{++}-free external medium (NMG replacement), the ATP-activated current was decreased in amplitude by 85% and 95%, and the reversal potential was shifted to -38 mV and -52 mV, respectively. α,β-Methylene ATP (50 μM) and ADP (200 μM) also activated an inward current, but were much less potent than ATP. Adenosine (100 μM), AMP (200 μM) and GTP (200 μM) had no effect. These observations suggest that interaction of ATP with a P2 type of purinergic receptor can activate a ligand-gated ion channel in rat nodose neurons. The inward current of this ATP-activated channel appears to be carried mainly by Na^+, but Ca^{++} may also contribute.

31.15

CHARACTERIZATION OF A PARTICULATE PROTEIN PHOSPHATASE IN NEURONS OF *APLYSIA*. S. Endo, S. Shenolikar*, and J.H. Byrne, Departments of Neurobiology and Anatomy, and Pharmacology, University of Texas Medical School, Houston, TX 77225.

The phosphorylation state of a given protein reflects the activities of both protein kinases and protein phosphatases (PrPs). Recent studies indicate that PrPs in *Aplysia* are similar to those of mammalian in many of their biochemical properties including substrate specificity and sensitivity to a variety of inhibitors, such as the mammalian inhibitor I-2 (a specific inhibitor of PrP-1), okadaic acid (OA) and Microcystin-LR (specific inhibitors of PrP-1 and -2A) (Endo et al., 1989, 1990). Moreover, OA and Microcystin-LR alter 5-HT- and cAMP-dependent membrane currents in voltage clamped pleural sensory neurons (Ichinose et al. 1990; Byrne et al. 1990). To characterize the PrPs which may be involved in the regulation of membrane proteins, we analyzed PrPs in a crude membrane fraction from *Aplysia* neurons.

Homogenate of the central nervous system was centrifuged at 1,000 xg and the resulting supernatant was again centrifuged at 100,000 xg to yield a particulate fraction. More than 70 % of PrP activity, measured using ^{32}P-phosphorylase *a* as substrate, was extracted from the particulate fraction by repeated washings with the buffer containing 0.5 M NaCl. Greater than 80 % of that PrP activity was PrP-1 as defined by its inhibition by I-2. Furthermore, over 75 % of PrP activities in membrane "ghosts" of R2 cells and PL1 cells was PrP-1. Salt-extracts of neural membrane were subjected to gel filtration on Superose-6, yielding multiple high molecular weight peaks of PrP activity. All the PrP activities in these peaks were completely inhibited by OA (1 μM), but one was only partially inhibited by I-2, suggesting the presence of multiple forms of PrP-1. These PrP-1 complexes retained the ability to reassociate with membranes and suggest the presence of additional components which confer membrane binding to PrP-1. In summary, these data confirmed that the major PrP activity associated with membrane from *Aplysia* neural tissue is PrP-1, and suggest that PrP-1 may be a candidate for the regulation of phosphorylation-dependent membrane processes.

31.16

PROTEIN PHOSPHATASE-1 INHIBITORS ATTENUATE 5-HT MODULATED K^+ CURRENTS IN *APLYSIA* SENSORY NEURONS. S.D. Critz, S. Endo, S. Shenolikar, and J.H. Byrne, Depts. of Neurobiology and Anatomy and Pharmacology, Univ. of Texas Medical School, Houston, TX 77225.

We previously reported that okadaic acid and microcystin-LR (MCYST) reduced serotonin (5-HT) and cAMP responses in sensory neurons of *Aplysia* (Ichinose et al. 1990a,b). These compounds, however, which potently inhibit both protein phosphatase-1 (PrP-1) and -2A, did not define the regulatory roles of the individual phosphatases. To address this issue, we have used the purified vertebrate inhibitor proteins, I-1 and I-2, which selectively inhibit PrP-1, to determine the physiological role of PrP-1 in the modulation of 5-HT responses.

Isolated clusters of sensory neurons from the pleural ganglion were perfused (0.5 ml/min) with 20 mM Tris-buffered artificial sea water (pH 7.4). Sensory cells were two-electrode voltage-clamped at a holding potential of -25 mV using 0.5 M KCl-filled electrodes. The current passing electrode also contained either I-1, I-2 or vehicle, which passively diffused into the impaled cell. Responses were produced by 'puffing' 5-HT (3.0 s, 5-20 p.s.i., 100 μM) from a third electrode every 6 min. Puffs of 5-HT produced transient inward shifts of the holding current that correspond to the cAMP-dependent protein kinase mediated suppression of the S-K^+ current (e.g., Siegelbaum et al. 1982, Ichinose et al. 1990a,b). Both thiophosphorylated I-1 (activated form, 20 units/μL, n=6) and I-2 (16 units/μL, n=5) gradually attenuated the responses to 5-HT. The kinetics of recovery from 5-HT puffs were unchanged and there was little change in baseline input conductance. In contrast, both okadaic acid and MCYST prolonged the recovery phase and gradually reduced baseline input conductance. These results suggest that PrP-1 dephosphorylates substrate proteins involved in the control of K^+ currents in *Aplysia* neurons. Since MCYST effects were not completely reproduced by either activated I-1 or I-2, PrP-2A may also participate in the regulation of K^+ currents.

32.1

WIDESPREAD DEGENERATION INDUCED IN THE DEVELOPING RODENT CNS BY D,L-2-AMINO-3-PHOSPHONOPROPIONATE (AP3). J. P. Tizzano, D. D. Schoepp, M. T. Price and J. W. Olney. Eli Lilly and Company, Greenfield, IN 46140 and Washington University Medical School, St. Louis, MO 63110.

AP3 is an aspartate analog known to cause acute gliotoxic changes in circumventricular organ (CVO) regions of infant rodent brain (Olney et al., 1971). Recently, AP3 was shown to inhibit the function of a unique phosphoinositide (PIn) hydrolysis-coupled glutamate (Glu) receptor subtype, the quisqualate metabotropic receptor. In rat brain, activation of this receptor by specific agonists (quisqualate or ACPD) stimulates PIn hydrolysis, and AP3 inhibits agonist-stimulated PIn hydrolysis (Schoepp & Johnson, 1989). Metabotropic receptor-coupled PIn hydrolysis is markedly enhanced in the neonatal rat CNS, suggesting a role for this receptor in CNS development. Tizzano et al, (NS Abstr., 1990) found that daily AP3 treatment of rats from postnatal days 3-12 inhibited ACPD-stimulated PIn hydrolysis and also caused developmental behavioral deficits, plus degeneration and/or dysplasia of the retina and optic nerve. We now report results of a more detailed histopathological analysis of the latter effect. A single i.p. treatment of infant mice with AP3 (500 mg/kg) caused rapid (within 2 hrs) edematous changes limited to glio-ependymal elements in CVO brain regions and slightly more delayed (within 6-8 hrs) edematous degeneration of small numbers of retinal neurons. Giving the same dose daily from postnatal days 4-8 consistently caused degeneration of the entire retina and in some animals induced degeneration of both glial and neural elements in scattered distribution over many brain regions, including CVO, cerebral cortex, caudate nucleus, hippocampus, thalamus, superior colliculus, cerebellum and cochlear nucleus. Thus, sub-chronic AP3 treatment of infant rodents can cause both inhibition of metabotropic receptor function and widespread neurodegenerative changes throughout the developing CNS. By several criteria, the neurotoxic action of AP3 appears different from that of Glu and related excitotoxins. Whether any relationship exists between the gliotoxic and neurotoxic actions of AP3 remains to be determined.

32.2

EXPOSURE TO trans-1-AMINO-1,3-CYCLOPENTANEDICARBOXYLIC ACID (trans-ACPD) INCREASES INTRACELLULAR FREE CALCIUM IN CULTURED TYPE I ASTROCYTES.
E.R. Whittemore, R.J. Bridges¶ and C.W. Cotman, Dept. of Psychobiology and Neurology¶, Univ. of Calif., Irvine, CA 92717

Biochemical studies have shown that trans-ACPD is the most selective known agonist at the G-protein-coupled excitatory amino acid receptor linked to the hydrolysis of inositol phospholipids. Although calcium imaging techniques have shown that activation of this receptor by other compounds induces increases in intracellular free calcium in cultured neurons and astrocytes, these studies have utilized less selective agonists such as quisqualate, which has known activity at a variety of other sites. To investigate whether activation of this receptor by the selective agonist trans-ACPD also induces increases in intracellular free calcium, we assayed cultured rat cortical astrocytes for responses to trans-ACPD using the calcium dye Fura-2 and digital fluorescence imaging microscopy. Type I astrocytes were prepared from the cortices of 4 day old rat pups. Oligodendrocytes and type II astrocytes were removed by shaking, and the purified type I astrocytes were plated onto polylysine-coated glass coverslips and allowed to reach confluency (14-21 days). Individual coverslips were incubated with 2 μM Fura-2 AM for 30-40 min at room temperature and transferred to the stage of an inverted fluorescence microscope. Drugs were applied at room temperature for 2-4 min. using a flow-through chamber, and the ratio of fluorescence at 340 and 380nm was observed. Clear increases in intracellular free calcium were observed in response to 200 μM trans-ACPD, which were characterized by a large initial spike, and subsequent smaller oscillations. In other experiments, 1 mM DL-2-amino-3-phosphonopropionic acid (AP3) was applied before and during exposure to 200 μM trans-ACPD. In accordance with biochemical studies, this compound was a weak antagonist in some cells, and had no effect in others. These results suggest that astrocytes express functional trans-ACPD receptors, which mediate calcium responses, and that DL-AP3 is a weak or ineffective antagonist at this site.

32.3

STEREOSELECTIVE *EX VIVO* INHIBITION OF PHOSPHOINOSITIDE-COUPLED EXCITATORY AMINO ACID RECEPTORS BY L-2-AMINO-3-PHOSPHONOPROPIONIC ACID (L-AP3) B.G. Johnson, E.C.R. Smith, L.A. McQuaid, and D.D. Schoepp., Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

During early postnatal ages in the rat, there is enhanced *in vitro* coupling of the metabotropic (phosphoinositide-coupled) excitatory amino acid receptor. Recently, we showed that i.p. administration of AP3 to neonatal rats produced long-lasting selective inhibition of excitatory amino acid-coupled phosphoinositide hydrolysis in the *ex vivo* brain slice (Neurochem. International 18: 411, 1991). This study further examined the stereoselectivity and pharmacology of *ex vivo* AP3 inhibition of the metabotropic receptors in the neonatal rat. When given to neonatal rats at doses of 125, 250, or 500 mg/kg i.p. for 3 consecutive days (6 days old at first dose), L-AP3 produced dose-related inhibition of trans-ACPD (100μM)-stimulated phoshoinositide hydrolysis in hippocampal slices. *In vivo* L-AP3 treatment significantly reduced the maximal trans-ACPD (100μM) stimulation (2148 ± 196 % of basal in saline group, and 1110 ± 134 % of basal following 250mg/kg x3 L-AP3), while having little effect on the trans-ACPD EC_{50} (saline control = 34.56 ± 6.18 μM; 250 mg/kg x3 L-AP3 = 28.93 ± 3.72 μM). *In vivo* treatment with the metabotropic agonist trans-ACPD (50 mg/kg i.p. x 3) or the inactive AP3 isomer, D-AP3, (500 mg/kg i.p. x3) produced no effect on *in vitro* trans-ACPD (100μM)-stimulated phosphoinositide hydrolysis. These studies show that the stereoselectivity and pharmacology of *ex vivo* L-AP3 inhibition is similar to that observed *in vitro*. *In vivo* L-AP3 administration to neonatal rats provides a selective pharmacological approach to prevent metabotropic excitatory amino acid receptor coupling in the neonatal rat brain.

32.4

EFFECTS OF EXCITATORY AMINO ACID ANTAGONISTS AT THE STRIATAL METABOTROPIC GLUTAMATE RECEPTOR IN VITRO. B.P. Symons and S. Weiss, Neuroscience Research Group, University of Calgary, Calgary, AB. Canada

The putative actions of the excitatory amino acid (EAA) antagonists 2-amino-3-phosphonoproprionate (AP3), 2-amino-4-phosphonobutyrate (AP4) and 2-amino-5-phosphonovalerate (APV) in blocking EAA agonist-induced [^{3}H]-inositol phosphate (InsP) accumulation were examined in mouse striatal neurons in primary culture. The agonists quisqualate (Quis), ibotenate (Ibo) and 1-amino-trans-1,3-cyclopentyldicarboxylate (ACPD) all induced dose-dependent increases in [^{3}H]-InsP accumulation with EC_{50} values of 0.5, 5, and 35μM, respectively. At saturating concentrations, Quis induced a 4-fold increase over the basal [^{3}H]-InsP accumulation, whereas Ibo and ACPD evoked a 3.5-fold increase. AP3 and AP4 (1mM) were partial agonists, inducing a 1.5 to 2-fold increase over the basal [^{3}H]-InsP accumulation. AP3 and AP4 stimulation was not additive, however, with the three other agonists tested and AP3 actually significantly attenuated 1μM Quis-induced [^{3}H]-InsP accumulation (IC_{50}, 500μM). APV (ineffectual alone) significantly attenuated Ibo- and ACPD-induced [^{3}H]-InsP accumulation in a dose-dependent manner (IC_{50}, 40μM); Quis actions were unaffected. These results suggest that agonist/antagonist interactions can distinguish multiple mechanisms mediating EAA-induced [^{3}H]-InsP accumulation in striatal neurons.

Supported by the Medical Research Council of Canada.

32.5

L-AP3 INHIBITS RECEPTORS ACTIVATED BY (±)TRANS-ACPD AND 1S,3R-ACPD IN XENOPUS OOCYTES EXPRESSING CEREBELLAR METABOTROPIC GLUTAMATE RECEPTORS. R.L. Berry, P.C. May, L. McQuaid, E.C.R. Smith, M.J. Valli*, J.A. Monn, and L.K. Simmons. Eli Lilly and Co., Indianapolis, IN 46285.

The metabotropic glutamate receptor (mGluR) increases cellular calcium levels by activating phosphoinositide metabolism. Recently, increasing research has focused on the mGluR, prompted in part by the possible role of this receptor in the mechanisms underlying neuronal plasticity. Using the Ca^{2+}-dependent chloride current as an assay of mGluR activity, we have pharmacologically characterized the activities of enantiomers of the selective mGluR agonist (±)trans -ACPD in Xenopus oocytes injected with rat cerebellar mRNA. Oocytes were exposed to increasing concentrations (30 μM-1mM) of the racemate (±)trans-ACPD and its enantiomers--1S,3R-ACPD and 1R,3S-ACPD. For any given concentration, the largest amplitude current was elicited with 1S,3R-ACPD, the racemate elicited an intermediate response, and 1R,3S-ACPD was virtually inactive.

We subsequently tested the ability of the antagonist L-AP3 to block the actions of (±)trans-ACPD and 1S,3R-ACPD. Three mM L-AP3 reversibly inhibited the responses to (±)trans-ACPD in all oocytes tested, but blocked responses to 1S,3R-ACPD only 58% of the time. These experiments show that cerebellar mGluRs expressed in Xenopus oocytes are qualitatively similar to mGluRs that have been described in other systems-- 1S,3R-ACPD is the active isomer of (±)trans-ACPD and L-AP3 inhibits the racemate. We are currently investigating possible explanations for L-AP3's inability to consistently block 1S,3R-ACPD at concentrations that significantly block the racemate.

32.6

EVIDENCE FOR METABOTROPIC EXCITATORY AMINO ACID RECEPTOR HETEROGENEITY:DEVELOPMENTAL AND BRAIN REGIONAL STUDIES. G.G. Vecil*, P.P. Li and J.J. Warsh. Clarke Institute of Psychiatry, University of Toronto, Canada, M5T 1R8.

To investigate whether multiple subtypes of the excitatory amino acid (EAA) receptor-linked to polyphosphoinositide (PPI) hydrolysis exist, we have pharmacologically characterized the metabotropic EAA receptor response in rat neonatal and adult hippocampus and cerebellum. Activation of PPI hydrolysis was determined by the accumulation of [^{3}H]inositol monophosphate (InsP) in brain slices prelabelled with [^{3}H]inositol. In neonatal hippocampal slices D,L-2-amino-3-phosphonopionic acid (AP3; 1 mM) inhibited the *cis*-1-aminocyclopentane-1,3-dicarboxylic acid (IUPAC, ACPD)- and quisqualate (Quis)-stimulated PPI hydrolysis by 73% and 66%, respectively, but did not affect ACPD- or Quis-stimulated [^{3}H]InsP accumulation in neonatal cerebellar slices. In adult hippocampus, AP3 stimulated PPI hydrolysis with comparable potency and efficacy to ACPD and Quis, and completely masked the Quis concentration-response curve. In adult cerebellum, Quis behaved as a full agonist, whereas ACPD and AP3 appeared to be weak or partial agonists. The Quis concentration-reponse curve was shifted to the right with a 4-fold increase in the EC_{50} value in the presence of a maximally effective concentration of ACPD. In contrast, the resultant accumulation of [^{3}H]InsP when AP3 (5 mM) and Quis (500 μM) were co-incubated was nearly additive with the response produced by either drug alone. Taken together, our data reveal significant developmental and brain regional differences in metabotropic EAA receptor responses and support the notion that the metabotropic EAA receptor is heterogeneous, both in a regionally specific and developmentally dependent manner.

32.7

EXCITATORY AMINO ACID STIMULATED IP_3 FORMATION IN RAT FOREBRAIN SYNAPTONEUROSOMES. A.K. Chaudhary*, J.M. Tuchek* and D.D. Johnson. Dept. of Pharmacology, College of Medicine, Univ. of Saskatchewan, Saskatoon, SK S7N OWO.

A considerable amount of work has been reported on the effect of excitatory amino acids (EAA) on phosphoinositide (PI) turnover. The majority of the studies have estimated PI turnover on the basis of accumulation of total inositol phosphates during lengthy incubations of brain preparations with an EAA agonist and LiCl. While the total accumulated inositol phosphates are mainly IP and IP_2, it is assumed that the source of these inositol phosphates is IP_3. Hence, it is believed that an increased accumulation of IP and IP_2 reflects increased amount of IP3 formation as well. However, it is also known that IP and IP_2 can be formed from the hydrolysis of PI and PIP respectively. In this report, we have examined the effect of various EAA agonists on the PI system by quantifying the amount of IP_3 formed using a radioreceptor assay kit (NEN). Rat forebrain synaptoneurosome preparations were pre-incubated at 37°C for 5 min in a buffer consisting of 110 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 1.3 mM $CaCl_2$, 10 mM glucose, 1 mM pyruvate, 0.25 mM DTT and 50 mM imidazole pH 7.4. After preincubation, various EAA agonists were added and the samples were further incubated for 2 min. The hydrolysis was terminated by the addition of TCA (5% final) and the precipitate was pelleted by centrifugation. The supernatant was extracted with trichlorotrifluoro-ethane/trioctylamine and assayed for IP_3. Using this method, concentration-effect curves for various EAA agonists including glutamate, quisqualate, t-ACPD and domoate can be demonstrated even when synaptoneurosomes from adult rats are used. (Supported by MRC).

32.8

BEHAVIORAL EFFECTS OF INTRATHECALLY ADMINISTERED ACPD AND AP4, AGONISTS FOR NOVEL EAA RECEPTORS. Kelley Kitto* and George Wilcox. Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

The involvement of traditional excitatory amino acid (EAA) receptors (NMDA and non-NMDA) in nociceptive neurotransmission in mouse spinal cord has been well documented. In particular, both NMDA and AMPA elicit caudally directed biting behavior in mice and excite nociceptive dorsal horn neurons in rats. We have observed a qualitative difference in the behavior elicited by quisqualate and AMPA. This result suggested the participation of receptors other than the AMPA-gated cation channel in quisqualate's action. Therefore, we chose to examine the participation of the G-protein-coupled (metabotropic) receptor activated by quisqualate and glutamate using (+/-)-1-amino-1,3-cyclopentane-*trans* -dicarboxylic acid (*trans*- ACPD) as an agonist. We observed that ACPD elicited behavior more similar to that elicited by substance P (SP) than to that elicited by NMDA. This result is consistent with the similarity of the intracellular events evoked by both ACPD and SP (i.e., mobilization of IP_3 and Ca^{++}). Biting and scratching behaviors similar to SP behaviors were elicited in a dose dependent manner (0.03, 0.1, 0.3, 1.0 μg/5μL). We tested whether ACPD-elicted behavior or tail flick responses would be inhibited by AP4. For comparison, we tested agents which inhibit SP-elicited behavior, α_2 adrenergic and δ or μ opioid agonists. ACPD-elicited behavior was inhibited by the α_2 adrenergic agonist UK-14304-18 (0.1 μg) and by the δ and μ opioid agonists DPDPE (1 μg) and DAMGO (0.5 ng) but not by AP4 (1 μg). (Supported by NIDA grants R01-DA-04274 and R01-DA-01933)

32.9

DELAYED QUISQUALATE NEUROTOXICITY IN RAT CORTICAL CULTURES. W. C. Zinkand, P. A. DeFeo*, Carolann Thompson* and J. Patel, ICI Pharmaceuticals Group, ICI Americas, Wilmington, DE 19897.

The excitatory amino acid quisqualate (quis) displays a type of neurotoxicity that is quite different from other excitatory amino acids. The ionotropic quis antagonists DNQX and NBQX are potent quis toxicity blockers when administered following quis washout but are ineffective when co-incubated with quis. Similarly, removal of the cell culture media 30 min. after quis washout is neuroprotective, and that media, when transferred to naive cells, is toxic. Quis is not toxic to the non-neuronal cells in our cell culture; however, those cells are able to reproduce this "transfer toxicity" phenomenon. We believe that quis is taken up by both neuronal and non-neuronal cells in rat cortical cell cultures and that a neurotoxic factor (likely quisqualate or slightly modified quisqualate) is then re-released to cause neurotoxicity by persistent activation of the ionotropic quis receptor. The time course of this neurotoxicity is similar to that of AMPA, which is toxic via the ionotropic quis receptor but has none of the peculiar properties of quis. The possible role of a neurotransmitter uptake site is implicated by the observation that quis is not toxic if incubated with neuronal cells in sodium-free buffer.

32.10

RETENTION OF QUISQUALIC ACID BY HIPPOCAMPAL SLICES FOLLOWING QUISQUALIC ACID INDUCED SENSITIZATION TO L-AP4. M.K. Schulte and J.F. Koerner. Dept. of Biochem. and Neurosci. Grad. Program, Univ. of Minnesota, Minneapolis, MN 55455

Previous work performed in our laboratory demonstrated that a brief exposure of pyramidal and granule cell neurons of the rat hippocampus to the excitatory amino acid agonist L-quisqualic acid results in a 30-100 fold increase in sensitivity to depolarization by L-AP4 (The QUIS effect). We have also reported that pre-exposure of slices to L-homocysteine sulfinic acid, L-serine-O-sulfate and L-α-amino adipic acid can block induction of this effect and reverse it once it has been induced. The pharmacological profile of the induction site and sensitized L-AP4 site have been extensively investigated, however, the molecular mechanisms involved are unknown. It has been proposed that a first step might involve neuronal uptake of quisqualic acid . Due to the unavailability of radio-labeled quisqualic acid, no direct uptake studies have been performed. We have, therefore, developed a sensitive assay system utilizing HPLC separation of o-pthaldialdehyde (OPA) derivatives of quisqualic acid which enables us to detect quisqualic acid retention by hippocampal slices. Slices were monitored via extracellular recording in the regio superior of the stratum radiatum and exposed to quisqualic acid. Following extensive washing with QUIS-free media, quisqualic acid was extracted from the tissue and samples were derivatized with OPA . Derivatized amino acids were separated via HPLC. Our experiments demonstrate a retention by hippocampal slices of 10 pMoles quisqualic acid per mg of tissue. This level of quisqualic acid remains throughout a 2 hour wash with QUIS-free medium. Treatment of slices with "pre-blocker" or "reverser" compounds had no observed effect upon levels of quisqualic acid within the slice. These results are consistent with the hypothesis that a sequestration step is involved in the induction of the QUIS effect.

32.11

CHARACTERIZATION OF L-AP4 ACTION FOLLOWING PRIMING BY QUISQUALATE IN HIPPOCAMPAL SLICE CULTURES OF RAT

Serge Charpak, Urs Gerber, & Beat Gähwiler, Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland

Several studies have shown that an enhanced excitatory action of L-2-amino-4-phosphonobutyric acid (L-AP4) at various CNS pathways is only observed following preexposure of tissue to quisqualate. However, the mechanism for this "priming" effect remains unclear. We have studied quisqualate priming of L-AP4 effects in voltage-clamped CA3 pyramidal cells using hippocampal slice cultures in TTX. Prior to quisqualate exposure, CA3 cells showed no response to L-AP4 (50-200 μM). Following exposure to quisqualate (500 nM for 30 sec) L-AP4 induced a complex response. At hyperpolarized membrane potentials an ionotropic inward current associated with a conductance increase was observed. This response was in part sensitive to CNQX and in part sensitive to L-APV and Mg^{++} ions. At depolarized potentials in the presence of CNQX and D-APV, L-AP4 induced a metabotropic excitation resulting from depression of K^+ currents. This indicates that the action of L-AP4 is mediated by three different receptor types: the NMDA receptor, the AMPA receptor, and a glutamatergic metabotropic receptor. The L-AP4 response appears to be calcium insensitive as it persists in low Ca^{++} high Mg^{++} medium with 200 μM Cd^{++}.

The L-AP4 response was markedly decreased or abolished by short applications of the endogenous excitatory amino acids glutamate, aspartate and homocysteate at concentrations of 10-100 μM. The response to L-AP4 following priming might be explained by two mechanisms: a presynaptic action involving (Ca^{++} insensitive) release of endogenous EAA by L-AP4 from presynaptic or glial sites, or a postsynaptic action whereby quisqualate would sensitize NMDA, AMPA and metabotropic receptors to L-AP4.

32.12

ROLE OF NITRIC OXIDE IN N-METHYL-D-ASPARTATE-STIMULATED NEUROTRANSMITTER RELEASE. A.K. Stout* and J.J. Woodward. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298.

N-methyl-D-aspartate (NMDA) stimulated the release of tritiated norepinephrine from hippocampal slices of adult Sprague-Dawley rats. N-nitro-L-arginine, a competitive inhibitor of nitric oxide (NO) synthase, had no apparent effect on NMDA-stimulated neurotransmitter release at concentrations between 0.1 and 100 μM. Hemoglobin, an iron-containing protein which binds NO, also had no significant effect on NMDA-stimulated transmitter release at concentrations of 0.001-1 μM. However, sodium nitroprusside (SNP), a vasodilator which generates NO upon reacting with sulfhydryl groups, inhibited transmitter release with an IC_{50} of approximately 100 μM. The inhibitory effect of SNP was not blocked by 1 μM hemoglobin and was not due to the formation of cyanide ions, since equivalent concentrations of potassium cyanide (10-300 μM) actually enhanced NMDA-stimulated release. Pretreatment of the slices with the sulfhydryl reducing agent dithiothreitol (5 mM) more than doubled NMDA-stimulated release and shifted the SNP inhibition curve upward. Pretreatment of the slices with the sulfhydryl oxidizing agent 5,5'-dithio-bis(2-nitrobenzoic acid) (0.5 mM) had no effect on NMDA-stimulated release by itself, but blocked the inhibitory effects of SNP. These results suggest that NO, either directly or indirectly, could feedback to inhibit NMDA-stimulated processes. Supported by NIAAA AA08089 and a grant from the Alcoholic Beverage Medical Research Foundation.

32.13

NMDA INCREASES PHOSPHATIDYLINOSITOL HYDROLYSIS VIA THE NOVEL SECOND MESSENGER NITRIC OXIDE IN NEONATAL RAT CEREBELLUM. J. Li*, M.C. Kennedy and S.S. Smith. Dept. of Anatomy, Inst. for Neurosci., Hahnemann Univ., Philadelphia, PA 19102.

NMDA receptor stimulation has been associated with development and plasticity of the CNS. Recent reports indicate that activation of this excitatory amino acid receptor subtype produces nitric oxide (NO) from arginine substrates (Garthwaite et al, 1989) and results in subsequent increases in cGMP. In the present study, the ability of NMDA to stimulate hydrolysis of phosphatidylinositol (PI) via production of NO was studied in neonatal cerebellar tissue, as sequelae of PI hydrolysis have also been linked with developmental processes in the CNS. Previous results from this lab indicate that the $GABA_B$ agonist baclofen (1μM) exerts potent permissive effects on the ability of NMDA (100 μM) to stimulate PI hydrolysis 70% above basal values ($P < 0.001$), an 80% increase above NMDA-stimulated levels of this parameter observed under control conditions. PI turnover was assessed using tritiated inositol in 160 μ cross-chopped slices of cerebellar tissue from 7-9 d.o. female rats. Following a 20 min incubation with agonists and chloroform extraction, total inositol phosphates were separated using anion exchange chromatography. Addition of 30 μM l-arginine to the incubation mixture produced NMDA-stimulated values of PI turnover which were 50% above basal levels ($P < 0.01$), an effect similar to that seen in the presence of 1 μM baclofen. In contrast, administration of 100 μM L-N^G-monomethyl arginine or N_ω-nitro-l-arginine, blockers of nitric oxide production, completely prevented the permissive effect of baclofen on the ability of NMDA to enhance PI hydrolysis. This blockade was reversed by addition of 200 μM l-arginine. In contrast, incubation with methylene blue (10 μM), which prevents formation of cGMP, did not alter NMDA-stimulated levels of this parameter, suggesting that stimulation of PI hydrolysis via NMDA-induced production of NO is not mediated by cGMP. Further, it is proposed by the results from this study that the ability of NMDA to produce NO and enhance PI hydrolysis is regulated by the degree of gabaergic tone via the $GABA_B$ receptor, actions which may be relevant for developmental processes in the cerebellum. *(Supported by NS 25809.)*

32.14

NITRIC OXIDE (NO) SELECTIVELY INHIBITS NMDA RECEPTOR ACTIVATION VIA A cGMP-INDENDENT PATHWAY Q.J.J Manzoni*, L Fagni,* P Marin*, L Prezeau*, A Sahuquet*, F Sladeczeck and J Bockaert* (spon: F Sladeczek) C.C.I.P.E. Rue de la Cardonille, 34094 Montpellier Cedex 5 France.

We examined the effects of NO-producing drugs on N-methyl-D-Aspartate (NMDA) receptor activation, measured by Fura-2 ratio imaging and whole cell patch clamp recording in striatal and cerebellar granule neurons in primary culture. Sodium Nitroprusside (SNP), a compound that spontaneously released NO, inhibited in a non competitive manner NMDA-induced [Ca2+]i. This effect was slow to develop (a 5 min preincubation was necessary) and reversible after a 10-15 min washout. NO inhibitory effect was inhibited by haemoglobin that strongly binds NO, and reproduced by glycerol trinitrate, showing that SNP effects were specifically due to NO production. Neither (RS)α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- nor 56 mM K+-induced [Ca2+]i increase were affected, suggesting that NO effect was specific to NMDA receptors. Both SNP and NMDA were able to induce cGMP formation in striatal neurons, but NO effects were not reproduced by application of cyclic GMP (cGMP) in the recording patch-pipette or by bath perfusion of 8-bromo-cGMP, showing that NO inhibitory action did not depend on cGMP accumulation. Interestingly, we found that NO inhibitory effects were not obtained when the NMDA receptor was already occupied by NMDA or by competitive antagonists (CPP or AP5). Furthermore, the specific binding of [3H]-CGS 17955 at the NMDA binding site was displaced by SNP pretreatment, strongly suggesting that NO could directly act at the NMDA binding site. Since NMDA receptor activation is known to induce NO synthesis, a feedback inhibition of NMDA receptor action is likely. NO could play a role in heterosynaptic depression and neuroprotection.

32.15

NITRIC OXIDE (NO) MODULATES NMDA-INDUCED INCREASES IN INTRACELLULAR Ca^{2+} IN CULTURED FOREBRAIN NEURONS. K.R. Gilbert, E. Aizenman[1] and I.J. Reynolds. Departments of Pharmacology and [1]Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

N-methyl-D-aspartate (NMDA) receptor responses are potentiated by chemical reduction and are inhibited by oxidation. In our search for potential endogenous redox agents which might physiologically modulate this receptor we tested NO, an oxidizing agent, for its effects on NMDA receptors. We recorded NMDA-induced changes in $[Ca^{2+}]_i$ in single cultured rat forebrain neurons using fura-2. Pretreatment with NO (1μM-300μM) for 200s followed by washing results in significant inhibition of NMDA-elicited calcium fluxes (63.8 $\pm$ 5.6% of control at 100μM; mean + SEM, $p < 0.05$ Student's t test, n=16). Pretreatment with NO (100μM) also causes a small, but significant inhibition of $[Ca^{2+}]_i$ fluxes in response to 50mM KCl or 50μM kainate. Additionally, sodium nitroprusside (1-300μM), which releases NO, mimics these effects of NO. Finally, this effect of NO probably is not a consequence of NMDA receptor oxidation because the effects of NO spontaneously reverse upon washing, which is in contrast to the actions of agents that oxidize the NMDA receptor. Because NO release is stimulated by NMDA receptor activation in neurons, this modulation of NMDA receptors by NO may represent a novel feedback mechanism for controlling synaptic NMDA responses.

32.16

AGONIST ACTION OF L-PROLINE AT GLUTAMATE AND GLYCINE RECEPTORS. V. Henzi and A.B. MacDermott. Dept. of Physiology and Center for Neurobiology and Behavior, Columbia University, New York City, NY 10032.

The capability of brain tissue for high-affinity reuptake and K^+-evoked release of L-proline (PRO) have implicated it as a potential neurotransmitter. However, to date it has remained unclear which neuronal receptors are sensitive to PRO. We have found that millimolar concentrations of PRO evoke an inward current in voltage-clamped rat embryonic spinal cord neurons in culture that is partially blocked by APV (30 uM) and CNQX (10 uM), the selective glutamate receptor antagonists (Helm et al, 1990). We now report that the remaining portion of the PRO-activated current is blocked by the selective glycine receptor antagonist strychnine (5 uM). Furthermore, using combinations of the selective antagonists, we isolated APV-, CNQX-, and strychnine-sensitive components of the PRO-evoked whole-cell currents and found that these had identical characteristics (reversal potential, desensitization, and other criteria) to those evoked by the selective agonists NMDA, kainate, and glycine respectively. This establishes an agonist action of PRO at strychnine-sensitive glycine receptors and at NMDA and non-NMDA glutamate receptors. No evidence was obtained for the proposal that PRO can act as an antagonist at these receptors or as an agonist at the strychnine-insensitive glycine site of the NMDA receptor. We went on to evaluate the agonist potency of PRO at each of these receptor subtypes. Detectable strychnine-sensitive currents were observed with [PRO] > 100 uM, while APV- and CNQX-sensitive currents were first observed with [PRO] > 300 uM. In each case, the responses did not saturate even at [PRO]= 50 mM. At 10 mM PRO, the APV-, CNQX-, and strychnine-sensitive currents were comparable to currents elicited by 15 uM NMDA, 5 uM kainate, and 35 uM glycine respectively. These actions may be physiologically significant if PRO functions as a neurotransmitter as well as in cases of hyperprolinemia, a metabolic disorder in which the [PRO] in cerebrospinal fluid reaches high micromolar concentrations.

32.17

STIMULATION OF NEURONAL N-METHYL-D-ASPARTATE RECEPTORS BY GLUTATHIONE. L.M. Brown, R.D. Trent*, Y.H. Lee*, P.K. Randall, S.S. Lau*, T.J. Monks*, and S.W. Leslie. Inst. for Neuroscience and College of Pharmacy, Univ. of Texas, Austin, TX 78712.

Reduced glutathione (GSH, γ-glutamylcysteinylglycine) contains three amino acids which have been demonstrated to interact individually with the NMDA receptor complex. Oxidized glutathione (GSSG) is composed of two molecules of GSH connected by a disulfide bond. This study examined the effects of GSH and GSSG on NMDA receptor function and binding. Dissociated brain cells were isolated from newborn rats and loaded with fura-2 (Dildy and Leslie, Br.Res. 499 (1989) 383-387). Concentration-dependent increases in free intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were observed upon the addition of GSH (10-3000 μM) or GSSG (16-8000 μM). Concentration effect curves were analyzed by the computer program ALLFIT (DeLean, et al., Am.J.Physiol. 235 (1978) E97-E102). The EC_{50}'s for GSH (980.6 $\pm$ 151.9 μM) and GSSG (908.4 $\pm$ 49.7 μM) were not significantly different. Maximum responses, expressed as percent change in $[Ca^{2+}]_i$ compared to resting levels, for GSH (24.4 $\pm$ 2.4 %) and GSSG (72.8 $\pm$ 1.6 %) were different ($p < 0.01$). The response produced by 2 mM GSH or GSSG was significantly prevented or reversed by the NMDA antagonists Mg^{2+} (1.0 mM), APV (100 μM), MK-801 (400 nM), and DGG (250 μM).

The interaction of GSH and GSSG with the NMDA binding site was also examined. These assays were performed according to the method of Sills et al.(Eur J. Phar. 192 (1991) 19-24) using the high affinity NMDA antagonist [3H]CGP-39653. Saturation curves for [3H]CGP-39653 (0.47-60 nM) generated a K_d of 9 $\pm$ 0.71 nM with a B_{max} of 1265 $\pm$ 69 fmoles/mg protein. When these experiments were performed in the presence of 1 μM GSH the K_d was significantly elevated to 13.3 $\pm$ 1.1 nM and the B_{max} was significantly decreased to 907 $\pm$ 62 fmoles/mg protein. Competition curves using 30 nM [3H]CGP-39653 demonstrated that GSH and GSSG inhibited binding with IC_{50}'s of 0.977 $\pm$ 0.18 and 11.2 $\pm$ 1.37 μM respectively. These results suggest the potential for modulation of the NMDA receptor site by GSH and GSSG. Supported by NIAAA grants AA05809 and AA08104.

32.18

PREGNENOLONE SULFATE: A POSITIVE MODULATOR OF THE NMDA-INDUCED CURRENT IN CULTURED NEURONS. F.-S. Wu, T.T. Gibbs and D.H. Farb. Dept. of Pharmacology and Experimental Therapeutics, Boston Univ. Sch. of Med., Boston, MA 02118.

We have shown previously that the neurosteroid pregnenolone sulfate (PS) is a negative modulator of both GABA and glycine responses. Here we examine the effects of this steroid on currents induced by N-methyl-D-aspartate (NMDA), kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) in cultures of chick spinal cord neurons. Using whole-cell recording methods, cells were voltage-clamped at -70 mV. Drug solutions were applied to single neurons by pressure ejection from 7-barrel pipets. PS (100 μM) rapidly and reversibly potentiated the response to 30 μM NMDA (by 197%) and slightly inhibited responses to 50 μM kainate (by 25%) and 25 μM AMPA (by 29%). Potentiation of the NMDA response by PS in the presence of a maximal concentration (10 μM) of glycine did not differ significantly from that measured without added glycine, indicating that glycine contamination cannot account for the observed effect. Similarly, potentiation of the NMDA response by 10 μM glycine was still observable in the presence of a near-maximal concentration (100 μM) of PS, indicating that enhancement of the NMDA response by PS is not mediated by the glycine modulatory site of the NMDA receptor. The effects of PS on NMDA and GABA were dose-dependent, with EC_{50} values of 57 and 7 μM and maxima of +256 and -100%, respectively. These results are consistent with the hypothesis that neurosteroids such as PS are involved in regulating the balance between excitation and inhibition in the central nervous system.

32.19

N-ACETYL-ASPARTYL GLUTAMATE (NAAG) AND ACTIVATORS OF THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR/CHANNEL COMPLEX AS REGULATORS OF [^{3}H]NOREPINEPHRINE ([^{3}H]NE) RELEASE FROM RAT HIPPOCAMPAL SLICES. L.L. Werling, D. Montgomery*, J.T. Coyle and P.S. Puttfarcken. Dept. Pharmacology, The GWU Med. Ctr., Washington, DC 20037 and Dept. Psychiatry, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

NMDA and other agonists at the NMDA receptor/channel complex have previously been reported to stimulate the release of NE from rat hippocampus. NAAG and NE are colocalized in cell bodies of the locus coeruleus, the origin of noradrenergic projections to the hippocampus. We have studied the interaction between NAAG and activators of the NMDA receptor/channel complex.

Hippocampi of rats were dissected, chopped into strips, washed in modified Krebs-HEPES buffer (MKB), and incubated with 15 nM [^{3}H]NE. Tissue was washed with MKB, resuspended in MKB containing 1 μM concentrations of desiprimine and yohimbine to prevent reuptake of and feedback inhibition by released [^{3}H]NE, and loaded into superfusion chambers. After establishment of a low, stable baseline release, tissue was stimulated by 2 min exposures to excitatory amino acid.

Under conditions in which activity of NAALadase, the enzyme responsible for the degradation of NAAG to N-acetyl aspartate (NAA) and glutamate, was inhibited, NAAG (1 mM) had no effect on release of [^{3}H]NE. NAA (1 mM) also had no effect on release. However, the other degradation product glutamate (50 μM-1 mM), as well as NMDA (25 μM) stimulated release of [^{3}H]NE. This release was sensitive to the non-competitive NMDA antagonist MK-801. The presence of 50 or 100 μM NAAG significantly reduced glutamate- and NMDA-stimulated release. This suggests a potential neuromodulatory role for NAAG in the release of NE from hippocampus. (Spported by an NRSA from NIMH to PSP and a PMAF grant to LLW).

EXCITATORY AMINO ACIDS: RECEPTORS I

33.1

CLONING OF, GLUR6, A GLUTAMATE RECEPTOR SUBUNIT ACTIVATED BY KAINATE BUT NOT AMPA. J. Egebjerg*, B. Bettler*, J. Hermans-Borgmeyer* and S. Heinemann. Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037

We have cloned a glutamate receptor subunit, GluR6, using GluR5 as probe in a low stringency hybridization screening approach. Sequence homology between cDNA clones encoding GluR1-GluR6 receptor subunits reveals the existence of two subunit classes: the GluR1 to GluR4 class, and the GluR5 and GluR6 class. Receptors generated from the GluR1-GluR4 class have a high apparent affinity for AMPA whereas the GluR6 subunit has the highest apparent affinity for kainate. Upon expression in Xenopus oocytes the homomeric GluR6 receptor is activated by kainate, quisqualate and L-glutamate but not by AMPA. The order of EC_{50}'s is kainate < quisqualate < glutamate. Desensitization of the receptor was observed in continuous application of agonist. The homomeric GluR6 glutamate receptor exhibits an outwardly rectifying current-voltage relationship. Finally, in situ hybridization reveals a pattern of GluR6 gene expression reminiscent of the binding pattern obtained with [^{3}H]kainate, showing high levels of transcript in regions which are most sensitive to kainate induced cell death.

Supported by The Danish Medical Research Council (J.E.), NINCDS, HFSP, Weingart Foundation and Fritz B. Burns Foundation (S.H)

33.2

FUNCTIONAL CHARACTERIZATION OF A THIRD ISOFORM OF RAT GLUR4. C.A. Winters*, V. Gallo, A. Buonanno, L. Vyklicky*, M.L. Mayer, J.T. Russell. Section of Neurophysiology and Biophysics, Unit on Molecular Neurobiology, Section of Neuronal Secretory Systems, LDN , NICHD, NIH, Bethesda, MD 20892.

Glutamate receptor subunits activated by kainate and AMPA have been shown to exist in flip and flop isoforms generated by alternative splicing, (Sommer, 1990). An additional isoform of the flop version of GluR-4, differing in sequence for 36 amino acids located at the C-terminus, was isolated by screening a rat cerebellar cDNA library, (see Gallo, Neurosci. Abstr.,1991).

Xenopus levis oocytes were injected with 10 ng of RNA synthesized from GluR-4c cDNA. Experiments were performed under two-electrode voltage-clamp 4-6 days after injection. Agonists were applied rapidly using a small chamber with a volume of $\approx$ 3 μl. AMPA-kainate receptor agonists but not N-methyl-D-aspartic acid induced inward currents. Dose response curves exhibited the following EC50 values and Hill coefficients: Kainate 44 $\pm$ 9 μM, 1.3 $\pm$ 0.1; domoate 6.1 $\pm$ 2.8 μM, 0.9 $\pm$ 0.1; quisqualate 0.36 $\pm$ 0.07 μM, 0.6 $\pm$ 0.02 and glutamate 1.8 $\pm$ 1.4 μM, 0.7 $\pm$ 0.1. Responses to kainate were blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) with a Ki = 0.43 $\pm$ 0.26 μM. These values are similar to those reported for the Glu-R1 flop clone (Dawson et al, 1990). No potentiation of the kainate response was observed with coapplication of zinc, however inhibition occurred with zinc concentrations greater than 30 μM. Coapplication of 0.3 μM to 30 μM quisqualate reduced the current evoked by 100 μM kainate with an IC50 of 3.5 $\pm$ 2.7 μM. The I/V relationship, recorded using 100 μM kainate, showed inward rectification: The slope was linear between -100 mV and -60 mV but decreased with further depolarization. No inward current was detectable above 0 mV.

Further studies characterizing the functional properties of this clone compared to those for other glutamate receptor subunits and the native receptor will be presented.

33.3

MOLECULAR CLONING OF NEURONAL AND MUSCLE SPECIFIC GLUTAMATE RECEPTOR SUBUNITS OF *DROSOPHILA*. C. M. Schuster*, A. Ultsch*, P. Schloss*, B. Schmitt* and H. Betz. (SPON: European Neuroscience Association). Zentrum für Molekulare Biologie, Universität Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg, FRG, and Max-Planck-Institut für Hirnforschung, Abteilung Neurochemie, Deutschordenstraße 46, D-6000 Frankfurt 71, FRG.

Insects and other invertebrates use glutamate as a neurotransmitter both in the central nervous system and at the neuromuscular junction. We have isolated cDNAs from *Drosophila melanogaster*, which encode two distinct glutamate receptor (GluR) subtypes, designated DGluR-I and DGluR-II. Primary structure analysis indicates a close evolutionary relationship between DGluR-I and the family of rat brain kainate/AMPA-sensitive GluR subunits, whereas DGluR-II represents a distant homolog of these proteins. Whole mount *in situ* hybridisation and Northern blot analysis disclosed a differential expression regulation for DGluR-I and DGluR-II mRNA: DGluR-I transcripts accumulate during major periods of neuronal differentiation and are localized in the developing central nervous system, whereas the embryonic DGluR-II gene expression seems to be restricted to somatic muscle tissue. Functional expression in *Xenopus* oocytes revealed that DGluR-II proteins form L-glutamate and L-aspartate gated cation channels of low agonist affinity. Our data indicate that this muscle-type GluR subunit shares structural and functional features with both the *Drosophila* neuronal DGluR-I and different excitatory amino acid receptor subtypes from mammalian brain. This suggests that the DGluR-II protein represents a distinct GluR subtype, and that all subtypes of the ionotropic glutamate receptor superfamily exhibit a common structural design. Supported by DFG (Leibniz Prog.), BMFT (BCT 365/1) and Fonds der Chemischen Industrie.

33.4

PURIFICATION, PHARMACOLOGICAL CHARACTERIZATION AND CLONING OF NMDA RECEPTOR. K.N. Kumar, N.T. Tilakaratne, P.S. Johnson, K.T. Eggeman, E.K. Michaelis. Departments of Pharmacology & Toxicology, Biochemistry and the Center for Biomedical Research, University of Kansas, Lawrence, Kansas 66045.

Our laboratory has recently purified a complex of four proteins with molecular weights of 70, 59, 47 and 36 kDa from rat brain synaptic membranes. The purification was carried out on a glutamate derivation affinity matrix and specifically eluted with NMDA. This complex of proteins had the ligand binding sites for the agonists glutamate and NMDA, the antagonists CPP and AP-7, the modulator glycine and the ion channel blockers TCP, PCP AND MK-801 that act on NMDA receptor. The same complex of proteins did not bind AMPA, kainate or quisqualate. The ligand binding constants for L-glutamate, NMDA and TCP were equivalent to those determined in synaptic membrane preparations. The 70 kDa subunit of this complex was recognized by antibodies raised against the previously purified 70 kDa glutamate-binding protein (Eaton, et.al., J. Biol. Chem. 265:16195, 1990). These antibodies were used to screen cDNA libraries from the rat brain hippocampus and a 2.3 kb cDNA for the 70 kDa subunit of the complex was cloned and sequenced. (Supported by ARO grant DAAL 03-88 K 0017 and NIAAA grant AA 04732)

33.5

ION CHANNEL CHARACTERIZATION OF A RECONSTITUTED GLUTAMATE/NMDA SENSITIVE RECEPTOR COMPLEX. Katy Eggeman, Gary Aistrup, K.N. Kumar, E.K. Michaelis, R. L. Schowen*. Departments of Biochemistry, Pharmacology and Toxicology, Chemistry, and the Center for Biomedical Research, University of Kansas, Lawrence, KS 66045.

In attempts to demonstrate ion channel function of an isolated protein complex that represents the NMDA receptor (K.N. Kumar, et. al., this meeting), planar lipid bilayer membrane (PLM) reconstitution studies were undertaken in our laboratory. These studies involved the identification of channel conductances, effector ligand concentration dependence, and kinetic analyses of the corresponding closed and opened states of the reconstituted channel. At present, much of the evaluation has been restricted to those responses evoked by L-glutamate, but agonist/antagonist studies have been initiated. Initial results indicate multiple conductance levels of 20-25 pS, 40-50 pS, and 60-80 pS for single-channel responses activated by 8-10 uM L-glu. Mean open/closed state lifetime distribution analysis shows fast, intermediate, and slow components of the time constant for each level. A simple three entity, two state model has been proposed to explain the fractional dwell time in each conductance state, and possibly the transition rates between them. (Supported by grants AA04732 and DAAL-03-88-K0017)

33.6

NMDA RECEPTOR PROTEIN IN CULTURED HIPPOCAMPAL NEURONS: DEVELOPMENTAL EXPRESSION, RELATION TO EXCITOTOXICITY, AND REGULATION BY BASIC FGF. E.K. Michaelis, H. Wang*, M.P. Mattson, Dept. Pharmacology and Toxicology, Univ. of Kansas, Lawrence, KS 66045. Center on Aging and Anatomy & Neurobiol., Univ. of Kentucky, Lexington, KY 40536.

We recently isolated, characterized and cloned a 71 kDa subunit of the NMDA receptor protein (NMDARP) from rat brain (Kumar, et.al., this meeting). We now report on the localization, function, and regulation by growth factors of this NMDARP in cultured embryonic rat hippocampal neurons. Immunocytochemistry and Western blots using monoclonal antibodies to the NMDARP demonstrated an increase in its expression in neurons with time in culture. NMDARP was localized to the somata and dendrites of pyramidal-like neurons and was sparse or absent in the axons. The developmental expression of NMDARP immunoreactivity closely paralleled the development of sensitivity to NMDA neurotoxicity, and neurons with high levels of NMDARP immunoreactivity were the most vulnerable to NMDA. A polyclonal NMDARP antiserum reduced NMDA neurotoxicity selectively. We previously found that FGF protected neurons against glutamate toxicity (J. Neurosci. 9:3728). Here we show that bFGF (but not NGF or EGF) causes a marked reduction in NMDARP levels in the cultured hippocampal neurons. We are currently examining the expression of mRNA for the NMDARP. This first demonstration of regulation of the expression of a glutamate receptor protein by a growth factor suggests that growth factors may play roles in modifying both adaptive and pathological actions of excitatory amino acids. Supported by AA04732, ARO DAAL-03-88-K0017 (E.K.M.); and NS 29001, AG05144, Alzheimer's Association (M.P.M.).

33.7

A CHIMERIC AMPA RECEPTOR SUBUNIT: DISCRETE SEQUENCE CHANGES TRANSFORM GLUR1 INTO GLUR2. P.Bochet*, A.Dutriaux*, B.Lambolez*, E.Nalivaiko*, J.Rossier and L.Prado de Carvalho*, Laboratoire de Physiologie Nerveuse, Centre National de la Recherche Scientifique, 91198 Gif sur Yvette Cedex, France.

GluR1, GluR2 and GluR3 are three highly homologous subunits of the glutamate AMPA receptor but with different functional properties. In ligand gated channels the second transmembrane domain is thought to form the wall of the ionic channel and determine its electrical properties. A chimeric AMPA receptor subunit was constructed by replacing the region comprising the putative first and second transmembrane domains in GluR1 by the corresponding region of GluR2. The functional properties of the resulting chimera, which is essentially GluR1 with a stretch of 143 amino-acids from GluR2, were studied in the *Xenopus laevis* oocyte expression system. Similar to GluR2, the chimera did not respond to agonists but greatly enhanced the response when coexpressed with GluR1, indicating the formation of heteromers. Furthermore, the current-voltage relationship of GluR1 + chimera was linear like that of GluR1 + GluR2 and did not display the strong inward rectification characteristic of GluR1 alone. Sequence comparisons suggest that the presence of an arginine in the chimera in the place of glutamine at position 600 in GluR1 and GluR3 is responsible for these properties.

33.8

ANALYSIS OF SIGNAL TRANSDUCTION AND LIGAND-BINDING OF CHIMERIC cDNAS WITH KBP AND GLUR1. K. Doi*, N. Yokotani*, R.J. Wenthold and K. Wada, Lab. of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892

cDNAs encoding a kainate-binding protein (KBP) and glutamate receptor subunits (GluR1-5) were isolated from frog, chick and rat brain, respectively (Wada et al., Nature, 342, 684, 1989; Gregor et al., Nature, 342, 689, 1989; Bettler et al., Neuron, 5, 583, 1990). We constructed four chimeric cDNAs with KBP and GluR1 to examine a possibility that KBP is a part of a kainate receptor-ionophore complex and to identify molecular regions that are responsible for the kainate-binding activity of KBP. Chimeric cDNAs are: 1) GluR-KBP had N-terminal half of GluR1 and transmembrane domains and C-terminal extracellular domain of KBP. 2) KBP-GluR had N-terminal extracellular domain of KBP and C-terminal half of GluR1. 3) short KBP had a truncation of N-terminal extracellular domain of KBP. 4) KBP-GluR-KBP had N-terminal extracellular domain, transmembrane domains of GluR1 and C-terminal extracellular domain of KBP. Two-microelectrode voltage clamp of the oocytes injected with chimeric cRNAs and receptor ligand-binding of the transfected cells with chimeric cDNAs revealed that: 1) GluR-KBP might form a channel that was weakly activated only by L-glutamate. 2) KBP-GluR did not form a functional glutamate receptor. 3) GluR-KBP had kainate-binding activity as much as KBP while KBP-GluR did not have any. 4) short KBP did not have any kainate-binding activity. The results suggest that KBP might require additional factors to be assembled into a receptor-ionophore complex and that multiple regions of KBP are required for its kainate-binding activity.

33.9

PARTIAL PURIFICATION AND SPECIFIC IMMUNOREACTIVITY OF THE AMPA-TYPE GLUTAMATE RECEPTOR FROM RAT BRAIN. R. Hall*, B.A. Bahr, M. Kessler, K. Sumikawa & G. Lynch, Ctr. Neurobio. of Learning/Memory, and Dept. of Psychobio., Univ. of Calif., Irvine, CA 92717

The subclass of glutamate receptors specifically activated by AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) appears to mediate most of the excitatory synaptic transmission in the brain and a recent study by Staubli *et al.*, (*Psychobio.* 18:377, 1990) has shown that a change in this receptor most likely accounts for the expression of long-term potentiation, a lasting increase in synaptic strength presumably involved in memory encoding. The present study is aimed at methods which may lead to the purification of this receptor. More than 50% of the [^{3}H]AMPA binding sites were solubilized from rat forebrain membranes (1 mg protein/ml) with 1% Triton X-100. Each of the following procedures produced a significant increase in specific AMPA binding (shown in parenthesis): (*a*) DEAE anion-exchange chromatography (up to 10 fold); (*b*) wheat-germ lectin affinity chromatography (10-15 fold); (*c*) heparin affinity chromatography (5-10 fold); and (*d*) hydroxyapatite adsorptive chromatography (up to 5 fold). Sequential application of these procedures produced as much as 40-fold purification. The partially purified AMPA receptor chromatographed on an HPLC size exclusion column with an apparent particle molecular mass (including bound detergent) of approximately 425,000 daltons, whereas on silver-stained polyacrylamide gels a single band was enriched with each purification step which had an electrophoretic mobility corresponding to 102 kDa. Using fusion techniques, rabbit antibodies (anti-AR) were developed toward a 19 amino acid sequence located in the large extracellular N-terminal domain of the 'Glu 1' receptor (based on the cloned sequence and nomenclature of Hollmann *et al.*, *Nature* 342:643, 1989). Through consecutive chromatographic steps, [^{3}H]AMPA binding and the immunolabeling of a 102 kDa band by anti-AR were comparably enhanced. The data suggest that anti-AR recognizes the AMPA-type glutamate receptor present in the central nervous system and that this receptor is a tetramer consisting of four subunits of about equal mass. (Supported by grants AFOSR #89-0383 and NIH #21860.)

33.10

SERINE AND TYROSINE PHOSPHORYLATION OF RECOMBINANT NON-NMDA GLUTAMATE RECEPTORS. S.J. Moss*, C.D. Blackstone, L. Raymond and R.L. Huganir, Dept. of Neuroscience, Howard Hughes Medical Inst., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

To examine the role of protein phosphorylation in the regulation of glutamate receptor function, we have been studying phosphorylation of recombinant non-NMDA glutamate receptor subunits transiently expressed in human embryonic kidney 293 cells. Using polyclonal anti-peptide antisera raised against the GluR1 subunit, we have immunoprecipitated a 106 kD phosphoprotein corresponding to the GluR1 subunit. The GluR1 subunit in this system is basally phosphorylated predominantly on serine residues. Treatment of the 293 cells with TPA, an activator of protein kinase C, increases phosphorylation of the GluR1 protein on serine residues. Phosphopeptide map analysis suggests that a single site is phosphorylated under both basal conditions and TPA stimulated conditions. Co-expression of the GluR1 subunit with v-src in 293 cells results in tyrosine phosphorylation of GluR1. These results suggest that the GluR1 protein is a substrate for protein kinase C and for protein tyrosine kinases. We are currently mapping these phosphorylation sites of the GluR1 subunit by site-specific mutagenesis. Comparisons between mutant nonphosphorylated receptors and the wild-type phosphorylated receptors will allow precise investigation of the effects of phosphorylation on ion channel function.

33.11

NMDA RECEPTOR PLASTICITY: SELECTIVE REGULATION OF RECEPTOR SUBTYPES BY KINDLING. D. W. Bonhaus, G. C. Yeh, J. V. Nadler and J. O. McNamara. Duke & V.A. Medical Centers, Durham, NC 27710

Kindling has been shown to upregulate hippocampal NMDA receptors. To characterize this receptor plasticity we examined the effects of kindling on the binding of NMDA receptor ligands ([^{3}H]CPP, [^{3}H]CGS-19755, [^{3}H]L-glutamate) and on the agonist-dependent binding of an NMDA channel blocker ([^{3}H]TCP). Binding was measured in hippocampal membranes of amygdala kindled rats sacrificed 28 days after the last kindling stimulation. Kindling produced a 40-46 % increase in the number of hippocampal [^{3}H]CPP and NMDA-sensitive [^{3}H]L-glutamate binding sites but did not significantly modify the number of [^{3}H]CGS-19755 binding sites. The binding affinity for [^{3}H]CPP was decreased while that for [^{3}H]glutamate and [^{3}H]CGS-19755 was unaltered. In parallel, kindling decreased the potency of CPP in inhibiting glutamate-stimulated [^{3}H]TCP binding but had no effect on the potency of CGS-19755. In contrast to the diminished potency of CPP, kindling markedly increased the potency of the NMDA antagonist APV in this paradigm. Thus, kindling upregulated hippocampal NMDA receptors in a manner detected by [^{3}H]L-glutamate and [^{3}H]CPP but not [^{3}H]CGS-19755. These data strongly suggest that kindling selectively upregulates specific NMDA receptor subtypes. The differing effects of kindling on the potency of CPP and APV indicates that the upregulation does not correspond to a change in the so called agonist or antagonist preferring conformational state of the receptor. These changes probably explain the enhanced ability of NMDA to depolarize CA3 pyramidal cells in kindled rats and may contribute to the maintenance of the kindled state.

33.12

Pharmacologically-Distinct NMDA Receptor Subtypes. D. T. Monaghan & J. A. Beaton*, Dept. Pharm., UNMC, Omaha, NE, 68198. Since the N-methyl-D-aspartate (NMDA) class of excitatory amino acid receptor plays a critical role in both normal and pathological processes throughout the vertebrate CNS, the identification of pharmacologically-distinct NMDA receptor subtypes is likely to be important for the therapeutic use of compounds active at the NMDA receptor. To evaluate possible subpopulations of NMDA receptors, several compounds were tested as inhibitors of L-[^{3}H]glutamate binding to NMDA receptors in various regions of rat brain by quantitative autoradiography (Eur. J. Pharm. 194:123). In addition to differing regional distributions in the potencies of agonists and antagonists, agonists displayed two patterns of regional variation; antagonists displayed three patterns of regional variation. While all agonists displayed higher affinities in the medial striatum than in the lateral thalamus, only two agonists, quinolinate and homoquinolinate, displayed markedly lower affinities in the cerebellar granule cell layer. Curve-fitting analysis indicated that quinolinate and homoquinolinate each displayed two affinity components (24μM & 275μM and 0.26 μM & 49 μM, respectively). While all antagonists displayed higher affinities in the lateral thalamus than in the medial striatum, certain antagonists, such as CPP (3-((+)-2-carboxy-piperazin-4-yl)-propyl-1-phosphonate), displayed a significantly lower affinity in the cerebellum than in other brain regions that was described by two affinity components. NMDA receptors of the medial thalamus also displayed relatively lower affinities specifically for homoquinolinate and CPP, but these binding sites were not pharmacologically identical to those found in the cerebellum and thus appear to represent a third class of pharmacologically-distinct NMDA receptor. (Support: NS 28966 & Eli Lilly Co.)

33.13

ZINC POTENTIATES DESENSITIZED KAINATE CURRENTS OF A GLUTAMATE RECEPTOR CLONE (GluR3). **J.C. Dreixler and J.P. Leonard** Dept. of Biol. Sciences, Univ. of Illinois at Chicago, Chicago, Il 60680.

Endogenous zinc may take part in modifications that may contribute to synaptic plasticity. It has been shown that low concentrations of zinc do potentiate non-NMDA currents in Xenopus oocytes expressing total rat brain RNA [Rassendren, F-A et al. Neuron, 4, 733-740]. We used Xenopus oocytes to express mRNA of a glutamate receptor clone (GluR3) [Boulter, J. et al Science, 249, 1033-1037]. Using a two electrode voltage clamp technique, desensitized currents were obtained upon application of 100 μM kainate for 5 minutes. One minute later, during constant kainate perfusion, a solution of 5 μM $ZnSO_4$ plus 100 μM kainate was applied. We confirmed the effects of zinc on currents in oocytes expressing total rat brain RNA. We also found that zinc enhanced the currents expressed from GluR3 mRNA by 70 ± 12% (s.e.m.) at -120 mV and by 82 ± 10% at -80 mV. Here we show that the receptor-channel formed by GluR3 alone may be responsible for this effect. The domain responsible for zinc potentiation and the role of other subunits is under investigation. (Supported by NIH NS26432)

33.14

L-BOAA (β-*N*-OXALYLAMINO-L-ALANINE) EXHIBITS AMPA-LIKE AGONIST PROPERTIES AT NON-NMDA EXCITATORY AMINO ACID RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES. D. Bednar, C. Maciver and W. Karbon, Nova Pharmaceutical Corporation, Baltimore, MD, 21224-2788.

The plant excitotoxin β-*N*-oxalylamino-L-alanine (L-BOAA) has been implicated as a causative agent in human lathyrism. In the present study electrophysiological responses to L-BOAA (EC = 23 μM), AMPA (EC_{50} = 5 μM) and kainic acid KA, EC_{50} = 90 μM) were characterized in *Xenopus laevis* oocytes injected with rat brain poly (A+) RNA. Although L-BOAA and AMPA produced inward currents having similar maximal amplitudes, they were substantially smaller than currents elicited by KA. In addition, L-BOAA, AMPA and KA demonstrated similar reversal potentials. The competitive non-NMDA antagonists DNQX (K_i = 411 nM) and NBQX (K_i = 118 nM) potently inhibited L-BOAA, with similar values being obtained for inhibition of AMPA. L-BOAA (100 μM) reduced the response to KA (100 μM) by 50% regardless of whether oocytes were pre-exposed to L-BOAA or simultaneously exposed to L-BOAA and KA. Similarly, AMPA (30 μM) inhibited the response to KA by 30%. Simulataneous exposure of oocytes to saturating concentrations of AMPA (30 μM) and L-BOAA (100 μM) produced a response no greater than those obtained with either agent alone. These findings are evidence that L-BOAA, AMPA and KA interact with a common non-NMDA excitatory amino acid receptor in *Xenopus* oocytes and that AMPA and L-BOAA are partial or desensitizing agonists at these sites.

33.15

CHARACTERIZATION AND DISTRIBUTION OF GLUTATHIONE RECEPTORS AND GLUTATHIONE UPTAKE IN VISUAL CORTEX

S. Bowlsby and C. Shaw, Depts. of Ophthalmology and Physiology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Glutathione (GSH) has been suggested to be an excitatory-amino-acid (EAA) neurotransmitter candidate in the CNS (Ogita and Yoneda, 1987). We have attempted to characterize GSH receptors and examine their distribution in rat and cat primary visual cortex using *in-vitro* radioligand methods on 20-um-thick sections. Saturation binding of radiolabeled GSH in rat primary occipital cortex sections revealed a high-affinity site (Kd = 5.4 +/- 0.8 nM; Bmax = 235 +/- 16 fmol/mg) and a much denser low-affinity site (Kd = 1.25 +/- 0.3 uM; Bmax = 1.26 +/- 0.7 pmol/mg), the latter in the affinity range for EAAs. Kinetic experiments yielded similar Kds. Displacement studies of the EAA-range GSH binding showed a separate GSH site as well as binding with affinity for the EAA-neurotransmitter candidates cysteine, aspartate, and glutamate. The only EAA-subtype specificity shown was for AMPA. Radiolabeled GSH binding in adult rat visual cortex showed relatively uniform distribution across all cortical layers. Characterization and distribution studies in cat gave similar results with the exception that radiolabeled GSH binding was densest in layer 4 at 13 and 32 days of age. *In-vivo* microinjection of radiolabeled GSH and of its primary metabolites into area 17 of rat and cat produced uptake to visual-system thalamic nuclei. The presence of GSH receptors in visual cortex and GSH uptake to the LGN may suggest a role for GSH as a geniculo-occipital neurotransmitter.

33.16

RELEASE OF GLUTATHIONE UPON DEPOLARIZATION OF RAT BRAIN SLICES. L. Zängerle*[1], M. Cuénod[1], K. Winterhalter*[2] and K.Q. Do[1]. [1]Brain Res. Inst., Univ. of Zürich, CH-8029 Zürich ; [2]Laboratory for Biochemistry I, ETH-Zentrum, CH-8092 Zürich, Switzerland

The functional roles of the abundant tripeptide glutathione (-glutamyl-cysteinyl-glycine) are still undefined in CNS. It has been shown to inhibit the binding of glutamate to the NMDA recognition sites (Yoneda et al., 1990). Moreover, the NMDA responses were sensitive to redox agents (Aizeman et al. 1989). We investigated the release of reduced and total (reduced + oxidized) glutathione from brain slices upon 50mM K^+-depolarization. Superfusates were derivatized with iodoacetic acid and o-phthaldehyde and analyzed by HPLC. The stimulated efflux of total glutathione was most prominent in cortex (3.9 pmol/mg protein/min; 3.0 x increase compared with resting efflux) and mesodiencephalon (4.0; 2.8 x), followed by striatum (2.6; 2.7 x) and hippocampus (2.6; 2.5 x), and lowest in medulla-pons (2.0; 2.4 x) and cerebellum (2.2; 1.8 x). The Ca^{++}-dependency of these increases varied from 90% to 60%. A large part of the endogenous glutathione released was detected in the reduced form (85-100%). Taken together, our results suggest a role for glutathione in synaptic transmission, possibly in the redox modulation of receptors and channels.

33.17

CONANTOKINS, LIGANDS FOR THE NMDA RECEPTOR. L. J. Cruz, R. A. Myers*, J-F. Hernandez*, J. Torres*, J. Rivier* and B. M. Olivera. Dept. of Biology, Univ. Utah, Salt Lake City, UT 84112; Marine Science Inst., Univ. Philippines, Diliman, Q. C. 1101; Salk Inst., La Jolla, CA 92057.

Conantokins, the first neuroactive peptides found to contain γ-carboxyglutamate (Gla), specifically inhibit glutamate receptors of the NMDA receptor subtype in several higher vertebrate systems. The conantokins isolated so far do not contain the multiple disulfide bonds characteristic of most *Conus* peptides; instead they have 4 conserved residues of Gla. An analysis of the natural peptides, as well as various analogs and derivatives suggest at least three structural motifs for full conantokin activity: the Gly-Glu residues at the amino end, an α-helical region stabilized by Gla residues, and the presence of positive charge(s) in the carboxyl terminal region.

One striking feature of these peptides is that they induce sleeping in 2-week old mice and cause hyperactivity in older mice. In the systems tested so far, such as *Xenopus* oocytes (Hammerland et al., *Neurosci. Abstr.*, 16: 540, 1990), rat cerebellar slices (Mena et al., *Neurosci Letters* 118:241, 1990), and rat cerebellar granule cells in culture (Haack et al., J. *Biol. Chem.* 265: 6025, 1990), conantokins act as antagonists of the NMDA receptor. However, the hyperactivity induced in older mice suggests that the peptides may also have some agonist effects in other systems. (Supported by NIH grants GM22737 and NS 27219.)

33.18

CONANTOKINS AND THEIR INTERACTION WITH THE NMDA RECEPTOR R. A. Myers*, J. Haack, J. S. Imperial*, T. Parks and B. M. Olivera. Depts. of Biology and Anatomy, University of Utah, Salt Lake City, UT 84112.

The conantokins, peptides from *Conus* venoms (McIntosh et al. (1984) *J. Biol. Chem.* 259: 14343) reported to target the N-methyl-D-aspartate (NMDA)-subtype of glutamate receptor (Olivera et al. (1990) *Science* 249: 217-332), undergo a Ca^{++}-dependent transition to a tight α-helix, which binds phospholipid membranes. The conantokins apparently interact with the receptor's NMDA agonist site as evidenced by displacement binding of [^{3}H]CGP-39653 (apparent K_i=3 μM) of postsynaptic densities (PSD). A similar interaction with the NMDA site has been demonstrated by monitoring NMDA-induced Ca^{++} entry into cerebellar granule cells by fura-2 fluorescence. Using this assay, no conantokin inhibition is observed on the co-agonist effects of glycine, nor do conantokins modulate the inhibitory effects of 7-chlorokynurenic acid. Saturating levels of conantokin do not completely abolish NMDA-induced Ca^{++} influx suggesting that conantokin induces an altered, but incompletely closed state of the receptor.

Photoaffinity and divalent crosslinking of synthetic radioiodinated conantokin analogs to PSDs consistently label electrophoretic bands with M_rs of 50-60 kd; size-exclusion HPLC suggests that these are part of a much larger complex. We are attempting purification of the putative complex using affinity chromatography and other methods. (Supported by GM22737. We thank Natural Product Sciences, Salt Lake City, UT, for use of the fura-2 assay system.)

33.19

NOVEL KAINATE DERIVATIVES: THEIR CHARACTERISTIC POTENT EXCITATORY ACTIONS IN THE RAT. M. Ishida and H. Shinozaki. The Tokyo Metro. Inst. Med. Sci., Tokyo 113, Japan.

Some kainate agonists have been available for pharmacological examinations. Recently we have found another kainoid, acromelic acid A, which is a more potent kainate-type agonist than kainic acid or domoic acid. Systemic administration of acromelate shows quite different behavioral signs and distribution of neuron damage from that of kainate in the rat. This finding prompted us to search for new powerful kainoids, and we obtained several new compounds. Among them, a methoxyphenyl kainate derivative (MFPA) showed a higher depolarizing activity than acromelic acid in the newborn rat spinal motoneuron, and depolarized isolated dorsal root fibers more significantly than kainate. In addition, the MFPA-induced depolarization was reduced by CNQX in a dose dependent manner, and MFPA and kainate induced cross desensitization of receptors on the isolated dorsal root fiber, suggesting that MFPA is a kainate-type agonist. This compound demonstrated behavioral signs in common with both kainate and acromelate in the rat. The discovery of potent kainoid compounds that are capable of producing marked depolarization in the CNS would provide a variety of new opportunities for neurobiologists, for example, classification of supposed subtypes of kainate receptors.

GABA RECEPTORS: STRUCTURE

34.1

USE OF SUBUNIT SPECIFIC ANTIBODIES TO DEFINE $GABA_A$ RECEPTOR SUBTYPE STRUCTURE AND PHARMACOLOGY P. Whiting, K. Quirk*, N. Gillard*, P. Cox*, R. Prince*, C.I. Ragan* and R.M. McKernan*. Biochemistry Department, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Eastwick Road, Harlow, Essex, CM20 2QR, U.K.

The $GABA_A$ receptor gene family consists of multiple forms of homologous subunits which are proposed to co-assemble to form multiple receptor subtypes in the brain. The subunits have high levels of sequence identity apart from the putative cytoplasmic loop domain between hydrophobic domains M3 and M4. These putative cytoplasmic loop domains have been expressed in bacteria and the recombinant protein used as immunogen to produce subunit specific antisera. Using these antisera as biochemical probes we have been able to define the benzodiazepine pharmacology of immunopurified native receptor subtypes. Additionally, we have immunopurified $GABA_A$ receptors using these antibodies and by western blot analysis we have begun to define which subunits co-assemble to form receptor subtypes.

34.2

GABA C RECEPTORS?: UNUSUAL PHARMACOLOGIC PROFILE AND HOMO-OLIGOMERIC EXPRESSION OF RETINA-ENRICHED RECEPTOR GABA RHO 1. G.R. Uhl, G.R. Cutting*#, S. Shimada, B. O'Hara, D. Donovan, and S. Kitayama*. Lab. Mol. Neurobiol., NIDA/ARC, Depts. of Neurol., Nsci. & #Peds., Ctr. Med. Gen., JHU Sch Med, Box 5180, Balto., MD 21224.

GABA A receptors are defined as GABA and bicuculline-sensitive ligand-gated chloride channels that are formed from combinations of alpha, beta, gamma, delta, and perhaps other subunits. Recently, we have used polymerase chain reaction techniques to identify a novel GABA receptor, GABA RHO 1, which is expressed at high levels in retina, and which displays more sequence divergence from the other receptor subunits then they display from each other.

This receptor robustly expresses GABA-gated chloride conductance when synthetic mRNA transcribed from the cDNA clone is injected into Xenopus oocytes. This conductance is potently blocked by picrotoxin. Neither bicuculline, pentobarbital, baclofen, nor benzodiazepines influence GABA-mediated current flows. Johnston and co-workers have suggested that bicuculline/baclofen-insensitive GABA receptors should be called "GABA C." We discussed the advantages and disadvantages of describing the cDNA as a GABA A receptor subtype, or as a prototype of a new class of GABA receptors. Inability to modify bicuculline insensitivity with co-expression of alpha and beta subunits underscores these points.

34.3

ULTRASTRUCTURAL IMMUNOLOCALIZATION OF $GABA_A$ RECEPTOR COMPLEX IN THE RAT THALAMUS. R. Spreafico 1, A. Amadeo 1*, S. De Biasi 2, A.L. de Blas 3
1 Dept Neurophysiology, Neurological Institute, Milano Italy; 2 Dept Gen Physiol & Biochem, University of Milano, Italy; 3 Div Mol Biol & Biochem, UMKC, Kansas City, MO 64110 USA

The subcellular localization of the $GABA_A$ receptor/benzodiazepine receptor/chloride channel complex ($GABA_AR$) was investigated in the rat thalamus by means of pre-embedding immunocytochemistry using the monoclonal antibody 62-3G1 (de Blas et al, 1988) directed to the β_2 and β_3 subunits of the $GABA_AR$. LM observation of vibratome sections showed that the immunoreactivity (IR) for $GABA_AR$ is expressed by neuronal processes throughout the dorsal thalamus, with regional differences. The EM investigation was performed on the anteroventral, central lateral, paraventricular nuclei and ventrobasal complex. In all the nuclei examined, $GABA_AR$ IR was observed along the membranes of a neuronal somata and dendrites, both at synaptic and non-synaptic sites. Labelling was intense close to synaptic junctions with terminals containing flattened vesicles, but absent from synapses made by large size terminals. The IR was also present in few endoplasmic reticulum cisternae and along the nuclear envelope of thalamic neurons. Myelinated fibers and the membrane of glial cells were always unlabeled. The results are in agreement with the subcellular distribution of $GABA_AR$ observed in the cat lateral geniculate nucleus using a different antiserum (Soltesz et al, 1990) and correlate with the intense GABAergic innervation of the investigated nuclei.

34.4

BINDING OF [^{3}H]RO 15-4513 TO CEREBELLAR MEMBRANES: ONTOGENY AND SUBSTRATE SPECIFICITY. M. Uusi-Oukari, E.R. Korpi, J. Kaivola', K. Wegelius'. Res. Labs, Alko Ltd., POB 350, SF-00101 Helsinki, and Dept. of Biomed. Sci., Univ. of Tampere, Tampere, Finland.

Cerebellar granule cells express a new $GABA_A$ receptor subtype that has poor affinity to classical benzodiazepine agonists. Its preferred ligand is an imidazobenzodiazepine, [^{3}H]Ro 15-4513 (sapmazenil), a partial inverse agonist.

Micromolar concentrations of diazepam displaced all bound [^{3}H]Ro 15-4513 from cerebellar membranes prepared from 1-to-2-day-old rats, whereas about 30% of the bound ligand was not displaced in 14-day-old and adult rats. Correspondingly, the saturable binding of [^{3}H]Ro 15-4513 in the presence of 10 µM diazepam was greatest to membranes from adult rats.

Binding studies with cerebellar membranes from alcohol-sensitive (which have enhanced diazepam affinity) and alcohol-insensitive rats, using benzodiazepine receptor ligands of various chemical structure and agonist, partial agonist, antagonist, and inverse agonist properties, indicated that most of the ligands with high affinity to the "diazepam-insensitive" [^{3}H]Ro 15-4513 site belonged to inverse agonists and antagonists. Only some of the partial agonists had equal affinity in both rat lines.

The results suggest that the expression of diazepam-insensitive [^{3}H]Ro 15-4513 binding sites begins after the cerebellar granule cells have moved to the internal granule cell layer. Further work is needed to establish the behavioral correlates of this $GABA_A$ receptor-associated binding site with unique substrate specificity.

34.5

IMMUNOAFFINITY PURIFICATION OF $GABA_A$ RECEPTOR α SUBUNIT ISO-OLIGOMERS. M.J. Duggan*, S. Pollard* and F. A.Stephenson, School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK.

Characterisation of cloned $GABA_A$ receptors has shown that the type of α subunit present in ($\alpha\beta2\gamma2$) expressed proteins confers the subtype of benzodiazepine receptor pharmacology (D B Pritchett et al., 1989, Science, 45, 1389). In agreement with this, we have previously shown by quantitative immunoprecipitation assays using antibodies specific for the $\alpha1$, $\alpha2$ and $\alpha3$ subunits of the $GABA_A$ receptor, evidence for the existence of native α variant iso-oligomers (Duggan and Stephenson,1990, J.Biol. Chem. 265, 3831). The sensitivity of these studies however was such that we could not exclude the possibility that in a minor population of receptors, the $\alpha1$, $\alpha2$ and $\alpha3$ subunits may coexist. We have now addressed this question by the development of $GABA_A$ receptor α subunit isoform immunoaffinity chromatography purification procedures. Thus $GABA_A$ receptors were purified from Na^+ deoxycholate extracts of bovine cerebral cortex by either anti-$\alpha1$ 324-341; anti-Cys $\alpha2$ 414-424 or anti-Cys $\alpha3$ 454-467 antibody affinity chromatography. The respective purified receptors were characterised by their ligand binding properties and by immunoblotting where each was compared to benzodiazepine affinity chromatography purified $GABA_A$ receptors. It was found that whereas the type of α subunit used for purification was enriched in the respective immunoaffinity purified material reactivity with the other subunit antibodies was also obtained. The use of two different specificity antibody affinity columns in series further substantiated these findings of co-existence. Thus we propose that although the predominant forms of native $GABA_A$ receptors contain one type of α subunit only, some classes are heterogeneous with respect to their α subunit complements.

This work was supported by the Medical Research Council (UK).

34.6

CORRELATION OF $GABA_A$ RECEPTOR mRNA SUBUNIT EXPRESSION AND SINGLE CHANNEL KINETICS IN THE DEVELOPING RAT CENTRAL NERVOUS SYSTEM. Poulter M.O.[1,3], A.-M. O'Carroll[2], L.C. Mahan[2], S.J. Lolait[2], and Barker J.L.[1] 1) Laboratory of Neurophysiology NINDS 2) Laboratory of Cell Biology, NIMH, NIH Bethesda, Maryland 20892. 3) McGill University, Montreal, Canada, H3G 1Y6.

Using *in situ* hybridization histochemistry the distribution of $GABA_A$ receptor subunit mRNAs was studied in the developing rat CNS. DNA oligonucleotide probes were utilized which hybridize to the mRNA sequences coding for the proposed cytoplasmic loop domains of the $\alpha_{1\text{-}4 \text{ and } 6}$, $\beta_{1\text{-}3}$, γ_2 and δ subunits. In particular, at embryonic day 15 in the cervical spinal cord region (myelencephalon) only α_3, $\beta_{2,3}$ and γ_2 subunit mRNAs could be detected. Based on these results we characterized the single channel kinetics of $GABA_A$ receptor coupled ion channels containing this relatively constrained subunit composition. Using acutely dissociated neurons from this region and on-cell patch clamp recording technique, ion channel activity could be detected in approximately 70-80% patches containing GABA. This activity could first be recorded at a threshold dose of 200 nM GABA. No apparent desensitization was observed in patches containing up to 5µM GABA. Channel currents were inward at patch pipette potentials less negative than -20 to -30 mV. Channel open and closed time duration frequency distributions could be fitted with up to 3 time constants. Brief (τ < 1.0 ms) channel openings predominated at all GABA concentrations whereas the frequency of opening increased with GABA concentration. This approach may therefore be an useful strategy for the correlation of $GABA_A$ receptor subunit composition and its ion channel properties.

34.7

DISTRIBUTION, RELATIVE ABUNDANCE AND CO-LOCALIZATION OF GENE TRANSCRIPTS FOR DIVERSE $GABA_A$ RECEPTOR SUBUNITS IN NEURONS OF RAT SPINAL CORD AND SPINAL GANGLION. E. Persohn*, P. Malherbe*, J.R. Martin and J.G. Richards, Pharma Division, Preclinical Research, F.Hoffmann-La Roche Ltd, CH-4002 Basel, Switzerland

Gene transcripts of diverse rat $GABA_A$ receptor subunits (α1-6; β1-3; γ1,2; δ) were localized by in situ hybridization histochemistry using 35S-labelled 60mer oligonucleotide probes. The $\gamma2$ mRNA was the most ubiquitous and abundant; $\alpha6$, $\beta1$ and δ were not detected. The labelling of motoneurons in layer IX was particularly strong for $\alpha2$, moderate for $\beta3$ and $\gamma2$ and weak for $\alpha1,3$ and 5. In layers VII, VIII and X the $\beta3$ and $\gamma2$ transcripts were moderately expressed whereas the $\alpha1$ and $\beta2$ transcripts levels differed markedly among the cells of these layers. Although the mRNAs of all subunit variants could be detected in layers IV-VI, only $\alpha3$, $\alpha5$, $\beta3$ and $\gamma2$ hybridization signals were observed in layers II and III. In spinal ganglia, $\alpha2$ transcripts were abundant in most large sensory neurons and to a much lower degree in the small diameter cells, whereas $\gamma1,2$ transcripts were confined to a subpopulation of large and small neurons. $\beta2$ and $\alpha1$ transcripts were even more restricted in their distribution. Although the subunit composition and stoichiometry of the native receptor protein(s) are not known, the cellular colocalization of $\alpha2$ $\beta3$ $\gamma2$ subunits suggests one likely combination. The findings provide a basis for the mediation of synaptic inhibition in the spinal cord by diverse $GABA_A$ receptors and further strong evidence that presynaptic inhibition of inter- and motoneurons, via axo-axonic synapses between GABAergic interneurons and primary afferent terminals, is mediated by $GABA_A$ receptors.

34.8

EXPRESSION OF $GABA_A$ RECEPTOR SUBUNIT mRNAs IN ETHANOL WITHDRAWAL SEIZURE PRONE (WSP) AND RESISTANT (WSR) MICE: ANALYSIS BY POLYMERASE CHAIN REACTION (PCR). K.J. Buck, L.D. Hahner', J.M. Sikela and R.A. Harris. University of Colorado Health Sciences Center, Denver, CO 80262, USA.

Molecular cloning has revealed that the $GABA_A$ receptor complex is composed of multiple subunits, and that the drug sensitivity of the complex depends upon its subunit composition (FASEB J. 4: 1469, 1990). Our recent studies indicate that modulation of the $GABA_A$ complex by benzodiazepine inverse agonists may be related to genetic differences in ethanol withdrawal severity (J. Neurochem., in press). In the present studies, levels of $GABA_A$ receptor subunit mRNAs were determined using the polymerase chain reaction. WSP and WSR mice were fed a liquid diet containing ethanol, or an equicaloric diet with sucrose substituted for ethanol, for 10 days. Poly(A)$^+$ mRNA was then isolated from mouse whole brain. Relative differences in accumulated mRNA levels of $GABA_A$ receptor subunits were analyzed by a reverse transcriptase-polymerase chain reaction (RT-PCR) assay using degenerate oligonucleotide primers recognizing conserved domains, and Southern hybridization with subunit-specific [^{32}P]oligonucleotide probes. In pair-fed controls, levels of α_5 and α_6 were 50% lower in WSP than in WSR mice. Chronic ethanol administration decreased α_1 mRNA levels by 40% in WSP mice, but did not significantly decrease α_1 mRNA levels in WSR mice. In contrast, α_6 mRNA levels were decreased by 50% in WSR, but not WSP mice. Chronic ethanol exposure increased expression of γ_2 mRNA by 100% in both WSP and WSR mice, but levels of γ_2L, γ_2S and α_5 mRNA were not changed. These results indicate that genetic differences in the regulation of α_1 subunit expression by chronic ethanol exposure may affect withdrawal severity. (Supported by the VA and AA06399 and AA03527.)

34.9

THE COMPLEMENT OF GABA-A RECEPTOR mRNAs IN RAT PITUITARY
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We have defined the complement of GABA-A receptor mRNAs in the anterior and intermediate lobes of the rat pituitary. In the anterior lobe, GABA receptors are composed of α1, α2, α5, β1, and β3, subunits as demonstrated by ribonuclease protection assays, and β2,γ1,and γ2 as shown by PCR. In the intermediate lobe, we find α2,α3, α5, β1, and β3 subunits by protection assay, and have found β2, γ1, γ2,and δ by PCR. α6 was not found in either lobe, in keeping with its presumed cerebellar localization. Both α and β subunit mRNAs are present at fairly low levels. The α1 mRNA in anterior lobe is of considerably higher abundance than the others; this finding parallels results obtained in the brain where α1 is typically seen to be the most abundant of these mRNAs. It is of interest that anterior lobe cells which recieve only a diffuse GABAergic innervation contain subunits associated with high affinity BZD Type I pharmacology, while melanotrophs receiving direct but non-synaptic innervation express BZD Type II associated subunits. The α5 subunit may account for spontaneous chloride currents previously observed in melanotrophs. (Taleb et al. Pflügers Arch,409) The occurence of both γ and δ subunits in melanotrophs implies that these cells may have a dual population of GABA receptors, consisting of BZD responsive and BZD non-responsive subsets. Studies are underway to test these predicted properties.

34.10

$GABA_A$ RECEPTOR SUBUNIT mRNA EXPRESSION IN THE DEEP CEREBELLAR NUCLEI OF THE PURKINJE CELL DEGENERATION MUTANT (Pcd) IS UNALTERED BY PURKINJE CELL LOSS.
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The $GABA_A$ receptor complex mediates the actions of GABA, the major inhibitory neurotransmitter in the mammalian CNS, as well as the actions of the benzodiazepines and barbiturates. The receptor is composed of multiple subunits and appears to exist in distinct compositions in different brain regions. While the signals regulating receptor expression are unknown, recent developmental studies have raised the possibility that synaptic interactions influence subunit mRNA levels in the cerebellum. To address this possibility, we have examined $GABA_A$ receptor subunit mRNA expression in the Pcd mutant. In these animals innervation to cells in the deep cerebellar nuclei is lost following the postnatal degeneration of Purkinje neurons. Our studies using hybridization histochemistry demonstrate that the mRNAs encoding the α1, β2, and γ2 subunit mRNAs of the $GABA_A$ receptor are present in the deep cerebellar nuclei of both the Pcd mutant and littermate controls. A 50% decrease in the level of α1 subunit mRNA is observed in the mutant soon after Purkinje cell loss (P24), but it returns to control levels at later stages (P50-90). In contrast, β2 and γ2 mRNA levels are similar in mutants and controls at all ages. These studies suggest that the maintenance of synaptic interactions is not required for $GABA_A$ receptor subunit mRNA expression in cells of the deep cerebellar nuclei.

34.11

DEVELOPMENTAL EXPRESSION AND MODULATION OF $GABA_A$ RECEPTOR SUBUNIT mRNAs IN RAT CEREBELLAR CELL CULTURES.
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Since the isolation of multiple $GABA_A$ receptor subunit mRNAs, much has been learned about receptor composition and function. In contrast, very little is known about the regulation of the expression of these subunit mRNAs in the mammalian CNS. Earlier studies in our laboratory have raised the possibility that cell-cell interactions may regulate subunit mRNA expression in the developing cerebellum. To test this possibility we established cultures of cerebellar granule and Purkinje neurons prepared from embryonic day 19 rats. $GABA_A$ receptor subunit mRNA levels were analyzed by the polymerase chain reaction with subunit specific primers. In granule cell cultures the α1 subunit mRNA was not detected at 4 hours but was present after 1 day *in vitro* (DIV). The γ2 subunit mRNA, however, was detected at 4 hours and was expressed at a similar level at 1 DIV. Both subunit mRNA levels remained constant from 1-21 DIV with γ2 being 4~6-fold more abundant than α1 at all times. To determine if activity could alter subunit mRNA expression in culture, granule cells were grown in depolarizing conditions (25mM KCl) for 14 days. Under these conditions α1 subunit mRNA expression was not significantly altered. Our findings *in vitro* differed from previously characterized patterns of expression *in vivo* where α1 and γ2 subunit mRNA levels increased 5-fold during the second postnatal week. These results are consistent with the hypothesis that interactions which normally regulate subunit expression have been disrupted in the culture system. Studies to analyze other cell-cell interactions which may influence $GABA_A$ receptor mRNA expression are underway.

34.12

PCR QUANTITATION OF $GABA_A$ RECEPTOR SUBUNIT mRNA CONTENTS IN RAT BRAIN AND IN CEREBELLAR GRANULE CELL CULTURES.
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The $GABA_A$ receptor is a heteropolymeric protein complex including a variable combination of different subunits (α, ß, γ, δ). In order to compare the expression of $GABA_A$ receptor subunit mRNAs in selected brain regions or cultured cells, we performed PCR in the presence of appropriate internal standards to provide a quantitation of the amplified signal. Total RNA was reverse transcribed and co-amplified with the internal standard which utilizes the same primers and has virtually the same sequence as the natural template, except for an internal restriction site created by site directed mutagenesis. We found that the amounts of α1 mRNA present in the cortex and cerebellum of adult rat are very similar (2.6 versus 2.1 fmoles/μg RNA). In cortex, the α4 mRNA abundance is about 1/10 and in cerebellum is less than 1/100 that of α1. In cerebellum we also monitored the changes in α1, α4 and γ2 (both the long, γ2L, and the short, γ2S, forms) mRNA contents during postnatal development. Their abundance changes with development and appear related to the changes occurring in granule cells developing in culture. Both the *in vitro* and *in vivo* expression of the α1, γ2L and γ2S mRNAs are similar in their temporal profiles and absolute amounts (α1 increases from about 0.1 to 2.5 fmoles/μg RNA). Postnatally, in cerebellum α4 mRNA continually decreases (from about 0.085 to 0.013 fmoles/μg RNA), while in cerebellar granule cells the change is biphasic: an initial increase is followed by a decrease. $GABA_A$ receptor subunits and, presumably, subtypes appear to be developmentally regulated in their abundance in brain structures and single neuronal cells.

34.13

CEREBELLAR GRANULE CELL AND GLIAL CELL CULTURES EXPRESS DIFFERENT PATTERNS OF $GABA_A$ RECEPTOR SUBUNIT mRNAs.
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The $GABA_A$ receptor is a heteropolymeric integral membrane protein that includes an anionic channel. Four different classes of subunits have been defined: α, β, γ, and δ and multiple mRNA variants of each class exist. To determine the mRNA levels of $GABA_A$ receptor subunits expressed in cerebellar granule cell and glial cell cultures, we used a variation of the polymerase chain reaction (PCR). Subunit specific oligonucleotides were used to amplifiy reverse transcribed mRNA isolated from each culture. In cerebellar granule cells maintained in culture for 8 days, the amount of the α1 mRNA subunit appears to be 5 times higher than that present in cerebellar glial cells prepared from the same rats and kept in culture until they reached confluence. The α3 and β3 subunit mRNAs are 10 fold more abundant in granule cell cultures, also the α4, β1, and δ subunit mRNAs content is from 15 to 20 times higher in granule cells than in glial cells. The α2 and β2 mRNA abundances in cerebellar cells are about 2 to 3 times that of glial cells. Comparable amounts of the γ1 subunit mRNA are expressed in both cultures; in contrast, in cerebellar glial cells the α6 and γ2 subunit mRNAs are not detectable for up to 40 PCR cycles, using from 0.5 to 1.5 μg glial cells total RNA.

Presently we are employing quantitative PCR, with the use of internal standards to determine the absolute amounts of different subunits in each culture type. Our results suggest that both cerebellar granule cells and cerebellar glial cells can express different $GABA_A$ receptor subunits, these differences support the inference that each cell type may include different $GABA_A$ receptor assemblies.

34.14

A QUANTITATIVE PCR ASSAY FOR $GABA_A$ RECEPTOR SUBUNITS.
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Northern hybridization has been widely used to assess mRNA concentrations, but it is difficult to quantify messages present in low abundance or of high molecular weights using this technique. We have developed a quantitative RNA-based polymerase chain reaction (PCR) assay to evaluate a number of $GABA_A$ receptor subunit mRNAs. Primer sequences were chosen from nonhomologous regions of 7 rat subunit cDNA sequences (alpha1-2, beta1-3, gamma1-2). Each primer set was demonstrated to produce correct size amplified fragments. For internal standards, synthetic RNA of differential length for individual subunit were prepared from plasmid constructs that contain each primer set plus poly A sequences. Total RNA of different regions of mouse brain were mixed with synthetic RNAs and complementary first strand DNAs were synthesized by reverse transcriptase. Amplification was performed in the presence of 5'-end-^{32}P labeled primers followed by gel electrophoresis, and bands of appropriate amplified fragments were excised and counted. Using 5 μg of total RNA and 5, 15, 25 pg of synthetic RNA, amplification was demonstrated to be exponential up to 30 cycles for all subunits. Using the slopes of the log-linear function of internal standards and of sample RNA (amplification efficiency), absolute amount of each subunit can be calculated. This technique offers a quantitative method to evaluate mRNA concentrations for $GABA_A$ receptor subunits.

34.15

PHARMACOLOGICAL CHARACTERIZATION OF THE $GABA_A$ RECEPTOR-CHANNEL COMPLEX COMPOSED OF RAT BRAIN α_1 AND β_2 SUBUNITS.

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The cloned rat brain α1 and β2 subunits of the $GABA_A$ receptor-channel complex were co-expressed in cultured fibroblasts and characterized utilizing whole-cell recording techniques with patch electrodes. The current-voltage curves of the passive membrane properties were linear and the baseline current fluctuations were fit by a 1/f function. The reversal potential of the current activated by the application of GABA corresponded to the reversal potential for Cl^- ions, and depended on the $[Cl^-]$ across the membrane. The GABA-activated currents had a K_d of $7x10^{-6}$ M, and were blocked by bicuculline and potentiated by (-)pentobarbital. Surprisingly, (-)pentobarbital didn't exert an effect of its own at 100 μM. Alphaxalone was relatively ineffective at 10-100 μM and GABA-activated currents were not modulated by 10 μM diazepam. The open times of the channels activated by either GABA, muscimol or alphaxalone were complex, but similar when evaluated using spectral techniques.

34.16

DEVELOPMENTAL CHANGES IN POLYADENYLATED VS TOTAL mRNA FOR THE $GABA_A$ RECEPTOR β1, α1 and γ2 SUBUNITS IN RAT.

DW Gallager and RJ Primus. Dept. of Psychiatry, Yale University, New Haven, CT 06508.

The ratio of polyadenylated (poly(A+)) to non-polyadenylated mRNA for the β1, α1 and γ2 subunits of the $GABA_A$ receptor complex was examined in rats as a function of age. While others have reported that levels of poly(A+) mRNA for the $GABA_A$ β1 subunit decrease with increasing age, preliminary evidence from this laboratory suggested that levels of total mRNA for this subunit did not change as a function of age. Therefore, a comparison of total vs poly(A)+ mRNA for the $GABA_A$ receptor β1, α1 and γ2 subunits was made in rat brain membranes as a function of age.

RNA was extracted from whole brain of rats that were either 0, 1, 3, 5 or over 60 days of age. Poly(A)+ RNA was purified by oligo(dT)-cellulose chromotography. Total and poly(A)+ mRNA for the β1, α1 and γ2 subunits were examined by Northern blot analysis using cDNA probes specific for these subunits. Levels of β1 subunit mRNA were also examined by solution hybridization with a β1 riboprobe. Analysis of Northern blots revealed that levels of poly(A)+ β1 subunit mRNA were highest at 0 days of age, but decreased and reached adult levels by 5 days of age. In contrast, levels of total mRNA for the β1 subunit were not significantly different at any of the ages examined, suggesting the existence of a population of β1 subunit mRNA that is not polyadenylated. This discrepancy between total and poly(A)+ β1 subunit mRNA expression was also observed using solution hybridization analysis. In contrast, levels of both poly(A)+ and total α1 subunit mRNA increased by nearly 95% from 0 days of age to adulthood. Similarly, levels of both poly(A)+ and total γ2 subunit mRNA increased by approximately 25% from 0 days of age to adulthood.

These findings demonstrate that 1) polyadenylation of RNA for the $GABA_A$ receptor β1, α1 and γ2 subunits is developmentally regulated, and 2) polyadenylated RNA for the β1 subunit is not representative of the entire RNA population for this subunit, as reflected by the lack of developmental change in levels of total mRNA for the β1 subunit. While the significance of a non-polyadenylated population of β1 subunit mRNA remains unknown, it is clear that both total and poly(A)+ RNA should be examined when evaluating developmental changes in receptor subunit expression.

34.17

$GABA_A$ RECEPTOR SUBUNIT mRNA EXPRESSION: DIFFERENTIAL REGULATION BY CHRONIC FG 7142 AND DIAZEPAM EXPOSURE IN RAT.

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Levels of mRNA for the α1, γ2 and β1 subunits of the $GABA_A$ receptor complex were examined in rats maintained on a chronic, continuous schedule of exposure to the benzodiazepine inverse agonist FG 7142. Recent evidence indicates that chronic diazepam (DZ) exposure downregulates $GABA_A$ α1 subunit mRNA, but does not change β1 subunit mRNA, in rat cortex. The present study was undertaken in order to determine if chronic FG 7142 exposure also influences levels of $GABA_A$ subunit mRNA. The effect of chronic DZ exposure on γ2 subunit mRNA was also examined.

FG 7142 (2 mg/ml of 100% DMSO) was administered continuously for eight days in the right lateral ventrical via an osmotic minipump (Alzet). At the end of the eighth day of exposure, cortex (CX), cerebellum (CB) and hippocampus (HP) were dissected and stored at -70°C until extraction of RNA. $GABA_A$ α1 and γ2 subunit mRNA was examined by Northern blot analysis with cDNA probes specific for these subunits. A significant increase in α1 subunit mRNA was measured in both CX and HP, but not in CB, of rats chronically exposed to FG 7142 relative to vehicle-treated rats. Likewise, a significant increase in γ2 subunit mRNA in CX was also evident in drug-treated rats; however, no change in γ2 subunit mRNA was observed in either the HP or CB. Examination of $GABA_A$ β1 subunit mRNA using a β1 riboprobe revealed no effect of chronic FG 7142 treatment on this subunit in either CX, CB or HP. In rats chronically exposed to DZ (21 days via silastic implants), levels of γ2 subunit mRNA were significantly decreased in CX, but not changed in either CB or HP.

The findings demonstrate that $GABA_A$ α1 and γ2 subunit mRNA is differentially regulated by chronic FG 7142 and DZ exposure. These two chronic treatments result in opposite functional effects on GABA sensitivity in some brain regions (subsensitivity following DZ, supersensitivity following FG 7142). Thus, the increase and decrease in subunit mRNA by FG 7142 and DZ, repectively, may underlie or at least reflect a compensatory response of the $GABA_A$ receptor complex to chronic occupation by these ligands.

34.18

MODULATION OF CLONED $GABA_A$ RECEPTOR CURRENTS BY CHRONIC EXPOSURE TO ELEVATED LEVELS OF THE FREE CATALYTIC SUBUNIT OF PROTEIN KINASE A.

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cDNAs encoding the bovine α1, β1, and human γ2 (short form) $GABA_A$ receptor subunits were subcloned separately into the vector pCMVNeo, which utilizes the cytomegalovirus promoter to drive expression in mammalian cells. These constructs were co-transfected (1:1:1 ratio by weight) using the Chen-Okayama modified calcium phosphate technique into wild type L929 cells and the stable L cell clone Cα12. The Cα12 cell line produces free Cα catalytic subunit of protein kinase A (PKA) at a level five times higher than that found in wild type L929 cells.

GABA-evoked currents were recorded in L929 and Cα12 cells at 48 hrs. post-transfection. In both cell types, peak whole cell GABA (0.3-100 μM) concentration-response curves produced Hill slopes averaging 1.5-2.0 with maximal current amplitudes occurring at 30 or 100 μM GABA. 10 μM GABA currents were enhanced 40-60% by 50 nM diazepam. Two different types of currents were observed, based upon current-voltage relationships. One group exhibited current amplitudes that did not differ at -50 and +50 mV, while the other was larger at +50 mV than -50 mV. The presence of these two currents was independent of cell type. One difference between cell types was the overall magnitude of the GABA-evoked currents; GABA receptor currents expressed in Cα12 cells were 2-3 times larger than those expressed in wild type L929 cells. This finding suggests that chronic exposure to elevated levels of PKA in Cα12 cells may modulate the assembly or functional expression of the $GABA_A$ receptor complex.

34.19

MULTIPLE CONDUCTANCE STATES OF RECOMBINANT $GABA_A$ RECEPTORS.

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The conductance levels of GABA activated single channel chloride currents differ among species and brain regions. Multiple subunits and subunit variants of the $GABA_A$ receptor have been identified. Different combinations of these subunits may underlie the diverse conductance properties observed. We have examined the conductance properties of single channel chloride currents activated by GABA in mammalian cells expressing recombinant $GABA_A$ receptors.

COS or CV-1 cells were acutely transfected with $GABA_A$ receptor bovine α_1 or α_3 subunit cDNA in combination with bovine β_1 and human γ_2 (short form) subunit cDNA. Subunit cDNAs were subcloned individually into the vectors pSVL and pCMVNeo. Transfections were done according to the method of Chen and Okayama. Single channel recordings were made from excised outside-out patches. Cells expressing the $\alpha_3\beta_1\gamma_2$ receptor responded to GABA with single channel currents which opened to conductance levels of 11, 19 and 27 pS. Substituting the α_1 subunit for the α_3 subunit resulted in GABA activated single channel currents which opened to the same conductance levels and an additional 33 pS level. The 27 pS conductance state was predominant for both recombinant receptors. Single channel kinetic properties were similar between the recombinant receptors for the 19 pS level but differed for the 27 pS level. These results suggest that the α subunit confers different conductance properties on the receptor. These differences in conductance properties may be due to structural differences between the individual α subunits. Alternatively, the α subunit could affect the stoichiometry of assembly of the receptor.

34.20

GABA-A RECEPTOR SUBTYPES: COMPARISON OF REGIONAL VARIATION IN BINDING HETEROGENEITY WITH SUBUNIT SUBTYPE POLYPEPTIDES AND mRNAs.

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Heterogeneity was observed for 15 ligands of the GABA-A/benzodiazepine receptor across regions of rat brain measured by autoradiography of [^{3}H]muscimol, [^{35}S]TBPS and [^{3}H]flunitrazepam binding. Regional variation was seen in the effects of allosteric modulators and competitive inhibitors. The same ligands showed differential interactions with [^{3}H]flu or [^{3}H]mus photolabeled polypeptides in purified receptors on SDS-PAGE. Barbiturates and steroids selectively enhanced binding to 51 and 55 compared to 53 and 58 kDa polypeptides; GABA selectively enhanced [^{3}H]flu binding in 53 vs. 51 kDa band; THIP and taurine selectively inhibited [^{3}H]mus labeling of 58 and 55 kDa bands respectively. Partial sequencing and subunit subtype-selective antibodies identified polypeptides as α1(51), α2(53), β2(55) and β3(58) gene products. In agreement with photolabeling specificity, areas rich in α1 plus β2 mRNAs are more sensitive to allosteric modulators, as shown by autoradiography; areas rich in α2 show good GABA-BZ coupling, areas rich in β2 or β3 are more sensitive to taurine or THIP. Thus pharmacological subtypes based on binding heterogeneity exist in the brain, apparently corresponding to the nature of the α and β subunits present.

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34.21

CYCLIC AMP-DEPENDENT PROTEIN KINASE PHOSPHORYLATES THE β_3 SUBUNIT OF THE RAT GABA-A RECEPTOR. G.B. Smith[1], M.D. Browning[2], S. Endo[1], & R.W. Olsen[1]. Department of Pharmacology, UCLA School of Medicine,[1] Los Angeles, CA 90024 & Department of Pharmacology, University of Colorado Health Sciences Center,[2] Denver, CO 80602.

Cyclic AMP-dependent protein kinase A (PKA) and protein kinase C (PKC) phosphorylate two subunits of the GABA-A receptor with Mr of 58 and 56 kDa, respectively, comigrating with muscimol-binding β subunits (Browning *et al.*, PNAS 87:1315 (1990). Here we report that a major substrate polypeptide phosphorylated with PKA is the rat β_3 gene product. Purified rat receptor was phosphorylated with purified PKA catalytic subunit and [^{32}P]ATP as described. The labeled protein was concentrated and digested sequentially with *S. aureus* V8 protease and TLCK-chymotrypsin for a total of 48 hrs. Peptides were separated on a 16.5% SDS gel and transferred to PVDF membrane. Autoradiography revealed two major labeled peptides of 11 and 9 kDa. N-terminal sequence analysis of the 11 kDa band gave a 16 residue partial sequence beginning at Met$_{353}$ of the rat β_3 clone. This peptide contains a consensus sequence for PKA phosphorylation, although the sequence does not extend that far. Supported by NS26377 (MDB) and NS22071, NS28772 (RWO).

34.22

STRESSOR-INDUCED CHANGES IN GABA RECEPTOR FUNCTION: ROLE OF HYPOTHALAMIC NOREPINEPHRINE PROJECTIONS. C.K. Kellogg, M.T. Taylor*, and J. Inglefield Dept. of Psychology, Univ. of Rochester, Rochester, NY 14627.

Environmental stressors markedly influence function of the GABA/benzodiazepine (BZD) receptor complex in the cerebral cortex. While biologic mechanisms mediating this influence are unknown, adrenalectomy abolishes the influence of stressors, implicating a role for corticosterone (CS). Considering that the norepinephrine (NE) projection to the hypothalamus (HYP) modulates the HYP-pituitary-adrenal axis, we examined the impact of this system on stressor-induced changes in GABA/BDZ receptor function. NE terminals in the paraventricular nucleus of the HYP of male Long Evans rats (60 days old) were destroyed using stereotaxic injections of 6-hydroxydopamine (6-OHDA, 9 µg in 1.5 µl). Sham-operated rats received saline injections. Stressor-responses were evaluated two weeks post-lesion. Rats were subjected to either 15 min of restraint or 10 min of forced swimming. Basal measures were made on nonstressed animals. Chloride (Cl) facilitation of BDZ binding, an index of responsiveness of the receptor complex , was measured in the cerebral cortex. The loss of NE terminals was estimated by either analysis of NE levels or by immunocytochemistry. Trunk blood was collected for analysis of plasma CS. 6-OHDA treatment induced a 63% decrease in NE levels in the HYP, and this manipulation markedly altered responsiveness of the GABA/BDZ complex in the cerebral cortex. In control rats, Cl facilitated BDZ binding was enhanced by 15 min of restraint, with maximal facilitation increasing from 20% in the basal state to 36%. The EC50 for Cl was decreased by the stressor, from 42 mM to 28 mM. In contrast, there was little stress-related change in Cl facilitated BDZ binding in 6-OHDA-treated animals. Maximal facilitation was 19% and 13% in basal and stressor states, while the EC50 was 27 and 22 mM, respectively. These studies are beginning to define neural interactions essential for integrated responses to stressors. Supported by grant no. DA 07080.

CATECHOLAMINE RECEPTORS: α AND β

35.1

ISOLATION OF NOVEL G-PROTEIN COUPLED RECEPTOR cDNAs FROM LOCUS COERULEUS BY PCR. J.D. Alvaro[1], J.W. Lomasney[2],R.J. Lefkowitz[2], E.J. Nestler[1,3,4], and R.S. Duman[1,3]. [1]Laboratory of Molecular Psychiatry, [3]Depts. of Psychiatry and [4]Pharmacology, Yale Univ. School Med.,New Haven, CT 06508; [2]HHMI, Duke Univ. Med. Ctr., Durham NC 27710.

Previous cloning studies have identified three α_2-adrenergic receptors (α_2-c2, -c4, -c10), but it is unclear whether any if these represent pre-junctional subtypes. We are attempting to isolate novel pre-junctional α_2-adrenergic receptor clones from cDNA derived from noradrenergic cell bodies of bovine locus coeruleus (LC). Oligonucleotide primers corresponding to highly conserved regions within the second and seventh transmembrane domains of the three mentioned receptors were constructed and used as primers to PCR amplify LC cDNA. This approach has lead to the amplification of 2 PCR bands which were purified and cloned into pBluescript. Partial sequence data suggest that these clones are novel G-protein coupled receptors, but neither one shares extensive homology with the α_2-adrenergic receptors. Northern blot analysis reveals that both clones are differentially expressed in both the central nervous system and peripheral tissues and that one of the clones is enriched in the brain. These clones are being used to probe a bovine LC cDNA library to isolate the full-length clones for each PCR product. The full-length cDNA clones will then be sequenced and expressed in vitro in order to study their functional properties.

35.2

DEVELOPMENT OF FUSION PROTEINS FROM ALPHA-1 ADRENERGIC RECEPTOR SUBTYPES FOR USE IN THE PRODUCTION OF SUBTYPE-SPECIFIC ANTIBODIES. D.C. Perry[1], R.P. Yasuda[2] and B.B. Wolfe[2]. Depts. Pharmacology, [1]George Washington Medical Center, Washington, D.C. 20037 and [2]Georgetown University, Washington, D.C. 20007

Three separate gene products have been demonstrated for α-1 adrenergic receptors, α-1$_a$, α-1$_b$ and α-1$_c$. We are employing clones of these receptor subtypes (courtesy of Drs. R. Lefkowitz and M. Caron) to produce subtype-specific antibodies. Polymerase chain reaction was used to clone a 432 base-pair segment from the C-terminus of the α-1$_a$ receptor and a 359 base pair segment from the C-terminus of the α-1$_b$ receptor. The segments were designed to contain BamH1 sites on the ends of both segments, permitting subcloning of each segment into the plasmid vector pET-3b immediately downstream from a T7 promoter site. Successful ligation was confirmed by DNA sequencing. The plasmid was transformed into the *E. coli* strain BL21(DE3), which contains the bacteriophage T7 gene under the control of the *lac*UV5 promoter. Bacteria were grown in the presence of 1 mM IPTG to activate the T7 gene, causing overproduction of the specific fusion proteins. The fusion proteins consist of the first 11 amino acids of the T7 gene 10 attached directly to the protein coded for by the cloned receptor sequences. SDS-PAGE analysis of timed samples confirmed the presence of induced protein bands at 18,000 daltons, agreeing with the predicted molecular weight for the two fusion proteins. The 4 hr induced culture was purified using a Rotofor automated isoelectric focussing apparatus followed by dialysis. Lowry and SDS-PAGE analysis indicated about 85-90% purity for each fusion protein, with a total yield of approximately 10 mg protein/liter culture. We have initiated production of antibodies in rabbits; such antibodies should prove to be valuable tools for analysis of α-1 receptor subtypes. (Supported by NS 26934)

35.3

STABLE EXPRESSION OF HUMAN RECOMBINANT α_2-ADRENOCEPTOR SUBTYPES IN A MOUSE MAMMARY TUMOR CELL LINE. A. Marjamäki, S. Ala-Uotila*, K. Luomala*, M. Jalkanen*, S. Leppä* and M. Scheinin. Depts. of Pharmacology and Medical Biochemistry, Univ. of Turku, SF-20520 Turku, Finland.

Cloning of the human genes encoding distinct α_2-adrenergic receptor subtypes now allows the use of recombinant expression systems to produce receptor material for structural and functional studies as well as for the testing of new, potentially subtype-selective pharmacological agents. We report here the expression of two human α_2-adrenoceptor subtypes, α_2-C4 and α_2-C10, in mouse mammary tumor cells.

The coding sequences from pSP65α_2C10NH and pSP65α_2C4BH (Kobilka et al., Science 238, 650; Regan et al, PNAS 85, 6301) were inserted into the G418-selectable expression vector pMAMneo, which contains the MMTV-LTR promoter. The resulting expression constructs were transfected into S115 cells using the Lipofectin^R reagent kit (Gibco). G418 (750 µg/ml) resistant clones from both cell populations were selected and examined for their ability to bind the α_2-adrenergic antagonist radioligand [^{3}H]rauwolscine.

Scatchard analysis of [^{3}H]rauwolscine binding revealed B_{max} values of about 700 and 600 fmol/mg protein and K_ds of 1 and 6 nM for the recombinant C4 and C10 receptors. Competition assays with oxymetazoline and prazosin yielded the expected K_i-ratios, 1.8 for C4 and 0.004 for C10. The K_i of norepinephrine was increased 6- and 4-fold in the presence of 10 µM GppNHp, suggesting efficient G-protein coupling of both receptor subtypes.

These recombinant cell lines appear suitable for pharmacological experiments and should be useful in the development of new, subtype-selective drug molecules.

35.4

RECEPTOR AUTORADIOGRAPHIC ANALYSIS OF p-IODOCLONIDINE BINDING IN DISCRETE BRAIN AREAS IN GENETICALLY OBESE ZUCKER RATS. M. Koulu, U. Pesonen, T. Miettinen, R. Huupponen* and M. Scheinin. Dept. of Pharmacology, Univ. of Turku, SF-20520 Turku, Finland.

The obese Zucker rat is a genetic model of obesity. Obese (*fa/fa*) animals are hyperphagic and hyperinsulinemic compared to lean (*Fa/?*) control rats. The mechanisms underlying hyperphagia of obese Zucker rats are not yet resolved, but impaired satiety signals may be involved. We have reported previously that the α_2-adrenoceptor mediated feeding response to clonidine is enhanced in obese rats (Neuroendocrinology 52:503-510, 1990). The present study was performed in order to investigate α_2-receptor binding characteristics in brain areas involved in the control of feeding behavior. Quantitative receptor autoradiographic analysis of ^{125}I-p-iodoclonidine (PIC) binding in obese and lean Zucker rats was performed.

Brain slices were incubated for 60 min in 180 mM Tris-HCl buffer containing 3 nM PIC. Non-specific binding was determined by 10 µM phentolamine. After washing, the slides were exposed on Fuji XJ film for 72 h together with plastic ^{14}C-standards. Images were analyzed with a MacIntosh-based image analysis system (Image, Wayne Rasband, NIMH, Bethesda, MD, USA). The brain areas where PIC binding was determined included the hypothalamic paraventricular nucleus (PVN), anterior hypothalamic nucleus, hippocampus, amygdala, and cerebral cortex. There were no differences between the Zucker phenotypes in PIC binding in any brain area examined.

It is concluded that PIC binding in brain is not altered in genetic obesity, suggesting that the enhanced hyperphagic effect of clonidine in obese Zucker rats is not due to increased α_2-receptor density. Instead, we propose that an imbalance between inhibitory serotonergic and stimulatory α_2-adrenergic tone in hypothalamic centers regulating food intake may account for the greater sensitivity of obese rats to clonidine.

35.5

THE ALPHA-2D ADRENERGIC RECEPTOR IN THE BOVINE PINEAL AND RAT SUBMAXILLARY GLAND. H.S. Blaxall, N.A. Hass* and D.B. Bylund. Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68198-6260.

Recently we have identified and characterized the alpha-2 adrenergic receptor in bovine pineal glands (Bylund *et al.*, Adrenoceptors: 27-36 Birkhauser Verlag, 1991). [^{3}H]Rauwolscine bound in a saturable manner with a K_D of 1.5 nM. Inhibition studies with twelve adrenergic antagonists indicated that the bovine pineal contained a single class of alpha-2 adrenergic receptor binding sites. The correlations between the pK_i values from the bovine pineal with cell lines expressing only one alpha-2 adrenergic subtypes are respectively: HT29 2A 0.80, NG108 2B 0.46 and OK 2C 0.61. These results suggest that the bovine pineal alpha-2 adrenergic receptor may represent a new pharmacological subtype, the alpha-2D. The alpha-2 adrenergic receptor in rat submaxillary gland has been characterized (Michel *et al.*, Br. J. Pharmacol. 98:890-897, 1989). The correlation coefficient for the rat submaxillary gland with the pK_i values for the bovine pineal gland is 0.93, suggesting that it may also contain the alpha-2D subtype. To further characterize this subtype, RNA was isolated from bovine pineal and rat submaxillary glands and subjected to RTPCR using oligonucleotide primers developed to transmembrane regions II and VII using the published sequences of the human α_2C4 and C10. This produced single products of approximately 1 kb which hybridized strongly to a 0.3 kb fragment which includes transmembrane regions I to V from HPα2GEN, a plasmid containing the coding sequence for alpha-2 C10. These 1 kb products are currently being used to obtain sequence data for the alpha-2D adrenergic receptor. (NIH grant GM40784).

35.6

INTERACTION BETWEEN ANGIOTENSIN II AND SUB-TYPES OF α_1-ADRENOCEPTORS. R. TABRIZCHI* and C. R. TRIGGLE. Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, AB, Canada, T2N 4N1.

Activation of different signal transduction processes by agonists suggests that a further sub-classification of membrane receptors may be necessary. Thus the α_1-adrenoceptors have been sub-classified into α_{1a} and α_{1b}. We have evidence which indicates that the alkylating agent phenoxybenzamine is capable of selectively inactivating the sub-class of vascular smooth muscle, α_{1b}, α_1-adrenoceptors coupled to the phosphoinositol pathway, whereas the other sub-type, α_{1a}, is sensitive to calcium channel blockade. Receptor mediated events due to the activation of α-adrenoceptors can also be influenced by peptides such as angiotensin II. We have shown that pretreatment of rats with captopril leads to the inhibition, *in vivo*, of pressor responses initiated by the α_1-adrenoceptor agonists cirazoline and St 587. Furthermore, the previous exposure to phenoxybenzamine leads to an enhanced inhibitory action of captopril. This suggests that phenoxybenzamine and captopril are acting by different mechanisms and that angiotensin II may act to potentiate receptor mediated responses via the influx of calcium. Moreover we have shown that a combination of captopril and nifedipine does not produce an additive inhibition of the pressor responses to α_1-adrenoceptor agonists. This also supports our hypothesis that angiotensin II may selectively enhance signal transduction which is mediated by the activation of α_{1a} receptors.
(Supported by Alberta Heart and Stroke Foundation)

35.7

FUNCTIONAL DISCRIMINATION BETWEEN CENTRAL AND PERIPHERAL ALPHA$_2$-ADRENOCEPTORS USING THE ADRENERGIC AGONIST ORG 9987. Th. De Boer[1*], G. Maura[2*], J.S. De Graaf[1*], G.S.F. Ruigt[1], J.A.D.M. Tonnaer[1] and M. Raiteri[2]. [1]CNS Pharmacology R&D Labs., SDG, Organon Int.B.V., 5340 BH Oss, The Netherlands, and [2]Istituto di Farmacologia e Farmacognosia, Univ. of Genova, 16148 Genova, Italy.

Heterogeneity of alpha$_2$-adrenoceptors has been studied using the unique discriminating property of the alpha$_2$-adrenoceptor agonist Org 9987. Functional studies included the inhibition of twitches evoked in the rat vas deferens by electrical stimulation and inhibition of ^{3}H-noradrenaline release from rat cortical synaptosomes, whereas displacement of ^{3}H-rauwolscine binding to three cloned human alpha$_2$-adrenoceptors was studied in CHO-cell membranes. The twitch response of the vas deferens was fully and concentration-dependently inhibited by azepexole (pD_2=6.3; slope=0.61), clonidine (pD_2=8.2) and Org 9987 (pD_2=7.5; slope=1.16). In contrast to clonidine, Org 9987 was unable to inhibit the release of ^{3}H-noradrenaline from rat brain synaptosomes. Org 9987 showed a 10-fold preference (pK_i=7.0) for a subtype of alpha$_2$-adrenoceptor preferentially expressed in peripheral organs. It is concluded that terminal autoreceptors differ pharmacologically from peripheral presynaptic alpha$_2$-adrenoceptors which are discriminated by the novel alpha$_2$-agonist Org 9987.

35.8

ACTIONS OF A-75200, A COMBINED ALPHA-2 ANTAGONIST/NOREPINEPHRINE UPTAKE INHIBITOR ON NOREPINEPHRINE-CONTAINING CELLS. G.A. Gerhardt, J. Firestone, M. Browning, P. Bickford-Wimer, T. Sebree, M. Pierce, J.F. DeBernardis, and J.F. McKelvy. Depts. of Pharmacology and Psychiatry, Univ. of Colorado Health Sci. Ctr., Denver, CO and Abbott Laboratories, Abbott Park, IL.

A-75200, (±)-(R'*,3R*)-3-phenyl-1-(1',2',3',4'-tetrahydro-5',6'-methylenedioxy-1-naphthalenyl)methyl)-pyrolidine, methanesulfonate is a racemic mixture which is a potent alpha-2 antagonist, and an inhibitor of the uptake of norepinephrine (NE) into central neurons which has potential as a novel antidepressant agent. The enantiomers of A-75200 have also been synthesized and these compounds exhibit differential potencies for both the uptake and alpha-2 effects. Receptor binding data showed that A-75200 possesses excellent affinity for the alpha-2 receptor with a K_I of 1.2 nanomolar and a good alpha-1/alpha-2 selectivity(~100 fold; ratio $K_{I\alpha1}/K_{I\alpha2}$). In synaptosomal preparations from rat hippocampus, A-75200 was found to inhibit the uptake of ^{3}-H-NE with an IC50 of 870 nanomolar. Preliminary studies conducted using bovine adrenal chromaffin cells showed that A-75200 inhibits the uptake of ^{3}-H-NE into these cells with a relative potency that is less than desipramine and nomifensine and with the reuptake inhibition conferred by the SS enantiomer. Interestingly, the SS enantiomer also appears to have effects on the uptake of ^{3}-H-NE into bovine adrenal chromaffin cells. We are currently conducting in vivo electrochemical measurements in rat cerebellum using Nafion-coated carbon fiber electrodes coupled with pressure ejection of A-75200 and other uptake inhibitors, to explore the effects of this novel compound on the diffusion/clearance of NE in the intact animal.

35.9

PHARMACOLOGICAL CHARACTERIZATION OF α-ADRENERGIC RECEPTORS COUPLED TO PHOSPHOLIPASE C IN CULTURED HUMAN RETINAL PIGMENT EPITHELIAL CELLS. S. Moroi and G.J. Jaffe*. Dept. of Ophthalmology, Duke University, Durham, NC 27710.

The retinal pigment epithelium (RPE), a monolayer of neuroectodermal cells between retina and choroid, is vital for retinal function. In RPE culture, α- and ß-adrenergic receptors have been identified, but the receptor subtypes have not been characterized pharmacologically.

Third to sixth passage human RPE cells were seeded in 24 well plates at 40,000-60,000 cells/ml and grown to confluency. Cells were labeled with [^{3}H]inositol (3 μCi/well) for 24 hr and stimulated for 30 min with varying concentrations of epinephrine in the presence of pargyline (10 μM). [^{3}H]inositol phosphate products were separated by anion exchange column chromatography. There was a concentration-dependent increase in [^{3}H]inositol phosphate products which was antagonized by prazosin, but not propranolol. Studies are underway to characterize the α_1-adrenergic receptor subtype in cultured human RPE. This receptor system may have a role in ion transport, proliferation, and rod outer segment renewal.

35.10

P_{2Y}-PURINERGIC AND α_1-ADRENERGIC ACTIVATION OF ARACHIDONIC ACID RELEASE. B.L. Firestein, D.A. Craig* and P.A. Insel*. Depts. of Pharmacol and Neurosci, UCSD, La Jolla, 92093.

We have used receptor-stimulated release of [^{3}H]-arachidonic acid (AA) from labeled Madin-Darby Canine Kidney cells (MDCK-D_1 line) as a model system to explore the possible interaction of ATP and norepinephrine (NE) co-released from sympathetic neurons. NE (0.1-3μM) activates α_{1b}-adrenoceptors and elicits a 5-6 fold maximal stimulation of AA release; ATP (0.1μM-3mM) evokes greater (12-15 fold) AA release. Responses to ATP are mimicked by nucleotides with the following relative potencies: ATP = UTP = 2-CH_3S-ATP > ADP = AMPPNP >>> α,β-CH_2-ATP. AMP, adenosine and cAMP (≤ 3mM) were ineffective. The agonist profile suggests action at a P_{2Y} purinergic receptor, but the broadness of the concentration-effect curves for ATP suggests involvement of multiple purinergic receptor subtypes. Both ATP- and NE-stimulated AA release are dependent on extracellular calcium, but are insensitive to 0.5 mM neomycin, an inhibitor of polyphosphoinositide-phospholipase C. Inhibition of ATP- and adrenergic-evoked release of AA by sphingosine (12 μM) and staurosporine (100 nM) suggests that protein kinase C (PKC) is involved in AA release. These data support the hypothesis that P_{2Y} receptors and α_1 receptors activate multiple phospholipases, including PLA_2. We further hypothesize that PLA_2 and/or PKC may represent sites of interaction between NE and ATP.

35.11

CHARACTERIZATION OF ALPHA-2A AND ALPHA-2B ADRENERGIC RECEPTORS IN HUMAN BRAIN. G.A. Ordway and A.E. Halaris, Depts. Psychiatry and Pharmacology, Case Western Reserve Univ. and MetroHealth Med. Ctr., Cleveland, Ohio 44109.

Subtypes of alpha-2 adrenergic receptors have been pharmacologically identified in human brain. Studies using ^{3}H-yohimbine demonstrate that oxymetazoline (OXY) has high and low affinity for alpha-2A and alpha-2B receptors, respectively, while prazosin has low and high affinity for alpha-2A and alpha-2B receptors, respectively. We further characterized alpha-2 receptor subtypes in homogenates of human brain. Competition experiments (30 to 35 point) using caudate (n=6) measured inhibition of binding of ^{3}H-yohimbine by OXY. Competition curves were best fit (LIGAND) by a two-site model ($p<0.005$) and were unaffected by GTP. Based on these curves, a concentration ($CONC_{opt}$) of OXY was calculated which would optimally antagonize binding of ^{3}H-yohimbine to high affinity sites, minimally inhibiting low affinity binding. In the presence of $CONC_{opt}$ of OXY, competition studies of remaining ^{3}H-yohimbine binding in caudate revealed pharmacology which was characteristic of alpha-2B receptors. Furthermore, ratios of alpha-2A to alpha-2B receptors were estimated in human frontal cortex (5:1), temporal cortex (5:1), caudate (1:1), hippocampus (5:1), amygdala (2:1), cerebellum (6:1) and locus coeruleus (5:1). Supported by MH42859 and NARSAD.

35.12

LOCALIZATION OF α_2-AGONIST BINDING SITES WITH p-[^{125}I]IODOCLONIDINE: COMPARISON WITH p-[^{3}H]AMINOCLONIDINE. L.L. Longlet, M.E. Alburges, D.B. Bylund*, M.A. Hunt, N. Narang and J.K. Wamsley. Neuropsychiatric Research Institute, 700 First Avenue South, Fargo, ND 58103; *Department of Pharmacology, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, NE 68198.

[^{3}H]Para-aminoclonidine (p-[^{3}H]AC) binds with high affinity and selectivity to α_2-receptors. Previous investigations utilizing this agonist may have overlooked some regions of α_2 binding due to the subtype selectivity of this ligand. In the present study, p-[^{3}H]AC was shown to be more effective at labeling α_{2A} sites than α_{2B}/α_{2C} sites. In contrast, p-[^{125}I]iodoclonidine (p-[^{125}I]IC) had only 1000 times more affinity for the α_{2A} than the α_{2B}/α_{2C} sites. The distribution of p-[^{125}I]IC binding was two-fold higher than the p-[^{3}H]AC binding in many brain areas analyzed. The results obtained with the guanine nucleotide, Gpp(NH)p, suggest that both radioligands bind preferentially to the guanine-nucleotide-sensitive sites of α_2-receptors. These studies indicate that p-[^{125}I]IC is less selective for α_2-subtypes than p-[^{3}H]AC and is a more precise indicator of the density and distribution of α_2-receptors.

35.13

QUANTITATIVE AUTORADIOGRAPHIC (QAR) COMPARISON OF α_2-ADRENERGIC RECEPTORS IN RAT BRAIN REGIONS USING MULTIPLE COMPETITORS. D.R. Wallace and N.R. Zahniser. Univ. Colorado Hlth. Sci. Ctr., Dept. Pharmacology, Denver, CO 80262.

As a prelude to investigating aging-related changes in α_2-adrenergic receptor subtypes in rat brain, binding of tritiated-idazoxan (^{3}H-IDAZ) and rauwolscine (^{3}H-RAUW) was examined using QAR. Brain regions investigated were the cortex, hippocampus, anterior thalamic nuclei, caudate/putamen, entorhinal cortex/amygdala, hypothalamus, inferior colliculus, locus coeruleus and nucleus tractus solitarius. Assays were carried out in 25 mM glycylglycine buffer (pH = 7.5) at room temperature. (-)Norepinephrine (NE; 1 mM) was used to define nonspecific binding. Specific binding constituted 75-80% of total binding for ^{3}H-IDAZ and 50-60% for ^{3}H-RAUW. Monophasic saturation curves for ^{3}H-IDAZ (0.1-15.0 nM) and ^{3}H-RAUW (0.1-10.0 nM) were obtained in all brain regions. The affinities for both radioligands ranged from 0.4 - 4 nM. The density of binding sites were 2- to 4-fold higher for ^{3}H-IDAZ compared to ^{3}H-RAUW. Competition curves were constructed using a concentration range of 10 pM - 1 mM for a nonselective α_2-adrenergic receptor agonist (NE), an α_{2A}-preferring compound (oxymetazoline), α_{2B}-preferring compounds (ARC-239 & prazosin) and an IDAZ-like α_2 agonist (guanabenz). For most competitors pseudo-Hill slopes were less than unity, and the curves were better fit by a two-site model. The exception was ARC-239 inhibition of ^{3}H-RAUW; these curves were fit to a one-site model. The higher density of ^{3}H-IDAZ binding sites suggests that throughout the brain ^{3}H-IDAZ binds to a larger population of α_2-adrenergic receptors than does ^{3}H-RAUW. Based on the competition curve analyses, the ratio of α_{2A}- and α_{2B}-adrenergic receptor subtypes was determined and will be presented for each brain region. Supported by USPHS AG 04418.

35.14

IN SITU HYBRIDIZATION ANALYSIS OF α-1B, α-2C10 AND α-2C4 ADRENERGIC RECEPTOR mRNAS DURING RAT BRAIN DEVELOPMENT. S.K. McCune, M.M. Voigt* and J.M. Hill, Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Multiple subtypes of alpha adrenergic receptors have been identified and characterized by pharmacologic and/or molecular biologic means. The regional distribution and functional significance of these subtypes have yet to be fully elucidated. Using pools of oligonucleotide probes specific for the α-1B, α2-C10 and α-2C4 subtypes, frozen sections of E14, E16, E19, newborn, P8, P14 and adult brains were examined by *in situ* hybridization. All three receptor subtypes were present in the brain as early as E14 and each had a unique regional and ontogenic expression.

The α-2C4 mRNA was expressed at higher levels throughout development than the other two subtypes. It was primarily localized in the cortex (especially the cingulate cortex in the postnatal rat), hippocampus, caudoputamen, pons, pineal and cerebellum. In general, the expression levels increased in these areas in the postnatal animal and the message was more abundant in fiber tracts in the adult animal. The α-2C10 receptor mRNA was present in cortex (particularly in the pyriform cortex on P14), hippocampus, pons and cerebellum. High levels of the receptor message were also found in the prenatal inferior olive and P14 locus coeruleus. Levels of α-2C10 mRNA were generally higher in the perinatal animal than in the adult. The α-1B receptor message was primarily expressed in the cortex (especially intermediate layers in the adult), thalamus, hippocampus, pineal, dorsal raphe, and cerebellum (particularly the cerebellar peduncles). In most regions, this receptor subtype mRNA was more abundant in the older animal.

These data confirm the previous observations made by Northern blot of the developmental expression of these three alpha adrenergic receptor subtypes. *In situ* hybridization allowed for more precise localization of these receptors during embryonic and postnatal development and may ultimately be used to correlate function with subtype specific expression.

35.15

IN SITU HYBRIDIZATION STUDY OF ALPHA AND BETA ADRENERGIC RECEPTORS IN THE RAT. A.P. Nicholas*, V.A. Pieribone, R.P. Elde and T. Hökfelt. Dept. of Histology and Neurobiology, Karolinska Institutet, Stockholm, Sweden, 104 01.

Previous autoradiographic studies have demonstrated the general location of alpha and beta adrenergic receptor binding by "specific" radioactive ligands; however, it is difficult to quantify or determine the pre- or postsynaptic location of these receptors using this technique. In the present study, we have designed specific 48-mer oligoprobes to recently-described sequences of the rat alpha-1B, alpha-2B, beta-1 and beta-2 adrenoreceptors for *in situ* hybridization experiments. Complementary DNA probes were made on a DNA synthesizer to the following nucleotide sequences: 1420-1467 rat alpha-1B receptor, 868-915 of rat brain alpha-2B receptor, 1401-1448 of beta-1 receptor and 2999-3046 of beta-2 receptor. All probes then were labelled at the 3'-end using terminal deoxynucleotidyltransferase and [^{35}S] dATP. Fresh rat tissues, including liver, kidney, lung, gut, brain and spinal cord, were rapidly removed, frozen, sectioned on a cryostat (14 µm), air dried and transferred to humidified boxes at 42° C for 18 hrs with 1 X 10^6 cpm of an end-labeled DNA oligonucleoide probe diluted in a hybridization solution consisting of 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 1% sarcosyl, 0.2 M sodium phosphate (pH 7), 10% dextran sulfate, 200 mM dithiothreitol and 500 µg/ml heat-denatured salmon sperm DNA. The sections then were washed 4 X 30 min in SSC at 55° C, dehydrated, dipped in Kodak NTB2 photographic emulsion and exposed for 3-6 weeks before development. Specific labeling with all probes was seen in various tissues. For example, beta-2 receptor signal was very high in the hippocampus, while alpha-1B receptor labeling was intense in the thalamus. Using this technique, not only will the specific localizations of the cells which manufacture these receptors be attained, but also various physiological or pharmacological manipulations can be performed in future studies to quantify any possible up or down regulation of these receptors.

35.16

GENETIC STRUCTURE OF THE ß1-ADRENERGIC RECEPTOR. R.P. Searles[1], V. Nipper[1*], and C. A. Machida[1,2], [1]Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006 and [2]Dept. of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR 97201.

The ß-adrenergic receptors are members of the superfamily of G protein-coupled neurotransmitter receptors and mediate physiological response to the catecholamines epinephrine and norepinephrine. Three major subtypes (ß1-ß3) have been identified on the basis of pharmacology and genetic structure. Our lab previously reported the isolation and characterization of the rat ß1-AR gene. To identify conserved elements within the flanking elements of this gene, the rhesus macaque ß1-AR gene was cloned and sequenced. This gene codes for a 480 amino acid receptor that is 98% homologous to the human ß1-AR and 94% homologous to the rat. Approximately 1.5 kb each of upstream and downstream flanking sequence of the primate gene was compared to corresponding sequences of the rat gene. Among the elements conserved between the two species are two potential polyadenylation sites, a consensus cyclic AMP response element, and a T-cell response element. Conservation of the gene for 1.5 kb downstream is greater than 76%, suggesting important untranslated and untranscribed sequences in this area. Overall conservation of the 5′ flank is 72% for the first 1.5 kb, but this similarity is unevenly distributed. 1.25 kb of rat ß1-AR upstream sequence (-1256 to -1 relative to the initiation codon) in a luciferase expression vector expresses highly when transfected into C6 glioma cells. Expression declines by 95% when the rat sequences -1033 to -1 are used to regulate transcription.

35.17

FUNCTIONAL EXPRESSION OF A HUMAN ß-2 ADRENERGIC RECEPTOR IN *XENOPUS* MELANOCYTES. M. N. Potenza and M. R. Lerner, Howard Hughes Med. Inst., Depts. of Int. Med., Pharm. & Cell Bio., Yale Univ. School of Med., New Haven, CT. 06510

Pigment translocation in *Xenopus* melanocytes is mediated by intracellular levels of cyclic AMP (cAMP): high levels induce dispersion whereas low levels induce aggregation. In order to investigate the feasibility of using the melanocytes to study G-protein coupled receptors not normally expressed in these cells, the human ß-2 adrenergic receptor (ß-2 AR) was selected. In its native environment, the ß-2 AR, when stimulated, produces an increase in intracellular cAMP levels. Cultured melanocytes transfected with the gene encoding the human ß-2 AR obtained the ability to disperse their pigment in response to the ß-2 specific agonist metaproterenol with an E.C.(50) value of 75 nM. Metaproterenol did not induce significant pigment translocation when applied to untransfected cells or cells transfected with vector alone. Metaproterenol-induced, but not MSH-induced, dispersion in the transfected cells was blocked by the ß-adrenergic antagonist propranolol. The demonstration of the functional expression of an introduced G-protein coupled receptor in cultured *Xenopus* melanocytes has implications for their use as a rapid visual means to study G-protein coupled receptors and the ligands with which they interact.

35.18

PURIFICATION AND CHARACTERIZATION OF THE ß-ADRENOCEPTOR FROM RAT CEREBRAL CORTEX. S.B. Lee*, K.N. Park*, J.S. Ahn* and K.H. Ko. Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul 151, Korea.

The ß-adrenoceptor of rat cerebral cortex was successfully solubilized by sequential treatment with cholate and digitonin. About 50% of the total receptor pool was released by this solubilization procedure. The ß-adrenoceptors in the digitonin extract were identified using the ß-adrenergic antagonist, (-)-[^{3}H]dihydroalprenolol ([^{3}H]DHA). The solubilized receptor retained all of the essential characteristics of membrane-bound receptor, namely saturability; stereoselectivity; high affinity to ß-adrenergic drugs. In order to facilitate effective purification, the affinity chromatography was adopted in this experiment. A ß-adrenergic antagonist, (-)-alprenolol, was immobilized to Sepharose CL-4B by using a hydrophilic spacer arm, bisoxirane. The affinity gel was prepared by the method of Caron *et al.* (1979). Finally, the ß-adrenoceptor of rat cerebral cortex was purified by sequential affinity chromatographic steps. Purified receptor preparations bind [^{3}H]DHA with a specific activity of 13.65 nmol/mg of protein, representing a 35,000fold purification from cortical membranes with a 3% overall yield. Sodium dodecylsulfate-polyacrylamide gel electrophoresis of purified receptor revealed a major band of protein of 52,000daltons and a minor band of 24,000 daltons. The binding of [^{3}H]DHA to purified receptor preparations displayed affinity, specificity, and stereoselectivity characteristic of solubilized receptor.

35.19

CHARACTERIZATION OF A SOLUBLE FORM OF THE β1- AND β2-ADRENERGIC RECEPTOR EXPRESSED IN BACTERIA. K. P. J. Dobrovský*, W.H.M.L. Luyten and J.E. Leysen. Dept. of Biochemical Pharmacology, Janssen Research Foundation, B2340 Beerse, Belgium.

Functional β1- and β2-adrenergic receptors (β-ARs) have been expressed in *Escherichia coli* as LamB or MalE fusion proteins (Marullo et al. Bio/Technology 7:923;1989). Although previous fractionation studies documented β-AR immuno-reactive material in the cytosol, this form did not appear to bind any ligand (Chapot et al. Eur. J. Biochem. 187:137; 1990).

Bacteria were grown as described by Marullo et al., and lysed (in 50 mM TRIS pH 7.6, 250 mM sucrose, 100 mM NaCl, 1.5 mM PMSF, 1 mM DTT, 1mM $MgCl_2$) by sonication after lysozyme treatment. The lysate was centrifugated for 30 min. at 150,000xg and ligand binding was performed on the supernatant.

Saturable 125I-iodocyanopindolol binding, displaceable by a variety of β-adrenergic agonists and antagonists was seen for the β2-AR after incubations for 60 min at 37°C, or for 2-16 hours at 4°C and for the β1-AR after incubation for 16 hours at 4°C. Prolonged incubations at 4°C yielded the maximum number of binding sites, which was still less than 1% of the specific binding to intact cells. Results of 125I-iodocyanopindolol binding to the soluble cytosolic β-AR form were independent of the 3 methods used to separate bound from free radio-ligand: polyethyleneglycol precipitation, filtration through polyethyleneimine pre-soaked GF-C filters or Sephadex LH-20 columns. Competition binding with 17 β-adrenergic agonists and antagonists (comprising hydrophilic and lipophilic agents) yielded IC_{50}s comparable to those measured on intact cells. On the whole, this strongly suggests that the soluble binding site is a β-AR.

We do not know how this β-AR remains in solution. The fact that this soluble receptor form could be obtained without the addition of lipids or detergents argues against it being solubilised from a membranous environment. In principle, the hydrophilic MalE part could keep the fusion protein in solution, but previous work showed that β-AR fails to bind ligands while fused to MalE.

35.20

NEURAL REGULATION OF BETA-1 AND BETA-3 ADRENERGIC RECEPTOR mRNA LEVELS IN BROWN FAT. J.G. Granneman and K. N. Lahners. Center for Cell Biology, Sinai Hospital and Clinical and Cellular Neurobiology Program, Wayne State Univ. Sch. of Med., Detroit MI 48235.

Beta-1 and ß-3 adrenoreceptor mRNAs levels of several tissues were determined simultaneously using a sensitive nuclease protection assay. The ß-1 receptor gene was expressed to varying degrees in all tissues examined. By contrast, the ß-3 receptor gene was abundantly expressed only in brown and white adipose tissues. Minor expression of the ß-3 receptor was also found in the adrenal gland. These data indicate that the ß-3 receptor is essentially fat tissue-specific.

Surgical sympathectomy of brown fat increased ß-3 receptor mRNA levels by 2.5 times, but did not affect ß-1 receptor mRNA levels. Exposing rats to 4°C, which increases sympathetic nerve stimulation of BAT, reduced ß-3 receptor mRNA levels in intact tissue by 50% but did not affect the denervation-induced increase in ß-3 receptor mRNA. Cold exposure slightly increased beta-1 receptor mRNA levels in both intact and denervated brown fat. These results indicate that ß-1 and ß-3 receptor mRNAs are differentially regulated and that sympathetic nerve activity reduces ß-3 recept- or mRNA levels in brown fat. (Supported by NIH Grant DK37006).

35.21

ESTRADIOL REGULATES α_{1B}-ADRENERGIC RECEPTORS WHICH POTENTIATE cAMP FORMATION IN THE HYPOTHALAMUS

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Estradiol potentiates α_1 receptor augmentation of β receptor-stimulated cAMP accumulation in slices of the preoptic area (POA) and middle hypothalamus (MH) (J. NEUROSCI. 10:2842-2849, 1990). Present studies examined: (1) whether α_{1A}- or α_{1B}-adrenergic receptors mediate the augmentation phenomenon, and (2) if ovarian steroids selectively alter the binding characteristics of either α_1-receptor subtype. The irreversible α_{1B} receptor blocking agent chlorethylclonidine eliminated phenylephrine (α_1 agonist) augmentation of isoproterenol (β agonist)-stimulated cAMP formation in POA and MH slices, whereas the α_{1A} antagonist 5-methyl urapadil did not. This suggests that the α_{1B} receptor subtype potentiates cAMP formation. POA and MH membranes from estradiol-treated rats, when compared to ovariectomized rats, had modestly (40-50%) but significantly elevated numbers of ^{3}H-prazosin (α_1) binding sites. Studies with chlorethylclonidine indicated that the estrogen-dependent increase in total α_1 binding sites in POA and MH membranes was attributable to a five- to six-fold increase in α_{1B} receptor number. Progesterone had no measurable effects on α_1 receptor binding. Thus the increased α_1 receptor augmentation of cAMP formation seen in estradiol-treated rats is correlated with increased α_{1B} receptor number.

35.22

IMMUNOHISTOCHEMICAL STUDIES OF NORADRENERGIC ACTIVATION OF C-FOS IN RAT CENTRAL NERVOUS SYSTEM. G. Bing, Y. Zhang*, D. Filer*, S. M. John* & E.A. Stone. Dept. Psychiatry, NYU Sch. Med., New York, NY 10016.

Previous experiments have shown that stimulation of brain beta adrenoceptors by i.p. yohimbine injection increases the mRNA of c-fos in the rat CNS. The present study examined the distribution of c-fos protein after this treatment. Rats were perfused 2 hr after injection of yohimbine (5 mg/kg) alone or with the beta blocker, propranolol (10 mg/kg). 30 u sections were stained immunohistochemically for c-fos, GFAP or neurofilament protein. Yohimbine treated rats were found to show marked increases in c-fos-like immunoreactivity in regions known to be either targets of noradrenergic output or noradrenergic neurons themselves. The most heavily stained regions included the neocortex (layers II and III), olfactory tubercle, piriform cortex, entorhinal cortex, paraventricular n. and locus coeruleus. Cotreatment with propranolol reduced c-fos-reactivity in all of the above areas. Double label experiments with GFAP and neurofilament protein indicated that the stained cells were predominantly neuronal. It is concluded that immunohistochemical staining of c-fos protein after yohimbine injection identifies postsynaptic beta adrenergic targets as well as presynaptic neurons of the central noradrenergic system. AFOSR 89-0208, MH45265 and MH08618.

35.23

MEASUREMENT OF ENDOGENOUS CENTRAL NORADRENERGIC NEUROTRANSMISSION FROM LEVELS OF cAMP EFFLUX IN VIVO.
E.A. Stone and S.M. John*. Dept. Psychiatry, New York Univ. Sch. Med., New York, NY 10016.

We have previously shown that microdialysis can be used to stimulate brain beta adrenoceptors with exogenous catecholamines and to detect the resulting increases in synthesis and outflow of cAMP. This method has enabled us to measure activation of beta receptors in vivo by exogenous catecholamines. The present experiments were undertaken to determine whether this technique can also detect receptor activation by endogenous norepinephrine (NE) release. Rats were implanted with microdialysis probes (3.5 mm) in the medial prefrontal cortex and given 48 hr to recover. The probes were perfused with Ringer's solution at 1 ul/min. Drugs were dissolved in Ringer's solution and infused via the input line. Portions of the dialysate were analyzed by HPLC for norepinephrine and by RIA for cAMP. It was found that infusion of the indirect sympathomimetic amine, amphetamine, at 10^{-2} M for 10 min elicited significant increases in NE and cAMP outflow. Studies of the effects of norepinephrine depleting agents on these responses will be reported. It is tentatively concluded that the microdialysis technique can detect endogenous noradrenergic neurotransmission at beta adrenoceptors in the cerebral cortex. Supported in part by grants AFOSR 89-0208, MH45265 and MH08618.

CATECHOLAMINE RECEPTORS: DOPAMINE I

36.1

MOLECULAR CLONING AND EXPRESSION OF THE RHESUS MACAQUE D1 DOPAMINE RECEPTOR GENE.
C.A. Machida[1,2], R.P. Searles[1], L.B. Kozell[3]*, K.A. Neve[3,4], J.E. Thornton[1], J.A. Brown[1]*, and V. Nipper[1]*. [1]Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006, Depts. of [2]Biochemistry and Molecular Biology and [3]Pharmacology, Oregon Health Sciences University, Portland, OR 97201, and [4]Veterans Administration Medical Center, Portland, OR 97201.

The dopamine receptors are members of the superfamily of G protein-coupled neurotransmitter receptors and serve as key biochemical modulators in motor activity, emotion, and cognition. Several subtypes of dopamine receptors (D1-D5) have been identified on the basis of their pharmacological properties, physiological effects, tissue and cell type specificity, and genetic structure. To begin analyzing the distribution and regulation of D1 dopamine receptors in the retina, we cloned and sequenced the rhesus macaque D1 dopamine receptor gene and expressed these receptors by transfection in C6 glial cells. Nucleotide sequence analyses of the coding region of the rhesus macaque D1 dopamine receptor gene show that it encodes a 446 amino acid protein, with several potential sites for N-linked glycosylation (2), and cAMP-dependent protein kinase phosphorylation. The primary amino acid sequence of the rhesus D1 receptor shows an extremely high degree of similarity (99.6%) to the human D1 receptor. Radioligand binding and adenylyl cyclase analyses of the transfectant demonstrate the expression of high-affinity (0.3nM, [^{3}H]SCH 23390), functional (stimulation of adenylyl cyclase by dopamine) rhesus macaque D1 dopamine receptors.

36.2

CLONING AND CHARACTERIZATION OF A NOVEL HUMAN D1 DOPAMINE RECEPTOR SUBTYPE AND ITS PSEUDOGENE.
K.R. Jarvie, C. Silvia, R.T. Fremeau Jr., J.A. Gingrich*, M.G. Caron. Dept. Cell Biology, Duke University Medical Center, Durham, NC. 27710.

Degenerate primers corresponding to conserved transmembrane domains of the dopamine D1 receptor were used with the polymerase chain reaction (PCR) to amplify sequences from sheared human genomic DNA. One of these products was homologous to the sequence between the 5th and 6th transmembrane regions of the human D1 receptor. This product was radiolabeled by PCR and used to screen a human genomic library. Two of the clones obtained (hg3-2 and hg4-2) were highly homologous to each other (~ 95 %) and the dopamine D1 receptor (~ 72 %). The apparently intronless coding region of one of these clones (hg4-2) revealed the presence of an in frame stop codon between the 4th and 5th transmembrane regions. This gene codes for a truncated receptor unlikely to be active and as such this sequence is presumably representative of a pseudogene. The open reading frame of the other clone (hg3-2) codes for a putative receptor which exhibits the seven transmembrane spanning helices typical of receptors coupled to guanine nucleotide regulatory proteins. Additionally, conserved amino acid residues associated with catecholamine receptors are conserved within this sequence. Based on the homology of this sequence to that of the D1 receptor, it is presumed that this sequence codes for a D1 receptor subtype. Potential sites of phosphorylation by protein kinases A and C are observed in the putative intracellular loops and may be involved in the regulation of receptor function. In situ hybridization in human brain tissue should allow for the comparison of the distribution of this receptor and its psuedogene, with that of the previously characterized dopamine D1 receptor.

36.3

EXPRESSION OF THE HUMAN D1 DOPAMINE RECEPTOR IN YEAST.
N. Din*[1], J.G.L. Petersen*[1], M.G. Caron[3] and P.H. Andersen[2]. Departments of [1]Microbiel Genetechnology and [2]Molecular Pharmacology, Bioscience, Novo Nordisk A/S, DK-2880 Bagsvaerd, Denmark and [3]Department of Cell Biology, Duke University Medical Center, Durham, NC 27710.

We have constructed a series of S. cerevisiae expression plasmids containing the human D1 receptor cDNA, using various regulated and constitutive promoters. In some of the constructs, the coding region for N-terminus of the receptor was exchanged with that of the N-terminus of S. cerevisiae α-Factor receptor (STE2), which belongs to the same super family of G-protein coupled receptors as the dopamine D1 receptor. Expression levels were measured in a number of host strains, using Northern blot analysis as well as ligand binding to whole cells or purified membrane fractions. Although mRNA levels were high, only low levels of functional receptors could be detected (10-200/cell), indicating that protein translation, stability and/or targeting were limiting factor(s). Analysis of cell extracts for the presence of non-functional receptors using antibodies are in progress. Also in progress are testing of various protease deficient host strains.

36.4

FUNCTIONAL EXPRESSION AND CHARACTERIZATION OF A NEW D1 RECEPTOR AND ITS PSEUDOGENE.
Nika Adham*, Mary Macchi*, Paul Hartig, Theresa Branchek, and Richard Weinshank* Neurogenetic Corporation, Paramus, N.J. 07652

Recently, an expansion in the number of receptor subtypes for dopamine has been forthcoming from molecular cloning techniques. We report the cloning of a novel intronless gene (GL30) encoding a G protein-coupled receptor of the dopamine D1 receptor family. Expression of this receptor in Cos-7 cells led to the high affinity binding of a number of dopamine D_1 antagonists, with a binding profile similar to that of the previously described dopamine D_1 receptor. In contrast, the agonist binding profile of this new receptor did not exactly match any previously defined dopamine D_1 receptor, and was notable for its unusually high affinity for its natural transmitter, dopamine. This new receptor was found to couple to adenylate cyclase activity, where addition of dopamine caused a 13-fold increase in activity. Messenger RNA encoding this new receptor appears to be distributed in a number of regions in the human brain, including cortical regions, choroid plexus, hippocampus, and brain stem. A second closely related gene, GL39, was isolated and shown to represent a pseudogene exhibiting 94% nucleotide sequence homology to the GL30 sequence. One of the amino acid substitutions which is found in transmembrane region V of pseudogene GL39 is an amino acid proposed to participate in the binding of catecholamine ligands. This mutation may have led to a loss of function and subsequent conversion of an active dopamine receptor gene to a pseudogene.

36.5

MOLECULAR CLONING OF A NOVEL D_1 DOPAMINE RECEPTOR FROM RAT KIDNEY. F.J. Monsma, Jr., Y. Shen, C.R. Gerfen, L.C. Mahan*, P.A. Jose, M.M. Mouradian & D.R. Sibley. Experimental Therapeutics Branch, NINDS and Laboratory of Cell Biology, NIMH, NIH, Bethesda, MD 20892.

We have used the rat kidney proximal convoluted tubule (PCT), which contains D_1 dopamine receptor subtypes linked to the activation of adenylyl cyclase as well as phospholipase C, to investigate the molecular biology of peripherally located D_1 receptors. In order to clone these receptor subtypes, the polymerase chain reaction (PCR) method was used to selectively amplify putative D_1 receptor cDNA sequences from rat kidney PCT mRNA. Poly $(A)^+$ RNA was used to synthesize cDNA by reverse transcription followed by PCR amplification using sets of highly degenerate primers derived from the transmembrane sequences of previously cloned dopaminergic, adrenergic, and serotoninergic receptors. This process resulted in the amplification of a novel cDNA sequence which exhibits considerable homology to dopaminergic and other members of the G protein-coupled receptor family. Within the transmembrane regions, this cDNA is >80% homologous with the previously cloned D_1 receptor suggesting that it belongs to this category of dopamine receptor subtypes. Northern blot analysis reveals a transcript size of ~3.3 kb which is located in kidney tissue as well as various regions in the CNS. Northern blots of different brain tissues reveals a relative abundance of hippocampus>hypothalamus = mesencephalon>olfactory tubercle>olfactory bulb>striatum. Little to no transcript was detected in the cerebral cortex, cerebellum, pituitary or retina. *In situ* hybridization analysis reveals a low abundance of this mRNA in CNS regions which agrees well with the Northern blot results. Full length cDNA and genomic clones have been isolated and are being expressed to establish the specific pharmacology and function of this novel D_1 dopamine receptor.

36.6

CHARACTERIZATION OF A DOPAMINE D1 RECEPTOR GENE FROM GOLDFISH. A.M. Manelli* and D.E. Frail. Corporate Molecular Biology, Abbott Laboratories, Abbott Park, IL 60064

We have isolated and characterized a gene from goldfish (Carassius auratus) that encodes a dopamine D1 receptor. A cDNA probe encoding the catecholamine receptor G-36 (FEBS Letters, 262, 8-12, 1990) was obtained by the polymerase chain reaction using degenerate oligonucleotides. This probe was used to screen a goldfish genomic library. One positive clone was plaque purified, mapped, and G-36 hybridizing DNA fragments were subcloned for sequence analysis. A complete open reading frame, apparently without introns, shares common features of G-protein coupled receptors and is 90% identical to the human D1 receptor in the transmembrane regions. However, the goldfish sequence lacks the final 79 amino acids of the human sequence. The goldfish and the human receptors were transiently expressed in eukaryotic cells and characterized by radioligand binding. The goldfish receptor showed a higher affinity for SCH23390, a classical D1 antagonist, than the human receptor. This receptor most likely represents the well-studied D1 receptor present in goldfish retina horizontal cells.

36.7

A Chimeric D1/D2 Dopamine Receptor Mediates A D1 Response To A D2 Ligand. D.E. Frail, N.J. Pollock, A.M. Manelli*, M. Steffey*, C.W. Hutchins* and R.G. MacKenzie. Abbott Laboratories, Abbott Park, IL 60064.

Dopamine (DA) stimulates adenylate cyclase via the D1 receptor and inhibits cyclase via the D2 receptor. DA receptors are coupled to G-proteins and model to seven transmembrane regions (TMs). Work on other receptors of the family show that subtype specificity is partly determined by the 6th and 7th TMs whereas the 3rd cytoplasmic loop specifies the transduction pathway. We constructed a chimeric DA receptor cDNA to have D1 sequence from the 5'-end through to the 5'-end of the 6th TM and to have D2 sequence for the remainder of the molecule. We predicted that, relative to wild-type (WT) D1, the chimera would lose affinity for D1 ligands, gain affinity for D2 ligands and mediate a D1 response to D2 agonists. WT and chimeric cDNAs were transiently expressed in mammalian cells and radioligand binding showed the chimera affinity to be 40-fold lower for the D1 ligands SCH23390 and SKF38393 and a 100-fold higher for the D2 agonist LY17155. Also, LY171555 was able to elevate cAMP levels only in cells transfected with chimeric cDNA.

36.8

LIGAND BINDING DOMAINS OF THE HUMAN DOPAMINE D1 RECEPTOR DETERMINED BY SINGLE SITE MUTATIONS. N.J. Pollock#, A.M. Manelli*#, C.W. Hutchins*+, R.G. MacKenzie+ and D.E.Frail# #Corporate Molecular Biology, +Pharmaceutical Products Div Abbott Laboratories, Abbott Park, IL 60064.

The dopamine receptors are a member of the G-protein coupled receptors that share homology in the seven transmembrane spanning regions. We are using site-directed mutagenesis followed by a pharmacological analysis of transiently transfected mammalian cells to determine which amino acids are important for ligand binding. There are three serine residues in helix five of the B-adrenergic receptor (a G-protein receptor) that are important for agonist binding (Strader et al., 1989). Analogous mutations, which changed serines to alanines in positions 198, 199 and 202 of the dopamine D1 receptor, were constructed. Using the D1 antagonist SCH 23390, the Kd for mutant Ala_{202} was equivalent to wildtype D1 (0.5nM); mutant Ala_{199} Kd=15.6nM; and mutant Ala_{198} was the same as mock transfected cells. Competition studies show that mutant Ala_{202} caused a decrease in affinity for dopamine, but not for SCH 23390. Alternatively, mutant Ala_{199} caused a decrease in affinity for both agonists and antagonists. Cells transiently transfected with wildtype D1 DNA or mutated receptor DNA show similar levels of mRNA by Northern analysis. Therefore, the lack of binding in cells containing mutant Ala_{198} is not due to the lack of available mRNA for translation into protein.

36.9

ISOLATION OF A cDNA CODING FOR THE OK CELL DOPAMINE D_1 RECEPTOR. D.R. Cerutis and D.B. Bylund. Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68198-6260.

Previous studies from our laboratory (Murphy and Bylund, Mol. Pharmacol. 34:1-7, 1988) have demonstrated the presence of the serotonin $5HT_{1B}$ receptor in the OK cell line, an established opossum renal proximal tubule epithelial cell line. We have also shown this cell line to contain the α_{2C}-adrenergic receptor (Bylund and Murphy, J. Pharmacol. Exp. Ther. 244:571, 1988) and in addition, it has been shown to express the D_1 receptor (Bates *et al.*, Neurosci. Abs. 16:1, 1990). To clone the $5HT_{1B}$ receptor, an OK cell λgt11 cDNA library was screened at reduced stringency with the human β_2-adrenergic receptor and a 4.2 kb clone was isolated. This clone is unreactive with α_2-adrenergic or 5HT receptor probes, and sequencing the region inclusive of transmembrane regions I - V reveals greater than 95-97% identity at the amino acid level with the human and rat D_1 receptors (Zhou *et al.*, Nature 347:76, 1990; Sunahara *et al.*, Nature 347:80, 1990; Dearry *et al.*, Nature 347:72, 1990). (NIH grant MH47354).

36.10

CHARACTERIZATION OF THE 5' FLANKING REGION OF THE HUMAN D-1 DOPAMINE RECEPTOR GENE M.T. Minowa*, T. Minowa*, F.J. Monsma, Jr., D.R. Sibley, and M.M. Mouradian, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

The D-1 dopamine receptor plays an integral role in propagating neural information in the basal ganglia and other brain structures. In an attempt to understand the molecular events leading to the transcriptional regulation of the adenylate cyclase-linked D-1 gene, a human genomic clone carrying the 5' flanking and coding regions of this gene was isolated using rat D-1 dopamine receptor coding sequence as a probe. The nucleotide sequence of this 5' flanking region extending 2.6Kb upstream from the first ATG codon was determined and the transcription start site defined using S1 nuclease mapping. The gene has neither a TATA box nor a CAAT box. It is rich in GC content with several consensus motifs for transcription factors, such as Sp1 and AP2.

Various fragments of the 5' flanking region were subcloned upstream of a CAT reporter gene to examine promoter/enhancer activity. A 1.1Kb fragment was found to have relatively high transcriptional activity in NS20Y cells known to express the D-1 receptor but not in other cell lines tested. The human D-1 dopamine receptor gene belongs to the category of tissue-specific genes lacking a TATA box.

36.11

POSTMORTEM STABILITY OF DOPAMINE D_1 RECEPTORS AND D_1 RECEPTOR mRNA. J.H. Gilmore[1,3], C.P. Lawler[3], A.M. Eaton[3], and R.B. Mailman[1,2,3]. Departments of Psychiatry[1] and Pharmacology[2], Brain and Development Research Center[3], University of North Carolina[1], Chapel Hill, N.C. 27599.

The D_1 dopamine receptor may have an important role in the development of symptoms associated with, and in the treatment of, schizophrenia. The recent cloning of the D_1 receptor has provided a basis for the study of D_1 receptor mRNA in human brain. The study of D_1 mRNA, especially of its cellular localization in the brains of normals and its possible alteration in the brains of people with schizophrenia, is potentially limited by postmortem degradation of the mRNA.

To assess the effect of postmortem interval on D_1 mRNA and on D_1 receptor binding, rats were decapitated, and the heads were allowed to cool in a controlled manner (to 25°C at six hours and to 4°C after 24 hours) to approximate the cooling and refrigeration of human postmortem brain tissue. Cellular localization of D_1 mRNA in the striatum was determined by *in situ* hybridization emulsion autoradiography in slide mounted sections of caudate, using a mixture of two ^{35}S-labeled oligoprobes. D_1 receptor density was determined in adjacent sections with receptor autoradiography using [^{125}I]SCH23982. We found a decrease in the overall density of D_1 mRNA after a 24 hour postmortem interval, resulting in a significant decrease (ca. 15%) in the number of labeled neurons ($p < 0.05$) compared to control rats (postmortem interval of 0 hours). Quantitative autoradiography of D_1 receptors indicated a corresponding decrease (ca. 20%) in receptor binding compared to controls. We conclude that a postmortem interval of 24 hours effects both D_1 mRNA concentrations and D_1 receptor binding. It it will be critical to characterize the time course of these decreases to provide a rational basis on which to interpret data from human postmortem brain tissue. Only then will it be possible to delineate the role of D_1 receptors in human diseases such as schizophrenia.

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36.12

REAL-TIME MEASUREMENTS OF HUMAN D_1 AND D_2 RECEPTOR ACTIVITY WITH A SILICON-BASED BIOSENSOR. J.A. Salon, R.A. Johnson*, and O. Civelli. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97201.

Classically, dopamine receptors have been functionally and pharmacologically classified into two major types, D_1 and D_2 which couple through G_S and G_i mechanisms respectively. It is known however, that the second messenger response depends not only on the specific properties of the receptor molecule itself, but also on the cell type in which it is expressed. It is likely that the cells overall metabolic response will also be different depending on the biochemical pathways involved in effector activity. However no direct measurements of cellular metabolic rates have been made in response to dopamine. It would therefor be of interest to examine the broad metabolic effects of D_1 and D_2 activation.

A silicon-based biosensor (microphysiometer, mfg. MDC Corp., Menlo Park, CA) has previously been described (Science 246, 243 (1989)) which measures the metabolic activity of cells by monitoring their rates of excretion of acidic metabolites. The ability to measure in real-time a broad cellular response to receptor activation provides us with a practical and general method of monitoring functional ligand-receptor interactions.

In the present study we have used the silicon microphysiometer to measure the dopamine responsive metabolic rates of cells transfected with human D_1 and D_2 receptors. The pharmacology of the metabolic responses are in good agreement with the predicted potency of various dopaminergic agonists and antagonists tested. Interestingly, the kinetic profile of the metabolic responses to D_1 and D_2 agonists appears to be different. Differences in the metabolic kinetics of these two classes of G protein-coupled receptors could be explained by the involvement of different effector coupling mechanisms and may provide insight into their relative roles in mediating a final response in the target cells.

36.13

UNCONVENTIONAL SECOND MESSENGER COUPLING FOR D1 AGONIST EFFECTS IN THE SUBSTANTIA NIGRA PARS RETICULATA (SNpr)? L.P. Martin and B.L. Waszczak. Pharmacol. Sect., Northeastern Univ., Boston, MA.

We have shown that iontophoreis of the D1 agonist SKF 38393 (SKF) markedly increases firing rates of rat SNpr neurons, whereas the D2 agonist LY 171555 lessens their inhibitory responses to GABA. The excitatory effect of SKF was later shown to require an intact striatonigral pathway. The current studies were undertaken 1) to determine whether the excitatory effect of SKF could be reproduced by another D1 agonist of a different chemical class, and 2) to examine the intracellular coupling mechanism by which D1 agonists increase SNpr firing. First, iontophoresis of the D1 agonist A-68930 (0.01M; 2-10 nA) produced current-related increases in SNpr cell firing similar to those seen with SKF, indicating that the effect is likely receptor-mediated and not a non-specific action of SKF. Second, we attempted to evaluate the role of Gs and cAMP as mediators of the D1 agonist effect. Accordingly, the Gi/Go protein inactivator pertussis toxin (PT) was injected into the nigra (1 ug/ul 0.9% NaCl) 24 hrs prior to recording, and the effect of SKF (0.01M, 2-10 nA) was re-evaluated. PT completely abolished the rate-increasing effect of SKF, suggesting that nigral D1 receptors mediating this response may be linked to their effector by a G-protein other than the conventional Gs. Next, we attempted to assess the role of cAMP in mediating the D1 agonist response by iontophoresing (5-20 nA) the membrane permeable cAMP analogs, dibutyryl-cAMP (db-cAMP), 8-Br-cAMP, and 8-(4-chlorophenylthio)-cAMP (CPT-cAMP), as well as the non-permeable analog, cAMP. While both db-cAMP and 8-Br-cAMP caused current-related increases in firing similar to SKF, CPT-cAMP and cAMP did not change baseline firing rates. Additionally, all four cAMP analogs were able to reduce the potency of applied GABA, an effect like that previously seen for the D2 agonist. Thus, cAMP analogs do not posses a profile identical to the D1 agonists. This may suggest either that cAMP does not mediate this response, or that cAMP analogs can exert effects outside the cell to lessen GABA inhibitions of SNpr cell firing. (Support: NS 23541)

36.14

D_1 DOPAMINE RECEPTORS DIFFERENTIALLY COUPLED TO PHOSPHOLIPASE C (PLC) AND ADENYLATE CYCLASE (AC) IN CNS. *B.D. Perry, M.S. Wainwright, H. Salti* J.T.Cuenco* and G. Farfel. Departments of Psychiatry and Pediatrics, The University of Chicago, Chicago, IL 60637*

Pharmacological and molecular characterization of the multiple dopamine receptor recognition sites (e.g., D_{1a}, D_{1b}, D_{2l}, D_{2s}, D_3, D_4, D_5) has preceded characterization of the transmembrane signal transduction mechanisms linked to these receptor subtypes in a given tissue or species. The present studies examined D1 mediated signal transduction in brain regions from rat and rhesus monkey. METHODS: D1 binding sites density ([125I]SCH 23982 saturation studies) and receptor-linked adenylate cyclase (AC) activity were determined in CNS membranes using standard methods. Receptor linked PLC activity in membranes was determined using a method developed in our lab. [3H]Inositol phosphates were determined following incubation of membranes @ 37 C using [3H]PIP2 as a substrate. RESULTS: In monkey hippocampus and rat cortex, containing relatively few D1 sites (approx. 100 fmol/mg prot), a consistent 50 % increase in basal PLC activity was observed following stimulation with SKF 38393, a selective D1 agonist, while D1 stimulated AC was less reliably observed. In rat caudate with a very high density of D1 sites, weak D1 stimulation of AC (50-75 % over basal) was observed. In other regions, low levels of D1 binding site were demonstrated with no reliable stimulation of either PLC or AC (e.g., rat olfact. tuber.) CONCLUSION: These studies demonstrate 1) a method for examining receptor linked PLC activity in CNS membranes and 2) heterogeneity of D1 mediated signal transduction across brain regions within a species, suggesting differential regional distribution of D1 'subtypes' or G-proteins or both. Utilization of clonal cell lines containing D1 receptors linked to both AC and PLC (see Wainwright et al., accompanying abstract) will allow examination of the relationships between receptor-linked signal transduction and the intramembrane stoichiometry of potential isoforms or subtypes of D1 receptor and various G proteins. (0-DA 00250/7)

36.15

D1 DOPAMINE RECEPTOR-MEDIATED PHOSPHOLIPASE C (PLC) ACTIVITY IN IMMORTALIZED MURINE CORPUS STRIATUM CELLS. *M.S. Wainwright, H. Salti*, A. Heller and B.D. Perry. Depts. of Pharmacological and Physiological Sciences and Psychiatry, The University of Chicago, Chicago IL 60637.*

The effects of a differentiating agent, n-butyrate (n-but), and a specific D1 agonist, SKF81297, on D1 receptor-mediated activation of PLC activity in immortalized corpus striatum (CS) cells were examined. We have demonstrated a 3 fold increase in D1 binding site density following n-but treatment in these cells (Wainwright, et al. Soc. Neurosci. Abstr. 16:646, 1990). Immortalized CS cells were prepared from embryonic (E18) murine brain using methods described previously (Hammond, et al. Science 234:1237-1240 1986). One of the fusion products was cultured in 1mM n-but with 10uM SKF38393 (a D1 agonist) for 5 days. Less than 0.1% of the cells survived this treatment and one colony of cells (E1X cells) was retained for further study.

E1X cells were treated for 5 days with either n-but (1mM) or 10uM SKF 81297 (SKF), a stable D1 agonist. PLC activity was measured in membrane fragments using [3H]PIP2 as a substrate. In the untreated E1X cells basal PLC activity was 16364 ± 2372 (values expressed as cpm/mg prot ± SEM). In E1X cells treated with n-but or SKF, the basal activity increased to 70891 ± 13254 and 67425 ± 16861 respectively ($p < 0.01$). D1 receptor-mediated stimulation of PLC activity in cell membranes was observed using 10uM SKF38393 (control cells 43 %; n-but 35%; SKF 32%; values are % increase from basal). Stimulation was inhibited by 100uM SCH23390. In the untreated parent N18TG2 line basal PLC activity was 23831 ± 5097 which was not increased following treatment with n-but (15868 ± 3235). As SKF38393 also stimulates adenylate cyclase activity in the E1X cells (ibid), these results suggest that in these immature cells, there are D1 receptors coupled to both second messenger systems. Further studies are required to determine whether these findings reflect expression of multiple D1 receptor subtypes or multiple G-proteins, or both. Supported in part by MH-28942, DA 00250/7 and the Brain Research Foundation of Chicago.

36.16

MULTIPLE D_1-LIKE DOPAMINE RECEPTORS NOT LINKED TO ADENYLATE CYCLASE EXHIBIT DIFFERENT AFFINITIES FOR [^{3}H]SCH23390. D.H. Mooney*, J.M. Petitto*, L.L. Cook, D.E. Nichols, and R.B. Mailman. Curriculum in Toxicology, Departments of Psychiatry and Pharmacology, University of North Carolina School of Medicine, Chapel Hill, N.C. 27599, and Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907.

Molecular studies in brain have identified at least two D_1-like dopamine receptors. ("D_1" and "D_5"). Both appear to be linked to adenylate cyclase stimulation, yet exhibit different recognition characteristics for some dopamine receptor ligands. Previously, this lab demonstrated that D_1-like receptors in amygdala are not linked to adenylate cyclase. In the present series of experiments, the recognition characteristics of these novel D_1-like receptors were compared to those of D_1 binding sites in striatum using the prototypical D_1 receptor ligand [^{3}H]SCH23390. In striatum, competition with SCH23390, as well as several phenothiazines (e.g., chlorpromazine), had kinetics which were consistent with interaction of the ligand with a single population of binding sites. Conversely, in the amygdala these same antagonists showed competition kinetics which clearly fit a model with two (or more) recognition sites.

Experiments were conducted to determine if these additional binding sites might be for other related receptor types. It was found that the binding of [^{3}H]SCH23390 to sites in the amygdala was not inhibited by ligands for serotonin (ketanserin and serotonin), opiate (naloxone), NMDA (MK801), or adrenergic (phentolamine and propranolol) receptors at concentrations of 1 μM or lower. This indicates that these multiple [^{3}H]SCH23390 binding sites were probably D_1-like dopamine receptors. Consistent with this, the new high potency, full efficacy D_1 agonist dihydrexidine competed for all of these [^{3}H]SCH23390 binding sites.

Together, these data provide evidence that there are subtypes of D_1-like receptors in the amygdala that can be partially discriminated by available phenyltetrahydrobenzazepine ligands. We have previously shown that [^{3}H]SCH23390 binding sites in the amygdala are not associated with dopamine adenylate cyclase. Thus, these data suggest additional multiplicity of this class of receptors, and offer the possibility that new drugs selective for D_1-like receptors can be designed. *(Supported by MH40537, MH42705, ES01104, and ES07126).*

36.17

NON-ADENYLATE CYCLASE COUPLED D_1 RECEPTORS IN THE BASOLATERAL AMYGDALOID NUCLEUS ARE COUPLED TO A GUANINE NUCLEOTIDE BINDING PROTEIN

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We have previously demonstrated that activation of D_1 dopamine receptors in the amygdaloid complex does not stimulate adenylate cyclase activity as in the caudate nucleus (Soc. Neurosci., Abstr., 15:1321, 1989). The present study was undertaken to determine whether D_1 receptors in the amygdaloid complex are coupled to a guanine nucleotide binding (G-) protein. Receptors which transduce signals via G-proteins exhibit decreased agonist affinity in the presence of GTP due to receptor-G-protein dissociation. This property was investigated for D_1 receptors in the basolateral amygdaloid complex using quantitative autoradiography of selective D_1 antagonist binding.

Dopamine (0-300 μM) displacement of [^{125}I]SCH 23982 (0.1 nM)binding in the presence and absence of GppNHp (10 μM), a non-hydrolyzable analogue of GTP, was measured on serial 10 μm thick coronal sections through the amygdaloid complex and caudate nucleus. GppNHp induced a rightward shift in the dopamine displacement curve for both nuclei, indicative of receptor-G-protein coupling. However, the magnitude of the shift is not as great in the basolateral nucleus as in the caudate. In addition, dopamine is more potent at displacing SCH 23982 in the basolateral nucleus than in the caudate nucleus, suggesting that amygdaloid D1 receptors may be structurally distinct from those in the caudate nucleus. (Supported by NIGMS-5T32GM07105 and MH-39967)

36.18

DARP-STIMULATED DA RELEASE FROM RAT STRIATAL FRAGMENTS IS BLOCKED BY SPECIFIC D-1 BUT NOT BY D-2 RECEPTOR ANTAGONISTS.

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DARP, a potent dopamine (DA) releasing protein was originally isolated from rat adrenal glands (Brain Research 463:385, 1988). The main actions of DA are mediated by at least two types of receptors, D-1 and D-2 which belong to the family of receptors acting thru G proteins. Herein, we report that a DARP preparation from the media of rat primary mesencephalic cell cultures releases DA and its action is blocked by SKF 83566, a specific D-1 antagonist. Rat striatal fragments were superfused in vitro as reported previously. After a 25-30 min equilibrium phase, DA in perfusates collected at 10 min intervals for an 80-90 min period was measured by HPLC-EC. The DARP or a control media extract was infused during interval 3 and 4. The data ($\bar{\chi}$ ±SEs, pg/mg/min of DA release) from each superfusion were expressed as the difference in DA release between the peak response and the lowest basal value. Curiously, an inverted U bell-shape dose-response was observed: CONTROL 200 µg protein = 8.1±4.6 (n=4). DARP: 14.6±6.9 (n=4); 17.7±6.6 (6); 33.5±6.6 (13); 25.3±11 (4) and 12.3±7 (4) for 25, 100, 200, 300 and 400 µg of protein, respectively. A dose of 200 µg of protein was selected to examine the effect of the antagonists. Spiperone dissolved in KRP and infused continuously from the beginning of the superfusion at concentrations ranging between $10x^{-10}$M to $10x^{-8}$M did not block the response to DARP. On the contrary, SKF 83566 blocked the action of DARP in a dose-dependent manner: $10x^{-10}$M = 36.6±7.9 (4); $5x10^{-10}$M = 19.3±3.4(4); 10^{-9}M = 6.5±3.4(8) and 10^{-8}M = 4.5±1.1(4). These results indicate that DARP interacts with D-1-like receptors and thereby controlling the release of DA.

36.19

SELECTIVE DOPAMINERGIC MECHANISM OF SKF38393-STIMULATED FORMATION OF INOSITOL PHOSPHATES IN RAT BRAIN SLICES.

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We have previously reported that dopamine (DA) and some D_1 receptor-effective agonists stimulate the formation of inositol phosphates (IPs) in rat brain slices. Because dopamine may activate α-noradrenergic receptors, and because some benzazepines have actions on serotonergic receptors, the present experiments were conducted to assess the dopaminergic selectivity of the observed stimulation of phosphoinositide hydrolysis. Rat striatal slices incubated with 0.48 μM (6 μCi/ml) [^{3}H]inositol in the presence of 10 mM LiCl were treated with up to 500 μM DA, serotonin (5HT), norepinephrine (NE), and the selective D_1 agonist, SKF38393 (SKF), and accumulated IPs determined after 90 min. All the agonists dose-dependently stimulated the formation of IPs; the greatest effects were obtained with the dopaminergic agents. The action of NE was dose-dependently blocked by prazosin but not by the selective D_1 antagonist, SCH23390, whereas the actions of DA and SKF were dose-dependently blocked by 1 to 100 μM SCH23390. Similarly, the effects of 5HT were blocked by the selective $5HT_2$ antagonist, ketanserin, the mixed $5HT_2/5HT_{1C}$ antagonist, mianserin, and, with much less potency, by the selective $5HT_{1C}$ antagonist, mesulergine, whereas neither ketanserin nor mianserin blocked the response to SKF. These observations indicate that the actions of DA and SKF in stimulating the formation of IPs is selectively mediated through a D_1-like DA receptor and not through the α_1-noradrenergic or the $5HT_2/5HT_{1C}$ receptors.

36.20

DOPAMINE (DA) STIMULATES [3H]PdBu BINDING TO CULTURED STRIATAL CELLS.

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The effect of DA on the binding of [3H]phorbol-12,13-dibutyrate ([3H]PdBu) in cultured rat striatal cells was examined. DA maximally increased specific [3H]PdBu binding by 70±10%, an increase comparable to that observed with norepinephrine(NE). This finding suggests that DA activates protein kinase C (PKC) in cultured striatal cells, since increases in [3H]PdBu binding reflect translocation of PKC. Half-maximal stimulation was observed with 10^{-6} M DA. The peak response was observed at two to three minutes after addition of 10^{-4} M DA, but [3H]PdBu binding was still increased above basal at 30 minutes. Prazosin (10^{-6} M) blocked the response to NE, suggesting mediation by an α_1 adrenergic receptor, but had little effect on the response to DA. Conversely, the D-1 receptor antagonist SCH23390 (10^{-6} M) blocked the response to DA but only partially inhibited the response to NE. Morphine (10^{-6} M) inhibited the response to DA by 46±14%, but did not significantly affect the response to NE. The DA effect on [3H]PdBu binding is apparently independent of the increase in cAMP seen on D-1 receptor activation. Forskolin, apomorphine, and the D-1 agonist SKF38393 all increased cAMP in striatal cells, but were less effective than DA in stimulating [3H]PdBu binding. The D-2 agonist quinpirole was ineffective in stimulating either cAMP or [3H]PdBu binding.

CATECHOLAMINES: DOPAMINE RELEASE

37.1

SIMULTANEOUS MEASUREMENT OF EXTRACELLULAR DOPAMINE IN THE MEDIAL PREFRONTAL CORTEX AND CAUDATE-PUTAMEN USING FAST-SCAN CYCLIC VOLTAMMETRY.

P.A.Garris and R.M. Wightman. Dept. of Chemistry and Curr. in Neurobiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3290.

The objectives of this study were to (1) establish the detection of extracellular dopamine (DA) in the medial prefrontal cortex (mPFC) using fast-scan cyclic voltammetry (FSCV) and (2) compare the release and reuptake of DA in this tissue with that in the caudate-putamen (CP). Carbon fiber microvoltammetric electrodes, stereotaxically placed in anethestized rats, were utilized to monitor changes in DA concentration as a result of medial forebrain bundle stimulation. The identification of DA was tentatively determined on the basis of cyclic voltammograms collected in the mPFC, CP and *in vitro*, and electrode placement. Evoked release in both the mPFC and CP was dependent on the frequency and duration of the pulse train. The maximum concentration of DA elicited by 120 pulses was 2- to 3-times higher in the CP (2 μM) than the mPFC (0.8 μM) at 60 Hz but similar at 30 Hz (0.5 μM). The experimental data derived from the CP but not the mPFC were successfully modeled using a Km of 0.14 μM. Conclusions: (1) FSCV is a suitable technique for the measurement of extracellular DA in the mPFC and (2) the dynamics of DA release and reuptake differ in the mPFC and CP.

37.2

NEUROCHEMICAL MODELING OF STIMULATED DOPAMINE OVERFLOW DETECTED USING *IN VIVO* VOLTAMMETRY.

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Electrically evoked overflow of dopamine in the rat striatum has been measured at low stimulation frequencies (10-20 Hz) in untreated rats and with single stimulation pulses in rats treated with nomifensine (20 mg/kg). These values are compared to overflow at higher frequencies (30-60 Hz) using a model based on a fixed dopamine concentration released per stimulus pulse ($[DA]_p$) and Michaelis-Menton uptake kinetics (1). The effects of diffusion and finite electrode response time are also considered. Dopamine overflow was measured using carbon-fiber microelectrodes stereotaxically placed in the striatum of anesthetized rats during electrical stimulation of the medial forebrain bundle. The value for $[DA]_p$ observed at low stimulation frequencies is 2-3 times greater than that obtained at high frequencies. Pharmacological evidence suggest this difference is, at least in part, a result of modulation of release by D_2 autoreceptors.

(1) R.M. Wightman, C. Amatore, R.C. Engstrom, P.D. Hale, E.W. Kristensen, W.G. Kuhr and L.J. May., *Neurosci.*, 25(2), 513-523, 1988

37.3

TEMPORALLY RESOLVED EFFECTS OF PRESYNAPTIC AUTORECEPTORS ON OVERFLOW OF DOPAMINE FROM SLICES OF CAUDATE NUCLEUS S.R. Jones, R.T. Kennedy and R.M. Wightman, Neurobiology Curriculum and Chemistry Department, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27499

Synaptic overflow of dopamine from rat caudate slices is detected using fast scan cyclic voltammetry at carbon fiber microelectrodes. The electrode potential is scanned at 300 V/s and the current resulting from dopamine oxidation is recorded every 100 ms. The current is directly proportional to the dopamine concentration. 5 s, 10 hz electrical stimulation was used to elicit overflow. The dopamine concentration profile consists of a peak of approximately 1.5 μM within the first second, and then a reduction to a plateau of approximately 0.2 μM for the duration of the stimulation. This unusual profile is due to autoreceptor activation. The D2 receptor antagonist sulpiride increases overflow by about 40% and alters the concentration profile so that the maximum concentration is maintained throughout the stimulation. The D2 agonist quinpirole decreases the peak height of stimulated dopamine overflow by approximately 50% and the concentration remains at a constant low level for the entire stimulation time. The kinetics of autoreceptor activation were deduced by modeling overflow with a kinetic model that takes uptake and different release rates into account. The data indicate that initial activation occurs within 100 milliseconds of stimulus onset. Autoreceptor off-rate kinetics were measured by the overflow response to a single electrical pulse at increasing times after a train of pulses.

37.4

DOPAMINE DIFFUSION AND UPTAKE IN SLICES OF RAT NEOSTRIATUM. C. Nicholson & M.E. Rice, Dept. Physiology & Biophysics, New York University Medical Center, New York, NY 10016.

Migration of dopamine (DA) in the extracellular compartment of the striatum is governed by the structural characteristics of the tissue and by a potent uptake system (Wightman et al., *Neuroscience*, 25: 513, 1988). We recently evaluated the diffusion properties of slices of rat striatum (*J. Neurophysiol.*, 65: 264, 1991), for which the tortuosity factor (λ) was 1.54, the extracellular volume fraction (α) was 0.21 and the concentration-dependent uptake term (k') was 0.01 sec^{-1}. Using these data, we have addressed quantitatively the contributions of diffusion and uptake to the clearance of DA from a local site of release. DA and α-naphthalene sulfonate, (ANS^-, an extracellular marker ion) were pressure ejected into striatum or adjacent cortex. DA was monitored with a carbon fiber microelectrode and high-speed cyclic voltammetry while ANS^- was detected with an ion-selective microelectrode. Diffusion distances were 90-150 µm. The resulting ANS^- diffusion profile, which was fitted to a solution of the diffusion equation, was used to estimate the volume ejected . In neocortex, DA diffusion was similar to that of ANS^-, with small ejected volumes (10-100 pL) that gave rise to micromolar DA increases. In striatum, no DA increase was detectable with the same ejected volumes. This avid uptake could be overcome by increasing ejected volume several-fold or by superfusing nomifensine (10-20 µM) or DA (10-100 µM). Under these conditions, k' was 0.1-0.2 sec^{-1}. That the DA curves could be fitted with this simple uptake term demonstrated that we were working in a linear portion of the DA uptake process. These data could be used to estimate *in situ* uptake parameters in an appropriate mathematical model. Supported by USPHS Grants NS-28642 and NS-28480.

37.5

ELECTROCHEMICAL EVALUATION OF THE MICROENVIROMENT SURROUNDING DIALYSIS PROBES *IN VIVO* AND *IN VITRO*. C.D. Blaha, R.K.Y. Lai, and A.G. Phillips. Department of Psychology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4.

Changes in extracellular dopamine (DA) concentrations were monitored in the striatum of the anesthetized rat using 1 s chronoamperometry (CA) with stearate-modified graphite paste electrodes placed 0-1mm away from a parallel 4 mm dialysis probe. 2 or 24 hrs following implantation of this assembly, dialysis with Ringers (1.3 mM Ca^{+2}) at various flow rates (0.1-5 ul/min) resulted in a rapid and maximal decrease in the DA baseline CA signal. DA in the dialysate decreased over a similar time-course but in contrast achieved steady-state values (10-50 nM) detectable by HPLC-EC. Maximal decreases in the CA signal were obtained at 40, 30, 25, 18 and 6 min using flow rates of 0.1, 0.2, 0.3, 0.5 and 5 ul/min, respectively. Stopping the flow of dialysate resulted in a gradual (30-40 min) recovery of the CA signal to pre-dialysis baseline levels. The zone of the dialysis-induced depletion of extracellular DA extended 1 mm distal to the dialysis probe (10% decrease in CA baseline). A 20 min dialysis pulse of 100 mM K^+ increased concentrations of DA in the dialysate by ~20 fold (10 nM to 200 nM). An increase in the CA signal above the dialysis-depleted CA baseline coincided with this change and corresponded to a 200-300 nM increase in extracellular DA concentrations. Inclusion of 5 uM DA in the perfusate resulted in a full recovery of the CA baseline signal at electrodes flush to the probe. Electrode calibration *in vitro* in combination with probe recovery values (~10%) indicated that the amount of dialysis-induced depletion of DA corresponded to ~500 nM. These results indicate that microdialysis induces a significant reduction of DA in the extracellular space surrounding the dialysis probe and may account for the discrepancy between estimations of basal DA concentrations using these two *in vivo* techniques.

37.6

QUANTITATIVE NEUROTRANSMITTER MICRODIALYSIS: EXTRACELLULAR DOPAMINE IN THE STRIATUM, A.D. Smith, R.J. Olson and J.B. Justice,Jr., **Department of Chemistry, Emory University, Atlanta, Georgia, 30322.**

An in vivo microdialysis method was used to estimate the basal extracellular concentration of dopamine (DA) in the anterior striatum of behaving rats. In this method, anesthetized rats (n = 5) were implanted with 20 gauge guide cannula in the striatum at coordinates AP +2.5, LR +2.7 from bregma and DV -2.7 from dura. Four millimeter dialysis probes were inserted in the animals 3-4 days after surgery. Animals were perfused overnight with artificial CSF (149 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.25 mM ascorbic acid and 5.4 mM d-glucose, pH 7.2-7.4) at a flow rate of 0.2 ul/min. Approximately seven hours after probe insertion, perfusate samples were collected at 20 minute intervals and a 0.5 ul aliquot was injected onto a smallbore HPLC system. Samples were analyzed until stable levels of dopamine were obtained (approximately 2-3 hours), at which time the perfusate containing zero DA was switched in a random order for each animal to an artificial CSF containing 0, 2.5, 5, 10 or 20 nM DA. These concentrations were chosen such that the expected value would fall within the range of the perfusate DA concentration so that the point of no net flux could be determined by linear regression. At this point, the dialysate concentration represents the basal extracellular concentration.

Using this method, the basal extracellular concentration of DA in the striatum was estimated to be 11.1 nM ± 1.57 (Mean ± S.E.). The in vivo recovery obtained from the slope of the linear regression was 70% ± 10%. The concentration of DA in the striatum is thus about twice that found in the nucleus accumbens (Parsons et al., Soc. Neuroscience Abs. 432.22, 1990) where the basal extracellular concentration of DA was found to be 5 nM.

37.7

IN VIVO STRIATAL DA RELEASE AND METABOLISM IN CHRONIC NEUROLEPTIC-TREATED RATS FOLLOWING ACUTE INJECTION OF QUINPIROLE AND PILOCARPINE. Ronald E. See and Mary Ann Chapman, Dept. of Psychology, Washington State University, Pullman, WA, 99164-4820.

The present study examined the effects of the dopamine D2 receptor agonist, quinpirole, and the muscarinic receptor agonist, pilocarpine, on DA release and metabolism using in vivo microdialysis in chronic neuroleptic-treated rats. Female, Sprague-Dawley rats were administered weekly subcutaneous injections of haloperidol (7.0 mg/kg), raclopride (7.0 mg/kg), or distilled water vehicle. After 28 weeks of neuroleptic treatment, rats were anesthetized with Equithesin and bilateral guide cannulae implanted into the striatum (A +0.2, L +3.0, V -5.0). Following one week of recovery, microdialysis probes with 3 mm of dialysis membrane were inserted into the cannulae (unilateral) and perfusion initiated. Samples were collected every 20 min before and after drug injection. Perfusates were directly injected onto an HPLC column and analyzed for DA, DOPAC, and HVA using electrochemical detection. For the first probe insertion, conducted 5 days after the weekly injection of neuroleptic, rats were administered quinpirole (0.03 mg/kg, SC). One week later, rats were reprobed contralateral to the first side and then administered pilocarpine (1.0 mg/kg, IP). Quinpirole produced a significant decrease in DA release and levels of DOPAC and HVA in all 3 groups. However, the duration of decreased DA release was significantly prolonged in the two neuroleptic treated groups as compared to the control group. Injection of pilocarpine had no significant effect on any measure in any of the 3 groups. Prior long term exposure to neuroleptics thus appears to alter autoreceptor feedback to a DA agonist while direct muscarinic receptor stimulation produces no discernible effects on striatal DA release and metabolism.

37.8

EFFECTS OF CLOZAPINE ADMINISTRATION ON STRIATAL NEUROTRANSMITTER OVERFLOW IN THE SQUIRREL MONKEY AND RAT MEASURED BY MICRODIALYSIS. L.W. Cooke, L.M. Ball, F.W. Ninteman, T.G. Heffner, M.D. Davis, Department of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Clozapine is an antipsychotic agent with improved efficacy and reduced neurological side effects. We have assessed the effects of clozapine on neurotransmitter overflow in rats and non-human primates, using the method of intracerebral microdialysis (ICMD). Guide cannulae were surgically implanted bilaterally in the striatum of Sprague Dawley rats and the putamen of squirrel monkeys (Saimiri Sciurea) to allow repeated, acute implantation of 4 mm microdialysis probes. Collected dialysates were analysed for neurotransmitter levels using HPLC separation and electrochemical detection. In rats single doses of clozapine (20 mg/kg S.C.) produced increases in dialysate DA levels of 68 ± 1.5% similar to published reports. Oral administration and i.p. dosing produced smaller changes. Levels of Dopac, HVA, and 5-HIAA in the dialysates appeared unaffected. In squirrel monkeys oral doses of clozapine (30 mg/kg), resulted in an increased DA overflow of 18 ± 5%. Again, metabolites appeared to be unaffected. In conditioned avoidance tests, the ED50 of clozapine was 4.2 mg/kg (p.o.) for squirrel monkey compared to 22 mg/kg for rat. These data demonstrate similarities between monkey and rat neurochemical responses to behaviorally active doses of clozapine, and support the feasibility of conducting ICMD studies in awake squirrel monkeys.

37.9

SYSTEMIC AMPHETAMINE INCREASES STRIATAL EXTRACELLULAR DA AND cAMP AS MEASURED VIA MICRODIALYSIS. A.T. Massey*, J.S. Randall, and P.K. Randall. College of Pharmacy and Inst. for Neuroscience, Univ. of Texas, Austin TX, 78712.

The stimulant actions of amphetamine are believed to result from release of endogenous dopamine (DA). In this study we utilized *in vivo* microdialysis to examine the relationship between amphetamine-induced increases in extracellular levels of DA and in its second messenger, adenosine 3'5' monophosphate (cAMP), following systemic injection.

Microdialysis probes with a 3mm membrane tip were calibrated *in vitro* (DA recovery=20%, cAMP recovery=23%) and stereotaxically inserted into the anterior striatum (AP +0.8, L +3.0, DV - 7.0 from Bregma) of chloral hydrate-anesthetized rats. Probes were perfused at 2 µl/min with artifical cerebrospinal fluid and sample collection began immediately after insertion. Samples were collected at 30 min intervals for 3 hr prior to IP injection of 0, 0.5, 2.0, or 8.0 mg/kg d-amphetamine sulfate in 0.9% saline. Post-injection samples were collected at 15 min intervals for 1 hr and at 30 min intervals thereafter. In each sample, DA levels were assessed via HPLC with electrochemical detection and cAMP by RIA.

Dialysate levels of DA and cAMP were highly elevated immediately following probe insertion and fell rapidly over the pre-injection period to a stable baseline (DA, 0.6±0.09 pg/µl; cAMP, 0.19±0.01 fmol/µl). As expected, amphetamine produced a pronounced dose-related increase in DA levels which peaked at all doses during the second 15-min post-injection collection period (i.e. 15-30 min following injection). A similar, though less dramatic, dose-related increase was observed in extracellular cAMP levels. The cAMP response consistently lagged behind the DA response by 1 to 2 time-periods (15-30min). Peak levels after the 8.0 mg/kg dose were elevated by approximately 2 fold over baseline values.

These data show that systemic administration of amphetamine produces a significant elevation of striatal extracellular cAMP *in vivo*. This increase is likely to be the result of increased DA release. cAMP, however, rises and disappears from the extracellular fluid at a less rapid rate than does DA itself. This may provide a convenient *in vivo* response for quantitative studies of DA and DA antagonist action at central D-1 receptors.

37.10

CHARACTERIZATION OF METHAMPHETAMINE-INDUCED DOPAMINE RELEASE IN MOUSE NEOSTRIATUM AS MEASURED BY *IN VIVO* MICRODIALYSIS. G. DeGeorge, A. Giovanni and P.K. Sonsalla. Department of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The administration of methamphetamine (METH) causes damage to nigrostriatal dopamine (DA) neurons. Although the mechanism by which METH produces neurotoxicity is unknown, there is a role for dopamine itself in mediating this toxicity. It has been shown *in vitro* in several preparations and *in vivo* in the rat by microdialysis that METH causes the release of DA. We have been studying mechanisms of METH-induced neurotoxicity in the mouse. In order to understand these mechanisms better, we have adapted the *in vivo* microdialysis technique to measure levels of monoamines in the extracellular compartment of the mouse striatum. A single i.p. injection of METH (5 mg/kg), produced a 400-500% increase in the amount of DA recovered through the probe, whereas levels of DOPAC fell to approximately 50% of basal values. The peak levels of DA release occurred approximately 60 min after METH treatment whereas DOPAC dropped to its lowest level within 90-120 min. Return to basal levels of DA was not complete at 5 hrs, nor did DOPAC levels fully recover during this time. This effect of METH on DA release *in vivo* in the mouse is similar to that which is seen in the rat. Thus the technique of *in vivo* microdialysis can be done in the mouse and will be useful for studying the many facets of neurotransmitter release in this species.

37.11

KAPPA-OPIOID AGONISTS DEPRESS RAT STRIATAL DOPAMINE OVERFLOW IN VIVO AND IN VITRO: AN INTRACEREBRAL MICRODIALYSIS AND NIGROSTRIATAL EXPLANT STUDY. M.D. Davis and L.M. Ball. Neurosciences Section, Parke-Davis Research Division, 2800 Plymouth Rd., Ann Arbor, MI 48105.

Opioid modulation of dopamine (DA) neurotransmission is a poorly understood phenomenon. Mu- and delta-opioid agonists facilitate brain DA release while kappa-opioid agonists attenuate release. Here we have focused on the selective kappa-agonists, U-50488H and CI-977, and their interaction with the nigrostriatal DA projection in the rat. In in vivo microdialysis studies, both anesthetized and awake male SD rats were acutely implanted with 4mm BAS/CMA probes into the striatum, while core explants of the intact nigrostriatal pathway were obtained for acute in vitro experiments. Dialysate and superfusate samples were collected and analyzed for catecholamine content via HPLC-EC. In some explant studies, microelectrode voltammetry was used to measure DA overflow. CI-977 administered 5 mg/kg ip, 0.1 mg/kg im, 10 mg/kg po or luM infused directly through the probe caused a marked inhibition of spontaneous DA overflow. DOPAC and HVA levels also dropped appreciably, while 5-HIAA levels were not significantly altered. Infusion of the kappa-antagonist, nor-binaltorphimine (luM), through the probe blocked the effect of CI-977 and, by itself, elevated DA levels above baseline. Electrical stimulation (5Hz) of the medial forebrain bundle evoked a five-fold increase in DA efflux which was almost totally inhibited by luM bath administration of CI-977 or U-50488H.

37.12

EVIDENCE FOR IMPULSE FLOW -DEPENDENT AND -INDEPENDENT RELEASE OF DOPAMINE IN THE RAT PREFRONTAL CORTEX AND STRIATUM. S.J.Chrapusta*, F.Karoum, M.F.Egan* and R.J.Wyatt. Neuropsychiatry Branch,, NIMH, Neuroscience Center at St. Elizabeths Hospital, , Washington, D.C. 20032.

Dopamine (DA) release from DA cell bodies is impulse flow-independent (IFI); some data suggest a similar mechanism may also exist in the terminals. We examined the effects of gammabutyrolactone (GBL) on DA content and release using accumulation of 3-methoxytyramine (3-MT acc.), 10 min after pargyline (75 mg/kg, i.p.), as an index of DA release. Thirty min after GBL (750 mg/kg, i.p.), DA content in the striatum (ST) and prefrontal cortex (PFC) was maximally increased, and 3-MT acc. was maximally decreased, suggesting maximal impulse flow blockade. GBL produced approximately 90% drop in the striatal 3-MT acc., while in the PFC only approx. 50% reduction was achieved. Changes in 3-MT acc., 30 and 90 min after GBL, suggested faster recovery of DA release in the PFC than in the ST. Our data indicate that most DA release in the ST is impulse flow-dependent, while in the PFC approximately 50% of DA is released via an IFI mechanism that may play an important role during chronic neuroleptic treatment.

37.13

EFFECTS OF MONOAMINE OXIDASE INHIBITION ON THE IN VIVO RELEASE AND METABOLISM OF DOPAMINE IN RAT BRAIN: COMPARISON BETWEEN STRIATUM AND SUBSTANTIA NIGRA. Dora Orosz (1) and James P. Bennett, Jr. (1,2,3). Departments of Neurology (1), Behavioral Medicine and Psychiatry (2) and Pharmacology (3). University of Virginia School of Medicine. Charlottesville, VA 22908.

L-Dihydroxyphenylalanine (L-DOPA), the amino acid precursor of dopamine (DA), is used in the treatment of the DA deficiency state in Parkinson's disease (PD). Selective inhibition of the B isoenzyme of monoamine oxidase (MAO-B) by l-deprenyl is used clinically in PD patients receiving L-DOPA.

We have used regional in vivo microdialysis in normal rats receiving L-DOPA to define the contributions of the two MAO-isoenzymes on the metabolism of DA in both the cell body and the axon-terminal region of the nigrostriatal system.

Dialysis probes were implanted unilaterally into the striatum and substantia nigra zona compacta of intact rats; both probes were simultaneously perfused in awake, behaving animals. Dialysate DA and metabolites were quantitated by HPLC-EC detection.

Non-selective inhibition of MAO by pargyline (40 mg/kg I.p.) inhibited DOPAC formation and increased basal efflux of DA and 3-methoxytyramine in striatum and nigra. MAO-B selective doses of l-deprenyl had less pronounced effects on basal DA efflux after acute administration, demonstrating a major role for MAO-A in regulating extracellular DA metabolism in the intact rat nigrostriatal system.

Additional experiments of the effects of MAO-inhibitors on L-DOPA-induced DA efflux demonstrate a similar major role of MAO-A in regulating extracellular DA and are examining the role of alternate DA catabolic pathways in the striatum and substantia nigra under conditions of increased DA synthesis and release.

(Supported by NIH-NINDS grants NS00978 and NS26581. L-deprenyl was a kind gift of Prof. J. Knoll, Semmelweiss Univ Med Sch, Budapest, Hungary.)

37.14

(-) DEPRENYL ALTERS GUINEA PIG STRIATAL DOPAMINE (DA) METABOLISM BUT DOES NOT ALTER EXTRACELLULAR DA LEVELS. I.A. Paterson, A.V. Juorio and M.Y. Zhu*. Neuropsychiatric Research Unit, University of Saskatchewan, Saskatoon, SK, Canada.

It has been shown that acute administration of (-) deprenyl, a specific monoamine oxidase (MAO) B inhibitor, does not alter rat striatal dopamine (DA) metabolism. The guinea pig striatum may be a better model of human DA metabolism since the ratio of MAO-B:MAO-A is higher. Studies were performed on English short-hair guinea pigs to determine the effects of acute administration of (-) deprenyl on striatal dopamine. Striatal MAO activities were determined by radioenzymatic assay. (-) Deprenyl (0.5-4 mg/kg, i.p., 2h) inhibited MAO-B to 29-8% of control without inhibiting MAO-A activity. Striatal levels of DA and the metabolites, DOPAC and HVA, were determined by HPLC-EC. Deprenyl (2-4 mg/kg, i.p., 2h) decreased DOPAC levels to 70% of control but did not alter HVA levels. The 4 mg/kg dose increased DA levels to 123% of control. Intracerebral microdialysis was performed in striata of anaesthetised guinea pigs and dialysates were assayed by HPLC-EC. Deprenyl (2-4 mg/kg, i.p.) did not alter levels of DA in the dialysate for upto 4 h where as pargyline, an inhibitor of both MAO-A and MAO-B, (75 mg/kg, i.p.) produced an increase in DA levels in the dialysate to 256-304% of baseline. It is concluded that in the guinea pig striatum, specific inhibition of MAO-B by (-) deprenyl significantly reduces the deamination of DA to DOPAC but does not result in a change in extracellular DA concentrations.

Supported by Saskatchewan Health and Saskatchewan Health Research Board.

37.15

DEPRENYL (SELEGILINE) AFFECTS DOPAMINERGIC NEUROTRANSMISSION IN RAT VIA METABOLITES. H. Nissbrandt, T. Elebring* and G. Engberg*. Department of Pharmacology, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden.

Utilizing behavioral, biochemical, and electrophysiological methods, central effects of the MAO-B inhibitor deprenyl (selegiline) were analyzed. Administration of deprenyl (3 - 30 mg/kg, i.p.) caused a dose-dependent increase in the spontaneous locomotor activity. In the striatum deprenyl (10 and 30 mg/kg) changed the DOPA accumulation following NSD 1015 in a biphasic manner. Deprenyl slightly decreased the firing rate of DA containing neurons in substantia nigra, zona compacta. However, the increases in locomotor activity and DOPA accumulation induced by deprenyl were almost totally prevented by pretreatment with the microsomal liver enzyme inhibitor SKF 525 A (50 mg/kg, i.p., 30 min), indicating that metabolites of the drug are of pharmacological significance for its central actions. Furthermore, administration of l-methamphetamine, a major metabolite of deprenyl, affected spontaneous locomotor activity, striatal DOPA formation and the firing rate of dopamine (DA) containing neurons in the substantia nigra within the same magnitude as deprenyl itself when given in doses relevant to the formation of l-methamphetamine from deprenyl. However, unlike the effect of deprenyl, the l-methamphetamine-induced increase in locomotor activity and striatal DOPA formation was not antagonized by pretreatment with SKF 525 A. The data suggest that the stimulatory effect of deprenyl on locomotor activity and DA synthesis is not related to a MAO-B blocking action of the drug or to a putative effect on DA re-uptake, but rather to effects of metabolites of the drug, e.g. l-methamphetamine. It is proposed that metabolites of deprenyl should not be disregarded to account for the clinical benefits of the drug.

37.16

ENDOGENOUS PHENYLETHYLAMINE & PHENYLACETIC ACID AS INDICATORS OF THE SPECIFICITY OF INHIBITION OF MAO IN THE RAT STRIATUM. L. E. Dyck and A. A. Boulton. Neuropsychiatric Research Unit, Dept. of Psychiatry, Univ. of Saskatchewan, Saskatoon, Canada S7N 0W0

Endogenous phenylethylamine (PE) in the rat striatum seems to be a substrate specifically for type B MAO, while tryptamine (TRA), m- and p-tyramine (mTA and pTA) appear to be substrates for both A and B MAO. In the following study, brofaromine (BFO), a reversible type A MAOI, and clorgyline (CLOR), an irreversible A inhibitor, were injected s.c. and i.p. acutely and chronically (7 days) into male Wistar rats and the levels of PE, mTA, pTA, TRA, phenylacetic, m- & p-hydroxyphenylacetic acids and DOPAC in the striatum quantified. While mTA, pTA and TRA levels were increased by BFO administration, PE levels were unaffected. The effects of acute BFO on the levels of these amines did not differ from those of chronic treatment. Similar results were observed with CLOR, but in this case, the effects of chronic CLOR on amine levels were greater than those of acute CLOR. Striatal levels of DOPAC, m- & p-hydroxyphenylacetic were decreased dose - dependently by acute BFO. By contrast, phenylacetic acid levels were not reduced even by 100 mg/kg BFO. It appears, therefore, that striatal PE and phenylacetic acid levels can be employed as endogenous indicators of the specificity of inhibition of MAO, striatal PE levels being increased and phenylacetic acid decreased only by type B and not by type A MAO inhibitors. Supported by Saskatchewan Health and Ciba-Geigy Canada Ltd.

CATECHOLAMINES: GENERAL

38.1

NEUROMELANIN ACCUMULATION WITH AGE IN THE MACACA FASCICULARIS BRAIN-STEM. M.T. Herrero[1,2], E.C. Hirsch[1], A. Kastner[1*], M.R. Luquin[2], F. Javoy-Agid[1], J.O. Obeso[2] and Y. Agid[1]. (1) INSERM U289, Hôp. Salpêtrière, 75013 Paris, France and (2) Neurologia Experimental, Univ. Navarra, 31080 Pamplona, Espana.

Neuromelanin (NM) an auto-oxidation by-product of catecholamine synthesis accumulates with age in the locus coeruleus and the substantia nigra in primate and dogs. The present experiments were undertaken in order to examine the time-course dependency of NM accumulation in the mesencephalon of Macaca fascicularis.

The intraneuronal presence of NM was analyzed in the mesencephalon of 5 monkeys (age : 7 days, 1.4, 3.5, 8 and 13 years). The brain-stem was fixed, frozen and cut in 40 µm serial sections covering the whole span of the mesencephalon from the subthalamic nucleus to the locus coeruleus. Regularly spaced (640 µm) sets of sections were stained by Fontana method in order to detect NM, by tyrosine hydroxylase immunocytochemistry to visualize catecholaminergic neurons or by thionin for cytoarchitectonic study. "Visible NM" was analyzed on an additional set of sections which were not stained. Each neurons was plotted and total number of neurons were counted using image analysis.

In the youngest monkey (7 days old) catecholaminergic neurons were not melanized. In the 1.4 years old monkey, 30% of the catecholaminergic neurons contained NM visible in Fontana stained sections but not detectable in unstained sections. In the older monkeys, NM-containing neurons represented 60% of the catecholaminergic neurons, without further modification of this proportion with age. Our results suggest that NM content increases with age in the neurons of the mesencephalon and that NM first appears as uncolored NM, the exact nature of which remain to be determined.

38.2

POSTMORTEM STABILITY OF MONOAMINES IN RAT BRAIN REGIONS P.J. Kontur, R.B. Innis, R.H. Roth. Depts. Pharmacology and Psychiatry, Yale U. Sch. Med. New Haven, CT 06510.

Two experimental paradigms that reflect handling of postmortem human brain tissue prior to neurochemical analysis were studied in postmortem rat brain tissue. Time and temperature dependent changes were analyzed by incubating rat brain tissue in situ for 0, 1, 3, 6, 9, 12 or 15 hours before it was dissected and stored. The incubation conditions mimicked the typical decrease in temperature of human brain left in situ for similar time periods. The effects of time of storage were analyzed by dissecting and storing rat brain tissue at -70°C for 0, 0.5, 1, 6 or 8 months. The levels of monoamines and metabolites in the striatum, cingulate and occipital cortex of these brains were measured using alumina extraction and HPLC methods. The gradual decrease in temperature over 15 hours resulted in 1) decreased levels of striatal DA and DOPAC and increased levels of HVA, 2) decreased levels of cingulate cortex NE and 5HT and increased levels of DA, DOPAC, HVA and HIAA, and 3) decreased occipital cortex NE levels, both increased and decreased 5HT levels and increased HIAA levels. Long term storage resulted in 1) decreased striatal DA levels and an uneven pattern of increase in DOPAC levels, 2) decreased cingulate NE levels and increased DA and DOPAC levels, and 3) decreased occipital cortex NE levels. These results demonstrate that there are time, temperature and storage dependent regional differences in the stability of monoamines and metabolites. The analysis of monoamines and metabolites levels in post-mortem human brain tissue should account for time, temperature and storage parameters in order to accurately interpret the effects of neurological disorders on neurotransmitter dynamics. Supported by USPHS Neuroscience Center grant #5-P50-MH44866.

38.3

NORADRENALINE RELEASE FROM RAT FRONTAL CORTEX AND HIPPOCAMPUS AFTER ELECTROCONVULSIVE SHOCK (ECS). D.N. Thomas*, D.J. Nutt* and R.B. Holman. Reckitt and Colman Psychopharmacology Unit, The Medical School, Bristol, BS8 1TD, UK.

The extracellular concentration of NA in frontal cortex (FC) is regulated primarily by presynaptic α_2 autoreceptors, while in hippocampus (HI) transmitter uptake is predominant (Thomas et al., 1990 J.Neurochem. 56). Chronic ECS is an effective treatment for depression. Biochemical and behaviourial evidence indicate that α_2-adrenoreceptors are downregulated with chronic treatment. The present study uses in vivo microdialysis to assess the effects of acute and chronic ECS on endogenous NA release and on α_2-adrenoreceptor sensitivity. Concentric dialysis probes were implanted into either the HI or FC of chloral hydrate anaesthetised rats. Basal NA concentrations in the HI and FC were 4.5 ± 0.3 (mean ± SEM, n=5) and 4.1 ± 0.3 pg/sample (n=4) respectively. Acute ECS significantly and immediately increased NA release in both regions [HI 21.4 ± 1.2 (n=5); FC 11.6 ± 1.2 (n=4) pg/sample] as compared with controls (ear clip). In the HI chronic ECS (100mA; 1sec x7 days) did not change either basal NA release or the increase in release following an additional ECS on day 8. In contrast, in FC chronic ECS significantly elevated basal release (6.9 ± 1.0 pg/sample, n=4) without affecting the ECS-stimulated NA release on day 8 as compared with the controls. In subsequent experiments, chronic ECS treated rats were challenged with the α_2 antagonist idazoxan (IDX). In the HI, IDX elicited significant increases in NA release in both the chronic ECS and control groups. However, in FC after chronic ECS the response to IDX was abolished as compared with the control. These data show that chronic, but not acute ECS causes a regionally (FC not HI) specific increase in basal NA release. Further, the lack of response to IDX in the FC after chronic ECS is consistent with a downregulation of presynaptic α_2-adrenoreceptors.

38.4

[^{3}H]-TOMOXETINE: A HIGHLY SELECTIVE, ENANTIOMERICALLY PURE RADIOLIGAND FOR NOREPINEPHRINE UPTAKE SITES IN BRAIN. D. T. Wong, D. W. Robertson, S. L. Gackenheimer, L. Reid, D. A. Schober, D. C. Thompson, and D. R. Gehlert. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

Studies of the presynaptic norepinephrine (NE) uptake carrier have been seriously hampered by the lack of radioligands which bind with high affinity and specificity for this site. Radiolabeled antidepressants, including [^{3}H]-desipramine and [^{3}H]-mazindol, have been used to label the NE uptake carrier, but these agents are fraught with high levels of nonspecific binding and also bind to multiple uptake and receptor sites within the CNS. Racemic [^{3}H]-nisoxetine was recently reported to be a specific radioligand for the NE uptake carrier. A related molecule, tomoxetine, is currently in widespread clinical trials as an antidepressant, and we now report that [^{3}H]-tomoxetine is a potent, highly specific, enantiomerically pure radioligand for the NE uptake carrier. Binding of [^{3}H]-tomoxetine was saturable and it labeled a homogeneous population of sites in synaptosomal membranes of rat cerebral cortex (K_d = 0.30 ± 0.01 nM, B_{max} = 47.1 ± 0.6 fmol/mg protein). Specific binding of [^{3}H]-tomoxetine was sodium dependent, and approximately 70% of total binding was specific at the K_d concentration. Binding was displaced by other compounds which inhibit the NE uptake carrier, including nisoxetine, tomoxetine, desipramine, and nortriptyline, but binding was not displaced by specific inhibitors of the 5HT uptake carrier, including sertraline and fluoxetine. Displacement of [^{3}H]-tomoxetine binding was stereospecific; for example, the (+) enantiomer of nisoxetine was considerably more potent than the (-) enantiomer (IC_{50} values were 1.4 and 26.9 nM, respectively). Autoradiographic studies indicated that [^{3}H]-tomoxetine bound to rat brain regions containing a high density of NE containing fibers and cell bodies, including the locus coeruleus, anterior dorsal thalamus, and the nucleus of the solitarus tract. These data suggest that [^{3}H]-tomoxetine is a useful radioligand for exploration of the physiology and pharmacology mediated via NE uptake carriers.

38.5

SYNTHESIS AND ADRENERGIC ACTIVITY OF A SEMI-RIGID ANALOG OF 6-FLUORONOREPINEPHRINE (6-FNE). S. N. Calderon,[1]* F. Gusovsky, H. M. Garraffo,* J. W. Daly, J-Y Nie,* D. C. Furlano,* A. H. Newman[1], K. L. Kirk,* Lab. of Bioorganic Chemistry, NIDDK, NIH, Bethesda , MD 20902. [1]NIDA Addiction Research Center, Baltimore, MD 21224.

We reported previously that 2-fluoronorepinephrine (2-FNE) is a selective β-adrenergic agonist while 6-FNE is a selective α-adrenergic agonist. Similar adrenergic selectivities are present in fluorinated epinephrines, phenylephrines and isoproterenols. These observations have led to the development of new pharmacological and potential medicinal agents. The mechanism of fluorine-induced adrenergic selectivities, although studied extensively, is still unknown. We now report further experimental evidence pertinent to this question. The use of semi-rigid analogs has proven to be an effective strategy to investigate the participation of conformational isomerism in drug activity. We have synthesized the semi-rigid analog of norepinephrine and 6-FNE, *trans*-2-amino-5,6-dihydroxy-1,2,3,4-tetrahydro-naphthalenol and *trans*-2-amino-5,6-dihydroxy-8-fluoro-1,2,3,4-tetrahydro-naphthalenol, respectively, by cyclization of the precursor arylbutanoic acids, followed by elaboration of the aminoalcohol functionality and deprotection of the catechol group. The α- and β-adrenergic activities of these analogs were evaluated through inhibition of binding of appropiate ligands for these receptors and through stimulation of cyclic AMP accumulation in C6 glioma cells. While the non-fluorinated analog had selective β_2-adrenergic activity the fluorinated analog had poor β-adrenergic activity.

38.6

PRESENCE OF CATECHOLAMINE TRANSPORT IN RAT BRAIN CELL NUCLEI. Nguyen T. Buu. IRCM, Department of Medicine, Université de Montréal, Montréal, Québec, Canada H2W 1R7

There is increasing evidence that catecholamines (CA) are involved in gene expression and growth although the exact mechanism of their regulation remains unclear. Growth regulation and gene expression occur in the cell nucleus and translocation of several peptides and hormones involved in gene regulation has been demonstrated in the nuclei of different cell lines. However, the translocation of CA into the cell nucleus is not known and is the aim of this study.

Uptake was studied using radiolabeled dopamine (DA) and norepinephrine (NE) in cell nuclei isolated from whole rat brain and verified for purity by electron microscopy. Cell nuclei were also incubated with L-DOPA and CA were measured by HPLC.

The results showed that cell nuclei from rat brain can uptake both DA and NE. This uptake is saturable and can be inhibited by N-ethylmaleimide (10 μM) but is not affected by reserpine. Incubation of cell nuclei with L-DOPA (1mM) generates DA and this production can be abolished by NSD-1015, suggesting the presence of dopa decarboxylase.

Thus cell nuclei from rat brain uptake CA and generate DA from exogenous L-DOPA. The meaning of the CA system in the cell nucleus remains to be investigated. Supported by the Canadian Heart and Stroke Foundation (Quebec) and le Fonds interne de la Faculté de Médecine, Université de Montréal.

38.7

THIOPHOSPHORYLATION OF CHROMAFFIN VESICLE MATRIX PROTEINS IN CULTURED BOVINE CHROMAFFIN CELLS. J.C. Brooks and M.H. Brooks. Marquette Univ. Sch. of Dent., Milwaukee, WI 53233.

Cultured bovine chromaffin cells were incubated with medium in which inorganic phosphate was replaced with inorganic [^{35}S]-thiophosphate. Within 5 min., label was found in a broad 97-121 kDa band on SDS-PAGE, with increasing incorporation over a 40 min period. Subcellular fractions were prepared from these cells and cells exposed for 3 days to inorganic thiophosphate. The 97-121 kDa radiolabeled proteins were present in isolated chromaffin vesicles and were released to the medium upon osmotic lysis of the vesicles. Vesicle membrane proteins were not thiophosphorylated.

Vesicles prepared from cells or adrenal medulla in isosmotic media were energized by ATP but not by the corresponding thiophosphorylated analog, adenosine-5'-O-(3-thiotriphosphate) (ATPγS). Neither ATPγS nor TP_i was incorporated into the 97-121 kDa proteins of isolated intact or disrupted chromaffin vesicles although there was incorporation into a 47 kDa protein.

We hypothesize that TP_i is rapidly taken up by chromaffn cells, synthesized into ATPγS and used for thiophosphorylation reactions. The physiological significance of the rapid and intense thiophosphorylation of vesicle matrix proteins is not yet evident. Supported by NIH Grant 1 A15 NS23101-01A2.

SEROTONIN RECEPTORS: $5HT_{1A}$ I

39.1

IN VIVO MICRODIALYSIS EVIDENCE FOR CENTRAL 5-HT_1 RECEPTOR BLOCKING PROPERTIES OF THE β-ADRENOCEPTOR ANTAGONIST (-)PENBUTOLOL. *S. Hjorth, T. Sharp* & D.G. Grahame-Smith*, Dept. of Pharmacol., Univ. of Göteborg, Box 33 031, S-400 33 Göteborg, SWEDEN, and MRC Unit of Clin. Pharmacol., Oxford, U.K.*

Selected β-adrenoceptor antagonists (*e.g.* pindolol, alprenolol, propranolol) are currently used as probes for 5-HT_1 receptors in various experimental paradigms. Recently, we found that (–)penbutolol (PB), another β_1/β_2-adrenoceptor blocking agent, prevents behavioural and biochemical actions of the specific 5-HT_{1A} agonist 8-OH-DPAT (Hjorth et al., in prep.). The putative 5-$HT_{1(A)}$ receptor antagonist profile of PB was further explored, using *in vivo* microdialysis to assess its effects on central 5-HT release. (–)Pindolol was included for comparison. Chloral hydrate-anaesthetized male S-D rats (250-350 g) were stereotaxically implanted with *in vivo* brain microdialysis probes into the ventral hippocampus. The probes were perfused with artific. CSF containing the 5-HT reuptake blocker citalopram (1 μM), and dialysates collected every 20 min for analysis of 5-HT using HPLC-EC. Drugs were initiated after a control period (2-3h), to establish stable baseline 5-HT output. In contrast to (–)pindolol (8.0 mg/kg SC), PB (2.0 or 8.0 mg/kg SC) treatment increased the hippocampal 5-HT output (max. ≈175-200% of control, t= +60'). The PB-induced response was dose-dependent, stereoselective and Ca^{2+}-sensitive, and was abolished by omitting citalopram from the perfusion medium; (–)-, but not (+)-, penbutolol pretreatment also prevented the 8-OH-DPAT-induced decrease of 5-HT release. The data indicate that (–)penbutolol possesses 5-HT_{1A} and/or 5-HT_{1B} receptor antagonist properties, and may be a useful tool in studies of central 5-HT_1 receptor-mediated function.

39.2

NEUROPHYSIOLOGICAL STUDIES OF WAY-100,135: A NOVEL AND HIGHLY SELECTIVE 5-HT_{1A} RECEPTOR ANTAGONIST. D. E. Jones* and J.T. Haskins. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000.

WAY-100,135 (WAY), binds with high affinity and selectivity to the central 5-HT_{1A} site and displays antagonist activity in functional models (Fletcher *et al.*, this meeting). WAY and several other putative 5-HT_{1A} antagonists were examined for effects on central 5-HT neuronal activity utilizing standard neurophysiological techniques in chloral hydrate-anesthetized rats. Among the compounds studied were the full agonist 8-OH-DPAT (DPAT), the partial agonists gepirone (GEP) and buspirone (BUS) and the putative antagonists BMY-7378 (BMY), NAN-190 (NAN), and MDL-73005 (MDL). Following i.v. administration, all compounds except WAY, markedly reduced the firing rate of 5-HT neurons recorded in the dorsal raphe nucleus. The rank order of potency (and ID_{50} μg/kg i.v.) for these effects was DPAT (2.05) > BMY (11.9) > NAN (16.0) > GEP (26.4) > BUS (30.0) > MDL (81.5) >>> WAY (>2500). To follow up these observations we examined the effects of pretreatment (500 μg/kg i.v.) with WAY on DPAT-induced inhibition of 5-HT neuronal activity. In these experiments, DPAT alone inhibited 5-HT neuronal activity with an ID_{50} of 2.6 ± 0.2 μg/kg, i.v. Following pretreatment with WAY, however, DPAT at doses up to 4.0 μg/kg i.v. reduced baseline activity by only 30%. Clearly, of the compounds examined here, WAY has a profile consistent with that of an antagonist at 5-HT_{1A} receptor sites.

39.3

WAY100135: A NOVEL AND HIGHLY SELECTIVE 5-HT_{1A} RECEPTOR ANTAGONIST. A. Fletcher*, D.J. Bill*, S.J. Bill*, N.T. Brammer*, I.A. Cliffe*, E.A. Forster*, Y. Reilly* and G.K. Lloyd. Wyeth Research (U.K.) Ltd., Taplow, Maidenhead, Berkshire SL6 0PH, U.K.

We present initial in vitro and in vivo data on WAY100135, a novel phenylpiperazine derivative [N-tert-butyl 3- 4-(2-methoxyphenyl) piperazin-1-yl-2-phenylpropanamide dihydrochloride] which binds with high affinity and selectivity to the central 5-HT_{1A} site, and which displays only 5-HT_{1A} antagonist activity in functional models.

The IC_{50} value of WAY100135 (displacement of rat hippocampal ^{3}H-8-OH-DPAT binding) was 25 nM. IC_{50} values (μM) of WAY100135 at other sites were:- 5-HT_{1B} >10; 5-HT_{1C} >10; 5-HT_2 6; alpha-1 adrenoceptor 1.8; alpha-2 >10; dopamine D_2 9. The pA_2 value of WAY100135 for antagonism of 5-carboxamidotryptamine in the transmurally-stimulated guinea-pig ileum was 7.2. The lowest effective dose of WAY100135 to antagonise 8-OH-DPAT-induced syndrome in the rat was 3mg/kg sc. WAY100135 did not display any agonist activity in these models. WAY100135 had no significant effect on raphe 5-HT neuronal firing in the anaesthetised rat, but blocked the inhibitory action of 8-OH-DPAT (Haskins et al., this meeting). Similarly, WAY100135 (up to 10mg/kg sc) had no effect on rat central 5-HT turnover (measured by cortical 5-HTP accumulation following decarboxylase inhibition) but antagonised the decreased turnover induced by 8-OH-DPAT (0.05mg/kg sc) at doses of 1-10 mg/kg sc. In an animal model of anxiety, the mouse two-compartment light:dark box, WAY100135 mimicked the activity profile of benzodiazepine positive controls by selectively increasing exploratory behaviour in the light compartment at doses of 3-10mg/kg sc.

Unlike previous 5-HT_{1A} 'antagonists' (eg: BMY7378, NAN-190), which display agonist activity at raphe autoreceptors, WAY100135 appears to be the first selective and anxiolytic 5-HT_{1A} ligand to act as an antagonist in both presynaptic and postsynaptic receptor models.

39.4

PATCH CLAMP ANALYSIS OF SEROTONIN RESPONSES IN SUBSTANTIA GELATINOSA NEURONS OF THE RAT SPINAL CORD SLICE. M. Yoshimura and S. Nishi. Dept. Physiol., Kurume Univ. Sch. of Med., Kurume, 830 Japan.

The effect of serotonin (5-HT) on substantia gelatinosa (SG) neurons in tissue slices of the adult rat spinal cord was studied with a patch clamp recording technique. Bath application of 5-HT (0.1-40 μM) produced an outward current in 30 % of SG neurons, an inward current in 10 %, a biphasic response consisting of an outward current and an inward current in 30 %, and no responses in 30 %. The 5-HT induced outward current was associated with an increase in membrane conductance and reversed in polarity at membrane potential near the potassium equilibrium potential, suggesting that the outward current was due to activation of a membrane potassium conductance. The outward current was unaffected by tetrodotoxin (0.5 μM) and was reduced but not abolished by Co^{++} (2 mM). Application of 8-OH-dipropylaminotetralin (8-OH-DPAT), a 5-HT_{1A} agonist, produced an outward current which was quite similar, if not identical, to the 5-HT induced outward current. The inward current caused by 5-HT was antagonized by the 5-HT_2 receptor antagonist ketanserin.

These observations suggest that SG neurons are endowed with 5-HT_{1A} and/or 5-HT_2 receptors and that the two receptor subtypes may be involved in modulation of nociceptive transmission in the dorsal horn of the spinal cord.

39.5

SEROTONIN TYPE 1A AND SEROTONIN TYPE 2 RECEPTORS MEDIATE DISTINCT BEHAVIORAL RESPONSES IN THE MONGOLIAN GERBIL. Robert N. Wright* and Arlene S. Eison. CNS Special Proj. Bristol-Myers Squibb Company, Wallingford, CT 06492.

Although the ability of serotonin (5-HT) receptor subtype activation to induce distinct behaviors has been well documented for the rat, it has thus far been largely unreported for the Mongolian gerbil. We have found that a 5-HT_{1A}/5-HT_2 agonist 5-Methoxy-N,N-dimethyltryptamine, the specific 5-HT_{1A} agonist 8-hydroxy-(di-n-propylamino) tetralin (8-OH-DPAT) and the 5-HT precursor, L-5-hydroxy-tryptophan all elicit a 5-HT syndrome in the gerbil consisting of: reciprocal forepaw treading (RFT), hindleg abduction (HA), body tremors and Straub tail. The putative 5-HT_{1A} antagonist NAN-190 blocked both RFT and HA in a dose-dependent manner suggesting that these behaviors are mediated via 5-HT_{1A} receptor activation.

We have also identified a unique, dose-responsive behavior in the gerbil induced exclusively by 5-HT_2 agonists such as (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and quipazine. This reciprocal hindleg body scratch (RHBS) is dose-dependently inhibited by pretreatment with the selective 5-HT_2 antagonist ritanserin.

Similar to that observed in the rat, we have shown interactions between 5-HT_{1A} and 5-HT_2 receptor subtypes. DOI-induced RHBS behavior is potently inhibited by pretreatment with 8-OH-DPAT. Conversely, simultaneous administration of DOI with 8-OH-DPAT potentiated the 5-HT syndrome.

39.6

FLESINOXAN, NAN-190 AND MOTION SICKNESS IN THE CAT. J.B. Lucot. Dept. Pharmacol., Wright State Univ., Dayton, OH 45435.

This study extends previous work on the ability of 5-HT_{1A} agonists to inhibit motion sickness by evaluating the effects of a new agonist, flesinoxan, and a putative antagonist, NAN-190. Ten susceptible subjects were tested biweekly in a motion device modelled after a Ferris wheel. Tests lasted 30 min followed by 1 min of observation at rest. As expected, flesinoxan suppressed motion sickness. However, unlike 8-OH-DPAT, this effect was not blocked by (-)propranolol. The dose of 3 mg/kg on NAN-190 weakly suppressed motion sickness and produced a strong nictitating membrane response, while lower doses had no effect on motion sickness. Doses of NAN-190 up to 1 mg/kg vs 8-OH-DPAT and up to 0.3 mg/kg vs flesinoxan did antagonize the agonists. Higher doses are being tested. The mechanism by which flesinoxan suppresses motion sickness is not yet clear. NAN-190 acted as a weak agonist when given alone but moderate doses did not reverse the effects of 8-OH-DPAT.

39.7

BENZODIOXEPIPERAZINE DERIVATIVES AS NOVEL 5-HT_{1A} ANTAGONISTS: A PHARMACOLOGICAL CHARACTERIZATION M.J. Millan, J.M. Rivet*, H. Canton, F. Lejeune*, M. Brocco*, K. Bervoets* and J.L. Peglion*. FONDAX, Groupe de Recherches Servier, 7 rue Ampère, 92800 Puteaux, Paris, France.

Currently, no selective, pure antagonists at 5-HT_{1A} receptors are known. Proposed antagonists have several problems; e.g., (-)-alprenolol is equipotent at 5-HT_{1B} sites, BMY 7378 and MDL 73005EF are partial agonists and NAN 190 is a potent α_1 antagonist; both BMY 7378 and NAN 190 are D_2 antagonists. In an attempt to improve upon these drugs, we synthesized a series of (benzocycloalkanyl)alkyl derivatives (in the N_1 position) of 4-(1,4-benzodiaxan-5yl) piperazine. These possessed extremely high affinity for 5-HT_{1A} receptors (pKis of 8.8-9.4). Their affinity for other 5-HT sites (1B/1C/2/3) was several hundred fold lower. In vivo, four tests of (post-synaptic) 5-HT_{1A} activity in rats were used; induction of corticosterone secretion, hypothermia, spontaneous tail-flicks and flat-body posture. In these tests, the 5-HT_{1A} ligand, 8-OH-DPAT, was a potent agonist: the benzodioxepiperazine derivatives blocked its action over a dose range of 0.1-2.5 mg/kg, s.c. in the absence of partial agonist effects. In vivo, their actions at α_1, α_2, D_1 and D_2 receptors were manifest only at significantly (≥10 fold) higher doses. With respect to their, 1) potency, 2) selectivity over other 5-HT receptor types as well as α_1, α_2, D_1 and D_2 receptors and, 3) purity of antagonism at post-synaptic 5-HT_{1A} sites, the benzodioxepiperazine derivatives offer advantages over other proposed 5-HT_{1A} antagonists such as (-)- alprenolol, BMY 7378, NAN 190 and MDL 73005EF. The activity of these benzodioxepiperazine derivatives at pre-synaptic 5-HT_{1A} receptors remains to be explored. These drugs should prove highly useful in exploring structure-activity relationships at 5-HT_{1A} receptors as well as in determining their functional significance.

39.8

S 14671: A NOVEL NAPHTYLPIPERAZINE 5-HT_{1A} AGONIST OF EXCEPTIONAL POTENCY POSSESSING 5-$HT_{1C/2}$ ANTAGONIST ACTIVITY H. Canton, J.-M. Rivet*, F. Lejeune*, M. Laubie*, M. Brocco*, K. Bervoets*, G. Lavielle* and M. J. Millan, Fondax, Groupe de Recherches Servier, 7 rue Ampère, 92800 Puteaux, France.

Serotonin (5-HT) is implicated in the control of mood and both 5-HT_{1A} agonists and 5-$HT_{1C/2}$ antagonists may possess anxiolytic properties. In an attempt to combine these properties, the naphtylpiperazine, S 14671 (4-[(thenoyl-2)aminoethyl]-1-(7-methoxynaphtyl)piperazine monochlorhydrate) was synthesized. Its profile was compared with the 5-HT_{1A} agonists, 8-OH-DPAT, (+)-flesinoxan and buspirone and the 5-$HT_{1C/2}$ antagonist, ritanserin.

pKi :	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}	5-HT_2	5-HT_3
S 14671	9.3	6.3	7.8	7.8	<6.0
8-OH-DPAT	9.2	5.2	5.0	5.0	<6.0
(+)-flesinoxan	8.7	5.1	5.0	<5.0	<6.0
buspirone	7.9	4.4	5.7	5.8	<6.0
ritanserin	6.0	5.4	8.6	8.6	<6.0

S 14671 had very high affinity for 5-HT_{1A} sites and its 5-$HT_{1C/2}$ affinity was only 30-fold less. In in vitro models of phosphoinositide turnover (rat choroid plexus, 5-HT_{1C} and rat cortex, 5-HT_2), S 14671 was a pure antagonist. In vivo 5-$HT_{1C/2}$ actions are under evaluation. In in vivo 5-HT_{1A} models in male rats (induction of corticosterone secretion, hypotension, hypothermia and spontaneous tail-flicks and inhibition of dorsal raphe firing) their relative potencies were S 14671 (= 1) >8-OH-DPAT (7) > buspirone (200) > (+)-flesinoxan (280). S 14671 was active at doses as low as 5 μg/kg, s.c. 8-OH-DPAT and S 14671 were full agonists and (+)-flesinoxan and buspirone were partial agonists. Thus, S 14671 is a potent 5-HT_{1A} agonist of high efficacy with 5-$HT_{1C/2}$ antagonist properties. The evaluation of its potential anxiolytic and other therapeutic properties will be of interest.

39.9

PHARMACOLOGICAL EVIDENCE FOR A MIXED AGONISTIC/ANTAGONISTIC PROFILE OF THE PUTATIVE 5-HT_{1A} RECEPTOR ANTAGONISTS BMY 7378 AND NAN-190. J.M. Greuel, T. Glaser and J. De Vry*. Institute for Neurobiology, Troponwerke, Berliner Straße 156, 5000 Köln 80, FRG

The present study characterized the putative 5-HT_{1A} receptor antagonists BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]-decane-7,9 dione dihydrochloride) and NAN-190 (1-(2-methoxyphenyl) 4-[4(2-pthalimido)butyl]piperazine) in a number of test paradigms reflecting activation of pre- and/or postsynaptic 5-HT_{1A} receptors. Both compounds bound with high affinity to 5-HT_{1A} sites in hippocampal membranes. In the hippocampus, the forskolin-stimulated adenylate cyclase assay revealed that both drugs were partial agonists. Increasing concentrations (10^{-9}-10^{-4}M) of NAN-190 or BMY 7378 partly reduced the adenylate cyclase activity and partly antagonized the effects of the 5-HT_{1A} agonist 8-OH-DPAT (1μM). Electrophysiological characterization at presynaptic sites in dorsal raphe neurons in rat brain slices showed that low concentrations (10 nM) antagonized and higher concentrations (> 30 nM) mimicked the effects of 8-OH-DPAT. Based on these results the intrinsic activities of both compounds were estimated to be in the range of 0.1 to 0.3. The suppression of neuronal firing by NAN-190 and BMY 7378 could be completely antagonized with propranolol, indicating that the inhibitory actions of both drugs were not primarily due to α_1-adrenoceptor antagonism. A mixed agonistic-antagonistic profile of BMY 7378 and NAN-190 was also obtained in a 8-OH-DPAT vs saline drug discrimination procedure. It is concluded that both BMY 7378 and NAN-190 are mixed agonists/antagonists at pre- and postsynaptic 5-HT_{1A} receptors. The apparent "full agonism" at presynaptic sites probably reflects the existence of a receptor reserve in the dorsal raphe nucleus.

39.10

INHIBITION OF SEROTONERGIC DORSAL RAPHE NEURONS BY THE 5-HT_{1A} LIGAND RK-153. Cam P. VanderMaelen and John P. Braselton. Bristol-Myers Squibb Co., CNS Neuropharmacology, 5 Research Parkway, Wallingford, CT. 06492.

Some 5-HT_{1A} antagonist compounds are effective, but are not selective (e.g. spiperone). Other compounds, such as BMY 7378 (Yocca et al., EJP, 1987, 137, 293-294) are more selective, but exhibit both agonist and antagonist actions. In general, compounds which are weak partial agonists exhibit antagonist activities in "postsynaptic" models of 5-HT_{1A} action (e.g. adenylate cyclase; forepaw treading), but exhibit agonist-like actions at 5-HT_{1A} autoreceptors on serotonergic dorsal raphe (DR) neurons. RK-153 (kindly provided by R. Glennon) is a possible "pure" antagonist highly selective for 5-HT_{1A} sites (Raghupathi et al., Soc. Neurosci. Abst., 1990, 16, 1036). We administered this compound i.v. to chloral hydrate anesthetized male rats, and measured its effects on the spontaneous discharge of serotonergic DR neurons, using standard extracellular single unit recording techniques. RK-153 potently inhibited the firing of these neurons (ED_{50}=14.5μg/kg). These results are similar to previous results with 5-HT_{1A} partial agonists like buspirone, and in combination with other data suggest that RK-153 is a partial agonist at 5-HT_{1A} receptors. Additional studies would help confirm that these electrophysiological effects are direct and 5-HT_{1A}-receptor mediated.

39.11

TANDOSPIRONE STIMULATES PROLACTIN RELEASE VIA SEROTONIN-1A (5-HT-1A) RECEPTORS IN RATS. S.E. Mulroney*, C.M. Skudlarek*, L.A. Pabreza*, A. Shemer[1] and K.J. Kellar. Georgetown University Medical Center, Washington D.C. and [1]Pfizer Inc., New York, N.Y.

Tandospirone (Tan), an azapirone drug with antidepressant activity, has high affinity for 5-HT-1A receptors. These receptors are involved in mediating the effects of 5-HT agonists on prolactin release in the rat (Hulihan-Giblin et al., Soc. Neurosci. Abst. 15:723, 1989). We have examined the effects of Tan on prolactin release in awake, freely moving rats in which an in-dwelling jugular vein cannula allowed multiple sampling of blood after injections of drugs. Following a single injection of Tan, plasma prolactin levels rose rapidly, peaked within 10 min at 4-6 times preinjection values and returned to baseline within 30 min. The ED_{50} for this effect of TAN was ≈ 0.3 mg/kg i.v. The Tan metabolite 1-PP did not release prolactin. Prolactin release by TAN was blocked in a dose related manner by pretreatment (90 min before Tan) with the 5-HT antagonist metergoline (ID_{50} ≈ 1 mg/kg). Pretreatment with 8-OHDPAT (0.2 mg/kg) released prolactin and diminished the response to Tan administered 60 min later. Similarly, pretreatment with Tan (1 mg/kg) decreased the subsequent prolactin response to 8-OHDPAT. These diminished responses appear to represent cross-desensitization and are thus further evidence that Tan releases prolactin via 5-HT-1A receptors. Repeated administration of Tan (10 mg/kg, i.p.) twice-daily for two weeks did not significantly alter the prolactin response to either Tan or 8-OHDPAT, indicating that receptor sensitivity was not altered by this chronic exposure. These results indicate that the prolactin response to Tan may provide a sensitive in vivo measurement of 5-HT-1A receptor function and that this function is probably preserved during chronic administration of 5-HT-1A agonists.

39.12

5-HT_{1A} RECEPTOR ANTAGONISTS DO NOT BLOCK THE HYPOTHERMIA CAUSED BY INCREASED PARTIAL PRESSURES OF OXYGEN. L. Fenton, G. Beck, S. Djali and M.B. Robinson. Children's Seashore House; Depts. Ped. and Pharm., U. of PA; Phila., PA 19104.

Rats exposed to oxygen at high pressure (OHP) develop hypothermia, bradycardia and several of the behaviors seen in the serotonin (5-HT) syndrome: head weaving, resting tremor and straub tail. These responses cannot be attributed to high pressure. The purpose of these studies was to investigate the possibility that the hypothermia is related to increased serotonin turnover. 5-Hydroxyindole acetic acid (HIAA) and other neurotransmitters were measured by HPLC in discrete brain regions. HIAA levels were increased (> 50% , $p < 0.05$) in the parietal-occipital cortex and striatum of rats exposed for 2.5 hours to 4 ATA O_2. Tryptophan, 5-HT, other biogenic amines, and their metabolites did not change. Pre-treatment with the 5-HT_{1A} receptor antagonists pindolol (0.1-10 mg/Kg) and NAN-190 (10 mg/Kg) did not block hypothermia induced by OHP at doses that block hypothermia induced by 8-OH-DPAT and 5-hydroxytryptophan (5-HTP, 50 mg/Kg). Previous studies have demonstrated that the Fawn Hooded (FH) rat is less responsive to hypothermia induced by 8-OH-DPAT. (Pharm. Bio. & Beh., 22:489, 1985) The FH rat did not become hypothermic during OHP. Hypothermia induced by OHP was not blocked by spiperone (3 mg/kg) and methysergide (50 mg/kg) and was additive to the hypothermia induced by 5-HTP. These data suggest that OHP induced hypothermia is not due to excess activation of 5-HT_{1A} receptors, and that the FH rat may have defects in thermoregulation that are independent of the 5-HT_{1A} receptor.

39.13

AGONISTIC PROPERTIES OF YOHIMBINE AND ITS STEREOISOMERS FOR 5-HYDROXYTRYPTAMINE$_{1A}$ RECEPTOR. N.Kawai*, T.Yamamoto*, A.Baba*, T.Moroji and Y.Hayashi. Dept. of Psychopharmacology and Molecular Biology, Psychiatric Research Institute of Tokyo, Tokyo 156, JAPAN.

Yohimbine is well-known as a selective alpha$_2$-arenoceptor antagonist. Recently it was reported that rauwalscine, one of stereoisomers of yohimbine, binds to 5-Hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors with high affinity (Convents et al.;Eur.J.Pharmacol.1986). In this study, the effects of yohimbine and a series of its stereoisomers on the binding of [^{3}H]8-OH-DPAT, a selective 5-HT_{1A} agonist, to rat hippocampal membranes were examined. In addition, we examined their effects on the forskolin-stimulated adenylate cyclase (A-C) activity. All of them inhibited the binding of [^{3}H]8-OH-DPAT to hippocampal membrane preparations as well as that of [^{3}H]idazoxan, a selective alpha$_2$-adrenoceptor antagonist, to membrane preprations of cortices. 5-HT and 8-OH-DPAT reduced the forskolin-stimulated A-C activity. Yohimbine and its stereoisomers reduced it to approximately the same extent as those for 5-HT and 8-OH-DPAT. This effect was inhibited by spiperone. They did not affect the inhibitory effect of 5-HT on the forskolin-stimulated A-C activity. The present findings suggest that yohimbine and its stereoisomers are not only alpha$_2$-adrenoceptor antagonists but also 5HT_{1A} receptor agonists.

39.14

EVIDENCE FOR A LARGE RECEPTOR RESERVE FOR INHIBITION OF DORSAL RAPHE (DR) CELL FIRING BY 5-HT1A AGONISTS. B.L. Waszczak[1], R.F. Cox[1] and E. Meller[2]. Pharmacol. Sect., Northeastern Univ., Boston, MA[1] and Dept. Psychiat., NYU Med. Ctr., New York, NY[2].

Previous studies (Meller et al., Mol. Pharmacol. 37: 231, 1990) have shown that DR cells exhibit a large receptor reserve for the inhibition of serotonin (5-HT) synthesis by full 5-HT1A agonists such as 8-OH-DPAT, whereas no reserve exists for the "partial" agonists ipsapirone (IPS) and BMY 7378 (BMY). Using identical methods for receptor inactivation, we carried out parallel single unit recording studies to determine if the above drugs exhibit similar relative efficacies and receptor reserves for inhibiting DR cell firing. I.v. dose-response curves (drc's) were constructed in untreated control rats, or in rats which received an injection of the receptor inactivator N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; 6 mg/kg, s.c.) 24 hrs before recording. All three drugs fully inhibited DR cell firing in control rats (ED50s: 1.5 ug/kg, 8-OH-DPAT; 30.0 ug/kg, IPS; 17.5 ug/kg, BMY). However, unlike effects on 5-HT synthesis, EEDQ treatments caused no depression of the maximal inhibitory response for any of the agonists, although all drc's were shifted rightward (ED50s: 10.1 ug/kg, 6.7-fold shift, 8-OH-DPAT; 139.9 ug/kg, 4.7-fold shift, IPS; 53.8 ug/kg, 3.1-fold shift, BMY). Since the degree of shift in the drc's after EEDQ is an indirect index of agonist efficacies, we conclude that the order of agonist efficacies is similar for both inhibition of 5-HT synthesis and DR cell firing (i.e. 8-OH-DPAT>IPS>BMY). However, a large receptor reserve exists for all three drugs in inhibiting DR cell firing. This suggests that the autoreceptor pools regulating synthesis and firing are either not identical or they have different receptor-effector coupling characteristics (i.e. unique G-protein transducers, effectors, and/or stoichiometries).

39.15

EFFECT OF TANDOSPIRONE (SM 3997) ON THE PRODUCTION OF GROWTH FACTORS FROM PRIMARY CULTURES OF ASTROGLIAL CELLS DERIVED FROM DIFFERENT BRAIN REGIONS. C. Rogers, A. Shemer, E.C. Azmitia and P.M. Whitaker-Azmitia. Dept. of Psychiatry, SUNY at Stony Brook, and Pfizer Incorporated, New York, New York.

Our laboratory has previously shown that the 5-HT_{1a} receptor active drug, 8-OH-DPAT, released a growth factor from astroglial cells which stimulated neurite outgrowth from serotonergic neurons in primary cultures. The current study uses a more selective 5-HT_{1a} drug which has clinical potential - SM 3997 or tandospirone. Primary cultures of astroglial cells were grown from cortex, hippocampus, and brainstem regions from 4 day old rat pups. When the cells reached confluence they were incubated for 24 hours with 50, 100 or 500 nM tandospirone. The conditioned media was added to the culture media of the neurons, and growth assessed after three days. Tandospirone released a growth factor, most likely the protein S-100, most potently from brainstem and to a lesser extent from hippocampus and cortex. The lowest concentration of the drug (50 nM) was the most effective.

39.16

5-HT1A but not 5-HT1D Agonists Increase Hippocampal Acetylcholine (ACh) Efflux in Conscious Guinea Pig. Wilkinson, L.O., Hutson, P.H., and Middlemiss, D.N. Merck, Sharp and Dohme NRC, Terlings Park, Eastwick Rd, Harlow, Essex CM20 2QR. U.K.

Serotonergic regulation of hippocampal acetylcholine (ACh) release was investigated using in vivo dialysis. An increase in ACh efflux was observed following administration of the 5-HT1A agonists 8-OH-DPAT (1 mg/kg, 137%: 10 mg/kg, 324%), buspirone (10 mg/kg, 249%), or NAN-190 (10 mg/kg, 79%) (maximal increase). Following pretreatment with NAN-190 (3 mg/kg), the effects of 8-OH-DPAT (1 mg/kg) were attenuated by 46%. Since NAN-190 is an ineffective antagonist at the 5-HT1A somatodendritic autoreceptor, it is unlikely that a 5-HT1A receptor mediated decrease in 5-HT release is responsible for the observed increase in ACh. In support of this hypothesis, the 5-HT1D agonist sumatriptan administered through the dialysis probe did not alter basal ACh efflux at concentrations which are reported to decrease 5-HT release (1 µM; 115% of control). Administration of 8-OH-DPAT through the dialysis probe (10 µM) did not increase ACh efflux (77% of control), thus 5-HT1A receptors in the hippocampus are not responsible for the effects of systemic 8-OH-DPAT. 5-HT1A agonists may increase ACh efflux through receptors on ACh cell bodies in the septal area.

39.17

EFFECTS OF ADRENALECTOMY AND CORTICOSTERONE ON 5-HT_{1A} AND 5-HT_{1B} RECEPTORS IN THE DORSAL HIPPOCAMPUS AND CORTEX OF THE RAT.

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Quantitative autoradiography was used to evaluate the effects of adrenalectomy (ADX) and corticosterone (CORT) on binding at 5-HT_{1A} and 5-HT_{1B} receptors in the dorsal hippocampus and cortex of male rats. ADX increased binding of [^{3}H]8-OH DPAT at 5-HT_{1A} receptors in the oriens and lacunosum moleculare layers of CA2 and CA3, in the lacunosum moleculare layer of CA4 region, and in the dentate. In restraint-stressed ADX rats, binding was decreased only in CA2. Restoration of baseline levels of CORT reversed the effects of ADX on 5-HT_{1A} receptors in the hippocampus, while chronic high levels of CORT decreased binding at 5-HT_{1A} receptors in the dentate. No treatment affected 5-HT_{1A} receptors in the CA1 region of the hippocampus or in the cortex. ADX increased binding of [^{125}I]iodocyanopindolol at 5-HT_{1B} receptors in the infrapyramidal dentate, but this effect was not observed in ADX rats that were restrained. In ADX and intact rats that received CORT, binding at 5-HT_{1B} receptors was lower than that in both control and ADX rats in the infrapyramidal dentate, and lower than that in ADX rats in the suprapyramidal dentate and CA4. In ADX and intact animals, CORT reduced binding at 5-HT_{1B} receptors in Area 2 of the cortex. Decreases in binding at 5-HT_{1A} and 5-HT_{1B} receptors after chronic exposure to high CORT may mimic changes in binding in animals that fail to adapt to severe stress, with an ensuing vulnerability to depression.

39.18

EFFECTS OF CHRONIC TREATMENT WITH 8-OH-DPAT (DPAT) AND GEPIRONE (GEP) ON THE SENSITIVITY OF SOMATODENDRITIC 5-HT_{1A} AUTORECEPTORS (5-HT_{1A}-AR). K. Bohmaker, A. Eison[1], F. Yocca[1] and E. Meller. Dept. of Psychiatry, NYU Medical Center, New York, NY 10016 and [1]CNS Neuropharmacology, Bristol-Myers Squibb Co., Wallingford, CT 06492.

The clinical anxiolytic response to the 5-HT_{1A} partial agonist buspirone requires several weeks of treatment, suggesting that receptor adaptation may be involved. Electrophysiological and autoradiographic binding studies indicate a selective desensitization and downregulation of 5-HT_{1A}-AR after treatment with the partial agonists GEP and ipsapirone. We previously showed that 5-HT_{1A}-AR display a large receptor reserve (RR) for agonist-mediated inhibition of 5-HT synthesis (5-HTP levels after NSD-1015) in terminal areas (cortex (CTX) and hippocampus (HIPP); Meller et al., Mol. Pharmacol. 37:231, 1990). In the present study the ability of chronic treatment with DPAT and GEP to modify the sensitivity of synthesis-inhibiting 5-HT_{1A}-AR was investigated as a function of agonist efficacy (full or partial), dose and route of administration.

Chronic treatment with the full agonist DPAT (2 weeks, 0.2 or 2.0 mg/kg/day, b.i.d., s.c.) did not alter the dose-response curve (DRC) to DPAT challenge (2 day washout) in CTX or HIPP. DPAT administration via ALZET minipumps (2 weeks, 0.4 or 4 mg/kg/day) also did not alter 5-HT_{1A}-AR sensitivity to the weak partial agonist BMY-7378. In contrast, GEP administration by minipump (2 weeks, 20 mg/kg/day) resulted in a small but significant rightward shift (2.4-fold) in the DRC to DPAT challenge (2 days after washout) in the CTX, and a slightly smaller shift (1.8-fold) in the HIPP. Furchgott analysis indicated a reduction in the extent of RR after chronic GEP treatment. These studies suggest that agonist efficacy may an important factor in vulnerability to desensitization. Additional studies will be reported. Supported by NS 23618.

SECOND MESSENGERS II

40.1

N-3 FATTY ACIDS MODULATE ARACHIDONIC ACID METABOLISM IN A PRIMARY CULTURE OF ASTROGLIAL CELLS

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The calcium-ionophore A23187 and platelet activating factor (PAF) activate arachidonic acid (AA) metabolism in a primary culture of astroglial cells, by generating cyclo (CO) and lipoxygenase (LO) products. These agents activate also, primarily, phospholipase $A_2$and phospholipase C , respectively, as judged on the type of products generated by ^{3}[H] AA labeled cells, after stimulation. CO metabolites were generated by cells stimulated with both agonists, but production of Leukotriene C_4 was triggered only by A23187. 12- and 15-hydroxy-eicosa-tetraenoic acids (HETEs) were formed after A23187 stimulation, but these products were detected only when [^{3}H] AA-labelled cells were used, after PAF stimulation. The PAF-induced formation of CO products, but not that induced by A23187, was blocked by PAF antagonists, which, however, did not affect the formation of HETEs from PAF-stimulated AA-labeled cells. Since the astroglial cell cultures, in our conditions, appeared to be depleted of the long chain n-3 fatty acids, especially of 22:6 (DHA) which is the major long chain polyunsaturated fatty acid in brain, we have added to the growth medium different µM concentrations of albumin-bound DHA for different time periods. We have then studied the incorporation of this fatty acid in cell lipids and the subsequent effects on the stimulated AA metabolism. After supplementation with DHA 5 µM for 48 hours, reduction of prostaglandin production was observed, and this was associated with a modified balance between 12- and 15-LO metabolites.Thus the eicosanoid system, which is activated in glial cells by endogenous products such as PAF, is modulated by the relative proportions of AA and DHA in cell phospholipids.

40.2

PLATELET-ACTIVATING FACTOR (PAF) RECEPTOR-MEDIATED SIGNAL TRANSDUCTION IN NEUROHYBRID NCB-20 CELLS. T-L Yue, JM Stadel, HM Sarau, E Friedman#, J-L Gu*, HY Wang*#, LC Shi*#, DA Powers* and G Feuerstein. Dept. of Pharmacology, SmithKline Beecham, King of Prussia, PA 19406 and #Dept. of Psychiatry and Pharmacology, Med. Coll. of PA, Phila., PA 19129.

In a previous study we reported that PAF induced a significant increase in intracellular free Ca^{++} [$(Ca^{++})_i$] in NCB-20 cells (Neurosci. 41:177, 1991). The present study was undertaken to further elucidate the mechanism involved in the regulation of PAF receptor-mediated signal transduction in NCB-20 cells. PI turnover was studied in NCB-20 cells prelabeled with myo-[^{3}H]inositol and [^{3}H]inositol phosphates were separated by anion exchange resin column, and confirmed by HPLC. PKC activity was assessed by phosphorylation of histone III-S with [^{32}P]ATP. ADP-ribosylation of membrane proteins was studied as reported previously (Proc. Natl. Acad. Sci. USA 83:7320, 1986). PAF stimulated PI metabolism with an EC_{50} of 1.96 nM and inhibited by PAF antagonist BN50739. PKC translocation was induced concentration-dependently by 0.001-10 nM PAF and also inhibited by BN50739. The PKC activator PDBu inhibited PAF-induced IP_3 formation and $(Ca^{++})i$ elevation which was partially reversed by PKC inhibitor H7. Pretreatment with pertussis toxin (PTX) inhibited PAF-induced IP_3 production and elevation of $(Ca^{++})_i$ with a maximal reduction of 67% and 63%, respectively, at 300 ng/ml of PTX. Pretreatment with PTX inhibited subsequent ^{32}P labelling of the toxin substrate (a 38-KDa protein) in the membranes and correlated with the uncoupling of the PAF-induced IP_3 formation. However, PAF-stimulated PI turnover was not completely blocked and Ca^{++} influx was only moderately inhibited by PTX. Our results reveal that neuronal cells possess PAFR linked to phospholipase C (PLC) and Ca^{++} channels which are regulated by PKC, and distinct G proteins may couple the PAF receptor to activation of PLC and the increase in $(Ca^{++})_i$.

40.3

AUTORADIOGRAPHIC AND BIOCHEMICAL STUDIES OF PLATELET-ACTIVATING FACTOR IN RAT AND FROG RETINA. J. Cluzel,*[1,2] M. Doly,*[2] D. Torbati, and N.G. Bazan[1]. [1]LSU Eye Center and Neuroscience Center, New Orleans, LA 70112 and [2]Laboratoire de Biophysique, Faculté de Pharmacie, Clermont-Ferrand, France.

Platelet-activating factor (PAF) exerts effects on the b-wave of the electroretinogram (ERG) obtained from the isolated rat retina. Furthermore, we recently reported the presence of a high-affinity retinal PAF-binding site (K_d = 2.9 ± 0.4 nM and B_{max} = 0.85 ± 0.61 pmol/mg retinal protein). In this study, autoradiography was performed on the sites of PAF binding in the retina. Preliminary experiments showed that the exposure to fixing and dehydrating agents resulted in inactivation of the binding site. Slide-mounted tissue sections from frozen frog or rat eyes were incubated with ^{3}H-PAF at 25°C in a buffer of 10 mM Tris, 2 mM EGTA, 10 mM $MgCl_2$ and 0.25% BSA. To prevent the loss of the radioactive material, we used double-step fixation with vapors of formaldehyde and osmium tetroxide. The autoradiogram was generated over a period of 2-3 weeks. Using low ^{3}H-PAF concentration (10 nM), specific binding was localized in the inner and outer nuclear layer of the retina. BN 52021, a PAF receptor antagonist, displaced more than 75% of the total binding. Taken together, the *in vitro* binding and autoradiography provide support for the existence of PAF binding in the synaptic region. In addition to its role as an inflammatory mediator in the retina, PAF may also be a functional mediator, acting on synaptic networks of the retina. Supported by EY05121.

40.4

CELLULAR ELECTROPHYSIOLOGICAL ACTIONS OF NANOMOLAR CONCENTRATIONS OF HEPOXILIN ISOMERS ON HIPPOCAMPAL NEURONS OF THE RAT.

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In previous studies we have shown that hepoxilin A_3 (HxA_3) caused neuronal membrane hyperpolarization, enhanced afterhyperpolarizations (AHPs), increased inhibitory postsynaptic potentials (IPSPs) and decreased spike threshold (Carlen et al., Brain Res., 497, 171, 1989). HxA_3 can be biotransformed into the glutathione adduct, HxA_3-C (Pace-Asciak et al., PNAS, 87, 3037, 1990). Both the 8R and 8S isomers of HxA_3 and HxA_3-C are found in the CNS. HxA_3 is more active than HxA_3-C in mobilizing intracellular free calcium in human neutrophils (Dho et al.,Biochem.J., 266, 63, 1990 and Pace-Asciak, unpublished). The 8R and 8S isomers of HxA_3 and HxA_3-C were tested on hippocampal CA1 neurons in vitro. Intracellular recordings were performed using the current clamp mode.

All Hx compounds were effective in causing a membrane hyperpolarization, enhancement of the AHPs and IPSPs and decrease of spike threshold in nanomolar concentrations as low as 5 nM. The compounds caused long-lasting effects and did not exhibit a concentration-response relationship. Due to the high variability of the responses obtained, it was difficult to conclude definitely that 8S isomers were more effective than 8R in influencing the hippocampal neuronal activity in the rat. A synthetic analogue of HxA_3-C in which the glutathionyl residue is located at the 9-carbon position instead of the 11-carbon position as in hepoxilin HxA_3-C was inactive in these neurons. These findings suggest that products in the hepoxilin pathway may function as neuromodulators.(Supported by the MRC, OMH and NIH).

40.5

SUPPRESSION OF VOLTAGE-DEPENDENT CALCIUM CURRENTS IN HIPPOCAMPAL NEURONS BY SODIUM NITROPRUSSIDE.

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A calcium (Ca)-calmodulin-dependent pathway for the enzymatic formation of the radical nitric oxide (NO) from l-arginine has been identified in neuronal tissue. NO activates guanylate cyclase and generates cGMP. Recent findings that cGMP can regulate ion channels, including Ca channels (Doerner and Alger, Neuron 1:693-699, 1988), suggest a role for this pathway in neurons. We now report that drugs, such as sodium nitroprusside (NaNP), that spontaneously generate NO depress voltage-activated Ca current in hippocampal neurons.

Ca currents were recorded under whole-cell voltage clamp using Ba^{2+} as the charge carrier in acutely dissociated guinea pig hippocampal neurons and in primary cultures of fetal rat hippocampus. Bath- or locally applied NaNP (100-500 μM) produced a 40-90% depression of voltage-activated I_{Ba} in responding neurons. Although onset of the effect occurred within 1-2 minutes, depression of I_{Ba} was long lasting and not always reversible. Furthermore, hippocampal cells were not uniformly responsive to NaNP. Neurons isolated from area CA1, for example, rarely responded, while NaNP had effect in a majority of those from CA3. An NO-stimulated suppression of voltage-activated Ca current could act as a regulatory mechanism that decreases Ca influx from extracellular space after a Ca-triggered event has occurred.

40.6

FORMATION OF NITRIC OXIDE FROM L-ARGININE IN ISOLATED BRAIN MICROVESSELS

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Nitric oxide (NO) , the major source of endothelium derived relaxing factor, mediates the effect of vasoactive substances on the blood vessels. NO activates guanylate cyclase in endothelial and/or the neighboring cells. We have previously shown the activation of guanylate cyclase by a number of vasoactive agents in isolated rat cerebral microvessels (Homayoun et al.,1989). This report shows that NO can be synthesized in brain microvessels from L-arginine.

Microvessels were prepared by bulk isolation from rat cerebra and cerebella. [^{3}H]citrulline formation from [^{3}H]arginine was assayed in the 20000g supernatant of microvascular homogenates according to the method described by Bredt and Snyder (1989).

[^{3}H]citrulline formation showed 2.2- and 3.3-fold increase over basal in cerebral and cerebellar microvessels respectively. The enzyme catalyzing this process is NADPH- and Ca^{++}- dependent.

[^{3}H]arginine transformation was completely blocked in the presence of N^G-monomethyl L-arginine at the concentration of 200μM.

These data suggest that hormonal stimulation of guanylate cyclase might be mediated through NO production in brain microvessels.

40.7

LOCALIZATION OF ARGININOSUCCINATE SYNTHETASE IN THE BRAIN: RELATIONSHIP WITH NITRIC OXIDE SYNTHASE.

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Argininosuccinate synthetase (ASS) is a key enzyme in the urea cycle where it catalyses the conversion of citrulline and aspartate to argininosuccinate which can be cleaved to yield fumarate and arginine. High levels of ASS are found in brain, however its role in the absence of the urea cycle enzymes responsible for citrulline synthesis has been puzzling. Recent studies have indicated that some neurons possess the capacity to synthesize citrulline from arginine directly via the enzyme nitric oxide synthase, which can be localized using NADPH-diaphorase histochemistry. We have used antibodies to localize ASS in the brain and determined its relationship with neurons containing nitric oxide synthase. ASS immunoreactivity was found in distinct populations of neurons in various brain regions, glial cells were not stained. Some ASS-positive neurons, i.e. those in the laterodorsal tegmental nucleus, corresponded to nitric oxide synthase-containing neurons identified histochemically. However, many ASS-positive neurons, including those in layer VI of the cortex, the endopiriform nucleus, and the thalamus did not contain NADPH-diaphorase. In addition, some diaphorase-positive cell groups were not ASS-immunoreactive. Thus in some cell groups ASS may be involved in recycling citrulline formed intracellularly via nitric oxide synthase, while in other neurons it may produce argininosuccinate from extracellular sources of citrulline.

40.8

A NOVEL METHOD FOR MEASURING PURIFIED NITRIC OXIDE (NO)-SYNTHASE ACTIVITY IN RAT BRAIN: KINETIC CORRELATION OF NADP AND CITRULLLINE FORMATION FROM L-ARGININE

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To establish the correlation between the formation of citrulline (CIT) and NADP from L-arginine and NADPH, steady state kinetics were measured under identical assay conditions using purified rat brain NO synthase (Proc. Natl. Acad. Sci. 88: 365, 1990). [3H]-CIT was separated from excess L-arginine on carboxymethyl-cellulose columns. NADP was measured using an enzymatic amplification procedure (alcohol dehydrogenase and diaphorase coupled with a redox dye, iodonitrotetrazolium, INT).

We found that during the 5 min. initial reaction, 8.2 picomoles of CIT and 42.2 picomoles of NADP were produced from the 50 uL assay mixture consisting of 3 nM enzyme, 8 uM arginine, 0.1 mM NADPH as well as Ca^{2+} and calmodulin. The ratio of NADP to CIT remained approximately 5 throughout the reaction. Biosynthesis of CIT from L-arginine by the rat brain enzyme shows a dependence on NADPH as reducing cofactor in the absence of exogenously added tetrahydrobiopterin.

The activity of the rat brain enzyme, as measured by the formation of NADP, was inhibited by the presence of the selective NO synthase inhibitors N^G-nitro-arginine and N^G-methyl-arginine.

CONCLUSION: Detailed kinetic analysis of this novel spectrophotometric assay validates the use of this rapid, sensitive and reliable method to measure the activity of purified NO synthase.

40.9

INHIBITION OF NITRIC OXIDE SYNTHESIS REDUCES THE HYPERTHERMIA INDUCED BY PROSTAGLANDIN INJECTION INTO THE PREOPTIC AREA IN RATS. S. Amir, E. De Blasio* and A. M. English*. Dept. of Psychology and Dept. of Chemistry and Biochemistry, Concordia Univ., Montreal, Quebec H3G 1M8, Canada.

Nitric oxide (NO) is a potent smooth muscle relaxing factor produced from L-arginine by the vascular endothelium. It is also synthesized in brain and has been implicated in neuronal signalling, and specifically in mediation of excitatory amino acid action on cyclic GMP production. We studied the involvement of NO in the thermogenic and hyperthermic actions of E_2 prostaglandin (PGE_2), a potent vasodialator and pyrogen involved in the brain fever mechanisms. PGE_2 (25 ng, 250 nl) was microinjected stereotaxically into the preoptic area of the anterior hypothalamus (POAH) of urethane-anaesthetized rats. The injection stimulated heat production in brown adipose tissue (BAT) and increased interscapular BAT (IBAT) and core temperatures by 2.26±0.07 and 1.22±0.05 °C, respectively (n=30). These thermogenic and hyperthermic effects were significantly attenuated by co-injection of N^G-monomethyl-L-arginine (NMMA, 25 µg), a competitive inhibitor of NO production from L-arginine (IBAT: 1.33±0.08; Core: 0.57±0.04 °C, n=22). Inclusion of L-arginine (50µg), though not D-arginine (50 µg) reversed the inhibitory effect of NMMA (25 µg) on intra-POAH PGE_2-induced increases in IBAT and core temperatures. Intra-POAH injection of NMMA (25µg) or L-arginine (50 µg) alone had no effect on IBAT and core temperatures. The results suggest that the effect on thermogenesis and body temperature induced by PGE_2 injection into the POAH is modulated by a local L-arginine-dependent and NMMA-sensitive NO-generating system.

40.10

MODULATION OF ENDOGENOUS ADP-RIBOSYLATION IN BRAIN. M. B. Williams, X. Li and R. S. Jope. Dept. of Psychiatry, Univ. of Alabama, Birmingham, AL 35294.

Post-translational modifications of proteins can regulate their function. One modification consists of attachment of an ADP-ribose donated by NAD, a process which appears to be limited to relatively few proteins. Many studies of this process have utilized exogenous toxins, e.g., pertussis toxin and cholera toxin, to induce ADP-ribosylation of G-proteins. Endogenous mono-ADP-ribosyltransferases are present in some cells, and may be important in modulating G-proteins and other proteins.

Endogenous ADP-ribosylation was studied in homogenates from rat brain regions by measuring the incorporation of label from [^{32}P]NAD into proteins. Sodium nitroprusside, which spontaneously produces nitric oxide, enhanced the ADP-ribosylation of 47 kD and 39 kD proteins and diminished the ADP-ribosylation of a 49kD protein in the cerebral cortex, hippocampus, striatum, thalamus and cerebellum. In the neonatal cerebral cortex, ADP-ribosylation of an additional 110kD protein was also enhanced by sodium nitroprusside. The effect of sodium nitroprusside was dependent on concentration and time. Each protein demonstrated differential sensitivities to sodium nitroprusside and the rate of ADP-ribosylation was different for each protein. Cyclic GMP did not mimic the effects of sodium nitroprusside. From these and previously published results it is suggested that nitric oxide generated from sodium nitroprusside or endogenous sources may have modulatory effects through regulation of the endogenous ADP-ribosylation of proteins.

40.11

IMMEDIATE EARLY GENE EXPRESSION IN ACRYLAMIDE NEUROTOXICITY. S.Kittur[1], H.Endo[1], G.A. Higgins[1], M. Sabri[2], P.Spencer[2], J.M.Stephens[3], P.H.Pekala[3] .[1]Mol. Neurobiol., NIA/NIH, Baltimore, MD, 21224. [2]Oregon Health Science Univ., Portland, OR 97201; [3]Dept. of Biochem., East Carolina Univ., Greenville, NC 27858.

Acrylamide has been shown to be a potent neurotoxin. However, the molecular mechanism by which acrylamide produces pathological changes in the CNS is unknown. Northern blot analysis was used to investigate the expression of mRNA coding for c-*fos* and c-*jun* in brain tissue from acrylamide-treated rats. Male Sprague-Dawley rats (10-12 weeks) were injected with 100mg/kg acrylamide dissolved in normal saline or with saline control for the study of acute acrylamide treatment. Chronic acrylamide treatment and dosage effects were studied on induction of c-*fos* and c-*jun* mRNA. There was a significant increase in the expression of c-*jun* mRNA and c-*fos* mRNA in brain tissue from acute acrylamide-treated rats as compared to control tissue. c-*jun* mRNA was increased in chronic acrylamide-treated rats, but no significant effects were observed on c-*fos* levels. These results indicate that acrylamide may act through immediate early genes in the central nervous system.

40.12

THE ROLE OF CYTOSOLIC FREE CALCIUM IN THE REGULATION OF PYRUVATE DEHYDROGENASE. H.-M. Huang, L. Toral-Barza*, K.-F. R. Sheu*, G.E. Gibson. Burke Med. Res. Inst. Cornell Univ. Med. Coll. White Plain, NY 10605

The pyruvate dehydrogenase complex (PDHC), a key mitochondrial enzyme, is regulated by phosphorylation (i.e. the PDHC activation state, $PDHC_A$). Studies in peripheral tissues have shown that the $PDHC_A$ is mediated primarily by extramitochondrial calcium. In brain, calcium has been implicated in physiological and pathological processes. Both altered calcium and $PDHC_A$ have been implicated in ischemic cell damage. To examine the relation between the $PDHC_A$ and cytosolic free calcium ($[Ca^{2+}]_i$), they were assessed in isolated nerve terminals under conditions that alter $[Ca^{2+}]_i$, including treatment with KCN, a model for studying ischemia-related changes. In the presence of external calcium, K^+ depolarization increased $[Ca^{2+}]_i$ and activated PDHC from 49.2% to 59.6% ($p<0.05$). Elevation of $[Ca^{2+}]_i$ is required for activation of PDHC by K^+, since omission of external calcium blocked activation and the elevation of $[Ca^{2+}]_i$. However, an increase of $[Ca^{2+}]_i$ is not necessarily correlated to the $PDHC_A$, since the calcium ionophore A23187 alone increased $[Ca^{2+}]_i$, but did not change $PDHC_A$. K^+ and A23187 elevated $[Ca^{2+}]_i$ more than K^+ alone, but did not further increase $PDHC_A$. Although KCN further increased the K^+-stimulated elevation of $[Ca^{2+}]_i$, it did not exaggerate the change in $PDHC_A$. Thus, activation of PDHC requires both external calcium and K^+.

40.13

PURIFICATION AND MOLECULAR CLONING OF GUANYLYL CYCLASE-ACTIVATING FACTOR SYNTHASE. M. Nakane, H. H. H. W. Schmidt* and F. Murad* Abbott Laboratories, Abbott Park,IL 60064 and Northwestern University Medical School, Chicago, IL 60611.

The soluble form of guanylyl cyclase-activating factor (GAF) synthase from rat brain was purified to homogeneity by 2',5'-ADP-Sepharose and Superose 6 gel-permeation chromatography. SDS-polyacrylamide gel electrophoresis revealed a single protein band with a molecular mass of about 155kDa. The purified GAF synthase was N-terminally blocked. We digested the purified protein by endoproteinase Lys-C, separated the peptides by HPLC, and obtained the amino acid sequences of several peptides. We also produced rabbit polyclonal antisera against GAF synthase from rat brain.

Complementary DNA clones corresponding to GAF synthase have been isolated from rat brain λgt 11 library by screening with the polyclonal antisera against GAF synthase. Blot hybridization of total poly(A)$^+$RNA from rat brain detected mRNA of about 8.5 kilobases. The partial amino acid sequences of GAF synthase showed a homology with some NADPH-dependent enzymes, indicating that GAF synthase may utilize a similar electron transfer mechanism from NADPH as these enzymes.

40.14

NMDA INDUCTION OF C-FOS IN DENTATE GYRUS NEURONS IS INHIBITED BY THE PHOSPHOLIPASE A_2 INHIBITOR MEPACRINE. L.S. LERREA & J.O. MCNAMARA, DUKE AND VA MED CTR, DURHAM, NC

Stimulation of N-methyl-D-aspartate (NMDA) receptors on dentate gyrus neurons causes an increase of intracellular calcium and expression of the immediate early gene (IEG) c-fos. The increase in c-fos is dependent on the rise in intracellular calcium but the calcium-dependent pathways involved in the induction of c-fos have not been defined. NMDA evokes a calcium dependent release of arachidonic acid from CNS neurons via phospholipase A_2 activation (Dumuis et al., Nature 347: 182-184, 1990; Sanfeliu et al., Brain Res. 526: 241-248, 1990). We therefore tested whether phospholipase A_2 is involved in NMDA induced increases in c-fos. Dentate gyrus neurons derived from 4 day old rats were maintained *in vitro* for 7-10 days. c-fos mRNA expression was measured by in situ hybridization using ^{32}P-dATP incorporated into a 50 base pair oligonucleotide complementary to the coding region of rat c-fos (Curran, T., et al., Oncogene 2: 74-87, 1987). Intracellular calcium in individual neurons was measured using the calcium sensitive dye Fura-2.

NMDA (50µM) produced a striking increase in intracellular calcium (75nM to 300nM) in dentate neurons. NMDA also caused a 10-15 fold increase in c-fos mRNA levels in individual neurons in a calcium dependent manner. The phospholipase A_2 inhibitor mepacrine (50µM) blocked NMDA induced increases in c-fos mRNA (12.8 ± 0.6 silver grains/cell vs. 1.9 ± 0.3 silver grains/cell) but had no effect on NMDA mediated calcium influx. Our results suggest that activating phospholipase A_2 is an absolute requirement for NMDA receptor mediated induction of c-fos. The absence of a mepacrine effect on calcium influx indicates that mepacrine did not inhibit NMDA receptor activation. Studies are in progress to further define the sequence of intracellular events coupling NMDA receptor activation to the induction of IEGs.

40.15

MOLECULAR SPECIES ANALYSIS OF PHOSPHATIDYLETHANOL (PE) FORMED IN PHORBOL ESTER-TREATED PC12 CELLS SUGGESTS A PHOSPHATIDYLCHOLINE (PC) PRECURSOR. P.G. Holbrook, L.K. Pannell* and J.W. Daly Laboratory of Bioorganic Chemistry; Bldg. 8, Rm 1A-15; NIH; Bethesda MD 20892

Phospholipase D-catalysed hydrolysis of a phospholipid produces phosphatidic acid (PA) and in the presence of ethanol, phosphatidylethanol (PEt). Activation of this enzyme is believed to underlie enhanced PC-turnover elicited the the tumor-promoting phorbol ester, 12-O-tetradecanoyl-13-acetate (TPA). PC12 cells were incubated for 5 or 30 minutes with ethanol (1%) in the presence or absence of TPA (80 nM). Phospholipids were extracted and separated by silica-gel thin layer chromatography. Molecular species analysis of phospholipids was performed by fast atom bombardment mass spectrometry (FAB) using a JEOL JMS-SX102 Mass Spectrometer. Fatty acid composition and head group structure were confirmed by link-scan of individual molecular ions. Material which represented 1% of the total lipid phosphorous and comigrated with PA had molecular ions corresponding in mass to phosphatidylglycerol and was shown to be its structural isomer, lysobisphosphatidic acid. The molecular species of PEt formed in TPA-treated cells were similar to those of PC isolated from untreated cells. These studies support the notion that PC is the precursor of PEt formed in TPA-treated cells.

BEHAVIORAL PHARMACOLOGY I

41.1

EFFECTS OF KAPPA OPIOID AGONISTS ON DOPAMINE. RECEPTOR-MEDIATED BEHAVIORS. C. Marin, T.M. Engber, P. Chaudhuri*, A. Peppe* and T.N. Chase. ETB, NINDS, NIH, Bethesda, MD 20892.

Current evidence suggests that striatal D-1 dopamine receptors reside largely on neurons which project to the substantia nigra pars reticulata and entopeduncular nucleus (internal segment of globus pallidus in primates). In contrast, striatal D-2 receptors occur primarily on neurons which project to the of globus pallidus (external segment in primates). The opiod perptide dynorphin, an endogenous agonist of kappa opiod receptors, is expressed in D-1 receptor-containing striatonigral cells. To evaluate the contribution of dynorphin-containing projections to motor function, we studied the acute effects of the kappa opioid agonists, spiradoline (U62066E; 0.5, 1 and 5 mg/kg, sc) and U50,488 (1, 10 and 25 mg/kg, sc) on D-1 and D-2 receptor-mediated behavior in rats. SCH 23390-induced catalepsy (0.5 mg/kg, sc) was significantly increased by spiradoline and U50,488 but raclopride-induced catalepsy (2.5 mg/kg, ip) was not affected. SKF 38393-induced grooming (10 mg/kg, ip) was attenuated by both kappa agonists in a dose-dependent manner. Quinpirole-induced stereotypies (1 mg/kg, sc) were also decreased by both drugs, but jerking was observed in these animals. Jerking was blocked by the selective D-1 agonist SKF 38393 (10 mg/kg, ip) and re-established with SCH 23390 (5 mg/kg, sc) pretreatment. These results suggest that kappa opioid receptor stimulation has functional effects on certain striatally mediated motor functions resembling those of D-1 receptor blockade.

41.2

EVIDENCE FOR TOLERANCE TO STIMULUS EFFECTS OF MORPHINE IN PIGEONS. S.A. Vanecek and A.M. Young, Department of Psychology, Wayne State University, Detroit, MI 48202.

Pigeons (N=8) were trained to discriminate among i.m. injections of saline, 1.8 mg/kg morphine (MS), and 10 mg/kg MS under FR 30 schedules of food reinforcement. MS (0.1-32 mg/kg) evoked dose-dependent substitution with both training doses during single trial test sessions. Similar results were obtained in tests of cumulative doses, with 0.18 mg/kg MS evoking only saline-appropriate responding, 1.8 mg/kg substituting for the low training dose, higher doses (5.6-32 mg/kg) substituting for the high training dose, and 32 or 56 mg/kg MS markedly suppressing rates. To assess development of tolerance, training was discontinued in four subjects, and 10 mg/kg MS, b.i.d., was administered for 14 days. After this treatment, the dose to suppress rates increased 3- to 5-fold, whereas the doses required to evoke substitution with either training dose did not change. Following an additional week of treatment with 56 mg/kg, b.i.d., the dose required to suppress rates showed no further tolerance, but the dose required to substitute for the low training dose increased by at least 3-fold in all subjects. The dose required to substitute for the high training dose increased 10-fold in one subject, but could not be determined in the remaining three due to severe rate suppression. Acute pretreatment with 56 mg/kg MS 24 h prior to a test did not change the doses required for stimulus or rate-altering effects. These results suggest that repeated MS treatment induces tolerance to discriminative stimulus effects of both low and high training doses of MS and to rate-altering effects of MS. (Supported by DA03796 and K02 DA00132)

41.3

THE KAPPA OPIOID AGONIST PD117302 FACILITATES BEHAVIORAL RECOVERY FOLLOWING FOUR-VESSEL CEREBRAL ISCHEMIA IN RATS. R.F. Genovese, X-C.M. Lu*, J.E. Moreton, K. Miller* and F.C. Tortella, Dept. of Med. Neurosci., Walter Reed Army Inst. of Res., Washington, DC 20307 and Univ. of Maryland, Sch. of Pharmacy, Baltimore, MD 21201.

Rats trained to lever press under a multiple schedule of food reinforcement (FR20, FI2') were prepared with bilateral vertebral artery occlusion and common carotid artery (CCA) snares. PD117302 (1.0 mg/kg, IP) (n=4) or saline (n=4) was administered after 15-min CCA occlusion (at reperfusion, and 2, 4, and 6 h post-occlusion). Sham occluded rats also received PD117302 (n=3) or saline (n=3). Although variability between subjects was observed, responding tended to return sooner in rats treated with PD117302 post-occlusion, as compared to rats treated with saline. Some behavioral disruption was also observed in sham occluded rats, however, substantial differences between PD117302 and saline treatments were not evident. These results suggest that PD117302 confers "behavioral protection" against global forebrain ischemia, and are consistent with previous studies demonstrating neuroprotective properties of PD117302 and other kappa opioid compounds.

41.4

BUPRENORPHINE DRUG DISCRIMINATION IN OPIATE-NAIVE ANIMALS: CROSS GENERALIZATION WITH MORPHINE. S. Pournaghash and A.L. Riley. The American University, Washington, D.C. 20016

The mixed opioid agonist/antagonist buprenorphine generalizes to morphine in animals trained to discriminate morphine from its vehicle in a drug discrimination procedure (see Shannon et al., J. Pharmacol Exp. Ther., 229: 768-774, 1984), an effect likely due to the fact that these compounds both act as mu receptor agonists. Because buprenorphine also has kappa antagonist properties, it is possible that this antagonist activity at the kappa receptor could influence the basis of its discriminative control and, thereby, affect the generalization of mu agonists such as morphine. In a test of this, animals were trained to discriminate buprenorphine from its vehicle and then were injected with various doses of morphine (0.56, 1, 1.8, 3.2, 5.6, 10 and 18 mg/kg). For all subjects, morphine dose-dependently generalized to buprenorphine. These effects are consistent with the previously-reported generalization of the two compounds and the position noted by Negus et al. (Psychopharmacology 98:141-143, 1989) that in opiate-naive animals buprenorphine's agonist properties overshadow its antagonist properties (which are evident only in opiate-dependent animals).

41.5

ANALYSIS OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE: INTERACTIONS WITH OPIATES. J. Broadbent and S.I. Dworkin. Department of Physiology and Pharmacology, Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1083.

While evidence now suggests that inhibition of dopamine (DA) uptake plays an important role in the discriminative stimulus properties of cocaine, DA is also known to interact with many other neurotransmitters, including opiates. Indeed, relatively recent data from both neurochemical and behavioral studies have highlighted this interaction; specific σ agonists increase DA release while the mixed μ agonist/antagonist buprenorphine inhibits cocaine self-administration. The present study therefore assessed potential interactions between opiate receptor systems and the stimulus properties of cocaine in rats trained to discriminate cocaine (10 mg/kg) from saline. Naltrindole, a purportedly selective σ antagonist, did not antagonize the cocaine cue even when tested at high doses (3-30 mg/kg, s.c.). Further, naltrindole did not significantly affect rates of responding. These preliminary data, suggest that blockade of σ opiate receptors does not influence the discriminative stimulus properties of cocaine.

41.6

DIFFERENT INVOLVEMENT OF D1 AND D2 RECEPTOR SUBTYPES IN DIFFERENT BEHAVIORAL RESPONSES TO DOPAMINE AGONSISTS. S. Cabib, S. Puglisi-Allegra
Istituto di Psicobiologia e Psicofarmacologia (CNR), via Reno 1, I-00198 Roma, Italy

High doses of the classic Dopamine (DA) agonist apomorphine induce in the mouse a typical stereotyped pattern of cage climbing behavior widely used as test for DA active substances. Neither the D2 nor the D1 selective agonists LY171555 and SKF38393 are able to induce this response. However, coadministration of the two selective agonists produce the same effect as apomorphine. 24 hrs after an interstriatal injection of the irreversible antagonist EEDQ, apomorphine-induced climbing was totaly suppressed, while no change in this respons was detectable in mice that received EEDQ injection in the nucleus accumbens septi. A 70% reduction of climbing was also observed in mice protected with α1, α2, 5HT2 antagonists and (-)-sulpiride (D2 antagonist) or SCH 23390 (D1 antagonist) before intrastriatal injection of EEDQ. These results indicate that apomorphine-induced steretyped climbing in the mouse is dependent on a positive interaction between D1 and D2 DA receptor subtypes at striatal level.

Cocaine, amphetamine, apomorphine at high doses as well as coadministration of high doses of selective D1 and D2 agonists, all induce increased horizontal locomotion in C57BL/6 mice; while selective agonists at D1 or D2 receptors are unable to produce this same response. However, although the selective D1 antagonist SCH 23390, at *per se* ineffective doses, is able to prevent both cocaine- and amphetamine- induced hyperlocomotion, the selective D2 antagonist (-)-sulpiride has no effect on this response even at hypokinetic doses. Taken together, these results support the view that different interactions between D1 and D2 DA receptor subtypes may be involved in the different behavioral effects of DA actve substances

41.7

THE STIMULUS PROPERTIES OF MU AGONISTS BUT NOT A KAPPA AGONIST SUBSTITUTES FOR THOSE OF DOPAMINE D2 AND DOPAMINE D1 AGONISTS IN A DRUG DISCRIMINATION PARADIGM. **S.C. Johnson, G.M. Samoriski and D.A. Cory-Slechta. Environ. Health Sci. Center and Program in Neuroscience, School of Med. and Dent., Univ. of Rochester, Rochester, N.Y. 14642.**

Neurotransmitter systems in the CNS are thought to interact with one another in a number of ways, both postsynaptically and presynaptically. In vivo and synaptosomal studies have shown that mu opioid agonists enhance dopamine (DA) release, while kappa opioid agonists decrease it. If such interactions have functional or behavioral properties, then mu agonists, but not kappa agonists would be expected to substitute for D2 postsynaptic receptor agonists or for D1 postsynaptic receptor agonists in a drug discrimination (DD) procedure. This hypothesis was tested in rats trained to discriminate an i.p. injection of 0.050 mg/kg of the DA D2 agonist quinpirole HCl from saline or an I.P. injection of 6.0 mg/kg of the DA D1 agonist SKF-38393 from saline in a standard two lever DD. Following acquisition of the discrimination, various doses of the mu agonists morphine, and fentanyl, or the kappa agonist U50,488 were substituted for the training drugs during generalization tests with drugs and doses tested in a random sequence. Morphine, in a dose range of 0.3 to 10.0 mg/kg, engendered SKF-38393 responding in a dose-dependent manner, with levels of generalization reaching > 90% for some individual animals at the highest dose. The mu agonist fentanyl, in a dose range of 0.025 to 0.3 mg/kg, substituted for both quinpirole and SKF-38393, reaching levels of drug lever responding of up to 80% in some rats. In contrast, the kappa agonist U-50,488 did not engender either quinpirole or SKF-38393 responding at any of the doses tested (2 and 4 mg/kg). These results support a role for mu agonists in modulating the behavioral functions of dopaminergic systems, including both D1 and D2 receptor function, and suggest that drug discrimination is a useful method for evaluating the functional properties of interactions between neurotransmitter systems. This work was supported by NIH grant R01 ES05017.

41.8

EFFECTS OF CHRONIC SCH 23390 OR ACUTE EEDQ ON THE DISCRIMINATIVE STIMULUS EFFECTS OF SKF 38393
Cao Gui-Hua*, Bruce D. Perry, William L. Woolverton, Drug Abuse Research Center, Pritzker School of Medicine, University of Chicago, Chicago, IL 60637

Three groups of rats (N=8/group) were trained in a two-lever, food-reinforced drug discrimination paradigm, to discriminate the D1 agonist SKF 38393 (SKF; 8.0 mg/kg, i.p.) from saline. After acquisition of the discrimination, the dose-response function for SKF (2.0-16 mg/kg, i.p.) was determined using a cumulative dosing procedure. In one group, the SKF dose-response function was redetermined 1 week after a regimen of 0.25 mg/kg SCH 23390 (SCH), i.p., once/day for 10 days, again 1 week after a second regimen of 0.5 mg/kg SCH, i.p., twice/day for 10 days and a third time after a regimen of 1.0 mg/kg SCH, i.p, twice/day for 21 days. SKF dose-response functions were redetermined in control rats after identical regimens of saline. In the third group, SKF dose-response functions were redetermined 24 hours after an injection of EEDQ vehicle; again 24 hours after an injection of 3.0 mg/kg; again 48 hours after 6.0 mg/kg EEDQ; and again 48 hours after 2 consecutive daily injections of 6.0 mg/kg EEDQ (12 mg/kg total). The dose-response function for the percentage of responses that occurred on the SKF lever (%DL) shifted significantly to the left following the second regimen of SCH; there was no further shift after the third regimen. The effects of SKF on response rate were unchanged. Repeated administration of saline did not alter the SKF dose-response function for %DL or response rate. Administration EEDQ vehicle or EEDQ also failed to alter the SKF %DL dose-response functions. However, EEDQ itself decreased response rate and enhanced the effects of SKF. Binding studies conducted 14 days after SCH or 48 hours after EEDQ exposure indicated an significant increase in Bmax for D1 binding sites in the corpus striatum (CS) in SCH-treated rats. Bmax tended to increase in the nucleus accumbens (NA) but did not achieve statistical significance (p=0.057). EEDQ treatment decreased Bmax in CS but had no effect in NA. Kd was unchanged in either region by either treatment. The results demonstrate that repeated administration of the D1 antagonist SCH can sensitize rats to the DS effects of the D1 agonist SKF, perhaps via an up-regulation of D1 receptors in the brain. Further, the results suggest that drug discrimination is a useful in vivo bioassay for measuring changes in CNS receptors. (Supported by NIH grant GM-22220).

41.9

DOPAMINE AUTORECEPTOR AGONISTS DECREASE LOCOMOTOR ACTIVITY OF 21-DAY- BUT NOT 10-DAY-OLD RATS. M.-Y. Lin* and D.E. Walters. Div. of Pharmacal Sciences, Auburn Univ., Auburn, AL 36849-5503.

In a previous study the DA autoreceptor agonist (-)3-PPP decreased locomotor activity of 28-day-old rats. In the present study, we examined the effects of (-)3-PPP and the putative DA autoreceptor agonists apomorphine (APO), B-HT 920, PD 128483 and SND 919 on locomotor activity in developing rats. 10- and 21-day-old rats were injected i.p. with normal saline or an agonist and placed in a 20 x 20 x 30 cm Plexiglass cage set inside a photocell activity monitor containing 8 infrared photocells in each horizontal direction. Their activity was monitored for a period of 15 minutes. All experiments were conducted in a quiet, dimly lit room. Each of the DA agonists produced a dose-related decrease in locomotor activity of 21-day-old rats. At higher doses, APO and B-HT 920 increased locomotor activity whereas (-)3-PPP, PD 128483 and SND 919 showed no evidence of postsynaptic agonist activity at any of the doses used. In contrast, at 10 days of age, there were no notable changes in locomotor activity in either direction following injection of each agonist at these doses. The results suggest that brain DA autoreceptors are functional behaviorally by 21 days of age and that the ability to exert control over locomotor activity develops between 10 and 21 days of age. Research supported by Auburn Univ. Research Grant-in-Aid.

41.10

EVIDENCE AGAINST DIFFERENT MOTOR BEHAVIOUR ELICITED BY SELECTIVE D-2 AND D-1 RECEPTORS STIMULATION IN MONKEYS
MR Luquin, J. Laguna* and JA Obeso
Department of Neurology, Movement Disorders Unit, Clínica Universitaria, Pamplona SPAIN

We have studied the motor responses elicited by independent administration of a D-2 agonist (+)-PHNO and of a D-1 agonsit (CY 208 243) to 5 parkinsonian monkeys. +-PHNO (0.04, 0.12, 0.3, 0.4 µg/kg) and CY 208 243 (0.1, 0.2, 0.4, 0.8 mg/kg) induced a similar relief of parkinsonism with regard to reduction in the disability score ($p>0.05$). Intensity and type of dyskinesias elicited by either drug were also similar for the whole range of doses ($p>0.05$).
Reserpine pretreatment (1.5 mg/kg) gave rise to a significant reduction in the duration of motor benefit induced by (+) PHNO ($p<0.0001$) and CY 208 243 ($p<0.05$) and increased the latency to the maximal effect ($p<0.001$). The magnitude of the motor response to each drug and the dyskinetic score did not change ($p>0.05$). These results indicate that selective D-1 or D-2 stimulation induces similar motor response in parkinsonian monkeys which seems to be independent of endogenous dopamine.

41.11

CAN SPONTANEOUS EYE BLINK RATE ASSESS CENTRAL DOPAMINE FUNCTION? M.S. Lawrence*, J.R. Taylor, J.D. Elsworth, R.H. Roth, J.R. Sladek, Jr.§, T.J. Collier§, and D.E. Redmond, Jr. Dept. of Psychiat. & Pharm., Yale Univ. Sch. of Med., New Haven, CT 06510, §Dept. of Neurobiol. & Anat., Univ. Rochester Sch. of Med. & Dent., Rochester, N.Y. 14642.

Previous studies have suggested a dopaminergic regulation of spontaneous eye blink rate in human and non-human primates. MPTP-treatment induces decreases in eye blink rates (Lawrence & Redmond, 1991). Dihydrexidine, a full D1 agonist, produced a rapid and dose-dependent (up to 1 mg/kg i.m.) increase in blink rate in 5 normal *Cercopithecus aethiops sabaeus* (St. Kitts green monkeys). The elevation in blink rate elicited by 0.3 mg/kg dihydrexidine was completely reversed by prior administration of a specific D1 antagonist, SCH 23390 (0.01 mg/kg i.m.), but was unaffected by prior administration of a specific D2 antagonist, remoxipride (1 mg/kg i.m.) The specific D2 agonist, (+)-4-propyl-9-hydroxy-naphoxazine (PHNO) (up to 0.01 mg/kg i.m.) also increased blink rate, reversed by pretreatment with D2 antagonists (Lawrence & Redmond, 1991; Elsworth, et al, unpublished). Since these data indicate that spontaneous blink rate is independently regulated by both D1 and D2 dopamine receptors, blink rate was measured from videotapes of 4 monkeys after possible reinnervation of the caudate nucleus by viable dopamine-containing grafts of fetal substantia nigra tissue, compared with 3 monkeys that received control grafts. A trend toward increases in blink rate was seen in the SN-caudate grafted monkeys, but not in the control subjects. However, spontaneous improvement in blink rates in mildly symptomatic monkeys has also been observed after MPTP-treatment alone, raising the question as to whether blink rates correlate with direct brain biochemical measures of dopamine function.

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41.12

EFFECT OF D1 AND D2 ANTAGONISTS ON COCAINE-INDUCED TASTE AVERSIONS. C.M. Ferrari and A.L. Riley. The American University, Washington, D.C. 20016.

Recently, subcutaneous (SC) cocaine has been shown to produce robust conditioned taste aversions (CTA), with subjects decreasing saccharin consumption by 95% by the fourth saccharin-cocaine (32 mg/kg) pairing (Ferrari, O'Connor & Riley, Pharmacol. Biochem. & Behav., 38: 267-271, 1991). To address the role of dopamine in this aversion, the present study compared the effects of SCH-23390, a D1 antagonist, and haloperidol, a D2 antagonist, on its acquisition. Water-deprived rats were given 20-min access to saccharin followed by an injection of SCH-23390 (0, 0.056, 0.10, 0.18, 0.32 or 0.56 mg/kg) or haloperidol (0, 0.42, 0.75, 1.3 or 2.4 mg/kg) followed 30 min later by a SC injection of 32 mg/kg cocaine. This procedure was repeated every fourth day for a minimum of five conditioning trials. On intervening days, subjects were given 20-min access to water. SCH-23390 antagonized the cocaine aversion at all doses except the lowest (0.056 mg/kg) for at least one trial. Haloperidol antagonized the cocaine aversion at all doses for at least seven trials. Although these data suggest that activation of either the D1 or D2 receptor is sufficient to induce the aversion (based on the antagonism with either SCH-23390 or haloperidol), some interaction between the subtypes in mediating the aversion to cocaine remains a possibility (see Wanibuchi & Usada, Psychopharmacology, 102: 339-342, 1990).

41.13

COMPARISON OF THE EFFECT ON LATENT INHIBITION OF DOPAMINE AGONISTS AND ATYPICAL ANTIPSYCHOTICS IN RATS. L.A.Dunn, R.J.Scibilia*, J.A. Franks*, C.D.Kilts. Dept. of Psychiatry, Duke Univ. Med. Ctr. Durham, NC 27710.

Latent inhibition (LI) of a conditioned response is a behavioral index of the stimulus filtering aspect of selective attention which is commonly observed to be defective in schizophrenia. LI is enhanced by typical antipsychotic drugs and is attenuated by amphetamine treatment in rats. Atypical antipsychotic drugs do not increase serum prolactin levels and have low or no liability for extrapyramidal side effects, neuroleptic malignant syndrome or tardive dyskinesia. We have reported previously that one atypical antipsychotic, clozapine, causes an amphetamine-like attenuation of LI (Dunn et al., Soc. Neurosci. Abstr., 16,2:1053, 1990). We have now evaluated the effects of the D_1 agonist SKF 38393, the D_2 agonist quinpirole and the atypical antipsychotics fluperlapine and amperozide on LI of the formation of a CER following 0 or 20 stimulus preexposures. A 2 x 3 ANOVA showed dose related attenuation of LI ($p<0.05$) by both the acute administration of SKF 38393 (0, 3 and 10 mg/kg sc.) and quinpirole (0, 0.1, 0.3 and 0.56 mg/kg). The atypical antipsychotics amperozide (1 mg/kg, i.p., 7 days) and fluperlapine (10 mg/kg, i.p., 7 days) also significantly decreased LI ($p<0.05$). The fact that the atypical antipsychotic drugs clozapine, fluperlapine and amperozide mimic the effects of D_1, D_2 and indirect dopamine agonists suggests that the therapeutic effects of these drugs are mediated by unique actions relative to typical antipsychotic drugs. (Supported by the Scottish Rite Foundation).

41.14

(+)3-PPP FAILS TO ANTAGONIZE AN APOMORPHINE-INDUCED MODEL OF PSYCHOSIS IN A PRIMATE SOCIAL COLONY. D.J. McGinness Grimes[1]*, R.F. Schlemmer,[1,2], N.L. Katz[1], D.P. Taylor[3] & J.M. Davis[1,2] [1]U. of Ill. at Chgo., [2]Ill. St. Psych. Inst., Chicago IL 60612 & [3]Bristol-Myers Squibb, Wallingford, CT 06492

The role of CNS sigma (σ) binding sites in the mediation of behavior has not been elucidated. Since many antipsychotic (AP) agents bind to σ sites as well as dopamine (DA) receptors, there is considerable interest in the potential role of σ ligands as AP. One model for screening compounds for potential AP activity is DA agonist-induced behavioral changes in nonhuman primate social colonies. In a stable adult stumptail macaque (Macaca arctoides) social colony, the DA agonist apomorphine (APO) induces behavioral changes that model human stimulant-induced psychosis. Known AP reverse the core behavioral changes in this model. The σ ligand BMY14802, an AP candidate, produces a dose-dependent antagonism of core behavioral changes induced by APO administration to members of a social colony. The purpose of this study was to examine the behavioral profile of another σ ligand with a low affinity for D_2 sites, +(3-(3-hydroxylphenyl)-N-(1-propyl)-piperidine, (3-PPP) in the APO model. After determination of baseline behavior, 4 doses of 3-PPP (0.1-3.0 mg/kg) were administered i.m. to 4 monkeys in a crossover design for 2 days/dose. 3-PPP was given 1.0 hr prior to the start of the first 60 min observation session on both days. APO (1.0 mg/kg, i.m.) was administered 15 mins after the first observation session and 15 min later a second observation session was conducted. Each session employed a "blind" observer who recorded > 40 behaviors. 3-PPP alone induced only minor changes (increased self-grooming) and did not induce movement abnormalities or sedation. APO increased the distance between monkeys in the cage, checking (visual scanning), submissive gestures and induced stereotypy. Unlike BMY14802, 3-PPP did not block the behavioral changes induced by APO administration. These results do not predict that 3-PPP has AP activity but they do not clarify the role of σ ligands as AP.

41.15

EFFECTS OF CHRONIC HALOPERIDOL AND CLOZAPINE ADMINISTRATION IN RATS WITH LESIONS OF THE FRONTAL CORTEX. G. Hussain*, G.E. Jaskiw, H.Y. Meltzer. Psychobiology Dept., Case Western Reserve University and Veterans Administration Medical Center, VAMC Cleveland, OH 44106.

Chronic administration of typical but not of atypical antipsychotic drugs (AAPDs), upregulates both mesolimbic and striatal dopamine (DA) type II (D2) receptors, and augments higher order stereotypic responses to apomorphine (APO). Such indices of DA transmission can also be modulated by the frontal cortex (FCx). The present study assessed effects of chronic AAPD treatment in rats with FCx lesions. Male Zivic-Miller rats (220-250 gr) had trephinations (SHAM) or knife cuts made across the frontal cortex (FCx) (AP $\pm$ 3.7, ML $\pm$ 4.0, VD $\pm$ 4.0). After 28d recovery, rats received a 21d treatment with haloperidol (HAL) 2 mg/kg/d, clozapine (CLZ) 20 mg/kg/d or vehicle in drinking water. Stereotypy after APO 0.75 mg/kg SC and catalepsy after HAL 1.0 mg/kg IP were assessed 3d and 7d after the last day of chronic treatment respectively. Overall, biting stereotypies were enhanced both by the FCx lesion and by HAL treatment but not by CLZ treatment. Furthermore, biting behaviors were not increased in the CLZ/FCx group. Overall the FCx lesion as well as HAL treatment reduced the intensity of HAL-induced catalepsy. The effects of CLZ on catalepsy were subtle and complex. Our data indicate that the differential effects of CLZ on stereotypy are not significantly altered by the type of FCx lesion induced here.

Supported by NARSAD.

41.16

HALOPERIDOL SLOWS LOCOMOTION AND INDUCES WITHIN-SESSION DECREMENTS IN EACH COMPONENT OF A RUN-CLIMB-RUN BEHAVIORAL CHAIN. **S.C. Fowler and L. Senyuz*. Depts. of Psychol. and Pharm., Univ. of Miss., University, MS 38677**

To determine if haloperidol differentially affects locomotor responses with different topographies, 10 hungry rats were successfully trained to traverse a 1.35-m-long rubber mat, climb a .59-m vertical rope, and cross a .8-m mat to reach and lick sweetened milk for 5 s. As recorded by video tape, absolute speed of locomotion was greatest in the first segment and least in the rope-climb link of the behavioral chain. Haloperidol (0.04 - 0.32 mg/kg, ip, 45 min) decreased locomotion speed and produced within-session decrements (across the 10 trials given per day) in all 3 components of the behavioral chain. Drug-induced failures to complete a trial increased as a joint function of dose and trial number. The drug did not attenuate normal increases in speed between trial 1 and trail 2. Taken together the results are more consistent with pseudoParkinsonism than with incentive/motivational deficits induced by haloperidol. Supported by MH43429.

41.17

DESENSITIZATION & CROSS-DESENSITIZATION TO THE BLADDER IRRITANT EFFECTS OF CAPSAICIN AND RESINIFERATOXIN IN RATS: BLOCKADE BY RUTHENIUM RED. V.J. Carlisi, A. Mattia and F. Porreca, Dept. of Pharmacology, Univ. of Arizona HSC, Tucson, AZ 85724

Capsaicin (CAP) and resiniferatoxin (RTX) may act at the same primary afferents to alter the micturition reflex. This study characterized the acute excitatory properties, development of desensitization and possible cross-desensitization of CAP and RTX after intravesical (*i.ves.*) administration to previously catheterized (4 d earlier) female, Sprague-Dawley rats. Additionally, the sensitivity of these effects to ruthenium red (RR) was studied. Lick/bite behaviors were scored during a 15 min observation period after *i.ves.* vehicle (VEH), CAP, or RTX. The incidence of lick/bite after CAP (1 µmol) was (41.2 ± 5.4%), a value significantly higher than that with VEH (15.3 ± 1.5%). Lower (0.1 µmol) and higher (3 µmol) doses of CAP were ineffective and behaviorally toxic, respectively. Following CAP (1 µmol), a second exposure to CAP (1 µmol) decreased the response to the control level (18.6 ± 1.4%), indicating desensitization. The irritant effects of RTX were also dose-related, with a lick/bite score of 34.6 ± 2.5, 67.0 ± 5.6 and 88.0 ± 2.4%, after 0.1, 1 and 3 nmol of *i.ves.* RTX, respectively; desensitization was produced by successive RTX (1 nmol) application. After *i.ves.* CAP (1 µmol) or RTX (1 nmol), the opposite treatment (RTX or CAP, respectively) after 30 min, failed to elicit a response differing from VEH, indicating cross-desensitization. Further, the desensitizing effect of CAP (1 µmol) was overcome by RTX (10 nmol). The acute irritant and desensitization responses for CAP and RTX were decreased by pretreatment with RR (1.5 mg/kg, s.c., -15 min), a treatment which did not itself produce effects. Thus, CAP and RTX appear to act on the same nerves in the bladder, via a RR-dependent mechanism.

41.18

SUBSTITUTION OF ELECTRICAL STIMULATION OF THE MEDIAL HYPOTHALAMUS FOR PENTYLENETETRAZOLE IN A TWO-CHOICE DISCRIMINATION TASK. R. Depoortere* and M. W. Emmett-Oglesby. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

The pentylenetetrazole (PTZ) discrimination paradigm, in which an animal (rat) is trained to press a lever in the presence of PTZ and an alternate lever in the presence of saline, has been proposed as an animal model of anxiety. The present study was aimed at assessing if the cue induced by electrical stimulation of the medial hypothalamus (MH), a brain structure whose electrical stimulation is known to induce aversive effects, would substitute for the discriminative cue induced by PTZ. Ten rats were trained to discriminate between PTZ (20 mg/kg i.p.) and saline, using a FR10, food reinforced choice-task. After acquisition of the discrimination task, these rats were unilaterally implanted with a pair of stimulating electrodes in the medial hypothalamus (4.5 mm AP, 0.3 mm ML, 9.5 mm DV with respect to lambda). After a further period of PTZ/saline discrimination training to habituate the rats to the electrical brain stimulation procedure, these rats were tested for stimulus substitution. During substitution sessions, rats were stimulated in the MH (monophasic cathodal pulses of 0.1 ms duration, frequency of 50 Hz, intensities varying from 10 to 80 µA), following a saline i.p. injection. Substitution of the cue induced by MH electrical stimulation was observed in 7 of 10 rats, at an average intensity of 50 µA (std 11 µA). This set of data further supports the hypothesis that the anxiogenic/aversive nature of PTZ is a major component of its discriminative properties. This study was supported by grants DA 03521 and AA 06890.

41.19

A DEMONSTRATION OF THE GRADED NATURE OF THE GENERALIZATION FUNCTION OF DRUG DISCRIMINATION LEARNING WITHIN THE CONDITIONED TASTE AVERSION PROCEDURE. A.L. Riley, M.A. Kautz, B. Geter, S.T. Smurthwaite, S. Pournaghash, P.M. Melton & C.M. Ferrari. The American University, Washington, D.C. 20016.

Prior work has suggested that generalization within drug discrimination learning is primarily quantal in nature with animals displaying either vehicle-appropriate or drug-appropriate responding, depending upon their detection of the drug stimulus. It has been questioned whether this reflects a characteristic response to drug stimuli or whether such responding is a function of the specific training and testing procedures used to establish and measure drug discrimination learning. The present experiment evaluated this issue by analyzing the generalization functions of individual subjects trained and tested within the conditioned taste aversion design under three different conditions. Specifically, animals were trained and tested with the same drug, trained with distilled water and tested with a drug or trained and tested with different drugs. The generalization functions under all three conditions were generally graded. Quantal responding, thus, is not a necessary outcome of drug generalization assessments. The nature of generalization in drug discrimination learning appears to be a function of the specific procedure utilized in training and testing the discrimination.

41.20

THE STRIATO-PALLIDAL-TEGMENTAL PATHWAY AND ORO-FACIAL DYSKINESIA: A BEHAVIOURAL AND ANTEROGRADE TRACING STUDY IN CATS. W. Spooren, J. Veening, B. Ellenbroek and A. Cools. Dept. Pharmacology, Univ. of Nijmegen, P.O. Box 9101, Nijmegen, The Netherlands.

Oro-facial dyskinesia (OFD), i.e. abnormal movements of the ear, eyelid and cheek in combination with tongue protrusions, can be elicited from the dorsolateral (r-CRM) but not the medial part (CRM) of the feline caudate nucleus. These effects are known to be funneled via the sub-commissural part of the globus pallidus (scGP), i.e. a first order output station of the r-CRM but not the CRM. OFD can also be elicited from the scGP by inhibition of GABA and stimulation of acetylcholine and dopamine D1 receptors. In order to find the destination of the r-CRM/scGP elicited OFD the efferent connections of the feline substantia innominata, which encompasses the scGP, were investigated using Phaseolus Vulgaris L-agglutinin (PHA-L). It has been found that the scGP projects, among other sites, to the peri-peduncular area (PPA). In contrast to the effects elicited from the scGP only tongue protrusions could be elicited from the PPA. Furthermore, OFD elicited from the scGP could, except for tongue protrusions, not be attenuated at the PPA suggesting that only tongue dyskinesias are funneled via the PPA but not ear, eyelid and cheek dyskinesias. These data suggest that at least tongue dyskinesias are funneled via the striato-pallidal-tegmental pathway.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: GLUCOCORTICOID RECEPTORS

42.1

Early Experience Alters Glucocorticoid Receptor Concentrations in the Hippocampus. L.A.Wilson, S.G.Warren, D.L.Amends, L.Nadel, and G.Wenk. Dept. of Psychology, Univ of Arizona, Tucson, AZ 85721.

Some events early in life alter long-term structural and functional changes in the nervous system. Handling of infant rat pups increased body and brain size, reduced emotionality and enhanced learning. Handling also increases glucocorticoid receptor (GR) number in the hippocampus, which has the net effect of providing a more rapid return to baseline corticosterone (CORT) levels following the termination of stress (Meaney, et al., Science, 1988). Isolation of young rat pups from their mother also interferes with the normal development of the hippocampus. Although the first 3 weeks of life are normally marked by a reduced tendency of animals to respond to stressful events with elevation of glucocorticoid hormones, pups do exhibit a stress response following a period of maternal isolation (Stanton, et al., Dev Psychobio, 1987). The presence of CORT during the developmental phase may interfere with cell production, particularly postnatal development in the hippocampus. We hypothesize that handling and isolating rat pups would alter behavior that depends on the hippocampus and CORT levels and glucocorticoid receptor concentrations. As previously reported (Wilson & Nadel, Neurosci Abst, 1990) handling and isolating animals impaired acquisition of a spatial task at 60 days of age, and handling and isolating combined enhanced performance. We now report that CORT levels differ between these groups, and that the behavioral changes are accompanied by alterations in the concentrations of GR in the hippocampus.

42.2

KAINIC ACID-INDUCED DECREASE IN HIPPOCAMPAL CORTICOSTEROID RECEPTORS. M.T. Lowy. Dept. of Psychiatry, Case Western Reserve University, Cleveland, OH 44106

The potential role of excitatory amino acids in the regulation of hippocampal glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) was examined using systemic administration of kainic acid (KA). KA (5,10,15 mg/kg) administered to 24 hr adrenalectomized (ADX) rats which were sacrificed 3 hr later produced large dose-related decreases in hippocampal GR (23-63%) and MR (50-71%). Significant decreases in hippocampal GR (26%) and MR (23%) could be detected as early as 1 hr after administration of KA (10 mg/kg). When ADX rats were sacrificed 24 hr after KA (10 mg/kg), large decreases in hippocampal GR (38%) and MR (47%) were still present. To assess the effects of KA under more physiological conditions, KA (10 mg/kg) was administered to adrenal-intact rats which were ADX 24 hr later and then sacrificed following an additional 24 hr. Under these conditions KA produced a 35% decrease in both hippocampal GR and MR. The KA-induced decrease in receptor binding was due to a decrease in the number of binding sites with no change in the dissociation constants. KA added in vitro failed to displace the radioligands from the GR and MR. These results indicate that excitatory amino acids play a prominent role in hippocampal GR and MR regulation. Supported by MH44699.

42.3

INCREASED LEVELS OF GLUCOCORTICOID RECEPTOR mRNA, GLUCOCORTICOID BINDING AND SENSITIVITY TO GLUCOCORTICOIDS IN CELL CULTURES AND IN MOUSE BRAIN FOLLOWING ANTIDEPRESSANT TREATMENT

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Increased cortisol secretion caused by hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and non-suppression of cortisol secretion following dexamethasone administration often characterize depression. A decreased number of glucocorticoid receptors (GR) in brain areas could explain this lack of feedback regulation seen in non-suppressors. Since non-suppressive responses of serum cortisol to dexamethasone revert to normal during successful antidepressant (AD) therapy, drug-induced modification of GR content and restoration of glucocorticoid sensitivity is possible. To test this hypothesis, we have investigated the effect of ADs on GR mRNA and glucocorticoid binding in both cell cultures (Ltk$^-$ and neuroblastoma) and in desipramine-treated (20mg/kgBW for 12 days) mouse brain. GR mRNA, isolated from cells or tissues fragments, was subjected to Northern blot analysis using a 1.8kb fragment of the GR cDNA as probe. In desipramine-treated cells we observed a two fold increase in GR mRNA compared to untreated cells and up to a seven fold increase in [^{3}H]-dexamethasone binding activity. Sensitivity to glucocorticoids was measured in cells stably transfected with a reporter plasmid carrying the chloramphenicol acetyl transferase (CAT) gene under MMTV-LTR (a glucocorticoid inducible promoter) control. When these cells were incubated in the presence of desipramine, a 2 to 3 fold increase in CAT activity was observed. *In vivo*, treatment of mice with desipramine increased the glucocorticoid binding in brain by 40%. These results suggest a mechanism whereby antidepressants could restore the sensitivity of cells to glucocorticoids and thus re-instate the normal feedback inhibition of glucocorticoids on the HPA axis.

42.4

Heterologous down-regulation of Type II adrenal steroid receptors by corticosterone-Type I receptor complexes in mouse brain. W. G. Luttge, M. E. Rupp* and M. M. Davda*. Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

In previous work (Endocrinology 125: 817-824, 1989), we found that adrenalectomy-induced increases in Type I adrenocorticosteroid receptor binding capacity in mouse hippocampus (HPC), cerebral cortex (CX), hypothalamus (HTH), brain stem (BS) and cerebellum (CB) could be inhibited by a subcutaneous injection of aldosterone (ALDO) given 24 h prior to tissue removal. The increases in Type II receptor binding capacity in HPC and CX, but not those in HTH, BS and CB, were also inhibited by ALDO. Since co-administration with a 50-fold excess of the Type I receptor antagonist, RU 26752, blocked ALDO-induced down-regulation of Type I and Type II receptors, we hypothesized that the Type II receptor down-regulation was mediated by ALDO-Type I receptor complexes. In the present study we extend this proposed mechanism, by examining the receptor-specificity properties of corticosterone (CORT)-induced Type I and Type II receptor down-regulation in female mice adrenalectomized for three days.

In our first experiment we used the same dose of CORT as in our earlier work with ALDO (0.5 μg/g) and, as expected, the magnitude of Type I and Type II receptor down-regulation was similar to that seen with ALDO. However, in contrast to our findings with ALDO, a 50-fold excess of RU 26752 was unable to block CORT-induced down-regulation of Type II receptors and it was only marginally effective against CORT actions on Type I receptors. In our second experiment we established the minimally-effective dose of CORT (0.1 μg/g) for Type I and Type II receptor down-regulation in hippocampus. In the third experiment we used this dose of CORT ± a 250-fold excess of RU 26752 to show that the Type I receptor antagonist could now not only block CORT-induced down-regulation of Type I receptors in HPC, CX, HTH, BS and CB, it also reduced the down-regulation of Type II receptors in HPC, but not in other brain regions. We interpret these findings as supporting a heterologous (*i.e.*, CORT-Type I receptor-mediated) mechanism for CORT-induced down-regulation of Type II receptors. The reduced efficacy of RU 26752 suggests that a homologous (*i.e.*, CORT-Type II receptor-mediated) mechanism is also operable. Follow-up studies examining Type I and Type II mRNA content are in progress. Supported by NS 24404.

42.5

ADRENAL STEROID RECEPTOR ACTIVATION IN BRAIN AND PITUITARY BY DEXAMETHASONE AND CORTICOSTERONE. A.H.Miller, R.L.Spencer, M.Pulera*, S.Kang*, B.S.McEwen and M. Stein. Mt. Sinai Sch. of Med., NY, NY 10029.

The 1 mg dexamethasone suppression test (DST) has been used extensively to evaluate feedback inhibition of the HPA axis by adrenal steroids (AS). Nevertheless, it remains unclear at what level of the HPA axis and through which AS receptor subtype dexamethasone (DEX) exerts its inhibitory effect. Type I and II AS receptor activation in the pituitary (PIT), hypothalamus (HYP) and hippocampus (HC) was assessed in rats after exposure to three separate doses of DEX. The low and medium doses were designed to reproduce DEX blood concentrations consistent with those found in humans following 1 mg of DEX. Receptor activation after steroid administration was determined by comparing AS receptor binding in the various treatment groups. Since AS receptors occupied and activated in vivo do not rebind radiolabeled steroid in vitro, a decrease in AS receptor binding as measured by an in vitro exchange assay reflects receptor occupation/activation by steroid in vivo. Results with DEX were compared to similar studies using corticosterone (CORT). All doses of DEX were associated with significant activation of type II receptors in the PIT, while only the high dose of DEX activated type II receptors in the HYP and HC. DEX did not significantly activate type I receptors in any tissue at any dose. In contrast, CORT significantly activated type I receptors in all tissues at all doses, while activating type II receptors primarily in the brain (especially HC) and not the PIT. These results suggest that the DST in humans may be primarily a test of PIT function, whereas CORT at low doses may provide an assessment of AS feedback inhibition at or above the level of the HYP. (Supported by MH00680)

42.6

GLUCOCORTICOID SUPPLEMENTATION, HYPOTHALAMIC-PITUITARY ADRENAL (HPA) AXIS REGULATION AND GLUCOCORTICOID RECEPTORS. E.A. Young, Mental Health Research Institute, University of Michigan, Ann Arbor MI, 48109

The HPA axis is under the negative feedback control of glucocorticoids, which are secreted in a circadian manner with a peak at lights-off and the nadir at lights-on. To determine the effects of raising the nadir level of corticosterone on circadian HPA function and glucocorticoid receptors, normal rats were implanted with subcutaneous pellets of corticosterone and cholesterol which serve as a slow release form of corticosterone supplementation. Two different concentration of corticosterone pellets were explored: 25% and 50%. Baseline hormones and receptors were examined in the a.m. and p.m. at two time points, days 3 and 6. The rats with low dose (25%) pellets demonstrated a circadian rhythm of corticosterone with increased corticosterone levels on day 3. By day 6 the levels of corticosterone were increased in the a.m. only. The adrenal weight and thymus weights were decreased, but not significantly. There were no changes in hippocampal Type II glucorticoid receptors by receptor binding. Type I receptors were too low to detect reliably in these intact rats.

The 50% pellets rats demonstrated an increase in a.m. corticosterone levels to ~5 μg/dl. This increase was enough to block the circadian p.m. rise in corticosterone, resulting in a flat corticosterone rhythm. This dose also resulted in a significant decreases in adrenal weight and thymus weight. Despite increased mean corticosterone levels resulting in decrease in thymus weight, an effect that is mediated via Type II receptors in thymus, there was no evidence of decreases in Type II glucocorticoid binding in hippocampus, hypothalamus or cortex. These data suggest that increased levels of corticosterone do not lead to changes in glucocorticoid receptor number in brain areas associated with negative feedback.

42.7

CRF-INDUCED DOWN REGULATION OF GLUCOCORTICOID RECEPTOR(GR) EXPRESSION IN AtT20 CELLS IS NOT POTENTIATED BY THE PROTEIN KINASE C ACTIVATOR PMA. K.E.Sheppard and D.J.Autelitano Baker Medical Research Institute, Melbourne, AUSTRALIA

In the rat pituitary corticotroph, AVP and phorbol esters potentiate CRF-induced secretion of POMC-derived peptides but not POMC gene expression. The major inhibitor of both POMC gene expression and peptide secretion are glucocorticoids. Studies on the regulation of corticotroph POMC suggest there is interplay between CRF and glucocorticoids, such that they influence the responsiveness of the corticotroph to each other. Recently we have demonstrated that CRF down-regulates GR mRNA levels and GR binding in a mouse corticotroph tumor cell line (AtT20). In the present study we have used AtT20 cells to determine the effects of PMA alone and the possible PMA potentiation of the CRF-induced decrease in GR expression. In addition, the effect of PMA and CRF on Bendorphin(βEP) secretion was measured. PMA (100nM) treatment for 1-24h did not alter levels of GR binding in AtT20 cells. To examine the possible synergism of CRF and PMA, cells were preincubated with CRF (100nM) for 23h. Following incubation, media were removed and fresh media added containing either CRF or CRF plus PMA(100nM); cells were then incubated for a further 1h. In these cultures, GR binding was significantly reduced to a similar extent by either CRF or CRF plus PMA. In contrast to GR binding, PMA significantly potentiated ir-βEP release from CRF pretreated cultures. These data confirm previous reports of synergism between CRF and PMA on POMC-derived peptide release, and in addition demonstrate that PMA does not potentiate the CRF-induced repression of GR expression.

42.8

SECOND MESSENGER REGULATION OF GLUCOCORTICOID (GC) RECEPTOR BINDING IN CULTURED BOVINE ADRENAL MEDULLARY CELLS. K. Betito, J. Diorio*, M.J. Meaney, & P. Boksa. McGill University, Depts. of Pharmacology & Psychiatry, Douglas Hospital Research Center, Montreal, Quebec H4H 1R3, Canada.

The sensitivity of target tissues to circulating GCs depends, to a large extent, on the concentration of GC receptors. Modulation of GC receptor number in response to cylcic nucleotides has been found in rat hippocampal, human skin fibroblast, murine lymphoma, and AtT20 cells in culture. In all but the AtT20 cell line, cAMP increases GC receptor binding and/or mRNA. Cyclic GMP either has no effect or slightly decreases GC receptor binding. In this study, we have investigated the regulation of GC receptor binding in bovine adrenal medullary cells in culture in response to cAMP and cGMP. GC receptors are present in adrenal medullary cells in culture and are involved in the regulation of catecholamine synthesis. Four day treatment of cells with 8 bromo cAMP (1 mM) or forskolin (10μM), decreases GC receptor binding by 60% and 54%, respectively. Preliminary results suggest that 1 mM 8 bromo cAMP requires as little as 1 day to reduce GC receptor binding. Four day 8 bromo cGMP (10 μM) treatment reduces GC receptor binding by 36%, and this effect is reversed by the cyclic nucleotide dependent protein kinase inhibitor H-8 (10 μM). The effects of both cAMP and cGMP on GC receptor binding are concentration dependent. Preliminary results suggest that 8 bromo cAMP can reduce GC receptor binding in highly purified chromaffin cell preparations, suggesting that 8 bromo cAMP acts directly on the chromaffin cells to modulate the GC receptor. These results demonstrate that GC receptor levels in adrenal medullary cells in culture can be regulated by the cyclic nucleotide second messengers cAMP and cGMP. Supported by FRSQ and MRC of Canada.

42.9

ESTROGEN (E) EFFECTS GLUCOCORTIOID RECEPTOR (GR) MEDIATED NEGATIVE FEEDBACK AND GR mRNA LEVELS IN THE RAT. L.H.Burgess and R.J.Handa. Dept. of Cell Bio., Neurobio., and Anat., Loyola Univ., Stritch Sch. of Med., Maywood, IL 60153.

We have previously shown that ACTH and CORT secretion following stress are elevated and prolonged in E treated ovariectomized (OVX) rats. This suggested an impairment of negative feedback by corticosterone (CORT). In these studies, we explored this possibility by examining feedback suppression by dexamethasone (DEX 100ug) or the synthetic GR agonist RU 28362 (100ug) in rats given E or sham treated for 17 days. Rats received daily sc injections of RU 28362, DEX, or oil for 4 days. One day later, the rats were subjected to a 1 min. ether stress. Plasma CORT levels at 0 min. and 20 min. post-stress showed an injection by E treatment interaction ($p < 0.0001$). At both time points CORT levels were decreased ($p < 0.01$ vs. oil controls) by DEX in both OVX and OVX + E rats, while RU 28362 decreased ($p < 0.01$) CORT levels only in OVX rats. This demonstrates that E interferes specifically with GR mediated negative feedback. We have also previously shown that MR mRNA levels (3.6 fmoles/mg RNA) in the 21 day OVX rat hippocampus (HIPP) were decreased ($p < 0.01$) by 1 day or 21 days of E treatment to 2.47 and 2.37 fmoles/mg RNA respectively. We have now examined GR mRNA levels, using an RNase protection assay, and found an increase ($p < 0.03$) in 21 day OVX rat HIPP GR mRNA from 2.78 to 3.22 fmoles/mg RNA following 1 day of E treatment . In the anterior pituitary gland of OVX rats, 21 days of E treatment resulted in a decrease ($p < 0.0005$) in GR mRNA from 1.86 to 0.79 fmoles/mg RNA. These data demonstrate a tissue specific effect of E on GR mRNA which may underlie the impairment of GR mediated negative feedback seen in the presence of E.

RESPIRATORY REGULATION I

43.1

HYPOXIA *DECREASES* INTRACELLULAR CALCIUM IN RAT CAROTID BODY GLOMUS CELLS. D. Kholwadwala and D.F Donnelly. Dept. Ped., Section of Respiratory Medicine, Yale Univ. School of Medicine, New Haven, CT 06510

Most theories of carotid body (CB) transduction postulate that hypoxia enhances secretion of neurotransmitters by raising intracellular calcium (Ca_i). We tested this hypothesis by examining changes in Ca_i of acutely isolated, adult rat glomus cells using fluo3 and confocal microscopy.

Glomus cells were isolated by repeated trituration after trypsin/collagenase digestion and plated on glass cover slips. Acute exposure to hypoxia (30-60s, $PO_2 \approx 0$ at nadir) caused a rapid and significant *reduction* in Ca_i (deltaF/F=-0.32±0.03, mean±SEM, n=43) which returned during reoxygenation. Acidity (pH 6.9, 5min) also caused a significant reduction in Ca_i (-.06±.02, n=12). Superfusion in Ca-free solution (+1mM EGTA) caused a reduction in Ca_i, but hypoxia continued to cause a reversible reduction in Ca_i from -0.33±0.06 to -0.64±0.04 (n=16). These results show that: 1) hypoxia causes a reversible reduction in Ca_i in rat glomus cells, 2) this reduction is likely due to sequestration to an intracellular site. Thus, it is unlikely that transmitter secretion from glomus cells is triggered by increases in Ca_i; rather, hypoxia may increase the binding of calcium to an intracellular regulatory site.

43.2

REGULATION OF INTRACELLULAR pH IN RAT GLOMUS CELLS. T.R. Cummins and D.F. Donnelly. Sect. Resp. Med., Dept. Pediatrics, Yale Univ. Sch. of Med., New Haven, CT 06510

Carotid body chemoreceptors transduce blood acidity and hypoxia into increased neural activity on the sinus nerve. It has been proposed that these stimuli may converge at the level of glomus cell intracellular pH (pH_i). The purpose of this study was to examine the mechanism(s) of pH_i regulation and examine how pH_i changes during chemostimulation. Glomus cells were acutely isolated from adult rat carotid bodies and plated onto glass cover slips. Cells were loaded with a pH sensitive dye, SNARF-1, and observed with a laser confocal microscope using single excitation/dual emission ratio technique. In HEPES saline, control pH_i was 7.21±0.04 (mean ± SEM, n=40), and cells recovered from an acid load. pH_i recovery was not blocked by amiloride (1 mM) or by Na^+ free solution, suggesting that these cells have a proton pump similar to renal tubule cells. Perfusion with Ringer's saline (HCO_3^- base) caused pH_i to acidify by 0.34±0.08 pH units (n=5). Removal of Cl^- in the presence of HCO_3^- caused pH_i to shift alkaline by 0.32±0.06 pH units (n=5).

Anoxia (5 min, $PO_2 \approx 0$) caused an acidification (-0.35±0.1 pH units, n=12) which recovered following reoxygenation. Extracellular acidity (pH 6.9) did not significantly effect pH_i. These results show that: 1) adult rat glomus cells may regulate pH_i by at least three mechanisms: a Na^+/H^+ exchanger, a H^+ pump and a HCO_3^-/Cl^- dependent mechanism. 2) Hypoxia causes an intracellular acidification.

43.3

NOREPINEPHRINE INDUCED BLOOD OXYGEN DESATURATION. D.G. Bernard, L.M. Sexcius*, R.M. Millis*, C.O. Trouth, and D.H. Wood*. Dept. of Physiol. & Biophysics, & Neurol., Coll. of Med., Howard Univ., Washington, D.C. 20059.

To determine the relationship between arterial oxygen desaturation and increased plasma norepinephrine (NE) levels during sleep apnea, we administered NE (3mg/Kg) to ten dogs anesthetized with Nembutal. Arterial blood pressure and ventilation were monitored continuously. Arterial blood and expiratory gas tensions were measured by spectrometry. Following a 30-minute control period, half of the animals (n=5) were given NE while breathing spontaneously and the remainder (n=5) were given NE during volume-controlled, positive-pressure mechanical ventilation at hypocapnic levels. Results showed that in the spontaneously breathing animals, intravenous NE produced an acute systemic vasopressor response with transient apnea, arterial oxygen desaturation, and hypercapnia. In the mechanically ventilated animals, arterial oxygen desaturation and hypercapnia were associated with a systemic vasopressor response and a decrease in the expiratory tidal volume. In sleep apnea endogenous NE may produce arterial oxygen desaturation. **SUPPORT: MBRS S06 GM 08016.**

43.4

ETHMOIDAL STIMULATION INHIBITS RESPIRATION RELATED NEURONES IN THE NUCLEUS TRACTUS SOLITARIUS (NTS) OF THE CAT. F.M. Boissonade and G.E. Lucier. Dept. of Medical Physiology, University of Calgary, Calgary, AB. T2N 4N1. CANADA.

Stimulation of the nasal mucosa initiates a number of respiratory protective reflexes, i.e. sneezing, coughing and apnea. Previous anatomical and electrophysiological studies have shown that afferents from the laryngeal mucosa of the cat, which are capable of initiating similar reflexes, project via the superior laryngeal nerve (SLN) directly to respiratory neurones in the NTS. The afferent fibres supplying the nasal mucosa travel to the brainstem within the ethmoidal nerve, a branch of the trigeminal nerve whose only direct projection is to the trigeminal nucleus. The purpose of the present series of experiments was to determine whether the activity of respiratory related neurones within the NTS could be inhibited by stimulation of the ethmoidal nerve. Extra-cellular recordings have been made from 133 respiration related neurones within the NTS of chloralose-anaesthetised cats and the effects of stimulation of the SLN and the ethmoidal nerve studied. Results demonstrate that the rhythmical firing of respiratory related neurones within the NTS can be inhibited by stimulation of the ethmoidal nerve. Previous reports that these neurones can be inhibited by SLN stimulation have been confirmed. The proportions of respiration related neurones within NTS inhibited by ethmoidal and SLN stimulation are similar (23% and 26% respectively). These results indicate that the NTS is a possible relay site for respiratory reflexes initiated by stimulation of the nasal mucosa, even though ethmoidal afferents do not project directly to this nucleus. (Supported by Canadian MRC.)

43.5

C-FIBER EVOKED RAPID SHALLOW BREATHING: EFFECTS ON MEDULLARY INSPIRATORY AND EXPIRATORY NEURONS. A.C. Bonham and J.P. Joad. Division of Cardiovascular Medicine and Department of Pediatrics, University of CA, Davis, Davis, CA 95616.

Phenyldiguanide (PDG) injected in the right atrium of spontaneously-breathing rats elicits the classic C-fiber response: rapid shallow breathing, bradycardia, and hypotension. The purpose of these studies was to extend previous work to determine the central neural mechanisms underlying PDG-evoked rapid shallow breathing. Studies were performed in urethane-anesthetized rats, in which phrenic nerve activity, arterial pressure, heart rate, and arterial blood gases were monitored. Action potentials were recorded extracellularly from neurons in the region of the ventral respiratory group (VRG) which discharged with inspiratory periodicity, expiratory periodicity, or phase-spanning activity. Neuronal discharge patterns were studied during the period of rapid shallow breathing evoked by right atrial injection of PDG (5 μg/kg). Neurons which discharged with inspiratory periodicity (n = 30) were inhibited; those with expiratory periodicity (n = 12) were excited and discharged throughout inspiration during the period of rapid shallow breathing. Neurons with respiratory phase spanning activity were first inhibited then excited (n = 3). These preliminary data suggest that PDG-evoked rapid shallow breathing is associated with complex inhibitory and excitatory effects on neurons with respiratory-related periodicities. Future studies will attempt to determine the projection and function of these neurons. (Supported by ALA RG007 and Tobacco-Rel. Dis. Res. Prog RT-411.)

43.6

MORPHOLOGY OF SLOWLY ADAPTING PULMONARY STRETCH RECEPTOR (SAR) AFFERENTS IN THE NUCLEUS TRACTUS SOLITARIUS OF THE RAT. S.K. Coles, F. Hayashi*, R. Behnia*, and D.R. McCrimmon. Depts. Physiology & Anesthesia, Northwestern University Medical School, Chicago, IL 60611

Activation of SAR afferent fibers gives rise to the Breuer-Hering reflex (*i.e.*, shortening of inspiration and lengthening of expiration). To identify potential central pathways responsible for the Breuer-Hering reflex, experiments were designed to study the axonal arborization patterns of SAR primary afferents and the distribution of synaptic boutons in the rat.

Glass pipettes (R = 20-30 MΩ) suitable for intracellular recording and dye injection were inserted into, or immediately medial to, the tractus solitarius at a level between 0.4 and 0.7 mm rostral to calamus scriptorius. Impaled axons were identified as SAR primary afferents if they discharged in phase with lung inflation and exhibited sustained firing of action potentials during maintained lung inflation. Neurobiotin (Vector Labs, 4% in 1.0 M KCl) was injected intra-axonally using depolarizing current pulses (1-2 Hz, 0.4 s) for 5-10 min. Tissue was sectioned (100 μm coronal) on a cryostat, processed using a conventional immunoperoxidase procedure and reacted with diaminobenzidine tetrahydrochloride.

SAR afferents arborized over about a 1 mm rostrocaudal extent at the level of the area postrema. Extensive axonal arborizations were observed with *en passant* and terminal varicosities appearing immediately medial, ventral and lateral to the tractus solitarius. Labelled primary afferent fibers could also be followed to their exit from the medulla in the vagus nerve several hundred microns rostral to the area postrema.

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43.7

DIFFERENTIAL EFFECTS OF VAGAL AFFERENT INPUTS ON VENTRAL AND DORSAL MEDULLARY INSPIRATORY-RELATED NEURONS. M.I. Cohen, W.-X. Huang*, R. Barnhardt* and W.R. See. Dept. of Physiol., Albert Einstein Col. Med., Bronx NY.

In decerebrate paralyzed cats, we observed the responses of inspiratory-related (I or IE) neurons to vagal (V) afferent inputs (lung inflation and/or electrical stimulation at 100 Hz). Stimulus trains started 100 ms after I onset and ended at I offset, with the stimulus current set to shorten I without net facilitation of phrenic discharge. 39/72 ventral respiratory group (VRG) neurons were excited, and 27 were inhibited by either type of V input; 10/17 dorsal respiratory group (DRG) neurons were excited and 4 were inhibited. Of those neurons excited by V electrical stimulation, stimulus-unit locking (indicated by significant coherence between stimulus and unit pulses) was shown by 6/7 DRG neurons but by only 4/38 VRG neurons. Further, excitation latencies < 20 msec were shown by 5/7 DRG neurons, but by only 3/38 VRG neurons. This difference indicates that the VRG neurons are more distant synaptically from the afferent inputs. Of the 39 VRG neurons that were excited by V inputs, 10 late-I neurons had patterns suggestive of an I off-switch function: a) V input produced earlier onset of firing as well as shortening of the I phase; b) the timing of discharge in relation to the end of I was very similar between control and stimulation phases. These neurons may play a role in I termination. (Supported by N.I.H. Grant HL-27300.)

43.8

PROPERTIES AND EFFERENT PROJECTIONS OF SECOND ORDER NEURONS ACTIVATED BY RAPIDLY ADAPTING LUNG RECEPTORS (RARs). J. Lipski, K. Ezure, K. Otake and R. B. Wong She. Dept of Physiology, Univ. of Auckland, New Zealand and Dept. of Neurobiology, Tokyo Metropolitan Inst. for Neurosciences, Japan.

RARs are responsible for a number of reflexes which affect respiration. Although the central terminations of afferents from these receptors have been traced to the caudal subdivisions of the nucleus tractus solitarius (NTS; Davies and Kubin, 1986, J.Physiol. 373: 63), the properties of the second order neurons are unknown. In Nembutal-anaesthetised cats, extracellular and intracellular recordings were made in the caudal NTS. RAR-activated cells (RAR-cells) were identified by: (a) electrical stimulation of vagal nerve afferents; (b) deflation and hyperinflation of the lungs; and (c) inhalation of ammonia vapour. The majority of the RAR-cells (85/100) were located in the commissural subnucleus of the NTS. Intracellular recordings of synaptic responses evoked by electrical stimulation of myelinated vagal afferents suggested monosynaptic EPSPs. Bilateral lesions of the commissural subnucleus abolished the reflex responses induced by ammonia inhalation or hyperinflations of the lungs, but not the Hering-Breuer reflex. A strong projection of the RAR-cells to the dorso-lateral pons was revealed by antidromic stimulation. This was confirmed in 3 cats by extracellular biocytin injection in the commissural subnucleus. Anterogradelly labeled terminals were also found in respiration-related areas of the medulla oblongata. These findings suggest that at least some commissural NTS projections to the rostral pons are involved in the reflex pathways from RARs. (Supported by the AMRF and the Wellcome Trust, U.K.)

43.9

CONCURRENT EFFECTS OF CAROTID CHEMORECEPTOR STIMULATION ON DISTRIBUTED BRAIN STEM RESPIRATORY NEURAL NETWORKS. K. F. Morris, A. Arata, R. Shannon and B. G. Lindsey. Dept. Physiol. & Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

We studied the responses of brain stem respiratory related neurons to carotid chemoreceptor (CCR) stimulation in anesthetized, vagotomized, artificially ventilated cats. CCRs were stimulated by injection of 200 ul of CO_2 saturated saline solution via the external carotid artery. Carotid sinus pressure was monitored and manipulated to control for baroreceptor effects. As many as five independently controlled arrays of electrodes (n=34) were used to monitor simultaneously single neuron spike trains in the ipsilateral n. tractus solitarius, rostral (region of retrofacial n.) and caudal ventral respiratory group (VRG), the contralateral VRG, and medullary raphe n. Spike train data files were analyzed with respiratory cycle-triggered and peristimulus-time histograms and correlational methods. Of 135 neurons, 69 increased and 23 decreased activity with CCR stimulation. Cells in all sampled domains responded to chemoreceptor stimulation; 34 of 38 pairs of neurons with short-time scale correlations included at least one responsive cell. The results include evidence for: (a) inhibitory connections from neurons excited by chemoreceptors to neurons that responded with decreased activity; (b) chemoreceptor mediated inhibition of rostral VRG inspiratory (I) - driver neurons and concurrent excitation of their caudal I neuron targets. The analysis of "many-neuron" recordings will be useful in elucidating the distributed actions of peripheral chemoreceptors on respiratory networks. Supported by NS19814 & BRSG S07 RR05749.

43.10

EXCITATORY AMINO ACID RECEPTORS IN THE COMMISSURAL NUCLEUS TRACTUS SOLITARIUS (SolC) MEDIATE CAROTID-BODY (CB) CHEMORECEPTOR REFLEX RESPONSES (CBCR) IN THE RAT. **A. Vardhan, A. Kachroo, J. Murugaian & H.N. Sapru, Section of Neurosurgery & Pharmacology, UMDNJ-New Jersey Medical School, Newark, NJ 07103.**

In spontaneously breathing, urethane-anesthetized rats, unilateral stimulation of CB chemoreceptors by saline saturated with 100% CO_2 produced an increase in minute ventilation (64%) and a decrease (17%) in mean arterial pressure (MAP). L-glutamate (GL; 0.88 nmole (nm)/site) was injected (20 nl) into the following areas of the SolC: (1) mid-line at the calamus scriptorius (CS) & 0.3 mm deep from the dorsal surface) & (2) midline, 0.3 mm deep & 0.5 mm caudal to the CS). GL increased minute ventilation (28.75%) and MAP (23%). Injections of carbachol (Carb; 50 pmole (pm)/site) in these areas produced similar respiratory and cardiovascular effects. GL and Carb did not elicit these responses from adjacent areas. Inhibition of areas (1) and (2) by injections of muscimol (1-2.5 nm/site; used as a tool to depress neurons) abolished the CBCR. Injections of kynurenate (Kyn; 500 pm; a non-selective excitatory amino acid receptor antagonist) into the areas (1) and (2) blocked the CBCR. The specificity of Kyn was indicated by its lack of action against the excitatory actions of Carb. These results indicate: (a) CB chemoreceptor afferents project to areas (1) & (2) in SolC and (b) excitatory amino acid receptors located in these areas mediate CBCR. Support: NIH (HL 24347) and AHA (NJ).

43.11

DIFFERENTIAL CHEMOSENSITIVITY TO CO_2 AND H^+ WITHIN THE IN VITRO PERFUSED ADULT BRAINSTEM PREPARATION. **M.P Morin-Surun, E. Boudinot and M. Denavit-Sauble, Laboratoire de Physiologie Nerveuse, CNRS, 91198 Gif sur Yvette, France.**

The brainstem contains chemosensitive elements which induce respiratory responses to changes in CO_2 and H^+ concentration. However, the question of the location of these central respiratory chemoreceptors is not solved: are they situated at the ventral surface or deeper into the brainstem? Recently we have developped an adult brainstem preparation perfused through the basilar artery. This preparation presents two advantages allowing this issue to be adressed. 1- The functional respiratory network is preserved (Morin-Surun et al, Soc. Neurosc. Abstr., p.226, 1989). 2- It is possible to control independently the extracellular fluid (ECF) by the perfusion through the basilar artery and the cerebrospinal fluid (CSF) by the bath perfusion. Under control conditions, the brainstem was perfused by a Krebs's solution saturated with 95% O_2 and 5% CO_2 (pH 7.4). The pH of the different solutions tested was adjusted to desired level (pH 7.0 and 7.8) by modifying the HCO_3 concentration without changing CO_2 content. Change in CO_2 concentration with constant pH was performed by modifying the HCO_3 concentration and the percent of CO_2 (HCO_3 60 mM, CO_2 9%, pH 7.4). Respiratory ouput was recorded from the hypoglossal roots. Changes of the hypoglossal integrated inspiratory activity were analysed. With different routes of application, the extracellular fluid or the cerebrospinal fluid, changes of the pH produced similar respiratory effects whereas changes of CO_2 induced different modifications. These results suggest different targets for CO_2 and H^+ respiratory chemosensitivity. Supported by the Fondation pour la Recherche Medicale.

43.12

VENTROLATERAL MEDULLA (VLM) CARBONIC ANHYDRASE (CA) INHIBITION: CHANGES IN PHRENIC NERVE ACTIVITY (PNA) AND LOCALIZATION OF CENTRAL CHEMORECEPTION SITES. E.L. Coates, A. Li, and E.E. Nattie. Dartmouth Medical School, Department of Physiology, Hanover, NH 03756

The purpose of this experiment was: 1) to determine the effect of VLM CA inhibition on baseline PNA, medullary tissue pH, and the response of PNA to transient and steady state increases in end-tidal CO_2, and 2) to localize sites of central chemoreception with acetazolamide (AZ) microinjections. AZ (10^{-4}M) pledgets applied to the surface of the VLM or AZ microinjected (1 nl, 10^{-3}M) beneath the medullary surface in chloralose-urethan-anaesthetized, vagotomized, carotid denervated, paralysed, servo-ventilated cats produced long-lasting increases in PNA. VLM AZ application by pledgets produced a long lasting decrease in extracellular pH but this was not a good predictor, in each individual animal, of changes in PNA. VLM AZ also reduced the slope and the half-time of the phrenic response to rapid step CO_2 increases but did not effect the phrenic response to steady state CO_2 increases. Localized induction of medullary tissue acidosis with AZ microinjections in the rostral, intermediate, and caudal chemosensitive areas of the VLM stimulated PNA. We conclude that localized inhibition of VLM CA causes a centrally mediated increase in ventilation attributable to medullary tissue acidosis, that medullary CA may play a role in central CO_2 chemotransduction, and that central chemoreceptors may be distributed throughout the VLM. (Supported by HL 28066 and HL 07449).

43.13

ENERGY SUPPLY OF THE RESPIRATORY NETWORK IN THE ISOLATED BRAINSTEM OF NEONATAL RATS

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We have measured tissue profiles of oxygen-pressure (pO_2), pH (pH_e) and K^+ activity (aK_e) to determine the microenvironment of respiratory neurons in the *in vitro* respiratory network of 1 to 4 day old rats. In the respiratory pool, ventrolateral to the N. ambiguus, rhythmic increases of aK_e (50-300 µM) and concomitant decreases of pO_2 (0.1-0.5 mmHg) occurred synchronously with the respiratory activity recorded from phrenic rootlets. Tetanic spinal cord stimulation elicited aK_e increases by up to 4 mM and decreases of pO_2 by up to 50 mmHg. These responses were reduced by 75 % after blockage of synaptic transmission by 5 mM Mn^{2+}. In the presence of 5-50 µM ouabain, there was a reversible increase of aK_e by 0.3-1 mM and a concomitant delay in the post-tetanic aK_e recovery.

The sensitivity of the respiratory neurons to hypoxia was tested by superfusion with a N_2-gassed solution. An initial increase in respiratory frequency was followed by a secondary slowing and, after about 10 min, by complete loss of activity. This disturbance of respiratory rhythm was accompanied by a fall in pO_2 to nominally 0 mmHg, a transient increase of aK_e by up to 1.8 mM and a decrease of pH_e by up to 0.3 units. These effects were reversible if hypoxia was shorter than 20 min.

It is concluded that the energy supply of the respiratory network is sufficient to maintain synaptic connectivity and Na^+/K^+-pump activity.

Supported by the DFG and A.v. Humboldt Foundation

43.14

EFFECTS OF HYPOXIA ON POSTSYNAPTIC POTENTIALS IN MEDULLARY RESPIRATORY NEURONS OF THE CAT. M.C. Bellingham, C. Schmidt*, U. Windhorst* and D.W. Richter. Physiol.Inst., Univ. Göttingen, 3400 Göttingen, F.R.G.

To investigate the reduction and eventual loss of rhythmic inhibitory inputs seen in cat medullary respiratory neurons in vivo during hypoxia, intracellular recordings were made from 16 expiratory neurons of the caudal ventral respiratory group, in 7 anesthetised, paralysed and artificially ventilated cats, which were vagotomised and glomectomised. Hypoxia was induced by ventilation with 5 or 10% O_2 in N_2, and continued until neuronal apnea occurred. IPSPs and EPSPs, evoked by ipsi- or contralateral superior laryngeal nerve stimulation (0.1 ms, 0.5-4 V, 2-3 Hz), were averaged (10-150 responses in the different phases of the respiratory cycle); their amplitude and neuron input resistance (Rn) were measured.

After 0.5-2 minutes of hypoxia and before the loss of rhythmic activity, IPSPs were decreased (mean change, -25%), EPSPs were decreased or increased (+12%), and Rn rose (+35%). During apnea (2-6 min. hypoxia), IPSPs were greatly reduced or abolished (-72%), EPSPs were reduced but not abolished (-18%), and Rn was increased (+60%).

These results indicate that decreased inhibition plays a significant role in the disruption of respiratory rhythm during hypoxia. The increases in Rn compared to reductions in PSP amplitude suggest that presynaptic factors, rather than postsynaptic properties, are involved in this decrease. Supported by SFB 330/B 10

43.15

VAGALLY MEDIATED, HEXAMETHONIUM-INSENSITIVE RELAXATIONS OF GUINEA PIG TRACHEALIS. B.J. Canning* A.C. Myers, B.J. Undem* Johns Hopkins Univ., Baltimore, MD 21224.

The guinea pig trachea with its extrinsic innervation intact was isolated and placed dorsal side down in a water-jacketed dissecting dish overfilled at a rate of 20 ml/min with warmed (37°C), oxygenated Kreb's solution containing 3 µM indomethacin, 1 µM atropine, 1 µM propranolol and 1 µM prazosin. The sixth and seventh tracheal rings caudal to the larynx were cut open opposite the trachealis and prepared for isometric tension measurements. The rings were contracted by adding 1 µM PGF2α to the buffer. Relaxations in response to nerve stimulation were measured and expressed as % reversal of this contraction. The nerves were stimulated with 10 sec trains of square pulses (1 msec, 150V) via suction electrodes at frequencies ranging from 1-32 Hz. Stimulation of the vagus nerves caudal to the nodose ganglia caused relaxations that were maximal at 32 Hz (55±17%) and abolished by hexamethonium (0.1 mM). Stimulating the vagi rostral to the nodose ganglia in preparations where the vagi were cut below the nodose also caused relaxations (26±5% at 24 Hz, n=8). These relaxations were, however, insensitive to 1 mM hexamethonium. By comparison, stimulating the rostral end of the cut cervical sympathetic trunks evoked relaxations (82±6% at 24 Hz, n=16) that were abolished by propranolol or hexamethonium. We conclude that the rostral trachealis of the guinea-pig receives two distinct types of nonadrenergic relaxant innervation from the vagi: 1) a hexamethonium-sensitive parasympathetic relaxant innervation, with preganglionic fibers carried by the recurrent laryngeal nerves, and 2) a hexamethonium-insensitive relaxant pathway projecting to the rostral trachealis from the vagus nerves via the superior laryngeal nerves. (Supported by NIH, HL 38095).

44.1

MECHANOSENSITIVITY OF REGENERATING SPROUTS OF GROUP I AND II MUSCLE AFFERENTS IN CATS. J.B. Munson and R.D. Johnson. Dept. of Neuroscience, College of Medicine and Dept. of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610.

We recently demonstrated that Group I and II muscle afferent fibers in cats were able to regenerate into the hairy skin and function as sensitive mechanoreceptors (Nishimura et al., Soc. Neurosci. Abst. 16:561, 1990). Almost all (98%) of the cross-innervated muscle afferents exhibited slowly adapting properties typical of Group I and II afferents in muscle but atypical of hairy skin afferents. This suggested that the slowly adapting pattern might be an inherent property of the muscle afferent axon terminal. We tested this hypothesis by axotomizing the nerves to the triceps surae muscles in cats and inserting the proximal cut end of each nerve into a Matrigel-filled Gore-Tex cuff. Six days later the properties of mechanosensitive nerve sprouts were studied by mechanically stimulating the cuff and recording from single afferents in L7-S1 dorsal root filaments. Of the 44 units that had developed mechanosensitivity, almost all exhibited a slowly adapting and regular discharge pattern to cuff stretch. Compared to Group I afferents, Group II afferents exhibited significantly lower stretch thresholds and were more likely to have a resting discharge. We conclude that the physiological signature of large diameter muscle afferents, namely a slowly adapting response to mechanical stretch, is an inherent property of the axon itself independent of its usual muscle spindle or musculotendinous target. After six days of regeneration, the axonal sprouts of Group II muscle afferents seem better able to transduce mechanical stretch stimuli. Supported by NS15913.

44.2

IMMUNOCYTOCHEMICAL PROPERTIES OF RAT DORSAL ROOT GANGLION (DRG) NEURONES INNERVATING MUSCLE, SKIN OR VISCERA. (SPON: J.Wood) M.J.Perry*, S.N.Lawson* and J.Wood. Dept. of Physiology, The Medical School, University of Bristol, Bristol, UK.

This study set out to correlate the peripheral innervation of DRG neurones with their immunocytochemical properties. The study extends the findings of previous workers and examines markers to carbohydrate (cbh) groups for the first time.

Female 10-12 week rats were anaesthetized with pentobarbitone (45mg/kg) and the gastrocnemius, saphenous or greater splanchnic nerve was exposed. The nerve was cut and placed in a tube containing 1µl 3% fast blue solution. After 2-3 days, the rats were re-anaesthetized and perfused with Zamboni's fixative. Appropriate DRGs were removed, frozen serial sections cut and immunocytochemistry performed. A computer system was used to count cells and record their labelling.

An interesting pattern was found with markers to cbh groups. The antibody 2C5 recognises a lactoseries cbh group and most labelling was found in skin afferents with less muscle and few visceral afferents labelled. Many of all three types of afferents showed binding for the lectins peanut agglutinin and soybean agglutinin but the highest percentages were for skin afferents and the lowest for muscle afferents. The antibodies against globoseries cbh groups, SSEA-3 and SSEA-4, were found to label a few muscle and skin afferents but no visceral afferents.

Over 80% of splanchnic visceral afferents were found to be neurofilament poor and were thus small dark (SD) cells whereas muscle afferents contained the greatest proportion of light (L) cells. A third of muscle afferents contained carbonic anhydrase which was present in very few skin and visceral afferents.

The results with all the markers examined in this study thus indicate that the chemical phenotype of DRG neurones is related to their peripheral innervation.

Supported by the Wellcome trust. The SSEA-3 and -4 antibodies were gifts from P.Goodfellow and the anti-carbonic anhydrase antiserum a gift from W.Cammer.

44.3

ROLE OF MECHANICS IN CUTANEOUS MECHANORECEPTOR RESPONSE. M. A. Srinivasan and K. Dandekar*, Research Lab. of Electronics and Dept. of Mechanical Engineering, MIT, Cambridge, MA 02139.

The relationship between the mechanical stimuli imposed on the skin and the resulting stresses and strains at the mechanoreceptor locations plays a fundamental role in the neural coding of tactile information. Although empirical determination of the stress or strain state of a mechanoreceptor is not possible at present, mechanistic models of the skin and subcutaneous tissues enable prediction and verification of the mechanisms of transduction. By using analytical models with simplifying assumptions for mathematical tractability, Phillips and Johnson (J. Neurophys. 46(6), 1981) demonstrated the power of mechanistic analysis in predicting peripheral neural response. In order to eliminate many of the limiting assumptions of these models, we employed numerical techniques of computational mechanics.

Finite element analysis was performed on a series of mechanistic models of the fingerpad under a variety of mechanical stimuli. The models ranged from a semi-infinite medium to cylindrical distal phalanx, composed of either a homogeneous elastic material or a thick elastic shell containing a fluid. Simulations of the mechanistic aspects of neurophysiological experiments involving mapping of receptive fields with single point loads, determination of spatial resolution of two-point stimuli, and indentations by single bars as well as periodic and aperiodic gratings were carried out. The results show, for example, significant differences in the loading imposed by a grating indented into the plane surface of a semi-infinite medium as compared to indenting a cylindrical finger. Also, the strain energy density at the receptor site emerges as a leading contender for the relevant stimulus that causes the responses recorded from slowly adapting afferent fibers. More generally, we demonstrate the power of computational mechanics in investigating the relationship between tactile stimuli imposed on the skin and the resulting peripheral neural response. (Supported by NIH Grant R29-DC00625)

44.4

FREQUENCY-RESPONSE FUNCTIONS IN THE PACINIAN CHANNEL USING THE PROPERTY OF TEMPORAL SUMMATION. C.M. Checkosky and S.J. Bolanowski, Institute for Sensory Research, Syracuse University, Syracuse, NY 13244

A model establishing a psychophysical-physiological link in the vibrotactile P channel (Bolanowski, et al, J. Acoust. Soc. Am.: 1988) was based on correlations among the psychophysical frequency response obtained on human glabrous skin and physiological functions measured on two Pacinian-corpuscle (PC) preparations: PC fibers innervating human glabrous skin (Johansson, et al, Brain Res.: 1982) and PCs isolated from cat mesentery. Although the three frequency responses were qualitatively similar, the low-frequency slope for the PC fibers differed from the other two functions by being 3 dB/octave less steep. The difference can be explained theoretically by differences in methodology and the property of temporal summation known to exist in the P channel (i.e., a 3 dB increase in sensitivity per doubling of stimulus duration). To test this, psychophysical experiments and physiological measurements on PCs isolated from cat mesentery were performed using two stimulus paradigms: a) constant stimulus duration and b) constant number of stimulus cycles (5) as used by Johansson, et al. When the stimulus duration was held constant at different test frequencies, the low-frequency (40-100 Hz) slopes of both the psychophysical and physiological curves were -12 dB/octave. However, when the stimulus duration was varied across frequency by holding the number of stimulus cycles constant, the low-frequency slopes were -9 dB/octave. The resulting low-frequency slopes agree with the theoretical expectations, the differences explained by methodology and the property of temporal summation. Supported by NS23933 and DC00380.

44.5

IMMUNOCYTOCHEMICAL STUDIES ON THE PACINIAN CORPUSCLE. J. E. Landcastle*, J. E. Savage*, N. B. Slepecky* and S. J. Bolanowski, Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

Using a variety of antibodies to cytoskeletal proteins (e.g., tubulin, filamin, glial fibrillary acidic protein (GFAP), tau, neurofilament 200, microtubule associated protein-2 (MAP-2), actin, S-100, and collagen) we performed immunocytochemistry on Pacinian corpuscles (PCs) obtained from cat mesentery. The purpose was to define and locate cell type specific proteins present in the different regions of this mechanoreceptor. Upon their removal, PCs were appropriately fixed (e.g., 2% paraformaldehyde, acetone, 95% ethanol, or Bouin's fix) and embedded in araldite. One-micron sections were cut, placed on gelatin coated slides and prepared for immunocytochemistry using commercially available primary antibodies and secondary antibodies coupled to FITC. The results showed: a) anti-S-100 protein specifically labeled the inner core of the PC but not the outer core or the neurite; b) anti-neurofilament 200 produced specific staining within the neurite, but no label was found in the inner or outer cores; c) anti-GFAP was found only surrounding the neurite; d) anti-tubulin was localized within the neurite. The results show that glial specific structural and calcium-binding proteins are found in the inner and outer cores surrounding the terminal neurite, and confirm the presence of cytoskeletal proteins within the neurite. Supported by NS 23933 and DC 00098.

44.6

VELOCITY DEPENDENT RESPONSES OF PRIMARY AFFERENT MECHANORECEPTORS TO BRUSHING OF FELINE HAIRY SKIN. J.D. Greenspan and S.L.B. McGillis*, Depts. of Neurosurgery and Physiology, SUNY Health Science Center, Syracuse, NY 13210

All types of cutaneous mechanoreceptors respond to a surface-parellel movement across the skin ("brushing"). The brushing velocity influences the neuron's mean firing rate in a systematic manner: a faster velocity produces a greater response up to at least 50cm/s (except for the C-mechanoreceptor).

The relationship between stimulus velocity and response rate is well fit by a power function. The exponent for the power function varies across mechano-receptor type, but is always less than 1.0. An analysis of 48 mechanoreceptors in the cat's distal hindlimb produced the following mean power function exponents: Field 1=0.72, Guard 1=0.54, SA1=0.46, Guard 2/Int.=0.43, Field 2/Int.=0.39, D-hair=0.26, PC=0.17.

A larger exponent can indicate a better ability to encode stimulus velocity information. However, consider-ation of the exponent alone does not take response variability into account. A discriminability estimate (d'e) was calculated for each mechanoreceptor's response to 5.0 vs. 10.0 cm/s stimuli. Only the Field 1 (mean d'e=4.3) and the Guard 1 (mean d'e=5.6) had values consistent with human perceptual d'e on hairy skin (4.3-5.1; Essick et al., 1988). All other afferent types showed d'e values below 3.5. Thus, the various classes of hairy skin mechanoreceptors have different capacities for encoding stimulus velocity information.

This research was supported by NSF grant #BNS-8808337.

44.7

AN UNBIASED METHOD RELATING CELL DIAMETER OR CROSS-SECTIONAL AREA TO CELL NUMBERS. C.M. Pover and R.E. Coggeshall, The Marine Biomedical Institute, Department of Anatomy and Neurosciences and Department of Physiology & Biophysics., The University of Texas Medical Branch, Galveston, TX. 77550

Studies using histograms to relate cell numbers to cell size will only be accurate if the cell profiles measured are representative of the population. Unfortunately, the assumption that measurements of profiles present in a random histologic section, the "classical" method, truly represent cell sizes is incorrect. This is because a large cell is more likely to be sectioned than a small one.

Using the "disector" method, we have obtained a random sample of rat dorsal root ganglion cells, which have been used to estimate total cell numbers and also to produce area and diameter histograms. If it is accepted that the disector identifies cells in an unbiased way, then comparisons show that the classical histograms over-represent large diameter cells. Supported by NIH grants NS10161 and NS11255.

44.8

RESPONSES OF TRIGEMINAL GANGLION CELLS PROJECTING THROUGH DEEP OR SUPERFICIAL VIBRISSAL NERVES. P.M.E. Waite & M.F. Jacquin. Anat., Univ. New South Wales, Sydney, Australia; Anat. & Neurobiology, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

The rat vibrissal follicle-sinus complex is innervated by a deep vibrissal nerve (DVN) and several smaller fascicles traveling in the dermis (conus or superficial vibrissal nerves, SVNs). The function of the SVNs is unknown, although it has been suggested (Rice et al., JCN 252:154, '86) that they form part of a diffuse, multivibrissal system. Anatomical and electrophysiological methods were used to test this hypothesis. No ganglion cells were double-labeled after HRP and DY injections in the DVN and SVNs of single follicles. In 15 rats, recordings were made from 58 ganglion cells with A-row receptive fields before and after cutting the DVN and/or SVNs to the responsive vibrissa. 83% of this sample projected through a DVN, 17% via a SVN. 15 other SVN cells were assessed after severing all A-row DVNs. No SVN or DVN cells were spontaneously active. All cells responded to single vibrissae; none were responsive to intervibrissal hairs. Latencies to electrical stimulation were similar for DVN (0.94 $\pm$ 0.25 ms) and SVN (1.0 $\pm$ 0.18) cells. In the DVN group, 40% were rapidly adapting and 60% were slowly adapting (SA); for SVNs, respective values were 20% and 80%. Directional sensitivity was not seen in any rapidly adapting cell, but it was found in all DVN and SVN SA cells. DVN SA cells had no group directional preference; SVN cells were more commonly responsive to caudal deflection. SVN SA cells had higher mechanical thresholds than DVN SA cells. No injury discharges occurred after cutting SVNs, but were present in 44% of DVN cells. Thus, DVN and SVNs differ in some response properties, although SVNs do not provide multivibrissal inputs. NIH DE07734, DE07662.

44.9

SENSORY ENDINGS IN THE INNER CONICAL BODY OF THE RAT MYSTACIAL VIBRISSA

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Previously we reported the existence of two types of sensory endings in the rat inner conical body (ICB), a superficial site of innervation in the mystacial vibrissa (Mosconi *et al.*, Proc. XII Int. Cong. Electron Microscopy, (1990):410-411). Lanceolate endings are supplied by myelinated axons, and blebbed endings are supplied by unmyelinated axon bundles. Both sets of endings are embedded in parallel sheaves of collagen, arrayed circumferentially within the ICB around the hair shaft. The two ending types are densely and reciprocally distributed with the lanceolate endings more prevalent in dorsal and ventral quadrants, and the blebbed endings more prevalent in rostral and caudal quadrants. Within the unmyelinated set of endings there are subsets identified immunohistochemically with antibodies to CGRP, galanin and substance P, as well as with the lectin GSA-IB$_4$. Taken together, these results support a wider role for thin caliber afferents in the ICB than simply nociception, possibly including low or high threshold mechanoreception and/or an efferent role in maintenance of the collagen and vascular matrix.

44.10

INNERVATION OF NONMYSTACIAL VIBRISSAE IN THE RAT. B.T.Fundin* and F.L.Rice. Anatomy dept., Albany Medical College, Albany, NY 12208.

Vibrissal follicle-sinus complexes (F-SCs) in the mystacial pad of mammals are heavily innervated by different types of sensory nerve endings (Rice et al.,JCN 252:154). One site - inner conical body (ICB) - in mystacial F-SCs was uniquely well innervated only in species such as the rat that rhythmically whisk their mystacial vibrissae. In this study we examined the innervation of F-SCs of rat vibrissae that are not whisked and are in other locations on the body. The Winkelmann silver technique was used on 100um thick frozen sections of F-SCs from: supraorbital (SO), temporal (T), lateral submental (LSM), antero-medial submental (aMSM), postero-medial submental (pMSM), median cervical (MC) and carpal forelimb (CF). Much of the innervation of the nonmystacial F-SCs was similar to that of mystacial F-SCs. All are innervated by a large deep vibrissal nerve (DVN) and several small superficial vibrissal nerves (SVNs). In all F-SCs, SVNs provide qualitatively similar distributions of Merkel endings to the rete ridge and circumferentially oriented endings to the ICB. DVNs provide Merkel and lanceolate endings at the level of the ring sinus and reticular endings at the level of the cavernous sinus. However, in comparison to others, F-SCs from SO,T,LSM,MC and pMSM have few lanceolates, aMSM have diffusely distributed Merkel endings, and CF have few Merkel endings that are diffusely distributed and limited to the superficial portion of the ring sinus. CF F-SCs also have a unique substantial contribution of DVN afferents to the ICB and numerous presumptive corpuscular endings in the cavernous sinus that are rarely seen in other F-SCs. These results failed to find variations in ICB innervation related to whisking but do suggest that some physiological differences should exist among vibrissae from different locations.

44.11

LOSS OF KNEE JOINT INNERVATION WITH AGING IN THE MOUSE. P. T. Salo and W. G. Tatton. Centre for Research in Neurodegenerative Diseases and Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8 CANADA

Loss of joint afferents with aging may result in degeneration of articular cartilage due to the failure of reflexes necessary to protect articular surfaces from abnormal loads. Some populations of murine neurons die progressively with age (Tatton et al, 1991). C57 mice develop knee osteoarthritis (OA) with age. Hence we sought to determine if knee joint afferents (KJ-DRGNs) die over the lifespan of C57 mice. Retrograde axonal tracing with Fluoro-Gold (FG, Fluorochrome, Inc.) was used to identify DRG neurons innervating the knee joint in young adult (3 months, n=6), middle aged (8 months, n=5) and aged (16 to 18 months, n=5) C57 isogenic mice (mean lifespan: about 24 months). Mice were injected with 2% FG (0.8 μL) into one or both knees. After 5-7 days DRGs from T13 to L6 were harvested. Serial sections were counter-stained with toluidine blue and examined alternately under bright field and UV illumination with appropriate filters (Leitz Filter Block A). FG-labelled somata innervating the mouse knee were distributed over 3 to 5 dorsal root ganglia in a quasi-normal distribution that was centered either at L3 or L4. We found that there are 158.2 $\pm$ 6.3 (mean $\pm$ S.E.) KJ-DRGNs per knee in 3-4 month old mice declining to 135.2 $\pm$ 10.7 at 8 months and 98.8 $\pm$ 10.7 by 16 to 18 months, representing a 37% loss over 15 months. Measurement of the KJ-DRGN somal diameters at the youngest and oldest ages indicated that all sizes are lost at similar rates.

Progressive loss of joint afferent innervation occurs in aging C57 mice which may contribute to the development of osteoarthritis in this species. Supported by MRC Grant #MA5218 and The Arthritis Society (Canada)

44.12

TRIGEMINAL SENSORY AXONS PROVIDE PEPTIDERGIC INNERVATION TO GLOMERULI OF THE OLFACTORY BULB. T.E. Finger, and B. Böttger*. Rocky Mountain Taste & Smell Center, Univ. Colorado Med. Sch., Denver, CO 80262.

Nerve fibers containing both substance P and CGRP occur in the olfactory nerves and glomeruli of the olfactory bulb. Although many investigators presumed that these were olfactory axons, speculation has arisen recently that these peptidergic fibers are derived from trigeminal sensory axons. In order to test this hypothesis, electrolytic stereotaxic lesions of the ophthalmic division of the trigeminal nerve and ganglion were produced in adult Sprague-Dawley rats. Success of the lesion was assessed by testing for abolition of a vigorous blink reflex. After survival periods ranging from 3 to 21 days, the animals were prepared for immunocytochemistry. In control animals and in animals which retained the blink reflex, numerous substance P-immunoreactive and CGRP-immunoreactive varicose fibers were present in the olfactory nerve and in glomeruli of the olfactory bulb. Transepithelial peptidergic axons, those that reach near the surface of the epithelium, also were present in the mucosa of the dorsal, caudal nasal cavity. In animals lacking the blink reflex, both CGRP- and substance P-immunoreactive fibers were absent from the olfactory nerve and glomeruli. Similarly, transepithelial peptidergic axons were sparse or absent in the nasal mucosa although subepithelial peptidergic fibers remained. The abolition of peptidergic fibers by trigeminal lesion indicates that these fibers originate from the trigeminal, not the olfactory system. Trigeminal peptidergic fibers also traverse the nasal epithelium and probably serve as the substrate for the sense of irritation produced by strong "odorants". If the peptidergic bulbar fibers are collaterals of those innervating the mucosa, these fibers would provide the basis for an axon reflex whereby irritating "odorants" might cause modulation of bulbar processing of subsequent pure olfactory stimuli.

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44.13

SENSITIVITY TO SMALL MAGNETIC VARIATIONS IN THE TRIGEMINAL SYSTEM OF THE BOBOLINK. R.C. Beason and P. Semm*. Biology Dept., State Univ. of New York, Geneseo, NY 14454

Electrophysiological recordings from the ophthalmic nerve and the trigeminal ganglion of the bobolink (Dolichonyx oryzivorus) indicate the presence of units that are sensitive to small or large changes in the magnetic field. The most sensitive units responded to changes of 200 nT (<0.5% of the earth's total field), but other units responded only to large changes of 30,000 nT or more. Most responses were to rectangular pulses of the magnetic field but other stimuli that units responded to included a 0.5 Hz sinusoidal variation of the magnetic field and a hand-held bar magnet. Fast adapting units responded to the retangular stimulus with a burst of spikes that was logarithmically correlated with the total change in maagnetic intensity. These sensitivities can be best accounted for by a magnetite-based receptor. Financial support was provided by NIH (1R15 NS2601601) to R.C.B. and the Deutsche Forschungsgemeinschaft (Heisenberg Program) to P.S.

PAIN MODULATION: BEHAVIOR

45.1

BEHAVIORAL AND PARAMETRIC ANALYSES OF THE TAIL-FLICK: FURTHER EVIDENCE THAT ALL FLICKS ARE NOT ALIKE. E.A. Bolan*, V.N. Iannone*, J.G. O'Neill*, and J.T. Cannon. Dept. of Psychology & Neuroscience Program, University of Scranton, Scranton, PA 18510-4596.

Several lines of evidence suggest that variations in the intensity of the heat stimulus can alter some neural substrates of the tail-flick and/or its sensitivity to antinociceptive mechanisms (e.g., Cannon et al., 1990; Jensen & Yaksh, 1986; Ness & Gebhart, 1986). The consequences of varying the inter-flick interval (IFI) for testing the tail-flick are less clear.

This study examined tail-flick latencies during 90 min sessions as they were elicited by one of the 9 permutations of 3 heat settings (generating latencies of approximately 2.5, 3.5, and 8.0 sec) and 3 IFIs (1, 5, and 10 min). Several topographical characteristics of the tail-flicks were also recorded.

Ten male albino rats (Holtzman) were individually housed and tested during the dark phase of a 12/12 hr light/dark cycle. On separate days, at about the midpoint of the testing sequence, 24 hr water and saccharin (3mM) intakes were measured. We previously found that the latter covaries with morphine analgesia and an opioid form of stress-induced hypalgesia.

Factor analysis across the 3 heat levels revealed 2 factors; mean latencies elicited by the lowest and highest settings loaded on factor 1 (.74 and .94, respectively), and those elicited by the middle setting loaded on 2 (.96). When saccharin and water intake were added, 3 factors emerged: 1--water (.95) and middle stimulus flicks (.94); 2--saccharin (.98) and low stimulus flicks (.81); and 3--high stimulus flicks (.99). A 3(heat) x 3(IFI) x 10(flicks obtained at 10 min intervals) ANOVA and subsequent analyses revealed that IFI significantly affected latencies at only the lowest heat setting at which 1 min IFIs produced a greater warmup effect with shorter tail-flick latencies occurring after 20 min. The speed of tail movement and most topographical measures varied significantly across heat levels, but not IFIs. At higher heat settings, the behavioral data suggest that animals continue to react to the test stimuli after tail withdrawal. Although the tail-flick occurs at a consistent tissue temperature regardless of the rate of heating (e.g., Ness & Gebhart, 1986), our observations suggest that with higher intensity test stimuli, residual heat in the skin can continue to stimulate nociceptors post-flick. Such suprathreshold stimulation could interact with nociceptive and antinociceptive mechanisms and thereby contribute to some conflicting observations across laboratories.

45.2

POWER SPECTRUM (PS) ANALYSIS OF ELECTROSTATIC WIRE DETECTOR DETECTS POST-SURGICAL MOVEMENT DEFICIT IN THE RAT. Vahn A. Lewis, Univ. of Texas, Dental Branch, Houston, Tx. 77030.

P.D. Wall proposed that following injury, animals experience a behavioral depression that is evident at 24 hours following surgery and may last for several days. J. Olds employed a simple wire movement detector which records movement induced electrostatic potentials. Here scoring of such signals by calculating the PS of the signal is reported. Resultant data can be used to assess movement and movement contamination of coincident EEG signals. 11 Sprague-Dawley rats were anesthetized with pentobarbital for surgery and implanted with recording pedestals for the purpose of recording EEG activity. The "wire" detector was fashioned from 34 cm. of Beldon 8430, audio-connecting cable circling the EEG recording cables. The movement signal was amplified, digitized and PS determined. Recordings were at 4, 8 and 27 hours post surgery, yoked control were made seven days later.

In controls, movement PS progressively increased following anesthesia, P<0.001. Post surgical rats had decreased movement at 27 hours (P<0.001, control=34X surgery). There was no correlation between movement PS and EEG PS.

45.3

EFFECTS OF PREPARATORY SURGERY ON THE THRESHOLD OF THE JAW OPENING REFLEX TO TOOTHPULP STIMULATION IN CONSCIOUS CATS. D. Banks,* M. Kuriakose* and B. Matthews. (SPON: Brain Research Association). Dept. Physiology, University of Bristol, BS8 1TD, England.

Previous experiments in this laboratory have shown that the threshold of the jaw opening reflex (JOR) to toothpulp stimulation is increased following noxious stimulation, including surgery, to other regions of the body in anaesthetised cats. In the present experiments we have investigated changes in this threshold during recovery from surgery. Three male cats were initially prepared under general anaesthesia (alphaxalone/ alphadolone, 18mg/kg I.M. and 5-8mg/kg/h I.V.). An occipital craniotomy was made and covered with a headpiece. A connector block was fixed to the skull over the frontal sinus. An indwelling cannula was inserted into an external jugular vein. Six stainless steel wires were passed subcutaneously from the connector block to the right anterior digastric muscle for recording EMG and Ag/AgCl fillings in the upper and lower right canine teeth for electrical stimulation. The JOR threshold was monitored every 2-10 hours for 7 days.

At the point of recovery from anaesthesia (usually 1-3 hours post operation) the JOR threshold to stimulating either the upper or lower canine tooth was >1000μA/ 1.0ms. In the following 2 hours both thresholds fell to 800-900μA/ 0.1ms and continued to decrease for 2.5 to 6 days, finally reaching a value of 17-34μA/ 0.1ms. The latter values are similar to those obtained in lightly anaesthetised animals without surgery. The threshold changes were similar for both teeth in each animal. In one animal requiring additional maxillary surgery several months after the first operation, there were similar changes in the JOR threshold but they were restricted to the upper canine tooth. In conclusion we suggest that the sustained nociceptor afferent barrage which follows preparation surgery leads to long lasting changes in the threshold of the jaw opening reflex.

Supported by the Medical Research Council, U.K.

45.4

PHENTOLAMINE SYMPATHETIC BLOCKS MISLEAD DIAGNOSIS. R. Verdugo*, S. Rosenblum*, J. Ochoa. Depts. of Neurology and Neurosurgery, Good Samaritan Hosp. and Oregon Health Sci. Univ., Portland, OR 97210.

Phentolamine sympathetic block (PSB) is reputed as pivotal in differential diagnosis of causalgiform neuropathic syndromes and in identifying "sympathetically maintained pain" (Anesthesiology 1989, 71:A732). Prevailing opinions on the matter recommend a "10-15 minute" observation period for placebo response (Arch Neurol 1987, 44:555). We evaluated both effectiveness of PSB and time course of placebo response (a: saline, b: propranolol) in a population of 46 patients, by extending the placebo phase to 30 minutes. Magnitude of spontaneous pain, mechanical hyperalgesia, cold hyperalgesia, and Tinel sign were tested every 5 minutes. Diminution of pain by 50% was considered significant response. Development of nasal congestion, red eyes and thermographic changes documented occurrence of sympathetic block.

Twenty seven of 46 patients did not respond to placebo or PSB; 13 responded significantly to placebo, and 6 to PSB but apparently not to placebo. In 12 of the 13 placebo responders, the effect was not saturated by 15 minutes; four of these would have been considered PSB responders if such short placebo phase had been used. In the 6 who appeared to respond to PSB, progressive pain relief was already witnessed during placebo phase, but by 30 minutes relief had not quite reached 50%. Phentolamine sustained, but did not change, the rate of placebo-induced relief, implying slow cooperative placebo response rather than specific effect of PSB on pain. There was no correlation between thermographic change and response to placebo or PSB.

Conclusions: 1) Placebo accounts for a high percentage of PSBs traditionally considered positive. 2) Placebo incidence in this population is greater than the assumed, 3) PSB is not a viable means for classifying causalgiform syndromes, 4) Proper evaluation of placebo effect demands at least a 30 minute observation period; placebo response develops slowly over time.

45.5

THE ROLE OF NORADRENERGIC NEURONS IN MEDIATING THE ANTINOCICEPTION INDUCED BY LOCUS COERULEUS STIMULATION IN TWO DIFFERENT SUBSTRAINS OF SPRAGUE-DAWLEY RATS. W.L. West*,D.C. Yeomans and H.K. Proudfit. Department of Pharmacology, University of Illinois at Chicago, Chicago,IL 60680.

There is evidence that electrical stimulation of noradrenergic neurons in the rat locus coeruleus (LC) produces antinociception (Jones and Gebhart, 1986a) that is mediated by the inhibition of nociceptive neurons in the spinal cord dorsal horn (Jones and Gebhart, 1986b). However, we have recently demonstrated that noradrenergic LC neurons innervate the ventral horn in one substrain of Sprague-Dawley rats (Sasco, Inc.) and the dorsal horn in another Sprague-Dawley substrain (Harlan, Inc.).

Behavioral experiments were performed in lightly anesthetized rats to define the functional significance of these anatomical differences. Electrical stimulation of the LC produced antinociception in both Harlan and Sasco rats, but only the antinociception in Harlan rats could be reversed by intrathecal injection of yohimbine, an alpha-2 adrenergic antagonist. In addition, the antinociception induced by LC stimulation in Sasco rats could not be reversed by either methysergide or naloxone. These results indicate that stimulation of the LC in Harlan Sprague-Dawley rats produces antinociception that is mediated by noradrenergic neurons. In contrast, the antinociception produced by LC stimulation in Sasco rats is not mediated by noradrenergic neurons. These results suggest that noradrenergic LC neurons are involved in modulating nociception in one rat substrain, but not in the other. Supported by USPHS Grant DA 03980 and in part by NIH Grant RR 07037.

45.6

THE DEVELOPMENT OF STIMULATION PRODUCED ANALGESIA (SPA) FROM THE PERIAQUEDUCTAL GRAY (PAG) IN NEWBORN RATS. H. van Praag* and H. Frenk. Dept. of Psychology, Tel-Aviv Univ., Ramat-Aviv 69978, Israel.

In adult animals, electrical stimulation of the PAG produces inhibition of nociceptive spinal reflexes, such as the tail-flick (TF). The present study investigated at which age SPA can be first evoked from the PAG in rats. In addition, naltrexone reversibility of SPA was tested. Pups aged 7, 14 or 21 days, and adult rats were anesthetized with Equithesin (3 ml/kg). A monopolar stimulating electrode was lowered into the dorsal or ventral PAG. After assessment of baseline TF latencies, constant current cathodal pulses (100 Hz, 100 μsec) were delivered, starting 10 sec before and continuing throughout each TF trial until a response occurred, or until cut-off (7 sec). Current intensity (3 to 200 μA) was increased stepwise for each 2 subsequent TF trials, which were separated by 1 min intervals. It was found that SPA can be elicited starting at 21 days, but not earlier. Effective current intensities were higher in 21-day-old animals (75-100 μA) than in adults (10-30 μA). Naltrexone inhibited SPA from the ventral PAG in 21-day-olds but not in adults. These findings indicate that supraspinal modulation of nociception develops only 3 weeks after birth and that the contribution of endogenous opioids to SPA does not remain constant throughout the ontogeny of rats.

45.7

ELECTRICAL STIMULATION IN THE MIDBRAIN: EFFECTS ON PSEUDAFFECTIVE RESPONSES TO COLORECTAL DISTENSION. A.Burnett and G.F.Gebhart. Department of Pharmacology, University of Iowa, Iowa City, IA 52242.

Colorectal distension (CRD) is a characterized noxious visceral stimulus which results in pseudaffective cardiovascular (CV) and visceromotor (VMR) responses (Brain Res. 450:153-169, 1988). While activation of descending inhibitory systems on cutaneous nociception have been extensively investigated, the effects of these systems on visceral nociception has not been systematically examined.

Present experiments examined descending modulation of visceral nociception from the midbrain periaqueductal gray (PAG). Experiments were conducted in male Sprague-Dawley rats anesthetized with halothane (0.5 - 0.9%). The pseudaffective responses to CRD (80 mmHg, 20s) were a pressor response, tachycardia and a vigorous VMR (measured from the integrated EMG of the abdominal musculature). Electrical stimulation in the PAG (100 Hz, 100 μs pulses) inhibited the VMR in an intensity-dependent manner (10 - 100 μA), while the pressor response to CRD was unaffected at any intensity tested.

These data suggest that the VMR and CV response to CRD are perhaps mediated via different mechanisms, and/or are not modulated by the same centrifugal systems.

45.8

EFFECTS OF LIDOCAINE INJECTION INTO THALAMIC NUCLEI IN THE FORMALIN TEST. J.E. McKenna and R.Melzack*, Dept. of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Quebec, Canada H3A 1B1.

Pain behavior expressed in the late, tonic phase of the formalin test is not solely dependent upon peripheral input, but may also rely to a large extent on changes within the CNS induced by nociceptor-generated activity which occured during the earlier phase. Since various thalamic nuclei have been proposed as mediators of separate aspects of the pain experience, this experiment was undertaken to determine if pain behaviors change within the formalin test after the temporary deactivation of ventrobasal, anterior, intralaminar or posterior nuclei of the thalamus. Long-Evans rats received 1.0 μl intracranial infusions of 2% lidocaine or saline, either before or after a 60 μl subcutaneous injection of 2.5% formalin acetate in the plantar surface of one hindpaw. The behavioral consequences of lidocaine infusion differed according to the site of delivery and time of administration relative to the formalin injection. Implications of these results are discussed. Supported by NSERC grant A7896.

45.9

EVIDENCE THAT THE INHIBITORY EFFECTS OF ROSTRAL HYPOTHALAMIC STIMULATION ON NOCICEPTIVE REFLEXES INVOLVE A RELAY IN THE PERIAQUEDUCTAL GREY IN THE RAT. B.M. Lumb* & F.M. Semenenko*. (SPON: Brain Research Association). Department of Physiology, Medical School, Bristol, BS8 1TD, U.K.

Activation of neurones in the anterior hypothalamus/preoptic area (AH/POA) reduces nociceptive responses in the spinal dorsal horn (Lumb, B.M. *J.Physiol.*, **424**, 427-444, 1990). Since there are no known direct projections from AH/POA to the spinal cord, these effects must be mediated via a supraspinal relay. One possibility is the periaqueductal grey (PAG).

We have demonstrated in a retrograde tracing study, using fluorescent latex microspheres, that individual neurones in AH/POA project to either the dorsal or the ventral aspect of the PAG and that very few neurones project to both. We have also tested, in six chloralose-anaesthetised rats, whether AH/POA stimulation that inhibits spinal reflexes also evokes activity in the PAG. Neurones in the AH/POA were activated by microinjection of the excitatory amino acid DL-homocysteic acid (DLH; 50nl, 0.5M). Descending influences on spinal nociceptive reflexes were assessed by monitoring changes in reflex activity recorded from a lumbar spinal nerve in response to stimulation of visceral afferent fibres in the splanchnic nerve (SPLN).

At 18 of 25 sites tested in AH/POA, microinjection of DLH led to a marked reduction in reflex activity which in all but 5 cases was accompanied by an increase in multi-unit activity in the dorsal and/or ventral PAG. Increases in PAG activity had a similar timecourse to the depression of viscero-somatic reflexes. Interestingly, any SPLN-evoked responses recorded at the same sites in the PAG were depressed over the same timecourse. These data indicate that descending inhibition evoked from the AH/POA involves an excitatory relay in the midbrain PAG. [Supported by the MRC, U.K]

45.10

BILATERAL LESIONS OF THE CENTRAL NUCLEUS OF THE AMYGDALA ATTENUATE ANALGESIA INDUCED BY ENVIRONMENTAL CHALLENGES. R.J. Fox* and C.A. Sorenson. Neuroscience Program, Amherst College, Amherst, MA 01002.

Studies involving shock controllability, exposure to natural predators, and classical conditioning indicate that higher brain regions are likely to be involved in stress induced analgesia. These environmental challenges also induce fear-related behavioral and physiological responses. Since the central nucleus of the amygdala (CNA) has been observed to be a very important brain region for fear-related responses (Davis et al. Psych. of Learning and Motiv. 21:263, 1987), it also may be an essential component of the neural circuitry supporting analgesia induced by some environmental challenges. This study tested this hypothesis by examining the effects of radiofrequency lesions of the CNA on analgesia induced by three different forms of environmental challenge: exposure to a cat, acute footshock (1.6 mA, 1 min), and classical conditioning to an environment previously associated with footshock. CNA lesions significantly attenuated analgesia induced by all three forms of environmental challenge, indicating that this nucleus may be critically involved in analgesia induced by environmental challenge as well as fear responses produced by these challenges.

45.11

THE EFFECTS OF DECORTICATION OR HEMISPHERECTOMY ON AUTOTOMY (AT) BEHAVIOUR IN RATS. N.E. Saadé, S.F. Atweh and S.J. Jabbur. Faculty of Medicine, American University of Beirut, Beirut, Lebanon.

Although lesions of the somatosensory cortex in rats have been reported to produce no effect on AT of the denervated contralateral hindleg (Wall, P.D. et al., Pain 35:327, 1988), various types of decortication have been reported to decrease pain in humans and experimental animals (Rampin, O. et al., Somatosensory Res. 4:237, 1987) In this study, two groups of rats (n=5 for each) were subjected under deep anesthesia, to either a massive decortication (group A) or to a massive hemispherectomy (group B) on one side and, after a recovery period (10-15 d), to denervation of the contralateral hindleg by sectioning and ligating the sciatic and saphenous nerves.

In group A, all rats exhibited a delay in onset of AT (27.4±0.87 days, control 12.7±4.14 days). In group B, rats did not show any signs of AT during 7 weeks of observation but a second denervation of the ipsilateral hindleg elicited an accelerated AT, starting simultaneously in both hindlegs (7.8±1.96 days). We conclude: (1) Hemispherectomy (including basal ganglia) abolished while decortication delayed AT. (2) The presence or absence of AT is not related only to neglect or awareness of an insensate appendage, because it was elicited in both hindlegs following an ipsilateral denervation. (Supported by grants from LNRC and DTSabbagh Fund).

PAIN MODULATION: OPIOIDS I

46.1

THE EFFECT OF NOCICEPTIVE FOOTSHOCK AND MORPHINE ON GLUCOSE UTILIZATION IN THE RAT. B. Gescuk*, S. Lang*, D. Huston-Lyons and C. Kornetsky. Boston University School of Medicine, Boston, MA 02118.

The present experiment determined the effects of morphine sulfate (MS) on local cerebral metabolic rates for glucose ($LCMR_{glu}$) in male F-344 rats required to turn a wheel manipulandum in order to escape from footshock. Four groups of animals were studied: control-saline, control-MS, footshock-saline, and footshock-MS. The two footshock groups were exposed to a maximum of 11 days of half hour sessions of footshock. All animals were administered MS (4mg/kg sc) or saline 7 days, 3 days, and 10 min prior to the start of the $LCMR_{glu}$ experiment. Footshock caused an increase in the medial prefrontal cortex (PFC) and the anterior pretectal area. This effect in the PFC appeared to be reversed by MS, suggesting an inhibition of cortical processing of pain. However, in other sites, MS in the presence of footshock caused an increase in metabolism when compared to footshock or MS alone. These areas were the subthalamus, mammillary bodies, red nucleus and several thalamic regions: VL/VPL, gelatinosus, and parafasicular nuclei. Results suggest that MS in the presence of pain increases metabolic activity in those regions associated with descending inhibitory pain pathways. (Supported in part by NIDA grant DA02326 and Research Scientist Award DA00099 to CK).

46.2

BEHAVIORAL EVIDENCE FOR THE EXISTENCE OF "ANTI-ANALGESIA" SYSTEMS: CONDITIONED REVERSAL OF OPIOID ANALGESIA. E.P. Wiertelak, L.Subel*, S.F. Maier & L.R. Watkins. Dept. Psych., Univ. CO, Boulder, CO 80309.

The existence of opiate analgesia systems has long been recognized. In recent years, it has been proposed that endogenous "anti-opiate" systems exist as well, serving to antagonize opiate analgesia. The existence of both opiate and anti-opiate systems raises the question of their relative physiological roles in the normal, intact organism. It has been previously hypothesized that endogenous analgesia systems serve to suppress pain in response to danger signals, to aid the animal in escaping life-threatening situations. We propose that the endogenous anti-analgesia systems serve to return the organism to basal pain sensitivity in response to safety signals, so that recuperative behaviors can ensue.

This idea suggests that analgesia elicited by learned danger signals should be reversible by learned safety signals. Therefore, we developed a paradigm to test this notion. Analgesia to learned danger signals was produced by repeatedly pairing contextual cues (general cues provided by the experimental room) with tailshock (see Subel et al., companion abstract). This paradigm rapidly elicits a powerful opioid conditioned analgesia, which is reversible by intrathecal naltrexone (1 μg), intracerebroventricular naltrexone (10 μg) & naltrexone delivered over the frontal cortex (1 ug). The safety signal was provided by a light which was illuminated at shock termination. The animals rapidly learned this safety signal as well, as evidenced by the ability of the light cue to induce the immediate & complete reversal of conditioned analgesia. Summation and retardation-of-acquisition tests established that the light cue served as a conditioned inhibitor of analgesia.

These data provide the first empirical behavioral evidence for the existence of endogenous anti-analgesia mechanisms. NSF grant BNS 88-09527.

46.3

"NON-OPIATE" ANALGESIA IS REALLY OPIATE: APPARENT PARALLEL ACTIVATION OF MULTIPLE SPINAL OPIATE SYSTEMS. L.R. Watkins, E.P. Wiertelak, L.H. Silbert* & S.F. Maier, Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

Repeated tailshocks (5 s, 1mA, 1 min ITI) elicit 3 sequential analgesias, as defined by s.c. naltrexone (tail flick test): an early opiate after 2 shocks, non-opiate after 10-40 shocks, & a late opiate after 80-100 shocks. The present study identified critical spinal & supraspinal opiate receptors using intracerebroventricular (ICV) & intrathecal (IT) injection of specific opiate antagonists (CTOP, naltrindole, binaltorphimine).

ICV (3rd ventricle injection; 4th ventricle occlusion) studies show that: (1) only the late opiate analgesia is blocked by 7 μg ICV naltrexone; (2) the late opiate analgesia involves supraspinal delta opiate receptors; & (3) neither the early opiate nor the "non-opiate" analgesia is mediated by supraspinal opiates.

IT (lumbosacral) studies show that: (1) 7 μg naltrexone "non-specifically" attenuates all 3 analgesias; (2) spinal kappa opiate receptors mediate both early & late opiate analgesias; & (3) none of the specific antagonists, when injected singly (equimolar to 7 μg naltrexone), affects "non-opiate" analgesia. (4) However, when a combined injection of mu, delta & kappa antagonists is made (combined dose equimolar to 7 μg naltrexone), "non-opiate" analgesia is abolished. (5) Mu-delta & mu-kappa antagonist pairs also abolish "non-opiate" analgesia; the delta-kappa pair had no effect.

Thus multiple opiate systems are activated during tail shock. The early opiate analgesia involves only spinal kappa receptors. The late opiate analgesia involves both spinal kappa & supraspinal delta receptors. "Non-opiate" analgesia is in fact opiate. Here, parallel spinal processes seem to be involved since analgesia is only blocked by combined mu-delta or mu-kappa receptor blockade. Other putative non-opiate analgesias are now being studied. To date, the data indicate that "non-opiate" analgesias are really opiate. NSF grant BNS 88-09527.

46.4

ENDOGENOUS OPIOID MEDIATION OF ANALGESIA CONDITIONED TO CONTEXTUAL VS. DISCRETE CUES. L. Subel*, E. P. Wiertelak, S. F. Maier, & L. R. Watkins, Department of Psychology, University of Colorado, Boulder, CO 80309.

Previous work has shown that animals can learn to activate endogenous analgesia systems in response to danger signals. This has been called *conditioned analgesia* (CA). While CA has been observed in response to both discrete (e.g. light cues) & contextual (e.g. room cues) signals, no clear comparison has been made of the two. The purpose of the present study was to: (1) develop paradigms in rats for eliciting CA (tail flick test) to discrete cues (discrete CA) & to contextual cues (contextual CA); & (2) begin defining whether contextual & discrete CAs are mediated by the same systems, by testing each for antagonism by subcutaneous (s.c.) naltrexone, intrathecal (IT) naltrexone, and IT specific opiate receptor subtype antagonists (CTOP, Naltrindole, Binaltorphimine).

In the 1st series of studies, contextual CA was acquired in response to room cues (conditioned stimulus; CS) paired with tailshock (unconditioned stimulus; UCS). Contextual CA was powerfully produced within 3 training sessions. Both s.c. (7, 1, & 0.1 mg/kg) & IT (1 μg) naltrexone completely reversed CA, compared to controls, strongly suggesting opioid mediation of this analgesia, with specific involvement of a critical opioid synapse at the level of the spinal cord. Further IT studies using specific opiate receptor-subtype antagonists showed that μ was primarily, δ was partially, and κ was not observably involved in contextual CA.

In the 2nd series of studies, discrete CA was acquired in response to a light cue (CS) paired with tailshock (UCS). Again, this association was powerfully and rapidly learned. Both s.c. (1 mg/kg) & IT (1 μg) naltrexone abolished discrete CA. IT studies with specific opiate antagonists are in progress. To date, the data indicate that the two analgesias are mediated by common systems. NSF grant BNS 88-09527.

46.5

WHETHER CONDITIONED ANALGESIA IS OPIOID MEDIATED DEPENDS ON SHOCK INTENSITY BUT NOT STIMULUS DURATION. A.R. Allen*, P.A. Illich, M.W. Meagher, & J.W. Grau. Dept. of Psychology, Texas A&M Univ., College Station, TX 77843.

Pairing a neutral stimulus (the CS) with an aversive event (the US) can endow the CS with the capacity to elicit a decrease in pain reactivity. Although it is commonly held that this conditioned analgesia is opioid mediated, it is clear that a CS can sometimes elicit an analgesia which is not affected by opioid antagonists, suggesting that nonopioid systems can also be engaged. Here we test whether US intensity or CS duration determines the form of the conditioned response (CR). A differential conditioning paradigm was employed in which one auditory stimulus (the CS+) was paired with shock while another (the CS-) was presented alone. Subjects received 6 CS+ and CS- trials spaced across two training sessions. Tail-flick latencies were then assessed during the CS+ and CS- over 2 days. Prior to testing, subjects received saline or naltrexone. Experiment 1 revealed that a 60 s CS elicits a nonopioid CR when paired with an intense (1.0 mA, 0.5 s) tailshock, but an opioid CR when it is paired with a weak tailshock (0.3 mA, 0.5 s). Experiment 2 showed that a 1.0 mA (0.5 s) US elicits a nonopioid CR irrespective of the CS duration (60 versus 300 s). Interestingly, naltrexone enhanced the nonopioid mediated CR in both experiments. The results suggest that US severity, and not CS duration, determine the form of the CR. Supported by BNS 881981 to J.W.G.

46.6

A PERIOD OF RESTRAINT IS NECESSARY TO OBSERVE A STRONG OPIOID ANALGESIA AFTER MILD SHOCK OR A LOW-DOSE OF MORPHINE IN RATS. M.K. Biles & J.W. Grau. Dept. of Psychology, Texas A&M Univ., College Station, TX 77843.

We have previously shown that exposure to mild shock (3, 0.75-s, 1.0 mA tail-shocks) can elicit both a transient nonopioid and a long-lasting opioid analgesia on the tail-flick test. Unlike the analgesia observed after other "brief shock" paradigms, the analgesia observed in our paradigm appears to be hormonally mediated since it is attenuated by adrenalectomy and dexamethasone (Biles et al., Neurosci. Abs., 16, 99, 1990). We hypothesized that the pituitary-adrenal axis may play a role in our paradigm because subjects are restrained for 20 min before they receive mild shock. The present experiments test this hypothesis. Experiment 1 showed that removing this 20 min period of restraint attenuated the opioid analgesia normally observed 6-10 min after mild shock. Experiment 2 demonstrated that this period of restraint has relatively little impact on the naltrexone-insensitive, nonopioid, analgesia observed 2 min after shock. The last experiment showed that the 20 min of restraint also potentiates the analgesia observed after a low dose (1 mg/kg) of morphine. The results suggest that a period of restraint acts to potentiate opioid mediated analgesic effects. Supported by a Tex. Adv. Res. Proj. (010366-097) to J.W.G.

46.7

DISSOCIATION OF ANTINOCICEPTIVE AND MOTOR EFFECTS PRODUCED BY INTRATHECAL ADMINISTRATION OF A SELECTIVE μ-OPIOID AGONIST L.Franck,* C.Miaskowski, K. Sutters,* J. Putris* and J.D. Levine*. University of California San Francisco, San Francisco, CA 94143

The purpose of this study was to determine whether the DAMGO-induced decrease in motor coordination (MC) was correlated with increases in nociceptive thresholds (NT); whether the decrements in MC were also opioid receptor mediated; and whether the time course of recovery of MC and NT differed. The Randall-Selitto paw-withdrawal (PW) test was used to measure mechanical NT. MC was tested using a Ugo Basile accelerating Rotarod treadmill. IT administration of DAMGO produced significant, dose-dependent increases in mechanical NT that correlated with decreases in MC ($R=-0.66$, $p<0.001$). IT naloxone (5 μg) reversed the decreases in MC produced by 5 μg of DAMGO by 90.73%. After administration of the highest dose (5 μg) of DAMGO, PW and MC testing were continued at 45 min intervals for 225 mins. Statistical analysis demonstrated that MC recovered significantly sooner than nociception. These data confirm spinally mediated motor as well as antinoci- ceptive effects of DAMGO and suggest that both these effects are mediated through an action at an opioid receptor. However, effects may be mediated at different populations of opioid receptors.

46.8

MICROINJECTION OF MU, BUT NOT DELTA, OPIOID RECEPTOR AGONISTS INTO THE MEDULLARY DORSAL HORN (MDH) PRODUCES NALOXONE-REVERSIBLE FACIAL SCRATCHING IN THE MONKEY. D.A. Thomas, G.M. Williams, K. Iwata, R. Dubner, and D.R. Kenshalo, Jr., Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Epidural opioid administration to humans can produce facial itch that is naloxone-reversible. We investigated the effects of selective mu and delta receptor agonists microinjected unilaterally into the MDH on facial scratching in cynomologus monkeys (microinjection method: Oliveras, et al., J. Neurosci. 6:3086, 1986; volume = 0.4 μl). Scratching was scored for 1-2 hrs following microinjection, and the number of scratches in each of the trigeminal nerve divisions (mandibular, maxillary, and opthalmic) was counted. The selective mu-receptor agonist, DAMGO (0.0031, 0.0125, 0.0250 μg), produced a dose-dependent increase in scratching with 0.0250 μg yielding 106 ± 11 scratches/5 min (saline = 3 ± 1 scratches/5 min) ipsilateral to the injection. Naloxone (0.5 mg/kg; IM) administered 10 min after DAMGO (0.0250 μg) reversed its effect. In contrast to the effect of DAMGO, the selective delta agonist, DPDPE (1.0, 2.5, 5.0 μg), produced little or no scratching. Additionally, the delta selective antagonist, naltrindole (1 mg/kg; IM), was ineffective in reversing morphine (5.0 μg) induced scratching. These data suggest that the activation of mu, but not delta, receptors within the MDH produces facial itch.

46.9

INTRATHECAL MORPHINE AND WITHDRAWAL RESPONSES TO A WIDE RANGE OF STIMULUS INTENSITIES IN THE RAT. M. Strimbu*, F. Guirimand*, J.C. Willer* and D. Le Bars*, P.Dutar INSERM U161 ,75014 Paris; Dep. Anesthesiologie, Hôp. Ambroise Paré, 92100 Boulogne-Billancourt; Lab. Neurophysiologie, Hôp. Pitié-Salpétrière, 75013 Paris.

Most data related to pain and analgesia deal with threshold measurements or studies of responses to a single suprathreshold stimulus. We aimed to determine the effects of various doses of intrathecal morphine on responses to a wide range of stimulus.

Electromyographic (EMG) activities were recorded from the biceps femoris muscle in halothane (0.8%) anesthetized rats. C-fiber evoked reflex responses were elicited by percutaneous electrical stimulation of the sural nerve receptive field applied every 6s in increasing intensities. EMG responses were digitized, full-wave rectified and integrated. Recruitment curves were built by plotting the integrated areas versus the current intensities in the 0-5 times the C-fiber threshold range before and following drug injections.

Below 0.04 μg, morphine did not modify the recruitement curves; in the 0.04-0,4 μg range, it produced both an increase in the threshold and a decrease in the slope of the recruitment curves in a dose-related and naloxone-reversible fashion; above 0.4 μg it completly erased the curves. Interestingly, systemic naloxone (0.4mg/Kg,i.v.)not only reversed these effects, but also facilitated the stimulus-response curves for the higher stimulus intensities, i.e. in the 3-5 times the C-fiber threshold range.

In conclusion, intrathecal morphine not only produces a shift of the encoding functions of the spinal cord but also reduces the gain of these functions, in a narrow range of doses.

46.10

SPINAL ANALGESIA IN FROGS: STUDIES WITH HIGHLY-SELECTIVE OPIOID AGENTS. C. W. Stevens, Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107.

Intraspinal (i.s.) administration of morphine in the amphibian, *Rana pipiens*, has been previously shown to produce a dose-dependent, naloxone-sensitive increase in the nociceptive threshold (NT). The present studies were conducted to examine the specific opioid receptor(s) mediating spinal analgesia. Male and female frogs (25-35 g) were obtained from commercial suppliers and maintained in individual plastic cages. NT was determined by the acetic acid test which consists of log-spaced concentrations of dilute acid applied dropwise onto the frog thigh until a wiping response is elicited. NT was determined 60 min before (baseline) and 60, 120, 180, and 240 min after the i.s. administration of morphine (0.3-10 nmol), the mu opioid, DAMGO (0.03-1 nmol), the delta opioid, DPDPE (1-10 nmol) or the kappa opioid, Dyn A_{1-9} (10 and 30 nmol). Maximum percent effect was calculated and dose-response curves constructed. ED_{50}'s (±95% C.I., in nmol/frog) were 2.3 (1.7-3.0), 0.1 (0.08-0.2), and 3.3 (2.3-4.7) for morphine, DAMGO, and DPDPE, resp. I.s. Dyn A_{1-9} (30 nmol) produced motor dysfunction of the hindlimbs which subsided after 60 min and mild analgesia at a lower dose (10 nmol). Naloxone (10 nmol/g) administerd s.c. 1 h before maximal doses of i.s. morphine, DAMGO, and DPDPE, significantly blocked the rise in NT.

These results suggest that specific mu and delta opioid receptors mediate the suppression of chemogenic pain in the amphibian spinal cord. Additionally, Dyn A_{1-9} appears to produce motor dysfunction in amphibians much like that previously reported for rats.

46.11

MORPHINE MODULATION OF STATIC VERSUS DYNAMIC RESPONSES OF COLD-RECEPTIVE NEURONS IN THE MEDULLARY DORSAL HORN. S.S. Mokha, Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

It has previously been reported that highly selective agonists at mu, delta (Mokha,S.S., J. Physiol.,394:152p,87; Mokha, S.S., J. Physiol., 398:85p, 87) and kappa (Soc. Neurosci.Abs.,16:409p) opioid receptors modulate the static responses of cold-receptive neurons. The present study investigated in detail the effects of morphine on the static versus the dynamic responses of these neurons. Extracellular single unit recordings were made in the superficial dorsal horn of the trigeminal nucleus caudalis in rats anesthetized with halothane. The effects of morphine (1-2 mg/kg,i.v.) were tested on the static and dynamic responses of cold-receptive neurons. Static responses were reduced in a majority of neurons (75%), however, responses of 25% of neurons were enhanced (25%). Effects of morphine, excitatory or inhibitory, were similar on the dynamic and static responses of neurons if the static firing frequency was low. However, morphine had little effect on the dynamic responses of neurons that had a high static firing frequency irrespective of the effects of morphine, excitatory or inhibitory, on the static responses. These latter results are consistent with the evidence obtained in behavioral studies showing little effect of morphine on the mean detection latency to cooling stimuli in the monkey (Oliveras et al., 1986). Supported by: NIH-RR03032.

46.12

MORPHINE ENHANCES THE ACTIVITY OF THERMORECEPTIVE-SPECIFIC LAMINA I SPINOTHALAMIC NEURONS IN THE CAT. A.D. Craig and S.J. Hunsley*. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

The actions of morphine on lamina I spinothalamic (STT) cells have not been determined, although these form half of the STT. The goal of the present work is to test the hypothesis that morphine has differential effects on nociceptive and thermoreceptive STT and "non-STT" lamina I cells. Units recorded with tungsten microelectrodes in L7 in barbiturate-anesthetized cats were characterized with natural stimulation and with antidromic activation from the thalamic sites previously identified anatomically as lamina I STT targets. Stimulus-response functions were measured with a computerized sequence applied with a Peltier thermode. The effects of increasing doses of systemic morphine (0.125-2.0 mg/kg iv) were examined on 9 thermoreceptive-specific (cold sensitive) lamina I STT cells, as well as on other nociceptive-specific and multireceptive STT and non-STT cells. Morphine significantly enhanced a portion or all of the response functions of 7 of 9 "cold" cells at nearly all doses. Mean overall increases to 105-163% of control across the temperature range of 6-34°C were observed. This result contrasts strongly with the predominant inhibition of nociceptive lamina I cells by morphine. It is consistent with reports that morphine does not affect thermoreception, that morphine in low doses induces choice of a warmer environment (and hyperthermia), and that morphine analgesia is greatly potentiated in a cold environment. These observations support the concept of selective opiatergic modulation of the integration of thermoreceptive and nociceptive activity in the lamina I STT projection system. (Supported by NIH grant NS25616)

SUBCORTICAL VISUAL PATHWAYS: MIDBRAIN

47.1

ORGANIZATION OF RETINOTECTAL FIBERS IN THE OPTIC CHIASM OF *Rana pipiens*. R. Waldeck, J. Tsai* and E. Gruberg. Biology Dept., Temple University, Philadelphia, PA 19122

In earlier work we found that after hemisection of the optic chiasm, frogs can accurately respond to visually presented prey throughout the visual field. The retinorecipient optic tectum mediates visually guided prey catching. We therefore investigated the distribution of retinotectal fibers in the optic chiasm of normal animals using horseradish peroxidase (HRP) histochemistry. In each animal we applied HRP to a circumscribed region of the dorsal tectum. After 6-17 days survival parasagittal sections were cut in a cryotome. In the sagittal plane the optic chiasm has roughly the shape of an obtuse triangle with a narrow ventral base and an obtuse angle between the base and caudal boundary. After medial injections labeled fibers are found in ventral areas of the chiasm distributed in a wide rostrocaudal band. After lateral injections labeled fibers are located in the narrower dorsal part of the chiasm. The fibers extend over a large rostrocaudal extent. In each case labeled tissue occupies a much greater proportion of the area of the chiasm section than of the retina. Larger tectal injections result in more than one band of labeled fibers in the chiasm. The bands are separated by unlabeled bands even though there is continuous labeling of retinal tissue within a circumscribed zone. Retinotectal fibers projecting to circumscribed locations in the tectum have a rostrocaudal spread in the chiasm thus accounting for the sparing of prey catching after chiasm hemisection. Supported by NIH Grant EY04366.

47.2

ABSENCE OF A RAPIDLY-CONDUCTING COMPONENT FROM THE IPSILATERAL RETINOFUGAL PATHWAY OF FERRETS. G.E. Baker* & M.P. Stryker. Dept. of Physiology and Neuroscience Graduate Program, University of California, San Francisco, California 94143-0444, U.S.A.

Anatomical evidence indicates that the ipsilaterally-projecting population of retinal ganglion cells in the ferret lacks alpha cells or large diameter axons (Vitek et al, 1985, *J.Comp.Neurol 241* 1-11; Reese & Baker, 1990, *Eur.J. Neurosci 2* 34-49). The present study sought physiological confirmation of these findings.

Electrodes placed in the optic chiasm and both optic tracts of adult sable ferrets were used for stimulation and recording. Antidromic responses to stimulation of these sites were recorded from the region of the optic disk. As previously reported (Baker & Stryker, 1990, *Nature 344* 342-5), stimulation of either the optic tract contralateral to the recording electrode or the optic chiasm resulted in optic disk potentials displaying two early peaks, a short latency (t_1) mode and a longer latency (t_2) mode, along with (at higher intensities of stimulation), much later peaks of much smaller amplitude. In contrast, responses evoked by stimulation of the optic tract ipsilateral to the recording electrode showed only one peak, with a latency comparable to that of the t_2 mode in the crossed projection. Similar observations were made when stimulating from the retina and recording orthodromic responses in both optic tracts: the uncrossed pathway lacked the short latency peak which was observable in the response of the crossed pathway.

The t_1 and t_2 conduction velocity peaks are believed to reflect respectively the responses of the Y and X retinal ganglion cell populations. We conclude therefore that the uncrossed projection of the ferret's retinofugal pathway lacks a significant Y cell component.

Supported by an MRC Travelling Fellowship and a grant from the NIH.

47.3

EXTRAGENICULATE RETINAL PROJECTIONS IN THE FERRET STUDIED WITH WGA-HRP. S.K. Itaya, E.H. Polley, and T.J. Drolsum*. Dept. of Biomedical Sciences, Univ. of South Alabama, Mobile, AL 36688, and Dept. of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL 60612.

The ferret visual system is a model which has been used in many anatomical and physiological studies, most of which have focused on retinogeniculate and geniculostriate pathways. We have examined the ferret retinal pathways in order to map extrageniculate projections and targets. Following intravitreal injection of WGA-HRP, brain sections were processed for TMB histochemistry. Three cases have been studied to date, with results showing retinal pathways in compliance with the general mammalian model, and several "nonvisual" retinorecipient areas. Extrageniculate retinal axons project to the suprachiasmatic nuclei, the pretectal area, the tectum, and the accessory optic system. All areas receive bilateral input, except the dorsal terminal nucleus.

There were six "nonvisual" retinal pathways, four rostral to the pretectum, and two caudal to the superior colliculus. Three groups of retinal axons continue rostrally from the brachium of the superior colliculus and form focal terminal areas. These axons project to: 1) an area between the dorsomedial and lateral dorsal nuclei, near the surface of the thalamus; 2) a caudal level of the dorsomedial nucleus adjacent to the lateral habenular nucleus; and 3) the parafascicular nucleus. A fourth group of axons continues from the lateral geniculate nucleus, along the stria terminalis; most of these fibers appear to terminate in the bed nucleus of the stria terminalis.

Labeled fibers also project from the caudal border of the superior colliculus into the inferior colliculus, where they appear to terminate in a scattered manner around the periphery. In addition, a sparse projection extends further caudally, to the parabrachial nucleus in the upper pons.

47.4

ULTRASTRUCTURAL ANALYSIS OF RETINAL, CORTICAL AND PARABIGEMINAL TERMINALS WITHIN THE SUPERFICIAL GRAY OF THE PRIMATE SUPERIOR COLLICULUS. S. Feig*, D.P. Van Lieshout* and J.K. Harting. Department of Anatomy, University of Wisconsin-Madison, Madison, WI 53706

We have used electron microscopic anterograde autoradiography to analyze the synaptic relationships of retinal, cortical and parabigeminal axon terminals within the superficial gray (SGS) of the Galago superior colliculus.

Following an injection of an equal mixture of 3H-proline and 3H-leucine into one eye, labeled retinal terminals within the SGS contain round vesicles, pale mitochondria, exhibit asymmetrical synapses and contact dendrites, some of which contain vesicles. Based upon terminal diameter and morphology, two populations of ipsilateral and contralateral retinal terminals are apparent.

Injections of tritiated amino acids into area 17 result in the labeling of terminals in the SGS which contain round vesicles, dark mitochondria, make asymmetrical contacts and terminate upon conventional spines (most frequently), dendrites and (occasionally) vesicle filled profiles. There appear to be two populations of cortical terminals. This distinction is based upon differences in size (small vs. large) and vesicle distribution (densely packed vs. dispersed).

Following injections of amino acids into the parabigeminal nucleus, labeled terminals within the SGS contain round vesicles and make asymmetrical contacts; such contacts are less pronounced than those associated with retinal and cortical terminals. Parabigeminal terminals contact spines, vesicle filled profiles and dendrites. The latter are more frequently contacted by parabigeminal terminals than by retinal or cortical terminals.

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47.5

LAMINAR DISTRIBUTION AND ORIGINS OF THE CATECHOLAMINERGIC INNERVATION OF THE OPTIC TECTUM IN THE PIGEON (*Columba livia*). H.R. Rodman and H.J. Karten. U.C. San Diego Department of Neurosciences, La Jolla, CA 92093.

Combined immunohistochemical and retrograde tracing methods were used to characterize the catecholaminergic (CA) innervation of the pigeon optic tectum (TeO), the major target of retinal projections in birds. Antibodies against tyrosine hydroxylase (TH) revealed that CA innervation is most dense in the superficial, retinorecipient layers of the dorsal TeO, where three bands are evident: 1) a band in layer 4 of Cajal; 2) a dense band of tortuous fibers with prominent varicosities in upper layer 5; and 3) a band of predominantly horizontal and oblique fibers in layer 7. Ventrally, the pattern of staining in the superficial layers becomes fainter and more uniform and a dense plexus of thick varicose fibers becomes evident in layers 10-11. Dense terminal-like labelling was also evident adjacent to the tectal ventricle (layer 15). Unilateral injections of fluorescent latex microspheres or cholera toxin into the TeO in combination with TH immunohistochemistry revealed double-labelled cells bilaterally in the locus coeruleus (LoC), the nucleus subcoeruleus, and in a zone immediately medial to and extending around the nucleus pretectalis. This pretectal CA group has not been previously described in birds and appears to have no mammalian equivalent. Intraperitoneal injections of the noradrenergic neurotoxin DSP-4 produced a moderate depletion of TH staining in all layers of the TeO at survival times of 2-3 mos. and no effect at shorter survival times, in contrast to the total depletion seen in mammalian superior colliculus after DSP-4 treatment (Fritschy & Grzanna, 1989). Thus, the CA innervation of the avian TeO appears to involve noradrenergic modulation of incoming visual information in the superficial layers by the LoC, as in mammals, but derives from other sources as well.

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47.6

THE SYNAPTIC ORGANIZATION OF THE RAT PRETECTO-COLLICULAR SYSTEM. AN ULTRASTRUCTURAL DEMONSTRATION OF GABAERGIC AND NONGABAERGIC PROJECTION NEURONS. J.J.L. VAN DER WANT*, J.J. NUNES CARDOZO* and C. VAN DER TOGT*. (SPON: EUROPEAN NEUROSCIENCE ASSOCIATION). Dept. of Morphology, The Netherlands Ophthalmic Res. Inst., P.O. Box 12141, 1100AC Amsterdam, The Netherlands.

Neurons in the pretectal Nucleus of the Optic Tract (NOT) have been investigated with retrograde WGA-HRP tracing from the Superior Colliculus (SC) and postembedding with an antiserum against GABA. The specific objective of this study was to identify and characterize the projection neurons that form part of the GABAergic circuitry interconnecting two primary visual, subcortical centers, that are involved in eye movement regulation.

Small iontophoretic injections of WGA-HRP were placed at different depths in the SC. Retrogradely labeled cell bodies were visualized with DAB and preembedding gold substituted silver peroxidase treatment. Identified neurons in the contralateral NOT are large to medium sized, show deeply indented nuclei, electron lucent cytoplasm with dark mitochondria, extensive Golgi apparatus and stacks of endoplasmic reticulum. GABA reactivity was observed in a substantial number of labeled cell bodies and dendrites in areas that contain also non GABAergic retrogradely labeled neurons. The pretecto-collicular neurons are partly surrounded by glial processes, but are also in receipt of axon terminals (F) that are nonGABAergic, in this respect they differ from the pretecto-olivary neurons that show limited glial coating and receive, apart from F terminals, also retinal (R) terminals and terminals of dendritic origin (P) on the soma. The dendrites of identified neurons are contacted by R, F and P terminals. The GABAergic projection is in agreement with light microscopical observations in the cat and supports the idea that GABAergic circuits might play an important role in the inhibitory control in subcortical visual centers related to eye movements.

47.7

GABAA SUBUNIT RECEPTOR DISTRIBUTION IN THE CAT SUPERIOR COLLICULUS USING ANTIBODY IMMUNOCYTOCHEMISTRY. Ranney Mize and Grace Butler*, Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN 38163.

GABA neurons are found throughout the cat superior colliculus (SC). They are distributed densely within the zonal (ZL) and superficial gray layers (SGL) and more sparsely within the deep layers. We have used the antibody mAb 62-3G1 directed against the ß subunit of the GABAA receptor/benzodiazepine recep- tor/Cl- channel complex to compare the distribution of this receptor subunit with that of GABA neurons. The antibody was localized using light and electron microscope immunocytochemistry. Particulate reaction product was found densely distributed through the ZL and SGL of SC and more lightly within the deeper layers. Within the ZL and SGL, there were few somata labeled by the antibody, although some cells contained a halo of reaction product on their outer membrane surfaces. Within the optic and deeper layers, numerous cells contained internalized cytoplasmic label as well as a dense rim of reaction product around the edge of the cell. At the ultrastructural level, label was found along the membrane surfaces of many cell bodies and dendrites. Often this label filled the synaptic cleft and coated the membranes at synaptic sites between axon terminals and dendrites. However, membrane associated label was also found at non-synaptic sites and sometimes coated much of the outer membrane surface of a cell. Often, but not invariably, the synaptic vesicles which were presynaptic to labeled membranes were flat- tened or pleomorphic in shape. Rarely, membrane associated label was found coating a presynaptic vesicle-containing profile. These results suggest that the GABAA receptor ß subunit is often postsynaptic in location and associated with flattened vesicle synapses, many of which are known to be GABAergic. However, it is also found at non-synaptic membrane sites and occasionally on presynaptic profiles.

Supported by NIH EY-02973. Antibody kindly supplied by Dr. A.L. de Blas.

47.8

LOCALIZATION OF THE m1 MUSCARINIC ACETYLCHOLINE RECEPTOR IN THE CAT SUPERIOR COLLICULUS. Chang-Jin Jeon [1], R. Ranney Mize [1], Gary R. Luthin [2] and Chia-Sheng Lin [2], [1] Dept. of Anatomy and Neurobiology, Coll. of Medicine, Univ. of Tennessee, Memphis, TN. and [2] Dept. of Physiology and Biophysics, Hahnemann Univ. Philadelphia, PA.

The m1 muscarinic acetylcholine receptor (mAChR) has been localized in the cat superior colliculus (SC) with antibody immunocytochemistry. Two patterns of labeling were found. Within the superficial SC, the receptor antibody formed a dense band of reaction product within the zonal (ZL) and upper superficial gray layers (USGL). The reaction product was evenly distributed through the neuropil and sometimes coated membrane surfaces of neurons. Within the deeper layers (DL), the antibody was internalized within the cytoplasm of cell bodies and proximal dendrites, where it formed a dense particulate reaction product. In some cases, the antibody also appeared to coat the outer membrane surfaces of these cell bodies. To better characterize these cells, we plotted their laminar position and measured their size. Only a few labeled neurons were found within the ZL and USGL. By contrast, the optic and DL contained numerous labeled cells with the densest distribution within the intermediate gray layer (IGL). Anti-m1 mAChR labeled neurons were medium to large size (range 9.3-80.8 µm) including some very large "predorsal bundle" cells in the DL. Adjacent sections labeled by antibodies to choline acetyltransferase (ChAT) showed no obvious correlation between the distribution of ACh fibers, which form distinctive patches in the IGL, and the m1 receptor labeled cell bodies. However, the dense band of m1 immunoreactivity in the ZL and SGL did partially overlap the band of ChAT fibers in that region of SC. This band may contain primarily presynaptic receptors given that very few cells in this region internalize the receptor. By contrast, the internalization in the DL suggests the cells may be ACh fiber recipient neurons.

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47.9

SOMATOSTATIN LOCALIZATION WITHIN THE PRETECTAL COMPLEX AND ADJACENT MIDBRAIN STRUCTURES: AN IN SITU HYBRIDIZATION STUDY. J.T. Weber, I.-L. Chen*, H. Shay*, and D.L. Hurley. Neuroscience Training Program and Department of Anatomy, Tulane University School of Medicine, New Orleans, LA 70112.

We previously used immunocytochemical (ICC) methods to demonstrate the locations of somatostatin (SOM) positive neurons within the pretectal complex of the cat. ICC revealed SOM-positive neurons within the oculomotor complex and the interstitial nucleus of Cajal which had not been previously reported. The present study employed *in situ* hybridization to detect preproSOM (ppSOM) mRNA in the regions of the midbrain. An ^{35}S-labelled antisense RNA probe was transcribed from a cDNA obtained from Dr. Richard Goodman (New England Med. Ctr.), and hybridized to tissue sections according to standard procedures. Specific ppSOM hybridization signals were noted in several regions, confirming ICC results. Within the pretectal complex, ppSOM mRNA containing neurons were located within the nucleus of optic tract, the pretectal olivary nucleus and the posterior pretectal nucleus. Neurons containing ppSOM mRNA were also found within the red nucleus, Edinger-Westphal nucleus, nucleus of Darkschewitsch, nucleus of the posterior commissure, superior colliculus, periaqueductal gray, and the interpeduncular nucleus. Neurons containing ppSOM mRNA were also located with the oculomotor nucleus and the interstitial nucleus of Cajal. These data furnish additional evidence that SOM mRNA and peptide is expressed within these important midbrain visual structures.

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47.10

CALBINDIN-D_{28K} AND PARVALBUMIN LOCALIZATION IN THE PRETECTAL COMPLEX AND ADJACENT VISUAL STRUCTURES OF THE SQUIRREL MONKEY. J. Scripter, L. Harrison, C.G. Cusick, and J.T. Weber. Neurosciences Training Program and Department of Anatomy, Tulane Medical School, New Orleans, LA 70112.

Standard immunocytochemical methods were used to localize calbindin-D_{28K} (CB) and parvalbumin (PB) within the pretectum complex and adjacent visual structures in the squirrel monkey. Within the pretectum, CB-positive neurons are located predominantly in the pretectal olivary nucleus and to a lesser extent in the nucleus of the optic tract and the posterior pretectal nucleus. PB-positive cell bodies are numerous within all pretectal nuclei except the pretectal olivary nucleus where only a few scattered PB-positive cell bodies are seen. The complementary nature of these two calcium binding proteins is also seen within other visual structures. Thus, in the dorsal lateral geniculate nucleus, CB-positive neurons are found primarily in the S-layers and the interlaminar layers: PB-positive cell bodies occupy the entire magno and the parvocellular layers. Within the parabigeminal nucleus, PB-positive neurons are abundant, and CB-positive neurons are absent. CB neurons within the superior colliculus are located in the superficial, optic and upper intermediate layers, while PB neurons were most numerous within the intermediate layers. These data suggest that PB and CB are concentrated within different functional pathways in the primate visual system.

Supported by NIH grant EY03731.

47.11

DISTRIBUTION OF CALCIUM BINDING PROTEINS IN THE CAT PRETECTAL COMPLEX. H. Shay, C.G. Cusick, F.R. Domer and J.T. Weber. Neuroscience Training Program and Department of Anatomy, Tulane Medical School, New Orleans, LA. 70112

Standard immunocytochemical methods were used to localize calbindin-D_{28k} (CB) and parvalbumin (PB) within the pretectal complex of the cat. The most significant finding is the presence of several dense plexuses or patches of immunoreactivity (IR). These patches of IR, in both the CB and PB tissue, 1) contain labeled axon terminals and cell bodies; 2) appear to overlap with retinal input; and 3) precisely overlap each other. The IR appears as continuous columns, rostrally within the pretectal olivary nucleus (ON) and nucleus of the optic tract (NTO) and caudally within the posterior pretectal nucleus and NTO. Within the patches, CB-labeled neurons are more numerous than PB-labeled neurons. IR in nonpatch pretectal regions includes scattered labeled CB neurons in the NTO and PB-labeled neurons in the NTO, ON, and anterior pretectal nucleus (APN). The most obvious difference in the CB and PB material is the abundance of PB terminals and PB-positive neurons in the APN, whereas this nucleus is almost devoid of CB staining. These data are in contrast to the locations of calcium binding proteins in the primate pretectum (see Scripter et al., Neurosci. Abstr., 1991); and underscores the non-complementary nature of CB and PB in some cat nuclei. While CB and PB can delineate certain nuclei in the cat, they do not discriminate different functional pretectal subsystems. Supported by grant EY03731.

47.12

CALBINDIN LABELED NEURONS RECEIVE SIGNIFICANT SYNAPTIC INPUT FROM RETINAL TERMINALS WITHIN THE CALBINDIN CELL CLUSTERS OF THE CAT PRETECTUM. S.Emami*, G.D. Butler*, B. Nabors, Q. Luo, R.F. Spencer, and R.R. Mize, Dept. of Anat. Neurobiol., Univ. of Tennessee, Memphis, TN 38163.

We have previously shown (Nabors and Mize, J. Neurosci., 1991) that the cat pretectum contains four clusters of neurons that are labeled by the calcium binding protein calbindin (CaBP). These clusters form four continuous zones that cross nuclear boundaries but perfectly overlap the retinal terminal zones found in the pretectum of cat. The CaBP neurons are medium to large in size and many project to the lateral geniculate nucleus (LGN). They are not labeled by GABA antibodies and GABA cells are almost always smaller. In this study, we have determined whether CaBP cluster neurons actually receive synaptic contacts from retinal terminals and whether they are the only neurons which receive these contacts. We examined thin sections treated with a CaBP antibody. At the electron microscope level, we found numerous somata, dendrites, and myelinated axons labeled by CaBP within the pretectal cell clusters. The somata were mostly medium to large neurons with plentiful cytoplasm. Many of the dendrites were quite thick with parallel arrays of labeled microtubules. Many of the axons were large and heavily myelinated. Retinal terminals were frequently found in contact with thick and thin labeled dendrites, some of which were found within retinal glomeruli. However, we did not find examples of CaBP labeled F2 presynaptic dendrites, which are thought to be GABAergic. F2 presynaptic dendrites frequently receive input from retinal terminals in both the pretectum and dLGN. We therefore conclude that CaBP labeled neurons are not the only cells within the pretectal clusters which receive retinal inner- vation, and retinal input must not be the sole determinant of whether a pretectal cluster cell will or will not contain calbindin. Supported by USPHS grants NIH EY-02973, NIH DK-07405, and the Howard Hughes Medical Institute.

47.13

NEURONAL AND SYNAPTIC ORGANIZATION OF THE OLIVARY PRETECTAL NUCLEUS IN THE RHESUS MONKEY INVESTIGATED BY THE IMMUNOHISTOCHEMICAL LOCALIZATION OF CALCIUM BINDING PROTEINS AND GABA. Robert F. Spencer and R. Ranney Mize. Dept. Anat., Med. Coll. of Virginia, Richmond, VA, 23298, and Dept. Anat. & Neurobiol., Univ. Tenn. Coll. Med., Memphis, TN 38163.

The olivary pretectal nucleus (OPN) is a retinorecipient area in the rostral dorsal midbrain as demonstrated by intraocular injections of WGA-HRP. Monoclonal antibodies to the calcium binding proteins calbinden D-28k (Calb) and parvalbumin (PA) reveal a distinct organization of neurones within the OPN. Calb-immunoreactive (IR) neurones are arranged as clusters and comprise the majority of neurones in the OPN. Fewer neurones are PA-IR, and, although also clustered, these are located predominantly along the medial border of the nucleus. GABA-IR neurones comprise only a small minority of the neurones in the OPN. By electron microscopy, the clustering arrangement of Calb-IR and PA-IR neurones is attributable largely to compartments of neuropil comprised of the dendrites of these neurones and synaptic endings, many of which are of retinal origin and establish axodendritic synaptic connections with both Calb-IR and PA-IR neurones. Most Calb-IR dendrites are conventional, whereas many PA-IR dendrites contain synaptic vesicles and establish dendro-dendritic synaptic contacts. The density of synaptic endings that contain pleiomorphic synaptic vesicles, many of which are GABA-IR, appears to exceed what might be expected from the few GABA-IR neurones within the nucleus, suggesting the existence of a significant extrinsic inhibitory input to the OPN in the monkey.

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47.14

AFFERENT AND EFFERENT PATHWAYS OF THE TURTLE BASAL OPTIC NUCLEUS IDENTIFIED USING FLUORESCENT TRACERS. A. E. Hurrianko and M. Ariel. Dept. of Behavioral Neuroscience, University of Pittsburgh, Pgh., PA 15260.

The connectivity of the basal optic nucleus (BON) of the turtle accessory optic system was investigated using fluorescent tracers. Solutions of tetramethylrhodamine dextrans (n=16) or 1,1'-dioctadecyl-3,3,3',3',-tetramethylindocarbocyanine perchlorate (di I, n=9) were injected with a micropipette into the BON or habenula of Pseudemys scripta elegans using an in vitro preparation of the turtle brain with eyes attached (Rosenberg & Ariel 1988). Tissues were maintained for 12-24 hrs. in oxygenated Ringers (dextrans) or 1-3 mos. in 4% paraformaldehyde (di I) before cutting 60 µm transverse sections.

BON injections retrogradely labeled many ipsilateral neurons in the oculomotor nuclei, pretectal nuclei, ventral tegmentum, ventral habenular nucleus and the dorsal and ventral nuclei of the posterior commissure (the latter receive input from the striatum). Fibers were seen which project to the contralateral BON via two paths: across the posterior commissure and through the tegmental decussation. Longitudinal fibers ran along the ventralmost aspect of the brain, some of which appeared to terminate in tiny puncta located in the Purkinje cell layer of the cerebellar cortex. In addition, a sub-population of ganglion cells were labeled in the retina.

The habenular injections labeled the small, round cells of the ventral nucleus, as well as fibers coursing in bundles toward the interpeduncular nucleus or BON, showing the anterograde aspect of the latter connection.

The pathways revealed by these experiments indicate the important role that the BON plays in relaying retinal information into the brainstem. The BON signals may be influenced by cortical projections, as well as relayed to the cerebellum for interaction with motor systems (Supported by EY05978).

47.15

DESCENDING PATHWAYS FROM THE NUCLEUS OF THE OPTIC TRACT IN THE FUSCATA MONKEY. S. Watanabe*, I. Kato, S. Sato*, T. Okada*, and M. Norita, Dept. Otolaryngol., St. Marianna Univ. Sch. Med., Kawasaki 213, and Dept. Anat. Niigata Univ. Sch. Med., Niigata 951, Japan.

The nucleus of the optic tract (NOT) is the visuo-motor relay between the retina and preoculomotor structures in the pathway mediating optokinetic nystagmus (OKN). In the present study, how OKN signals are transmitted from the NOT was investigated using biocytin as an anterograde tracer. After identifying the position of the NOT electrophysiologically, biocytin (Sigma, 5% in 0.05 M Tris-HCl buffer at pH 7.6, total 0.3-0.7 µl) was injected mechanically through a micropipette into this nucleus. Descending fibers from the NOT consisted of the following three major pathways: 1) the NOT-contralateral NOT fibers which traversed in the posterior commissure into the contralateral NOT, the superior colliculus, and the dorsal terminal nucleus; 2) the NOT-dorsolateral pontine nucleus (DL) fibers which descended along the lateral border in the ventral part of the lateral terminal nucleus, the parabigeminal nucleus, mesencephalic reticular formation and terminated in the DL; and 3) the NOT-NRTP-PH and IO fibers which descended into the frontal tegmental field ventromedially and reached the medial lemniscus. At the level of the nucleus reticularis tegmenti pontis (NRTP), fibers terminated partly in the rostrocaudal 2/3 of the nucleus and passed through it, forming two separate fiber bundles at the level of 7th nucleus and terminated in the rostral part of the prepositus hypoglossi (PH) and the dorsal cap of the inferior olive (IO), respectively. In conjunction with the physiological data, OKN tract from the NOT may terminate directly in the prepositus, sending bifurcating their axons to the pontine nuclei.

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47.16

ELECTRON MICROSCOPIC EVIDENCE OF CAPSAICIN-INDUCED DEGENERATION IN RAT BRAIN AND RETINA. T.T. Dinh and S. Ritter. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Using various silver stains, we have demonstrated capsaicin-induced argyrophilia in specific sites throughout the rat central nervous system. The purpose of this experiment was to confirm the presence of capsaicin-induced degeneration in several of these sites with electron microscopy. To date, we have examined two capsaicin-sensitive sites, the nucleus of the solitary tract (NTS) and retina. Twenty-day-old rat pups were anesthetized, treated with 90% dihydrocapsaicin (Sigma, 75 mg/kg, s.c.) or the alcohol/Tween 80 vehicle solution, and killed 6 hrs later by anesthetic overdose. In the NTS, degenerating terminals appeared as highly condensed, electron dense aggregations of disorganized intracellular organelles, devoid of cytoplasm and frequently surrounded by or in close proximity to microglial processes. In the retina, degenerating bipolar and ganglion cell soma were recognizable by their highly condensed cytoplasm, nuclei and organelles. Degenerating soma appeared to be shrunken away from adjacent normal cells. Results further demonstrate the neurotoxicity of capsaicin for specific neuronal populations in the central nervous system.

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48.1

Physiology and morphology of identified projection neurons in rat visual cortex studied *in vitro*

Ekkehard Kasper *, Alan U. Larkman *, Colin Blakemore and Stuart Judge, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

We have investigated the correlation between the electrophysiology and the morphology of identified projection neurons in an *in vitro* slice preparation of adult rat visual cortex. Cells were prelabelled via stereotactic injections of rhodamine conjugated latex microspheres into either the ipsilateral superior colliculus or the primary visual cortex of the contralateral cortical hemisphere two days prior to the date of slice preparation. Prelabelled pyramidal neurons were visualized in the slice by fluorescence microscopy and injected carboxyfluorescein during the recording procedure to obtain intracellular staining for subsequent morphological classification.

Cells projecting to the superior colliculus fired action potentials in bursts in response to depolarizing suprathreshold current pulses and all had an apical dendrite arborizing in layer I. Callosal projecting cells had an apical dendrite that did not arborize in layer one and these cells did not fire action potentials in bursts but had a regular spiking pattern. The two populations differed significantly in some other features of their electrophysiology, e.g. cells projecting to the superior colliculus had shorter membrane time constants and lower input resistances compared to cells projecting to the contralateral hemisphere.

48.2

SPATIAL INPUT SPECIFICITY OF SYNAPTIC POTENTIATION AND DEPRESSION IN THE VISUAL CORTEX. J.P. Burke*, Y. Fregnac and M.J. Friedlander. Neurobiology Research Center and Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama, 35294.

We previously reported (Soc. Neurosci. Abstr.,16:798,1990) that temporal conjunctions of pre- and postsynaptic activity in the visual cortex can change synaptic efficacy. To determine the degree to which these effects are confined to the active synapses, we tested individual cortical neurons' responses to activation of different synaptic inputs, including those that were or were not paired with current injection into the postsynaptic cell. Conventional slices (500 μm thick) of visual cortex were obtained from anesthetized guinea-pigs and kittens. Intracellular recordings were made in bicuculline-free ACSF from neurons in layer 3/4 of cortical area 17. Compound post-synaptic potentials (PSPs) were evoked by microstimulation at two spatially separate sites. Separability of synaptic inputs from the two stimulation sites was evaluated by testing for occlusion. Postsynaptic membrane potential was altered by intracellular current injection through the recording micropipette. Protocols included temporal pairings of 80 ms membrane depolarization (S^+) or hyperpolarization (S^-) with activation of one synaptic pathway at 0.2 Hz for 30-80 trials, while the other synaptic pathway was inactive. To date, 23 experimental pairing protocols with dual electrode stimulation have been characterized for 11 cells. In 3 of 14 cases, the synaptic pathway that was paired with membrane depolarization was significantly potentiated (K-S test, $p<0.05$) while the unpaired pathway was not. In 1 of 9 cases, the synaptic pathway that was paired with membrane hyperpolarization was significantly depressed while the unpaired pathway was not. The average peak amplitude change of the PSP for the significant cases was +41% during S^+ pairing and -33% during S^- pairing. Thus, temporal conjunctions of pre- and postsynaptic activity in visual cortex seem to be specific for the active synapse. Supported by NIH EY05116 and HFSP.

48.3

TRANSMISSION OF NEURAL EXCITATION IN RAT VISUAL CORTICAL SLICES VISUALIZED BY OPTICAL RECORDING. M. Tanifuji*, A. Yamanaka*, and K. Toyama. Natl. Inst. Physiol. Sci., Okazaki, 444, JAPAN

Transmission of neural excitation in rat visual cortical (VC) slices stained with a voltage sensitive dye (RH482) was visualized using a cooled CCD video camera as time-lapse images taken by strobe illumination at different time-lags after white matter (WM) stimulation. A sequence of events were found: 1) impulses propagating from WM to the cortical surface, 2) monosynaptic excitation produced in layers IV and V-VI, 3) polysynaptic excitation in layer II-III transferred from layer IV, 4) higher order polysynaptic excitation transmitted back to layers V and VI. The main component of excitation propagation was restricted to a vertical stripe probably corresponding to columner structure with some lateral spread in layers II-III and V-VI. The lateral spread was abolished by blockade of synaptic transmission with DNQX and APV, but the columnar component remained unaffected, indicating that the latter component represents impulse propagation. The spread of excitation was greatly affected by pharmacological modification of cortical inhibition by GABA antagonists and agonists.

48.4

OPTICAL RECORDINGS OF EVOKED ACTIVITY IN GUINEA PIG NEOCORTICAL SLICES. B. Albowitz* and U. Kuhnt. Dept. of Neurobiology, Max-Planck-Institute for biophys. Chemistry, 3400 Göttingen, FRG.

The distribution and flow of activity in neocortical slices was studied by use of voltage sensitive dyes. Coronal slices were prepared from guinea pig visual cortex and stained with the fluorescence dyes RH 414 or RH 795. Voltage dependent light intensity changes following electrical stimulation of the white matter (WM) or layer I (LI) were monitored from a 10x10 photodiode matrix. The system provided a spatial resolution of 60µm or 94µm and a temporal resolution of 0.2ms or 0.4ms.

Following stimulation of WM, peaks of activity were reached in layers V and III. Activity in layer III appeared only with higher stimulation intensities in an almost all-or-none mode, indicating a predominantly polysynaptic pathway. Potentials in layer III were of shorter duration than those in lower layers and were occasionally followed by hyperpolarizations. Following stimulation of LI, peaks of activity were also found in layers III and V, but activity in layer V was very low. Potentials in layer III increased gradually with stimulation intensity and were never followed by hyperpolarizations. Thus, although stimulation of LI and WM both activate predominantly neurons in layer III, different synaptic pathways and/or neuronal populations seem to be involved. This is also reflected in the horizontal distribution of activity, which was wider for potentials elicited by stimulation of LI than for those elicited by stimulation of WM, even though presynaptic signals were more restricted following stimulation of LI. Also, potentials elicited by stimulation of WM frequently had an asymmetric horizontal amplitude distribution. Conduction velocities for potentials elicited by stimulation of LI were slower than for potentials elicited by stimulation of WM, measuring both post- and presynaptic activity. The latter shows that stimulation of LI activates slower conducting fibers.

48.5

SYNAPTIC PROPERTIES OF NEURONS IN TURTLE VISUAL CORTEX IN AN APPARENT MOTION PARADIGM STUDIED *IN VITRO*. P. S. Ulinski, L. J. Larson-Prior and N.T. Slater. Dept. Organismal Biology and Anatomy, Univ. Chicago, Chicago, IL 60637, and Dept. Physiology, Northwestern University, Chicago, IL 60611.

Apparent motion properties of neurons in visual cortex of turtles (*Pseudemys scripta*) were studied in an *in vitro* preparation of isolated cortex by sequential, electrical stimulation of geniculocortical fibers carrying information from two different regions of visual space. Postsynaptic potentials elicited by stimuli applied to two bundles of geniculocortical fibers in sequence were recorded intracellularly from cortical neurons. The ratio (P) of the second PSP to its control value was plotted as a function of interstimulus interval (t) between 25 and 1,000 msec. These P(t) curves show a fast facilitatory peak, an intermediate inhibitory peak and a slow facilitatory peak. P(t) curves could be modeled as a function of the form:

$$P(t) = 1 + N_1\, t\, \exp(-a_1 t) + N_2\, t \exp(-a_2 t) + N_3\, t \exp(-a_3 t).$$

The times to peak ($1/a_i$) for the three components were 25-50, 75-100 and 200-500 msec. Bath application of the NMDA receptor antagonist AP-5 (100 µM) significantly reduced the amplitude of the late component. These experiments indicate that circuitry within the cortex generates a complex form of synaptic interaction that likely plays an important role in the temporal integration involved in visual motion analysis. Supported by grants EY68352 (PSU) and NS17489 and NS25682 (NTS).

48.6

MEDIAL AND LATERAL DIFFERENCES IN POPULATIONS OF GABAERGIC NEURONS IN LAYER 3 OF TURTLE VISUAL CORTEX. J.M. Nicolaus and P. S. Ulinski. Dept. Org. Biol. and Anat., U. Chicago, Chicago, IL 60637.

Distinct medial-lateral differences exist in the organization of turtle visual cortex (e.g. Ulinski, 1990, Soc. Neurosci. Abstr.). It is known that GABA-positive neurons are found in layers 1 and 3 of this cortex, but previous studies (Blanton, et. al., 1987, JCN, 259:277-297) have focused on the medial portion of cortex. We used a flattened, *whole-mounted* cortex to analyze distributions and morphologies of neurons showing GABA-like immunoreactivity in the medial and lateral parts of cortex. Cortices were incubated with anti-GABA antibody (Sigma) and visualized with an avidin-biotin-HRP protocol. Most GABA-positive cells in medial and lateral caudal cortex fell along two morphological continua of fusiform cells with spine-free, varicose or beaded dendrites showing limited branching. One continum consisted of cells having an asymmetrically oriented dendritic field, with an extensive dendrite of large diameter at one pole and two smaller dendrites extending from the opposite pole. The somata and dendrites were compressed in the horizontal plane, so these cells were termed "horizontal cells." The second continum consisted of "multipolar cells" whose 3-5 dendrites extended both horizontally and vertically from the somata. At least twice as many GABA-positive cells are present medially as laterally. The size spectra of somata in medial and lateral populations are the same; therefore the two continua of horizontal and multipolar cells are present in both parts of cortex in about the same proportions but with a different density distribution.

Supported by PHS grant EY 68352 and by an NSF graduate fellowship.

48.7

MOVING VISUAL STIMULI INDUCE cFOS EXPRESSION IN THE VISUAL CORTICES OF THE RAT. S.L. Craner, J.S. Lund, G.E. Hoffman, and R.D. Lund. University of Pittsburgh School of Medicine, Pittsburgh PA 15261.

Previous work has shown that stationary flash stimulation of the rat eye induces cFos expression in the superior colliculus (SC) and pretectal region (PT) but not in the dorsal lateral geniculate nucleus (dLGN) or the visual cortices (VC). Here we examine cFos activation patterns in rat brain in response to motion stimuli. Anesthetized rats were subjected to either a moving grating or a moving random pattern (14.3°/sec) for one hour. The animals were euthanized after a further 30 mins and the brain processsed for immunohistochemical labeling of the cFos protein. Appropriate control groups (bilaterally enucleated and nonstimulated animals) were also processed.

Similar to animals stimulated with a stationary flash, animals subjected to a motion stimulus exhibited stimulus-specific cFos expresssion in both the SC and PT but not in the dLGN. We postulate that the lack of label in the dLGN may be due to the strong postexcitatory inhibition normally experienced by its cells. Unlike animals stimulated with a stationary flash, motion-stimulated animals did express cFos in the VC. With monocular stimulation, labeled cells in the contralateral area 17 were found predominantly in layers II, IV, and VI, with fewer cells in layer V. Labeled cells were also present in area 18 and 18a. Nonstimulated animals and animals stimulated with light flash exhibited labeling only in deep layer VI of visual cortex. These data suggest that 1) motion, but not flash, is effective for evoking cFos expression in the VC, 2) cortical cFos expresssion may be stimulus specific and 3) dLGN neurons, though activated, may not readily express cFos. Supported by NIH grants EYO6194 (SC), EYO5282 (JL), NIH-284-77 (GH), and EYO5283 (RL).

48.8

DISTRIBUTION OF EXCITATORY AMINO ACID BINDING SITES IN ADULT FERRET VISUAL CORTEX. A.L. Smith* and I.D. Thompson* (SPON: Brain Research Association). University Laboratory of Physiology, Oxford University, Parks Road, Oxford OX1 3PT, U.K.

We have used radioligand binding techniques to characterize the distribution of glutamate receptor subtypes in adult ferret visual cortex. Incubation of tissue homogenates prepared from posterior neocortex with the appropriate tritiated ligands revealed four of the five putative receptors for excitatory amino acids. Saturation analyses were performed by centrifugation binding assay with [3H]-kainate, [3H]-AMPA, [3H]-CGP39653 and [3H]-L-glutamate. These demonstrated the presence of kainate (high and low affinity), quisqualate-ionotropic (AMPA), N-methyl-D-aspartate (NMDA) and chloride-sensitive glutamate binding sites. In this preparation no quisqualate-metabotropic receptor could be detected.

The binding of these radioligands to cryostat sections of adult ferret brain was then investigated by quantitative *in vitro* autoradiography. Visual cortex was found to express all five known excitatory amino acid receptor subtypes. The distribution of these binding sites is not uniform, with the different subtypes exhibiting markedly different patterns of lamination. These differences may be indicative of the different roles fulfilled by the receptors.

This work was supported by a grant from the Wellcome Trust.

48.9

DISTRIBUTION OF GLUTAMATE NEURONS AND TERMINALS IN STRIATE CORTEX OF NORMAL AND MONOCULARLY DEPRIVED MONKEYS. R.K. Carder, E.G. Jones and S.H.C. Hendry. Dept. of Anatomy and Neurobiology, Univ. of California, Sch. of Med., Irvine, CA 92717

The morphology and distribution of glutamate immunoreactive neurons and terminals was examined with reference to the well defined laminar and columnar subdivisions of monkey striate cortex (area 17). Observations were made in normal adult monkeys and in adults deprived of input from one retina by intravitreal injection of tetrodotoxin (TTX) to determine whether select populations of cortical glutaminergic neurons are regulated by changes in visual input.

A large proportion of the glutamate immunoreactive neurons display morphological features characteristic of pyramidal neurons while other small, round glutamate positive neurons are presumably spiny stellate neurons. In normal monkeys glutamate immunostaining is dense in layers II-IVA, IVC and VI, and light in layers I, IVB and V. Staining of tangential sections through layers II-III reveal no preferential distribution within or surrounding the cytochrome oxidase patches. Staining in layer IVC of normal monkeys is also homogenous. However, following TTX injections, glutamate immunoreactive somata and puncta are greatly reduced in layers IVA and IVC of deprived eye columns. These data indicate that relative levels of glutamate immunostaining in monkey visual cortex vary across layers and are acutely sensitive to changes in afferent input.
Supported by NIH grants EY 06344, EY 06432, and EY 07193.

48.10

DIFFERENTIAL INTRANEURONAL REGULATION OF GAD AND β-PREPROTACHYKININ mRNAs IN MONKEY VISUAL CORTEX FOLLOWING MONOCULAR DEPRIVATION. D.L. Benson and E.G. Jones. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717

Neural activity can profoundly affect detectable levels of certain neurotransmitters, neuropeptides, receptors and protein kinases in the primary visual cortex of adult monkeys (Benson et al. J. Neurosci., 11:31, 1991). Following monocular deprivation by tetrodotoxin injection, in situ hybridization revealed that β-preprotachykinin (β-PPT) mRNA decreased in ocular dominance columns corresponding to the deprived eye. This change in β-PPT mRNA expression or stability was most apparent in the middle layers of cortex and parallels deprivation dependent decreases in tachykinin and GABA immunoreactivity (Hendry et al. J.Neurosci., 8:1225, 1988). Tachykinin immunoreactive neurons in these layers are also immunoreactive for GABA. However, unlike β-PPT mRNA, GAD mRNA levels do not change in response to monocular deprivation. To determine whether β-PPT mRNA levels were decreasing in the same cells demonstrating an apparent maintenance of GAD mRNA levels following monocular deprivation, we conducted double labeling in situ hybridization studies using radioactive β-PPT and nonradioactive GAD antisense RNA probes. We found that neurons in layer IVC colocalized both mRNAs, indicating that GAD and β-PPT mRNAs are differentially regulated by activity in the same neuron. This suggests that although the immunodetectable products of these neurons are coincidently decreasing, the mechanism dictating this change is targeting different levels of gene expression. Supported by USPHS grant EY 07193.

48.11

DISTRIBUTION OF IMMUNOCYTOCHEMICALLY LOCALIZED $GABA_A$ RECEPTOR SUBUNITS IN MONKEY AND HUMAN VISUAL CORTEX. M.M. Huntsman, E. G. Jones, H. Möhler and S. H. C. Hendry. Dept. Anatomy & Neurobiol., Univ. Calif., Irvine, CA, USA 92717 and Institute of Pharmacol., Univ. Zurich, Switzerland

The $GABA_A$ receptor is a complex molecule that is composed of several subunits and that contains binding sites for GABA, benzodiazepines and barbiturates. We have found that three of these subunits, localized by immunocytochemistry, are unevenly distributed in the visual cortex (area 17) of monkeys and humans. The greatest density of ß2/ß3 subunits in monkey area 17 is in the zones containing the densest geniculocortical terminations and GABA terminations (layer IVC, the honeycomb of layer IVA and the cytochrome oxidase patches of layers II-III; Hendry et al., 1990; J. Neurosci. 10: 2438). Most of the ß2/ß3 staining is punctate and present in the neuropil. Immunostaining for alpha1 and gamma2 subunits produces a similar laminar pattern and a similar punctate distribution. In addition, the gamma2 subunit is localized very densely to the cell bodies and proximal processes of a small population of neurons. Within area 17 of humans the immunostaining for all three subunits is densest in layer IVC, where they form strips of intense immunoreactivity, separate by narrow regions of light immunoreactivity. For none of the three is there any evidence of intense staining in layer IVA. As in the monkey, the gamma2 subunit in human area 17 has a distinct pericellular localization along a small population of neurons. These data demonstrate that three subunits of the $GABA_A$ receptor have a similar laminar distribution in monkey area 17 and an identical distribution in human area 17. However, the subcellular localization of the three subunits differs, which may give rise to variations in the pharmacological properties of GABA receptors in the primate visual cortex. Supported by USPHS Grants EY 06432 and EY 07193

48.12

NEUROTRANSMITTER PLASTICITY IN ADULT MONKEY VISUAL CORTEX: THE ROLE OF INTEROCULAR INTERACTIONS. S. H. C. Hendry and K. K. Welty* Dept. of Anat. & Neurobiol., Univ. of California, Irvine, CA 92717

Elimination of input from one retina in an adult monkey reduces the immunoreactivity for GABA and its synthesizing enzyme, GAD, in neurons of the visual cortex (area 17; Hendry and Jones, 1986; Nature 320: 750). The most robust changes are seen in the major geniculo-recipient layer, IVC, of the deprived-eye columns. These and other neurochemical changes in cortical neurons deprived of normal visual input are interpreted as activity-dependent events. To determine whether activity changes alone or interactions between inputs from the two eyes produces the changes in GABA and GAD immunoreactivity, two sets of experiments were performed: 1) area 17 in the two hemispheres of adult monkeys were compared following complete lesions of the dorsal lateral geniculate nucleus (LGN) on one side; 2) the monocular segments in area 17 contralateral to an eye injected with tetrodotoxin (TTX) were compared with injected-eye and normal-eye columns of the same hemisphere. With both kinds of experiments the GABA and GAD immunostaining in regions without competing inputs were found to be qualitatively and quantitatively normal. Thus, in area 17 denervated of LGN inputs for 5 or 7 days and in the monocular segments dominated by the TTX-injected eyes (retinal activity blocked for 5-20 days) the immunostaining is identical to that in the normally innervated or active area 17. Stereological analyses demonstrate that in layer IVC of the denervated area 17 and of the deprived monocular segment, GABA-immunoreactive cells make up the same proportion of neurons as in the normally innervated and active hemispheres and columns. These data indicate that interactions between inputs from the two eyes play an important role in the neurotransmitter plasticity of the adult monkey visual cortex. Supported by NIH Grants EY 06432 and EY 07193.

48.13

LATERAL INHIBITION BETWEEN BASKET CELLS IN THE VISUAL CORTEX OF CAT (AREA 18) Z.F.Kisvárday[1],C.Beaulieu[2] and U.T.Eysel[1]
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Somata of GABAergic large basket cells receive dense synaptic input from GABAergic terminals, suggesting that they are strongly inhibited by other basket cells. We tested this hypothesis using extracellular ionophoretic injections of biocytin in combination with immunostaining for parvalbumin (PV) in the same material.

The injected tracer labelled many large basket cells around the injection sites in their entire morphology while PV labelled somata of a subpopulation of GABAergic cells, including cells with large soma size characteristic of large basket cells. The axonal fields of two biocytin - labelled large basket cells, BC_1 and BC_2, were reconstructed in 3 - dimensions from horizontal sections in layers III and V, respectively. They contacted 58 and 33 PV+ somata distributed at about 100 µm intervals up to 1.6 mm from the somata of the parent basket cells. The synaptic contacts to 5 - 5 PV+ somata provided by BC_1 and BC_2, respectively, were additionally verified by electron microscopy.
Quantitative soma size distribution showed that the PV+ cells targeted by BC_1 and BC_2 comprise the largest cells of the total population of PV+ cells and they do not differ significantly from that of identified large basket cells, including BC_1 and BC_2.

The results show that large basket cells in addition to their well - known pyramidal cell targets mutually inhibit each other. This indicates that along their oriented long axons they result in a cascade of disinhibition of certain pyramidal cells and direct inhibition of other pyramidal cells. Z.F.K. is supported by the A. von Humboldt Foundation.

48.14

SPECIFICITY OF LATERAL INHIBITORY CONNECTIONS IN CAT VISUAL CORTEX U.T. Eysel and Z.F. Kisvárday. Department of Neurophysiology, Ruhr - University Bochum, P.O. Box 102148, D - 4630 Bochum 1, FRG.

Small microiontophoretical injections of the neuronal tracer biocytin into areas 17 or 18 of the cat were made to visualize a detailed pattern of excitatory and inhibitory connections. The tracer was delivered in a small volume occupying about one orientation column only (100 µm). Orientation and direction selectivity within a cortical region containing the injection site were recorded in detail at 50 - 200 µm steps.Somata, dendritic trees and axonal arborizations of cells connected with the injection site are filled by the tracer. Labelling and recording sites were analyzed in horizontal sections of the supragranular layers. In addition to pyramidal and spiny stellate cells a number of large basket cells with characteristic smooth dendrites and perisomatic axons were connected with the injection sites. The somata of the inhibitory (large basket) cells were distributed predominantly within 1 mm from the recording site in area 17 and 18 and the majority was situated in the range between 200 to 500 µm. Most of the basket cells and their dendrites fell outside the regions occupied by the excitatory network formed by pyramidal cells and their axons. Some basket cells, however, were sharing the territory with the patchy excitatory network.

The map of orientation selectivities superimposed to the anatomical reconstruction of the irregular mosaic of basket cells indicated that the basket cells connected with the injection sites originated from regions comprising the whole range of orientation selectivities, including iso - as well as cross - orientation with respect to their target regions. This suggests that basket cells can be involved in neuronal computations contributing to directionality as well as orientation selectivity in visual cortex. Supported by grants of the DFG and the European Communities.

48.15

TRANSNEURONAL TRANSPORT OF TETANUS TOXIN C-FRAGMENT REVEALS SECOND-ORDER LOCAL CIRCUITS IN CAT VISUAL CORTEX. J.A. Matsubara,[1,2] J. Zhang[2]* and J.D. Boyd[2]. Depts of [1]Anatomy and [2]Ophthalmology, Univ of British Columbia, Vancouver, BC, Canada, V5Z 3N9.

Locally projecting neurons in area 18 of the cat are organized into discrete groups, or patches, which exhibit an interpatch spacing of about 1 mm. Previous studies from our lab showed that the intrinsic patches of neurons interconnected sites of different preferred orientation. We further studied the local connectivity amongst these patches by utilizing the retrograde transneuronal tracer, C-fragment of tetanus toxin (TTC). Solutions containing a mixture of 1% TTC and 0.5% WGA-HRP were injected into area 18. Following a 1-4 day survival, the animal was euthanized and perfused with standard fixatives. Blocks of tissue were cut in the horizontal plane, tangential to the cortical surface. Every other section was reacted for HRP (1st order neurons), while the alternate sections were first incubated in a solution of 3% H2O2 and 10% methanol to deactivate peroxidase activity, and then placed into a primary antibody solution against TTC. Subsequent incubations in aviden biotin solutions allowed us to immunohistochemically identify TTC labeled (2nd order) neurons.

Our study revealed two types of labeling derived from the transport of TTC: dark spots of heavily labeled neuropil and clusters of labeled cell bodies. The dark spots aligned with the WGA-HRP patches and probably represent 1st order labeling. TTC cell bodies were found both within and outside of the dark spots. Cells falling inside the dark spots may represent unlysed 1st order cells, or more interestingly, 2nd order neurons contacting 1)cells in the same column or 2) cells in the other WGA-HRP patches of a reciprocally connected network. TTC cells located outside the dark spots either formed their own separate clusters at the outskirts of, thus extending, the WGA-HRP pattern or they formed clusters which adjoined the WGA-HRP patches. The significance of these disynaptic circuits to visual processing of receptive field properties will be discussed. This work was funded by MRC 5-99150 (Canada).

48.16

THE CALLOSAL PATHWAY IN CAT VISUAL CORTEX IS PATCHY AND ORIENTATION SPECIFIC. J.D. Boyd[1] and J.A. Matsubara[1,2]. Depts of [1]Ophthalmology and [2]Anatomy, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

Although the patchy nature of local connections is well known, the spatial organization of most callosal connections is not. We examined callosal connections between the area17/18 border regions, representing the vertical meridian.

1% wheat germ agglutinin conjugated horseradish peroxidase (HRP) and/or 1% cholera toxin subunit B (CTB) was injected through micropipettes into the lateral gyrus, in most cases after making an electrophysiological map. The cortex was flattened, sectioned tangentially and the tracers visualized with tetramethylbenzidine histochemistry (HRP) and/or diaminobenzidine immunohistochemistry (CTB).

Large injections (10-12 µl at 18 sites) of HRP gave retrograde callosal labeling which, while uniform on a large scale, contained fine scale fluctuations in labeling at 200-250µm intervals. Focal injections (.02µl) resulted in patches of callosal labeling with an interpatch distance of about 1mm. The patches often aligned parallel to the mediolateral axis. When injections of different tracers were made into areas of similar preferred orientation, the labeled patches from the two tracers overlapped with an abundance of double labeled cells. After injections into areas of orthogonal orientation, the two sets of labeled patches mostly interdigitated and fewer retrogradely labeled cells contained both tracers.

Orientation bands near the 17-18 border tend to run perpendicular to the border, and have a periodicity of about 1mm. Thus, the orientation selectivity of callosal connections in this region could account, in part, for the patchy distribution described above. The making of patchy connections by zones of all orientation preferences would interdigitate to such an extent as to explain the large scale uniformity when large injections were made. The explanation of the 200-250µm density fluctuations is less clear. This work was funded by MRC(Canada).

48.17

MORPHOLOGY OF AREA 18 NEURONS OF CAT VISUAL CORTEX STUDIED BY CONFOCAL MICROSCOPY OF LUCIFER YELLOW FILLED NEURONS IN PRELABELLED, FIXED BRAIN SLICES. D.M. Thejomayen[1] and J.A. Matsubara[1,2]. Depts of [1]Anatomy and [2]Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

Cells projecting from one cortical area to another cortical area and to given sites within an individual cortical area are distributed in a number of discrete clusters or patches, as shown by horseradish peroxidase (HRP) tracing experiments. We used intracellular dye injections in lightly fixed cortical slices to examine the morphology of layer II/III neurons of these patchy, columnar systems. Neurons of area 18 that project to area 17 and the ones which participate in local connections within area 18 were retrogradely labeled with fluorescent latex microspheres. 300 µm thick vibratome sections of the perfusion fixed brains were transferred to a epifluorescence microscope equipped with long working distance objectives. Under visual guidance prelabelled layer II/III neurons were selectively impaled and intracellularly injected with lucifer yellow (LY) until all dendrites appeared brightly fluorescent.

We used a confocal laser scanning microscope (Biorad MRC 600) to collect serial optical sections and to perform three-dimensional reconstructions and rotations of LY filled neurons in wholemount preparations. This technique provided us an efficient alternative to tedious camera lucida reconstuctions as well as a means to study the spatial arrangement of the dendrites in three dimensions. When layer II/III neurons of area 18 were examined, we saw that the dendritic trees of many cells, which appear symmetric in the coronal plane, were in fact, quite asymmetric with processes extending further antero-posteriorly. Other neurons in our preliminary sample exhibited a 'slab-like' or planar arrangement of dendrites. These differences in the spatial arrangement of the dendritic arborizations will be related to the corticocortical and intracortical patches. This work was funded by MRC 5-99150 (Canada).

48.18

EXTRACELLULAR BIOCYTIN STAINING OF CELLS IN THE VISUAL CORTEX SLICE PREPARATION. G. Kenan and H. Katz*, Center for Neurosciences, The Weizmann Institute of Science, Rehovot 76100, Israel.

Using the cortical slice preparation, Biocytin (2.5%) was applied at different layers of rat visual cortex by diffusion from a broken micropipette inserted into the slice (10-30um tip diameter) for 1-5min, or by drop application (5-10ul). After 20min-3hrs incubation, the slices were fixed over night (4% paraformaldehyde) and processed for HRP staining.

Biocytin was taken by cells at the area of injection and revealed the detailed morphology of dendritic trees of pyramidal and none pyramidal cells. The quality of neuronal staining, i.e. spine density and fine dendritic branches was comparable to intra-cellular injections of Biocytin. In some cases parts of the axonal arbor could be traced. Stained cells were not limited to the area of injection e.g. injection in layer I revealed pyramidal cells in layer V. Injection sites varied in size from few cells to several hundred um. When Biocytin was introduced by drop application, Golgi-like staining of cells could be observed throughout the cortical layers.

48.19

RELATIONSHIP OF BIOCYTIN LABELED NEURONAL PROCESSES TO THE CYTOCHROME OXIDASE (CO) RICH BLOBS IN MONKEY STRIATE CORTEX. R. Malach Weizmann Inst. Rehovot, Israel 76 100.

How segregated are the CO blobs from the surrounding interblob tissue? To address this question, the tracer biocytin was injected at numerous sites in macaque monkey striate cortex resulting in Golgi-like staining of neuronal processes. These were related, in tangential sections, to the pattern of CO blobs. As was found previously in Golgi material of squirrel and marmoset monkeys, (Malach 1990, Soc. Neurosci. Abst. 130.2) dendritic arbors of upper layer neurons appeared to cross freely through blob boundaries. The axons of these neurons, studded with boutons, ramified extensively for about 100 μm around the injection sites. Further away, and up to a distance of 1-1.2mm, axons tended to form clusters 150-200 μm wide. The same axon could often be traced to more than one cluster.

In agreement with previous reports (Livingstone and Hubel 1984, J. Neurosci.4:2830) axons from centers of blobs and interblobs tended to arborize in similar tissue compartments. Individual axons, labeled by border injections, crossed freely through blob boundaries and ramified in both blob and interblob territories. These results suggest that processes of upper layer neurons are organized in periodic, blob-related but smoothly shifting patterns. (BSF 88-275)

48.20

CELL MORPHOLOGY AND BLOB PATTERN IN MONKEY STRIATE CORTEX. M. Hübener and J. Bolz. Friedrich-Miescher-Labor der Max-Planck-Gesellschaft, Spemannstr. 37-39, 7400 Tübingen, Germany.

Cytochrome oxidase staining reveals a regular pattern in primate striate cortex, the blobs. Neurons within the blobs often have unoriented, color selective receptive fields, whereas cells in the interblob region usually have oriented receptive fields and respond to all wavelengths. In addition, cells in the blob and interblob compartment receive different input and project to different sites. We studied the relationship between the blob pattern and the morphology of single cells in the striate cortex of macaque monkeys. Lucifer yellow was injected into individual cells in lightly fixed tangential sections from area 17. Adjacent sections were stained for cytochrome oxidase, radially running blood vessels were used as landmarks to determine the position of each stained cell with respect to the blob pattern. We confined our study to the analysis of pyramidal cells in layer 2/3. A comparison of the morphology of blob and interblob cells showed that there are no differences between the two groups with respect to soma size, spine density, and dendritic field structure. The dendritic field elongation of blob and interblob cells, determined by a quantitative statistical analysis, was found to be similar, indicating that cortical orientation selectivity is not generated through dendritic field elongation. We found many cells with dendrites freely crossing the borders between blob and interblob regions. However, a number of cells had a tendency to stay within one compartment. A quantitative analysis revealed that on average cells in the vicinity of the borders tend to avoid crossing a border. Thus there is a weak bias for the basal dendritic fields of layer 2/3 pyramidal cells to be segregated into blob and interblob compartments. Our observation that many cells send dendrites into a blob as well as into the interblob region demonstrates that a structural basis for crosstalk between the different channels exists already at an early stage of cortical processing.

48.21

PATCHY INTRINSIC CONNECTIONS IN MACAQUE AREA V4.
J.B. Levitt, T. Yoshioka, and J.S. Lund. Center for Neuroscience, University of Pittsburgh, Pittsburgh, Pa. 15261.

We have examined the pattern of intrinsic connectivity of area V4 in the macaque monkey by making focal (200-500 μm) iontophoretic injections of the neuronal tracer biocytin into the dorsal portion of V4. We noted prominent, focused interlaminar projections at each injection site. In addition, we found laterally-spreading fibers to be restricted to two bands, one in lower layer 3 and one in layer 5. These laterally-spreading fibers were conspicuously absent from layer 4, even in cases where the injection zone included layer 4. Neuronal processes could be found over 3 mm from an injection site, though the majority of fibers spread within 2 mm of an injection. Long-distance connections travelled in the layer 3 band; lateral connections in layer 5 seemed more restricted in extent. The spread of label around injections seemed preferentially elongated in the mediolateral direction.

These lateral connections were not uniformly distributed across V4, but often clustered into discrete patches of terminal label. These patches were between 250-500 μm in diameter, and the spacing between the patches varied from 0.5 to 1.5 mm. Patches of label were generally found from 0.3 to 2 mm from an injection site, though we occasionally observed patches over 3 mm away. These injections also retrogradely labelled clusters of cells in V4. These cells were predominantly located in layer 3, and were pyramidal in morphology. The clusters of labelled cells often, but not always, coincided with the patches of terminal labelling.

These results indicate that area V4 contains focused columnar projections, as well as more widespread and divergent lateral projections, a pattern of intrinsic connectivity previously observed in other visual areas.

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CHEMICAL SENSES: PERIPHERAL MECHANISMS I

49.1

NEUROCHEMICAL ORGANIZATION OF CAROTID BODY (CB) AFFERENT NEURONS. J.C.W. Finley[1], J. Polak*[2] and D.M. Katz[1,2]. Depts. Medicine[1] and Neuroscience[2], CWRU Sch. of Med., Cleveland, OH 44106.

A large subset of CB afferents in the rat petrosal ganglion (PG) express tyrosine hydroxylase (TH) and catecholamine fluorescence; these cells do not express dopamine beta hydroxylase and therefore appear to be dopaminergic (Katz, et al., 1983; Katz & Black, 1985). To further characterize transmitter properties of these cells we examined expression of dopa decarboxylase (DDC), the dopamine synthesizing enzyme. The majority of TH+ CB afferents in the distal PG specifically co-localized DDC, consistent with a dopaminergic phenotype. Ultrastructural studies demonstrated that TH+ afferents synapse directly with CB glomus cells, supporting a role for these fibers in chemoreception. Parallel studies on the expression of peptidergic properties in CB afferents examined the localization of Substance P (SP) neurons retrogradely labeled by microinjection of Fluorogold into the vascularly isolated CB. In contrast to TH+/DDC+ cells, SP+ CB afferents were localized primarily to the proximal PG and jugular ganglion (JG) of the glossopharyngeal nerve. SP+ afferents were also larger than most TH+/DDC+ cells. The CB is therefore innervated by at least two neurochemically and morphologically distinct populations of afferents that are also topographically segregated within the glossopharyngeal sensory ganglia. Parker B. Francis Foundation Pulmonary Fellowship (JCWF) and HL-39921 (DMK).

49.2

MECHANISM OF ACTION OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN RABBIT CAROTID BODY. L. He*, J. Chen, B. Dinger and S. Fidone. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

Our previous studies have shown that ANP is stored in type I (glomus) cells in the carotid body and that submicromolar concentrations of the synthetic ANP fragment, atriopeptin III (APIII), inhibit carotid sinus nerve (CSN) activity evoked by hypoxia. Furthermore, APIII elevates the content of cyclic GMP (cGMP) in the tissue in a dose-related manner. In the present study we have: 1), evaluated CSN inhibition and cGMP production in the presence of the biologically inactive ring-deleted analog of ANP, C-ANP; 2), examined the effects of APIII on nerve discharge produced by direct depolarization of CSN endings; and 3), examined whether met-enkephalin (ME), an agent known to be inhibitory in the carotid body, may be involved in the mediation of the ANP-produced inhibition of CSN activity.

cGMP levels were increased 20-fold in carotid bodies incubated in vitro for 10 min in the presence of 100nM ANP. C-ANP (100nM), however, failed to alter cGMP levels in the tissue. Similarly, C-ANP (100nM or 1000nM) also failed to inhibit CSN activity evoked by hypoxia in vitro, while in the same preparations APIII (100nM) reduced the integrated nerve discharge by 44.1% ± 4.6%. APIII did not alter CSN activity evoked by 20mM K^+, either in the presence or absence of Ca^{++}. Finally, APIII inhibition of CSN activity was blocked by a concentration of naloxone (10^{-6}M) sufficient to occupy delta opiate receptors in other peripheral and central nervous tissues. Our data are consistent with the hypothesis that APIII inhibition in the carotid body is mediated by preneural type I cells via specific ANP receptors coupled to guanylate cyclase. Furthermore, the ability of naloxone to antagonize APIII inhibition suggests the involvement of endogenous ME. Supported by USPHS grants NS12636 and NS07938.

49.3

LOCALIZATION OF ATRIAL NATRIURETIC PEPTIDE RECEPTORS IN THE CAROTID BODY. Z.-Z. Wang, S. Hirose*, B. Dinger, S. Fidone and L.J. Stensaas. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108, and Dept. of Biol. Sci., Tokyo Inst. of Technology, Tokyo, Japan.

Previous studies in our laboratory have shown that atrial natriuretic peptide (ANP) is stored in preneural type I cells of the cat carotid body and that the synthetic ANP analog, atriopeptin III (APIII), applied in vitro inhibits chemoreceptor activity evoked by hypoxia. Our recent RIA and immunocytochemical experiments also showed that APIII significantly increased the level of cyclic GMP in type I cells. Although the mechanisms of ANP-mediated inhibition of carotid sinus nerve discharge and the accompanying activation of guanylate cyclase observed in our experiments remains unclear, the striking correspondence between the effective dose-range for ANP actions in the carotid body and its effects in other tissues known to contain ANP receptors (Rugg et al. BBRC 162: 1339, 1989) strongly implicates the involvement of specific ANP receptors in our experiments. In the present immunocytochemical study, receptors for ANP were localized to type I cells of the pig carotid body using a specific polyclonal antibody combined with the avidin-biotin-peroxidase method. Staining was absent in the type II cells and nerve fibers in the tissue. Using a double labeling post-embedding immunocytochemical technique, we further demonstrated that the cGMP produced in response to APIII stimulation is co-localized with ANP in type I cells. These data are consistent with the notion that endogenous ANP released by type I cells modulates chemoreceptor function by acting at specific autoreceptors coupled to guanylate cyclase. Supported by USPHS grants NS12636 and NS07938.

49.4

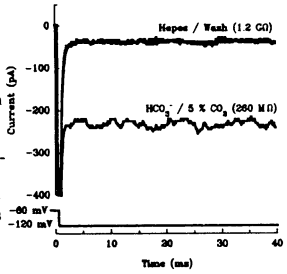
ALTERATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) IMMUNO-STAINING IN THE RAT CAROTID BODY EVOKED BY HYPOXIA. L.J. Stensaas, Z.-Z. Wang, B. Dinger and S. Fidone. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

Our previous immunocytochemical and electrophysiological studies demonstrated that type I cells in the carotid body contain ANP, and that submicromolar concentrations of the synthetic ANP analog, atropeptin III (APIII), inhibit carotid sinus nerve discharge evoked by hypoxia. These findings suggest that endogenous ANP may modulate chemoreceptor activity during physiological conditions that promote ANP release from type I cells. However, the ability of the carotid body to synthesize and release ANP has never been demonstrated. In the present investigation we have utilized immunocytochemical techniques to monitor the relative content of ANP in rat carotid body type I cells exposed to prolonged periods of hypoxia. Following 3 hr of breathing 10% O_2 (balance N_2), ANP immunostaining was significantly reduced in type I cells; ANP staining intensity recovered in rats which subsequently breathed room air for 2 hr. Parallel experiments were performed in vitro to eliminate possible interference due to uptake of circulating ANP. Carotid bodies incubated for 3 hrs with superfusates equilibrated with 10% O_2 likewise demonstrated marked depletion of ANP immunoreactivity, whereas ANP levels appeared normal in similarly treated tissue samples which were allowed to recover for 2 additional hrs in superfusates equilibrated with 100% O_2. The observed reductions in ANP immunoreactivity are consistent with the hypothesis that hypoxia evokes the release of ANP from type I cells. Furthermore, the recovery of immunostaining in vitro (and in vivo) suggests that carotid body type I cells synthesize ANP de novo following depletion of the peptide. Supported by USPHS grants NS12636 and NS07938.

49.5

DO CHLORIDE CHANNELS MEDIATE BOTH CO_2-CHEMOSENSITIVE AND OSMOSENSITIVE FUNCTIONS IN ARTERIAL CHEMORECEPTORS? C.A.Nurse and A.Stea. Dept.of Biology, McMaster University, Hamilton, Ontario, L8S 4K1.

Elevated arterial CO_2 is thought to excite carotid body chemoreceptors via intracellular acidification of (receptor) glomus cells, which contain the enzyme carbonic anhydrase (Nurse,Cell Tiss.Res.261:65). Using the perforated patch technique we recently showed (submitted) that an acidic pH_i depressed voltage-activated currents in cultured rat glomus cells, at constant pH_o maintained by Hepes-buffered media (HBM). To compare the effects of CO_2 stimuli we have shifted to bicarbonate-buffered media (BBM). Unexpectedly, we found that in 10 of 13 cells tested, simply switching the perfusate from HBM to 5% CO_2/BBM resulted in a substantial (up to 5-fold) decrease in input resistance and the appearance of large conductance single channel events superimposed on whole cell leakage currents (see figure). We are testing the hypothesis that cell volume changes or osmotic stress in CO_2/BBM activated these channels and that they correspond to the HCO_3^--permeable Cl^- channels (≈ 296 pS) we characterized in inside-out patches (Stea and Nurse, AJP 257:C174). This hypothesis incidentally provides an explanation for osmosensitivity in glomus cells. These preliminary findings raise further questions about the physiological interpretation of recordings based solely on the use of HBM. Supported by NIH -R01 HL 43414.



49.6

CO_2 STIMULANTS DIFFERENT CLASSES OF LINGUAL TRIGEMINAL NEURONS. B.P. Bryant[1], M. Komai[1,2,*] and M. Wachowiak[1*], [1]Monell Chemical Senses Center, Philadelphia, 19104 and [2]Tohoku University, Sendai, Japan.

CO_2 is a potent trigeminal stimulus. When applied orally at a high enough concentration, it produces a tingling or slight burning sensation. As a first approach to understanding the neural basis of oral CO_2 sensation, we examined single unit responses in the lingual nerve of the rat to a panel of stimuli: 8°, 24° and 53°C H_2O, solutions of CO_2 (5-6,000 ppm = 114-136 mM) at 8°, 24° and 28°C, 0.5 M HCl, 2.5 M NH_4Cl and tactile stimulation. In agreement with previous findings (Kawamura, 1967), some units that were sensitive to CO_2 were also sensitive to cold stimuli. However, in the present study, not all cold units were sensitive to CO_2. The pattern of chemical sensitivity was not identical across all units tested; responsiveness to CO_2 was not a good predictor of responsiveness to HCl and/or NH_4Cl. Several units responded solely to both hot and CO_2 stimuli. In spontaneously active cold units, CO_2 caused suppression followed by an excitatory response. In most CO_2 sensitive neurons, the excitatory response to CO_2 was delayed 4-9 sec relative to stimulus onset and thermal responses. This complex response suggests, among other possibilities, a temperature-dependent diffusion delay. Acetazolamide (15 mg/kg b.w.) was used to demonstrate the possible role of carbonic anhydrase (CA) and intraepithelial acidification in trigeminal sensitivity to CO_2. Responses to CO_2 were specifically inhibited whereas responses to thermal and other chemical stimuli were unaffected.

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49.7

ELECTRIC PROPERTIES AND CHEMICAL REACTIVITY OF NODOSE GANGLION NEURONS IMPLANTED ONTO CHICK EMBRYO CHORIOALLANTOIS. J. Eugenín and C. Eyzaguirre. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108, U.S.A.

Electric properties and responses to chemical stimuli of adult rat nodose neurons grafted for 4-10 days onto the chorioallantoic membrane of chick embryos were compared with those from normal neurons.

Normal and implanted ganglia were placed in vitro under flowing saline equilibrated with 100% O_2. Only neurons showing resting potentials (E_m) larger than -40 mV and action potentials (APs) with overshoot followed by hyperpolarizing afterpotentials (AHPs) were included in this analysis. Comparisons were made with the Mann-Whitney (U-test) using a significance level, $p < 0.01$.

Implanted nodose cells (n=18) had a mean E_m of -57.7 mV ± 2.5 (SE), input resistance of 72.7 MΩ ± 10.9, input capacitance of 67.1 pF ± 15.2 and membrane time constant of 3.5 ms ± 0.5. These parameters were similar to those from control nodose neurons (n=20). Neither control nor implanted neurons showed spontaneous APs. However, both groups showed time-dependent rectification to inward current and responded with single or multiple APs to depolarizing pulses. The APs of grafted nodose cells were smaller, with lower overshoot, and longer than those of control neurons. The AHPs were of a lower amplitude in implanted nodose cells.

Implanted and normal neurons responded to chemical stimuli. Switching to saline equilibrated with 100% N_2 and applications of 5-10 µl of lactic acid (6.8 to 13.4 µg/µl) depolarized the neurons by 5 to 20 mV. NaCN (10-50 µg), on the other hand, induced a transient dose-dependent hyperpolarization (5 to 30 mV).

Results show that implanted nodose neurons retain their passive electric properties and respond to chemical stimuli. The increase in AP duration and decrease in the amplitude of AHPs may be an effect secondary to chronic axotomy. Supported by grants NS05666 and NS07938. J.E. is a Fogarty International Fellow.

49.8

ELECTROPHYSIOLOGICAL PROPERTIES OF LIGHT AND DARK CELLS ISOLATED FROM MUDPUPPY TASTE BUDS. M.McPheeters, A.J.Barber, J.C.Kinnamon, and S.C.Kinnamon. Dept. of Anatomy and Neurobiology, Colorado State Univ., Fort Collins, CO 80523 and Dept. of Molecular, Cellular and Developmental Biology, Univ. of Colorado, Boulder, CO 80309.

Cells within taste buds have been characterized as light cells or dark cells based on their appearance in the electron microscope. The physiological significance of these cell types, however, remains unclear. Light cells constitute a minority of taste cells, and previous recordings have been almost exclusively from dark cells. We now report recordings from both light and dark cells, using quinacrine staining to select light cells prior to giga-seal whole cell recording (Delay et. al. Soc. Neurosci. Abstr. 16:878, 1991). Depolarizing voltage steps and pharmacological agents were used to examine the current profiles of different cells. After recording, cells were fixed and prepared for electron microscopy. Dark cells were distinguished by dense granules in the supranuclear and apical regions. Mitochondria and microtubules were plentiful in these cells. Light cells were identified by abundant stacks of smooth endoplasmic reticulum found throughout the cell and the lack of apical granules. Light cells often had empty-appearing cytoplasmic inclusions which may reflect metabolic instability during isolation and/or recording. Results show that both light and dark cells contain voltage-sensitive Na^+ and K^+ currents. To date, Ca^{++} currents have been observed only in dark cells. Thus, both light and dark cells have the capacity to generate action potentials in response to taste stimuli.

49.9

CALCIUM-RELATED CONDUCTANCES IN FROG OLFACTORY RECEPTOR NEURONS. S.J. Kleene*, R.Y.K. Pun, & R.C. Gesteland. Anatomy & Cell Biology, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267-0521.

An influx of Ca^{2+} may play a role in odorant transduction in some olfactory receptor neurons. We have measured the effects of cytoplasmic Ca^{2+} on the conductance of single cilia excised from frog olfactory neurons. When free cytoplasmic Ca^{2+} is buffered at or below 0.1 μM, ciliary conductance is low. As Ca^{2+} is increased, ciliary conductance increases. Maximal conductance averages 7-fold higher than that measured in the absence of Ca^{2+}. We estimate that the $K_{1/2}$ for Ca^{2+} is about 5 μM. Activation by Ca^{2+} is rapid and fully reversible. The maximal Ca^{2+}-activated conductance averages 3.6 ± 0.2 nS (n=55, range 1.3 to 7.9 nS). The reversal potential of the Ca^{2+}-activated current shifts 57 mV for each tenfold change in cytoplasmic Cl^- concentration, suggesting that Cl^- is the principal current carrier. Replacement of Na^+ and K^+ by $choline^+$ has no detectable effect on the Ca^{2+}-activated conductance. Analogues of the Cl^--channel inhibitor diphenylamine 2-carboxylate reduce the Ca^{2+} current by as much as 90%. We cannot yet define the role of the Ca^{2+}-activated ciliary Cl^- conductance, since the intraciliary concentrations of Ca^{2+} and Cl^- are unknown.

To look for Ca^{2+} currents in the intact neuron, we apply 25 mM $tetraethylammonium^+$ externally. This depolarizes the membrane and prolongs the duration of the action potential several-fold. Under voltage-clamp with K^+ and Na^+ currents blocked, a sustained inward current is recorded in the presence of 5 mM extracellular Ca^{2+}. This current activates near -20 mV and peaks between +5 and +20 mV. The sustained current shows little inactivation even with 500-msec pulses, but shows marked rundown, disappearing within 10 min after the onset of whole-cell recording. Rundown of the fast inward current is not seen. External application of 50 μM Cd^{2+} reversibly blocks most of the sustained inward current, indicating that it is Ca^{2+}-dependent.

This work was supported by NIH grants PO1 DC00347 and RO1 DC00352.

49.10

AMILORIDE MODULATION OF MEMBRANE ELECTRICAL PROPERTIES OF RAT FUNGIFORM TASTE CELLS. M.S. Herness. Lab. of Neurobiology & Behavior, Rockefeller University, New York, NY 10021.

The salt taste quality is hypothesized to utilize amiloride-sensitive (AS) sodium channels as a transductive utensil. The effect of amiloride (10^{-4} M) was tested on dissociated rat fungiform taste cells using the patch clamp technique in the whole cell configuration. The most common response was a decrease in a stationary-inward current when an amiloride-ECF solution was applied to the bath. This is likely to be an amiloride block of a depolarizing sodium leak from the extracellular fluid (containing 126 mM NaCl) occurring via the AS sodium channel. This response was often accompanied by diminished voltage-sensitive outward currents. At least part of this diminished current appears to be carried by outward sodium currents occurring above E_{Na+}. Another possibility may be a previously unnoticed influence of apical AS sodium channels on basolateral g_{K+}. In other cells amiloride increased a stationary inward current and augmented outward currents supporting the notion of receptor cell heterogeneity. Both depolarizations and hyperpolarizations of the resting potentials (measured by the zero current) were observed.

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49.11

BIOCHEMICAL CHARACTERIZATION OF A NOVEL INOSITOL-1,4,5-TRISPHOSPHATE (IP_3) RECEPTOR PROTEIN IN OLFACTORY CILIA. D.L. Kalinoski[1], A.G. Boyle[1*], J.F. Marecek[2*], G.D. Prestwich[2*] and D. Restrepo[1*], [1]Monell Chemical Senses Center, Phila, PA and [2]SUNY, Stony Brook, NY.

Certain olfactory stimuli elicit increases in IP_3 in olfactory cilia of catfish (Huque & Bruch, BBRC 137:36, 1986) and rat (Breer & Boekhoff, Chem. Sens. 16:19, 1991). In catfish IP_3 is believed to cause opening of an IP_3-gated channel located in the ciliary membrane (Restrepo et al. Science 249:1166, 1990). Opening this channel presumably causes membrane depolarization leading to generation of action potentials. To determine the biochemical properties of this channel we characterized the binding of [^{3}H]-IP_3 to olfactory cilia from the catfish. We find a single saturable binding site for 1,4,5-IP_3 with a K_d of 1.1±0.31 μM and B_{max} of 17.6±5.8 pmol/mg. The rank order for potency of inhibition of [^{3}H]-IP_3 binding is 1,4-IP_2 < 1,3,4-IP_3 < 1,3,4,5-IP_4 = 1,4,5-IP_3 < 2,4,5-IP_3. IP_3 binding increases slightly when free [Ca^{2+}] is increased from < 10 nM to 1 mM. These characteristics are different from those of an IP_3 receptor characterized in cerebellum and other peripheral tissues (Guillemette et al. PNAS 84:8195, 1987, Spat et al. Nature 319:514, 1986, Worley et al. JBC 262:12132, 1987). In addition, we have used a photoactivatable analogue of IP_3 (^{125}I-ASA-IP_3) to identify the olfactory IP_3 receptor protein. Catfish and rat cilia were exposed to short-wavelenght UV light in the presence of ^{125}I-ASA-IP_3 plus or minus excess 1,4,5-IP_3 or analogues. After SDS-PAGE separation of the membrane proteins autoradiograms detected several labeled bands. ^{125}I labeling was specifically displaced from a band of 100 kDa by addition of 50 μM 1,4,5-IP_3 or 1,3,4,5-IP_4 or of 100 μg/ml heparin. Labeling could not be displaced by 1,4-IP_2 or 1,3,4-IP_3. In control experiments we show that ^{125}I-ASA-IP_3 specifically labels the IP_3 receptor protein in rat brain (200 kDa). These results indicate that olfactory cilia possess a novel IP_3 receptor protein which is presumably the IP_3-gated calcium channel recently identified by us in catfish olfactory cilia.

49.12

REDUCTION OF OLFACTORY NEURON ADENYLATE CYCLASE ACTIVITY BY AMITRIPTYLINE IN VITRO

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Adenylate cyclase (AC) plays an important role in olfactory transduction. Because the tricyclic antidepressant, amitriptyline (AMI), inhibits AC activity in embryonic chick neurons (Wong et al., J. Neurochem. In Press), we measured the effect of AMI on forskolin-stimulated AC activity in olfactory membrane preparations from normal and unilaterally bulbectomized (OB-X) adult rats. On unoperated animals AMI (0.5μM-8.0μM) inhibited forskolin-stimulated AC activity in the presence of Gpp(NH)p. To determine whether this effect was specific for neurons in olfactory epithelium we made membrane preparations from OB-X animals 4 days after surgery. In these preparations we saw significantly ($p<.005$) lower AC levels and no significant AMI effect, in comparison to unoperated animals. Our data support the hypothesis that AMI lowers AC activity via a GTP-mediated mechanism and also indicate that in olfactory tissue this decrease is specific for olfactory neurons.

Supported by NIH Grants #DC 00566 and DC 00080.

49.13

THE ROLE OF ADENOSINE IN STIMULUS-EVOKED CYCLIC AMP PRODUCTION IN THE RABBIT CAROTID BODY. J. Chen, B. Dinger and S. Fidone. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

Previous studies have shown that adenosine and its related agonists increase respiratory minute volume and excite carotid body chemoreceptors (McQueen and Ribeiro, Br. J. Pharmacol. 88: 615, 1986; Monteiro and Ribeiro, N.S. Arch. Pharmacol. 340: 230, 1989). Furthermore, adenosine antagonists inhibit the excitatory effects of adenosine and reduce the sensitivity of carotid chemoreceptors to hypoxia. Pharmacological studies have indicated that adenosine actions in the carotid body are mediated by the A-2 subtype of adenosine receptor, which in other tissues is positively coupled to adenylate cyclase. Because our recent studies have shown that hypoxia elevates cyclic AMP (cAMP) in the carotid body, we have investigated the possible role of adenosine in this hypoxia-mediated response.

cAMP levels in carotid bodies superfused in vitro for 10 min were increased in a dose-related manner in the presence of adenosine (10μM, 100μM and 1000 μM adenosine; maximum increase = 127%, $p<0.01$). Basal cAMP levels were also increased 46% ($p<0.01$) by the adenosine uptake blocker dipyridamole (100nM), and this drug potentiated the hypoxia-induced increase in cAMP by 65% ($p<0.01$; 10 min incubation in solution equilibrated with 10% O_2). Finally, the adenosine receptor antagonist 1,3-dipropyl-8(p-sulfophenyl)xanthine (DPSPX, 1μM) blocked the effect of hypoxia in increasing cAMP in the carotid body. Our data suggest that the carotid body contains specific adenosine receptors positively coupled to adenylate cyclase. Furthermore, the data are consistent with the notion that increases in cAMP associated with hypoxia may be mediated in part by the release of endogenous adenosine. Supported by USPHS grants NS12636 and NS07938.

49.14

CYTOCHEMICAL LOCALIZATION OF ECTO-ATPASE/PHOSPHATASE ACTIVITY IN OLFACTORY SENSILLA OF THE SPINY LOBSTER.

R.A. Gleeson,* L.M. McDowell,* H.G. Trapido-Rosenthal, H.C. Aldrich* and W.E.S. Carr,* Whitney Laboratory, University of Florida, St. Augustine, Florida 32086.

Electrophysiological studies have shown that the olfactory organ (antennule) of the spiny lobster, Panulirus argus, has chemoreceptors which are selectively stimulated by adenine nucleotides in seawater. Biochemical studies have revealed that these same nucleotides can be rapidly dephosphorylated by ecto-enzymes associated with the olfactory sensilla (aesthetascs). In this study the distribution of ecto-ATPase/phosphatase activity within aesthetascs was examined by monitoring the deposition of cerium phosphate. The distribution of ATP-dephosphorylating activity was similar to that shown previously for AMP and β-glycerol phosphate (Gleeson et al. Cell and Tissue Res. in press); i.e., cerium phosphate reaction product was specifically localized to the transitional zone where the sensory dendrites develop cilia and branch to form the outer dendritic segments. Reaction product was found within extracellular spaces adjoining both dendrites and auxiliary cells in this region. Unlike the dephosphorylation of AMP and β-glycerol phosphate, Mg^{++} or Ca^{++} was required in the incubation medium for ecto-ATPase/phosphatase activity. Degradative ectoenzymes in the transitional zone may be of importance in clearing exogenous chemical stimuli from this region. Supported by NSf Grant BNS 8908340 and Univ. of Fla Interdisciplinary Center for Biotech. Research.

49.15

INDIRECT EVIDENCE FOR A CALMODULIN REGULATED CALCIUM PUMP IN PARAMECIUM CHEMOSENSORY TRANSDUCTION. J. L. Van Houten, N. Elwess, M. V. Wright, C. Lamoureux, M. Frantz. Univ. of Vermont, Dept. Zoology, Burlington, VT 05405, USA.

There appear to be two chemosensory transduction pathways in *Paramecium*. One involves the possible coupling of receptors to a Ca-ATPase pump that generates a hyperpolarizing current. Hyperpolarization, in turn, changes ciliary beat, behavior of the cells, and accumulation in attractant stimuli. Our evidence for the involvement of this pump in chemosensory transduction is indirect and includes: the correlated effects of lithium on both chemoresponse and calcium efflux from whole cells (both are inhibited) and the failure of a calcium homeostasis mutant (courtesy of T. Evans and D. Nelson) to show normal chemoresponse. We have been able only indirectly to demonstrate calmodulin regulation of the putative Ca-ATPase pump activity through calmodulin inhibitors and calmodulin overlays of blots. Therefore, in order to study the role of the calcium pump further and to gather more indirect evidence for the regulation of the Ca-pump by calmodulin, we turned to mutants that harbor alterations in the gene for calmodulin and consequently have different conductances affected by their altered calmodulin molecules (Kink et al., Cell 62: 165-174, 1990). These mutants present an unusual opportunity to study a variety of changes in calmodulin, some of which may affect the activity of the plasma membrane Ca-ATPase. Two mutants show abnormal efflux of ^{45}Ca from preloaded cells and altered Ca-ATPase activities, consistent with the interpretation that their calmodulins regulate the Ca-pump abnormally. Additionally, these mutants have abnormal responses to chemical stimuli, reinforcing the notion that the calmodulin-regulated pump plays a role in chemosensory transduction. (NSF, NIH, VRCC)

49.16

CHARACTERIZATION AND DETERGENT-SOLUBILIZATION OF THE PUTATIVE HYDRA* GLUTATHIONE CHEMORECEPTOR. S.L. Bellis, G. Kass-Simon and D.E. Rhoads*. Univ. of R.I., Kingston, RI 02881.

Feeding behavior in the coelenterate, Hydra, is initiated by the association of glutathione (GSH) with an external chemoreceptor. In the present study, the binding of ^{35}S-GSH to Hydra membranes has been characterized. Seventy-one percent of the specific binding was eliminated by treating membranes with inhibitors of the GSH-metabolizing enzyme, γ-glutamyl transpeptidase. The remaining 29% of the specific binding demonstrated all of the characteristics expected of a ligand-receptor interaction. The binding was rapid, reversible and saturable. A Scatchard analysis of saturation isotherms indicated a dissociation constant (K_D) of 3.0 μM, a value which is a good agreement with concentrations of glutathione which are known to induce feeding behavior. The detergent, CHAPS, was used to solubilize the membrane preparation, resulting in release into the soluble fraction of 45% of membrane proteins and 40% of the saturable, reversible GSH binding activity. The K_D for GSH binding to the solubilized preparation was estimated as 2.7 μM, a value which is not appreciably different from the K_D for GSH-binding to intact membranes. The fidelity of GSH-binding in the solubilized preparation suggests that this preparation will be useful in further characterization of the receptor.

CIRCUITRY AND PATTERN GENERATION I

50.1

PROPERTIES OF PROPRIOSPINAL NEURONS INVOLVED IN THE RHYTHMIC EXCITATION OF MOTOR POOLS IN THE ISOLATED EMBRYONIC CHICK SPINAL CORD. Stephen Ho and Michael O'Donovan. Laboratory of Neural Control, NIH, Bethesda, MD 20892.

Previous studies of rhythmic motor activity in the isolated lumbosacral cord of the chick embryo have shown that motoneurons in different segments discharge synchronously at the onset of each cycle. The aim of this study was to identify the mechanisms that coordinate motoneurons during rhythmic activity.

After cutting the ventrolateral white matter (LW) tracts, the discharge of motoneurons caudal to the lesion was delayed when compared to the contralateral control. Caudal motoneurons were no longer active when a Co^{++} gel was applied over the lesion site to block synaptic transmission in the remaining grey matter. The Co^{++} gel alone did not affect the timing of rostral and caudal motor activity if the LW tracts between them were left intact. A brief burst of discharge at the onset of each cycle and rhythmic electrotonic potentials in synchrony with motoneuron deplorization could also be recorded from the LW axons across a Co^{++} gel. These findings suggest that the tight coupling between rostral and caudal motoneurons is mediated by the LW axons.

Retrograde labelling at LS5 was used to locate the cell bodies of the axons traveling in the LW tracts and revealed a column of cells located dorsomedial to the motor column extending from the injection site to LS1. Electrical stimulation of the LW axons produced a powerful synchronized discharge in motoenurons, especially in the extensors. Whole cell voltage clamp recordings in motoneurons and other spinal neurons showed that the synaptic currents arising from LW tract stimulation were facilitated during episodes of motor activity. This suggests the last order interneurons responsible for the LW tract evoked synaptic currents are also driven by the central pattern generator, or alternatively, the synaptic efficacy of these propriospinal neurons is modulated during rhythmic activity.

50.2

REAL-TIME IMAGING OF IDENTIFIED PROPRIOSPINAL NEURON ACTIVITY FOLLOWING BATH APPLICATION OF FURA-2AM AND MICROINJECTION OF FURA2-DEXTRAN INTO FIBER TRACTS. Michael O'Donovan, Stephen Ho, Wayne Yee & Miklos Antal. Laboratory of Neural Control, NINDS,NIH,Bethesda,MD 20892.

Real-time Fura-2 imaging during motor activity in the isolated chick spinal cord has revealed a high proportion of rhythmically active cells in and around the lateral motor column (LMC). The goal of the present experiments was to identify these cells and to characterize their behavior in more detail. Retrograde labelling was used to show that many of these cells are propriospinal neurons that project their axons in the ventro-lateral white matter (LW) over several segments. Propriospinal neurons could be imaged in Fura-2AM loaded spinal cords by high frequency stimulation of LW in the presence of excitatory amino acid antagonists, and were found dorsal to the LMC. Neurons in this region can receive a powerful synaptic excitation from the LW and exhibit synchronized fluorescence oscillations during rhythmic motor activity. These optical findings were corroborated by imaging the activity of propriospinal neurons that had been selectively loaded with Fura2-Dextran by microinjection into LW at LS4-LS5. This procedure results in an optically active population of labelled neurons in a rostrocaudal column similar in distribution to that found with retrograde labelling techniques. Whole cell patch recording from ventrolateral interneurons located outside the LMC revealed their depolarizing synaptic drive was in phase with that of motoneurons, confirming the optical findings.

Our findings support the hypothesis that propriospinal neurons may play an important role in the origin or coordination of motoneuron activity in the developing spinal cord.

50.3

PHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERIZATION OF RHYTHMIC EXCITATORY AND INHIBITORY INPUTS ONTO MOTONEURONS IN THE CHICK EMBRYO SPINAL CORD. Evelyne Sernagor and Michael J. O'Donovan. Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Whole-cell patch clamp recordings from identified motoneurons (MN's) in the isolated chick embryo spinal cord (E7-E12) have revealed rhythmic synaptic currents during motor activity. From E10 both sartorius (flexor) and femorotibialis (extensor) MN's express a common sustained excitatory drive and firing in sartorius MN's is modulated by a more transient inhibitory input. At E7-E8 the predominant current expressed in sartorius MN's is inhibitory. The pharmacological nature of these inhibitory and excitatory currents has been investigated either with pressure ejections of antagonists on the ventral surface of the spinal cord (1 or 10 mM) or with injections of these drugs in different segments of the cord, in the vicinity of the lateral motor column (100 μM, 1 or 10 mM) while recording from the muscle nerves. This experimental approach allowed us to study the effects of the drugs without blocking the motor rhythm. The sartorius inhibition was completely blocked by the $GABA_A$ antagonists bicuculline and picrotoxin and also by curare, a cholinergic nicotinic blocker. The inhibition was blocked to a lesser extent by strychnine, a glycinergic antagonist, by atropine, a cholinergic muscarinic blocker and also by the NMDA antagonists AP5, 7Clkynurenic acid and kynurenate, but not by pancuronium bromide, a specific nicotinic antagonist. On some occasions kynurenate also reduced the sartorius excitation. However, it was possible to reduce the sartorius excitation significantly with 2,4DHPAA, a specific glutamate antagonist. The duration of activity in femorotibialis MN's was significantly reduced by AP5 and the activity was almost abolished by kynurenate. These results suggest that (1) the inhibition expressed in sartorius MN's during motor activity is mediated by the effect of GABA on $GABA_A$ receptors. The effects of curare, atropine and strychnine may result from a non-specific blockade of these receptors, while the action of AP5, kynurenate and 7Clkyn suggest that these drugs block the excitation of the GABAergic interneurons; (2) the excitatory inputs onto femorotibialis and sartorius MN's during motor activity is mediated by the release of glutamate.

50.4

THE DEVELOPMENT AND VISUALIZATION OF MOTOR ACTIVITY IN THE ISOLATED BRAINSTEM/SPINAL CORD PREPARATION OF THE CHICK EMBRYO. G.N. Sholomenko and M.J. O'Donovan. Laboratory of Neural Control, NINDS, NIH, Bethesda, MD.

The isolated brainstem/spinal cord preparation (iBST/SC) was used to study the development of descending pathways involved in the regulation of motor activity in the chick embryo. The cooled embryo (E5-E14) was removed either from the egg or from the dish in which a shell-less embryo (*ex-ovo*) was incubated. The embryo was decerebrated and placed in a dissecting dish containing recirculating oxygenated Tyrode's solution. The brainstem/spinal cord was removed from the surrounding tissue and fore- and hindlimb muscle nerves or ventral roots were dissected for recording motor activity. The iBST/SC was then placed in a double partition bath which allowed isolation of the brainstem, cervical and lumbosacral cord into independently perfused chambers. In some experiments, the fluorescent tracer dextran tetramethylrhodamine (RH) was injected into the cervical (iBST/SC) or lumbosacral (*ex ovo*) spinal cord to retrogradely label cells of origin of supraspinal pathways. In preliminary studies, the calcium indicator Fura-2AM (Fura) was bath applied to examine the activity of cells on the cut face of the brainstem. Our results indicate that: **1)** in E6-14 embryos, the iBST/SC produced spontaneous bouts of alternating activity in flexor and extensor muscle nerves indistinguishable from that seen in the isolated spinal cord preparation. **2)** electrical stimulation of relatively discrete regions of the brainstem in E6-14 embryos consistently evoked episodes of rhythmic neural activity closely resembling spontaneous activity, but no activity could be elicited in the E5 preparation. **3)** the addition of a 0 calcium/ high magnesium solution to the middle bath (cervical cord) did not abolish brainstem stimulation-evoked rhythmic activity recorded from hindlimb muscle nerves. **4)** the cell bodies, axons and dendrites of neurons retrogradely labelled with RH injected into the cervical and lumbosacral spinal cord could be visualized *in vitro* on the cut face of the brainstem. **5)** Fura-labelled neurons on the cut brainstem face oscillated synchronously with the lumbosacral motor activity.

These results suggest that in the chick embryo, supraspinal pathways make functional connections with the lumbosacral spinal cord as early as E6. Further, by combining retrograde tracing and optical methods, the means now appear available to examine the relationship of individual brainstem neurons to the central pattern generator for locomotion present in the spinal cord.

50.5

FICTIVE SPINAL MOTOR OUTPUT ELICITED BY STIMULATION OF THE MIDBRAIN OF GOLDFISH. J.R. Fetcho. Dept. Neurobiology and Behavior. SUNY at Stony Brook, NY 11794.

Kashin et al., (1974) stimulated the midbrain tegmentum in teleost fish and produced rhythmic axial EMGs and tail movements. I have confirmed their observations in goldfish and extended their techniques to produce fictive motor output. Rhythmic alternating movements of the tail, similar to swimming, were elicited in nine decerebrate goldfish by midbrain stimulation (50-100Hz square pulses, 0.2 msec duration, 50-100μA) at sites along the midline just rostral to the cerebellum and about 2 mm deep. After locating optimal sites for producing these movements, I paralyzed the fish and recorded extracellularly from ventral roots or branches of roots from the trunk and tail. During midbrain stimulation, motor axons in the nerves were active in repetitive bursts. The frequency of bursts varied with stimulus strength and among fish, ranging from 1.5 to 12.5 Hz. The duration of bursts was correlated with cycle time, with longer bursts associated with longer cycles. The bursts occupied a mean of 0.45 of the cycle times (n = 24; s.d.= 0.086). Bursts from nerves on opposite sides of the same segment alternated, with one side active when the other was silent. Thus, as in other vertebrates, the CNS in teleosts can produce rhythmic motor output- in this case with some similarities to swimming- in the absence of movement. This fictive preparation will allow intracellular studies of the circuitry for comparison with the spinal networks for other motor behaviors such as escape. Supported by NIH NS26539.

50.6

Multiple electrode recordings along the isolated lamprey spinal cord during fictive swimming reveal strong coupling among segmental oscillators. N.M. Mellen[1*], T. Kiemel[2*], and A.H. Cohen[3]. [3]Department of Zoology, University of Maryland, College Park, MD. 20742, [1]Department of Psychology, Cornell University, Ithaca, NY 14853, [2]Mathematical Research Branch, NIDDK, NIH, Bethesda, MD. 20892.

A method of positioning 16 electrodes ca. 5mm apart along the isolated spinal cord of the lamprey has been developed. This technique allows for the investigation of temporal regulation of drug-induced (D-glutamate) fictive swimming at a higher spatial and temporal resolution than previously possible. Through the use of the these methods we observed that during stable fictive swimming, the correlations between phase delays measured one or more cycles apart are small, suggesting that the coordinating system is sufficiently strong to damp out intrinsic perturbations within one cycle of activity. In addition, data obtained with the new method reveals that stable phase delays can also be preserved in the absence of stable periodicity even between segments separated by some distance. Taken together, these data suggest that both long and short coordinating neurons provide strong coupling among the spinal segments.
Supported by MH44809 to AHC.

50.7

INTEGRATIVE PATTERNS OF DORSAL NECK AND HINDLIMB MUSCLE ACTIVITIES DURING THE SCRATCH CYCLE. P. Carlson Kuhta and J.L. Smith. Neuromotor Control Lab., Dept. Kinesiology, UCLA, Los Angeles, CA 90024-1568.

The normal cat scratch response consists of cyclic hindlimb motions at an average frequency of 5-7 cycles/s, with each cycle containing a contact phase that occupies about 50% of the cycle period. The paw accurately contacts the stimulated area of the head suggesting a coordination between the hindlimb and neck muscles to correctly position the paw and head. To gain insight into this coordination, we implanted wire electrodes in four dorsal neck muscles, bilaterally (i, ipsilateral; c, contralateral): biventer cervicis (BC), splenius (SP), complexus (CM), and obliquus capitis caudalis (OC). In addition three hindlimb muscles, medial gastrocnemius (MG), soleus (SOL), and tibialis anterior (TA) were implanted, and ankle extensor, tendon-forces were measured with a buckle force transducer. Scratches were elicited by ear stimulation and EMG & force measurements were synchronized with 100 fr/s ciné film; for further methods see Carlson Kuhta and Smith (*J. Neurophysiol.*, 64:1653-1667, 1990).

Of the neck muscles, the iSP, iBC, and cOC were phasically active most often during the cyclic period of scratching, while the other neck muscles usually had tonic low-level activities. The iSP was active during the contact phase with onset occurring after the onset of paw contact and iSP offset occurring at or just after peak tendon-force. Also, bursting in iSP appeared to occur only after a threshold tendon-force level (~7N) was reached. The iBC was also coactive paw contact but onset during the precontact phase and offset near peak tendon-force. The cOC bursts were brief and occurred during the postcontact phase.

Our preliminary results suggest that several neck muscles help coordinate head movement with the paw. The iBC appears to anticipate paw contact, while the iSP appears to compensate for paw contact, possibly via proprioceptive or cutaneous feedback. Activity in both iBC and iSP would tend to reduce head motion evoked by paw contact and hindlimb extension. The cOC, a neck rotator, would tend to move the pinna away from the paw and may thus help terminate the contact phase. Funded by NIH NS19864.

50.8

DISCHARGE DYNAMICS OF RED NUCLEUS NEURONS IN RESPONSE TO SPINAL CORD STIMULATION IN THE *IN VITRO* TURTLE BRAIN. R.R. Carter, J.B. Andersen*, S. Hansen*, and J.C. Houk Department of Physiology, Northwestern University School of Medicine, Chicago, IL 60611.

In several species, red nucleus neurons have demonstrated action potential discharge patterns which are linked temporally with several parameters of limb movements. These discharge patterns are postulated to be the result of reverberating activity within a positive feedback loop formed by the red nucleus, cerebellar nuclei, and other brainstem nuclei. Loop activity is thought to be sculpted into motor programs by the inhibitory action of Purkinje cells located in the cerebellar cortex. We have employed an *in vitro* preparation from the turtle (Keifer and Houk, *Neurosci. Lett.*, **97**: 123-128, 1989) in which the cerebellum, brainstem and spinal cord are maintained intact to study the dynamic characteristics of these loop generated motor commands. Electrical stimulation of the spinal cord by single pulses or brief trains was used to simulate a sensory input to the cerebellorubrospinal loop and the evoked discharge pattern of single red nucleus units was recorded extracellularly. Our results show that the discharge patterns were repeatable given identical stimuli. This suggests that the response does not depend appreciably on initial loop conditions which could be a function of highly variable spontaneous Purkinje cell activity. The magnitude of the discharge (average firing rate over the 1 s period following the stimulus) was graded at low levels of stimulus amplitude and saturated for higher levels of stimulus amplitude. The stimulus typically drove a unit to a higher firing rate from which it began to decline immediately to its resting rate in a manner well described by a two-exponential model. We propose a two-neuron reciprocal neural network model of the cerebellorubral circuit based on these results.

50.9

ORGANIZATION OF THE PRE-ECDYSIS MOTOR PATTERN IN *MANDUCA SEXTA*. A. Novicki and J.C. Weeks. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Pre-ecdysis behavior serves to loosen the old cuticle before it is shed at ecdysis. The larval pre-ecdysis motor pattern includes rhythmic bursts in two motor neurons, MN-2 and MN-3, which are synchronous in all abdominal ganglia and on the left and right body sides. The organization of the motor pattern in the isolated abdominal nerve cord was investigated by cutting the connectives or by using low calcium saline to block synaptic transmission in single ganglia. The working hypothesis resulting from these experiments is that the pre-ecdysis motor pattern is coordinated and maintained along the nerve cord by a bilateral ascending projection from the terminal ganglion. This projection appears to ascend the nerve cord without interposed chemical relays, and may synapse directly on MN-2 and MN-3, as well as acting through premotor interneurons. Previous work [*J. Comp. Physiol.* (1991) **168**:179] has shown that the larval pre-ecdysis motor pattern is severely attenuated at larval-pupal pre-ecdysis, and this model of the organization of the pre-ecdysis motor pattern will be useful for investigating the sites of developmental plasticity of this behavior.

Supported by NIH and NSF.

50.10

Chaos in the Lobster Stomatogastric Network

Norman Herterich and Al Selverston, *UCSD*

The pyloric system normally produces simple "1:1" rhythms; i.e., each neuron produces one burst of activity for each pacemaker burst, and successive bursts in each cell are similar. Under various experimental paradigms, dynamic complexity is observed as a consequence of network interactions. Increasing the strength of LP's synapse onto the PDs slightly induces 2:2 rhythms (i.e. successive bursts differ, but a repeating cycle consisting of 2 PD and 2 LP bursts is observed); 1:2, 1:3, and 2:3 rhythms are frequently observed at greater strengths. Plots of pacemaker burst period versus synaptic strength reveal bifurcations similar to those seen in transitions to chaos. For the greatest synaptic strengths, the period return diagram, though complex, exhibits structure which may be indicative of chaotic dynamics. Additionally, 2:1 oscillations are often observed in the IC neuron when VD is killed, due to sequential phasic inhibition from the pacemakers and the PY neurons.

50.11

JAW MOVEMENTS AND MUSCLE (EMG) ACTIVITY DURING DRINKING IN THE PIGEON. R. Bermejo, M. Remy and H. P. Zeigler. Biopsychology Program, Hunter College (CUNY), New York, NY 10021

In birds, variations in gape during ingestive behavior are produced by the coordinated movements of the two beaks and the jaw muscles are innervated by both the trigeminal and the facial cranial nerves. To relate these movements to neuromotor mechanisms, the displacement of each beak was monitored during drinking, while recording electromyographic activity in representative jaw and tongue muscles.

Individual drinking cycles begin with a rapid closing movement of the lower beak, followed, first, by elevation of the upper beak and then by depression (opening) of the lower beak. Beak movements during drinking are correlated with nearly synchronous activity in the protractor (opener) of the upper jaw and several jaw closer muscles as well as with alternating activity in tongue protractor and retractor muscles, suggesting that opening movements of the lower beak are passively produced and related to concurrent tongue movements. The results represent a first step in relating the generation of cyclic drinking movements to activity in specific motoneuronal pools.

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50.12

MOTOR NEURON FEEDBACK TO THE VENTILATORY CENTRAL PATTERN GENERATOR IN THE CRAB. R. A. DiCaprio. Dept. of Zoological & Biomedical Sciences, Ohio University, Athens, OH 45701.

Previous studies have demonstrated that motor neurons as well as interneurons are involved in the generation of the ventilatory motor pattern in Crustacea. This conclusion is based on the observation that perturbation of the membrane potential of individual motor neurons with intracellular current injection can start, stop and reset the ventilatory motor output and also modulate the frequency of the ventilatory rhythm (Simmers & Bush, J. Comp. Physiol., 150:1-21, 1983; DiCaprio & Fourtner, J. Comp. Physiol., 155:397-405, 1984).

To further define the role of motor neuron feedback in the organization of this system, single motor neurons were injected with the fluorescent dye Lucifer Yellow and then killed by photoinactivation (Miller & Selverston, Science, 206:702-704, 1979). Analysis of the motor pattern before and after photoinactivation indicates that the basic structure of the ventilatory motor pattern is unaltered, but that some parameters of the motor pattern such as burst duration, burst phase and intraburst frequency are altered. These results support the conclusion that a functional role of motor neuron feedback is to fine-tune the temporal pattern of the ventilatory rhythm. Supported by NIH grant NS25002 and the Whitehall Foundation.

50.13

Effects of serotonin on NMDA, kainate, and sensory induced locomotor activity in the lamprey. J. L. Schotland, T. Matsushima, and S. Grillner, Nobel Institute for Neurophysiology, Karolinska Institute, Stockholm, Sweden.

The isolated lamprey spinal cord can generate a pattern of neural activity that closely resembles swimming, consisting of reciprocally organized bursts of activity with a rostro-caudal intersegmental phase lag. By changing the excitability of the locomotor circuitry, a physiological frequency range may be covered (0.2-10 Hz). Low frequencies may be elicited by applying NMDA to the bath whereas higher frequencies are induced by kainate. The overall activity is determined by the segment with the highest frequency and the phase lag by the frequency differences between this segment and other segmental oscillators (Matsushima and Grillner 1990). 5-HT has pronounced effects on the locomotor network. While a great deal is known about the morphology, ultrastructure, and cellular mechanisms of action of the 5-HT system, little is known of the physiological significance of this ubiquitous transmitter. Previous studies of 5-HT locomotor effects have been performed on locomotor activity elicited by bath application of excitatory amino acids. It was therefore important to test the effects during a physiological activation of the network by using sensory stimuli (Brodin and Grillner 1985). 5-HT could elicit the same type of effects under these conditions. In a spinal split bath preparation, 5-HT (0.1-1 uM) or the 5-HT reuptake blocker, citalopram (10 uM) applied to the rostral half of the cord reversed the intersegmental phase lag in NMDA-mediated locomotor activity. This action is not mediated by altering intersegmental synaptic strength, since EPSP amplitudes were unchanged following 5-HT. The 5-HT induced reduction in the after hyperpolarization may account for these effects. The relative contribution of 5-HT effects on synaptic versus plateau potentials was evaluated by applying 5-HT (.2-.5 uM) to kainate-induced (10-25 uM) fictive swim in which NMDA receptor mediated effects were blocked (50 uM APV). In contrast to 5-HT effects when NMDA receptors were not blocked, the pattern of swimming could be severely disrupted. Often two frequencies of activity were present; burst frequency could increase on one side (e.g. 5 Hz) without contralateral burst activity, while a slow alternation (e.g. 0.01 Hz) was induced between the two sides.

50.14

MECHANISMS UNDERLYING RHYTHM INITIATION AND GENERATION BY NEUROMODULATORS OF THE GASTRIC MILL NETWORK IN LOBSTERS. R.C. Elson* and A.I.Selverston. Department of Biology, UCSD, La Jolla, CA 92093-0322.

Central generation of gastric mill motor rhythms by the lobster stomatogastric ganglion (STG) depends on modulatory inputs from higher centers. When isolated from these inputs the gastric central pattern generator becomes non-rhythmic. We studied mechanisms by which specific modulators (proctolin, muscarinic agonist) initiate gastric rhythms from the quiescent network in the isolated STG. Rhythm initiation is correlated with induction of bursting pacemaker potential properties in particular neurons. Induction of such non-linear membrane properties enables individual cells to burst endogenously and specific subcircuits of neurons to generate patterned rhythmic activity. Gastric rhythmicity may arise from several loci within the network. Burst oscillations are coordinated by graded synaptic transmission and slow or biphasic postsynaptic potentials. Supported by NIH grants 09322 and P01NS25916.

50.15

ARTIFICIALLY COUPLED STOMATOGASTRIC NEURONS IN PRIMARY CELL CULTURE. Andrew A. Sharp* and Eve Marder. Biology Department, Brandeis University, Waltham MA 02254.

Our goal is to determine how the connections among cells influence the functioning of individual neurons and the networks that they comprise. In ganglia, the complexity of the interconnections makes it difficult to determine what effect any of the connections has on the network. We have devised techniques which allow us to artificially couple physically isolated neurons which may be located next to each other in the same dish or in separate chambers. To mimic electrical coupling, the potential from two cells is compared and equal and opposite currents are injected into the cells based on the magnitude of the intercellular potential difference. To mimic chemical synapses, iontophoretic application of transmitter is controlled by the membrane potential of the presynaptic neuron.

One of our interests is how electrical coupling affects the frequency and waveform of coupled oscillators. Computer modeling has indicated that when an oscillatory cell is electrically coupled to a more hyperpolarized cell it slows down if the oscillation is dominated by the inward current phase and speeds up if dominated by the outward current phase (Kepler et al.,1990,Science,248:83). When neurons from the stomatogastric ganglion of the crab Cancer borealis are dissociated and placed into primary cell culture, many behave as conditional oscillators. We are now studying artificially coupled pairs of neurons to determine how oscillators with various intrinsic properties and waveforms are modulated by oscillatory or non-oscillatory neurons. Supported by NSF BNS 9009251.

50.16

EFFECTS OF LOCAL OSCILLATOR FREQUENCY ON INTERSEGMENTAL COORDINATION IN THE LAMPREY LOCOMOTORY CPG: THEORY AND EXPERIMENT. K.A. Sigvardt, N. Kopell*, G.B. Ermentrout* and M.P. Remler. Neurology, University of California-Davis, VAMC, Martinez CA 94553; Mathematics, Boston University, Boston MA 02215; Mathematics, University of Pittsburgh, Pittsburgh PA 15260.

The mathematical analysis of chains of coupled oscillators developed by Kopell and Ermentrout (SIAM J. Appl. Math. 50:1014, 1990) makes predictions about the effects upon intersegmental phase lags of varying the intrinsic frequency of the oscillators within the chain. In a series of experiments on intersegmental coordination in the in vitro lamprey spinal cord, intersegmental phase lags were examined following changes in the intrinsic frequency of the local segmental oscillators by changing the concentration of excitatory amino acid bathing the rostral half of the spinal cord relative to the concentration bathing the caudal half. In experiments where the concentration of D-glutamate was higher in the caudal compartment than in the rostral compartment, the intersegmental phase lags in the caudal half of the spinal cord remained the same as controls whereas the phase lags in the rostral half of the cord decreased. Within the context of coupled oscillator theory, this result indicates that in the lamprey spinal cord ascending intersegmental coupling is dominant over descending coupling and determines the intersegmental phase lags.

50.17

A NEUROPEPTIDE EXERTS ITS EFFECTS ON THE LOBSTER CARDIAC SAC MOTOR PATTERN AT MULTIPLE SITES. P. S. Dickinson and J. Hetling*, Dept. of Biology, Bowdoin College, Brunswick, ME 04011

The foregut of decapod crustaceans is controlled by four semi-discrete motor networks, each of which generates characteristic patterns of rhythmic activity. We have previously shown that the neuropeptide Red Pigment Concentrating Hormone (RPCH) activates one of these networks, the cardiac sac network, in the spiny lobster, *Panulirus interruptus* (Dickinson and Marder, J. Neurophysiol. 61: 833-844, 1989). We now show that RPCH exerts its effects directly on one element of the cardiac sac motor pattern generator, the inferior ventricular nerve (*ivn*) fibers. The *ivn* fibers pass through two ganglia, the oesophageal ganglion and the stomatogastric ganglion. When synapses in either ganglion are blocked and RPCH is applied to that ganglion, rhythmic activity in the *ivn* fibers is induced, even though neither ganglion contains the *ivn* cell bodies. In addition to inducing rhythmic activity in the *ivn* fibers and thus in the cardiac sac network, RPCH causes the post-synaptic potentials from the *ivn* fibers to increase significantly in amplitude. This increase is mediated partly through facilitation and partly through a direct effect of RPCH on the terminals of the *ivn* fibers in both the oesophageal and the stomatogastric ganglia. Supported by NSF BNS9008816.

50.18

POCKET SCRATCH, CAUDAL SCRATCH AND FLEXION REFLEX MOTOR PATTERNS PRODUCED BY THE *IN VITRO* TURTLE SPINAL CORD. Scott N. Currie and Paul S.G. Stein, Department of Biology, Washington University, St. Louis, MO 63130.

The turtle spinal cord responds to tactile stimulation within specific areas on the body surface by generating either a flexion reflex or one of three forms of the scratch reflex in hindlimb motor neurons (Stein, Ann. NY Acad. Sci. 563:1, '89). The turtle CNS is ideally suited for *in vitro* studies because it is extremely resistant to anoxia (Chan and Nicholson, J. Physiol. 371:89, '86); previous work demonstrated that an *in vitro* turtle spinal cord can generate the rostral form of the scratch reflex (Keifer and Stein, Brain Res. 266:148, '83). We have extended this work by developing an *in vitro* spinal cord-peripheral nerve preparation that expresses pocket scratch, caudal scratch and flexion reflex motor patterns. The spinal cord hindlimb enlargement and several adjacent spinal segments are exposed by dorsal laminectomy, removed along with associated peripheral nerves, and placed in flowing, oxygenated turtle saline. We record motor output from selected hindlimb muscle nerves and electrically stimulate specific nerves containing cutaneous afferents to elicit the pocket or caudal scratch. Stimulation of specific dorsal roots evokes the flexion reflex. Preparations continue to express robust motor responses for up to several days *in vitro*. These motor patterns display a prolonged excitation after brief sensory input similar to that previously demonstrated *in vivo* (Currie and Stein, J. Neurophysiol. 60:2122, '88 and 64:1134, '90). The technical advantages provided by the *in vitro* preparation will assist future studies aimed at revealing the cellular mechanisms responsible for the selection and generation of these motor patterns. Supported by NSF Grant BNS-8908144 to PSGS.

50.19

BILATERAL ACTIVATION OF INTERNEURONS DURING FICTIVE SCRATCHING IN SPINAL TURTLES. A. Berkowitz and P.S.G. Stein, Dept. of Biology, Washington University, St. Louis, MO 63130.

The turtle spinal cord contains sufficient neural circuitry to generate an appropriate scratch reflex in response to cutaneous stimulation (Ann. NY Acad. Sci. 563:1, 1989). In a low-spinal, immobilized turtle, many spinal interneurons with axons descending in the hindlimb enlargement are activated during all fictive scratching evoked by ipsilateral stimulation (Abstr. Soc. Neurosci. 16:1091, 1990). We extended investigation of such neurons, using ipsilateral or contralateral stimulation and bilateral motor nerve recording.

Units were recorded with a microsuction electrode from descending axons in the white matter in the caudal face of a multisegmental spinal cord preparation (ibid.). Many interneurons were activated during either ipsilateral or contralateral stimulation. Others were activated by stimulation of one side only; of these, some were inhibited by stimulation of the opposite side. Some units were tonically activated during all fictive scratching. Many others displayed rhythmically modulated firing in phase with motor output. The strength of modulation varied, with the activity of some units restricted to bursts during a particular phase of fictive scratching. For many units, when ipsilateral stimulation evoked unit activity in phase with a particular ipsilateral motor pool, contralateral stimulation evoked activity out of phase with the homologous contralateral motor pool.

This work suggests that a large percentage of cutaneous-activated spinal interneurons with a descending axon in the hindlimb enlargement either participate in scratch motor pattern generation or receive feedback from scratch pattern generators during stimulation of either side. Supported by NSF Grant BNS-8908144 to PSGS.

50.20

DEVELOPMENT OF MOTOR PATTERNS IN NEUROLOGICAL MUTANT MICE. V.J. Bolivar*, E.M. Coscia*, W. Danilchuk*, J.C. Fentress, and K. Manley*. Department of Psychology, Dalhousie University, Halifax, N.S., Canada, B3H 4J1.

Neurological mutant mice have been employed with success in a number of cellular and circuit level analyses. However, few systematic behavioral studies of these animals exist. We examined the ontogeny of natural movement sequences of swimming and grooming in four strains of neurological mutant mice: jimpy (jp), reeler (rl), staggerer (sg), and weaver (wv). Double recessive and littermate controls were video-taped at 2 day intervals from the 3rd to the 21st postnatal day. Primary tests included both supported and free swimming in 38±2 C water for 10-30 s. and subsequent self-grooming in a heated chamber. We report data that have been examined quantitatively with a video/computer-based motion analysis system (Peak), capable of generating stick figures, from which limb trajectories, accelerations, velocities and angles of movement were calculated. Myelin (jp), cortical laminar (rl), cerebellar (sg), and striatal (wv) disorders in these mice are differentially expressed in movement.

CIRCUITRY AND PATTERN GENERATION: MODELS

51.1

MODEL OF LOBSTER GASTRIC MILL CPG CONSTRUCTED FROM RELAXATION OSCILLATORS. P.F.Rowat and A.I.Selverston Biology 0322, U.C. San Diego, La Jolla, CA 92093-0322.

We use a one-compartment cell model with one fast current, one slow current, and two parameters. One parameter controls whether the fast current has a region of negative resistance, while the other sets the amplitude and gain of the slow current. By appropriately setting the parameters, the model cell may exhibit plateau potentials, post-inhibitory rebound (PIR), or endogenous oscillations. Synapses were modeled by a post-synaptic current calculated as the product of a post-synaptic conductance varying with the presynaptic potential, and a driving potential given as the difference between the post-synaptic potential and the synaptic reversal potential. A network with all known gastric connections was constructed. Very wide ranges of parameter values are sufficient for the model to produce approximately correct patterns (ACP). The model is deterministic but exhibits bounded chaos similar to the biological variability. When parameters that control the network oscillation frequency were adjusted, so that network frequency varied by an order of magnitude, ACP was still obtained. When parameters were set so that each cell, if isolated, had a frequency randomly assigned in a broad band ($T \pm 10\%$), and the network connections then re-introduced, the cells quickly entrained to a common frequency with ACP. It is not necessary for any individual cell to be an oscillator. Provided each isolated cell has PIR, the fully connected network oscillates with ACP. The cell model is a latent, generalized, relaxation oscillator. The effect of connections is to convert cells that are quiescent in isolation into an ensemble of relaxation oscillators, with chaotic variability. It is proposed that the pattern generating properties of the gastric mill should be understood in terms of the entrainment and phase-locking properties of networks of relaxation oscillators. Supported by ONR N00014-88-K-0328.

51.2

THE LAMPREY LOCOMOTOR CPG AS A CHAIN OF COUPLED OSCILLATORS: ANALYSIS OF NETWORK SIMULATIONS. Thelma L. Williams. Dept. of Physiology, St.George's HMS, London, SW17 ORE, U.K.

Experiments on the lamprey spinal cord, interpreted in light of the behaviour of coupled nonlinear oscillators, have revealed properties of intersegmental coupling (Williams et al,1990: J.Neurophysiol.64,862; Kopell & Ermentrout,1988: Math.Biosci.90,87). In such analysis, coupling is mediated by "H-functions", which describe the effects of neighbouring oscillators upon each other. This approach will be illustrated by experiments on simulated chains of coupled oscillators. The unit oscillators have the structure proposed by Buchanan & Grillner (1987:Science 236,312), and the intersegmental connections are between cells in neighbouring oscillators (Buchanan,1990:Eur.J.Neurosci.Supp. 3,184). The H-functions for various coupling combinations have been determined, and the resulting behaviour of the oscillator chain has been shown to obey the predictions of the mathematical theory. In particular, uniform phase lags can be produced by any of a number of coupling mechanisms, without the need of extra excitation at the rostral end of the spinal cord.

51.3

NEURAL NETWORK SIMULATIONS OF FICTIVE LOCOMOTION. J.T. Davis and L.E. Moore. Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Fictive locomotion is defined as the response pattern of spinal cord neurons that occurs during the application of exogenous excitatory amino acids. This pattern consists of alternating individual activities of neurons on either side of the cord leading to oscillatory outputs of action potential bursts in the ventral roots supplying the musculature, similar to that of normal swimming in the intact animal.

Neural network simulations of a single spinal cord segment were made for specific conditions of fictive locomotion, namely, those induced by different concentrations of excitatory amino acid agonists and antagonists. The specific measures of the network consist of records of (1) intracellular potentials of the neurons involved in the pattern generation and (2) the bursting discharge of motoneurons. Under these conditions both the frequency and waveforms of the intracellular potentials are dependent on the relative activation of different amino acid receptors. Especially noteworthy are the relative effects of NMDA and non-NMDA receptor activation and the ensuing fictive locomotion.

Single cell voltage clamp and constant current experimental data were used to constrain the parameters used in neural network simulations of fictive locomotion. Specific cable models of neuronal units with voltage dependent membrane properties were constructed to reflect anatomical and physiological behavior. The network simulations for three classes of models are compared with the fictive lomotion data using a limited number of free parameters. The three classes of models are:

1) Passive, simple summation units with sigmoidal activation functions,
2) Hodgkin-Huxley somas with passive integrating dendrites,
3) Non-linear units based on experimental data from spinal cord neurons.

This work was supported in part by NS-11255.

51.4

PHASE RESETTING AND FIXED-DELAY STIMULATION OF A SIMPLE MODEL OF NEURAL RHYTHMOGENESIS: IMPLICATIONS FOR RESPIRATORY RHYTHM GENERATION. J.E. Lewis*, L. Glass*, M. Bachoo and C. Polosa. Dept. of Physiol., McGill Univ., Montréal, Québec, Canada, H3G 1Y6.

The aim of this study was to investigate the response of a simple three-phase neural network model to perturbation, in the context of previous experimental studies on the effects of superior laryngeal nerve (SLN) stimulation on the respiratory rhythm in cats. SLN stimuli produced a phase-dependent shortening or prolongation of the respiratory cycle. These effects were illustrated in the form of phase resetting curves (PRCs). Fixed-delay stimulation, which entails delivering stimuli at a constant delay after the onset of a cycle, was also used. Certain combinations of delay, stimulus intensity, and number of cycles between stimuli, resulted in (1) a variable, rather than consistent, response, and (2) a transient increase in cycle duration during and after stimulation. PRCs of the model were similar to those obtained experimentally. The results of fixed-delay stimulation in the model were comparable to the experiments in that variable combinations of shortened and prolonged cycles were observed. However, the increases in cycle duration during and after stimulation were not evident in the model. Thus, in the model, stimulus dependent properties with a longer time course are needed to account for the transient increases in unstimulated cycle duration observed experimentally. These comparisons show that several properties of oscillators in addition to phase resetting must be considered in the evaluation of theoretical models of rhythmogenesis. (This work was supported by M.R.C. and N.S.E.R.C. of Canada, and F.C.A.R. du Québec.)

51.5

OFF-LINE SORTING OF SPIKES USING AN ARTIFICIAL NEURAL NETWORK. I. Espinosa E. and J. Quiza T.*, Cybernetics Lab., Physics Dept., Faculty of Sciences, UNAM, México, DF 04510

We are developing a computer environment for transforming and analyzing multi-recording spike trains (SPT). The SPT can come from multi-recording experiments with multiple microelectrodes as well as from neural network simulators. This facility will be used for functional connectivity analysis in neural assemblies recorded simultaneously in the brain of the rat.

We have produced software (ENTRENADOR) that classifies noisy action potentials by means of a 3-layer neural network using the back-propagation learning algorithm (Espinosa and Quiza, Soc.Neurosci.Abstr., Vol. 16, Part 2, p.1092, 1990). Now we have improved the network's architecture and added off-line classification of action potentials embedded in a SPT. The digitized version of the SPT is fed to the program (CLASIFICADOR) that detects spikes using the Haar transform and then classifies them with the trained neural network. The program delivers a list containing the class and points of occurrence of the spikes and also produces a separate list for each class of spikes as well as special format files for cross-correlation and gravitational analyses (Lindsey et al., Brain Research 483: 373-378, 1989). The system works reasonably fast and with a low error percentage. However, it has problems with overlapping spikes. We expect to improve this and add on-line analysis and sorting of SPT. Supported by IN-206189-UNAM

51.6

BISTABLE NEURONS AND MEMORY PATTERNS IN THE INHIBITORY FEEDBACK MODEL INSPIRED BY NEOSTRIATUM. A.B. Kirillov*, C.D. Myre and D.J. Woodward. *Dept. of Cell Biology and Neuroscience, UT Southwestern, Dallas, Texas, 75235.*

We have reported previously that a three dimensional array of mutually inhibitory model neurons (inspired by the extensive inhibitory feedback network of the medium spiny neurons in the neostriatum) is capable of maintaining patterns of activity and exhibits bistable neuronal firing. The neurons either fire repeatedly (the "on" state), or do not fire at all (the "off" state). Global noise can induce spontaneous switching between states: some "on" neurons switch "off" and some "off" neurons switch "on". Further analysis of this model revealed the following features. 1) Spontaneous switches of neuron states can occur in a wide range of model parameters. In a typical switch, only two mutually inhibitory neurons interact and "swap" their states. 2) If inhibition is extended only to nearest neighbors, an artificial checkerboard pattern of "on" and "off" neurons appears. Often two alternative checkerboard patterns arise in different regions with most switching found at the border in space between these patterns. 3) When the domain of local inhibition for each neuron is increased, which makes the model more realistic, a great variety of stable patterns of "on" and "off" neurons is possible. A specific local pattern may then be set by stimulatory inputs with weighted projections, and the network can maintain the pattern over several seconds (functioning as a short-term memory register). 4) The local connection strengths can be adjusted so that the network has several patterns as "preferred" long term memory states. When a part of such a pattern is set "on" by stimulation, the network can recall the rest of the pattern if the global noise is powerful enough to allow spontaneous switches. Our hypothesis is that such an array may operate as a form of local temporary memory in a motor control system.

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51.7

METHOD FOR INCORPORATING DISCONTINUOUS ELEMENTS IN CONTINUOUS-TIME NEURONAL NETWORK MODELS. C.D. Myre and D.J. Woodward. *Dept. of Cell Biology and Neuroscience, UT Southwestern, Dallas, Texas 75235.*

A strategy has been devised for computational simulation of both continuous- and discrete-time neuronal network models. Discrete-time simulations are suitable for networks with large numbers of elements, but suffer form limited accuracy, whereas continuous-time simulations require excessive time to determine values for more than a few elements. Our approach in modeling is to replace some components (especially the "stiff", or slow-computing components) within a complex network model with discontinuous elements, so that continuous components run with stepsizes relatively independent of the stepsizes of the discontinuous components. This allows hybrid networks to be assembled using combinations of neuronal definitions. The strategy allows efficient computation of temporal properties of channel conductances, electrotonic properties of dendrites, or critical time-dependent interactions. The influence of such physiological properties can be examined within large neuronal ensembles which include many elements which are more appropriately computed at large, discrete time steps. We are employing this approach to characterize influences of detailed physiological mechanisms on memory phenomena in models inspired by neostriatum, which include interactions between mutually-inhibitory medium spiny neurons. In our model, the basal ganglia are observed to operate as a system for asynchronous accumulation of information from cortical signals, with storage and output of sets of analog values employed in motor programs.

Support from the Texas Adv. Res. Prog., Biol. Humanics Found., MH44337, DA02338, and AFOSR 90-146.

51.8

SYNAPTIC INTERACTIONS AMONG PATTERN GENERATING NEURONS IN BUCCAL GANGLIA OF *APLYSIA*. D.A. Baxter and J.H. Byrne. Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

Several neurons have been identified that are believed to be members of a central pattern generator (CPG) in the buccal ganglia. Susswein & Byrne (1988) identified cells B31/32 and B35 on the caudal surface of the buccal ganglia, and Plummer & Kirk (1990) identified cells B51 and B52 on the rostral surface. To further examine how these neurons might function as a CPG, we have characterized the synaptic interactions among B31/32, B35, B51 and B52.

Simultaneous intracellular recordings were made from either B31/32 or B35 on the caudal surface of the left buccal ganglion and from B51 and/or B52 on the rostral surface of the right buccal ganglion. Stimulation of B51 produced an IPSP in B31/32 and B35. In contrast, stimulation of B52 had no effect on the membrane potential of either B31/32 or B35. Stimulation of B31/32 produced IPSPs in B51 and a mixture of IPSPs and EPSPs in B52. Stimulation of B35 produced EPSPs in B51 and IPSPs in B52. As previously reported, we observed that B51 and B52 reciprocally inhibited each other and that B35 excited B31/32. In addition, we recorded from these neurons during patterned activity. Activity in B52 coincided with activity in B31/32, whereas activity in B51 occurred while B31/32 and B52 were inhibited.

This pattern of connectivity is only one of the factors determining how this neural circuit may function as a CPG. Additional factors, such as the intrinsic properties of the neurons, also must be considered. We are using the simulation program SNNAP (Ziv et al., this volume) to construct a model representing the properties and interconnections of these identified neurons, and to examine how this neural circuit may function as a CPG. The simulations indicate that elements of this neural circuit can produce patterned activity, but that additional elements are probably necessary to account fully for the CPG.

51.9

SIMULATOR FOR NEURAL NETWORKS AND ACTION POTENTIALS (SNNAP): APPLICATION TO A CENTRAL PATTERN GENERATOR. I. Ziv, D.A. Baxter and J.H. Byrne Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

An understanding of the causal role that individual neurons play in complex behaviors can be facilitated by computer simulations of the neural circuit that includes descriptions of both the detailed properties of each neuron and the pattern of synaptic connectivity. SNNAP was developed to achieve this goal.

The program is capable of simulating a network of up to 20 fully connected neurons. Intrinsic voltage-dependent membrane currents are described by a H-H type membrane model that may include up to 20 different ionic conductances which can be individually specified for each cell. Each conductance is described by a first order ordinary differential equation (ODE) for activation and inactivation with voltage-dependent steady-state values and time constants. Connections to other cells can be made by either electrical or chemical synapses. Chemically mediated postsynaptic conductances are simulated by the numerical solution of a second order ODE driven by a pulse equal to the duration of the presynaptic spike. Using this solution, the postsynaptic cell can respond to a change in the duration of a presynaptic spike. Moreover, PSP's from successive presynaptic spikes summate in real time. Operational features of SNNAP include callable files for each cell which include its unique membrane properties and parameters, and files of cell connectivity matrices.

We are using SNNAP to simulate a central pattern generator (CPG) in the buccal ganglia of *Aplysia* in order to understand the mechanisms that produce and modify the pattern. As a first step we simulated the intrinsic properties of cells B51 and B52 (Plummer & Kirk, 1990) including the postinhibitory rebound in B52 and regenerative activity of B51. Depolarizing both cells leads to simulated cyclic activity, but the activity is different from that of the CPG. Currently, we are incorporating additional elements, such as B31/32 (Susswein & Byrne, 1988; Baxter & Byrne, this volume) into the network, to simulate the CPG and evaluate the contribution that each element of the network makes to the pattern.

51.10

***ISLANDS IN THE MIND:* Dynamic Subdivisions in "Association" Cortex as a Darwin Machine that Augments Intelligence.** WILLIAM H. CALVIN. University of Washington NJ-15, Seattle WA 98195 USA

The population-shaping known from darwinism provides a conceptual model (a "Darwin Machine") for the second-to-second activities of multifunctional cerebral cortex. The temporary cortical "map" might resemble a patchwork quilt, decision-making involving competition between islands of differing patterns against a background sea of inhibition. An island's spatiotemporal pattern of neural activity might successfully resonate with a schema's long-term memory (widespread spatial-only patterns, similar to a bar code on a grocery package representing apples or oranges; movement schema patterns might be more like the roll for a player piano). Because adjacent islands could merge, with takeover by one pattern, the number of schemata held simultaneously in this **cache memory** would depend on regulating inhibition. Creating **sequences of schemata** such as unique sentences, while approachable as a travelling salesman problem, is best done with a specialized sequencing area, such as the one at the core of language cortex. In analogy to eukaryotes, each island in the sequencer archipelago would contain a potpourri of patterns drawn from a few islands in the cache; each island **chimera** would reproduce depending on its near-resonance with episodic memories and grammar rules, the population evolving for generations until one multipattern took over (the sentence "understood," the movement plan "good enough" to initiate). Island biogeography shows that small-island species evolve more quickly than those on large ones when climate or sea level fluctuates; attention shifts and EEG rhythms might similarly speed word or face recognition.

Many intelligence tests stress recognition speed; individuals who are fast also tend to be among those who can readily maintain many items "in mind" simultaneously. This fast-and-many correlation (part of **intelligence's general factor *g***) could be aided by a learned or innate ability to subdivide a workspace into many small islands.

51.11

ANALYTICALLY DERIVED FUNCTIONAL CONNECTIVITY USING NEURAL NETWORKS S. Shah*, W.E. Faller and M.W. Luttges. Departments of Electrical and Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309-0429.

Can the analysis of "simple units" in a trained artificial neural network reveal the "functional connectivity" of a biological neural network? The analysis of an artificial neural network capable of reproducing the spike train activity of many individual neurons suggests it is possible. Spike histories of over 50 cells in the dragonfly mesothoracic ganglion were simultaneously recorded and decomposed into individual spike trains. These raw spike train data were transformed to a stochastic spike train representation and used in training a standard backpropagation artificial neural network. Training yielded a mean sum squared error for the network that indicated successful network learning. The trained network contained the information required to predict neurobiological spiking activity. The artificial network model accurately reproduced the relative timings of spike events among cells as well as the intrinsic complex temporal patterns of individual spike train histories. To evaluate the functional connectivity developed between trained units a lesioning technique was used to remove individual cells from the artificial network. By lesioning an input unit and measuring the change in each of the output units we were able to compute the "functional influence" of each unit on the stochastic behavior of the network. Different units clearly made different overall contributions to network performance. Units may have both positive and negative (facilitatory and inhibitory) influences within the network. And, individual units can apparently have both global and specific influences on network performance. Since such information is difficult to obtain directly from analyses of the neurobiological data the neural network lesioning technique may provide important information about the function of biological nervous systems.

51.12

USING NEURAL NETWORKS TO GENERALIZE A NEURAL CODE W.E. Faller, S. Shah and M.W. Luttges. Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309-0429.

Neural network simulations of experimental neural data have been implemented and tested in our laboratory. The developed networks retained the experimentally resolved spatial distribution of cells, the physiologic operating range and stochastic firing history of each neuron. In the present work the firing histories from 55 simultaneously recorded cells in the dragonfly mesothoracic ganglion were used to determine if such a network, following training, could generalize the cell spiking histories for time periods not used in training. A 20 second record of the firing histories from these cells was separated into 10 consecutive records (1-10) of 2 seconds each. The network was then trained using a gradient spike train representation to predict the cell firing histories at time (t+5 msec) given the cellular firing patterns at time (t) for 5 alternating (1,3,5,7,9) records in this 10 record set. Following 1000 cycles of training the network was "asked" to predict the firing patterns of the 55 cells in all of the 10 records. The results indicated that the network accurately predicted the future cell firing patterns based on past firing history information for all 10 data sets. Although slightly worse performance was evident for previously "unseen" data sets, in all cases the mean sum squared error was less than 0.03 and the majority of spike train temporal histories were accurately reproduced. Despite this it seems highly unlikely that the solution of the network is a direct reproduction of the biological synaptic connectivity patterns between cells within the ganglion. However, it is asserted that the weight matrices of the network must contain a generalized statistical representation of the neural coding "rules" through which these neural cells interact with each other within this biological assembly. This assertion is strongly supported by the ability of the network to generalize solutions of cell firing patterns for "unknown" data sets. Detailed analyses of the weight matrices in turn should permit this neural code to be defined.

51.13

REPETITIVE DISCHARGE IN A COMMAND NEURON.
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Dept. of Physiol. & Pharmacol., Vermillion, SD 57069

A simple model was constructed for initiation of repetitive discharge in the earthworm medial giant fiber (MGF). The MGF receives two types of synaptic inputs: a long duration synaptic depolarization (SL) which is larger in amplitude than a heterogenous group of brief synaptic depolarizations (SB). Alone, SL can initiate one MGF action potential (AP) but can initiate repetitive discharge with great difficulty. SB pulses are always subthreshold unless they summate with another depolarization. The function of SL, besides directly initiating only one AP, is to enhance the probability that SB pulses will initiate discharge. The initiation of repetitive discharge is primarily dependent upon the occurrance of SB pulses *during* the time of SL. The equation for the enhancement of responsiveness to SB pulses under these conditions is

$$\ln(SB) = 0.078 - 0.597\,(SL),$$

($r = 0.992$) where the stimulation strength of SL is given in relative terms, with values being 1.0 for AP initiation. Values calculated for SB will initiate an AP. The discharge rate initiated in the MGF is dependent upon the rate of delivery of SB pulses during SL *PLUS* the inherent ability of the MGF to also excite itself once activated. SUPPORTED BY PARSONS TRUST FUND.

51.14

A NOVEL VECTORIAL MEASURE FOR DETECTING TEMPORALLY CORRELATED FIRING PATTERNS IN MULTIPLE SPIKE TRAINS. D. C. Tam and G. T. Kenyon Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

A novel vectorial measure, the "cross-interval vector", is developed to detect temporally correlated firing patterns in multiple spike trains recorded simultaneously. The scalar quantity of this cross-interval vector is similar to the analogous first-order "conditional cross-interval" measure introduced by Tam *et al (J. Neurosci. Methods* 23:23-33, 1988). The x- and y-components of this vector are given by the reciprocal of the cross-intervals between the neighboring spikes in another train and the reference spike. The direction of this vector is given by the arctangent of the ratio of the preceding cross-interval and the succeeding cross-interval. For n spike trains, there are a total of n such vectors for any pair of neurons. The resultant vectorial sum of the n vectors provides a quantitative measure of a specific firing pattern in these n neurons based on any reference spike in any train. The time evolution of these resultant vectors describes quantitatively the changes in firing patterns in these n neurons. Clusters of the resultant vectors delineate the sets of repeated firing patterns. The Euclidean distance between consecutive resultant vectors can be used to quantify the repeated firing patterns in the set of neurons. This method captures not only a *scalar* measure for determining any repeated firing patterns, but also a *vectorial* measure for determining the *specific* neurons contributing to the repeated patterns. Furthermore, this vectorial measure encapsulates the correlated patterns among all n neurons, not just the pair-wise correlations between any two neurons. (Supported by ONR N00014-90-J-1353 and NIH NS07182-10).

51.15

AN OBJECT-ORIENTED PARADIGM FOR SIMULATING INTERCONNECTED NEURAL SYSTEMS. D. G. Boney, L. J. Feinswog, R. K. Hutson, G. T. Kenyon and D. C. Tam Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The dynamics of realistic neuronal networks are implemented in two simulators using an object-oriented programming paradigm. The first simulator (MacNeuron) employs a detailed reconstruction of physiological nerve cells and is appropriate for the investigation of underlying physiological properties. The second simulator (MacNerveNet) describes the dynamics of simplified interconnected neurons and is optimized for studying the collective computational properties of large neuronal systems. The former simulator implements a conventional compartmental model, incorporating known biophysical and biochemical properties. The membrane "patches" and ionic mediums are decomposed into an arbitrary number of "compartments" forming the basic computational elements. The latter simulator implements a generalized integrate-and-fire neuron model, such that the idealized neuron itself forms the basic computational element; summing synaptic potentials with different time constants in different "compartments" to generate a spike when a threshold level is reached. Common to both simulators is the incorporation of hierarchical structures using an object-oriented paradigm. This greatly reduces the complexity of the model and allows easy incorporation of additional capabilities into the simulation. For instance, different ionic channels (i.e., Na^+,K^+,Ca^{++}) and (voltage/ligand-gated) conductances are implemented as subclasses of a generic conductance. Both simulators employ customized numerical algorithms designed to enhance computational efficiency. (Supported by ONR N00014-90-J-1353 and NIH NS07182-10).

51.16

STATISTICAL COMPARISON OF COMPLEX WAVE FORMS. J. A. Schetz, R. M. Friedman and C. M. Leonard. *Department of Neuroscience and The Whitney Laboratory, University of Florida, Gainesville, Florida 32610*

Simple wave form functions can be adequately compared by a combination of Fourier transformation and correlation techniques. Since correlation methods either rely upon a normal distribution or a linear assumption, they are not applicable to comparison of complex wave form functions whose differences are non-parametrically distributed. The transformation step for a complex wave form can become problematic if a mathematical expression for a given complex wave form is not known or obvious. For these reasons I have developed a simple and rapid method for comparison of complex wave forms that does not depend upon transformations or correlations.

A novel computer-aided method for statistically comparing complex wave forms (Statistical Wave Form Comparator version 1.54 S copyright (C) J. Schetz 1991) will be presented. This method employs a two-step algorithm that first finds the region(s) of best fit for two complex wave forms slid past one another, and then calculates a non-parametric matched pair signed-rank statistic for the differences over this entire region of mutual overlap. Comparison values are reported as an overall confidence interval for regions of best fit. Additional comparison information is displayed graphically and as bins of positive and negative point distributions.

Example wave forms include: (1) hydrophobicity plots of peptide neurotoxins that act on the voltage-dependent sodium channel, (2) voltage clamp records from single cells, (3) somatosensory evoked potentials prior to and following lesion, (4) and anatomical patterns of normal verses pathological brain gyri.

LIMBIC SYSTEM I

52.1

DO HIPPOCAMPAL CA3 PYRAMIDAL CELLS PROJECT INTO THE DENTATE HILUS? J. Pokorný* and P.A. Schwartzkroin. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Although there has been much recent interest in the role of the dentate gyrus, and particularly of dentate hilar neurons, we still know relatively little about the connectivity of the hilus with other parts of hippocampus. Morphological studies suggest a strong interaction between some hilar neurons - the mossy cells - and the granule cells; physiological data have shown a potent excitatory projection from granule cells onto these mossy cells. There are, in addition, some tantalizing indications that CA3 pyramidal cells send axon collaterals into the hilus. Physiological data suggest a potent CA3-to-hilar connection, but morphological studies have failed to support such a pathway.

In the present study, we have combined intracellular injections of CA3 pyramidal cells (with neurobiotin and lucifer yellow) and hilar stimulation to explore the connectivity between the hilus and CA3 regions. In rat hippocampal slices, most CA3 pyramidal neurons tested for antidromic activation from the hilus showed no spike invasion, even with relatively strong stimulation. However, when cells were depolarized, stimulation at localized hilar sites sometimes evoked antidromic action potential invasion. We hypothesize that antidromic invasion normally does not occur because of the fine caliber of the hilar-projecting axonal branch. When antidromically-activated cells were dye-injected, segments of fine axon collaterals were found projecting toward the hilar region. The extent of their projection, and their postsynaptic targets have still to be determined.

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52.2

INTRASEPTAL CONNECTIONS REDEFINED: LACK OF A LATERAL SEPTUM TO MEDIAL SEPTUM PATH. T. Deller,[1*] C. Leranth,[1] and G. Buzsáki.[2] [1]Section of Neuroanatomy, Department of Obstetrics and Gynecology, Yale University, New Haven, CT 06510 and [2]Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

The integrity of the septohippocampal system is essential for memory formation and spatial behavior as well as for the electrical stability of the hippocampus. For many years it has been presumed or explicitly stated that the reciprocal septohippocampal loop is closed by a massive lateral septum-medial septum path. In the present study we reexamined the intraseptal connectivity in the rat using phaseolus vulgaris leucoagglutinin tracing combined with choline acetyltransferase and immunohistochemistry at both the light and electronmicroscopic levels. We found that the previously hypothesized lateral septum to medial septum projection is extremely sparse or non-existent and that the major medial septum to lateral septum path is GABAergic. The main targets of the lateral septal area is the lateral preoptic area, lateral hypothalamus, and amygdala. The redefined circuitry has important implications for the understanding of the septal regulation of hippocampal electrical activity and the operations of the septo-hippocampal system.

52.3

PRESENCE OF SOMATOSTATIN OR NEUROTENSIN IN LATERAL SEPTAL AREA (LSA) DOPAMINERGIC AXON TERMINALS OF KNOWN ORIGINS. R.L. Jakab[1] and C. Leranth[1,2]. Dept. of Obstetrics and Gynecology[1] and Section of Neurobiology[2], Yale University, School of Medicine, New Haven, CT. 06510

A new approach is proposed to demonstrate the coexistence of dopamine (DA) and neuropeptides in axon terminals, using targeted injection of 6-hydroxydopamine (6-OHDA) to DA cell groups and immunocytochemistry. We have studied the possible presence of somatostatin (SOM) and neurotensin (NT) in dopaminergic boutons of the rat LSA, because of the earlier reports that some periventricular hypothalamic DA neurons contain SOM (Sakanaka et al. '90), while a population of ventral tegmental area (VTA) DA neurons contain NT (Hökfelt et al. '84), and both DA systems project to the LSA (Lindvall and Stenevi '78). We focused our study on the afferents of the LSA somatospiny neurons which are surrounded by both DA- and SOM-containing pericellular baskets (Jakab and Leranth '90, '91). The animals were treated with 25 mg/kg desmethylimipramine i.p. to protect the noradrenergic fibers, and 45 min later 6-OHDA (1μg in saline-L-ascorbic acid) was unilaterally injected into the periventricular hypothalamus (*group1*) or the VTA (*group2*). After 48 hours the rats were sacrificed, and the septal area of *group1* was immunostained for SOM, while that of *group2* was immunostained for NT. Electron microscopy revealed numerous axon terminals on the treated side of the LSA which were immunopositive for SOM (*group1*) or NT (*group2*), and contained autophagous cytolysosomes. These organelles represent characteristic signs of catecholamine fiber degeneration which can be induced by 6-OHDA (Onteniente et al. '87) or catecholamine pathway transection (Leranth et al. '88). Between these axon terminals and somatospiny neurons synaptic contacts were detected. In conclusion, LSA neurons receive double innervation from a "somatostatinergic" DA system of the hypothalamus and a "neurotensinergic" DA system of the midbrain. (Supported by NIH grants NS 26068 and HD 23830, C.L.).

52.4

SEIZURE-SENSITIVE SUBSTANCE P (SP) NEURONS IN THE HIPPOCAMPAL FORMATION OF THE NON-HUMAN PRIMATE AND HUMAN. C. Leranth[1] and N. de Lanerolle[2] Dept. of Obst. & Gyn.[1] and Sects. of Neurobiol.[1,2] and Neurosurg.[2] Yale Unive. New Haven, CT 06510.

We examined the distribution and synaptology of SP cells and axons in the vervet monkey (*Cercopithecus aethiops*). The majority of these cells are concentrated in the dentate hilar area while others were found in all layers of the ammon's horn. SP boutons in the dentate gyrus formed exclusively asymmetric synaptic contacts with granule cell dendrites in the external molecular layer, as well as with parvalbumin (PA)-immunoreactive neurons. In the ammon's horn, where the CA2 subfield was the most heavily innervated, the majority of the contacts were asymmetric; however, symmetric synapses could also be observed.

In order to determine the physiological significance of this rich hippocampal SP innervation, we examined the distribution of SP in human hippocampal seizure foci obtained from patients with intractable temporal lobe epilepsy (TLE). In the dentate hilar area of cryptogenic epileptic patients a loss of SP-immunoreactive neurons was observed. Although there was a major loss in CA1 pyramidal neurons, SP interneurons of this area were unaffected. In comparison, the hippocampi of TLE patents with extrahippocampal temporal lobe lesions, were essentially similar to those of normal human and primate hippocampi.

SP is a well known excitatory transmitter. In addition to innervating the excitatory granule cells, SP boutons terminate on PA-containing (presumably GABAergic) cells. Therefore, one can speculate that their loss affects the initiation of GABAergic inhibitory mechanisms. (Supported by NS-26068, C.L. and NS-27081, N. de L.)

52.5

LIGHT AND ELECTRON MICROSCOPY OF PARVALBUMIN-IMMUNOSTAINED NEURONS AND AXON TERMINALS IN THE AMMON'S HORN AND DENTATE GYRUS OF THE PRIMATE HIPPOCAMPUS. C. E. Ribak, L. Seress* and C. Leranth. Dept. of Anatomy and Neurobiol., Univ. of Calif., Irvine, CA 92717 and Sect. Neurobiol. & Ob/Gyn, Yale Univ. Sch. Med., New Haven, CT 06510.

Previous studies have described the localization of parvalbumin-immunoreactive (PARV-ir) neurons in the rodent hippocampus but similar descriptions of PARV-ir neurons in monkeys have not appeared. The present study examined light and electron microscopic preparations obtained from PARV-ir sections of hippocampus from six green monkeys. Light microscopy showed PARV-ir neurons and axon terminals in all layers of the dentate gyrus and Ammon's horn with some regional differences in the latter site. For example, the stratum moleculare in only CA2 showed many PARV-ir terminals but no PARV-ir cells were ever found in this layer in this region or in any of the other subfields of Ammon's horn. Most of the PARV-ir cells were located in or adjacent to the principal cell layers and had features that were described previously for PARV-ir cells in the rat. Except for CA2, the hippocampus displayed the most PARV-ir axon terminals among the somata of the principal cells both in the dentate gyrus and Ammon's horn. Electron microscopy showed that the PARV-ir axon terminals formed symmetric synapses with somata, dendrites and axon initial segments of principal and PARV-ir cells. Of the cells in the principal cell layers, pyramidal cells in CA2 showed the greatest number of axosomatic synapses formed by PARV-ir axon terminals whereas granule cells displayed the least. These results indicate areal differences in the GABAergic inhibitory innervation of hippocampal neurons. (Supported by NIH grants NS-15669, NS26068 and MH 44866)

52.6

POSTSYNAPTIC TARGETS OF TIMM-LABELED MOSSY FIBERS IN RAT AND MONKEY HIPPOCAMPUS: A LIGHT AND ELECTRON MICROSCOPIC STUDY. L. Seress*, R.A.E. Bakay and C. E. Ribak. Dept. of Anatomy and Neurobiol., Univ. of Calif., Irvine, CA 92717 and Div. Neurosurgery, Emory Clinic, Atlanta, GA 30322

A comprehensive study of Timm-labeled terminals and their postsynaptic targets has not been described in electron microscopic preparations of the rat and monkey hippocampus. The present analysis used a modified Timm method that employed unosmicated tissue and postembedding histochemistry. In the electron microscopic preparations, axon terminals of granule cells including the large mossy fibers were selectively labeled with numerous silver grains. The distribution of these terminals in thin sections was identical to that found in light microscopic preparations using the routine Timm method. The hilar neurons displayed two different distributions of mossy fibers; some displayed numerous terminals on their cell body and dendrites whereas others were much less frequently contacted by these terminals. Within the granule cell layer, smooth dendrites and somata of basket cells as well as unidentified spines were targets of Timm-labeled axon terminals, but not granule cell bodies. In the CA3 region, mossy fibers in stratum lucidum were labeled and they formed synapses with the typical thorny excrescences of the apical dendrites of rat pyramidal cells whereas both apical and basal dendrites were contacted by these terminals in monkeys. Also, Timm-labeled terminals formed synapses with dendrites and spines of unknown origin in the stratum oriens of the CA3 in rats. In conclusion, the main targets of the granule cells for both normal rats and monkeys are the pyramidal cells of CA3, the inhibitory neurons of the dentate gyrus and other hilar neurons. (Supported by NIH grant NS 15669)

52.7

EVIDENCE FOR A PRESYNAPTIC CO-LOCALIZATION OF KAINIC ACID RECEPTORS AND ACETYLCHOLINESTERASE IN THE DENTATE GYRUS OF THE RHESUS MONKEY. K. J. Rhodes, D. L. Rosene and M. B. Moss. Dept. of Anat. and Neurobiology, Boston Univ. Sch. of Med., Boston, MA 02118.

The aim of this study was to determine the cellular localization of kainic acid (KA) receptors and acetylcholinesterase (AChE) and that occupy the inner third of the molecular layer (ML) of the dentate gyrus (DG). To accomplish this, we examined the distribution of AChE and KA receptors in nine rhesus monkeys that received unilateral experimental lesions (14-21 day survivals) designed to destroy specific afferent axons or cell populations while sparing afferent fibers. Brains were fresh-frozen, cut into 15μm sections, and processed histochemically for AChE and by *in vitro* [^{3}H]KA binding and autoradiography for KA receptors. Quantitative analysis of the histochemically stained sections and autoradiograms revealed the following: 1) fornix transections (n=2) reduced the density of AChE in the inner third of the ML by 15-20% but had no effect on the density of KA receptors, suggesting that only a small proportion of this AChE is associated with septohippocampal afferents; 2) ibotenic acid lesions (n=3) that destroyed dentate granule cells and polymorph cells but spared afferent fibers reduced the density of KA receptors in the inner third by 35%, but had no effect on the density of AChE, suggesting that some KA receptors are located postsynaptically on DG neurons; and 3) hippocampal transections that destroyed axons of the intrinsic, caudally directed dentate gyrus association (DGA) pathway reduced the density of AChE and KA receptors in the inner third at levels caudal to the lesion, suggesting that the two markers are co-localized presynaptically on axons of the DGA pathway. Interestingly, the density of both AChE and KA receptors is increased in the DG of Alzheimer's brains and following long-term entorhinal lesions. The results of the present study suggest that sprouting of intrinsic DGA fibers rather than septohippocampal afferents may account for this "plasticity". "Supported by NIH 16841, and 04321"

52.8

ORGANIZATION OF EFFERENT PROJECTIONS FROM THE ENTORHINAL CORTEX TO THE POSTERIOR PARAHIPPOCAMPAL GYRUS IN THE RHESUS MONKEY. D. L. Rosene, G. J. Blatt, K. J. Rhodes and D. N. Pandya, Dept. of Anatomy and Neurobiology, Boston Univ. School of Medicine, Boston, MA 02118.

The entorhinal cortex (EC, area 28) is widely recognized as the major link by which input from cortical association areas reaches the hippocampal formation (HF). The posterior parahippocampal gyrus (PPHG) provides the major source of this cortical association input. Recent data from this and other labs demonstrates that the EC receives direct projections from the HF, amygdala and presubiculum and in turn sends projections back to the association cortices. To investigate the organization of EC projections to the PPHG (areas TH, TL, and TF), complementary retrograde and anterograde tracers were used. To localize the cells of origin in the EC, injections of fast blue and diamidino yellow were made into subdivisions of the PPHG. To determine the laminar and areal pattern of EC termination in the PPHG, injections of ^{3}H-amino acids were made into the EC. A large anterograde tracer injection into the EC labeled layers I and II in all three PPHG subfields, but in TL and TF there was additional label in layers V and VI. An injection centered in area 28L labeled layers I and II in TH and layers II and superficial III and V and VI in TL and TF. An injection centered in area 28I labeled primarily layer II in all three PPHG subfields and layer V and VI in rostral TL and TF. Retrograde tracer injections into isocortical area TF or proisocortical area TLc labeled cells in layer V in the caudal two-thirds of areas 28L and 28I, and throughout area 28S. In contrast, retrograde tracer injections into proisocortical area TH labelled cells in layers III and V throughout the entire extent of the EC. Since hippocampal efferents terminate heavily in layer V while layer III receives major input from the amygdaloid and the presubiculum, the information flow to the different subdivisions of the PPHG may be quite different. Thus the EC should be viewed as an important *bidirectional* link between the hippocampal formation and the PPHG as well as a *relay* for the amygdala and presubiculum to both the hippocampus and PPHG. "Supported by NIH grants NS16841 and AG04321."

52.9

TERMINAL ORGANIZATION OF PROJECTIONS FROM PRESUBICULUM AND PARASUBICULUM IN RELATION TO IDENTIFIED ENTORHINAL-HIPPOCAMPAL PROJECTION NEURONS. M.P. Witter and M. Caballero Bleda*, Dept. Anatomy, Vrije Universiteit, Amsterdam, The Netherlands.

Cells in layers II and III of the entorhinal cortex give rise to projections to the hippocampus and distribute their fibers to dentate gyrus/CA3 and CA1/subiculum, respectively. Since layer II receives fibers from the parasubiculum (PaS) and layer III from the presubiculum (PrS; Kohler, '85), it is conceivable that layers II and III convey different types of information to the different subdivisons of the hippocampus. As a first attempt to elucidate this presumed specificity in the relation between the terminal distribution of inputs from the PrS and PaS and the origin of the two components of the entorhinal-hippocampal projection, we used a combination of anterograde and retrograde tracing and subsequent intracellular filling of retrogradely identified projection neurons in lightly fixed slices of the entorhinal cortex. Subsequently, the cell type, its position in the cell layer, its relation to the anterogradely labeled afferents from PrS/PaS, and the number of presumed synaptic contacts between the afferents and the projection neurons was assessed.

Our results show that the dendrites of neurons that are located close to the border between layers II and III cross this border into the adjacent, non-parent cell layer. Since also the terminal plexuses from PrS and PaS are not confined to the cell layer of preference, such "borderline" neurons appear to be innervated by both the inputs from PrS and PaS. We noted that projection neurons, classified as "horizontal" cells, are also located in the most superficial part of layer IV. Their dendrites are innervated at regular intervals by fibers from the PrS that course through layers VI-IV to the superficial layers of the entorhinal cortex. Whether these cells receive similarly organized input from PaS is subject of further study.

52.10

CORTICAL AFFERENTS TO THE RAT ENTORHINAL CORTEX STUDIED WITH AXONAL TRACERS. R. Insausti. Dept. of Anatomy, University of Navarra, Apdo. 273, 31080 Pamplona, Spain.

The rat entorhinal cortex is the main source of afferents to the hippocampus. The exact origin of the information to the entorhinal cortex is relevant to understand the hippocampal mnemonic function. Cortical afferents to rat entorhinal cortex has been studied by both pressure and iontophoretic injection of WGA-HRP after direct surgical exposure of its surface. Series of 50 μm sections were obtained through the brain and reacted according to Mesulam's protocol. Caudally placed injections resulted in retrogradely labeled cells in temporal and temporo-ocipital cortices, but in little labeling in perirhinal cortex. In contrast, injections of rostral entorhinal cortex gave heavy labeling in perirhinal cortex, as well as in medial frontal (infralimbic) and orbital cortices, but not in temporal cortex. Our study shows that the cortical afferents to the entorhinal cortex are less numerous than subcortical afferents, and that they are arranged in a differential rostrocaudal topographic fashion (supported by a grant from the Government of Navarra, Spain).

52.11

SPATIOTEMPORAL ORGANIZATION OF THE ENTORHINAL (E) INPUT TO THE HIPPOCAMPUS: MULTIPLE ELECTRODE RECORDING FROM THE ADULT GUINEA PIG BRAIN MAINTAINED *IN VITRO*. D. Paré and R.R. Llinas, Dpt. Physiol. and Biophys., New York Univ.

In order to test the lamellar hypothesis, field potential responses to E stimulation (STM) were recorded with multiple electrodes from the CA1 pyramidal layer of the arterially perfused adult guinea pig isolated brain maintained *in vitro*. Only tri-synaptic population spikes (PSs) were evoked by low-intensity E STM. However, they were widely distributed along the septotemporal (ST) and transverse (T) axes. Irrespective of the E region stimulated, the latency of CA1 responses gradually increased from the CA3/CA1 border toward the subiculum. In the ST axis, the E projection was organized topographically, each ST segment of the CA1 region responding at a shorter latency to a particular rostrocaudal level of the E cortex. This early response then propagated further septally and temporally with a conduction velocity roughly proportional to the stimulation intensity (0.5-2 m/sec). Transections interrupting the connections between the various E STM sites did not prevent the propagation of the response. Moreover, direct stimulation of the dentate gyrus (DG) at different ST levels gave rise to a similar pattern of activity in the CA1 region. These results suggest that each region of the E cortex projects preferentially at a particular ST level of the DG and that the associative pathways linking CA3-CA1 regions in the ST and T axes are powerful enough to transmit DG influences to the whole hippocampal formation. These results call for a revision of the lamellar hypothesis. Supported by NIH grant NS-13742 and MRC of Canada.

52.12

COMPARISON OF THE INTRINSIC ORGANIZATION OF MONKEY AND HUMAN ENTORHINAL CORTEX. M.J. Beall and D. A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213.

Inferences about the connectivity of human entorhinal cortex (ERC) arise mainly from tracing studies conducted in monkey. The accuracy of such inferences may depend upon the degree to which monkey and human ERC have a similar intrinsic organization. Left ERC was obtained at autopsy from 6 humans (ages 32-63) with no known neuropsychiatric disorders and both ERCs were obtained from 5 perfused adult monkeys (*M. fascicularis*). Comparisons of cytoarchitectonic features were made between monkey and human using Nissl stained sections and the criteria of Amaral et al. (JCN 264: 326, 1987) for ERC subdivisions in the monkey. In general, the cytoarchitecture of ERC was similar in both species although specific laminar differences were observed. As a result, modifications of the monkey criteria were necessary for the identification of human ERC subdivisions. Immunohistochemical markers were also used to compare the distribution of distinct subpopulations of neurons across species. Antibodies labeling neurofilament proteins (NF) and two calcium binding proteins, calbindin and parvalbumin, revealed both similarities and differences in the distribution of immunoreactive neurons in the two species. For example, the gradient in NF immunoreactivity in layer II neurons was comparable in both species, however the two species had dissimilar patterns of labeling in the deeper layers that paralleled species differences in cytoarchitecture. Although many similarities were present in the organization of human and monkey ERC, significant differences were also evident and should be considered when using monkey ERC as a model for the human.

52.13

THE DOPAMINERGIC INNERVATION OF MONKEY ENTORHINAL CORTEX. M. Akil and D.A. Lewis. Depts. of Psychiatry and Behav. Neurosci., Univ. of Pittsburgh, PA 15213

The dopaminergic (DA) system and the entorhinal cortex (ERC) have both been implicated in the pathophysiology of schizophrenia. Little is known, however, about the DA innervation of primate ERC. In this study we used immunohistochemical methods to characterize the distribution of tyrosine hydroxylase (TH)-containing axons in monkey *(M. fascisularis, M. mulatta)* ERC. The relative density and laminar distribution of TH-immunoreactive (IR) axons differed among cytoarchitectonic regions of ERC. In general, fiber density decreased following a rostral-caudal gradient; some changes in density were stepwise whereas others were gradual. In most regions of ERC, TH-IR axons were present in greatest density in layers deep I and VI. In contrast, in the densely innervated olfactory ERC, TH-IR axons were more evenly distributed across all cortical layers although the highest density was in layer I. Dense TH-immunoreactivity was also noted in temporal regions rostral to ERC where vertical columns of TH-IR fibers were seen. Dual-label experiments with TH and dopamine-β-hydroxylase antibodies, as well as preliminary studies with DA antisera confirmed that the majority of TH-IR axons described in this study are DA. These data suggest a lamina- and region-specific role of the DA system in the function of monkey ERC.

52.14

CONVERGENCE OF PROJECTIONS FROM THE RAT HIPPOCAMPAL FORMATION, THALAMUS, AND BASAL FOREBRAIN ONTO NEURONS OF THE ANTERIOR CINGULATE CORTEX: AN *IN VIVO* PHYSIOLOGICAL STUDY. D.M. Finch and A.M. Tan*, Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024.

A number of anatomical and physiological studies have shown projections from the hippocampal formation, from the medial dorsal nucleus of the thalamus, and from the cholinergic basal forebrain to the anterior cingulate cortex. We examined the possibility of synaptic convergence from these inputs onto single cingulate neurons. Sprague-Dawley albino rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), and stimulating and recording electrodes were placed stereotaxically. Extra- and intracellular responses from anterior cingulate neurons were recorded *in vivo* after electrical stimulation of the hippocampal formation (dentate gyrus, hippocampal fields CA3 and CA1, subicular complex, and entorhinal cortex); thalamus (medial dorsal nucleus and vicinity); and basal forebrain (diagonal band, ventral pallidum, septum). The predominant response to stimulation of each of these regions was inhibition, though short latency excitatory responses were also seen in some cells. Individual cingulate neurons could show responses to two, or all three, of the stimulation sites. This indicated that synaptic influences from these areas converges onto single cingulate neurons, and provides a basis for synaptic integration. Supported by NIH Grants NS 23074 and NS 16721.

52.15

THE CORTICOTHALAMIC PROJECTIONS OF THE ANTERIOR CINGULATE AND PRECENTRAL AGRANULAR CORTICES IN THE RAT. G.D. Fisk, T. van Groen and J.M. Wyss. Dept. of Cell Biology, The University of Alabama, Birmingham, Alabama 35294.

The present study characterizes the corticothalamic projections of anterior cingulate (area infraradiata; anterior, IRα; posterior, IRβ) and precentral agranular cortices using the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L). In agreement with previous findings, the present data demonstrate that IRα, IRβ and precentral agranular cortices project to anteromedial, mediodorsal, laterodorsal, lateroposterior, ventromedial, centrolateral, paraventricular and reuniens thalamic nuclei. In addition to these similarities, each cortical area has distinct projections. IRα, but not IRβ, has a dense projection to the parataenial nucleus, while IRβ, but not IRα, projects to anterodorsal and anteroventral nuclei. Further, while both IRα and IRβ project most densely to anteromedial, ventromedial and mediodorsal nuclei, precentral agranular cortex has denser projections to ventrolateral, laterodorsal and lateroposterior nuclei. Within IRα and IRβ, dorsal and ventral regions have different projection patterns. Compared to ventral IRα, dorsal IRα has denser projections to parataenial and anteromedial nuclei, but a less dense projection to the paraventricular nucleus. In IRβ, dorsal (compared to ventral) IRβ projects more densely to mediodorsal and ventromedial nuclei. Together, these data demonstrate that while the subregions of the anterior midline cortex project to many common thalamic targets, each cortical subregion has a distinct pattern of termination.

52.16

AGE RELATED BREAKDOWN OF DENDRITIC BUNDLES IN THE RETROSPLENIAL CORTEX OF THE RAT. J.M. Wyss and T. van Groen. Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294.

In retrosplenial granular cortex (Rg), the apical dendrites of layer II pyramidal neurons bundle together tightly in layer Ic and Ib and spread out in layer Ia, where they appear to receive the projection from the anteroventral thalamic nucleus. In the present study, we tested the hypothesis that these dendritic bundles become disorganized in aging rats. At 3, 6 and 14 months of age, male Sprague Dawley rats received a large, unilateral injection of the retrograde tracer, Fluorogold, in Rg. In the 3 month old rats, nearly all densely labeled, layer II neurons in Rg contralateral to the injection have apical dendrites that are confined to the layer I dendritic bundles. This pattern is relatively undisturbed in the 6 month old rat. In contrast, in 14 month old rats, the dendritic bundles are somewhat disorganized. In these rats (compared to the younger rats), a similar number of layer II neurons are labeled, but fewer of them have dendrites in the bundles. In some locations, the apical dendrites do not collateralize in layer Ia, while in other locations a apical dendrites appear to be broken off in layer Ic, and short collaterals arise from the remaining shaft. These characteristics are not observed in younger rats. In the 14 month old rats, labeled apical dendrites arising from layer II-VI of Rg display no obvious signs of age-related changes. Preliminary data suggest that this disorganization progressively worsens in rats that are older than 14 months. These data suggest that compared to other neurons, the layer II neurons in Rg are more vulnerable to the deleterious effects of aging, an effect that could impair learning in aging individuals.

52.17

IMMATURE-LOOKING DENDRITES IN MATURE HIPPOCAMPAL CA1. T. Esch,* M. S. Heydenreich,* W. B Levy and N. L Desmond. Dept. Neurosurgery, Univ. Virginia Hlth. Sci. Ctr., Charlottesville, VA 22908.

Using rapid Golgi-impregnated material from a developmental series of rat hippocampi, we find that some pyramidal cell dendrites in adult CA1 s. moleculare (s. mol.) have morphological characteristics similar to immature dendrites. At 7 dpn, s. mol. dendrites consist of asymmetric varicose segments interconnected by filiform segments. Thin, zigzagging processes longer than dendritic spines at this age, and sometimes possessing small varicosities, may extend from these dendrites. Few dendritic spines are seen at 7 dpn. With maturation, the varicose segments become smaller and more symmetric, and the intervaricose, filiform segments become shorter and less frequent. Dendritic spines increase in number. In adult (45-60 dpn) CA1 s. mol., dendrites have a fairly uniform diameter. Dendritic varicosities do exist, but their appearance is suggestive of sessile dendritic spines extending from the parent dendrite. Overall, dendritic spines are more numerous and more uniformly dispersed across the dendritic length in the adult. 'Immature'-looking dendrites sometimes appear in the mature CA1 s. mol., generally in the most distal s. mol. Like the dendrites from immature animals, the 'immature' adult dendrites have both asymmetric varicose segments and filiform segments. An 'immature' adult dendrite can either be a terminal branch or can bifurcate into 2 additional 'immature' terminal branches. The young appearance of these adult CA1 s. mol. dendrites suggests on-going dendritic growth and synaptogenesis in the adult hippocampus.

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52.18

AN ANATOMICAL MARKER FOR ENDOGENOUS LTP IN THE DENTATE GYRUS EXTENDS TO HIPPOCAMPAL CA1. N. L Desmond. Dept. of Neurosurgery, Univ. of Virginia Hlth Sci Ctr, Charlottesville, VA 22908.

Previously[1] we hypothesized that concave spine synapses, with their set of defining features, were a structural marker for endogenous LTP in the hippocampal dentate gyrus (DG). Here the ultrastructural features of concave and nonconcave spine synapses in normal, adult rat CA1 s. radiatum (s. rad.) and s. moleculare (s. mol.) were compared with existing DG data to assess whether the hypothesized marker extends beyond the dentate gyrus. Concave spine synapses, on average, differ from nonconcave spine synapses in both CA1 s. rad. and s. mol. in the same manner as they do in the DG. Concave spine synapses have larger spine heads (0.199 μm^2; 0.273 μm^2 area) than do nonconcave spine synapses (0.097 μm^2; 0.172 μm^2) in CA1 s. rad. and CA1 s. mol., respectively. Concave spine synapses have larger PSDs than do nonconcave spine synapses in both CA1 s. rad. and CA1 s. mol. As in the DG, the probability of a polyribosome associated with a dendritic spine is less for a concave spine synapse (0.034; 0.086) than for a nonconcave spine synapse (0.055; 0.146) in both s. rad. and s. mol., respectively. In all three regions, the same relative characterization exists for concave and nonconcave spine synapses. Thus, by virtue of the structural similarity with synapses of the DG, it is conceivable that these structural distinctions in the two CA1 regions reflect the existence of a normal, ongoing DG-like LTP there.

[1]Desmond & Levy. Soc. Neurosci. Abstr. 14 (1988) 833.

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52.19

CHARACTERIZATION OF "DARK" CELLS INDUCED BY NORADRENERGIC DEPLETION IN THE RAT HIPPOCAMPUS. Y.-P. Lee and H.-M. Hwang. Dept. Anatomy, Chang Gung Medical College, Taoyuan, Taiwan, R.O.C.

Noradrenergic depletion, caused by neonatal administration of 6-hydroxydopamine (6-OHDA), 10 mg/kg dissolved in 0.9% saline containing 0.04% L-ascorbic acid for 4 days, was found to produce an increase of beta-adrenergic receptor activity in the hippocampus and an occurrence of "dark" cells in the dentate gyrus and CA4 area. In order to examine the ultrastructure of "dark" cells and to search possible correlation to the increase of beta-adrenergic receptors, electron microscopy was performed on hippocampi treated by 6-OHDA or DSP-4. The "darkness" appeared to be due to fine and fuzzy particles distributed over the whole neuron, including nucleus, somata, axon, and dendrite. They revealed the features as neurons in an active metabolism. In comparison with usual neurons with "light" appearance, their soma seemed to contain more mitochondria, rough endoplasmic reticulum, and ribosomes. Their nuclei appeared to be vesicular with invagination. Study on possible correlation of "dark" cells with receptor activity is in progress.

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52.20

DEGENERATION OF THE HIPPOCAMPAL PYRAMIDAL NEURONS IN THE SOCIALLY STRESSED TREE SHREW. H. Uno, G. Flügge, C. Thieme*, O. Johren*, and E. Fuchs. Wisconsin Regional Primate Research Ctr., Madison, WI 53715, and German Primate Center, Gottingen, Germany.

The tree shrew (*Tupaia belangeri*) has provided a useful model for studying the consequences of psycho-social stress. Male cagemates often behave in a hostile, territorial-defending manner and develop a dominant/subordinate relationship. The subordinate animal, harassed and attacked by the dominant, loses body weight and catecholamine and glucocorticoid urinary levels increase. The behavior and physiological parameters in nine pairs of male tree shrews were studied. After the establishment of hierarchical order all the animals were euthanized at 1, 2, and 4-week periods. The hippocampuses cut from perfusion-fixed brains were studied in both light and electron microscopy. Degeneration of the pyramidal neurons in CA3 and CA1 regions was observed in 6 subordinates kept under the stress conditions for 2 to 4 weeks. Ultrastructurally, the degenerated neurons showed highly condensed axoplasmic organella and nuclear chromatin, and vacuolization and shrinkage of perikarya, nucleus, and dendritic branches. This model provides a study of the correlation between the degree of stress and brain damage. (Supported by NIH Grant RR00167.)

LEARNING AND MEMORY—ANATOMY I

53.1

DISSOCIABLE ROLES OF HIPPOCAMPUS AND ORBITOFRONTAL CORTEX IN AN ODOR-GUIDED DELAYED NON-MATCH TO SAMPLE TASK. T. Otto & H. Eichenbaum. Dept. of Psychology, University of North Carolina, Chapel Hill, NC 27514.

Converging evidence indicates that rats' excellent abilities for odor-guided learning can be exploited for the development of rodent models of primate memory capacities. The present study focused on development of an odor-guided analog of the visually-guided delayed non-match to sample (DNM) task commonly used in studies of the neural substrates of memory formation in humans and monkeys.

Successful performance in our continuous DNM task (cDNM) requires the rat to remember the odor presented on the immediately preceding trial, and to respond for water reinforcement only if the odor presented on the current trial is a *non-match*. In normal rats performance in this task is sensitive to increased delay or interference.

The acquisition of cDNM and subsequent performance across increasing delay and levels of interference in separate groups of rats with lesions of the fornix (FX,n=6), of the orbitofrontal cortex (OFX,n=4), or of the entorhinal cortex (ECX,n=7) was compared to that of controls. FX rats performed as well as normal rats on all aspects of cDNM. In contrast, OFX rats were severly impaired in cDNM acquisition, but subsequent performance across even long delays or increased interference was unaffected. Conversely, ECX rats acquired cDNM normally, but their memory decay was abnormally rapid and sensitive to interference. Thus it appears that, like primates, rats with FX lesions are unimpaired on cDNM. Moreover, the frontal and temporal cortical targets of olfactory projections play critical and complimentary roles in cDNM: The orbitofrontal cortex is required for odor discrimination or acquisition of the cDNM rule, while entorhinal projections to the hippocampus are critical to maintaining a memory representation across a delay.

53.2

TRANSVERSE, BUT NOT LONGITUDINAL HIPPOCAMPAL CONNECTIONS ARE NECESSARY FOR SPATIAL LEARNING. E.I.Moser*, M.B.Moser* & P.Andersen. Institute of Neurophysiology, Univ. of Oslo, Box 1104 Blindern, 0317 OSLO 3, Norway.

We investigated whether spatial learning in the rat requires integration of impulses from different septo-temporal levels of the dorsal hippocampus. Longitudinal connections were severed by exposing the dorsal hippocampus and placing transverse cuts through the dorsal hippocampus at 1 or 3 septo-temporal levels. The knife penetrated either the CA3 or the CA1 and the underlying dentate gyrus. In a separate group of rats, longitudinal lesions were made by aspiration of a 5mm long, 1 mm wide strip within the CA1 and the dentate gyrus of the dorsal hippocampus. Following the lesions, place learning was tested in a Morris water-maze.

Longitudinally oriented CA1 lesions gave the expected increase in escape latency and abolished spatially biassed swimming. Rats with one, or three separate, transverse sections of the CA3 or the CA1/dentate gyrus all had escape latencies similar to the neocortical control rats. However, on removal of the platform, rats with transverse cuts spent somewhat less time in the central one-third of the target quadrant, suggesting less precise spatial navigation.

In conclusion, spatial learning seems to depend primarily on transmission of impulses in the lamellar direction. Longitudinal integration of impulses may improve the precision of spatial performance.

53.3

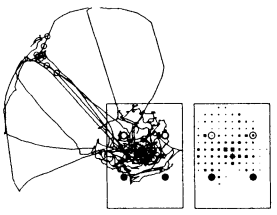
A TEST BATTERY FOR ASSESSING NONSPATIAL MEMORY IN BRAIN-DAMAGED RATS. D.G. Mumby, J.P.J. Pinel, & D.S. Anzarut*, Dept. of Psychology, University of British Columbia, Vancouver, B.C., Canada V6T 1Y7.

Studies of brain-damage-produced amnesia in monkeys often employ a battery of nonspatial memory tasks to assess the mnemonic abilities of individual subjects. We tested normal rats on a battery of tasks that were designed to mimic some of those used with monkeys: (1) Object discrimination and reversal, (2) concurrent object discriminations, (3) delayed nonmatching-to-sample with various retention delays (4,15,60, & 120 s) and list lengths (1,3,5, & 7 items), and (4) temporal-order recognition. Each task made use of the same apparatus and, like the monkey versions of these tasks, each one used similar test stimuli (objects). The rats acquired each of these tasks and their performance on each paralleled that of monkeys (e.g., scores on delayed nonmatching-to-sample decreased as either the delay or list length increased). In previous experiments, rats with brain lesions similar to those that cause anterograde amnesia in monkeys (e.g., mediodorsal thalamus, hippocampus plus rhinal cortex) were impaired on concurrent object discriminations and delayed nonmatching-to-sample. The resemblance that these tasks bear to those used in studies of brain-damage-produced amnesia in monkeys suggests that this test battery might be useful in similar models of amnesia in rats.

53.4

THE MANHATTAN MAZE: A NEW TECHNIQUE FOR ANALYSING SPATIAL LEARNING. R.G.M.Morris, R.Biegler and R.Spooner, SPON: B.R.A. Dept. of Pharmacology, Univ. of Edinburgh, Edinburgh, EH8 9JZ, Scotland.

Research on the neurobiology of spatial learning and memory would benefit from better understanding of the strictly psychological processes involved, notably, by analogy with studies of associative conditioning, of the *content* and *conditions* of learning. Following the work of Collett et al (*J. Comp. Physiol.*, 1986), we have developed a technique in which rats are trained to find food reward within a large arena (3.6 m diameter) containing wood-shavings (4 cm depth). Landmarks ("skyscrapers") are placed within the arena at various predefined positions which move between trials, with the rewards hidden in the shavings at specific locations near the landmarks. The environment is directionally polarised. The animals' searching movements are tracked automatically using an image-analyser, and a computer is used to plot time spent near the landmarks (Fig *left*: paths of 7 rats from periphery to vicinity of landmarks; *right*: cumulative pixel time plot). The results show that rats concentrate their search in specific locations relative to the landmarks, perhaps by computing vectors specifying distance and direction, and we are now investigating whether "classical" phenomena such as *discrimination, overshadowing* and *blocking* occur within the spatial domain. For the latter, rats are trained to find food whose location is defined by two cues, two further cues are then added, and a final test trial is used to examine control of behaviour by the added cues.



53.5

CONTEXTUAL RETRIEVAL OF ASSOCIATIVE INFORMATION IS IMPAIRED IN RATS WITH IBOTENIC ACID LESIONS OF THE HIPPOCAMPUS. M.A. Good and R.C. Honey, (SPON: Brain Research Association) Dept. Pharmacology, University of Edinburgh, Scotland, EH8 9JZ, and Dept. Psychology, University of York, England, YO1 5DD.

Using a Pavlovian contextual switching paradigm, Good and Honey (Behav. Neurosci., in press) recently showed that rats with electrolytic lesions of the hippocampus (HPC) are impaired in the use of contextual cues to retrieve associative information (see Hirsh, 1974, Behav. Biol.,12, 421-444). The aim of the present study was to determine whether rats with ibotenic acid lesions of the HPC are impaired in contextual retrieval using the same switching procedure. In this task subjects receive reinforced presentations of stimulus X (train of clicks) in context A and of stimulus Y (offset of a light) in context B and nonreinforced presentations of both X in context B and Y in context A. The contexts (Skinner boxes) were discriminated by odour and visual cues. Initial results indicate that ibotenic lesioned animals are impaired in the acquisition of contextual switching ($F=10.29$, df 1/14, $p<0.01$), but showed no impairment in the acquisition of a Pavlovian contextual discrimination in which food is delivered in context A but not in context B. These findings imply that damage to hippocampal cell fields are responsible for the impairment in contextual retrieval, but it is not yet possible to determine whether the magnitude of the deficit following ibotenic acid lesions is comparable to that observed following electrolytic lesions of the HPC.

53.6

CONCURRENT SPATIAL AND NONSPATIAL DISCRIMINATION LEARNING: DISRUPTION OF LEARNING BY INTERFERENCE AND HIPPOCAMPAL LESIONS. C.A.Stewart, M.A.Good and R.G.M.Morris, (SPON: B.R.A.). Dept. Pharmacology, Univ. Edinburgh, Edinburgh EH8 9JZ, Scotland.

Concurrent discrimination tasks have proved a puzzling part of test batteries for examining the contributions of the medial temporal lobe to memory in primates (Malamut et al, 1984, *Behav. Brain Res.*; Zola-Morgan and Squire, 1985, *Behav. Neurosci.*). The usual procedure involves simultaneous training on a number of problems of the *same* class (eg discrimination learning) with each problem having *distinct* stimulus items. However, the concurrent learning of *different* types of task has rarely been studied in either primates or rats.

Rats were trained in a watermaze on both a 2-platform spatial task and a similar 2-platform visual discrimination. Each task employed a rigid platform providing escape from the water and a floating platform which did not. These platforms were identical in appearance for the former task or visually distinct for the latter. Both tasks could be learned by normal rats (N=8) to a criterion of 90% correct when training was conducted on each task alone (10 trials/day). However, when training on the two tasks was combined on the same day (10+10 trials/day), normal rats learned the spatial task but failed to learn the visual discrimination. One possible explanation could be interference with visual learning by spatial information. As rats with aspiration hippocampal (HPC) lesions are impaired in learning this spatial task (Morris et al., 1986, *Q.J.E.P.*), we investigated whether rats with ibotenate HPC lesions (N=4) would, when trained concurrently, learn the visual task because they would fail the spatial version. However, the results showed that control rats (N=4) again learned the spatial but not the visual task, while HPC-lesioned rats failed to learn either task. These findings imply that either HPC-lesioned rats process spatial information (even though they fail to learn the spatial task), or that other aspects of the training procedure are responsible for the interference between tasks.

53.7

THE ROLE OF THE HIPPOCAMPUS IN OLFACTORY DISCRIMINATION LEARNING IN RATS. I.C. Reid, D.M. Bannerman and R.G.M. Morris, SPON: B.R.A. Dept. of Pharmacology, 1 George Square, University of Edinburgh EH8 9JZ, Scotland.

We have previously reported that although olfactory discrimination learning in rats is both very rapid and shows progressive improvement across serial novel problems, these characteristics of olfactory learning do not, as has been claimed, represent true 'learning set' acquisition (Reid and Morris, 1989, *Soc. Neurosci. Abstr. 15*). Rodent olfactory discrimination learning may, nevertheless, be a useful 'model system' for investigating specific issues in the neurobiology of learning and memory. As studies of the effects of indirect interference to the hippocampal formation (e.g. fornix, entorhinal and lateral olfactory tract lesions) have produced conflicting results, we examined the effects of selective hippocampal lesions.

Rats (n=20) were trained on a simultaneous 2-odour discrimination problem and, after matching for initial discrimination score, half the subjects were given bilateral ibotenate hippocampal lesions (Jarrard, 1989, *J. Neurosci. Meth.*). They and sham lesioned rats were then trained on a further 5 different 2-odour problems, followed by a reversal of the final problem. In conflict with findings using fornix lesions (Eichenbaum et al, 1988, *Behav. Neurosci.*), no significant impairment was found on any of the 5 postoperative problems ($F<1$). The reversal training revealed no evidence of forgetting over 24 hours, nor any enhancement in reversal learning. The same hippocampal lesioned rats were, however, impaired in acquisition of a watermaze spatial reference memory task and in searching the correct quadrant during a transfer test after training ($F=5.4$, df 2/36, $p < 0.005$).

These findings call into question the notion that the integrity of the hippocampus is required for simple olfactory discrimination learning.

53.8

7-CHLOROKYNURENIC ACID, AN NMDA RECEPTOR ASSOCIATED GLYCINE SITE ANTAGONIST, IMPAIRS PLACE NAVIGATION PERFORMANCE. D.M.Bannerman, S.P.Butcher and R.G.M.Morris, (SPON: B.R.A.). Dept. of Pharmacology, Univ. of Edinburgh, Edinburgh, EH8 9JZ, Scotland.

Blockade of NMDA receptors by the competitive antagonist D-AP5 impairs spatial learning across a comparable dose range to inhibition of hippocampal LTP *in vivo* suggesting that LTP-like events in hippocampus may be part of the neural mechanisms of spatial learning (Davis et al, 1991, *submitted*). Antagonism of the NMDA-associated glycine receptors provides a different method of investigating this issue. 7-Chlorokynurenate (7CK), which limits the induction of hippocampal LTP *in vitro* (Bashir, Tam and Collingridge, 1990 *Neurosci. Letts* 108, 261-266), was chronically infused via osmotic minipumps into the right lateral ventricle. Performance of drug treated animals in a transfer test after spatial reference memory training in a watermaze was impaired relative to CSF infused and unoperated controls ($F=11.63$, $p<0.005$). However, when LTP was subsequently examined *in vivo* under urethane anaesthesia (1.5gm/kg) in the left hippocampus, a proportion of drug treated animals showing behavioural deficits failed to exhibit any blockade of LTP. Tissue levels of 7CK from various brain regions were assayed using a novel analytical technique involving reverse phase HPLC followed by fluorescence detection enhanced by post-column application of zinc acetate. Preliminary measurements indicate that 7CK levels were substantially lower in the left hippocampus (28.9 pmoles/mg wet weight) than in the right hippocampus (50.1) and lower still in other brain regions (mean for visual cortex, frontal cortex and anterior striatum = 3.9), suggesting that 7CK may be rapidly removed from the brain and only found in high concentrations adjacent to the infusion cannula. The uneven cerebral distribution of 7CK may explain our failure to block LTP in the left hippocampus in animals which, nevertheless, show a behavioural deficit. Further investigations are underway.

53.9

EFFECTS OF ENTORHINAL LESIONS ON TESTS OF SPATIAL AND NONSPATIAL MEMORY IN THE RAT. L.A. Rothblat, A.M. Brown*, T.C. Gleason*, A.D. Davis*, N. Vnek*, and L.F. Kromer, Dept. of Psychology, The George Washington University, Washington, D.C., 20052 and Dept. of Anatomy and Cell Biology, Georgetown University School of Medicine, Washington, D.C., 20007.

Entorhinal cortex is a key relay station between the neocortex and the hippocampus, and as such is thought to play a critical role in mnemonic functions. To further explore the role of the entorhinal region, we tested rats with aspiration or excitotoxic (NMDA) lesions on tests of spatial (discrete trial rewarded alternation) and nonspatial (concurrent object discrimination) memory. The concurrent task has previously been used to assess mnemonic disturbances in monkeys with hippocampal system damage and amnesic patients, including those with Alzheimer's Disease.

Both entorhinal groups were impaired on the concurrent task. Rats with aspiration lesions were also impaired on the spatial task, whereas rats with NMDA lesions did not differ from controls.

The results indicate that concurrent object discrimination may be a particularly sensitive assay of entorhinal/hippocampal dysfunction, and that these circuits in the rat may serve mnemonic functions which are qualitatively similar to those of human and nonhuman primates. Supported by ONR N00014-88-K-0227.

53.10

DIFFERENTIAL EFFECTS OF ENTORHINAL CORTEX AND HIPPOCAMPAL LESIONS ON PERFORMANCE OF A SPATIAL LOCATION RECOGNITION TASK. D.L. Johnson and R.P. Kesner, Dept. of Psychology, Univ. of Utah, Salt Lake City, Utah 84112.

Rats were trained on an 8-arm radial maze on a spatial location recognition task. In this task, on each trial animals were allowed to visit 5 arms in a sequence predetermined by the experimenter. This was followed by a test phase in which the animals were required to choose between an arm visited and an unvisited arm. Tests were balanced across all 5 serial positions. A win-stay rule was required to receive additional reinforcement.

After the animals displayed better than chance performance for all serial positions, they received electrolytic lesions of either the entorhinal cortex (ENTO) or the hippocampal formation (HIPPO).

Following small (medial and lateral) ENTO lesions, performance for all serial positions except the first was at chance levels. Conversely, the small HIPPO lesions resulted in chance performance at all serial positions except the last. These results imply that the ENTO mediates the primacy component of the serial position curve, whereas the HIPPO mediates the recency component. Large lesions of the ENTO and the HIPPO resulted in chance performance at all serial positions. Based on the small lesion data , there appears to be a clear dissociation between the entorhinal cortex and the hippocampus, suggesting that even though the two structures are closely interrelated, each may play a unique role in a spatial location recognition task.

53.11

CONTINUOUS RECOGNITION OF 3-DIMENSIONAL OBJECTS IN RATS WITH HIPPOCAMPAL AND MEDIAL PREFRONTAL CORTEX LESIONS. P. Jackson-Smith, R. P. Kesner, and A. A. Chiba. Dept. of Psychology, Univ. of Utah, Salt Lake City, UT 84112.

The purpose of the present experiment was to compare performance on a spatial continuous recognition task (Jackson-Smith & Kesner, 1990) with performance on an analogous nonspatial task with the same processing demands, in hippocampal and medial prefrontal cortex lesioned rats. Each session involved sequential presentation of eight new objects, and four repeated objects (chosen from the eight). The objects consisted of 3-dimensional 'toys' in various shapes, sizes, textures and brightnesses. Repeated objects had lags ranging from 0 to 4 (from 0 to 4 different object presentations between the first and the repeated presentation). An object was presented on one side of a long table divided in half by an opaque Plexiglas guillotine door, and latency to move the object upon opening the door was measured. The first presentation of the object resulted in food reinforcement; however, no food was available upon the second presentation of the same object. For each block of 15 sessions the objects were randomly presented, and 8 new objects were chosen for each session without repetition (120 distinct objects). Rats were assigned to groups and received surgery upon completion of 3 blocks of 15 sessions. The latencies at each lag prior to surgery were shorter for object continuous recognition as compared to the analogous spatial task, however, all of the rats showed a decreasing function with long latencies for the shorter lags and decreasing latencies with increasing lag. There were no deficits following sham lesions, and, in contrast to the spatial continuous recognition task, there were mild deficits following lesions to the hippocampus and no deficits following lesions to the medial prefrontal cortex. In conclusion, hippocampus has a disproportionately greater involvement in recognition of spatial locations versus recognition of 3-dimensional objects.

53.12

A DOUBLE DISSOCIATION BETWEEN IMPLICIT AND EXPLICIT SPATIAL MEMORY FOLLOWING HIPPOCAMPAL AND PARIETAL CORTEX LESIONS. A.A. Chiba, P. Jackson-Smith and R.P. Kesner,
Department of Psychology, University of Utah, Salt Lake City, UT 84112

Rats were trained on a 12-arm radial maze using a continuous recognition procedure. Animals were allowed to visit a sequence of 12 arms per day in an order predetermined for that trial. Of the 12 arms visited, either 3 or 4 of the arms were repeated within the running sequence. The arms selected for repetition varied according to lag (0-6), or the number of arms which occurred between the first visit to an arm and its repetition. In order to gain access to each arm, the animal was required to orient to a cue on the Plexiglas door at the entrance of the arm. Once the animal oriented to the cue, the door was lowered and the latency for the animal to reach the end of the arm was measured. Two groups of animals were trained, one on an implicit training procedure and one on an explicit training procedure. The implicit group received reinforcement at the end of each arm regardless of whether the arm was a novel arm or a repeated arm. This group showed decreased latencies when visiting repeated arms. The explicit group received reinforcement only when visiting an arm for the first time in a given sequence. This group showed increased latencies for repeated arms. Following total hippocampal ablation, the performance of the animals in the implicit condition did not differ significantly from preoperative performance, whereas animals in the explicit condition showed a deficit i.e., a significant decrease in latency to return to an arm. Following parietal lesions, animals in the implicit condition showed a deficit i.e., an increase in latency to return to an arm, whereas the performance of animals in the explicit condition did not differ significantly from preoperative performance. Sham operated control animals and cortical control animals did not differ significantly from preoperative performance in either reinforcement condition. Thus, a double dissociation appears to exist between parietal cortex and hippocampus for implicit versus explicit memory for spatial location.

53.13

HIPPOCAMPAL LESIONS DO NOT DISRUPT ACQUISITION OF LEARNING IN AN OCCASION SETTING PARADIGM. D.M. Skinner, G.M. Martin*†, C. Harley†, B. Kolbt, A. Pridgar*, A. Bechara and D. van der Kooy, Dept. of Anatomy, University of Toronto, Toronto, M5S 1A8, †Dept. of Psychology, Memorial University, St. John's, A1B 3X9, ‡Dept. of Psychology, University of Lethbridge, Lethbridge, T1K 3M4.

Hippocampal lesions do not disrupt simple Pavlovian conditioning, but performance on more complex conditional and configural tasks is impaired. We examined whether removal of the hippocampus would impair performance on a discrimination learning task that employed conditioned taste aversions. On some days rats were injected s.c. with a drug (either 15 mg/kg sodium pentobarbital or 5 mg/kg morphine sulfate) 15 min prior to 15 min access to a flavored solution. Removal of the solution was followed by an i.p. injection of LiCl. On alternate days injections of physiological saline preceded and followed access to the same flavored solution. Animals rapidly acquire the discrimination, consuming significantly more of the flavored solution after saline injections than after drug injections. Animals with aspiration or ibotenic acid lesions of the adult hippocampus or neonatal aspiration lesions of the cortex (a major source of afferents to the hippocampus) learned the task as well as controls. However, these lesions did disrupt spatial learning in the Morris water maze in the same animals. Animals with hippocampal lesions also learned, as well as controls, a discrimination task in which visual and textual cues, rather than drug states, predicted whether access to a flavored solution would be followed by an injection of LiCl or saline. We have shown previously that animals do not learn the discrimination tasks on the basis of simple Pavlovian associations (J. Exp. Psych.- Anim. Beh. Proc. 16: 56-68, 1990), suggesting that these may be configural tasks. The present data clearly demonstrate that the hippocampus is not necessary for the acquisition of such discrimination learning. The hippocampus may mediate only a subset of learning classed as configural. The robust (and non-cortically mediated) nature of taste-sickness associations may permit configural learning involving these associations that is independent of the hippocampus.

53.14

HIPPOCAMPAL LESIONS IMPAIR ACQUISITION OF THE TRANSVERSE PATTERNING PROBLEM BUT NOT OF SIMPLE DISCRIMINATIONS. M.C. Alvarado and J.W. Rudy, Psych. Dept., University of Colorado, Boulder, CO 80309

Sutherland and Rudy (Psychobio., 17, 1989) have proposed that the hippocampal formation (HF) contributes to learning and memory by providing the neural basis for the acquisition of configural associations. Such associations are necessary when the conditional relations among groups of stimuli are more predictive of reward than the individual elements. To test this theory, we have developed a task that demands a configural solution and have tested the effects of HF lesions on performance of that task. We have reported that animals with lesions of the HF are impaired on retention of the Transverse Patterning (TP) problem (Alvarado & Rudy, Soc. Neurosci. Abst., 1989). Briefly, this problem requires animals to solve three simultaneous visual discriminations as follows: A+ vs B-; B+ vs C-; C+ vs A-. Animals trained to solve this problem were impaired on reacquisition when subjected to post-training lesions of the HF. The present study examined the effects of HF lesions on acquisition of the TP problem and of three simultaneous non-configural discriminations (A+ vs B-; C+ vs D-; E+ vs F-). Twenty male Long-Evans rats received combined colchicine/kainic acid lesions of the HF or sham-lesions. After recovery, animals were trained to solve either the (TP) problem or three simultaneous simple discriminations. HF lesioned animals were unable to solve the TP problem, although they could learn any two of the TP discriminations concurrently. HF lesions did not affect learning of simple discriminations. These results are consistent with the hypothesis that the HF is necessary for acquisition of configural but not simple discriminations. (Supported by NIMH 1 T32 MH18882-03 to M.C.A. and NSF 9008251 to J.W.R.)

53.15

HIPPOCAMPAL LESIONS DO NOT IMPAIR INSTRUMENTAL CONDITIONING WITH DELAYED REINFORCEMENT. R.L. Port, K.S. Curtis*, C. Inoue*, J. Briggs* and K.S. Seybold. Departments of Psychology, Slippery Rock University, Slippery Rock, PA 16057 and Grove City College, Grove City, PA 16127

Damage to the hippocampus has been reported to abolish or marginally affect classical trace conditioning. Recent data suggests that the level of impairment may be related to the difficulty inherent to the specific task. Hippocampectomized animals show no impairment in instrumental conditioning when reinforced immediately after the response is given. The present experiment evaluated the ability of lesioned animals to associate a response with reinforcement given after a 5 sec delay.

Adult male rats were randomly assigned to nonoperated control, cortical or hippocampal lesioned groups. Aspiration lesions were accomplished while the animals were anesthetized. After a two week recovery period, animals underwent daily 20 min sessions of instrumental bar press conditioning using a 5 sec delay procedure. Results showed that all groups acquired the response at equivalent rates.

53.16

MK-801 FAILS TO ALTER INSTRUMENTAL CONDITIONING IN RATS. K.S. Seybold, R.D. Miller* and R.L Port. Departments of Psychology, Grove City College, Grove City, PA 16127 and Slippery Rock University, Slippery Rock, PA 16057.

Long-term potentiation is a form of synaptic plasticity that appears to be mediated by NMDA-receptors and is a primary candidate for a neurobiological mechanism underlying associative learning. MK-801, a potent and selective non-competitive NMDA receptor antagonist, has been shown to impair various forms of associative learning (classical conditioning, spatial cognition and passive avoidance). The present experiment evaluated the effects of MK-801 on instrumental conditioning of adult, male hooded rats. Animals were given daily, i.p. injections of either isotonic saline (CONT), .025 mg/kg MK-801 (LOW DOSAGE) or .05 mg/kg MK-801 (HIGH DOSAGE). Training consisted of daily 30 min sessions in which a bar-press response was autoshaped. Animals were trained to criteria and subsequently placed on extinction training. Results showed that MK-801 had no effect on acquisition or extinction rates. Consequently, this form of learning does not appear to be dependent on NMDA receptors.

53.17

QUINOLINIC ACID LESIONS OF THE DORSAL HIPPOCAMPUS IMPAIR ACQUISITION, BUT NOT WORKING MEMORY IN RATS .G.R. Dawson*, P. Bayley* A.C. Foster & S.D. Iversen. Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow. UK. CM20 2QR.

Lesions of cholinergic innervation to the hippocampus by neurotoxin- infusion into the medial septal area or by transections of the fimbria-fornix, induce working memory deficits in rats. We trained rats on a partially-reinforced-discrete-trial Fixed Interval 30s (prd FI30s) schedule prior to bilateral infusions of quinolinic acid into the dorsal hippocampus to destroy intrinsic neurons. Successful performance on the prd FI schedule requires intact reference memory and working memory. Post-lesion and pre-lesion performance on the prd FI 30s was comparable indicating that reference and working memory were intact and functioning normally. However, when the animals were transferred to a prd FI 8s schedule sham lesioned rats readily transferred to the new schedule requirements while lesioned rats transferred only after extensive training. It appears that a dorsal hippocampal lesion with quinolinic acid impairs the transfer of information to reference memory which is necessary for learning, but is without effect on working memory or retrieval from reference memory.

53.18

LATENT LEARNING AFTER RETROHIPPOCAMPAL CORTEX LESIONS IN RATS. H.Sundberg* and A.Bøkenes*. Inst. of Biological and Medical Psychology. University of Bergen, NORWAY. (SPON: European Brain and Behavior Society)

O'Keefe and Nadel (1978)[1] suggest that hippocampus might function as a neural substrate for a tolmanian cognitive map. Thus, destruction of the hippocampal area would abolish the latent learning effect in a maze.

By large lesions in the retrohippocampal area hippocampus was deprived of its main cortical input in 2 groups of rats. After operation one (N=8) explored a Lashley III maze for 10 min during 4 days, while the other (N=7) explored an open field. Two non-lesioned control rats were treated identically. Immidiately after the last pre-exposure the rats were water deprived. After 24 hours of deprivation the rats were tested on 4 consecutive sessions with 5 reinforced trials in the maze. Numbers of errors were scored.

The opportunity to explore the maze significantly reduced the number of errors during training in both the lesioned and non-lesioned rats as compared to their non-exposed controls.

We therefore conclude that rats with hippocampus deprived by its main cortical input can establish 'maps' of its environment.

[1] O'Keefe,J. and Nadel,L. 1978. The hippocampus as a cognitive map. Oxford Univ.Press, Oxford.

53.19

EFFECTS OF UNILATERAL ENTORHINAL CORTEX LESION AND GM1 GANGLIOSIDE TREATMENT ON WATER MAZE PERFORMANCE. **M. Glasier*, X. Chen*, R. Sutton and D. Stein. Brain Research Laboratory, I.A.B., Rutgers University, Newark, NJ 07102.**

Adult male rats with sham (S) or electrolytic lesion (L) of the left entorhinal cortex received GM1 (30 mg/kg, i.p.) or saline (SAL) injections for 14 days after surgery. From day 7 to 16 rats were given 2 daily trials (T), spaced 1 hr apart, in a water maze task where the escape platform position changed daily (clockwise rotation over 4 quadrants). Daily starting positions remained constant with respect to platform, with T2 beginning on the pool side opposite T1. S+GM1 and S+SAL groups did not differ on any of the dependent measures (swim distance on T2, perseverations, T1 - T2 latency). ANOVAs revealed that L+SAL rats were significantly (p's <.05) impaired (vs shams) on all measures whereas L+GM1 animals did not differ significantly from shams or L+SAL. However, L+GM1 were better than L+SAL rats in all measures on 9 of 10 test days (p's <.01; binomial and chi-square analyses). Location and size of L did not differ between groups.

Supported by FIDIA and RO1NS25685

53.20

RETROGRADE AMNESIA OF LONG-TERM FEAR MEMORY FOLLOWING HIPPOCAMPAL LESIONS IN THE RAT. J.J. Kim and M.S. Fanselow.
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The hippocampus seems to serve a temporary memory storage function because when damaged, recent but not remote memories are selectively impaired. This retrograde amnesia has been demonstrated in monkeys in a positively reinforced two-choice object discrimination task (Zola-Morgan & Squire, 1990). In the present study, we examined whether retrograde amnesia can be demonstrated in rats by using fear conditioning as assessed by the freezing response. Animals were placed in a novel context where they received 15 tone-footshock pairings (tone: 2000 Hz, 90 dB, 30-s; footshock: 1 mA, 2-s). A 3-min fixed ITI was used to ensure reliable conditioning to both tone and context. After training, electrolytic lesions were made in the hippocampus either 1, 7, 14 or 28 days after training. Contextual fear was completely abolished in the rats which received lesions 1 day after training. However, significant contextual fear was shown when lesions were made 7 days after training. The fear response to the tone was not affected by the lesions at any time. Thus, retrograde amnesia, selective to contextual fear, can be demonstrated in hippocampal lesioned rats.

54.1

THE RESPECTIVE ROLES OF THE HIPPOCAMPUS, AMYGDALA AND CAUDATE NUCLEUS IN LEARNING AND MEMORY: A TRIPLE DISSOCIATION. R.J. McDonald and N.M. White, Department of Psychology, McGill University, 1205 Dr. Penfield Ave, Montreal, Quebec, Canada H3A 1B1.

A standard set of experimental conditions for studying the roles of the hippocampal formation, the amygdala and the caudate nucleus in learning and memory was used employing the 8-arm radial maze: (1) a win-shift version that is sensitive to damage to the hippocampal system (Olton, Walker & Gage, 1977). (2) a win-stay version that is sensitive to damage to the cortico-striatal system (Packard, Hirsh & White, 1989). (3) a conditioned cue preference (CCP) version that is sensitive to damage to the amygdalo-ventral striatal system (Hiroi, McDonald & White, 1990). The three tasks used visual cues to guide behaviour and food as the reinforcer. Electrolytic lesions of the fornix/fimbria impaired performance on the win-shift task but not the win-stay or CCP tasks. Neurotoxic (NMDA) lesions of the hippocampal formation also impaired performance on the win-shift task. Electrolytic lesions of the lateral amygdala impaired performance on the CCP task but not the win-shift or win-stay tasks. Neurotoxic lesions that included the lateral amygdala also impaired performance on the CCP task. Electrolytic lesions of the caudate nucleus impaired performance on the win-stay task but not the win-shift or CCP tasks. The results support the notion that learning and memory in the mammalian nervous system is mediated by anatomically distinct structures that may acquire different kinds of information in parallel about the organism's internal and external environment.

54.2

RADIAL-ARM MAZE RESPONSE STRATEGIES IN JUVENILE AND ADULT RATS. **J.L. Hall & R.F. Berman. Department of Psychology, Wayne State University, Detroit, MI 48201.**

Previous research demonstrates that juvenile rats solve the radial arm maze by selecting adjacent arms, while adult rats use a less restrictive response strategy. The present experiment replicates and extends these findings to include a third group of rats tested both as juveniles and as adults, and compares these response patterns in two strains of rats.

Radial 8-arm maze performance was assessed daily for 10 days in juvenile and adult Long Evans and Sprague-Dawley rats. In both strains of rats, juveniles made significantly more adjacent arm entries than did adults. Additionally, both strains of rats when re-tested as adults, continued to use the adjacent arm entry strategy established during juvenile training, rather than adopting the maze-naive adult strategy.

Together, these results support previous findings demonstrating that juvenile rats use an adjacent arm entry strategy to solve the radial 8-arm maze, while adult rats do not. Additionally, these findings suggest that juvenile maze experience produces a long lasting change in the response patterns used by rats in the radial 8-arm maze. (Supported by NIAAA Research Grant #P50AA07606).

54.3

ACQUISITION OF SPATIALLY CUED LEARNING BEHAVIORS FOLLOWING HIPPOCAMPAL LESIONS IN NEWBORN RATS. K. L. Altemus and C. R. Almli. Devel. Neuropsychobiol. Lab., Washington University, St. Louis, MO 63110.

Neuropsychological evidence obtained from amnesic patients and behavioral data from studies on hippocampally lesioned adult rodents and primates suggest that the hippocampus mediates some types of learning and memory, including object localization in space.

This spatial learning ability develops as early as postnatal day 18 in rats, and the early emergence of this ability is influenced by previous experience on the task.

The present research evaluated the effects of electrolytic hippocampal lesions sustained on postnatal day 1 on the development of spatial learning abilities of albino rats in the Morris water maze. As suggested by previous reports of neurochemical lesions, the present results indicate that spatial learning abilities are disrupted (delayed or abolished) by neonatal hippocampal damage. (Conducted under NIH Guide for Care and Use of Laboratory Animals)

54.4

THE EFFECTS OF NEONATAL LIMBIC LESIONS ON LONG-TERM RECOGNITION MEMORY IN THE ADULT RHESUS MONKEY. R.C. Saunders, R.S. Richards*, and J. Bachevalier, Clinical Brain Disorders Branch, NIMH, Washington, D.C., 20032 and Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

When tested on a paired comparison recognition memory task, normal adult monkeys perform at a high level of proficiency even at delays of several weeks (>80%), whereas monkeys with amygdalo-hippocampal removals remain at chance with delays of only a few min. Equally impaired on the task are infant monkeys with the same limbic lesions. Here, we re-evaluated the performance of adult monkeys with neonatal removals of the amygdala (A), hippocampus (H), or both (AH) in a paired comparison task. Preference for novelty was assessed for delays of 10s, 5-10 min, 24-48 hrs, and 1-2 wks. Although neonatally operated monkeys in all 3 groups performed at chance when they were 1 month old, they all showed substantially better performance on this task when they were retested as adults (6-7 years). Indeed, the animals in Groups A and H performed nearly as well as normal controls at all delays, whereas our preliminary data indicate that those with the combined AH removal, though improved, never reached the level of proficiency of controls, even at the short delays. These data indicate that there exist some level of recovery of visual recognition memory in adult monkeys with neonatal limbic lesions.

54.5

DEFICIT IN RESPONSE SUPPRESSION IN THE JUVENILE RAT AFTER NEONATAL HIPPOCAMPAL X-IRRADIATION J. L. Diaz-Granados, P. L. Greene, N. J. Lobaugh & A. Amsel. Department of Psychology and Institute for Neuroscience, University of Texas, Austin, Texas, 78712

Response suppression (inhibition) has been related to mature and intact hippocampal function (Altman, Brunner, Bayer, 1973; Douglas, 1972). One way to test for response suppression is to use discontinuously negatively correlated reinforcement (DNC), differential reinforcement of slow responding. In our case, the rat is reinforced for traversing a straight runway, but only if it takes 4 s or longer. Weanling-age rats, with still developing hippocampi, do not suppress responding on a DNC schedule as adult rats do (Chen, Gross, Stanton, & Amsel, 1980). Adult rats, treated with a glutaminergic antagonist (into hippocampus), are deficient in response suppression on a DRL task, an operant analog of DNC (Tonkiss, Morris, and Rawlins, 1988). We examined the effects on DNC in juvenile rats of x-irradiation-induced hippocampal insult. Neonatal rats were x-irradiated from postnatal day (P) 2 to P15, resulting in severe agenesis in postnatally arising granule cells of the hippocampal dentate gyrus. Controls were sham-irradiated. Rats were tested at P35-39. X-irradiated subjects received a significantly lower percentage of reward throughout training than the controls. Running speeds were also significantly higher in the x-irradiated animals. This finding suggests that hippocampal damage in infancy, which reduces the capacity to inhibit or suppress behavior may be related to hyperactivity. Supported by NSF grant BNS-8609877 and NIAAA grant AA07052.

54.6

GRANULE CELL LOSS FOLLOWING ADRENALECTOMY HAS NO EFFECT ON THE ACQUISITION, RETENTION AND REVERSAL OF PLACE LEARNING IN THE MORRIS WATER MAZE. J. N. Armstrong D. C. McIntyre, S. Neubort and R. S. Sloviter. Department of Psychology, Carleton Univeristy, Ottawa, Ontario, K1S 5B6; Neurology Research Center, Helen Hayes Hospital, West Haverstraw, New York 10993.

Adrenalectomy of adult male rats results in substantial granule cell loss in the dentate gyrus 3 to 4 months after surgery. We report here that 4 months after surgery, 45 adrenalectomized (ADX) rats, with weight gain 50-200 grams below controls, displayed behaviour in the Morris water maze which was similar to 16 sham-operated control rats and 16 ADX rats maintained on corticosterone throughout the study. The loss of granule cells in the ADX rats varied from minimal to extensive, with the latter characterizing those with lowest weight gain. In spite of extensive granule cell loss, these ADX animals performed similar to controls in acquisition and retention of place learning. They also quickly learned a new location (reversal), resisted manipulation of a subset of extramaze cues and maze rotation (intramaze cues), but like controls, performed poorly when the hidden platform was randomly varied. Thus, under these conditions, a normal compliment of granule cells is not necessary for spatial learning or retention.

54.7

PATTERNS OF NATURAL SPATIAL BEHAVIOR PREDICT HIPPOCAMPAL SIZE IN KANGAROO RATS. L. F. Jacobs and W. Spencer*. Department of Psychology, University of Utah, Salt Lake City, UT 84112.

The size of the hippocampus, a forebrain structure in birds and mammals that mediates spatial learning, correlates with foraging demands on spatial memory in passerine birds and with sex-specific patterns of space use in microtine rodents (Jacobs et al.1990. *PNAS*, 87, 6349-6352). Here we report that natural space-use patterns predict hippocampal size within and between two species of kangaroo rats (*Dipodomys*). Differences in foraging behavior suggest that Merriam's kangaroo rat (*D. merriami*) experiences greater selective pressure on spatial abilities than bannertail kangaroo rats (*D. spectabilis*); sex-specific differences in mating strategy suggest that males of both species experience greater selection pressure on spatial cognition than females. As predicted, hippocampal size (relative to brain size) is larger in Merriam's than in bannertail kangaroo rats (19%); allometric arguments suggest the difference is closer to 31%. Also as predicted, males have larger hippocampi than females (12% in *D. merriami*, 16% in *D. spectabilis*). This suggests that two types of selection pressures, natural selection for foraging efficiency and sexual selection for the efficient location of mates, operate on hippocampal size. These results also suggest that sex differences in hippocampal size may be the dominant mammalian pattern. Supported by NSF Environmental Sciences Postdoctoral Fellowship BSR-880271 to L.F.J.

54.8

ANATOMICAL CONNECTIONS AND ELECTROPHYSIOLOGICAL INTERACTIONS BETWEEN PREFRONTAL AND PARAHIPPOCAMPAL CORTICES IN RABBIT. D.G. Harden+, N.E. Coniglio*+, T.W. Berger+, and D.J. Weisz*. Departments of Behavioral Neuroscience+ and Neurological Surgery*, University of Pittsburgh, Pittsburgh, PA 15260.

We have begun to investigate the anatomical connections and electrophysiological interactions between the prefrontal cortex and parahippocampal regions. Anatomical studies were conducted using intracranial injection of WGA-HRP, with subsequent reaction of tissue sections using tetramethylbenzidine. In the first experiment, we injected tracer into the mediodorsal nucleus of the thalamus, to establish the boundaries of the prefrontal cortex in rabbit. Both retrograde and anterograde label were present in the prelimbic area, the medial and lateral precentral area, and the anterior cingulate cortex. In the second experiment, we investigated the connectivity between prefrontal cortex and parahippocampal regions by injection into the prefrontal cortex. Retrogradely labeled cells were observed in the CA1 pyramidal layer, the subiculum, and the entorhinal cortex. Terminal fields were observed in the entorhinal cortex.

In electrophysiological experiments, the effects of prefrontal stimulation on dentate granule cell field potentials elicited by perforant path stimulation were examined. The dentate population spike could be facilitated by prior stimulation of the prefrontal cortex. Results of these studies indicate that reciprocal connections exist between prefrontal and parahippocampal cortices in rabbit and that these connections can alter the excitatory influence of entorhinal afferents on intrinsic hippocampal neurons. (Supported by MH45156 and MH00343)

54.9

ULTRASTRUCTURAL LOCALIZATION OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN THE RAT HIPPOCAMPAL FORMATION. T.A. Milner and E. Veznedaroglu. Dept. of Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021

The immunocytochemical localization of neuropeptide Y (NPY) was studied quantitatively in each lamina of CA1 & CA3 of the hippocampus and the dentate gyrus of adult rats. Intraperikaryal localization of NPY-like immunoreactivity (NPY-LI) was seen in Golgi complexes & rough endoplasmic reticulum. Neurons with NPY-LI often lacked astrocytic appositions and were contacted mostly on the shafts of large and small dendrites by unlabeled terminals forming asymmetric synapses. The types of associations and distribution of NPY-labeled terminals on targets were similar in each lamina. NPY-labeled terminals were associated (1) with one unlabeled perikaryon or dendrite; (2) with two unlabeled perikarya or dendrites simultaneously; or (3) with one NPY-containing perikaryon or dendrite. Most terminals with NPY-LI formed symmetric synapses or appositions without any glial intervention with small dendritic shafts. The remaining NPY-labeled terminals were closely apposed to unlabeled & NPY-labeled terminals or had no neuronal associations. These results provide cellular substrates for the inhibition (symmetric synapses) and modulation through presynaptic interactions of hippocampal neurons by NPY terminals. Moreover, they support the concept that hippocampal NPY neurons may be vulnerable to damage due to the presence of numerous presynaptic terminals which form excitatory (asymmetric) synapses and the sparsity of astrocytic invagination. Supported by MH42834 & HL18974.

54.10

MORPHOLOGY OF NEURONS IN THE ENTORHINAL CORTEX THAT PROJECT TO NEOCORTEX IN THE RAT AND MONKEY P.E. Good[1], P.R. Hof[1,2] and J.H. Morrison[1,2] [1]Fishberg Res. Ctr. for Neurobiology, and [2]Dept of Geriatrics, Mt Sinai Med. Ctr. N.Y.,N.Y, 10029

The entorhinal cortex (ERC) is the cortical link between neocortical and parahippocampal cortices and the hippocampal formation. Transport studies have demonstrated that polymodal and limbic areas such as the superior temporal gyrus, cingulate and insular cortices project to the ERC. However, few data are available concerning the reciprocating projections in particular, as well as the general organization of the ERC efferents to the neocortex. We have injected the retrograde tracer fast blue (FB) into premotor cortex of the rat and the dorsal bank of the superior temporal sulcus (STS) in the cynomolgus monkey. FB containing neurons within the ERC were subsequently filled with the fluorescent dye lucifer yellow and neuronal morphology analysed by confocal microscopy. The projection from ERC to STS arises from layer V of the caudal-lateral region of the ERC; fields Ei, Elc, and Ec of Amaral et.al. (J. Comp. Neurol, 1987), along with a few neurons in the medial region. In the monkey, these neurons can be grouped into three morphological types: 1) pyramidal, some with apical dendrites reaching layer I; 2) multipolar with dendrites confined to layer V, and 3) vertical fusiform with a long basal dendrite reaching the white matter. ERC neurons projecting to premotor cortex in the rat displayed a similar pattern of laminar distribution and morphologies with many neurons having their dendrites in a strict laminar confinement and extending 1000-2000 microns in the horizontal axis. Thus, some of these neurons may sample only a restricted set of afferents, likely to be hippocampal in origin while other neurons are in a position to receive afferents synapsing in all lamina of the entorhinal cortex and may monitor either hippocampal and/or neocortical input. Supported by NIH grants AG05138 and AG06647.

54.11

THE ENTORHINAL CORTEX IN HUMANS: A CYTOARCHITECTONIC AND COMPARATIVE STUDY WITH NON-HUMAN PRIMATES. T.M. Hyde and R.C. Saunders. Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.

The entorhinal cortex has long been considered a relay for cortical sensory information into and out of the hippocampal formation. There is renewed interest in the entorhinal region because recent experimental work with monkeys has demonstrated extensive subcortical projections and the ability of these connections to support some level of cognitive memory function. In addition, recent clinico-neuropathological reports have found neurodegenerative changes in the entorhinal cortex related to Alzheimer's disease and developmental abnormalities associated with schizophrenia. As part of our ongoing work studying the entorhinal cortical region in primates we have examined 7 normal human brains. Nissl, AChe and fiber staining methods were used to identify morphologically the architectonic regions of area 28. For comparison we have emphasized the cytoarchitectonic features previously described in the Rhesus monkey. Like the monkey the different regions of entorhinal cortex are distinguished largely by the appearance of layer II and the lamina dissecans, and can be divided into five main regions. Differences between the monkey and human entorhinal region are addressed.

54.12

PROJECTION OF THE ENTORHINAL LAYER II NEURONS AS REVEALED BY INTRACELLULAR PRESSURE-INJECTION OF NEUROBIOTIN

N. Tamamaki and Y. Nojyo* Dept. of Anatomy, Fukui Medical School, Matsuoka, Fukui 910-11 Japan

The hippocampus plays a key role in short term memory formation and its information processing is initiated by input conveyed through the perforant path. In this study, the major part of the perforant path, projection of the entorhinal layer II neurons was investigated by neurobiotin pressure intracellular-injection in anesthetized rats. Thus far, three spinous neurons were well labeled including their axonal arbors in the hippocampus. Some collaterals originating from the axon project also to the subiculum. An axon of the single spinous neuron covers the hippocampus more than 2 mm in septo-temporal direction, and the infrapyramidal blade, the suprapyramidal blade, the str. moleculare of field CA3 and CA2 at the same time by making a sheet-like form. The sheet of axon branches was thin in the suprapyramidal blade, the str. moleculare of field CA2 and CA3. However the sheet became not clear in the crest and in the infrapyramidal blade. This study shows that the entorhino-hippocampal circuit is not a simple circle but more complex than ever thought.

54.13

THREE DIMENSIONAL MAPPING OF BRAIN LESIONS IN THE PRIMATE BRAIN. H. Damasio, R. Frank*. Dept. of Neurology (Div. of Behavioral Neurology and Cognitive Neuroscience) and the Image Analysis Facility, The University of Iowa, Iowa City, IA 52242.

We have developed a technique for 3-dimensional reconstruction and analysis of brain lesions *in vivo*, based on the manipulation of high resolution magnetic resonance raw data obtained with a particular protocol. The technique permits the direct visual identification of neuroanatomical landmarks such as sulci and gyri in each individual brain specimen and eliminates the need to rely on averaged templates of standard brain sections, the use of which is a source of inevitable lesion localization error. The technique allows for the bi-directional cross reference between marked data points in the 2-dimensional sections and in the volume reconstructions, and for the projection of marked subcortical structures onto the 3-D cortical surface. The technique also allows for volume or surface measurements of individual brain structures or lesions. We will present examples of the application of this technique which include, among others: (1) research with the lesion method in both human and nonhuman primates; (2) planning of neurosurgical approach to abnormal tissue to be excised in humans; (3) anatomical analysis of P.E.T. data; (4) the teaching of neuroanatomy.

54.14

HUMAN-LIKE SHORT-TERM MEMORY FOR TEMPORAL ORDER IN NON-HUMAN PRIMATES. E.C. Gower. Boston VAMC and Boston University School of Medicine, Boston, MA 02130.

A normal adult cynomolgous monkey tested in a manually operated testing apparatus (WGTA) mastered an order discrimination problem of the following type: if the presentation of object A is followed by object B, choose A when the two objects are presented simultaneously. The performance rule is isomorphic with the formulation "choose the first object in the list". An expanded version of the two-object problem was then used to evaluate memory for temporal order. The paradigm was modeled on a two-alternative, forced-choice judgment of recency (JOR) protocol used to study serial order memory in human subjects. The discriminanda were familiar objects randomly drawn from a pool of 200. On any trial, 5 objects were presented successively at 10 s intervals followed by a single test probe. The probe was a pair of objects representing one of all possible pair-wise comparisons of the order of objects in a series of 5, and the subject signaled 'which came first' by making a choice of the least recent of the two. A single object from each list position was also tested against a foil. A foil was an object that did not appear in the list, and therefore was always the least recent object when it appeared in the test probe. Eighty trials were accumulated in each of the 15 possible probe combinations.

The accuracy of order discriminations depended on the lag of both objects in the probe. Probe lag is the serial distance of an object in the presentation list from its appearance at test: the last object in the list has a lag of 1, and the penultimate object a lag of 2, etc. For any lag of the most recent object in the probe, accuracy increased directly with *increasing* lag of the distant object in the probe (the one that was chosen); and for any lag of the distant object, accuracy increased directly with *decreasing* lag of the most recent object. This dual dependence on probe lag is characteristic of human JOR. The monkey, like human subjects in a comparable design, also treated the foils as if they were objects with a lag greater than the longest lag of any object in the list. These findings suggest that both monkeys and humans rely upon similar cognitive processes in recalling the order of random events.

54.15

METABOLIC ACTIVATION OF DORSOLATERAL PREFRONTAL AND INFERIOR PARIETAL CORTEX BY COGNITIVE PROCESSING: A 2-DG STUDY IN RHESUS MONKEYS PERFORMING MNEMONIC TASKS.
H. R. Friedman, S. Bhalla and P. S. Goldman-Rakic, Sect. of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

The dorsolateral prefrontal cortex (PFC) and the inferior parietal lobe (IPL) of the rhesus monkey are related both anatomically and functionally. In the course of our 2-DG studies of the pattern of metabolic activation underlying working memory processing in the rhesus monkey, we examined these cortical regions to elucidate their contribution to cognitive performance in the monkey. For the present analysis, we also examined activity in the lateral posterior (LP)-pulvinar complex of the thalamus-components of which are known to be reciprocally connected with both IPL and, to a lesser extent, with dorsolateral PFC. In these 2-DG experiments, monkeys were trained to perform on either 1 of 3 working memory tasks (Work group) or on 1 of 2 control tasks that do not engage working memory (Cont group). ^{14}C-2DG (100μCi/kg) was administered before the final 45 min test session. Local cerebral glucose utilization (LCGU) rates were determined for specific brain regions using a computerized image analysis system and were compared across groups using the analysis of covariance.

The results of this analysis showed that LCGU in the Principal Sulcus region of the dorsolateral PFC and in several regions of IPL (7a,b, 7ip, 7m) was significantly enhanced in the Work group relative to the Cont group. However, LCGU in the LP-pulvinar complex was not enhanced. These findings for the LP-pulvinar complex contrast with our previous report that LCGU in the mediodorsal thalamus (MD) is significantly enhanced in monkeys performing working memory tasks (Friedman et al., J. Cog. Neurosci., 2, 18-31, 1990). As MD is the principal thalamic connection of the dorsolateral PFC and, the dorsolateral PFC and IPL are reciprocally interconnected, the present data, taken together with our previous findings, suggest that working memory processing in the monkey activates a selective complement of brain regions which can be characterized by their association with the prefrontal cortex. Supported by USPHS grants.

54.16

TASK SPECIFIC MODULATION OF CEREBRAL BLOOD FLOW WITHIN THE HUMAN FRONTAL CORTEX. M.Petrides, B.Alivisatos*, A.C.Evans* and E.Meyer*. Montreal Neurological Institute, McGill Univ., Montreal, Quebec, CANADA H3A 2B4.

Patients with frontal cortical excisions are impaired on tasks in which they must monitor self-ordered responses and on tasks in which the responses are conditional upon the stimulus presented. Regional cerebral blood flow (rCBF) was measured by means of positron emission tomography in nine normal male volunteers in a) a control condition involving pointing to an abstract design, b) a self-ordered task in which the subjects had to monitor their pointing responses to abstract designs, and c) a conditional task requiring pointing to designs in response to differently colored stimuli. The normalized CBF in the control condition was subtracted from that in the two experimental conditions. Within the frontal cortex, there was, in the self-ordered task, a focal increase in rCBF in the right mid-dorsolateral frontal region and an increase extending over a slightly larger area within the left dorsolateral frontal cortex. By contrast, in the conditional task there were focal decreases in rCBF within the right lateral frontal cortex.

54.17

ALTERED SERIAL POSITION LEARNING AFTER HUMAN FRONTAL LOBE LESIONS. **L.M. Grattan & P.J. Eslinger. Depts. of Neurology, Univ. of Maryland, Baltimore, MD. 21201 & Penn State Univ., Hershey, PA 17033.**

The serial position function refers to the more rapid learning of the early (primary effect) and late (recency effect) items of a list relative to middle items. Human and experimental animal studies have demonstrated impaired primacy but not recency following hippocampal lesion and in most amnesias. However, serial position effects after frontal lobe lesion are less well known. Such data may provide insight into the learning difficulties of frontal lesion patients. Furthermore, several processes associated with the frontal lobe, such as learning in the presence of interference and temporal ordering, may be important contributors to normal serial position effects. To test this hypothesis, we studied 40 patients with CT/MR verified frontal and non-frontal lesions with a standardized measure of word list learning. Findings indicated that despite a similar number of free recall responses, the groups differed in serial order effects. A prominent, significant serial position curve was found in the non-frontal lesion group. In contrast, the frontal lesion group demonstrated no significant differences among primacy, middle and recency items. This change in the serial position function suggests that altered learning processes are evident after frontal lobe damage.

54.18

RELATIONSHIP BETWEEN CT MEASURES OF REGIONAL BRAIN ATROPHY AND PET MEASURES OF REGIONAL GLUCOSE UTILIZATION IN ALCOHOLIC KORSAKOFF'S SYNDROME. E.M. Joyce*, D.E. Rio* M.J. Eckardt. Lab. of Clin. Studies, NIAAA, DICBR, Bethesda MD 20892.

In a previous report, patients with alcoholic Korsakoff's syndrome were shown to have relatively reduced rates of 18FDG uptake in cingulate, precuneate and right sensorimotor cortex and right thalamus, as well as increased uptake in cerebellum (Soc. Neurosci. Abstr. 16:571,1990). To assess whether this might be due to reduced neuronal mass rather than reduced neuronal function, regional brain atrophy was estimated on the CT scans of the same subjects (9 Korsakoff, 10 normals) by measuring sulcal and ventricular CSF content. Korsakoff patients had increased ($p < 0.01$) CSF in third and lateral ventricles, Sylvian fissures, and sulci of sensorimotor cortex, and had a trend ($0.01 < p \leq 0.05$) towards increased CSF over frontal cortex and cerebellar vermis. When group differences in relative rates of regional glucose utilization were re-analysed using appropriate regional estimates of tissue atrophy as covariates, the differences in right thalamus, sensorimotor cortex and cerebellum disappeared whereas those for cingulate and precuneate cortex remained significant. The data suggest that the structural lesions in this disorder give rise to dysfunction in a neural circuit which includes cingulate cortex and which is thought to mediate arousal and memory.

54.19

THE ROLE OF THE FRONTAL LOBES IN FREE RECALL: INTERFERENCE, ORGANIZATION, AND SERIAL POSITION EFFECTS. F.B. Gershberg and A.P. Shimamura. Department of Psychology, University of California, Berkeley, CA 94720.

Free recall ability was tested in patients with unilateral damage in dorsolateral prefrontal cortex. Patients and control subjects learned two lists of 15 unrelated items, with five study-test trials for each list and a break between lists. Frontal patients recalled fewer items overall but showed learning across trials. To assess interference effects, performance on the first trial of each list was analyzed, revealing a significant Group (patients vs. controls) X List (first list vs. second list) interaction. Specifically, the recall deficit shown by the frontal patients was greater for the second list than for the first list. The pattern of results suggests that the interaction was due to both the control subjects' "learning to learn" and the frontal patients' increased susceptibility to interference. A measure of subjective organization (the extent to which the same items are reported together across trials, corrected for level of recall) was used as an indicator of organizational strategies. Frontal patients were found to use less subjective organization, which may reflect a general deficit in the development and use of strategies. Serial position effects were also examined. Frontal patients showed a significant recency effect, but their recall of recency items was significantly lower than control subjects' recall of those items, indicating that short-term memory might be disrupted. These results suggest that dorsolateral prefrontal cortex normally plays a role in short-term (working) memory, developing and using learning strategies, and reducing the effects of interference. (Supported by NIH grant AG09055 to APS and an NSF Predoctoral Fellowship to FBG.)

54.20

DATA-BASED AND KNOWLEDGE-BASED MEMORY FOR TEMPORAL DISTANCES IN HYPOXIC BRAIN INJURED SUBJECTS. R.O. Hopkins and R.P. Kesner. Dept. of Psych., University of Utah, Salt Lake City, Ut 84112.

Subjects who have experienced a hypoxic episode and normal age matched control subjects were tested for temporal distance in both data-based and knowledge-based memory. Temporal distance is determined by the number of items that occur between the two test items. Temporal distances for words and spatial locations for new data-based memory were assessed. Subjects were shown a series of 8 words or 8 spatial locations. The items are shown one a time and then in the test phase subjects are shown 2 words or 2 X's each of which occurred in the study phase. Subjects are asked to choose the item that occurred earlier in the sequence. Choices occur across temporal distances of 0 to 6, with eight observations for each distance. Assessment of memory for temporal distances in the knowledge-based system is carried out using scripts and map tasks. Scripts are overlearned standardized episodes which occur both for linguistic and spatial information. Subjects are asked to remember 4 structured scripts (e.g. restaurant) and familiar routes (Salt Lake City) and 4 unstructured scripts (e.g. parade) and unfamiliar routes (Santiago). Data are collected for temporal distances of 0, 2, 4, and 6 with 4 observations for each distance.

Results show that hypoxic subjects are impaired across all distances for words and spatial locations. On the structured scripts and maps tasks hypoxic subjects are similar to control subjects across all distances. On the unstructured scripts task hypoxic subjects are impaired relative to control subjects for distances of 0 and 2, and then are the same as control subjects for distances of 4 and 6. On the unstructured maps task hypoxic subjects perform poorly relative to controls for all temporal distances. Subjects with hypoxic brain injury are impaired for new data-based temporal distance information but show no impairments for knowledge-based temporal distance information.

LEARNING AND MEMORY--PHARMACOLOGY: ACETYLCHOLINE I

55.1

SCOPOLAMINE DISRUPTS MAZE RETENTION IN TURTLES. C. A. Ritter* and A. S. Powers. St. John's University, Jamaica, NY 11439.

Recent studies from our laboratory have shown that lesions of the basal forebrain in turtles produce deficits in learning and memory. The basal forebrain of turtles contains cholinergic cells. The present study was conducted to determine whether scopolamine, a muscarinic receptor blocker, would disrupt maze retention in turtles.

Turtles (n=25) were trained in an X-maze for water reward. After reaching criterion, they were given five days rest and then retested on the maze to be certain their performance was at criterion. On the day following the second achievement of criterion, they were injected 30 min before running with either saline, scopolamine (1.6, 3.2, or 6.4 mg/kg IP), or methylscopolamine (6.8 mg/kg) (n=5 in each group). They were run on the maze under the drug condition on that day and then, without injection, until they again achieved criterion.

The results showed a dose-dependent disruption of maze retention by scopolamine. Methylscopolamine had no effect on performance. A supplementary study showed that the highest dose of scopolamine had no effect on three measures of unlearned behavior. The results suggest that acetylcholine plays an important role in associative functions in turtles.

55.2

BEHAVIORAL CONSEQUENCES OF THE DIFFERENTIAL EFFECTS OF EXCITOTOXIC LESIONS IN THE NUCLEUS BASALIS MAGNOCELLULARIS. Cheryl A. Harrington and Gary L. Wenk. Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Loss of cholinergic (CH) neurons in the nucleus basalis magnocellularis (NBM) is seen in Alzheimer's Disease (AD) and may underlie the associated memory impairment. Animal models of AD use excitotoxins to produce NBM lesions that mimic this CH cell loss. Ibotenic (IBO) and quisqualic (QUIS) acids produce a differential loss of NBM CH cells. We attempted to manipulate the differential vulnerability of CH NBM cells to IBO and QUIS by co-injection of selected ions (e.g. Zn & Mg), anti-oxidants (e.g. cystine & glutathione), receptor antagonists (e.g. CNQX) and neuromodulators (e.g. muscimol). The consequences of these injections on spatial memory was determined using a continuous non-match-to-sample task in a T-Maze. Performance was correlated to the loss of CH and non-CH neurochemical markers in the NBM, amygdala, and neocortex. The pattern of results suggests that non-CH NBM cells may play a role in learning and memory. Supported by the National Science Foundation (BNS 89-14941).

55.3

THE EFFECTS OF SCOPOLAMINE, MECAMYLAMINE, AND QUINPIROLE ON RADIAL-ARM MAZE PERFORMANCE OF RATS WITH FORNIX TRANSECTIONS. B. Osborne, R. Liddell* and J. Feeley*. Department of Psychology, Middlebury College, Middlebury, VT 05753-6145.

Both cholinergic manipulation and hippocampal damage disrupt memory function as measured by deficits in radial-arm maze performance. In the present experiment, rats with fornix transections and sham control rats were trained to asymptote on the radial arm maze and then tested following injections of the muscarinic blocker scopolamine (0.04 mg/kg; Sigma) the nicotinic blocker mecamylamine (2.5 mg/kg; Merck) and the D_2 agonist quinpirole (0.01 mg/kg; Lilly), both alone and in combination. All rats received all treatments separated by recovery days. Cholinergic blockers given singly or in combination reduced accuracy as measured by trials to repeat and increased run time; quinpirole had no effect alone. Quinpirole, however, reversed the effects of mecamylamine and scopolamine and mecamylamine in combination. The response to the drugs for the fornix transected rats differed significantly from those of controls but were fundamentally similar. The results suggest major differences between the hippocampal and cholinergic syndromes.

55.4

MUSCARINIC ACTIVATION OF THE MEDIAL SEPTAL AREA: IMPROVEMENTS IN WORKING MEMORY AND MODULATION OF HIPPOCAMPAL PHYSIOLOGY IN AGED RATS. B. Givens, A.L. Markowska and D.S. Olton. Deptartment of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Cholinergic dysfunction in the septohippocampal system may underlie age-related mnemonic impairments in aged rats. This study investigates whether activation of the septo-hippocampal pathway by infusion of the muscarinic agonist oxotremorine (OXO) into the medial septal area (MSA) can improve working memory (WM) and modulate hippocampal physiology in male Fisher rats (24 mo old). Intraseptal OXO (0.1, 0.5, 2, 5 & 10 μg) produced a dose-dependent increase in choice accuracy in a spatial alternation task. The lowest effective dose, 0.5 μg OXO, did not improved WM performance when infused into the lateral ventricles. Intraseptal OXO increased 3 measures of the population response (EPSP) of the dentate gyrus to perforant path stimulation: the initial slope, the amplitude and the area. The values returned to baseline levels by 90 min after infusion. The hippocampal theta rhythm was shifted to a lower frequency following intraseptal OXO infusions. These data suggest that (1) changes in hippocampal physiology may underlie changes in WM, and (2) the cholinergic input to the MSA may be a useful target for drugs designed to improve WM in aging.

55.5

OPIOID SYSTEM IN THE MEDIAL SEPTAL AREA: THE EFFECT OF MICROINFUSION OF BETA-ENDORPHIN ON SPATIAL WORKING MEMORY IN RATS. R.Q.Wan, B.Givens and D.Olton. Dept. of Psychology, The Johns Hopkins University, Baltimore, MD. 21218.

Systemic and central administrations of opioid antagonists facilitate, whereas opioid agonists impair, memory in a variety of tasks. The present study investigated the role of the opioid system in the medial septal area (MSA) on spatial working memory (SWM). SWM was evaluated in a T-maze continuous alternation task. Each session had 20 trials with a 20-sec intertrial interval. Hippocampal θ rhythm (θ) was recorded while rats performed the task. β-endorphin (β-END, 50, 250, and 500 ng) was infused into the MSA immediately prior to behavioral testing. Muscimol (20 ng), a GABAergic agonist, was infused for a comparison. Both β-END (500) and muscimol (20) suppressed the power of θ. However, muscimol (20) greatly impaired choice accuracy, whereas β-END (250, 500) only slightly reduced choice accuracy. The behavioral impairment following intraseptal muscimol indicates a role of the MSA in SWM. The difference in magnitude of behavioral impairment produced by muscimol and β-END suggests a differential role of opioid and GABAergic systems in the MSA on SWM.

55.6

BLOCKADE OF MUSCARINIC RECEPTORS IN MEDIAL SEPTAL AREA OR NUCLEUS BASALIS MAGNOCELLULARIS IMPAIRS SPATIAL MEMORY. A.L. Markowska, A. Caprioli*, K. Pang, and D.S. Olton. Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Intraseptal administration of scopolamine, a muscarinic antagonist, impairs working memory. The present experiment reports the results of intraseptal infusion of scopolamine on another type of memory, reference memory, and in another cholinergic area, the nucleus basalis magnocellularis (NBM). Fisher-344 rats were tested in a spatial discrimination task in the water maze. Saline (SAL), scopolamine 30 μg (SCOP-30), or 60 μg (SCOP-60), was infused into the medial septal area (MSA) before each training session. SCOP-30 and SCOP-60 decreased the rate of acquisition and impaired asymptotic performance. The magnitude of these decreases was dose-dependent. All rats were then trained to criterion performance without infusions. Subsequently, retention was tested after infusions into the MSA or NBM. Both MSA and NBM infusions of SCOP-60 and SCOP-30 produced a dose-dependent impairment in performance. These results suggest, that the memory deficits produced by scopolamine are due to impairment of retrieval, and that both cholinergic systems, MSA and NBM, contribute to processing of spatial information.

55.7

ACETYL-L-CARNITINE: CHRONIC DIETARY TREATMENT IMPROVES SPATIAL MEMORY IN AGING RATS. A. Caprioli and D. S. Olton. Dept. of Psychology., The Johns Hopkins Univ., Baltimore, MD 21218.

Acetyl-L-carnitine (ALCAR) may be capable of reducing age-related memory impairments. The present experiment examined the effect of chronic ALCAR treatment on spatial memory in Fisher-344 rats. Each rat, 18 months old, was trained in a place discrimination in a water maze. Performance in this task was used to assign each rat to one of two groups, good performance or poor performance, based on their latency to reach the platform. Half of the rats in each group received 100 mg/kg/day of ALCAR for the next 4 months; the other half received water. At 22 months of age, during the first two sessions of retention, the ALCAR treated rats performed better than the control rats. The effect of ALCAR was more evident in the poor performance group than in the good performance group. Acquisition of a new spatial discrimination was tested two weeks later in a new environment. Neither control nor ALCAR rats improved their performance in the new environment, but ALCAR rats performed better than controls. These data indicate that ALCAR can improve spatial memory; the magnitude of the improvement is more pronounced in poor performance rats than in good performance rats. Supported by Sigma Tau, Institute for Research on Senescence. Italy.

55.8

INTRASEPTAL INFUSION ALTERS ACETYLCHOLINE RELEASE IN THE HIPPOCAMPUS OF THE RAT. L.K. Gorman, K. Pang, B. Givens, C. Kwon, & D.S. Olton. Neuromnemonics Laboratory., Psychology Dept. The Johns Hopkins Univ., Baltimore, MD 21218.

To study the kinetics of acetylcholine release in the hippocampus, cholinergic and GABAergic drugs were infused directly into the medial septal area (MSA). ACH release in the hippocampus and overlying cortex was assayed using in vivo microdialysis and HPLC with electrochemical detection. Rats were anesthetized with a gas mixture containing 2% ethrane. A 3 mm microdialysis probe containing a Ringers solution and 100 μM neostigmine bromide was perfused for 30 min prior to drug infusion and for 1 hr after the start of the drug infusion. Dialysate samples were collected over 5 min intervals. Drugs were infused into the MSA at a rate of 0.1 μl/min for 5 min. The anatomical placement of the microdialysis probe and drug infusion cannula was verified. Muscimol, 30 μg, a gabaergic agonist, increased ACH release between 15-20 minutes after the infusion. Carbachol, 2 μg, a muscarinic agonist increased ACH release 10 min. after the infusion. In both cases, ACH returned to baseline. Oxotremorine, 5 μg, a muscarinic agonist, steadily decreased ACH release which started at the onset of the drug infusion and continuing through the entire session. These results demonstrate that cholinergic and GABAergic systems in the MSA alter ACH release in the hippocampus.

55.9

ATTENTION AND THE NUCLEUS BASALIS MAGNOCELLULARIS. K. Pang, D.S. Olton and H. Egeth. Dept. of Psychology, Johns Hopkins University, Baltimore, MD 21218.

The nucleus basalis magnocellularis (NBM) may have an important role in attention. The present experiment assessed the role of the NBM in attention as measured in a two-choice reaction time task. The task had two stimuli (light and tone), each of which had a specific response (left or right) rewarded. On each trial, a single stimulus was presented. The rat responded by releasing a lever. Attention to each stimulus was manipulated by varying the probability of stimulus occurrence (20%, 50% or 80%). The stimulus probability remained constant for a block of 7 sessions (1 session/day). Muscimol (MUS,1 and 15 ng), a GABA agonist, was infused into the NBM prior to two sessions within each block of seven sessions, with two or more days between infusions. These infusions increased reaction time to both stimuli. The slowing due to MUS was inversely related to the probability of the stimulus: greatest with 20% stimulus, moderate with 50% stimulus and smallest with 80% stimulus. This same pattern occurred for both light and tone stimuli, suggesting that the observed impairment was not due to a sensory deficit. MUS decreased the number of correct responses for 20% stimuli, but not for 50% and 80% stimuli. The greater impairment observed for unexpected stimuli than for expected stimuli suggests that the NBM is involved in shifting of attention or the ability to divide attention.

55.10

BEHAVIORALLY INDUCED CHOLINERGIC ACTIVITY:ROLE OF ASSOCIATIVE AND NONASSOCIATIVE PROCESSES. S.Golski, L.K.Gorman, D.S.Olton. Department of Psychology, The Johns Hopkins University, Baltimore, MD, 21218.

The role of the basal forebrain cholinergic system in different kinds of memory was assessed. Changes in [3]hemicholinium-3 (HC3) binding were measured in four conditions which differed in their mnemonic requirements. In two conditions, rats learned a discrimination on a radial arm maze. The spatial working memory group (SWM) used space as a discriminative stimulus. The cued reference memory group (CRM) used a single cue as the discriminative stimulus, irrespective of space. In two other conditions, no discrimination was required. The Forced choice group (FORC) was variably reinforced for visiting the single arm to which the gate was raised. No contingencies existed between space, cue, and food. The Cage (CAGE) group had no experience with the maze. The SWM group had more cholinergic activity than the CRM group, suggesting that the type of discrimination influenced cholinergic function. The FORC group had more cholinergic activity than the CAGE group, suggesting that maze experience also influenced cholinergic function. Mean HC3 binding (and SEM) for dorsal hippocampus (HD) and ventral hippocampus (HV) immediately following the last trial was:

	CAGE	FORC	SWM	CRM
HD	10.7(1.5)	16.2(1.3)	13.3(1.7)	10.4(.8)
HV	12.9(1.2)	18.1(1.6)	18.0(2.0)	14.3(1.6)

The results suggest that cholinergic activity is influenced by associative and nonassociative processes and that the hippocampus may process spatial information even when such information is noncontingent with reward.

55.11

MEDIAL SEPTAL AREA AND HIPPOCAMPAL SYNAPTIC PLASTICITY: EFFECTS OF OXOTREMORINE IN AGED RATS. A. Balogh, K. Pang and D.S. Olton. Depts. of Psychology and Biomedical Engineering, The Johns Hopkins University, Baltimore, MD 21218.

Impaired memory in aged rats may be due to changes in synaptic plasticity in the hippocampus. Interventions that ameliorate age-related mnemonic impairments, including microinfusion of the cholinergic agonist oxotremorine (OXO) into the medial septal area (MSA), may do so by affecting hippocampal plasticity. The present study examines long-term potentiation in aged rats following infusion of OXO into the MSA. Fisher 344 rats, 25 months old, were implanted with cannulae dorsal to MSA, recording electrodes in the hilar region of the dentate gyrus and stimulating electrodes in the perforant path (PP). High frequency stimulation of PP was preceded by one of the following treatments: no infusion (CON-HFS), saline infusion (SAL-HFS), or OXO (2ug) infusion (OXO-HFS). Each rat received each of the treatments. Percentage increase in EPSP slope following OXO-HFS treatment was greater than that for either CON-HFS or SAL-HFS, suggesting increased hippocampal plasticity following intraseptal microinfusion of OXO. This increase may contribute to the mnemonic improvements seen in aged rats after MSA infusions of OXO.

55.12

GALANIN-INDUCED AMNESIA: EFFECTS OF DOSE AND TIME OF ADMINISTRATION. D.H. Malin, S.J. Radulescu*, B.J. Novy*, J.R. Lake, R.E. Plotner*, A. Lett-Brown*, P.J. Schaefer*, M.K. Crothers*, B. May* and L.D. Osgood*. University of Houston-Clear Lake, Houston, TX 77058.

Galanin (GAL) negatively modulates cholinergic transmission in the septohippocampal pathway. Previously, 8 µg GAL i.c.v. prior to a single rewarded acquisition trial prevented subsequent retention. The present study determined the dose-relatedness of this impairment, and whether a similar impairment would result from injecting GAL before the retention trial. Apparatus and procedure for one-trial discriminative reward learning are described in Malin et al. Neurobiol. Aging 12:181,1991. Each rat's retention scores are its decrease in errors and increase in speed from the single training trial to the retention trial 24 hrs. later. Groups of 9 rats were injected i.c.v. with either 0, 0.5, 2, or 8 µg GAL in saline 20 mins. prior to the training trial. Retention was impaired in a non-monotonically dose-related manner.

(M ± SEM) µg GAL:	0	0.5	2	8
Error Decrease	10.8 ± 4.0	0.4 ± 1.7	3.6 ± 1.2	-1.0 ± 1.0
Speed Increase	4.3 ± 1.4	1.7 ± 1.3	3.3 ± 0.9	0.1 ± 0.8

In a second experiment, 8 µg GAL (n=9) or saline alone (n=9) was injected i.c.v. 20 mins. prior to retention trial. Both groups showed high and not significantly different retention, suggesting that GAL impairs memory formation rather than retrieval or performance.

55.13

DA-ACH INTERACTIONS IN THE HIPPOCAMPUS AND MEMORY IN THE RAT. G.N.O. Brito and L.S.O. Brito*. Setor de Neurociencias, UFF, Brasil.

We showed that intrahippocampal injections of the muscarinic antagonist scopolamine (SCO) impair the performance of rats on a working-memory task (WM -- T-maze alternation), whereas injections of the D2 antagonist sulpiride (SUL) do not. We additionally demonstrated that intrahippocampal injections of either SCO or SUL have no effects on performance of a reference-memory task (RM -- visual discrimination) in the same maze. We now report data on the effects of bilateral 1ul intrahippocampal injections of scopolamine (7.5 ug), SCH-23390 (a D1 blocker -- 1.5 ug) or a combination of the same doses of scopolamine and SCH-23390, on performance of the same T-maze tasks. We found that injections of SCH-23390 had no effect on performance of either task. Importantly, combined injections interfered with performance of the WM task as much as SCO and the two drugs interacted to lower performance of the RM task to chance. We conclude that the hippocampal ACH system is critically involved with WM mechanisms and interacts with the hippocampal DA system in the mediation of RM processes.

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55.14

PHYSOSTIGMINE FAILS TO ATTENUATE SPATIAL WORKING AND REFERENCE MEMORY DEFICITS IN THE MORRIS WATER MAZE IN RATS WITH MEDIAL SEPTAL LESIONS. C.P.J. Dokla[1] and J. J. Boitano[2]. Depts. of Psychology, [1]Saint Anselm College, Manchester, NH 03102 and [2]Fairfield Univ., Fairfield, CT 06430.

F-344 rats sustained electrolytic lesions (1.0 mA/10 s) of the medial septal nucleus (MS) at two sites or sham-operations (SHAM). Reference memory testing in the Morris water maze was conducted for 12 consecutive days using a single, fixed escape platform location. One group of MS rats received physostigmine (PHYSO; 0.06 mg/kg, ip) 15 min prior to daily two-trial tests, whereas MS-control and SHAM groups received distilled water (vehicle) only. MS groups were highly deficient on acquisition of the task, particularly during days 7-12 of testing. PHYSO did not improve performance and probe-trial behavior was normal for all groups. Spatial working memory was assessed using a procedure similar to the one described by Wesierska et al (Behav. Neurosci., 104: 74, 1990). Escape platform location was changed daily and a single training trial (60 s limit) was followed 5 min later by two successive retention trials. Four-day blocks of testing followed by 3-day washout periods were used to evaluate 5 doses of PHYSO (0.015, 0.03, 0.06, 0.12, 0.24 mg/kg). Retention measures were highly impaired in the MS groups, and PHYSO, at all doses, did not improve performance.

55.15

SEPTAL GABA-ERGIC INTERNEURONS AND THE TRANS-SYNAPTIC CONTROL OF BASAL ACTIVITY AND MEMORY TEST-INDUCED ACTIVATION OF SEPTO-HIPPOCAMPAL CHOLINERGIC NEURONS IN MICE. T.P. DURKIN* and J. KOENIG. Laboratoire de Neurosciences Cognitives et Comportementales,* URA CNRS n°339, and Lab. Neurobiol. Cellulaire, Universités de Bordeaux*I et 2, 33405 Talence Cédex, France.

A neurochemical study of the trans-synaptic interaction established between septal GABA-ergic interneurones and the cholinergic septo-hippocampal neurones was conducted using mice. The effects of acute *in vivo* injections of either muscimol (20-500 ng/0.2 µl), bicuculline (100 ng-1 µg/0.2 µl) or saline vehicle (0.2 µl) into the medial septum on septo-hippocampal cholinergic activity was evaluated using measures of hippocampal high affinity choline uptake 30 min post-injection in two main groups of mice. The first (quiet control) remained in their home cages during the post-injection period whereas the second (active) were submitted, 10 min following injection to a 20 min period of spatial working memory testing. Both muscimol and bicuculline produced significant (25-35%) inhibition of hippocampal cholinergic activity in quiet conditions (basal) as compared to intact or saline-injected mice. In the active groups whereas memory testing induced significant cholinergic activation (+15-20%) in intact and saline injected mice at 30-sec post-test no such activation was observed in either muscimol or bicuculline-injected mice at any dose. The role of septal GABA-ergic mechanisms in the trans-synaptic control of septo-hippocampal cholinergic activity is discussed with respect to the concept that this neuronal interaction contributes to the modulation of working memory performance. (Supported by CNRS, URA 339)

55.16

AMPA-INDUCED CHOLINERGIC LESIONS OF THE VERTICAL LIMB OF THE DIAGONAL BAND (VDB) IMPAIR PERFORMANCE OF A CONDITIONAL VISUAL DISCRIMINATION (CVD) TASK. H.L. West, T.W. Robbins, and B.J. Everitt. Dept. of Expt. Psych., Univ. of Cambridge, Downing Street, Cambridge CB2 3EB, UK.

Rats were trained on a CVD task in which they were required to discriminate two temporal frequencies (0.83 and 5 Hz) of flashing lights matched for flux. Training continued until a stable baseline of at least three consecutive sessions averaging over 75% correct responding was attained. Subjects were then exposed to modified versions of the CVD task in which either the duration of the stimulus (ST-DUR) was gradually decreased or the delay between stimulus presentation and opportunity to respond (DEL) was gradually increased within a session in order to titrate the minimum ST-DUR and maximum DEL at which the subjects could successfully perform the task. Animals achieved a baseline success rate of 77.7%, minimum ST-DUR of 1.2 seconds, and maximum DEL of 8.4 seconds on average before surgery. Rats then received bilateral injections of either buffer or the cholinergic neurotoxin AMPA (0.015 mM, 0.5 µl/side) in the VDB (AP+0.2 from bregma, ML±0.6, DV-6.8 mm from dura), an intervention that has been demonstrated in experimentally lesioned animals to produce an average reduction in ChAT activity of 60% on average in cingulate cortex but only 24% in hippocampus. After a 14-day recovery period, rats were retrained to criterion and again challenged with the ST-DUR and DEL titration tests. VDB lesioned animals took signi-ficantly longer to reacquire the CVD to a 75% correct criterion than the sham controls but performed as well as controls on both the ST-DUR and DEL challenges. These results suggest that the disruption in use of decision rules produced by AMPA-induced cingulate cholinergic depletion evidently does not result from long-lasting deficits in attentional or working memory function.

55.17

BEHAVIORAL EFFECTS OF CHOLINERGIC HYPOFUNCTION IN RHESUS MONKEYS: VISUAL VS SPATIAL DISCRIMINATION. E. Grauer and Y.Kapon*. Dept of Pharmacology, IIBR, Ness-Ziona, Israel.

Rhesus monkeys were tested in a system that allows testing of monkeys in their home cage, while in the presence of other caged monkeys. An array of 12 press keys is used to present either a visual (V) or a spatial (S) discrimination task. All trials are initiated by the monkey. In the V procedure, key lights signal the "correct" keys. In the S procedure, some unsignalled keys are randomly designated as "correct" keys. In both tests only one press per key is required and it is secondarily reinforced by a brief blink of the lights above the reinforcement levers. Initial responses on "incorrect" keys are indicative of "reference memory" errors (RME) and repeated presses are indicative of "working memory" errors (WME). Scopolamine, a cholinergic antagonist, differentially affected these two tests. While no cognitive decrements were detected in the V task, the S task was susceptible to scopolamine. A specific increase in WME with no changes in RME was seen following administration of 1µg/kg of scopolamine. The data demonstrate the cholinergic involvement in the working memory process, with specific reference to spatial requirments.

55.18

POST-TRAINING ADMINISTRATION OF CHOLINERGIC M2 RECEPTOR ANTAGONIST AFDX 384 MODULATES MEMORY PERFROMANCE ON WIN-STAY AND WIN-SHIFT TASKS. A. Wilson*, N.M. White, & R. Quirion. Department of Psychology and Douglas Hospital Research Centre, McGill University, 1205 Dr. Penfield Avenue, Montreal, Quebec, Canada H3A 1B1.

Previous evidence indicates that post-training injections of a cholinergic M2 receptor antagonist, AFDX 116, enhances retention and acquisition on two tasks: One sensitive to lesions of the caudate nucleus (win-stay); the other sensitive to lesions of the hippocampal system (win-shift). The extent to which a similar but more selective M2 receptor antagonist, AFDX 384, modulates performance on these tasks was examined. In the win-stay task rats were given one trial per day and injected with AFDX 384 (0.50 mg/kg) and d-amphetamine (3.0 mg/kg) following trial seven. Rats in these groups required significantly more trials to reach criterion than saline treated controls. While alternative interpretations of the results are possible the results suggest that these doses of these drugs may have impaired acquisition of this task. In the win-shift task post-training administration of a low dose of AFDX 384 (0.25 mg/kg) enhanced retention while a higher dose (1.0 mg/kg) had no effect. These findings support the view that presynaptic mechanisms which increase cholinergic activity can modulate performance on memory tasks associated with hippocampal and/or caudate function.

55.19

STRIA TERMINALIS LESIONS ATTENUATE MEMORY ENHANCEMENT INDUCED BY POST-TRAINING INTRACAUDATE INJECTION OF OXOTREMORINE. J. L. McGaugh, M. G. Packard *, I. B. Introini-Collison. Center for the Neurobiology of Learning and Memory and Dept. of Psychobiology, University of California, Irvine, CA 92717.

Lesions of the stria terminalis (ST), a major afferent-efferent pathway of the amygdala, attenuate the memory enhancing effect of peripheral post-training injection of the cholinergic agonist oxotremorine (OXO) [Introini-Collison et al., 1989]. One possible explanation of these findings is that the amygdala modulates the memory improvement produced by OXO via ST innervation of some other cholinergic brain system. The caudate nucleus receives amygdala input via the ST. Further, several previous studies have shown that post-training manipulation of cholinergic function in the caudate nucleus affects memory. The present study examined the hypothesis that amygdala output is necessary for the memory improvement produced by post-training intracaudate injection of OXO.

Groups of rats with either sham operations or bilateral lesions of the ST were trained on a one-trial inhibitory avoidance task and received a unilateral post-training intracaudate injection of either vehicle or OXO (0.3 µg/0.5 µl) into a region of the caudate nucleus innervated by the ST. Intracaudate injection of OXO improved memory in sham-operated rats. Although ST lesions did not affect retention in rats that received intracaudate injection of vehicle, the memory improvement produced by post-training intracaudate injection of OXO was blocked by ST lesions. The findings show that amygdala output via the ST is a cofactor required for memory enhancement by intracaudate injection of a cholinergic agonist, and suggest that the role of the amygdala in memory may involve modulation of memory processes during the post-training period in brain loci receiving amygdala efferents.

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NEURAL PLASTICITY I

56.1

CALCIUM-BINDING PROTEINS IN ORGANOTYPIC SLICE CULTURES OF A LEARNING-RELEVANT FOREBRAIN AREA IN CHICKS. K. Braun[1], A. Malouf[2], C. Robins[2], P Schwartzkroin[2], H.Scheich[1]. [1]Inst. Zool., Techn. Univ. Darmstadt, Schnittspahnstr. 3, 6100 Darmstadt, FRG; [2]Dept. Neurosurg., Univ. of Washington, Seattle, WA 98195, USA

Parvalbumin (PV), calbindin-D28K (CaBP) and calretinin (CaR) characterize distinct neuron populations forming an intrinsic network in the medial neostriatum/hyperstriatum (MNH), an area which undergoes dramatic synaptic plasticity during auditory filial imprinting. Using these proteins as neuronal markers, we are now investigating critical parameters underlying these changes in cultures of MNH taken from newly hatched chicks. Many PV- and CaBP-immunoreactive(IR) neurons, and numerous PV-, CaBP- and CaR-IR puncta on dendrites and somata of immunostained neurons indicate the presence of a similar intrinsic neuronal network in culture and in vivo. However EM-analysis reveals numerous synapses with some unusual ultrastructural features. In vitro, dendritic spines contain reduced amounts of Ca-binding proteins. Given the activity-regulated expression of either Ca-binding protein, these differences may reflect different activity levels within in vitro networks.

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56.2

TIME COURSE OF CHANGES IN NERVE GROWTH FACTOR IMMUNOREACTIVITY (NGFi) IN THE DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESION. J.M. Conner, B. Fass-Holmes and S. Varon, Department of Biology, 0601, UCSD, La Jolla, CA 92093

Removal of the entorhinal input to the hippocampal formation provokes a sprouting response by many adjacent afferent systems. The molecular cues regulating this response are unknown at present, although a possible role by trophic factors has been suggested (Crutcher and Collins, 1986). The cholinergic basal forebrain neurons, which have a well documented sprouting response following entorhinal cortex lesion (ECL), presumably rely upon NGF for their trophic support. Using affinity purified antibodies to NGF, we have previously shown that NGFi is exclusively localized within cholinergic neurons of the basal forebrain and in the hilus of the dentate gyrus (DG) (Conner, et. al. 1990). To determine whether NGF plays a role in lesion-induced cholinergic sprouting, we examined changes in NGFi in the DG of adult female rats at 3,8,16 and 30 days following unilateral ECL. At 3 days post-lesion (PL3), prior to any reported sprouting, a band of NGFi appeared in the denervated zone of the ipsilateral DG. At PL8, a time at which cholinergic axons have begun sprouting, NGFi was most intense. At PL16 and PL30 NGFi within the denervated zone began to diminish but still remained more intense than in the corresponding region contralateral to the lesion. The pattern of NGFi was discretely localized within the denervated zone but could not be seen in association with cell bodies or fibers. NGFi most likely was not attributable to lesion-induced shrinkage since the time course of NGFi intensification does not parallel the time course for tissue shrinkage. Our findings demonstrate that ECL is associated with an increase in NGFi within the denervated zone of the DG and suggest that NGF may play a trophic/tropic role in regulating lesion-induced cholinergic sprouting. Supported by NINCDS grants NS-16349 and NS-25011.

56.3

CONTINUED EXPRESSION OF POLYSIALYLATED ('EMBRYONIC') N-CAM IN CENTRAL STRUCTURES CAPABLE OF STRUCTURAL PLASTICITY IN ADULTHOOD. D.A. Poulain*, D.T. Theodosis, L. Bonfanti *and G. Rougon*. Lab. de Neurendocrinologie Morphofonctionnelle, Univ.Bordeaux II, F33076 Bordeaux, France.

The neural cell adhesion molecule, N-CAM, changes at the cell surface during development, from an isoform highly enriched in polysialic acid (PSA), to 'adult' isoforms containing much less PSA; the type and content of N-CAM thus varies as a function of tissue source and age. The timing of the conversion has suggested that N-CAM and especially its PSA may serve as overall regulators of contact-dependent cell-cell interactions during development. The use of a monoclonal antibody that specifically recognises PSA-N-CAM, with immunochemical and immunocytochemical procedures at the light and electron microscopic levels, revealed that neurons and astrocytes in discrete areas of the rat CNS continue to show high PSA-N-CAM immunoreactivity in adulthood. One of the most striking is the hypothalamo-neurohypophysial system, which undergoes important, reversible neuronal-glial and synaptic changes in response to physiological stimuli, such as lactation. PSA-N-CAM immunoreactivity was also present in other areas, including the arcuate nucleus of the hypothalamus and the dorsal horn of the spinal cord, known to undergo physiological- or lesion-induced plasticity, respectively. Our observations thus support the hypothesis that the continued expression of PSA-N-CAM in certain areas of the adult CNS enables them to undergo morphological plasticity in adulthood.

56.4

MEMORY ALTERS PROTEIN KINASE C SUBSTRATE (MARCKS) PHOSPHORYLATION. B.J. McCabe*[1], F.-S. Sheu[2], G. Horn*[1] and A. Routtenberg[2]. 1. Dept. Zoology, Cambridge Univ., CB3 3EJ (UK). 2. Northwestern Univ., Evanston, IL 60208.

Phosphorylation of acidic proteins F1/GAP-43 and MARCKS are involved in synaptic plasticity, including rat hippocampal long-term potentiation (J. Neurosci. 9:381). We have enquired whether a measure of learning is related to F1/GAP-43 and MARCKS phosphorylation in the left IMHV, a forebrain region crucial for imprinting in the domestic chick. Imprinting entails the learning of an object's characteristics.

Chicks were exposed to an imprinting (training) stimulus and then given a preference test by exposure to the training stimulus and a novel object; the preference score (proportion of approach activity during the test directed towards the training stimulus) is a measure of learning. Control chicks were dark-reared. Left IMHV samples were incubated with [^{32}P]ATP and subjected to 2-dimensional gel electrophoresis. Phosphorylation of a heat-stable, 67 kDa protein kinase C substrate, having two acidic components with pIs of ~5.0 and ~4.0, and provisionally identified as MARCKS protein, was measured. Also measured were F1/GAP-43 (50 kDa) as well as a 48 kDa and a 52 kDa phosphoprotein. Of these phosphoproteins, only the phosphorylation of the less acidic component of MARCKS with pI ~5.0 was significantly greater in trained than dark-reared chicks ($t=4.10$, 22 d.f., $p<0.001$), and was significantly correlated with preference score in trained chicks ($r=0.62$, 10 d.f., $p<0.05$). The partial correlation between preference score and phosphorylation, holding constant the effect of training approach, was significant ($r=0.75$, 9 d.f., $p<0.01$). Phosphorylation of this acidic component (pI~5.0) of MARCKS was therefore specifically related to an index of learning. Phosphorylation of the more acidic component (pI~4.0) of MARCKS was not significantly affected by training and was not significantly correlated with preference score, even after correcting for the the effect of training approach. The left IMHV probably functions as a long-term store (Exp. Brain Res. 48:22). Hence the learning-related increase in phosphorylation of MARCKS (pI~5.0 component) may be implicated in mechanisms of recognition memory. (Supported by NIMH 25283 and AFOSR 90-0240).

56.5

DIFFERENTIAL mRNA DISTRIBUTION IN CHICK BRAIN OF TWO PROTEIN KINASE C (PKC) SUBSTRATES, F1/GAP-43 AND MARCKS. P.J.Meberg*[1], B.J.McCabe*[2], G.Horn*[2], J.P.Rosenfeld[1] and A.Routtenberg[1] 1. Northwestern Univ., Evanston, IL 60208. 2. Univ. of Cambridge, Cambridge, U.K.

Using in situ hybridization the most striking difference in F1/GAP-43 expression was the low level of hybridization in the paleostriatal complex in comparison to robust hybridization in the hyperstriatal complex, the neostriatum and a ventromedial telencephalic region. The hyperstriatal complex includes the intermediate and medial part of the hyperstriatum ventrale (IMHV). Hybridization was weak in the ectostriatum. Hybridization was strong in the tectum, the hippocampus and the mitral cell layer of the olfactory bulb, and moderate in cerebellar granule cells--similar to that seen in the rat (Meberg and Routtenberg, 1991, Neuroscience, in press).

The pattern of MARCKS mRNA expression differed from F1/GAP-43. MARCKS hybridization was apparent in the Wulst, IMHV and neostriatum, with medial structures more strongly labelled than lateral ones. Hybridization was weaker in the paleostriatal complex. Hybridization was also apparent in the tectum and over cerebellar granule cells. It was surprisingly strong over cells immediately adjacent to the ependyma of the lateral ventricles.

These data, together with recent evidence which indicates increased PKC substrate phosphorylation with imprinting (B. J. McCabe et al., this meeting), provide a basis for studying transcriptional regulation of such substrates in chick learning. (Supported by NIMH 25283 and AFOSR 90-0240)

56.6

INTRACELLULAR LOCALIZATION OF PKC SUBSPECIES IN HIPPOCAMPUS AND ITS INVOLVEMENT IN LTP. N. Saito, A. Kose*, T. Tsujino*, S. Tominaga, and C. Tanaka Dept. of Pharmacology, Kobe University School of Medicine, Kobe 650, Japan.

Protein kinase C (PKC) is a key enzyme for various transmembrane signalling systems and also known to be associated with long term potentiation (LTP) in the hippocampus. Recent molecular cloning revealed that PKC is a large family consisting of more than eight subspecies, but the physiological difference between the function of each subspecies is not identified. We demonstrate here the intracellular localization of each PKC subspecies of PKC in the hippocampus to elucidate the PKC subspecies which is involved in LTP. In situ hybridization demonstrated that considerable amount of mRNAs for α-, βII-, γ-PKC and ε-PKC were present in the hippocampus. The precise localization in the synapse of the PKC subspecies was studied in rat and monkey hippocampus by immunocytochemical technique. In rat, γ-PKC was seen in the dendritic spine as well as peripheral dendrites, while βII-PKC was present only in the peripheral dendrites but not in the dendritic spine. Both γ- and βII- PKC was found in the axon but neither was seen in the presynaptic terminals. Contrary, in monkey hippocampus, γ-PKC was present in the presynaptic terminals and in the dendritic spines. These findings suggests that in rat, both βII- and γ-PKC may be involved in LTP postsynaptically and γ-PKC but not βII-PKC may act within dendritic spine.

56.7

SELECTIVE REDUCTION OF B-50/GAP43 PHOSPHORYLATION IN HIPPOCAMPUS OF RATS TREATED WITH METHYLAZOXYMETHANOL AT GESTATIONAL DAY 19 Di Luca M.*, Cimino M.*, De Graan P.N.E., Gispen W.H.* and Cattabeni F. Inst.of Pharmacol.Sci.Univ.of Milano 20133 Milano, Italy, and Rudolf Magnus Inst., Inst. Mol. Biol. Univ. of Utrecht 3584 CH Utrecht, NL.

The prenatal treatment at different gestational days with a potent alkylating agent (Methylazoxymethanol acetate, MAM) produces hypoplasia of specific brain areas associated with different patterns of cognitive impairments. We have previously demonstrated that the administration of MAM to pregnant rats at gestational day 15 (GD 15) produces a dose-dependent reduction in cerebral cortex and hippocampus in the offspring. GD 19 corresponds to the active mitotic phase of granule cells of hippocampus whereas the formation of cortical interneurons is completed. The treatment with MAM at GD 19 produces little morphological changes in brain; however, these animals show specific behavioural abnormalities. We have, therefore, studied in brain areas of GD 15 and GD 19 treated animals, the in vitro phosphorylation of B-50/GAP43.In membrane preparations of GD 15 MAM-treated rats B-50/GAP43 phosphorylation is reduced of 42±5% if compared to control levels both in cortex and in hippocampus (1); in GD 19 treated rats the 32P incorporation into B-50/GAP43 is reduced of 44±3% in the hippocampus whereas no modifications are observed in the cortex of these animals. In situ hybridization studies, performed utilizing an oligonucleotide probe designed on aminoacid sequence 8-21 of B-50, revealed no statistically significant differences in B-50 expression in GD 15 and GD19 MAM-treated animals with respect to controls. These data indicate that the phosphorylation of B-50/GAP43 is correlated with different patterns of cerebral damage induced by MAM administration at different gestational days and therefore these animals are an interesting model to study the involvement of the protein in synaptic plasticity, since these animals show selective changes in LTP (2).

References: 1. Di Luca M.et al. Brain Res., 538 (1991):95-101.
2. Ramakers, G.M.J et al. Abstr. Soc. Neurosci. 1991

56.8

EXPRESSION OF HUMAN TYROSINE HYDROXYLASE AND UNREGULATED SIGNAL TRANSDUCTION ENZYMES IN NEURONS IN THE MAMMALIAN BRAIN, FROM HSV-1 VECTORS. M.J. During, A.I. Geller, A.Y. Deutch, R.L. Neve, K.L. O'Malley. Sect. Neurosurg., Yale U. Sch. Med., New Haven, CT 06510; Div. of Cell Growth and Regulation, Dana Farber Can. Inst., Boston, MA 02115; Dept. Psychiatry, Yale U. Sch. Med.; Dept. Psychobiol., U. Cal., Irvine, CA 92717; Dept. Neurobiol., Wash. U. Sch. of Med., St. Louis, MO 63110.

We have recently developed Herpes Simplex Virus (HSV-1) vectors that can stably express a gene in cultured neurons, and in vivo, following stereotactic injection into the adult rat brain. A HSV-1 vector that stably expresses human tyrosine hydroxylase (pHSVth) in cultured striatal cells directs regulated release of L-dopa and dopamine from these cells. Direct, intrastriatal injection of pHSVth, and the subsequent expression of TH in striatal neurons and glia surrounding the injection site, is a promising approach to Parkinson's Disease (PD). The efficacy of intrastriatal injection of pHSVth on behavioral and biochemical recovery in the 6-hydroxydopamine rodent model of PD is being investigated.

Additional groups of unlesioned rats received intranigral stereotactic injection with HSV-1 vectors expressing the catalytic domains of either protein kinase C or adenylate cyclase; these unregulated enzymes stably activate a particular signal transduction pathway. Both vectors increase long term basal and/or stimulated neurotransmitter release (e.g. dopamine, glutamate) from neurons *in vitro;* a similar effect *in vivo* may be realized, perhaps causing asymmetric rat rotation. These studies, currently in progress, represent a first step towards using genetic intervention techniques to clarify the mechanisms by which specific signal transduction pathways might modify behavior.

56.9

HIPPOCAMPAL SYMPATHETIC INGROWTH ALTERS CARBACHOL STIMULATED INOSITOL PHOSPHOLIPID HYDROLYSIS.

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Following cholinergic denervation of the hippocampus, via medial septal (MS) lesions, peripheral sympathetic fibers, originating from the superior cervical ganglia, grow into the hippocampus. To assess the functional significance of hippocampal sympathetic ingrowth (HSI), hydrolysis of inositol phospholipids (InsP) was examined, in control, MS lesions plus sham ganglionectomy (HSI group) and MS lesions plus ganglionectomy groups, three to four months after lesioning. Both NE and carbachol were found to produce a dose-dependent increase in the hydrolysis of InsP in hippocampal slices from all groups. However, the presence of HSI was found to significantly elevate the hydrolysis of InsP when stimulated by carbachol but not NE. In further studies, the time course of this effect was found to parallel the ingrowth of HSI, so that by 4 weeks after lesioning, carbachol stimulated InsP hydrolysis was equivalent between controls and animals with HSI but greater than that found in MS ganglionectomized animals ($p < .05$). This effect was blocked by atropine suggesting mediation through muscarinic cholinergic receptors. The results of these studies suggest that HSI is accompanied by functional metabolic alterations within the hippocampus.

56.10

INDUCTION OF IMMEDIATE EARLY RESPONSE GENES IN SOMATOSENSORY CORTEX AFTER TACTILE STIMULATION.

K.J. Mack and P.A. Mack*
Waisman Center On Mental Retardation, University of Wisconsin, Madison, WI 53705

Immediate early response genes (IEGs) have been shown to be inducible in subcortical regions of the CNS after a variety of stimuli. In this study it was demonstrated that brief tactile stimulation can induce IEGs (c-fos, NGFI-A, and NGFI-B) in somatosensory cortex.

Adult male rats were lightly anesthetized with urethane. Tactile stimuli was delivered by an artist's paint brush gently stroking an animals whiskers on one side of its face intermittently over a fifteen minute period. Two hours later, the animals were sacrificed and tissue sections were prepared for immunohistochemical analysis. Cortex contralateral to the stimulation was compared with ipsilateral cortex using antibodies raised against the IEG products of NGFI-A, NGFI-B, and c-fos. The different transcription factors showed slightly different patterns of induction to the tactile stimulus. Cell counts indicated that the increase in the number of positively staining cells was approximately half of that observed after a metrazole-induced seizure. In contrast to seizure stimulation, the induction of immunohistochemical staining after tactile stimulation was most prominent in layer 4 with all antibodies under study.

These data demonstrate that physiologic stimulation can induce IEGs in cortical cells, and that multiple IEGs react differentially to a stimulus.

This research was supported by a grant from the PMA.

56.11

INDUCTION OF THE IMMEDIATE EARLY GENE ZIF-268 IN THE CORTEX AND HIPPOCAMPAL FORMATION OF WEANLING RATS AFTER BRIEF EXPOSURE TO ENVIRONMENTAL COMPLEXITY.

G.S. Withers, C.S. Wallace, I.J. Weiler and W.T. Greenough. Neurosci. Prog., Depts. of Psych. and Cell & Struct. Biol., Beckman Inst., Univ. of Illinois, Urbana, 61801.

Modifications in neuronal structure have been reported following various learning experiences. Differential housing, in which littermate rats are assigned to either a complex environment (EC) or an individual cage (IC), has been valuable in exploring structural plasticity related to long term information storage. We have detected significant increases in cortical thickness and dendritic length in occipital cortex of EC animals after only 4 days in EC (Kilman et al., 1988). This suggests that neurons undergo very rapid structural remodeling after behaviorally relevant experience. However, many questions remain about the mechanisms by which interaction with the environment might rapidly activate the coordinated gene expression required for dendritic growth. Transcription factors (proteins which bind to specific DNA sequences and regulate the transcription of other mRNA species) seem well suited to this process. A family of genes encoding transcription factors known as immediate early genes (IEG) has been described. One of these, ZIF-268 (known also as Egr-1, NGFI-A, Krox 24) has been correlated with NMDA receptor activation during the induction of LTP using electrical stimulation (Cole et al., 1989; Wisden et al., 1990). To investigate the sensitivity of an IEG to more natural stimulation, we used in situ hybridization to measure ZIF-268 mRNA levels after brief exposure to environmental complexity.

Preliminary results indicate that after 4 days exposure to EC/IC, expression of ZIF-268 is detectably elevated in the EC occipital cortex and hippocampal formation. This is one of the first demonstrations that behaviorally relevant experience can induce a transcription factor. Work is ongoing to further examine the regional activation of ZIF-268. Supported by ONR and NIMH 35321

56.12

FOS IS EXPRESSED IN THE RAT DURING A FORELIMB REACHING TASK.

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Most studies showing expression of the immediate-early gene, c-*fos* and its phosphoprotein product, Fos in the adult nervous system have shown its induction in response to invasive stimuli such as direct electrical stimulation. The purpose of this study was to examine whether Fos expression could be behaviorally induced. Animals were trained in a forelimb reaching task to reach for small pellets of food. (Peterson, Comp. Psychol. Monogr., 9:1, 1934; Whishaw et al., Brain. 109:805, 1986). Fos expression was detected by an immunoperoxidase procedure using the Alu antiserum. Animals that reached as few as 30 reaches on training day 1 revealed intense Fos immunoreactivity in cortico-fugal fibers within the corpus striatum. These fibers include sensorimotor cortex projections which govern forelimb movement. It is probable that Fos expression of glial cells may be contributing to this labelling since Fos positive glial cells have previously been identified in white matter tracts. In cerebellar lobules, particularly the paramedian lobule (PML), an area specifically involved in forelimb movement, Fos was detected in nuclei of Purkinje cells and inhibitory interneurons. Animals which reached on 3 consecutive days, showed an absence of labelling which resembled naive, nonreaching animals. This suggests that Fos expression in these regions may be involved in the earlier but not later stages of acquision of the task. Parietal cortex, which receives forelimb cortex input, and other association cortices showed Fos expression. Cortical labelling was most pronounced in cell nuclei of layer II/III cells, which are involved in cortico-cortico input. This would further suggest an associational learning component of the pattern of Fos expression during the forelimb reaching task.

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56.13

FOS-LIKE IMMUNOREACTIVITY IN DEVELOPING RAT HIPPOCAMPUS, CEREBRAL CORTEX, AND CEREBELLUM.

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Cellular immediate-early gene (cIEG) influences in the nervous system have been described extensively (Greenberg et al., J. Biol. Chem., 260:14101, 1985; Curran and Morgan, BioEssays, 7:255, 1987). The nuclear phosphoprotein product (Fos) of the IEG, c-*fos* binds as a heterodimer with the product of c-*jun* to specific DNA regulatory sequences, thereby modulating transcription of target genes. c-*fos* expression in the adult is induced by a variety of external stimuli. Fos and Fos-related antigens (FRAs) expression was examined in this study in intact developing animals ages P5-P10, a time when the brain is naturally undergoing many plastic changes. 10μm alternate cryosections were immunolabelled with either Alu antiserum which appears specific to Fos or with M5 antiserum which recognizes Fos as well as several FRAs such as Fra-1, Fra-2, and FosB (Cohen et al., Genes & Develop., 3:173, 1989). In general immunolabelling was either cytoplasmic or nuclear and showed region and cell-specificity. Labelling changed across ontogenetic development. Fos-like immunoreactivity detected with the Alu antiserum was developmentally regulated in the cerebellum, whereas immunoreactivity detected with the M5 antiserum was expressed in hippocampus, cerebral cortex and cerebellum. M5 labelling in hippocampal pyramidal cell nuclei was intense at P5 and became virtually absent by P10. Cerebral cortex pyramidal cells showed exclusive cytoplasmic M5 labelling whereas, layer VI cells displayed nuclear labelling. Immunoreactivity was very intense at P5 and reduced or absent by P10. Cerebellar Purkinje cells showed nuclear M5 and Alu immunoreactivity at P5. Labelling of these cells became increasingly cytoplasmic. Purkinje cell dendrites became intensely labelled by P7 and was greatly reduced or absent by P10. **Supported by MH-40631, MH-35321, and MH-18882.**

56.14

SPATIALLY DISTRIBUTED INCREASES IN C-FOS mRNA IN ODOR-ACTIVATED REGIONS OF THE MAIN OLFACTORY BULB.

K.M. Guthrie, A.J. Anderson, M. Leon and C.M. Gall, Depts. of Anatomy & Neurobiology and of Psychobiology, Univ. of Calif., Irvine, CA 92717

Odor responsive regions of the main olfactory bulb can be revealed with ^{14}C 2-deoxyglucose (2-DG) autoradiography. This mapping technique is limited in some aspects, particularly in its lack of cellular resolution. The expression of the immediate early gene (IEG) *c-fos*, which encodes a transcription regulatory protein, has been shown to respond to neural activation in a number of different neuronal systems. We report here the mapping of odor responsive regions of the rat olfactory bulb using cellular localization of mRNA for *c-fos*. Young rats (PN20-22) were injected with ^{14}C-2DG (20 μCi/100g, s.c.) and exposed to either air, peppermint odor or isoamyl acetate odor (1:10 dilution in air; flow rate = 2L/min) for 30 min. Animals were then decapitated and the brains dissected out and frozen in methylbutane (-50°C). Alternate sections were processed for 2-DG autoradiography and *in situ* hybridization with *c-fos* ^{35}S-cRNA. Periglomerular, tufted and granule cells were labeled with *c-fos* riboprobe. In air-exposed animals, low levels of hybridization were seen throughout the glomerular layer (GL), while in odor-exposed animals, dense cell labeling occurred in discrete regions of the GL. Analysis of adjacent sections indicated that these glomerular areas corresponded to regions of high 2-DG uptake. Differential cRNA hybridization also occurred in the granule cell layer of odor-exposed animals, with those areas underlying the activated glomeruli exhibiting the heaviest cell labeling. These data demonstrate that odor stimulation activates *c-fos* expression in cells of the olfactory bulb, and that this technique is a more sensitive and precise means of identifying odor-responsive regions of the olfactory bulb than 2-DG autoradiography.

Supported by HD24236 from NICHD to M.L. and C.G.

56.15

ODOR-STIMULATED INCEASES IN C-JUN mRNA IN THE RAT MAIN OLFACTORY BULB. A.J. Anderson, K.M. Guthrie, M. Leon and C.M. Gall. Depts. of Anatomy & Neurobiology and of Psychobiology, Univ. of Calif., Irvine, CA 92717

The protein products of the immediate early genes (IEGs) *c-fos* and *c-jun* combine to form a transcriptional regulatory factor. We have shown previously that areas of increased *c-fos* mRNA expression in the olfactory bulb correspond to areas of odor activation revealed by ^{14}C 2-deoxyglucose (2-DG) autoradiography. We report here the mapping of odor responsive regions of the rat olfactory bulb using cellular localization of mRNA for *c-jun*. Young rats (PN20-22) were injected with ^{14}C 2-DG (20μCi/100g) and exposed to either air, peppermint, or isoamyl acetate odor (1:10 dilution in air) at a flow rate of 2L/min for 30 minutes. Animals were sacrificed and their brains frozen in methylbutane (-50ºC). Alternate cryostat sections were cut and processed for 2-DG autoradiography and *in situ* hybridization with ^{35}S-cRNA probes for *c-fos* and *c-jun*. Periglomerular, tufted, and granule cells labeled with *c-jun* cRNA; unlike *c-fos* cRNA, *c-jun* cRNA also labeled mitral cells. Odor-stimulated animals exhibited increased levels of *c-jun* expression in the glomerular, mitral, and granule cell layers compared to air controls. Examination of adjacent sections revealed that areas of increased cell labeling with *c-jun* cRNA in the glomerular layer corresponded to regions of elevated *c-fos* mRNA expression. Areas of elevated *c-jun* expression in the mitral cell layer were broader than those labeled with *c-jun* cRNA in the glomerular layer, or *c-fos* cRNA in the glomerular and granule cell layers. Mitral cells are responsive to odor-stimulation, however, changes in the mitral cell layer have not been observed using 2-DG autoradiography. These data demonstrate the differential expression of IEGs by olfactory bulb cell populations, and suggest that these genes may be co-activated by odor-stimulation in some neurons. Moreover, the induction of *c-jun* but not *c-fos* mRNA in mitral cells suggests the odor-activation of transcription factors with different regulatory specificities. (HD24236 to C.G. and MH45353 to M.L.)

56.16

GENE REPRESSION IN THE AVIAN SONG CONTROL SYSTEM. J. M. George and D. F. Clayton. Lab of Animal Behavior, The Rockefeller University, NY, NY 10021, & Dept. of Cell & Structural Biology, University of Illinois, Urbana, IL 61801

We have identified several mRNAs which are generally enriched in the forebrain but specifically repressed in parts of the song control circuit of canaries and zebra finches. These RNAs were identified using a combination of differential cDNA cloning and in situ hybridization. One of these, HAT-3, predicts a novel protein with unusual structural properties. Decreased gene expression in the song circuit was first observed in birds when song is stable and behavioral plasticity is low. Thus we hypothesize that regulation of plasticity in the song system may be achieved through repression and activation of specific genes such as HAT-3. To test this, we are measuring the expression of these genes in the song control centers of young zebra finches during the critical period for song development, and in adult canaries at different points in the seasonal cycle of song learning and production.

INGESTIVE BEHAVIOR: MONOAMINES

57.1

INHIBITION OF FEEDING BY THE NON-SPECIFIC 5-HT AGONISTS, 5-HTP AND FLUOXETINE. M.A. Prendergast*, K. Skaar*, S.E. Hendricks, D.P. Yells*, and D.F. Fitzpatrick*, Dept. of Psychology, Univ. of Neb. at Omaha, and Dept. of Psychiatry, Univ. of Neb. Med. Ctr., Omaha, NE 68182.

We assessed the effects of 5-HT agonism on sucrose consumption in food-deprived and free-feeding rats. In Experiment 1, food-deprived, but not free-feeding, rats receiving 2 mg/kg i.p. injections of the 5-HT precursor, 5-HTP, showed reduced consumption of a 10% sucrose solution. Both food-deprived and free-feeding rats receiving 20 mg/kg i.p. injections of 5-HTP showed reduced consumption of the sucrose solution. In Experiment 2, free-feeding rats receiving 10 mg/kg i.p. injections of fluoxetine showed reduced consumption of a 10% sucrose solution. These results support the conclusion that at low doses, 5-HT agonists inhibit feeding motivated by metabolic need and that higher doses inhibit feeding motivated by both metabolic need and palatability. The methodology described in Experiment 1 will be employed to assess the effect of Fluoxetine on both food-deprived and free-feeding rats and the results will be presented.

57.2

EFFECTS OF THE 5-HT$_1$ AGONISTS TFMPP AND RU 24969 ON FOOD INTAKE FOLLOWING PERIPHERAL OR MEDIAL HYPOTHALAMIC INJECTION. P.J. Fletcher, Z.H. Ming*, M.H. Zack* and D.V. Coscina. Clarke Institute of Psychiatry, Toronto, Ontario M5T 1R8, Canada.

It is well established that manipulations of 5-HT alter feeding behaviour. For example, indirect 5-HT agonists such as fenfluramine and fluoxetine reduce food intake. Previous studies have indicated that activation of 5-HT$_{1C}$ and/or 5-HT$_{1B}$ receptors are important for mediating these effects and that the medial hypothalamus, particularly the paraventricular nucleus (PVN) may be an important site of action in the brain. Experiments were conducted to test these hypotheses by examining the effects of activating 5-HT$_{1B/1C}$ receptors (using TFMPP) or 5-HT$_{1B}$ receptors (using RU 24969) on feeding in rats following peripheral or intra-hypothalmic injection. For intra-hypothalamic injections rats were equipped with chronic, indwelling, stainless steel guide cannulae aimed at the PVN. When injected peripherally RU 24969 and TFMPP (0.3-5 mg/kg, IP) dose dependently reduced feeding stimulated by three separate stimuli: (1) infusion of 25 nmol noradrenaline (NA) into the medial hypothalamus, (2) a palatable wet mash diet or (3) 20 hr of food deprivation. However, following microinjection into the medial hypothalamus neither drug (12.5-50 nmol in 0.4μl) altered food intake. In contrast both 5-HT and fluoxetine infused into the medial hypothalamus induced a mild attenuation of the feeding response to NA. The results indicate that while activation of 5-HT$_{1B/1C}$ receptors following peripheral drug injection leads to marked reductions in food intake, the site of action of this effect is not the medial hypothalamus. However, it is possible that activation of multiple hypothalamic 5-HT receptor sub-types as occurs with 5-HT, and presumably fluoxetine, may suppress food intake.

57.3

AGONISTS AT 5-HT$_1$ AND 5-HT$_2$ RECEPTORS SUPPRESS EATING WITHOUT ADVANCING THE BEHAVIOURAL SATIETY SEQUENCE. L.M. Dixon, A.M.J. Montgomery*[1] and P.J. Fletcher. Clarke Inst. Psychiatry, Toronto, Ontario M5T 1R8, Canada and [1]City London Poly., London E1 7NT, England.

As rats feed to satiety they exhibit a characteristic sequence of behavioural changes. The cessation of feeding is followed by activity and grooming which, in turn, give way to resting. Enhancing satiety by prefeeding advances the behavioural satiety sequence (BSS): feeding ends earlier and resting starts sooner. Activation of 5-HT$_{1B}$ receptors (using RU 24969), 5-HT$_{1B/1C}$ receptors (using TFMPP) or 5-HT$_2$ receptors (using DOI) have each been shown previously to suppress food intake. The present study sought to examine the effects of these manipulations on feeding and the BSS. Drug effects were assesssed in non-deprived rats during 1h meals of palatable wet mash. Throughout the meals each rat was observed every 15s and its behaviour scored into one of four mutually exclusive categories (eating, active, grooming and resting). Both RU 24969 and TFMPP (0,0.23,0.7 and 2.1 mg/kg; IP) dose dependently suppressed intake, eating time and eating rate. Activity was enhanced and resting suppressed, with the effects of RU 24969 being more pronounced. DOI enhanced activity and almost totally suppressed resting even at sub-anorectic doses (0.33 and 1 mg/kg; IP). Eating time was not altered because animals showed low levels of feeding throughout the 1h test. At an anorectic dose (3 mg/kg) DOI reduced feeding by decreasing eating rate. In summary although all three drugs suppressed eating RU 24969 and DOI disrupted the BSS while TFMPP left the sequencing of behaviours intact without advancing the termination of eating or the start of resting. The data show that none of these drugs suppress intake by enhancing satiety, but illustrate that activation of different 5-HT receptor sub-types induces different behavioural profiles.

57.4

EFFECT OF AN ANORECTIC AGENT - DEXFENFLURAMINE (DF) - ON NEUROPEPTIDE Y (NPY) RELEASE IN THE PARAVENTRICULAR NUCLEUS (PVN) OF FOOD-DEPRIVED RATS. M.G. Dube, [1]C. Phelps, S.P. Kalra and P.S. Kalra, Dept. of Obstet. and Gynecol., Univ. of Fla. Gainesville, FL 32610, [1]Dept. Anat., Univ. So. Fla., Tampa, FL 33612

Our studies indicate that NPY release in the PVN may be a physiological signal for initiation of feeding in the rat. This is based on the demonstrations that (a) central administration of NPY evokes robust feeding; (b) NPY levels and release in the PVN are significantly increased after 3 days of food deprivation and return to control levels after refeeding, and (c) NPY levels rise and fall in association with food availability in rats on a scheduled feeding regimen. Since DF is an anorectic agent, we tested the hypothesis that DF's effect may be due to changes in NPY release in the PVN.

In a preliminary experiment, adult male rats were food-deprived for 24 h and then injected with either DF (10 mg/kg, IP, n=8) or saline, IP (n=8). After 30 min rats were allowed access to preweighed rat chow for 1 h. While control rats ate 6.15 ± 0.7 g, the DF-treated rats failed to eat. To test DF's effect on NPY release, rats were implanted with push-pull cannula aimed at the PVN. Following 3-5 days of recovery, they were food-deprived for 3 days, then injected with either DF or saline and perfused with aCSF. Samples, collected at 10 min intervals, were lyophilized and NPY measured by RIA. NPY levels in samples collected from DF and controls fluctuated considerably (range 2.0 - 218.3 pg/10 min). The mean rate of NPY efflux in the PVN 30-90 min after injection was 42.7 ± 9.7 pg/10 min in the DF group and 31.7 ± 12.7 pg/10 min in control rats. Thus, despite suppression of food intake by DF there was no significant change in NPY output in the PVN suggesting that inhibition of NPY release in the PVN may not be necessary for the anorectic effect of DF. It is likely that DF may act within the PVN or at other sites in the brain to inhibit the action of NPY at target sites in the PVN (Supported by NIH DK 37273).

57.5

THE ANORECTIC ACTION OF PERIPHERAL SEROTONIN (5-HT) IS NOT ATTENUATED FOLLOWING BLOCKADE OF ENDOGENOUS CCK-A RECEPTORS WITH DEVAZEPIDE IN RATS. K. Eberle-Wang and K.J. Simansky, Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

There is evidence that 5-HT-ergic antagonists can reverse anorexia following systemic cholecystokinin (CCK-8) (Stallone et al., 1989). Conversely, Cooper et al. (1989) demonstrated that the selective CCK-A receptor antagonist, devazepide (DVZ), reversed anorexia produced by fenfluramine. We examined the effect of devazepide pretreatment on anorexia produced by **peripheral** administration of serotonin as well as CCK-8 in rats given a 30-min test meal of sweetened mash following 3h of food deprivation. 5-HT (4.0 and 10.0 µmol/kg) or CCK-8 (4.0 nmol/kg) was administered (i.p.) 6 min prior to mash presentation and 24 min following pretreatment with either DVZ (.75 µmol/kg, i.p.) or its vehicle (0.1% DMSO in 0.5% carboxymethylcellulose). In these experiments DVZ did not alter anorexia produced by either 4.0 or 10.0 µmol/kg 5-HT. However, under the identical testing conditions, DVZ reversed CCK-induced anorexia [n=7-8; veh/veh=7.8±0.6; dvz/veh=8.3±0.8; veh/cck=3.5±1.0 ; dvz/cck=7.3±0.8 g/30-min]. Subcutaneous injection of the same dose of DVZ also failed to alter the anorectic response to peripherally-administered 5-HT (4.0 µmol/kg, i.p.) [n=8-9; veh/veh=10.6±0.8; dvz/veh=8.9±1.4; veh/5-HT=4.5±1.0 ; dvz/5-HT=5.4±1.0 g/30-min]. Therefore, a dose of DVZ which completely blocked CCK-8-induced anorexia did not alter 5-HT-induced reductions in food intake under these testing conditions. These results suggest that peripherally-administered 5-HT produces anorexia in rats independently of CCK-A receptor stimulation. Supported by MH41987 to KJS.

57.6

FEEDING SPECIFICITY OF 8-OH-DPAT. D.V. Coscina, J. Jarry*, and P.J. Fletcher. Section of Biopsychology, Clarke Institute of Psychiatry, and Department of Psychology, University of Toronto.

8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) is a serotonin$_{1A}$ agonist known to increase solid as well as powdered food intake in satiated rats but does not enhance intake of sucrose or glucose solutions and can increase gnawing of wooden blocks. Therefore, some question exists surrounding its feeding specificity. This study investigated the ability of 8-OH-DPAT to elicit feeding in rats allowed to run in wheels as an alternative to eating. If this drug truly produces feeding specific effects, we expected the amount of food consumed to be the same in rats offered this choice compared to other rats wherein eating was the only option. Subjects were 24 adult male Sprague-Dawley rats adapted to living in running wheels with separate side cages equipped with food cups containing powdered lab chow. On different days, each animal received one of four doses of 8-OH-DPAT (0, 60, 120 and 240 ug/kg s.c.) in saline 30 min prior to a 1 hr test. Half of the animals were allowed to both eat and run during these tests while the other half were confined to their side cages so that only eating was possible. One-way analyses of variance showed no reliable dose-dependent feeding in either test condition nor dose-dependent running in the choice group, due to high levels of baseline behaviours. However, when data across drug trials were averaged and compared to the saline condition, paired t-tests showed that rats only allowed to eat consumed more food after 8-OH-DPAT. Although paired t-test of running between saline and mean drug conditions revealed no reliable activity effects, rats offered the choice of eating and running did not overeat in response to the drug. These results suggest that when another source of motivated behaviour is made available to rats, 8-OH-DPAT does not reliably enhance intake. Such findings continue to question the specificity of feeding produced by this agent.

57.7

D-FENFLURAMINE: EFFECTS ON MICROSTRUCTURE OF LICKING FOR WATER AND SUCROSE. D.A. Morris* and S.J. Cooper, Lab. of Psychopharmacology, University of Birmingham, B15 2TT.

We investigated the effects of d-fenfluramine (F) (0.3-3.0 mg/kg; i.p.), on i) characteristics of licking responses ii) microstructure of licking iii) temporal patterns of drinking in two groups of rats. Group I was adapted to water deprivation and trained to consume water, while Group II was not deprived but was trained to consume a palatable 3% sucrose solution. (F) at 1.8 and 3.0 mg/kg, significantly reduced sucrose and water consumption during a 20 min. test period ($P<0.01$; N=10). The groups showed no sign of impaired oral motor function except for a significant reduction in lick rate for Group I at 3.0 mg/kg, but these animals also displayed flattened body posture. Microstructural analysis showed that (F) reduced the mean duration of bursts of licking for water at 3.0 mg/kg ($P<0.05$; N=10), and sucrose at 1.8 mg/kg ($p<0.05$; N=10) and 3.0 mg/kg ($P<0.01$; N=10). The temporal distribution of drinking was different for the two groups. Group I showed a high initial level of drinking followed within 7.5 min. by clear signs of satiation; little drinking occurred after 10 mins. Group II consumed sucrose throughout the test period without signs of satiation. In both groups, after 3.0 mg/kg, drinking occurred more intermittently. Thus, (F) reduced water and sucrose intake by altering drinking patterns, and not by any general motor impairment.
(This research is supported by a grant from IRIS).

57.8

A COMPARISON OF THE ANORECTIC EFFECTS AND RECEPTOR SPECIFICITY OF MK212 OR QUIPAZINE IN PREVIOUSLY FOOD-DEPRIVED RATS S. M. Snyder, F. R. Davis*, L. L. Van Dyke*, R. A. Smith*, L. R. Reid*, and D. T. Wong. Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, IN 46140.

The arylpiperazine class of serotonin (5-hydroxytryptamine, 5-HT) agonists is known to suppress food consumption in rats. In the present study, food-deprived male Sprague-Dawley rats were treated with 0.3-10 mg/kg of either MK212 or quipazine. A dose-related suppression of food intake was observed in both groups at 1 and 3 hours after food access. The anorectic effect of MK212 or quipazine at 5-10 mg/kg was reversed by pretreatment with 0.1-5 mg/kg i.p. of metergoline (a nonselective antagonist of 5-HT1 and 5-HT2 receptors) and partially reversed by pretreatment with 1-5 mg/kg i.p. of 1-naphthyl-piperazine (an antagonist of 5-HT1a,b,c and 5-HT2 receptors), or 3-10 mg/kg i.p. of mianserin, (an antagonist of 5-HT1c and 5-HT2 receptors). The anorexia was not reversed by 0.01-0.3 mg/kg i.p. of spiperone (a 5-HT1a and 5-HT2 antagonist). The anorectic effect of quipazine, but not MK212, was partially reversed by 1-10 mg/kg i.p. of ketanserin or 1-5 mg/kg s.c. of ritanserin (antagonists of 5-HT2 receptors). The present findings suggest that the 5-HT1 receptor is involved in mediating the anorectic effect of MK212, while the 5-HT1 and 5-HT2 receptors are involved in mediating this effect of quipazine.

57.9

CIRCADIAN EFFECTS OF d-AMPHETAMINE ON THE MEAL PATTERNS OF RATS R. Bauman and R. Pastel. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307.

The meal patterns of non-deprived rats were used to evaluate the effects of d-amphetamine (AMP) on food intake. A 12 hour light/dark cycle was maintained in each rat's cage and at any time a rat could eat by pressing a lever once for each 45mg food pellet. A meal was defined as a bout of eating that was not interrupted by a pause longer than 10 mins. At first, either saline or a (0.5, 1.0, or 2.0 mg/Kg) dose of AMP was delivered intraperitoneally within the 30 minutes that preceded light offset; subsequently, the same doses were delivered before light onset.

During the dark, AMP caused a dose-dependent reduction of meal size and duration, but increased meal frequency. Similar, but non-significant effects were evident during the light. Despite an increase in meal frequency, AMP selectively reduced intake during the dark. Similar doses of DOI, a serotonergic agonist, also reduce meal size and duration during the dark, but only AMP induces a compensatory increase in meal frequency.

57.10

EFFECTS ON FEEDING OF THE ALPHA1-ADRENOCEPTOR AGONIST CIRAZOLINE IN RATS. P.J. Wellman and B.T. Davies*. Dept. of Psychology, Texas A&M University, College Station, TX 77843-4235.

Activation of alpha1-adrenoceptors within the hypothalamic paraventricular nucleus (PVN) in rats results in the suppression of feeding. Injections into the PVN of the alpha1-adrenoceptor agonist phenylpropanolamine suppress feeding whereas antagonism of alpha1-adrenoceptors within the PVN prevents the feeding suppressive action of systemically administered phenylpropanolamine (*Pharmacol. Biochem. Behav.*, 1991, 38: 905-908). In this study, we extended these experiments to assess the effects on feeding of intra-PVN and systemic administration of cirazoline (RAZ), a potent alpha1-adrenoceptor agonist. Rats underwent stereotaxic placement of unilateral guide shafts aimed at the PVN and after recovery were shown to reliably eat (> 3.0 grams) following 25 nMol norepinephrine administered into the PVN. Feeding trials were 30 min in duration and were conducted in the late afternoon in non-food deprived rats. In Phase 1, the rats were treated with either 0.0, 0.05, 0.1, 0.2 or 0.4 mg/kg RAZ (IP) 15 min prior to each feeding trial. In Phase 2, intra-PVN injections of either 0, 3, 6, 12 or 24 nMol RAZ were given 6 min prior to each feeding test. Systemic injections of RAZ significantly suppressed feeding (ED_{50} = 0.05 mg/kg) as did intra-PVN administration of RAZ (ED_{50} = 23.4 nMol). Systemic RAZ administration suppressed consumption of water but to a lesser degree than the effect of systemic RAZ on food intake (ED_{50} = 0.22 mg/kg). Intra-PVN administration of RAZ did not reduce water intake. These results support the hypothesis that alpha-1 adrenoceptors within the PVN act specifically to suppress food intake.

57.11

INTRACEREBROVENTRICULAR INJECTIONS OF α_2-NORADRENERGIC AGONISTS INCREASE CALORIC INTAKE IN GENETICALLY OBESE *(ob/ob)* MICE. P.J.Currie and L.M.Wilson. Dept. of Psychology, University of Manitoba, Winnipeg, MB, CANADA R3T 2N2.

Hypothalamic paraventricular injections of norepinephrine [NE] and clonidine [CLON], the α_2 agonist, elicit feeding, particularly of carbohydrate, in the satiated rat. In the genetically obese *(ob/ob)* mouse, which exhibits hyperphagia and increased hypothalamic NE, we have previously shown that peripheral injection of CLON produces a biphasic effect on caloric intake (in 6-h meal-feeding *ob/ob* mice). The stimulatory effect of CLON (at doses of 25-50 μg), associated with a preferential increase in carbohydrate ingestion, was blocked by α_2 receptor antagonism. The present studies examined the impact of ICV CLON and NE on feeding in obese and lean mice maintained under free-feeding conditions. Following adaptation to carbohydrate, fat, and protein diets, pre-satiated obese *(n=8)* and lean *(n=8)* mice were injected with CLON [10-20 nmol ICV] or sterile physiological saline, in counterbalanced order, approximately 1 h (16h00) before dark onset, in a 12 h L-D cycled colony room. (Fresh diets were given 1 h before testing to ensure maximal satiation). Macronutrient intakes were assessed at 1 h and 2 h postinjection. Similar tests were conducted with l-norepinephrine-d-bitartrate [40-80 nmol ICV]. Drug effects were also examined in mice maintained on standard rodent chow diet. CLON and NE increased feeding of chow as well as macronutrient (caloric) intake in both *ob/ob* and lean mice, although carbohydrate intake was particularly affected in obese mice. This resulted in a substantial increase in the proportion of carbohydrate ingested, relative to total caloric intake, an effect that was less pronounced in lean mice, suggesting enhanced pharmacological sensitivity in the *ob/ob*. *[Supported by NSERC #7907 to LMW and MHRC Graduate Studentship to PJC.]*

57.12

AUTORADIOGRAPHIC ANALYSIS OF ALPHA2-NORADRENERGIC RECEPTORS IN HYPOTHALAMIC NUCLEI: RELATION TO LIGHT/DARK CYCLE AND NUTRITIONAL STATE OF ANIMALS. G.B Kovachich[1], M. Jhanwar-Uniyal[2], S.F. Leibowitz[2] VA. Med. Ctr.[1], Philadelphia PA, Dept. of Psychiatry, Univ. Pa.[1], Philadelphia PA and The Rockefeller Univ.[2], New York NY.

Alpha2-noradrenergic receptors in the paraventricular nucleus (PVN) of rats are believed to be involved in the control of feeding behavior, particularly at the onset of the dark cycle. Binding procedures with [3H]p-aminoclonidine have shown that the density of alpha2-adrenoceptors in the PVN increases at dark onset and declines after food deprivation (FD). The present study used quantitative autoradiography to examine further the alpha2-adrenoceptor density in the PVN and other hypothalamic areas under similar conditions.

Male Sprague-Dawley rats, maintained on 12 hr light/dark cycle, were sacrificed at light and dark onset of period and after 48 hrs FD. Binding was done using alpha2-adrenoceptor antagonist [3H]idazoxan (3nM), with non-specific binding determined in the presence of phentolamine. The results show: **a)** at the dark onset, relative to light onset, there occurs an increase in alpha2-adrenoceptors in the magnocellular PVN (PVNm; $p<0.05$) and a trend towards an increase in the parvocellular PVN (PVNp; $p<0.09$); **b)** a decline in alpha2-adrenoceptors in both the PVNm and PVNp after FD, as compared to satiated condition; **c)** opposite changes in alpha2-adrenoceptor density in the median eminence (ME), namely an increase at the light onset and after FD. The findings confirm, through autoradiographic measurements, that PVN as well as ME alpha2-adrenoceptors shift in relation to the diurnal cycle and nutritional state.

57.13

PARAVENTRICULAR HYPOTHALAMIC CLONIDINE INCREASES RATHER THAN DECREASES SUSCEPTIBILITY TO ACTIVITY-BASED ANOREXIA IN THE RAT. T.S. Rieg, S.N. Downing, and P.F. Aravich, Dept. of Anat. & Neurobio., Eastern Virginia Medical School, Norfolk, VA 23501, and Veteran Affairs Medical Center, Hampton, VA 23667.

It has been proposed that anorexia nervosa is related to a reduction in the activity of the paraventricular hypothalamic (PVN) noradrenergic-feeding system. The purpose of this investigation was to determine if chronic administration of the alpha-2 adrenergic agonist, clonidine (CLON), into the PVN reduces susceptibility to activity-based anorexia (ABA) in the rat. Male Sprague-Dawley rats were stereotaxically implanted with a PVN cannula attached to an osmotic minipump. Exp 1 used seven groups of adolescent (192 g) rats chronically infused with either 0, .75, 3, 6, 12, or 48 nM CLON/hr. Following recovery from surgery all animals were exposed to ABA (1.5 hrs/day access to food; 22.5 hrs/day access to running wheels without food). It was found that CLON increased susceptibility (number of days to 25% weight loss) to ABA with the highest dose animals taking the least amount of time to reach the weight-loss criterion. There was also a dose-related suppression of food intake. No differences in activity levels were found. Exp 2 was similar to the first, except adult (292 g) rats were infused with three drug doses (0, 6, or 48 nM CLON/hour). No differences were found in the amount of food consumed or the amount of activity. However, both CLON-treated groups took less time to reach the weight loss criterion than the saline treated group. These data indicate that CLON infused chronically into the PVN does not produce hyperphagia and that, under these conditions, it exacerbates rather than attenuates susceptibility to ABA. Support: NIMH NRSA #1F32MH09805-01A1 (TSR); Dept. Vet. Affairs Merit Award (PFA).

57.14

STIMULATORY EFFECTS OF THE ALPHA-2-ADRENOCEPTOR ANTAGONIST IDAZOXAN ON FOOD AND WATER INTAKE IN RATS H. C. Jackson* and I. J. Griffin*. (SPON: Brain Research Association). Reckitt & Colman Psychopharmacology Unit, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, England.

Idazoxan is an alpha-2-adrenoceptor antagonist which binds with high affinity at non-adrenoceptor idazoxan binding sites (NAIBS). These sites are localised in the hypothalamus and area postrema (Hudson et al. Br. J. Pharmacol. 102:4P, 1991) and may play a role in the control of appetite. Hence we have compared the effects of idazoxan on food and water intake with those of its 2-ethoxy and 2-methoxy analogues RX811059 and RX821002, which act more selectively at alpha-2-adrenoceptors, having only minimal affinity at NAIBS (Mallard et al. Br. J. Pharmacol. 102:221P, 1991), and with those of the peripherally-acting alpha-2-antagonist L-659,066 (Clineschmidt et al. J. Pharmacol. Exp. Ther. 245:32, 1988).

Experiments were performed during the light phase on singly-housed male Wistar rats (250-350g) with free access to powdered diet and water at all times. Feeding jars and water bottles were weighed at the time of drug administration (i.p.) and after 1, 2 and 4 hours to enable calculation of mean group cumulative intakes g/kg rat weight ± s.e. mean (n=6).

Idazoxan (1, 3, 10 mg/kg) significantly increased both food (over five-fold) and water (two-fold) intake in the first 1-2 hours after injection. RX811059 (0.3, 1, 3 mg/kg), RX821002 (0.3, 1, 3 mg/kg), and L-659,066 (1, 3, 10 mg/kg) had no effect on feeding during the 4 hour test but the highest doses of each antagonist significantly increased drinking (over two-fold) in the first 1-2 hours following drug administration. These results suggest that the effects of idazoxan on water intake may be mediated by blockade of peripherally-situated alpha-2-adrenoceptors whereas its effects on feeding may be related to its high affinity at NAIBS.

57.15

AN ANORECTIC ACTION OF 2-METHYLSEROTONIN (2-Me-5-HT) WITHOUT APPARENT BEHAVIORAL TOXICITY. K. J. Simansky, F. C. Sisk* and J. Jakubow*, Dept. Pharmacology, Med. Coll. Pennsylvania, Philadelphia, PA 19129.

The serotonin (5-HT) analog, 2-Me-5-HT, is the prototypical **agonist** at 5-HT_3 receptors. 5-HT_3 **antagonists** are antiemetic agents. This study compared the actions of 2-Me-5-HT, given i.p., on feeding with its ability to act as an unconditional stimulus in a taste aversion paradigm (CTA). In a 30-min test using milk after overnight food deprivation, 2-Me-5-HT (.33-40 µmol/kg) produced anorexia with 20 and 40 µmol/kg reducing intakes by 38% and 59%. In a separate study using time-sampling analysis, 20 µmol/kg 2-Me-5-HT decreased milk intake by 27% (16.8 ± 1.6 ml vs. 23.1 ± 1.8 ml for controls, $p< .05$). 2-Me-5-HT reduced feeding frequency, shortened meal length and increased resting without altering nonfeeding activity or the normal periprandial sequence of behavior. This dose of 2-Me-5-HT was not an aversive stimulus in a two-bottle CTA although 20 µmol/kg of **5-HT** (but not 10 µmol/kg) does produce a taste aversion. Neither the 5-$HT_{1/2}$ antagonist, methysergide (METHY) nor the 5-HT_3 antagonist, ICS 205-930 antagonized anorexia after 2-Me-5-HT in the milk test. METHY did prevent the anorectic effect of the ID_{50} dose of alpha-Me-5-HT, a 5-HT_2 agonist. 2-Me-5-HT also decreased 30-min intakes of mash after deprivation (16.5 ± 1.6 g vs. 32.8 ± 1.4 g, con, $p< .01$) and METHY and ICS (20 µmol/kg) failed to reverse the anorexia. It appears that 2-Me-5-HT can inhibit feeding without producing behavioral toxicity. A receptor not of the serotonin-1, 2, 3 or 4 subclasses seems to mediate this action. Grant MH41987 to KJS.

57.16

THE STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR ANTAGONIST 7-CHLOROKYNURINIC ACID INDUCES FEEDING IN SATIATED RATS. T.L. Sorrels and E. Bostock. Department of Psychology, Queens College-CUNY, Flushing, NY 11367.

We tested whether the strychnine-insensitive glycine receptor antagonist, 7-chlorokynurinic acid (7-CK), induced feeding, stereotypies, and locomotor deficits following icv administration. Using a within subjects design, six rats were administered vehicle (v), 10, 20, or 30 ug of 7-CK icv 1/wk for 4 weeks in a random order. Immediately following the injections food intake was measured for 2 30 min intervals, and the presence of stereotypies and locomotor deficits was rated 1/min for 60 min. 7-CK induced a significant dose-dependent increase of food intake. The mean intake for the v, 10, 20, and 30 ug groups was .34, 1.5, 1.9, and 3.6 g respectively. The mean latency for the onset of this effect was 4.1 min in the 30 ug group. There was also a dose-dependent increase in locomotor deficits. No stereotypies were observed. A second group of rats received icv v, .015, .15, 1.5, or 3.0 uM D-serine (D-s), a strychnine-insensitive glycine receptor agonist, 2 min prior to icv injection of 20 ug 7-CK, and food intake was measured for 60 min. D-s produced a significant dose-dependent reduction of 7-CK induced intake, such that the 3 uM D-s/20 ug 7-CK group did not significantly differ from the v/v group. These findings suggest a role of the strychnine-insensitive glycine receptor, either via modulation of the NMDA receptor or otherwise, in the regulation of food intake.

57.17

CYCLIC AMP REDUCTION IN THE PREPYRIFORM CORTEX AFTER AN AMINO ACID IMBALANCED DIET. D.W. Gietzen, S.M. Wimberg* and Q.R. Rogers. Depts. VM:Physio. Sci., Med: Psychiatry and Food Intake Lab., Univ. Calif. Davis, Davis, CA 95616.

The anorectic responses to imbalanced amino acid diets (IMB) have been associated with an increase in norepinephrine (NE) activity and protein synthetic processes in the prepyriform cortex (PPC), an area essential for the initial feeding responses of rats to IMB. Clonidine, an $alpha_2$ autoreceptor agonist increased intake of IMB, suggesting that NE acts at the $alpha_2$ receptor in the feeding responses to IMB. Because clonidine can inhibit the production of cyclic AMP (CAMP) via the $alpha_2$ receptor and cAMP has been linked to protein synthesis, we measured cAMP concentrations in the PPC of rats fed either threonine IMB or a basal control diet (BAS). After prefeeding BAS for 10 days, rats were given IMB or BAS for 2 hours at dark onset. They were rapidly decapitated, the brains frozen in liquid N_2, and stored at -80°C. The PPC and the anterior cingulate cortex (AC), which does not show the changes in NE after ingesting IMB, were dissected, sonicated in ETOH·HCl, centrifuged and the supernatant evaporated under N_2. The pellet was assayed for the concentration of cAMP using a kit (Amersham). To control for any generalized changes in cAMP, the data for the PPC were compared to those for the AC. In animals fed IMB, the CAMP in PPC= 0.84±0.12 (mean±SE, pmol/mg wet tissue wt) AC= 2.22±0.16; for the BAS group PPC= 1.2±0.22, AC= 1.82± 0.31; $p < 0.05$ due to differences between tissue values in IMB. This decrease in cAMP in the PPC after eating IMB suggests a link between the NE system and protein synthesis in the PPC upon ingestion of imbalanced amino acid diets.
Supported by USDA: CRCR-1-2418 and NIH: BRS-RR05457.

STRESS, HORMONES AND THE AUTONOMIC NERVOUS SYSTEM

58.1

THREE SESSIONS OF TAILSHOCK PRODUCE AN INCREASE IN THE PLASMA CHOLESTEROL LEVELS OF RATS MAINTAINED ON A LOW-FAT DIET. Francis X. Brennan, Jr., R. F. Soames Job*[1], Linda R. Watkins and Steven F. Maier. Psychology Department, University of Colorado, Boulder, CO. 80309, [1]Psychology Department, University of Sydney, Sydney, Australia.

Increases in plasma cholesterol have been demonstrated to be a risk factor for the development of atherosclerosis, the most common form of Coronary Heart Disease (CHD). Animal subjects exposed to stress typically show elevated levels of cholesterol in plasma, relative to nonstressed controls. These studies, however, generally utilized relatively long stress procedures, and also used animals maintained on a high-cholesterol diet. As a first step towards analyzing the mechanisms responsible for stress produced increases in cholesterol, we sought to determine whether a brief stress paradigm might produce reliable increases in plasma cholesterol in animals maintained on standard lab chow.

Our experimental animals were male Sprague-Dawley rats, approximately 90 days old at the beginning of the stress procedure. Subjects had free access to standard lab chow and water, with food being removed from all animals four hours prior to all blood samples in order to minimize acute feeding-induced variations in cholesterol levels. A stress session consisted of 100 1.6 mA 5 sec tail shocks, presented on the average of one shock per minute. Stressed animals were given one stress session a day for three consecutive days. Blood samples were taken 2 hrs after the end of each shock session. Results indicated that stressed animals had significantly higher plasma cholesterol levels than did nonstressed controls after the third stress session.

These results differ from prior reports in the literature in both the length of the effective stress procedure, and the lack of a high cholesterol diet. We believe that this paradigm will allow us to begin to efficiently investigate the neural and hormonal mechanisms responsible for the cholesterol increases observed in our stressed subjects. Preliminary research suggests that the endogenous opioid peptides, for example, may be involved in mediating stress-induced hypercholesterolemia.
Supported by NSF BNS 88-09527.

58.2

STRESS DEPRESSES [^{125}I]MELATONIN BINDING IN RAT SUPRACHIASMATIC NUCLEI. C.Tenn* and L.P. Niles. Dept. of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

The effect of swim-stress, which increases circulating melatonin, was examined on high-affinity binding of [^{125}I]melatonin ([^{125}I]MEL) in rat suprachiasmatic nuclei (SCN). Twenty min after a 10-min swim at 20 ± 2°C, stressed animals and controls were rapidly decapitated and trunk blood and brains collected. Serum melatonin levels were significantly higher in stressed animals than in controls ($p<.001$). Saturation binding was measured in washed hypothalamic sections (20µm) in which binding is predominantly confined to the SCN. There were no differences in binding affinity between the two groups (control k_d = 52 ± 2 pM; stress K_d = 47 ± 3 pM), but the density of receptor sites in the stress group was significantly lower (control B_{max} = 6.4 ± 0.3 fmol/mg protein; stress B_{max} = 3.3 ± 0.2 fmol/mg protein; $p<.001$, $n=4$).

These findings indicate that an acute stress-induced increase in circulating melatonin causes rapid down-regulation of SCN receptors for melatonin.
Supported by NSERC Canada.

58.3

STRESSFUL INTERVIEW INCREASES PLASMA LEVELS OF THYROID STIMULATING HORMONE (TSH) AND GROWTH HORMONE (GH) IN HUMANS. E.H. Mougey, M.A. Oleshansky and J.L. Meyerhoff. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

As part of a continuing effort to study neuroendocrine responses to psychological stress in humans, we examined plasma TSH and GH responses in normal male military volunteers (N=16) undergoing a 30-minute job-related structured interview. The soldiers gave written informed consent prior to appearing before a panel of senior non-commissioned officers to compete for the honor of "Soldier of the Month". This type of interview has been shown to elicit marked plasma ACTH and cortisol increases in these subjects as previously reported [Psychosomatic Medicine, 50, 295-303 (1988)]. Blood samples were taken at three time points prior to entering the room, twice while participating in the interview and two times after leaving the room. Samples were drawn from an indwelling catheter into EDTA tubes and the plasmas were stored frozen until assayed. IRMA kits (Nichols Institute) were used to determine TSH and GH in unextracted plasmas. ACTH (INCSTAR Corp.) and cortisol were determined by radioimmunoassay in extracted plasmas.

ACTH and cortisol showed significant increases as a result of the interview, replicating our previous report. Mean plasma TSH levels increased 13% during the interview and returned to baseline levels immediately afterward. Mean plasma GH levels were slightly elevated at the end of the interview and continued to increase to four times baseline levels immediately afterward.

58.4

EFFECT OF STRESS ON CHOLECYSTOKININ (CCK) RECEPTOR LEVELS IN THE RAT BRAIN. K.D. Richardson Morton, L.D. Van de Kar+, J.B. Ackman and N.J. MacLusky. Div. of Reproductive Sci., Univ. of Toronto, ONT M5G 1L7, and +Dept. of Pharmacology, Loyola Univ. Med. Ctr, Maywood, IL 60153.

Adrenalectomy (ADX) increases CCK immunoreactivity in corticotrophin-releasing factor (CRF) neurons of the paraventricular (PVN) nucleus (Mezey *et al* PNAS 83:3510, 1986) suggesting that, in addition to its role in the control of appetite, CCK may also be involved in neuroendocrine responses to stress. In female rats, CCK receptor levels are down-regulated by estrogen (Morton et al., *Neurosci. Abstr* 451.3,1990; Akesson et al. *Neuroendo* 45:257, 1987). We tested the hypothesis that steroids involved in stress responses might also modulate CCK receptor levels in the brain. Male rats were either ADX, sham-ADX, or ADX and treated with dexamethasone (DEX, 200µg/kg/day, s.c., for 3 d). Animals were killed on day 3 and their brains immediately frozen. CCK receptors in brain sections (10 µm) were labelled by incubation with 0.1 nM ^{125}I-CCK-8 (1h, r.t.) in 50 mM Tris-$MgCl_2$ buffer (pH 7.7) containing 0.2% bovine serum albumin, 1mM dithiothreitol, and 0.2% bacitracin, then exposed against Hyperfilm (Amersham) for 2 weeks. Film densities were assessed using computerized densitometry in the following brain regions: cingulate cortex, hippocampus, ventromedial nucleus and PVN. No differences were observed in CCK binding between ADX and sham-ADX rats, for any brain region. However, in the ADX DEX-treated group, CCK binding was slightly but significantly lower than in controls. Addition of DEX (10^{-6}M) to brain sections in vitro had no effect on CCK binding. To study the effects of increased endogenous glucocorticoid levels, male rats were stressed by immobilization for 20 min. on 5 consecutive days and sacrificed immediately following the last immobilization. Control animals were handled identically, but did not undergo the stress procedure. The stressed rats had significantly increased corticosterone levels and gained less weight than controls. However, no significant differences were observed in CCK receptor levels between control and stressed rats, in any brain region. These data suggest that high doses of DEX may have a slight inhibitory effect on CCK binding in the brain; but that neither physiological levels of adrenal steroids nor 5-day immobilization stress have a significant impact on brain CCK receptor levels (Supported by MRC Canada).

58.5

BASAL SYMPATHOADRENAL FUNCTION IN POST-TRAUMATIC STRESS DISORDER. M. M. Murburg, M. E. McFall*, E. Petrie*and R. C. Veith*. Seattle VAMC, University of Washington, Seattle WA 98108

Previous findings of elevated resting blood pressure (BP) and heart rate (HR) in patients with Post-traumatic Stress Disorder (PTSD) suggest that basal sympathetic nervous system (SNS) activity may be increased in this disorder. We have measured Epinephrine (EPI) and Norepinephrine (NE) in arterialized venous plasma (Study #1) and assessed plasma norepinephrine kinetics in arterialized plasma using a radioisotope dilution technique (Study #2) in order to investigate basal SNS function in patients with PTSD compared with asymptomatic normal controls. In the first study, eleven Viet Nam combat veterans (mean age=40.3 years) with PTSD were compared with eleven asymptomatic normal controls (mean age=40.2 years). PTSD patients did not exhibit statistically significant differences from controls in HR (62±7 vs 62±1 beats per minute), systolic (124±11 vs 120±7 mm hg) or diastolic BP (79±8 vs 76±7 mm hg) or in resting plasma levels of NE (237±52 vs 232±70 pg/ml) or EPI (67±33 vs 81±36 pg/ml). In the second study, six Viet Nam combat veterans with PTSD diagnosed by the SCID (mean age=40) and five asymptomatic normal controls (mean age=37) have been compared to date. Neither the rate of appearance of NE into plasma (0.211±0.033 vs 0.351±0.109 $\mu g/min/m^2$), nor the rate of clearance of NE from plasma (1.259±0.128 vs 1.363±0.207 $L/min/m^2$) differed significantly between the PTSD patients and the control subjects. The results of these two studies do not lend support to the hypothesis that SNS activity is tonically increased in patients with PTSD.

58.6

SWIM STRESS INDUCED ALTERATIONS IN REGIONAL 2-DEOXYGLUCOSE (2-DG) UPTAKE AND C-FOS PROTEIN INDUCTION: DIFFERENTIAL MODIFICATION BY IMIPRAMINE. G.E. Duncan, K.B. Johnson, W.E. Stumpf, and G.R. Breese Brain and Development Research Center, School of Medicine, University of North Carolina, Chapel Hill, NC 27599

The effects of imipramine were assessed on regional brain 2-DG uptake and c-fos like immunoreactivity in rats that were processed in the forced swim test. Swim stress induced a marked increase in 2-DG uptake in a specific region of the lateral septal nucleus (LSN). In rats given IMP, the stress-induced activation of the LSN was almost completely abolished. Swim stress induced c-fos in a select region of the LSN that corresponded in part to the region that displayed increased 2-DG uptake. However, IMP treatment did not antagonize the induction of fos in the LSN. Swim stress also induced fos protein in the parvocellular paraventricular nucleus (PVN) and IMP antagonized this response. Swim stress had no apparent effect on 2-DG uptake in the PVN. The results show that IMP antagonizes swim stress induced activation in select brain regions and that 2-DG uptake and c-fos induction provide distinct and complementary information. Supported by MH 39144 and MH-33127.

58.7

NEUROCHEMICAL RESPONSES TO ADRENALECTOMY. K. Kolasa and R. S. Jope. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294.

The hippocampus appears to be one of the principal targets of glucocorticoids in the brain. Removal of circulating glucocorticoids by adrenalectomy of adult male rats results in degeneration of granule cells in the dentate gyrus (Sloviter et al., Science 243:535, 1989). Therefore, we examined behavioral and neurochemical responses to adrenalectomy in rats.

Adrenalectomy caused a slight deficit in the acquisition of a conditioned avoidance response measured after 4 weeks. Choline acetyltransferase activity, a marker of cholinergic cells, was not different from controls 7 or 28 days after adrenalectomy. Therefore, the learning impairment did not appear to be due to a cholinergic lesion.

Phosphoinositide hydrolysis is an important second messenger-generating system which can modulate calcium concentrations and protein kinase C activity. Several types of brain lesions have been reported to result in enhanced receptor-activated phosphoinositide hydrolysis. Adrenalectomy caused increased stimulation of phosphoinositide hydrolysis induced by norepinephrine or by excitatory amino acid agonists when measured 4 weeks after adrenalectomy in hippocampal slices, but did not alter the response to carbachol and had only slight effects in cortical slices.

These results show that adrenalectomy alters the modulation of phosphoinositide metabolism in rat hippocampus.

58.8

STRESS-INDUCED CHANGES IN DAILY ACTIVITY IN THE RAT ARE MODULATED BY DIFFERENT FACTORS THAN ARE STRESS-INDUCED ESCAPE LEARNING DEFICITS. W.W. Woodmansee, L.H. Silbert*, & S.F. Maier. Dept. of Psychology, Univ. of Colorado Boulder, CO 80309

Exposure to stressful stimuli produces a variety of behavioral alterations in the rat. The escape learning deficit that follows exposure to some stressors is one of the best studied. Escape learning deficits in tasks such as shuttlebox escape occur following inescapable shocks (IS), but not following equal amounts of escapable shock (ES). This behavioral change persists for 48-72 hours after IS, can be blocked by administration of opiate antagonists before IS, and can be produced by anxiogenics such as FG-7142.

More recently, we have found a stress-induced behavioral change that follows a more prolonged time course. Daily running wheel activity (RWA) was depressed for 14-42 days following exposure to IS. Experiments were undertaken to determine whether factors known to be important in the development of the short-term behavioral changes following IS would also be important in the production of the long term effect of IS on daily RWA. We assessed the impact of three factors on daily RWA: escapability of the stressor, reversal with naltrexone pretreatment, and induction with FG-7142. The results indicated that daily RWA is not sensitive to escapability of the stressor. IS and ES produced the same degree of depression of daily RWA. Naltrexone (10 mg/kg) given 20 min prior to IS did not prevent the stress-induced reduction in daily RWA. Additionally, rats given the anxiogenic β-carboline, FG-7142 (10 mg/kg), did not differ from controls. FG-7142 did not mimic IS in its ability to reduce daily RWA. These results suggest that the prolonged reduction in daily activity following IS is a fundamentally different phenomenon than many of the behavioral consequences of IS that are of shorter duration. Supported by NSF Grant BNS 88-09527.

58.9

SUBCHRONIC CORTICOSTERONE TREATMENT LEADS TO MINOR LEARNING IMPAIRMENT IN THE RADIAL ARM MAZE IN YOUNG RATS. A. Levy and S. Dachir*. Pharmacology Dept., Israel Inst. Biological Research, Ness Ziona, 70450, ISRAEL.

High levels of corticosterone are associated with stress, and may lead to hippocampal damage and memory impairments. Corticosterone slow release pellets, implanted subcutaneously into four months old Fischer rats (200 mg released over 3 weeks), resulted in elevated corticosterone plasma levels, comparable to those found in rats under stress. Radial arm maze training which followed this treatment revealed significant difference between corticosterone and placebo treated groups only in one of the monitored parameters (first error entry, n=12 in each group, $p<0.05$ by ANOVA). Only minor morphological changes were found in the hippocampal area under histological observations. Longer period of treatment might be needed to substantiate a more robust cognitive impairment.

58.10

CHRONIC STRESS: EFFECTS OF STRESS CONTROLLABILITY. G.J. Kant, R.A. Bauman, S.M. Anderson and E.H. Mougey. Dept. Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307-5100.

Our laboratory has been characterizing the physiological and behavioral effects of chronic stress utilizing a rodent paradigm in which rats are maintained 24hr/day in operant cages and required to avoid/escape signalled intermittent footshock. We have shown that rats exposed to this stressor have elevated levels of plasma corticosterone, decreased leverpressing for food pellets and disturbed circadian temperature rhythms. However, after several days, food intake, corticosterone levels and temperature rhythms gradually return toward control levels. In the present study, a yoked stressed group, which was given no control over shock delivery, was added to the paradigm. Each yoked rat was paired to a controlling stressed rat and the controlling rat could avoid/escape for both rats by pulling a chain. After 3 days of stress, yoked animals were found to have higher levels of plasma corticosterone than the paired controlling rats, greater disruptions of temperature rhythms and more marked decreases in leverpressing for food.

58.11

ANIMAL MODELS FOR POST-TRAUMATIC STRESS DISORDER. B.I. Diamond, M.B. Hamner*, T.L. Chalker*, and R.L. Borison. Medical College of Georgia, Augusta, GA 30912

It has been hypothesized that exposure to inescapable stress resulting in learned helplessness (LH) may provide an animal model for post-traumatic stress disorder (PTSD). Changes in noradrenergic function may occur in both LH and PTSD. Male Sprague Dawley rats (160g) underwent either acute stress (SA) or 6 days of either chronic variable (CVS) or chronic homogeneous stress (HMS). They were then challenged with either yohimbine (YB) (7.5mg/kg, i.p.) or clonidine (CL) (0.1mg/kg, i.p.) to stimulate or inhibit norepinephrine release. The effect of these drugs on immobility during a 5 min. swim test was quantified. In all groups CL antagonized the effects of stress (SA 1.2 $\pm$.2 (mean $\pm$sd);CVS 1.0$\pm$1.4;HMS 3.1$\pm$.1;$p<.05$). There were no differences between the types of stress on immobilization. Moreover, SA produced the same effects as did chronic. The differential effects of YB (4.25 $\pm$ .84) and CL (1.18 $\pm$.25) were most noticeable in the the SA group, indicating that SA may be a better model for PTSD than chronic stress.

58.12

GASTRIC EROSION FORMATION INDUCED BY LESIONS OF THE SUBSTANTIA NIGRA OR VENTRAL TEGMENTAL AREA. C.V. Grijalva, B. Roland, G. Burcham*, K. Branch*, T.M. Nguyen*. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Lateral hypothalamic (LH) lesions produce a host of behavioral and autonomic symptoms including anorexia, sensorimotor deficits, and glandular gastric erosions. Many of these symptoms have been attributed to disruption of dopamine systems originating in the pars compacta, substantia nigra (SN) or ventral tegmental area (VTA). The present study examined the possibility that direct damage to the SN or VTA induces gastric erosions. Groups of rats were food deprived (18 h), anesthetized, and then given control operations (N=9) or electrolytic lesions of the SN (N=7) or VTA (N=11). Animals were sacrificed 24 h postoperatively. Only Group SN display significantly more gastric erosions than controls ($p<.05$). A second experiment demonstrated that the formation of erosions induced by SN lesions could be blocked by atropine methyl nitrate. The results suggest that the nigral striatal system may be involved in parasympathetically mediated gastrointestinal function.
(Supported by UCLA Univ. Research Grant PZ-06)

58.13

BEHAVIOR AND PHYSIOLOGY OF SOCIALLY STRESSED RATS: ANTI- AND PRO- STRESS EFFECTS OF ANXIOLYTICS. W. Tomatzky and K.A. Miczek. Dept. of Psychology, Tufts University, Medford, MA 02155

In an intense social confrontation an intruder rat is briefly forced to the limits of its physiological and behavioral capacities. The function of these acute reactions is to cope with the high demand of this significant situation. However, potentially detrimental long-term consequences of such stressfull confrontations persist. In an attempt to delineate the magnitude and temporal sequence of social stress responses, we implanted adult male Long Evans rats with senders. The transmitted heart-rate (HR) and temperature (T_c) data were monitored via telemetry (Mini-Mitter, Dataquest III) and evaluated together with the recorded data on behavior and ultrasounds. Repeated dayly defeats on 5 consecutive days changes circadian rhythmicity (cosinor analysis) of HR and T_c. The amplitude of these rhythms remains decreased for 10 days after the last contact with the resident rat, indicating the longlasting and severe impact of the challenge on physiological regulation. In order to investigate the potential anti-stress effects of anxiolytic drugs, we pretreated intruder rats with diazepam (1 - 10. mg/kg, IP) , gepirone (0.3 - 6.0 mg/kg, IP), and clonidine (0.01 - 0.1 mg/kg, IP). In contrast to the sedative effects on locomotor activity, non of the compounds affected the prominent defensive postures and acts of the experimental rat in reaction to intense threats of the resident rat. The large elevations of T_c and HR during the threat period are dose-dependently decreased by clonidine. The hyperthermia is attenuated at the higher gepirone and diazepam doses. Diazepam as well as gepirone do not effectively decrease the HR response. Ultrasonic vocalizations (20-30 kHz range) emmitted at high rate the are not decreased by clonidine nor diazepam. At 3.0 and 6.0 mg/kg these vocalizations are almost abolished by gepirone.
Physiological and behavioral stress markers are alleviated differentially by the compounds. However, the prevention to cope within a relevant behavioral context by a compromising dose may by itself induce an increased stress response.

58.14

SELECTIVE BREEDING OF RATS FOR SUSCEPTIBILITY TO THE EFFECTS OF UNCONTROLLABLE SHOCK: ELECTROPHYSIOLOGICAL AND BEHAVIORAL EFFECTS. P.A. Scott, C.A. Lorenz*, E.F. Hargrove*, and J.M. Weiss. Dept. of Psychiatry. Duke University Medical Center, Durham, NC 27710

One effect of exposure to uncontrollable electric shock in rats is to decrease motor activity as measured in a swim test; however, not all animals within a given population show this effect. We have previously reported the results of three generations of selective breeding of Sprague Dawley rats to be either susceptible or resistant to this effect of uncontrollable shock (Scott, Cierpial, & Weiss, Soc. Neurosci. Abstr., 15, Part 1, p.801, 1989). In the current (5th) generation, heritability remains a significant factor; rats bred for susceptibility (SUS) continue to show significantly lower levels of activity in the swim test after stress while rats bred for resistance (RES) show no such decrease in activity.

Decreased swim activity is correlated with increased levels of spontaneous and evoked activity of locus coeruleus (LC) neurons (Simson & Weiss, Neuropsychopharmacology, 1, 287-295, 1988). When electrophysiological recordings of single unit activity were made, LC neurons of SUS rats showed significantly higher levels of both spontaneous and evoked activity following stress compared to nonstressed SUS rats, while LC neurons of RES rats did not show significant increases in either spontaneous or evoked activity compared to nonstressed RES rats. Interestingly, the evoked activity of nonstressed RES rats was significantly higher than the evoked activity of nonstressed SUS rats.

SUS and RES rats were tested for differences on a number of behavioral measures known to be affected by shock exposure, including 24 hr homecage activity and food and water intake. Differences in these measures did not match the differences seen in the swim test. For example, male SUS rats displayed significantly depressed homecage activity (during dark period) for 3 days after stress, while male RES rats showed depressed activity for a full two weeks after stress.

58.15

THE EFFECTS OF MUSIC TEMPO VERSUS MUSICAL STLYES UPON MUSCLE TENSION REDUCTION. D. F. Harris*, and S. R. Harris. Harris and Associates, 715 W. Sherman, Harrison, AR 72601

The primary purpose of this study was to discover if there was a difference in relaxation states while listening to fast versus slow music and secondarlily, if there was a difference between rock music versus classical music. Eight subjects listened to either fast classical, fast rock, slow classical, or slow rock conditions. An Autogenic 1100 feedback myograph was used to measure muscle tension across the frontalis muscle. Relaxation was infered by lowered muscle tension levels after a five minute session. Significance was found at the $p < .01$ level concerning musical tempo but not stlye. Further statistical analysis indicated a slow tempo would significantly ($p < .01$) decreased muscle tension. These results could indicate a specific tempo or rhythm may synchronize with a biological homeostatic rhythm.

59.1

EFFECTS OF THE 5-HT 1A AGONIST GEPIRONE DEPEND UPON PRE-TREATMENT TIME. J.B. Richards, D.C. Jolly, K.E. Sabol, L.S. Seiden. University of Chicago, Dept. of Pharm./Phys. Sci., Chicago, Ill 60637

The differential reinforcement of low rate 72-s (DRL 72-s) schedule reinforces only those responses which occur > 72-s since the last response. In rats trained to bar press for water, the contingency of reinforcement enforced by the DRL 72-s schedule generates a pattern of responding which is very sensitive to drug effects. This pattern of responding is reflected in the distribution of interresponse times (IRTs) which has a peak (modal point) near the 72-s criterion for reinforcement. The effects of drugs on the IRT distribution can be quantitatively characterized by measuring the temporal location of the peak and the area under the curve formed by the IRT distribution. Antidepressant drugs cause the temporal location of the peak to shift toward longer IRT durations without decreasing the area under the curve.

Previously, we reported that in rats trained on the DRL 72-s schedule (1hr sessions) the 5-HT 1A agonist gepirone causes dispersion of the IRT distribution as indicated by a decrease in the area under the curve. Here we report that this effect is dependent upon pre-treatment time. When given with a pre-treatment time of 0 min, gepirone (2.5, 5.0, 10.0 mg/kg) disperses the IRT distribution toward random, decreasing the area under the curve. In contrast, gepirone (2.5, 5.0, 10.0 mg/kg) given with a pre-treatment time of 1 hr does not decrease the area under the curve and shifts the peak location to the right. This latter effect is consistent with the effects seen with antidepressant compounds.

Dispersion of the IRTs associated with gepirone (0 min) could be mediated by decreased 5-HT release produced by stimulation of 5-HT 1A autoreceptors. (Supported by: MH-11191; RSA-10562 L. Seiden)

59.2

DEPLETION OF BRAIN SEROTONIN WITH 5,7-DIHYDROXYTRYPTAMINE IS ASSOCIATED WITH A PERSISTENT BEHAVIORAL DEFICIT IN RATS PERFORMING ON THE DIFFERENTIAL REINFORCEMENT OF LOW RATE-72 SECOND OPERANT SCHEDULE OF WATER REINFORCEMENT. D. C. Jolly, J. B. Richards, and L. S. Seiden, Dept. of Pharmacol. and Physiol., University of Chicago, Chicago, Illinois. 60637.

The Differential Reinforcement of Low Rate-72 second (DRL-72s) operant schedule of water reinforcement is a behavioral screening procedure for antidepressant drugs. The purpose of this experiment was to assess the involvement of the brain serotonergic system in the performance of DRL-72s behavior. Trained rats were given bilateral, intracerebroventricular (icv) infusions of 200 µg of 5,7-dihydroxytryptamine (5,7-DHT). A control group received icv infusions of vehicle. After the rats recovered from surgery, DRL-72s performance was continually assessed for a total of 17 weeks. At 15 weeks post-lesion, a 5-hydroxytryptophan (5-HTP) dose-effect determination was made. Throughout the experiment, under drug free baseline conditions, the 5,7-DHT rats made more responses and obtained fewer reinforcers than they did in pre-lesion baseline sessions. The behavioral deficit in the 5,7-DHT treated rats was, apparently, secondary to a derangement of the temporal distribution of operant responding. In the sham operated control rats, the DRL-72s baseline response and reinforcement rates remained unchanged, and the temporal distribution of responding was unaltered. Administration of 5-HTP to the 5,7-DHT treated rats was associated with a partial amelioration of the behavioral deficit. The neurochemical assay results indicated specific, extensive depletion of serotonin (5-HT) in the 5,7-DHT treated rats, in all brain regions examined. These results suggest that a certain level of 5-HT system activity is necessary for normal response timing in the performance of DRL-72s behavior.

This work was supported by NIDA DA-00085, MH-11191, and RSA-10562 (L. Seiden).

59.3

ESTROUS CYCLE MODULATION OF THE HYPERPHAGIA INDUCED BY THE 5-HT_{1A} AGONIST, 8-OH-DPAT. S. Salamanca*, M. Pastuszka-Caldarola* and L. Uphouse, Department of Biology, Texas Woman's University, Denton, Texas, 76204.

Treatment with the 5-HT_{1A} agonist, 8-OH-DPAT, produces several behavioral syndromes characteristic of increased serotonergic activity; other behaviors appear paradoxical. Since 5-HT inhibits eating behavior, the unexpected hyperphagia elicited by 8-OH-DPAT has been attributed to the presence of 5-HT_{1A} autoreceptors on raphe neurons. This is of particular interest since 8-OH-DPAT's ability to decrease firing of raphe neurons is attenuated by estradiol treatment. Thus, variations in raphe autoreceptor activity could contribute to known estrous cycle variations in eating behavior. 8-OH-DPAT (0.1-1.0 mg/kg, s.c.) -induced hyperphagia was examined in male rats and in female rats on the days of diestrous 2, proestrus and estrus. Hyperphagia was most evident in males and in diestrous females and least evident in proestrous and estrous females. Studies of 8-OH-DPAT-induced hypothermia (thought to result from an action at postsynaptic sites) demonstrated a significant sex difference, but changes during the estrous cycle were small. Thus, the present findings suggest that the somal/dendritic 5-HT autoreceptor may be functionally modulated during the estrous cycle.

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59.4

EFFECTS OF ISOLATION REARING ON INDICES OF 5-HYDROXYTRYPTAMINE FUNCTION IN RAT HIPPOCAMPUS. L.S. Wilkinson*, F.S. Hall, T. Humby* and T.W. Robbins. **SPON: Brain Research Association.** Experimental Psychology, University of Cambridge, Cambridge CB2 3EB. U.K.

Isolation rearing has been shown to precipitate a variety of behavioural changes in the rat, including increased reactivity to novelty, deficits in rule learning, hyperphagia and enhanced responding in reward-related situations. Most work on the neurochemical changes underlying the "isolation syndrome" has centred on dopamine. The present study examined the effects of isolation rearing on indices of 5-hydroxytryptamine (5HT) function in hippocampus. In the first experiment male weanlings (21 days postnatal) were divided into isolates and social controls. After 90 days the animals were killed and tissue levels of indoleamines in hippocampus determined. 5HIAA/5HT ratios were significantly lower in isolates than in controls. In the second experiment weanlings were isolated as above, anaesthetised with halothane and hippocampal extracellular 5HT monitored using *in-vivo* microdialysis. Potassium-evoked release of 5HT (intraprobe, 100mM, 20 min.) was significantly reduced in the isolated group. These data are consistent with there being a reduction in the releasable pool of 5HT in the hippocampus of isolates.

59.5

8-OH-DPAT DISRUPTS PREPULSE INHIBITION OF ACOUSTIC STARTLE IN WISTAR RATS. R.F. Kucharik*, J.A. Moyer and K.L. Marquis. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

Prepulse inhibition (PPI) is a measure of sensorimotor gating that may be elicited in humans as well as in animals. In rats, previous studies have described the ability of dopaminergic agonists and glutamatergic antagonists to disrupt PPI. In addition, these animal studies have shown that GABA antagonists and cholinergic agonists also disrupt the PPI response. The role of serotonin (5-HT), however, has not been examined in depth. In the current study, the selective 5-HT1A agonist 8-OH-DPAT (0.1-3.0 mg/kg sc) was given to male Wistar rats (Charles River) 30 minutes prior to being placed in a San Diego Instruments startle apparatus. Startle responses to a 114 dB acoustic stimulus (PULSE) were collected (digitized over 100 ms) during trials with and without a 71 dB stimulus (PREPULSE occurring 100 ms prior to PULSE) against a 65 dB background. Trials consisting of no stimulus and prepulse alone were also given. Data collected on PREPULSE/PULSE trials were expressed as a percent of PULSE alone. Vehicle-treated rats had a startle response on PREPULSE/PULSE trials which was 50-60% of that occurring in PULSE alone trials. 8-OH-DPAT, at 0.3 and 1.0 mg/kg, disrupted PPI in a manner similar to that observed in rats treated with apomorphine (0.3-3.0 mg/kg sc). Likewise, PPI was disrupted in rats treated with BMY-7378 (1.0 and 3.0 mg/kg sc), a weak 5-HT1A partial agonist. Haloperidol (1.0 mg/kg sc) blocked the disruption of PPI induced by apomorphine (0.6 mg/kg sc), but not that induced by 8-OH-DPAT (0.3 mg/kg sc). BMY-7378 (0.3 mg/kg sc), at a dose which did not affect PPI per se, was also unable to block a 8-OH-DPAT-induced disruption of PPI. In contrast, WAY-100135, a novel 5-HT1A antagonist (see J.T. Haskins *et al.*, and P. Mitchell *et al.*, this meeting), given at 10.0 mg/kg sc, attenuated 8-OH-DPAT-induced PPI disruption. Thus, these data support a potential role for the 5-HT1A receptor in this sensorimotor gating phenomenon. Moreover, as PPI is disrupted in schizophrenic patients, it is possible that serotonergic systems may contribute to these PPI deficits.

59.6

FACILITATED ACQUISITION OF A CONDITIONAL VISUAL DISCRIMINATION. FOLLOWING 5,7-DIHYDROXYTRYPTAMINE-INDUCED LESIONS OF FOREBRAIN SEROTONINERGIC PROJECTIONS IN RATS. B. Ward,*[1] B.J.Everitt,[1] T.W.Robbins,[2] L.S.Wilkinson,[2] (Spon: Brain Research Assn). [1]Dept Anatomy, [2]Dept Experimental Psychology, University of Cambridge, Cambridge CB2 3DY, England.

The role of forebrain serotonin (5-HT) in cognition is unclear, although previous studies suggested that depleting 5-HT centrally may facilitate learning. In these experiments, the effects were investigated of forebrain 5-HT depletion on the acquisition of a conditional visual discrimination, which is known to depend on frontal cortical mechanisms. Lesions were made by infusing a selective 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), into the lateral ventricles (icv) of adult male Lister hooded rats that were pre-treated with desipramine and nomifensine to protect noradrenergic and dopaminergic neurons, respectively, from damage. Infusion icv of 5,7-DHT resulted in 90% reductions in forebrain 5-HT, but minimal effects on noradrenaline and dopamine, concentrations. The results showed that animals with 5-HT depletions acquired the task markedly and significantly faster than sham-operated controls, both in terms of errors and sessions to criterion. This was true at all criteria of acquisition up to the most stringent of 85% correct responses. Performance on-the-baseline showed that rats with lesions were significantly worse when a delay was introduced between stimulus presentation and the opportunity to respond, but significantly better at short inter-stimulus intervals. Rats with lesions were also slower in extinction of the task. The results will be discussed in the context of functions of 5-HT in learning, memory and attention.

59.7

5-HT1A AGONISTS, 8-OH-DPAT AND IPSAPIRONE, LOWER THE EJACULATORY THRESHOLD OF RHESUS MONKEYS. S.M. Pomerantz, Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Although numerous studies in rats have demonstrated an influence of serotonin on male copulation, no studies have yet to demonstrate whether such a relationship exists in primate species. The present study evaluated two 5-HT_{1A} agonists, 8-OH-DPAT (DPAT) and ipsapirone (IPSAP), on male copulatory behavior of rhesus monkeys.

DPAT (1-200 µg/kg, N=9). Compared to vehicle-based performance, administration of 5 or 10 µg/kg DPAT lowered the ejaculatory threshold of the monkeys as evidenced by a shortening of the ejaculation latency (time from initiation of copulation to ejaculation) and a reduction in intromission frequency (number of intromissive copulatory mounts prior to ejaculation). At higher doses (>50 µg/kg), DPAT had an inhibitory effect on sexual behavior, with a number of males failing to mount the female and an increase in intromission latency (time from the start of the test to first intromission) being observed.

IPSAP (50-800 µg/kg, N=7). At all doses being tested, administration of IPSAP resulted in a shortening of ejaculation latency. Intromission frequency was reduced following either 200 or 400 µg/kg IPSAP. No adverse effects of IPSAP on copulation were observed, even at the highest dose being administered.

The lower ejaculatory threshold of the monkeys following 5-HT_{1A} agonist administration is presumeably due to presynaptic/autoreceptor agonist activity of these compounds, whereas the broader dose-response effect with IPSAP compared to DPAT may be related to differences in postsynaptic activity (partial versus full agonist) of these compounds.

59.8

EFFECTS OF 5-HT_3 RECEPTOR SELECTIVE DRUGS ON RAT SEXUAL BEHAVIOR. N.V.Watson, S.Tanco and B.B.Gorzalka, Dept. Psychology, Univ. British Columbia, Vancouver, B.C., Canada V6T 1Z4

Serotonin (5-HT) may facilitate or inhibit rat sexual behavior, depending on the subtypes of 5-HT receptors activated (for review: Gorzalka, Mendelson and Watson, *Ann N.Y. Acad Sci*, **600**:435-446, 1990). In contrast to work on the 5-HT_1 and 5-HT_2 receptor subtypes and rat sexual behavior, little is known concerning effects mediated by 5-HT_3 receptors. Among other central effects, activity at these sites has been implicated in social interaction, suggesting a role of 5-HT_3 receptors in the effects of 5-HT on rat sexual behavior.

In male rats, intracerebroventricular administration of the putative 5-HT_3 agonist 1-phenylbiguanide (1-PBG) significantly decreased ejaculation latency at all doses tested (0.4, 2.0, 10.0 and 50.0 µg/rat). However, the putative 5-HT_3 agonist 2-methyl-5-HT was without effect in males (1.5 - 40.5 µg/rat, icv). Neither 1-PBG nor 2-methyl-5-HT altered the sexual behavior of female rats. Furthermore, the 5-HT_3 antagonists GR38032F and BRL 43694 did not affect male rat sexual behavior. Overall, these data suggest that 5-HT_3 receptor activity may have only slight effects on rat sexual behavior. It is possible that these effects are mediated by dopamine, as 1-PBG has been reported to induce carrier-mediated dopamine release.

59.9

EFFECTS OF SEROTONIN TYPE 3 (5-HT_3) RECEPTOR ANTAGONISTS AND OPIATES ON REPRODUCTIVE BEHAVIOR IN MALE AND FEMALE RATS. S.A. TANCO, N.V. WATSON, AND B.B. GORZALKA, Dept. of Psychology, University of British Columbia, Vancouver, B.C. V6T 1Y7

Serotonergic effects on reproductive behavior vary as a function of sex and subtype of receptor involved (Gorzalka et. al, *Ann N.Y. Acad Sci: 600*:435-446, 1990). The influence of 5-HT_3 activity on reproductive behavior is unknown; however, 5-HT_3 antagonists are known to antagonize morphine-induced place-preference conditioning (Carboni et. al, *Eur J Pharmacol, 151*:159-160, 1988) and morphine-induced stimulation of dopamine release in the nucleus accumbens (Carboni et. al, *Eur J Pharmacol, 164*:515-519, 1989). Opiates inhibit sexual behavior in both males and females. Thus, 5-HT_3 antagonists may attenuate opiate-induced sexual inhibition independent of their potential effect when administered alone. In both male and female rats, low doses of morphine (1.5mg/kg) profoundly inhibited copulatory behavior. In females MDL 72222 alone (.05-5mg/kg) did not affect lordosis nor did it attenuate morphine-induced sexual inhibition. Similarly, GR38032F (0.8-20mg/kg) alone and in conjunction with morphine did not affect copulatory behavior in males or females. Analogous studies employing ICS 205-930 are currently underway. At present, results obtained with 5-HT_3 antagonists do not support the idea that 5-HT_3 activity is implicated in reproductive behavior.

59.10

PYRIDOXINE FACILITATION OF COPULATORY BEHAVIOR: POSSIBLE SEROTONERGIC MEDIATION? B.B. Gorzalka and I.V. Moe*, Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z4.

Serotonin type 2 (5-HT_2) receptor activity has been reported to have an inhibitory effect on sexual behavior in the male rat (for review see Gorzalka, Mendelson and Watson, Ann. N.Y. Acad. Sci., 600, 435-446, 1990). Recent research has revealed that chronic pyridoxine (vitamin B_6) administration decreases the number of 5-HT_2 receptors in areas such as the cerebral cortex, the hypothalamus, and the brainstem (Dakshinamurti, Sharma and Bonke, Klin. Wochenschr., 68, 142-145, 1990). Therefore, pyridoxine may act to facilitate sexual behavior in the male rat by inhibiting 5-HT_2 receptor activity.

In the present experiment we recorded the sexual activity of male Sprague-Dawley rats during 17 days of chronic pyridoxine or saline administration. Sexually naive males were given 100 mg/kg pyridoxine or saline intraperitoneally. Behavioral testing commenced on day 8 of the chronic injections and continued at 3 day intervals. Pyridoxine significantly reduced ejaculation latencies ($p<.05$) and increased the proportion of animals ejaculating ($p<.05$). All other measures of sexual behavior revealed trends toward a facilitatory effect of pyridoxine. Taken together, these findings confirm our hypothesis that pyridoxine acts to facilitate sexual behavior in the male rat. It should be noted however, that pyridoxine's effect on sexual behavior is not necessarily selective to 5-HT_2 receptor inhibition. For example, pyridoxine has been shown to elevate serotonin and dopamine levels. It remains to be confirmed that 5-HT_2 activity mediates the effects of pyridoxine on sexual behavior.

59.11

NON-INVOLVEMENT OF 5-HT_2 RECEPTORS IN DEFENSIVE ANALGESIA IN MICE. R.J. Rodgers, P. Donat*[1] and J.K. Shepherd*. Dept. of Psychology, University of Leeds, U.K. and [1]Institute of Pharmacology, Czechoslovak Academy of Sciences, Prague.

Recent evidence from our laboratory has delineated a major role for serotonin (5-HT) in the non-opioid analgesic consequences of social defeat in male mice. This form of adaptive pain inhibition is potently and dose-dependently inhibited by 5-HT_{1A} receptor agonists (e.g. 8-OH-DPAT, buspirone, (+) MDL 72832) and by 5-HT_3 receptor antagonists (e.g. ondansetron, ICS205-930, MDL 72222). In the present study, we have assessed the effects of two 5-HT_2 receptor antagonists (ritanserin & ICI 169,369) on basal nociception and defeat analgesia. Although both antagonists were found to have intrinsic analgetic effects at higher doses, neither compound specifically influenced defeat analgesia over the dose ranges tested (0.05-10 mg/kg ritanserin; 0.3-10 mg/kg ICI 169,369). It is concluded that 5-HT_2 receptor populations do not play an important role in this ecologically-relevant component of the defensive repertoire.

We thank the Y.R.H.A. & The Royal Society for financial support, and Janssen Pharmaceutica & ICI Pharmaceuticals for the gifts of compounds used.

59.12

BETA-NORADRENERGIC ANTAGONIST IMPAIRS ACQUISITION OF A NOVEL LOCOMOTOR TASK. Christine Heron, Kevin Poth, and Paula Bickford-Wimer. Vet. Admin. Med. Ctr. and Dept. of Pharmacology, Univ. Colorado Health Sci. Ctr. Denver, CO 80262.

Several theories of motor learning implicate a role for cerebellar structures as being critical for learning to occur. In addition, noradrenergic innervation of this structure has been shown to be necessary for the acquisition of novel locomotor tasks. Depletion of central stores of NE results in impairment of acquisition in a motor learning task. NE is known to act as a neuromodulator in the cerebellar cortex and it is this modulatory action which may be important for its effects on the rate of learning motor tasks. The action of NE to increase the effect of GABAa receptor activation is mediated via a β noradrenergic receptor. Thus, it was the purpose of this study to determine if β noradrenergic receptors were important in the acquisition of this task. Fischer 344 rats were trained to run on regularly spaced pegs for a water reward. After a two week delay, animals were then tested for performance on irregularly spaced rods. Prior to daily testing, rats were injected with propranolol (10 mg/kg, ip) or saline. The rats injected with propranolol were significantly impaired with respect to the rate of acquisition of this novel motor task ($p<0.05$, ANOVA). Thus, these data support the hypothesis that beta noradrenergic receptors are important in motor plasticity. This work was supported by the VA Medical Research Service, USPHS grant AG04418, and the American Federation for Aging Research.

59.13

EXCITATION AND INHIBITION OF SYMPATHETIC PREGANGLIONIC NEURONS (SPGNs) BY AMPHETAMINE: POSSIBLE CLUE TO MECHANISMS IN ADDH. D.N. Franz, C. Sangdee* and L.C. Miner*, Pharmacology, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

The mechanism by which amphetamines reduce symptoms of ADDH are poorly understood although they are known to depress locus coeruleus (LC) neurons which may be hyperactive. Recent evidence that clonidine is effective in ADDH suggests that alpha-2 receptors may account for the effects of amphetamines. Both LC neurons and SPGNs are markedly depressed by activation of their alpha-2 receptors which are negatively coupled to adenylate cyclase and are innervated by C1 epinephrine (EPI) neurons. SPGNs are also innervated by descending NE pathways to alpha-1 receptors that are positively coupled to adenylate cyclase.

In the present study, i.v. dextroamphetamine was tested on intraspinal transmission to SPGNs in spinal cats, activated by cervical spinal stimulation and recorded from preganglionic rami. Small doses (0.25-0.5 mg/kg) doubled or tripled transmission whereas larger doses (1-4 mg/kg) produced progressively smaller and briefer increases. Changes after the 4 mg/kg dose alone were insignificant but, after yohimbine, it increased transmission to 300% for more than 4 hrs. After blocking alpha-1 receptors with chlorpromazine, this dose only depressed transmission by 80%. The larger doses appear to release more EPI or sufficient NE to reach inhibitory alpha-2 receptors to counteract its effects on excitatory alpha-1 receptors. Likewise, amphetamine may inhibit LC neurons by enhancing release of EPI from its terminals or of NE from LC neurons to activate their alpha-2 receptors. Such a mechanism suggests a defect in one of these inhibitory processes in ADDH.

59.14

NOREPINEPHRINE-INDUCED DEPRESSION OF POPULATION SPIKE: DIRECT MICROAPPLICATION OF LIGAND TO STRATUM PYRAMIDALIS OF THE RAT HIPPOCAMPAL SLICE. D. Dahl, D. J. Hayes, P. J. Watson, E. Rich-Bennett, J. Easley, and C. J. Frederickson. The University of Texas at Dallas, and Microfab Technologies, Inc., Plano, TX.

A novel system (the TISSUE-JET), adapted from the printer ink jet, has been developed for dispensing micro-quantities of water-soluble ligands directly onto brain slices. The TISSUE-JET applies ligands as individual droplets, each 50 μm in diameter, at any rate, to any location on the slice surface. Concurrent electrophysiological recordings provide an index of ligand-induced effects on neuronal activity.

Norepinephrine (NE)-induced potentiation or depression of field excitatory postsynaptic potentials (EPSPs) and population spikes, or unit activity have been reported. These results were obtained from studies in which NE and related ligands were applied by perfusion or iontophoresis. In this work, we report results obtained with TISSUE-JET application of NE on activation of CA1 pyramidal neurons.

In an interface chamber, recordings of CA1 population spikes were taken from a hippocampal slice preparation to orthodromic or antidromic activation of Schaffer collateral afferents. Following a control period, droplets of *l*-NE (1 mM) were directed toward the pyramidal cell layer, near the location of the recording electrode tip. A dose-dependent, NE-induced reduction in population spike amplitude was obtained. This reduction persisted for up to 10 min. The NE-induced reduction was reversibly blocked by concurrent perfusion of the slice with the α-adrenergic antagonist phentolamine (50 μM). Although the population spike amplitude was reduced with NE application, there was no concurrent reduction in the slope or amplitude of the pyramidal cell layer EPSP.

These results are interpreted as an α-adrenergic-induced reduction of excitability of CA1 pyramidal cells. This is in contrast to perfusion studies, in which the concurrent exposure of the whole slice to NE and phentolamine was associated with an increase in CA1 population spike amplitude. The α-adrenergic agonist clonidine, however, induced a reduction of CA1 population spike amplitude with no change in EPSP.

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59.15

THE EFFECTS OF NORADRENERGIC NEUROTOXIN, DSP-4, ON HIGH VOLTAGE SPINDLE ACTIVITY DIFFER BETWEEN YOUNG AND AGED RATS. P. Riekkinen Jr, J. Sirviö, R. Lammintausta*, T. Ekonsalo, M. Aaltonen and P.J. Riekkinen. Dept. of Neurology, Univ. of Kuopio, P.O.B. 1627, Kuopio, Finland. Orion Co, Farmos Group, Turku, Finland*

The present study investigated the role of alpha2-adrenoceptors in the regulation of neocortical EEG activity. The EEG effects of D-medetomidine (DMED, an alpha2-agonist; Farmos Group, Finland) were studied in intact and dorsal noradrenergic bundle (DNB)-lesioned rats. Waking-immobility related high voltage spindle (HVS) activity was increased dose-dependently (0.3< 3.0 µg/kg=30 µg/kg; no HVS at 300 µg/kg) in intact rats. Synchronized slow wave activity was increased dose-dependently by DMED: 0.3 µg/kg had no effect on quantitative EEG (qEEG); 3.0 µg/kg increased waking immobility related theta (4-8 Hz) activity, but had no effect on running-related qEEG values; 30 µg/kg increased delta (1-4 Hz), theta, alpha (8-12 Hz) and beta (12-20 Hz) activity during waking-immobility and increased delta and theta during running: 300 µg/kg produced greatest increase in waking-immobility related qEEG activity. Atipamezole, and alpha2-antagonist (Farmos Group, Finland), dose-depently reversed DMED induced HVS and slow wave activity. Pinching of the tail blocked slow wave and HVS activity, and produced desynchronized EEG activity in rats injected with DMED at 0.3, 3.0 and 30 µg/kg. Rats treated with DMED at 300 µg/kg showed no change in the qEEG values after tail pinching. In DNB-lesioned rats, DMED produced similar effects on HVSs and slow waves. The present study demonstrates that EEG slow wave activity and HVSs are regulated by alpha2-adrenoceptors located post-synaptically.

59.16

EFFECT OF LESION SIZE AND REMOTE DAMAGE ON MOTOR RECOVERY AFTER SENSORIMOTOR CORTEX ABLATION. K Nishino, H Ooyu, M Kowada, Dept of Neurosurgery, AKITA University School of Medicine, JAPAN

Norepinephrine (NE) is considered to play a critical role in amphetamine-facilitated beam walking recovery after a sensorimotor cortex ablation (Feeney 1982, Goldstein and Davis,1988). In order to characterize the phenomenon and try to determine the site at which NE act and facilitate the recovery, a selective alpha-2 adrenergic antagonist, yohimbine(5mg/Kg) was administrated after the same and larger lesions. L-DOPS(400mg/Kg, Sumitomo, Pharma. Co Ltd, Japan) and benserazide (1-2 mg/Kg, extracerebral decarboxylase inhibitor), and L-DOPA(30mg/Kg) were also tested in this model. Male Sprague Dawley rats (270-350gm) were subjected to training for beam walking and the subsequent ablation. The agents were administrated 24 hours after the injury. And then beam walking was consecutively evaluated using a seven grade scale until the 14th days. All animals were decapitated and the removed brains were photographed for measurement of lesion size. The results showed single dose of yohimbine or L-DOPS facilitated the beam walking recovery as compared to the control injected the vehicle (t-test: yohimbine [N=9] vs control [N=8] at 6 and 24 h ; $p<0.025$, $p<0.05$, L-DOPS [N=11] vs control [N=9] at 1, 2, 3, 4, 5, 6 d ; $0.005 < p < 0.05$). Yohimbine, however, was less effective when the cortical lesion surface was larger than approximately 35mm^2. There were no statistically significant difference in the lesion size between treated and control groups in each experiment. With HPLC measurement, the NE levels did not increased after administration of L-DOPS (400mg/Kg) plus benserazide [N=5] as compared to the control [N=5], while MHPG levels increased in the cerebellum and the hippocampus after the administration [N=10] (t-test; $p< 0.005$, $P< 0.025$, respectively). We conclude that 1) these data are consistent with the hypothesis of NE-facilitated recovery, 2) the cerebral cortex adjacent the lesion and the cerebellum would be relevant to the facilitated recovery, and 3) such agents would predict the functional prognosis at acute stage of brain injury.

59.17

NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF DEXMEDETOMIDINE IN NEONATAL RATS. K. Laitinen, E. MacDonald* and L. Tuomisto. Dept Pharmacol and Toxicol, Univ Kuopio, 70211 Kuopio, Finland.

Dexmedetomidine (d-MED), a selective α_2-agonist causes sedation in adult rats. Its effects in neonatal animals, however, are unknown. We have studied behavioral and neurochemical effects of a single injection of d-MED in 7-d-old pups. Animals were injected s.c. at doses 0, 10, 30 and 100 µg/kg. Pups were divided in two groups (kept at 30°C or at 21°C). Oral temperature, righting reflex and behavior were monitored and biogenic amines were determined using HPLC methods.

All doses of d-MED caused hypothermia in pups kept at 21°C but not at 30°C. Instead of sedation, d-MED induced hyperactivity in 7-d-old rats. Hyperactivity, including intense movements of limbs while animals lying on their back and clonic-like convulsions, increased in a dose-dependent manner. The righting reflex in those animals was negative. d-MED increased tissue levels of noradrenaline (NA), serotonin (5-HT) and histamine (HA) and decreased tissue level of MHPG-SO$_4$. Neurochemical changes were more significant in animals kept at 21°C suggesting a connection between thermoregulatory impairment and NA, HA and 5-HT metabolism.

59.18

POSSIBLE MECHANISMS UNDERLYING ENHANCED NOREPINEPHRINE RELEASE AND SYNTHESIS IN HIPPOCAMPUS OF CHRONICALLY STRESSED RATS L.K. Nisenbaum and E.D. Abercrombie. *Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pgh., PA and Center for Molecular and Behavioral Neuroscience, Rutgers Univ., Newark, NJ.*

We have investigated the effect of chronic cold stress on the release and synthesis of norepinephrine (NE) in hippocampus using the technique of microdialysis. Whereas the basal levels of NE release and synthesis are unchanged in chronically stressed rats, exposure to a novel stressor elicits a greater increase in these parameters (*Nisenbaum et al., J. Neurosci., in press*). To determine whether alterations at the terminal level might be involved in the enhanced noradrenergic response, we infused aCSF containing 30 mM K+ through the dialysis probe. In naive rats, a 20-min exposure to 30 mM K+ produced 104% and 63% increases in extracellular NE and DOPAC, respectively. In chronically cold-stressed rats, significantly greater increases in NE (254%) and DOPAC (84%) were observed. Since extracellular DOPAC may provide an in vivo measure of NE biosynthesis, these data suggest that alterations at the terminal level are sufficient to maintain enhanced evoked NE release and synthesis in chronically stressed rats. Given that presynaptic alpha-2 receptors modulate both release and synthesis of NE, we examined whether a change in receptor sensitivity might contribute to the chronic stress-induced alterations we have observed. In chronically cold-stressed rats, 0.1 µM clonidine applied through the dialysis probe produced a significantly greater decrease in extracellular NE than in naive controls, suggesting that an increase in the sensitivity of presynaptic alpha-2 receptors had occurred. Thus, while an alteration in alpha-2 receptor sensitivity cannot explain the enhanced evoked noradrenergic response, the observed increase in this variable may allow for a normalization of basal levels of NE release in the chronically stressed rats. (Supported by USPHS grants MH43947 and MH09920.)

59.19

STRESS-INDUCED SENSITIZATION OF NOREPINEPHRINE RELEASE IN MEDIAL PREFRONTAL CORTEX. J.M. Finlay and E.D. Abercrombie. *Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pgh., PA, and Center for Molecular and Behavioral Neuroscience, Rutgers Univ., Newark, NJ.*

Prior exposure to a chronic stressor results in enhanced efflux of hippocampal norepinephrine (NE) in response to a novel stressor (Nisenbaum et al., *J. Neurosci.*, in press). The present experiment assessed whether the enhanced responsiveness of noradrenergic neurons following chronic stress also occurs in the medial prefrontal cortex (mPFC). We examined the effect of prior exposure to chronic cold stress on cortical NE efflux elicited in response to a novel stressor (30 min of tail-pressure), using *in vivo* microdialysis in freely moving rats. Tail-pressure stress elicited a greater increase in NE efflux in the mPFC of rats previously exposed to chronic stress than in naive rats (196% and 150% of baseline, respectively). NE levels remained elevated longer in chronically stressed rats such that, at 30-60 min after cessation of the tail-pressure, NE efflux was 147% of baseline in chronically stressed rats whereas it had returned to basal values in naive controls. The enhanced stress-induced efflux of NE in the mPFC of chronically stressed rats was not attentuated by pretreatment with the anxiolytic benzodiazepine diazepam (2.5 mg/kg, IP); tail-pressure stress increased NE levels to 182% of baseline in chronically stressed rats given an injection of diazepam 1 h prior to presentation of the acute stressor. These data demonstrate that prior exposure to chronic stress results in enhanced release of NE in mPFC in response to a novel stressor. This finding corroborates previous observations regarding the impact of chronic stress on hippocampal NE efflux and suggests that NE projections to forebrain may respond in a homogenous manner to chronic stress. *(Supported by an MRC of Canada Postdoctoral Fellowship to JMF, USPHS Grant MH45156 and MH43947, and a gift of diazepam from Hoffman-La Roche.)*

59.20

[^{3}H]DIHYDROALPRENOLOL BINDING TO B-ADRENERGIC RECEPTORS IS UNCHANGED IN THE BRAINS OF CHRONICALLY STRESSED RATS. S.M. Anderson, E.H. Mougey and G.J. Kant. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

It has been reported that repeated immobilization stress induces down-regulation of brain B-adrenergic receptors. To evaluate this phenomenon in a model of chronic stress we used a behavioral paradigm in which 300 g male S-D rats, habituated to press a lever to receive food, were trained in an active shock avoidance/escape task. Trials were presented around the clock using a 5 min intertrial interval. Animals housed in identical operant chambers but not exposed to footshock were used as controls. We found no difference in the amount of 0.9 nM [^{3}H]dihydroalprenolol ([^{3}H]DHA) bound to membranes from olfactory bulbs, frontal cortex, motor/somatosensory cortex, caudate/putamen, hippocampus, or hypothalamus between controls and animals stressed for either 4 or 14 days in this "sustained performance" stress paradigm. A small decrease in 0.9 nM [^{3}H]DHA binding was found in the mesolimbic area (olfactory tubercle and nucleus accumbens) in stressed rats in some, but not all, experiments. However, saturation binding data indicate no differences in either the density of B-adrenergic receptors or the affinity of those receptors for [^{3}H]DHA. We suggest that the stress-induced down-regulation of brain B-adrenergic receptors reported previously by other investigators may be due to transient factors and/or experimental specific parameters, but are not related to chronic stress, in general.

DRUGS OF ABUSE--BENZODIAZEPINES

60.1

ANXIOLYTIC AND ACTIVATING EFFECTS OF DIAZEPAM IN DS AND DR MICE TESTED ON THE PLUS MAZE. N.D. Courtney, G.E. Jones* and E.J. Gallaher. VA Medical Center and Oregon Health Sciences University, Portland, OR 97201.

Diazepam-sensitive (DS) and -resistant (DR) mice were previously developed using a selective breeding technique, with matings based on the duration of diazepam-induced rotarod impairment. DS mice are about 10-15 fold more sensitive to diazepam when tested on the rotarod. A finding that DS mice are also more sensitive to anxiolytic effects would support the hypothesis that rotarod impairment and anxiolysis have common genetic bases.

In the current study we used the elevated plus maze to monitor the anxiolytic effect of diazepam (DZ) in DS and DR mice. In general mice explore the maze, but tend to avoid the open arms. A count of total entries (TEs) provides a measure of activiy, while an increase in open-arm entries (OEs) following treatment with known anxiolytic drugs is interpreted as an anxiolytic effect.

Vehicle-treated DS and DR mice performed similarly, with about 5-8 TEs and 15-20% OEs during a 5-min trial. Following doses of 0.1 to 10 mg/kg DZ, DR mice exhibited a dose-dependent increase in activity, which decreased at higher doses (30-170 mg/kg) due to frank sedation, OEs also increased in a dose-dependent manner in DR mice, from 17% (vehicle) to 70% (30 mg/kg); results with higher doses were unreliable due to sedation. DS mice exhibited no changes in either TEs or OEs, in opposition to the expected result.

Antagonism of the activating and anxiolytic effects was tested in DR mice with the competitive antogonist R0 15-1788. The antagonist had no significant effect on TEs, but produced a dose-dependent decrease in OEs. These results suggest a dissociation of the activating and anxiolytic effects in DR mice.

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60.2

EFFECTS OF 5-HT1A AGONISTS ON BENZODIAZEPINE WITHDRAWAL IN RATS. A.J. Goudie and M.J. Leathley*. Psychology Dept., Liverpool University, Liverpool, L69 3BX, U.K.

Effects of ipsapirone and gepirone in rats withdrawn from chlordiazepoxide were studied. Subjects were injected for 21 days with saline or CDP. Subsequently, some animals received saline, others received ipsapirone (3, 10 or 30 mg/kg b.i.d.) or gepirone (3, 9 or 27 mg/kg b.i.d.). Withdrawal indices recorded were weight and food intake. Significant withdrawal signs were seen. At 30 mg/kg, but not lower doses, ipsapirone potentiated withdrawal. Gepirone potentiated withdrawal at 9 and 27 but not 3 mg/kg. These effects were not due to drug-induced "malaise", because animals pretreated with saline gained weight on gepirone. The likely cause of the potentiation of withdrawal involves the metabolite 1-PP. Ipsapirone and gepirone did not attenuate withdrawal. Patients withdrawn from benzodiazepines will therefore experience withdrawal if treated with ipsapirone and gepirone. At high doses, withdrawal may be exacerbated. These findings may be related to reports that buspirone-type anxiolytics are poorly tolerated by patients with a history of benzodiazepine treatment.

60.3

TOLERANCE TO THE DISCRIMINATIVE STIMULUS EFFECTS OF MIDAZOLAM (MDZ) IN RATS. C.A. Sannerud & R.R. Griffiths*. Dept. of Psychiatry. Johns Hopkins Univ. Medical School, Baltimore, MD 21205.

The ability of behavioral variables to modify the development of tolerance to the discriminative stimulus (DS) effects of MDZ were evaluated. Rats were trained to discriminate 0.32 mg/kg MDZ s.c. from no-drug in daily experimental sessions consisting of multiple discrete 20 min trials: 15 min time-out (TO), 5 min fixed-ratio 15 schedule of food-delivery. Generalization testing was accomplished by administering progressively increasing doses of MDZ prior to each TO period. During the chronic phases, twice daily injections of 10 mg/kg MDZ or saline were given while discrimination training was either suspended or continued; generalization gradients for MDZ were determined weekly for 4 weeks. Chronic saline given when training was continued or suspended produced slight fluctuations in the MDZ minimal discriminable dose (MDD) (the first dose of MDZ in an individual generalization gradient to produce ≥ 90% drug-lever responding). Tolerance developed to the DS effects of MDZ when it was given while training was suspended: at week 4 chronic MDZ produced 0.5-2 log-unit increases in the MDD of MDZ. In contrast, continued training during chronic MDZ produced no tolerance to MDZ's DS effects: at week 4 chronic MDZ the MDD of MDZ was not different than pre-chronic and not different than either saline condition. (Supported by NIDA Grant DA 04147).

60.4

ACUTE AND CHRONIC EFFECTS OF DIAZEPAM ON BRAIN NORADRENERGIC NEURONS. R. Shiekhattar, S.F. de Boer, R.J. Valentino, and G. Aston-Jones. Div. Behav. Neurobiol., Dept. Mental Health Sciences, Hahnemann Univ., Philadelphia., PA 19102.

Chronic treatment with benzodiazepines (BZ) induces tolerance to most acute behavioral effects, and withdrawal reactions when the drug is abruptly discontinued. Given the possible involvement of the noradrenergic nucleus locus coeruleus (LC) in stress/anxiety processes as well as in addictive behaviours (e.g. opiate tolerance, dependence and withdrawal), we examined the acute and chronic effects of BZ-treatment on the spontaneous and sensory-evoked discharge of LC neurons in anesthetized rats. Acute i.v. injection of diazepam (DZ; 2.5 mg/kg, n=4 rats) decreased LC spontaneous discharge rate by 24% and suppressed responses evoked by subcutaneous electrical stimulation of the rear footpad (FS) by 51%. In 6 rats chronically administered DZ (2.5 mg/kg, b.i.d. for 14 days) LC spontaneous and sensory-evoked discharge rates were decreased 14% and 50%, respectively. Acute i.v. injection of DZ (2.5 mg/kg) in 6 chronically treated rats attenuated (29%) LC spontaneous firing rate but, in contrast to drug-naive rats, had no effect on their FS-evoked responsiveness. Administration of the BZ receptor antagonist, flumazenil (FLU;10 mg/kg, i.v.), to a vehicle-treated rat had no effect on LC discharge rate or FS responses, but completely reversed the DZ-induced depression of LC spontaneous activity and FS responses of 3 cells in 3 rats. However, in chronically DZ-treated rats, FLU-injection caused a robust increase in the discharge rate in 3 of 7 cells (7 rats). These findings suggest that 1) acute DZ-injection attenuates both the spontaneous and sensory-evoked firing rate of LC neurons in drug-naive rats, 2) chronic DZ treatment results in decreased LC spontaneous and sensory-evoked discharge rates, 3) tolerance may develop with chronic administration to the effects of DZ on LC sensory-evoked discharge characteristics, 4) the flumazenil-induced activation of LC neurons may be indicative of precipitated withdrawal. Supported by PHS grants NS 24698, DA 06214 and by a NATO-science fellowship (SFdB).

60.5

MODULATION OF AGGRESSIVE BEHAVIOR AND $GABA_A$-BENZODIAZEPINE RECEPTORS IN MICE SELECTIVELY BRED FOR AGONISTIC BEHAVIOR. E. Weerts[1], L.G. Miller[2], K.E. Hood[3] and K.A. Miczek[1]. Dept. Psychology[1] and Pharmacology[2], Tufts University, Medford, MA 02155 and Boston, MA, 02111 and College of Health and Human Development[3], Pennsylvania State University, University Park, PA 16802

The $GABA_A$-Benzodiazepine receptor complex is important for both inhibition and enhancement of aggressive behavior. Three lines of male ICR mice that had been selectively bred at Penn. State for either low levels of aggressive behavior, or high levels of aggressive behavior, as well as unselected controls were isolated for 21 days. In 5-minute confrontations with group-housed, CFW outbred mice in a novel test cage, low-aggressive mice spent more time walking, rearing and interacting socially than high-aggressive mice. Interestingly, high-aggressive mice treated with chlordiazepoxide (17 and 30 mg/kg) display similar reductions in aggressive behaviors and increased levels of walking, rearing and social behavior as untreated low-aggressive mice. In a separate group of animals, these lines also differ in benzodiazepine binding *in vivo*, as determined by specific uptake of [^{3}H] Ro15-1788 per fmol/g brain tissue, and in $GABA_A$ receptor function as measured by Cl^- uptake (nmol/mg protein) in cortical synaptoneurosomes treated with muscimol (1-50 μM) and [$^{36}Cl^-$]. The low-aggression line showed increased benzodiazepine binding in cerebral cortex, hypothalamus and hippocampus and increased $GABA_A$ receptor function when compared to the high-aggression line. Thus, enhanced benzodiazepine receptor binding and $GABA_A$ receptor function are related to suppression of aggressive behavior. The similarity between the low-aggressive line and animals treated with benzodiazepines is consistent with this hypothesis.. This suggest a direct relationship between $GABA_A$-benzodiazepine receptor function and inhibition of aggressive behavior.

60.6

ANXIOLYTIC EFFECTS OF INTRASEPTAL BENZODIAZEPINES IN THE CONFLICT TEST AND THE ELEVATED-PLUS-MAZE. H. Grishkat. Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

In the conflict test thirsty rats were permitted to lick water for 2 min and were then trained to suppress licking for 2 min during which a tone was presented, in order to avoid foot-shock. This sequence was repeated for a total session length of 8 min. The number of unpunished and punished licks was recorded. Guide cannulae were implanted unilaterally into the lateral septum. One week after surgery, animals were retested in conflict. Intraseptal administration of chlordiazepoxide (CDP, 60 ug/ul) or midazolam (MDZ, 30 & 60 ug/ul) produced a significant increase in punished licking.

Following conflict testing, the exploratory behavior of these same rats was examined in the elevated-plus-maze after intraseptal administration of MDZ (30 or 60 ug/ul) or vehicle. Rats were placed in the center of the maze facing an enclosed arm. The number of open and enclosed arm entries and the time spent in those arms was recorded during a 5-min test session. Animals given intraseptal MDZ showed a tendency to enter the open arms of the plus maze more frequently and remained in those arms significantly longer than vehicle injected rats.

These findings support the lateral septal area as a site of action of benzodiazepine anxiolytics.

60.7

SEROTONERGIC MEDIATION OF THE ANXIOGENIC EFFECTS OF DIAZEPAM WITHDRAWAL. N. Andrews* and S.E. File Psychopharmacology Research Unit, UMDS Division of Pharmacology, London University, Guy's Hospital, London SE1 9RT, UK.

There is both neurochemical and behavioural evidence for a serotonergic role in the benzodiazepine withdrawal syndrome. We found significant increases in [^{3}H]-5-HT release from hippocampal slices taken from rats 24h after the last of 21 daily injections of diazepam (2 mg/kg i.p.). This increased release was reversed by (±) baclofen (1 mg/kg i.p. 30 minutes before sacrifice), which has been previously found to reverse the increased anxiety detected on diazepam withdrawal (File et al. 1991). Both 5-HT_{1A} and 5-HT_3 receptors are implicated in the serotonergic modulation of this anxiogenic response. Low doses of the 5-HT_{1A} receptor partial agonist buspirone (0.2 mg/kg s.c.) and the 5-HT_3 receptor antagonist, zacopride (0.01 mg/kg i.p.) reversed or significantly reduced the anxiogenic responses detected on withdrawal from diazepam. The 5-HT_3 receptor agonist 1-(3-chlorophenyl)-biguanide (1 & 10 mg/kg i.p.) exacerbated this anxiogenic response. We therefore suggest that during diazepam withdrawal there is increased serotonergic function, at least in some brain areas such as the hippocampus. We suggest that the reversal of the anxiogenic withdrawal response by buspirone is due to an action on 5-HT_{1A} autoreceptors in the dorsal raphé, and that drugs acting at post-synaptic 5-HT_3 receptors can also modulate this anxiogenic response.

File. S.E., Mabbutt, P.S. & Andrews, N. (1991) Psychopharmacology (in press)

60.8

TOLERANCE/CROSS-TOLERANCE TO THE DISCRIMINATIVE STIMULUS EFFECTS OF CHLORDIAZEPOXIDE AND RO16-6028. M.E.Bronson, M. Picker and L.Dykstra. The University of North Carolina, Dept. of Psychology, Chapel Hill, NC 27599.

Rats were trained to discriminate 10 mg/kg chlordiazepoxide (CDAP), from saline in a 2 lever drug discrimination procedure. Following training, substitution tests were conducted in which cumulative doses of CDAP and the non-sedative benzodiazepine partial agonist, Ro16-6028, were evaluated. Both CDAP and Ro 16-6028 dose-dependently substituted for the training dose of CDAP. Training was then suspended, and half of the rats were placed on chronic CDAP and the other half received vehicle. During this period, the CDAP dose-effect curve was redetermined once a week. Tolerance to the discriminative stimulus effects of CDAP developed after one month and a final dose of approximately 110 mg/kg/day, as evidenced by the fact that the training dose of CDAP no longer produced drug-appropriate responding. Tolerance also developed to Ro16-6028, as no rat responded on the drug-appropriate lever at doses as high as 56 mg/kg, whereas in the pre-chronic dose-effect curve, 1 mg/kg of Ro16-6028 produced 100% drug-appropriate responding. Ten days after chronic CDAP was discontinued, the dose-effect curve for CDAP was comparable to that obtained in the pre-chronic phase, indicating that the rats were no longer tolerant to CDAP. In contrast to CDAP, the Ro16-6028 dose-effect curve did not return to its pre-chronic position, as no dose produced 100% drug-appropriate responding in all of the rats. In the chronic vehicle group, the dose-effect curves for CDAP and Ro16-6028 were essentially the same before, during and after the chronic regimen. Thus, suspension of training for more than two months does not result in loss of the discriminative stimulus properties of benzodiazepines. Chronic exposure to CDAP, however, does result in tolerance to the discriminative stimulus effects of CDAP with cross-tolerance to the non-sedative benzodiazepine, Ro16-6028. Supported by USPHS grants DA02749 & DA00033 and Biomedical Research Support Grant S07 RR07072, Div. of Research Resources, NIH.

60.9

EFFECTS OF TRIAZOLAM ON MEMORY CONSOLIDATION IN RATS ON A RADIAL MAZE NON-MATCH-TO-SAMPLE TASK. S.A. McBride, G.R. Sessions, J.J. Pilcher and E.L. Closser-Gomez*. Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Triazolam, a short-acting benzodiazepine hypnotic, has been associated with memory impairments in humans. A delayed non-match-to-sample radial maze paradigm was used to study the effects of this drug on memory consolidation in rats. Rats were first trained to obtain food rewards from each of eight arms of a radial maze (one trial/day). Working memory errors were scored when the rats re-entered an arm after having obtained the food pellet from that arm during a particular trial. Following training, the rats were tested in a memory consolidation task. After obtaining the fourth of eight available rewards, the animals received drug injections (0, 0.05 or 0.20 mg/kg ip), followed by a 3 hr delay, after which the trial was resumed. Retroactive errors were scored when the rats re-entered an arm from which a reward was obtained prior to the delay. Drug injections did not increase either error score. Triazolam did not impair memory consolidation nor produce residual effects on working memory for a spatial task.

60.10

EFFECTS OF TRIAZOLAM ON SCHEDULE CONTROLLED BEHAVIOR IN A RADIAL MAZE MODEL OF FORAGING. J.J. Pilcher, G.R. Sessions, S.A. McBride and E.L. Closser-Gomez*. Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Drug-induced performance deficits and disruptions of scheduled-controlled behaviors involving memory were studied in rats injected with the benzodiazepine triazolam. Rats were allowed one hour access to a radial maze where delivery of food was controlled by concurrent fixed interval schedules of reinforcement in each of 8 arms, ranging from 55 to 759 sec. Triazolam (0.025-0.3 mg/kg ip) produced dose-dependent decreases in measures of performance at doses above 0.05 mg/kg, but the overall patterning of responding was altered only at doses which produced severe motor deficits. The proportion of time spent in each arm relative to proportion of rewards obtained (time matching) was relatively unaffected. The response matching also remained relatively unaffected at doses below 0.3 mg/kg. Triazolam produced performance decrements consistent with its hypnotic actions, especially at the highest doses. The relative robustness of the matching functions across doses free of severe motor impairments argues against impairments of memory processes required to perform this task.

60.11

BENZODIAZEPINES MODULATE ULTRASOUNDS IN RESPONSE TO SOCIAL STRESS AND ACOUSTIC STARTLE. J.A. Vivian, William J. Farrell* and K.A. Miczek. Dept. of Psychology, Tufts University, Medford, MA 02155.

The role of benzodiazepine receptors in affective behavior was studied in adult rats that emitted ultrasonic vocalizations. Ultrasounds ("low": 20-30 kHz, and "high": 40-50 kHz) are emitted by rats as part of their submissive responses to an attacking opponent and when exposed to intense sensory stimuli. The effects of full agonists, antagonists and inverse agonists at benzodiazepine receptors were investigated in male Long-Evans rats (*Rattus norvegicus*) exposed to: (1) 9 105 dB and 9 115 dB startle stimuli, or (2) attack and threats by a resident opponent while displaying defensive and submissive responses followed by protection from physical contact with a wire mesh. Diazepam (0.1-6.0 mg/kg IP) dose-dependently decreased the rate of 22 kHz ultrasounds during startle; this rate decreasing effect was reversed with flumazenil (10 mg/kg IP). Conversely, diazepam had no effect on the rate or duration of 22 and 50 kHz vocalizations during the threat of attack. However, flumazenil (3-30 mg/kg IP) increased the rate and duration of 22 kHz vocalizations, and 10 mg/kg flumazenil increased the rate of 50 kHz vocalizations during the threat of attack. There was no effect on startle amplitude or tail flick analgesia with the drugs and doses studied. Because the startle (hindbrain) and tail flick (spinal cord) reflexes were unaffected, a mid- or forebrain site of diazepam action is suggested; this is consistent with neural sites mediating affect and ultrasound production. Furthermore, the differential diazepam effects may result from the eliciting stimulus intensity or behavioral salience.

DRUGS OF ABUSE--COCAINE: BINDING AND NEUROPHYSIOLOGY

61.1

ELECTROPHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF REPEATED COCAINE ADMINISTRATION ON THE MESOACCUMBENS DOPAMINE SYSTEM: FURTHER EVIDENCE FOR ENHANCED D1 DA RECEPTOR TRANSMISSION. D.J. Henry, R.J. Brooderson and F.J. White. Wayne St. Univ. Sch. Med., Dept. Psychiatry, Cellular and Clinical Neurobiology Program, Neuropsychopharmacol. Lab., Lafayette Clinic, Detroit, MI 48207.

Repeated cocaine (COC) administration to rodents increases the locomotor stimulating effect of subsequent COC challenge. Although augmented dopamine (DA) release within the nucleus accumbens (NAc) is clearly involved in this sensitization, we have recently used single-unit electrophysiological recordings to demonstrate that NAc neurons from COC treated rats (2 x 10 mg/kg, i.p., 14 days) exhibit enhanced inhibitory responses to iontophoretic application of DA or the D1 agonist SKF 38393. The present studies extend these findings, demonstrating that this COC treatment regimen results in augmented locomotor responses to COC challenge (10 mg/kg, i.p.) and enhanced grooming reponses following SKF 38393 challenge (8.0 mg/kg, s.c.). In addition, NAc neurons from COC treated rats displayed enhanced inhibitory responses to both iontophoretically applied and i.v. COC challenges. Although this COC treatment decreases the inhibitory effects of DA and direct DA agonists on A10 DA neurons (autoreceptor subsensitivity), the inhibitory responses of these neurons to i.v. COC were not significantly different from controls. This may be due to enhanced NAc-A10 feedback inhibition via sensitized NAc D1 responses since a subset of A10 DA neurons from COC-treated rats displayed partial inhibitory responses to i.v. SKF 38393 (max 4 mg/kg dose), an effect not observed in control rats. These studies provide additional support for enhanced NAc D1 receptor transmission following repeated cocaine treatment. (Supported by MH 40832 and DA 04093).

61.2

COCAINE BLOCKS LONG TERM POTENTIATION IN THE CA1 REGION OF THE RAT HIPPOCAMPUS D. A. Smith, M.D. Browning, and T. V. Dunwiddie. Dept. of Pharmacology, University of Colorado Health Sciences Center and VA Medical Center, Denver, CO 80262.

High frequency (1 sec., 100Hz) stimulation of the Schaffer collateral/commissural pathway in the hippocampal slice produces robust long term potentiation (LTP). 30 uM cocaine, by itself, produced a 0-15% suppression in the amplitude of the population spike, but if present during stimulation totally blocked the LTP measured at 20 min. Two local anesthetics, lidocaine (30uM) and procaine (60uM) also blocked LTP. D-amphetamine sulfate (30-60 uM), a drug that blocks dopamine reuptake, had no effect on LTP when administered under these conditions. In our attempt to explain the mechanism for cocaine's antagonism of LTP, we are currently examining several hypotheses: a) Cocaine selectively blocks the NMDA receptor; b) Cocaine, at the doses tested, is a calmodulin inhibitor; c) Cocaine blocks phosphorylation of synapsin I, a presynaptic protein that may also be involved in LTP (See E.M. Dudek et al., this meeting). We have found that cocaine significantly reduces the ability of the tetanized axons to fire during the full length of the tetanizing stimulation. Use dependent blockade of presynaptic axonal conduction may, therefore, be one reason for the inhibiting effect of cocaine on LTP. However, we also find that cocaine blocks the potentiation of LTP produced by 25mM TEA. This suggests that the suppression of high frequency firing cannot entirely account for cocaine's action on LTP.

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61.3

ELECTROPHYSIOLOGICAL EFFECTS OF COCAINE AND PROCAINE ON NEURONS OF THE CENTRAL NUCLEUS OF THE AMYGDALA. H. Ni, J. Zhang, R.K. Harper and R.M. Harper. Brain Research Institute and Department of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

Local administration of cocaine to the central nucleus of the amygdala (ACE) suppresses ACE neuronal discharge in half of recorded cells, and causes 45% of respiratory and 37% of cardiac related cells to change their dependency relationships. These effects may be elicited by local anesthetic properties of cocaine, or may result from cocaine effects on neurotransmitter action.

The effects of procaine were compared to those of cocaine on neuronal spontaneous activity within the ACE in the freely moving cat using extracellular single neuron recording and microinjection techniques. Microinjection of both agents (100µg/0.2ul) into the ACE elicited a potent and reversible inhibition of firing rate with cocaine (16/30, 53%) and procaine (4/9, 44%). Neither agent altered spike waveform duration or amplitude. However, the inhibitory effects of cocaine on ACE neurons were slower in onset and longer in duration than those of procaine. The maximal suppression of discharge from procaine occurred within 3-7 minutes, while the maximal cocaine effect occurred as late as 17 minutes. The duration of procaine induced suppression lasted 16-38 minutes, while that of cocaine was sustained as long as 91 minutes. The data suggest that cocaine exerts effects with substantially different time courses on ACE neurons than procaine.

Supported by R01-DA04913.

61.4

SENSITIZATION OF LOCUS COERULEUS NEURONS FOLLOWING CHRONIC EXPOSURE TO STIMULANTS AND ANTIDEPRESSANTS. G.C. Harris and J.T. Williams. *Vollum Institute, Oregon Health Sciences University, Portland OR 97201*

These experiments examined the effects of chronic exposure to drugs that block the reuptake of noradrenaline (NA) using intracellular recordings from locus coeruleus (LC) neurons in a slice preparation. Rats were treated with either cocaine (20 mg/kg/i.p./daily), amphetamine (6 mg/kg/i.p./every third day), or desiparmine (10 mg/kg/i.p./daily) for a total of 14 days. Following 7 days of abstinence from drug treatments, experiments were performed that evaluated the sensitivity of a NA mediated inhibitory postsynaptic potential (IPSP) to amphetamine and cocaine and the sensitivity of the α_2 autoreceptor to clonidine. Cells recorded in slices taken from animals in all three treatment groups showed a significant ($p<.01$) dose related shift to the left in sensitivity to the effects of cocaine and amphetamine on the NA IPSP and also in response to the hyperpolarizing effects of clonidine as compared to saline injected controls. All drug treatments led to a 10-20% increase in the maximum response to clonidine. These data suggest that exposure to drugs that block the reuptake of NA can lead to significant increases in the sensitivity of NA neurons to the blockade of NA reuptake. Supported by USDHHS DA 04523 and DA 05387.

61.5

EFFECTS OF COCAINE ON FIRING RATES AND OLFACTORY-EVOKED RESPONSES IN NUCLEUS ACCUMBENS AND OLFACTORY TUBERCLE UNITS IN RATS.

Charles H.K. West and Richard P. Michael. Department of Psychiatry, Emory University School of Medicine, and Georgia Mental Health Institute, 1256 Briarcliff Road, Atlanta, GA 30306.

The mesolimbic dopamine terminal regions of the nucleus accumbens (NAC) and olfactory tubercle (OT) have been associated with reward produced by psychostimulant drugs such as amphetamine and cocaine. We have previously reported that amphetamine differentially affects the firing rates of NAC and OT neurons in anesthetized rats. Here we report that cocaine, like amphetamine, decreased the firing rates of NAC units but increased those of OT units. Since many units in these brain regions respond to olfactory stimuli, we also tested if cocaine affects sensory-evoked responses. Single units were recorded in adult male urethane (1.3 g/kg, ip) anesthetized rats (n = 21). Cocaine was administered intravenously in a cumulative dose range of 0.25 to 4.0 mg/kg such that each dose doubled the previous dose. The firing rates of the 12 NAC units recorded to date were significantly ($P < 0.001$) decreased in a dose-related fashion, with the 4.0 mg/kg dose reducing the mean firing rate to 26% of the pre-drug rate. In contrast, the firing rates of OT units (n = 6) were significantly ($P < 0.01$) increased by cocaine. These effects were antagonized by the dopamine antagonist haloperidol (0.5 mg/kg, iv). Firing rates of some additional units in the border between NAC and OT were unaffected by cocaine. The magnitude of the responses evoked by olfactory stimuli was reduced by cocaine in most of the NAC and OT units tested. Differences in the effects of cocaine on NAC and OT neurons may help to separate the roles of these two structures in drug-induced reward. (Supported by the Emory University Research Committee and by the Georgia Department of Human Resources.)

61.6

EVIDENCE THAT THE HIGH AFFINITY DA REUPTAKE INHIBITOR GBR12909 IS LESS EFFECTIVE AT ENHANCING MESOLIMBIC DOPAMINE FUNCTION THAN COCAINE.

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Dopamine (DA) uptake inhibitors appear to stimulate locomotor activity by elevating extracellular levels of mesolimbic DA. In the present study, rats received i.p. injections of behaviorally equi-effective doses of cocaine (COC, 20 mg/kg), GBR12909 (GBR, 20 mg/kg), nomifensine (NOM, 5 mg/kg), WIN-065-2 (WIN, 1 mg/kg) or saline (SAL). Locomotor activity and stereotopy were measured for 30 min. The subjects were sacrificed. Brain supernatants were prepared and kept frozen at -70c. Aliquots of the supernatants were serially diluted, and assayed for inhibition of [^{3}H]DA reuptake by a striatal synaptosomal preparation. The percent inhibition produced by 200 µl of supernatants were 0,13.5, 37.9, 25.2, 90.3 for SAL, COC, NOM, WIN, and GBR groups. The brain level of GBR was calculated to be about 1 µM. Control studies determined that brain supernatant did not metabolize these agents. In vivo binding assays demonstrated 61.2, 78.1, 58.1 and 100 % occupancy of the DA transporter in the COC, NOM, WIN, and GBR groups, respectively. These data support the hypothesis that COC, WIN and NOM enhance mesolimbic DA function at substantially lower occupancy of the DA transporter than does GBR.

61.7

WITHDRAWAL OF CHRONIC COCAINE DECREASES BINDING OF [^{3}H]WIN 35,428 IN THE RAT NUCLEUS ACCUMBENS.

N.S. Pilotte, J.W. Boja, L.G. Sharpe, E.P. Kornak*, M.J. Kuhar. NIDA, Addiction Research Center, Baltimore, MD 21224.

Cocaine increases the extracellular concentrations of dopamine (DA) by inhibiting DA uptake at transporter sites. Although the binding of the transporter appears normal immediately after repeated administration of cocaine, fewer sites in the nucleus accumbens (Nac) are evident 10 days after withdrawal of cocaine than after withdrawal of its vehicle. To assess the time course of the recovery of the binding of transporter sites in the Nac, we treated male Lewis rats repeatedly with infusions of cocaine (1 mg/kg IV) or 0.15 M NaCl (0.1 ml/kg IV) every 12 min for 2 hr per day for 10 days. The rats were killed within 15 min of the last infusion (Day 0), or 10 or 30 days later. The binding of [^{3}H]WIN 35,428 to the membranes from the striatum and the Nac was assessed in each animal. The [^{3}H]WIN 35,428 binding was significantly reduced in the Nac but not in the striata of rats withdrawn from cocaine. Reductions of 30% were found after both 10 and 30 days of withdrawal when compared to the vehicle controls. Additionally, binding decreased by 40% in both withdrawn groups when compared with cocaine-treated rats killed on Day 0. The reduction of [^{3}H]WIN 35,428 binding during cocaine withdrawal thus appears to be persistent and may be involved in sensitization or other long-lasting effects of cocaine.

61.8

SODIUM REGULATION OF [^{3}H]COCAINE BINDING TO THE DOPAMINE TRANSPORTER.

A.J. Eshleman*, A.T. Eldefrawi, and M.E. Eldefrawi*. Dept. Pharmacology and Experimental Therapeutics, Univ. Md., Baltimore, Md. 21201.

The interaction between Na^+ and buffering ions in regulating cocaine binding to the dopamine transporter in bovine striatum was studied. [^{3}H]Cocaine binding to membranes was measured in several buffers with varying concentrations of Na^+ but constant osmolarity. Na^+ decreased significantly the transporter's affinity for [^{3}H]cocaine in either 10 mM Na_2HPO_4 or 20 mM $NaHCO_3$. Little change was observed in maximal number of binding sites (B_{max}). However, in 50 mM Tris, the B_{max} of [^{3}H]cocaine increased in a dose-dependent manner in presence of NaCl, peaking at 70 mM Na^+. There was little or no change in the affinity. In absence of Na^+, [^{3}H]cocaine binding in Tris was extremely suppressed; the addition of Na^+ increased B_{max} to the levels observed in the other two buffers. These results show that there is an interaction between the Na^+ and cocaine binding sites and that Na^+ is inhibitory in the physiologically relevant bicarbonate buffer. It is suggested that Tris may bind to the Na^+ site and inhibit cocaine binding allosterically. (Supported by NIDA grant #DA036805.)

61.9

COCAINE BINDING SITES IN MOUSE STRIATUM, DOPAMINE AUTORECEPTORS, AND COCAINE-INDUCED LOCOMOTION.

M.E.A. Reith and G. Selmeci*. Division of Neurochemistry, N.S. Kline Institute, Orangeburg, New York, NY 10962.

A possible regulation of cocaine (COC) binding sites and dopamine (DA) autoreceptors was studied in a COC sensitization model. Male BALB/cBy mice were treated once daily with saline (SAL) or COC (25 mg/kg IP) for 3 days. The locomotor response to COC on day 3 was significantly higher than on day 1 as opposed to the locomotion following SAL. One day after the last session, the striatum was prepared for the measurement of the binding of the COC analog [^{3}H]WIN 35,428 (CFT) under conditions of single-site kinetics. There was no difference between the SAL and COC group in the K_d (12 nM) or Bmax (5.8 pmol/mg prot.). In a similar experiment, mice that showed sensitization to the locomotor stimulatory effect of COC on day 3, responded on day 4 to a low dose of apomorphine (0.03 mg/kg SC), targeted at DA autoreceptors, with the same reduction in locomotor behavior as mice pretreated for 3 days with SAL, indicating the importance of mechanisms other than regulation of striatal COC binding sites or DA autoreceptors.

Although C57BL/6ByJ mice showed a greater locomotor response to acute COC administration (25 mg/kg IP) than BALB/cByJ mice, the affinity and density of [^{3}H]CFT binding to striatal membranes was the same in the two strains. Differences other than striatal COC binding appear to be involved, with our previous work suggesting a role for brain cocaine disposition. (Supported by NIDA 03025)

61.10

CHARACTERIZATION AND LOCALIZATION OF ^{3}H-WIN 35,428 BINDING SITES IN RABBIT BRAIN.

V. J. Aloyo, A. L. Kirifides*, S.E. McMaster*, J. Guo* and J.A. Harvey. Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

The binding of the potent cocaine analog, WIN 35,428 (WIN) was investigated using adult rabbits. Coronal brain sections incubated with ^{3}H-WIN in the presence or absence of 30 µM [-] cocaine revealed a high density of specific WIN binding sites in the caudate-putamen. Binding assays were performed using a crude membrane fraction prepared from caudate-putamen at 0° C in 20 mM phosphate buffer, pH 7.4 containing 0.32 M sucrose and ^{3}H-WIN. Nonspecific binding was defined as the binding remaining in the presence of 1 µM WIN. Scatchard analysis revealed a single binding site with a K_d of 3 nM and a B_{Max} of 2.3 pmoles/mg tissue. [-]Cocaine completely displaced bound WIN and Hill plot analysis demonstrated a single site (n = 0.89) and an IC_{50} of 44nM. Dopamine also competed with WIN binding at a single site; Hill analysis (n = 0.92 and an IC_{50} of 3.3 µM). The presence of a single WIN binding site in rabbit brain, in contrast to other species, will facilitate investigation of the properties of WIN binding. (Supported by NIDA Grant DA04944-04).

61.11

COMBINING PCR AMPLIFICATION WITH SUBTRACTION HYBRIDIZATION TO DETECT COCAINE-REGULATED G-PROTEIN COUPLED RECEPTORS IN RAT NUCLEUS ACCUMBENS. J.R. Walker, R.S. Duman, E.J. Nestler, and K.A. Sevarino. Lab. of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.

Behavioral, electrophysiological, and neurochemical studies indicate that the nucleus accumbens (NAc) plays a major role in the reinforcing actions of cocaine. To define the biochemical changes that underlie this reinforcement we are developing methods to identify mRNA species regulated in the NAc by chronic cocaine.

We initially used subtraction hybridization (SH) and a lambda-based NAc cDNA library to identify cocaine-regulated mRNAs. We could detect moderately abundant and more common mRNAs, but not rarer species. To overcome this limitation, we optimized electrotransformation of the bacterial strain DH10B and synthesized plasmid-based libraries that were an order of magnitude more complex than in lambda phage (10^7 versus 10^6 per μg cDNA).

A second difficulty encountered in SH is poor selectivity resulting in many false-positive clones. To reduce this problem we utilized degenerate primers in a PCR amplification step to detect G-protein coupled receptor sequences in an SH-enriched cDNA population. Preliminary studies indicate that this approach allows for detection of cocaine-regulated sequences that otherwise would escape PCR amplification if nonsubtracted material were used.

SH and PCR offer novel ways to identify some of the molecular adaptations to cocaine that underlie its addictive actions.

61.12

MULTIPLE DOPAMINE TRANSPORTERS: DIFFERENT MOLECULAR WEIGHTS IN RAT STRIATUM AND NUCLEUS ACCUMBENS. R. Lew, R. Vaughan*, R. Simantov and M.J. Kuhar. Neuroscience Branch, National Institute on Drug Abuse/Addiction Research Center, Baltimore, MD 21224.

The molecular weight of the dopamine transporter in rat striatum and nucleus accumbens was examined using the photoaffinity probe ^{125}I-DEEP and SDS-PAGE electrophoresis. Using molecular weight standards, analysis revealed that the apparent molecular weight of the dopamine transporter in the nucleus accumbens (77 kDa) was significantly higher than that in the striatum (72 kDa; $P < 0.004$, $n = 5$). In some experiments, ^{125}I-DEEP binding to the dopamine transporter in the nucleus accumbens and striatum was performed in the absence or presence of mazindol, nomifensine, GBR 12909, (-) & (+) cocaine, desipramine and citalopram. The pharmacological binding characteristics of ^{125}I-DEEP binding in the nucleus accumbens was identical to that in striatum and was consistent for binding to the dopamine transporter. Treatment with neuraminidase (2 U/ml) removed terminal sialic acid residues from ^{125}I-DEEP labeled transporter from both regions resulting in proteins of similar apparent molecular weights. It appears a major reason for the difference in the apparent molecular weight of the dopamine transporter in these two regions is due to a difference in glycosylation. Additional experiments examining this hypothesis is in process.

61.13

IN VIVO BINDING TO DOPAMINE AND SEROTONIN UPTAKE SITES IN THE MOUSE WITH THE COCAINE ANALOG [^{125}I]-RTI-55; AUTORADIOGRAPHY, REGIONAL DISTRIBUTION, AND PHARMACOLOGY. E.J. Cline, U. Scheffel[1], J. W. Boja, W.M. Mitchell, F.I. Carroll[2]*, and M.J. Kuhar. Neuroscience Branch, N.I.D.A./A.R.C., Baltimore, MD 21224, [1]Dept. of Radiology, The Johns Hopkins Med. Inst., Baltimore, MD 21205, and [2]Research Triangle Inst., Research Triangle Park, NC 27709.

[^{125}I]-RTI-55 is a cocaine analog with high in vitro affinity for dopamine transporters and potent behavioral effects. In vivo binding studies were carried out to assess the possibility of using radiolabelled RTI-55 for visualizing cocaine binding sites by PET or SPECT. Mice were injected i.v. with 2-4 μCi [^{125}I]-RTI-55 and binding was determined in whole brain autoradiograms and in several dissected brain regions. Autoradiography revealed highest binding in DA rich areas and lesser binding in 5-HT rich areas. Highest regional radioactivity concentrations (%dose/gram) occured at 1-2 hours. Specific to non-specific (cerebellar) ratios in DA rich areas increased from 0.88 at 15 min to 14.60 at 8 hrs and decreased significantly by 24 hrs. WIN 35,428 and unlabelled RTI-55 produced dose dependent inhibition of [^{125}I]-RTI-55 accumulation in dopamine rich brain areas. Paroxetine and citalopram produced a similar effect in 5-HT rich areas. Results with RTI-55 suggest it will be a useful tool for labelling DA and 5-HT uptake sites in vivo.

61.14

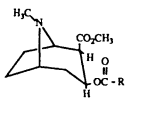
In Vitro Autoradiographic Characterization of 3β-[4-Iodophenyl]-tropane-2β-carboxylic Acid Methyl Ester ([^{125}I]RTI-55) Binding to Dopamine and Serotonin Uptake Sites in Rat Brain. W.M. Mitchell, J.W. Boja, E.J. Cline, R. Lew, F.I. Carroll*[1], A. Lewin*[1], M.J. Kuhar. NIDA/ARC, Baltimore, MD 21224. [1]Research Triangle Inst., Research Triangle Park, NC 27709.

The cocaine analog ([^{125}I]RTI-55) binds with high affinity to both dopamine and serotonin uptake sites in rat brain. Brain sections incubated with [^{125}I]RTI-55 (50pM) alone or together with 10 μM mazindol revealed mazindol displaceable binding in the striatum, nucleus accumbens, olfactory tubercle, ventral tegmental area, and substantia nigra. Citalopram (50 μM) inhibited [^{125}I]RTI-55 binding in cortex and hindbrain. Competition studies with [^{125}I] RTI-55 (50pM) and citalopram, (-)cocaine, (+)cocaine, mazindol, GBR 12909, haloperidol, and desipramine in coronal sections through striatum/accumbens resulted in IC_{50} values corresponding closely with those reported in rat striatal membrane preparations. These findings indicate that the high affinity and specific activity of [^{125}I]RTI-55 make it an excellent ligand for autoradiographic studies of cocaine binding site.

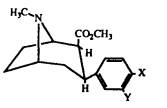
61.15

ANALOGS OF CFT AND CIT FOR IMAGING COCAINE RECEPTORS. J.L. Neumeyer, S.Y. Wang*, R. Zong*, R. Baldwin*, Y. Zea-Ponce*, M. Laruelle, and R. Innis. Research Biochemicals Inc., 1 Strathmore Road, Natick, MA 01760 and Yale University School of Medicine, West Haven, CT 06516.

Our recent finding that the potent cocaine analog, 2β-carboxymethoxy-3β-(4-fluorophenyl)-tropane (CFT, also known as WIN 35,428) when tritiated or labeled with $^{11}CH_3$ was a superior radioligand probe for cocaine receptors [*Mol. Pharmacol* **36**, 518 (1989)] led to the development of analogs suitable for PET and SPECT imaging. The corresponding 3β-(4-iodophenyl)tropane analog [*Eur. J. Pharmacol.* **194**, 133 (1991)] designated as CIT, the iodo analog of CFT, was found to be the most potent ligand so far prepared for binding to the dopamine transporter and was selected as an *in vivo* labeling ligand. The receptor affinities of CIT, CFT and related iodo-fluoro analogs as well as a series of carbamates related to cocaine were determined from displacement studies using brain tissue homogenates prepared from baboon striatum and ^{3}H-CFT as the radioligand.



Compound	R	IC_{50} (nM)
Cocaine	C_6H_5	221
SW 2-69	NHC_6H_5	>1000
SW 2-71	NH-*m*-ClC_6H_5	>1000
SW 2-75	NH-*p*-$CH_3OC_6H_4$	>1000
SW 2-74	NH-*p*-FC_6H_4	>1000



	X	Y	
CFT	F	H	18.8
CIT	I	H	1.11
CFIT	F	I	25.1
C-*m*CF_3T	CF_3	H	262

Supported by NIMH (MH 48243) and NIDA (DA 5648).

61.16

A ROTATING DISK ELECTRODE VOLTAMMETRIC INVESTIGATION OF THE INHIBITORY MECHANISM OF COCAINE ON THE DOPAMINE UPTAKE CARRIER *IN VITRO*

J.S. McElvain and J.O. Schenk. Depts. of Biochemistry and Chemistry, Washington State University, Pullman, WA 99164.

A rotating disk electrode (RDE) system was used to study the effects of cocaine on the kinetics of exogenous and endogenous dopamine (DA) uptake into rat striatal homogenates. The homogenates were incubated at 37°C in 500 μL of physiological buffer in the presence or absence of increasing amounts of cocaine (0.5-8 μM). Approximately two minutes after the addition of cocaine, either exogenous (by direct addition) or endogenous (by KCl stimulation) DA was introduced into the incubation medium. The resulting DA uptake signal was monitored by the RDE, and the raw uptake rate data was analyzed graphically using the Eadie-Hofstee linear transformation of the Michaelis-Menten kinetic mechanism to determine the kinetic parameters, V_{max} and K_m. Under resting conditions it was found that cocaine inhibits exogenous DA uptake uncompetitively however endogenous DA uptake was inhibited noncompetitively in homogenates depolarized with KCl. Hill plot analyses showed that there was one DA, one sodium, and one chloride binding site on the DA uptake carrier. Isosmotic replacement of sodium in the incubation medium with choline in the presence and absence of cocaine demonstrated that cocaine is noncompetitive with the sodium site on the DA uptake carrier under resting conditions. In contrast, it was shown that cocaine competes directly with the chloride site on the DA uptake carrier under resting conditions when chloride was substituted isosmotically with isethionate.

Supported by the Washington Alcohol and Drug Abuse Program and MH 42759 (JS).

61.17

IN VIVO AUTORADIOGRAPHIC DISTRIBUTION OF COCAINE RECOGNITION SITES IN MONKEY BRAIN. M.J. Kaufman, R.D. Spealman and B.K. Madras. Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772-9102.

The cocaine congeners [^{3}H]CFT ([^{3}H]WIN 35,428) and [^{125}I]CIT ([^{125}I]RTI-55) were used to map cocaine recognition sites in squirrel monkey (*Saimiri sciureus*) brain by in vivo autoradiography. Cocaine recognition sites labeled by [^{3}H]CFT were distributed heterogeneously in primate brain. The regions of highest density were the caudate-putamen, nucleus accumbens-olfactory tubercle, substantia nigra-ventral tegmental area and amygdala. Lower densities were found in the subthalamus including the zona incerta and the hypothalamus. This distribution pattern parallels in vitro autoradiographic distribution reported previously (Soc. Neurosci. Abstr. 16:746). The distribution of [^{125}I]CIT binding in vivo was similar to that observed for [^{3}H]CFT. The striatal:cerebellar ratios for both radioligands were at least 3:1. Higher levels of binding were detected with [^{125}I]CIT in the thalamus, cortex, and brainstem nuclei. The results suggest that [^{3}H]CFT and [^{125}I]CIT label similar though not identical populations of binding sites in squirrel monkey brain, and both can be useful imaging probes for detecting cocaine receptors in vivo. Supported by DA06303, DA00499, RR00168 and MH14275.

61.18

PHARMACOLOGICAL CHARACTERIZATION OF SOLUBILIZED COCAINE RECEPTORS FROM RHESUS STRIATUM. L.M. Gracz and B.K. Madras. Harvard Med. Sch., NERPRC, Southboro, MA 01772.

High- and low- affinity binding components for [^{3}H]cocaine and [^{3}H]CFT (2β-carbomethoxy-3β-(4-fluorophenyl)-tropane) have been identified in homogenates of monkey caudate-putamen. The heterogeneity of binding sites in membranes was not due to ligand degradation. Cocaine receptors were solubilized from the caudate-putamen of rhesus monkey with 1% digitonin in Tris-HCl buffer (≈60% yield). Binding properties were determined in competition experiments with [^{3}H]CFT. [^{3}H]CFT binding was fully inhibited by cocaine congeners, monoamine uptake inhibitors, and monoamine neurotransmitters with the same order of potency observed in membranes ($r = 0.994$, $p < 0.001$), suggesting that [^{3}H]CFT binding sites are associated with the dopamine transporter. CFT, cocaine, and (-)norcocaine inhibited [^{3}H]CFT binding with shallow competition curves (n_H 0.61-0.89) in both membrane and solubilized material. Monoamine uptake inhibitors structurally unrelated to cocaine (GBR 12909, Lu 19-005, and bupropion) also inhibited [^{3}H]CFT binding but with steeper competition curves (n_H 0.94-1.1). This suggests that these drugs bind differently to the [^{3}H]CFT labeled sites. Thus the nature of [^{3}H]CFT binding heterogeneity can be studied in solubilized preparations. Supported by USPHS grants DA06303, DA00499, RR00168, and MH14275.

61.19

PRELIMINARY EVIDENCE FOR MULTIPLE [^{3}H]GBR12935 BINDING SITES OR STATES ASSOCIATED WITH THE DOPAMINE TRANSPORTER IN RAT STRIATAL MEMBRANES. H. C. Akunne[1], G. C. Char*[2], B. R. de Costa*[1], K. C. Rice[1] and R. B. Rothman[2]. [1]LMC, NIDDK, Bethesda, MD 20892; [2]NIDA Addiction Research Center, PO BOX 5180, Baltimore, MD 21224

We conducted quantitative binding studies using [^{3}H]GBR12935 and [^{3}H]mazindol to label the dopamine transporter in rat striatal membranes. Binding surface analysis of the interaction of GBR12935, CFT and BTCP with [^{3}H]GBR12935 demonstrated that a two site model fit the data better than a one site model. The Bmax values of the [^{3}H]GBR12935 binding sites were about 3000 (site 1) and 15,000 fmol/mg protein (site 2). The Ki values of mazindol for sites 1 and 2 were about 600 nM and 50 nM. GBR12935 had similar affinity for both sites (Kd about 1 nM). [^{3}H]Mazindol binding surfaces were best described by a one site model: Bmax about 16,000 fmol/mg protein, Kd about 150 nM, Ki of GBR12935 about 1 nM. Given the low affinity of mazindol for site 1, these data support the hypothesis that [^{3}H]mazindol selectively labels site 2. These preliminary findings suggest that single ([^{3}H]mazindol) or multiple ([^{3}H]GBR12935) affinity sites/states of the DA transporter can be resolved depending on which radioligand is used.

DRUGS OF ABUSE--COCAINE: DEVELOPMENT

62.1

PRENATAL COCAINE EXPOSURE EFFECTS THE LOCOMOTOR RESPONSE TO ACUTE AMPHETAMINE ADMINISTRATION IN WEANLING RATS. H. E. Hughes and D. L. Dow-Edwards. Laboratory of Cerebral Metabolism, Department of Pharmacology, SUNY Health Science Center, Brooklyn, N. Y., 11203

Our lab has previously demonstrated that gender differences in the baseline activity of 21-22 day old control rats were absent following prenatal cocaine exposure. The present study investigated the effects of prenatal cocaine exposure on locomotor activity following acute amphetamine injection in weanling rats. Pregnant rats were gastrically intubated with 30 or 60 mg/kg/day cocaine HCl or vehicle during gestational days 8-22. Vehicle-treated rats were pair-fed/watered to rats receiving 60 mg/kg cocaine. A non-treated control group was maintained. At parturition, litters from all four groups were surrogate fostered. At 21-22 days of age, four offspring per litter were sequentially placed in a Digiscan Activity Monitor. Activity counts were collected in one minute intervals over a 15 min. baseline period. Subjects then received either 0.1 or 0.25 mg/kg amphetamine sulfate sc followed by a 95 min. period of activity monitoring. Pending completion of the statistical analysis, preliminary data indicate that weanling rats in the 60 mg/kg group traveled more distance than pair-fed controls in response to amphetamine. In particular, pair-fed males tended to remain at rest throughout the session. Therefore, prenatal cocaine administration appears to enhance the locomotor response to amphetamine in weanling rats. Supported by ADAMHA grant #DA04118.

62.2

PRENATAL COCAINE IN RATS ALTERS IMMUNOCOMPETENCE IN OFFSPRING. G.D. Royall, L. McGurk, R.F. Smith, M.Coss*, P. Naylor*, and K. K. Oates. Dept of Psychology, George Mason U. Fairfax, VA 22030, and *Dept of Biochemistry, Geo. Washington U., Washington, D. C. 20037

Since we previously found that prolactin (PRL) levels are reduced in neonatal rats dosed prenatally with cocaine, we dosed rats with 5, 10, 20, or 40 mg/kg/d cocaine from GD7 through GD20, and evaluated components of cellular immune functioning in the offspring at several ages. At P1, high dose offspring had reduced thymus weights but no significant changes in thymosin alpha-1 (TA1). At P21, high dose offspring had substantial thymic hypertrophy, high TA1 levels, and reduced mitogen-induced T cell proliferation. At approximately P95, phagocyte-killing index following C. albicans infection was reduced in high dose animals. These data suggest that low PRL levels in neonates experiencing withdrawal after prenatal cocaine may have lasting effects upon a number of components of the immune system.

62.3

SEX DIFFERENCES IN RESPONSIVENESS TO AMPHETAMINE IN ADULT RATS: THE ROLE OF TESTOSTERONE EXPOSURE DURING THE NEONATAL PERIOD FOR SEXUAL DIFFERENTIATION. M.L. Forgie and J. Stewart. Center for Studies in Behavioral Neurobiology, Dept. of Psychology, Concordia University, Montreal, Quebec, Canada, H3GlM8.

Sex differences in both acute responsiveness to amphetamine (AMPH), and sensitization to its behavioral effects following repeated administration, have been reported (e.g., Camp & Robinson, 1988). Females show higher levels of behavioral activation than males. These sex differences have been suggested to be due in part to differences in circulating levels of gonadal hormones in the adult animals (i.e. an "activational effect"). In the present studies, the extent to which adult sex differences in responsiveness to AMPH can be attributed to differential exposure to testosterone (T) during the critical period for sexual differentiation was investigated. In Experiment 1, at birth, male pups were sham-operated or gonadectomized, while female pups were given T or a control injection. At 80-90 days of age all animals were gonadectomized or sham-operated, and were tested in the absence of circulating gonadal hormones. In Experiment 2, the same procedure was followed except that all animals received 5.0 ug estradiol benzoate (s.c.), 30-40 min. prior to each behavioral test. Locomotor activity in response to either 1.5 mg/kg AMPH (i.p.) or the saline vehicle was measured for 2 h. every 3 days, on 5 occasions. On the 6th occasion all animals received 0.75 mg/kg AMPH (i.p.) in a test for sensitization.

The results of the first experiment suggest that neonatal exposure to T suppresses responsiveness to AMPH in adulthood. The results of Experiment 2, were similar, but further suggest that in the presence of circulating estrogen (E), female animals may be more responsive to AMPH regardless of neonatal T exposure. These results suggest that lifetime exposure to E alters responsivity to this hormone in adult animals.

62.4

PRENATAL COCAINE ADMINISTRATION AFFECTS RADIAL-ARM MAZE PERFORMANCE IN YOUNG ADULT RATS. F. J. Seidler and E. D. Levin. Departments of Pharmacology and Psychiatry, Duke University Medical Ctr., Durham, N.C. 27710

In utero cocaine exposure has been shown to cause delays in early behavioral development. However, it is not known if this exposure affects performance on cognitive tests in older animals. The current study examined the effects of prenatal cocaine administration (30 mg/kg, s.c. from gestational day 8 though 20) on 8-arm radial maze performance in young adult rats. Starting approximately day 50, animals were given 18 training sessions; these sessions were grouped into 3 blocks of 6 sessions each for analysis. Three-way ANOVA (factors = treatment x sex, repeated measure = session block) indicated that, while there was no significant main effect of cocaine treatment, there was a significant cocaine x sex x session block interaction in choice accuracy ($p<0.005$). Further analysis revealed choice accuracy deficits in cocaine-treated females during the third phase of training ($p<0.005$); in contrast, cocaine-treated males exhibited improved choice accuracy during this phase ($p<0.05$). Response latency was not significantly affected by cocaine exposure. These results demonstrate that prenatal cocaine exposure can cause long-lasting alterations in cognitive task performance. (Supported by grants from the United Way, Duke University Medical School Research Fund and USPHS DA05031)

62.5

BRIEF MATERNAL D-AMPHETAMINE EXPOSURE PRODUCES HYPERDOPAMINERGIC BEHAVIORS IN ADULT OFFSPRING M. Lyon and W.O. McClure. Center for Neuropsychiatry and Schizophrenia Research, Univ. Arkansas for Medical Sciences, Little Rock, AR 72205 and Neurosciences Program, Univ. Southern California, Los Angeles, CA 90089.

This experiment was designed as a rodent model of brief drug abuse during pregnancy, hence treated mothers raised their own young. D-amphetamine was chosen for its abuse potential, anorexic effect, and dopamine stimulation. Procedure: 14 pregnant Sprague-Dawley rats received either saline (N=4), d-amphetamine 2.0 mg/kg (N=5), or 5.0 mg/kg (N=5). Treatment occurred on gestational days 11-14, during early fetal neurotransmitter formation and cell migration. Results: At weaning, there was a dose-related weight reduction in both drug groups compared to the saline control group (Total N=110). The 5.0 mg/kg group weighed less than the 2.0 mg/kg group $P<0.002$), and the 5.0 mg/kg group remained lighter as adults. As juveniles, drug exposure reduced eating time in both sexes ($P<0.006$), but not foraging ability in the hole-board. In the 5.0 mg/kg group, hole-board behavior showed decreased rearing at the wall ($P<0.03$) and less mid-field locomotion ($P<0.0005$), but in females only. Drug-exposed young adults had strongest conditioned turning preference from the startbox in both sexes ($P<0.05$). Perseveration in turning occurred in the 5.0 mg/kg group even when food location changed ($P<0.04$; Chi-square). Turning preferences peaked at 2PM in all groups, paralleling seasonal ultradian rhythms in striatal dopamine receptor-binding (Kafka et al., 1981). Amphetamine challenge (1.0 mg/kg) significantly increased rearing at the wall in females, yet reduced rearing in mid-field for both sexes.
Conclusion: Taken together, these results indicate that hyperdopaminergic behavior results, especially in female young, from even brief maternal abuse.

62.6

EFFECTS OF PRENATAL COCAINE EXPOSURE ON FETAL RAPHE DEVELOPMENT. E.M. Factor, R.P. Hart and G.M. Jonakait. Dept. Biol. Sciences, Rutgers Univ., 101 Warren St., Newark, NJ 07102.

Disorders among cocaine-exposed infants suggest the medullary raphe nuclei (MRN) as potential targets for cocaine-induced disabilities. Serotonin (5-HT) and substance P (SP) are colocalized in individual neurons of the MRN. Colocalization is an early-appearing and enduring feature of these cells (Ni and Jonakait, Neuroscience 30:257). In order to determine possible neurochemical abnormalities resulting from prenatal cocaine exposure, we measured levels of mRNA coding for the SP preprohormone preprotachykinin (PPT), SP peptide levels, and the activity of tryptophan hydroxylase (TPH, the rate-limiting enzyme in 5-HT synthesis) throughout perinatal and early postnatal development.

Pregnant rats at embryonic day 7 (E7) were implanted (s.c.) with Alzet osmotic minipumps dispensing either 10 or 40 mg/kg cocaine HCl dissolved in sterile saline daily for two weeks. Controls received sham surgery, but no pumps. Maternal plasma cocaine concentrations one and two weeks after pump implantation were $\geq$30 ng/ml for the lower dose and $\geq$160 ng/ml for the higher dose. Maternal weight gain, duration of pregnancy and fetal viability were normal at both doses. Levels of PPT mRNA in the MRN, assessed from E17 through postnatal day 14, were found to be normal in pups receiving the lower dose. SP peptide levels in the ventral spinal cords (sites of raphe termini) and TPH activity in the first postnatal week were also unchanged by this dose. These data suggest that exposure throughout gestation to low concentrations of the monoamine uptake inhibitor, cocaine, have negligible consequences for the development of these neurotransmitters.
Supported by DA05964 (GMJ,RPH) and the NJDHS (EMF).

62.7

THE EFFECTS OF GESTATIONAL COCAINE EXPOSURE ON LOCOMOTOR ACTIVITY IN ADULT RATS. L. Rajachandran, C.J. Heyser, and L.P. Spear. Center for Developmental Psychobiology, Dept. of Psychology, SUNY, Binghamton, NY 13902-6000.

In a recent study in our laboratory examining cocaine-induced conditioned place preference, adult offspring exposed gestationally to cocaine were observed to exhibit more crossing between chambers on the test day (Heyser et al., submitted). Although hyperactivity has been observed early in life following gestational cocaine treatment (Hutchings et al., 1989; Spear et al., 1989; Henderson & McMillen, 1990) exposed offspring have been reported to exhibit normal levels of activity as adults (Giordano et al., 1990; Henderson & McMillen, 1990; Sobrian et al., 1990). The present study examined open field activity in adult offspring using a treatment regimen previously observed to produce neurobehavioral alterations in developing as well as adult offspring (e.g., Spear et al., 1989; Scalzo et al., 1990; Heyser et al., 1990). Adult female offspring from Sprague Dawley dams injected s.c. from gestational day 8-20 with 40 mg/kg/3cc cocaine HCl, offspring from two types of nutritional controls, and untreated dams were tested in a 56 x 66 x 45 cm open field for 15 min/day over three consecutive days. No differences among the prenatal treatment groups were observed in open field activity, with all groups showing comparable levels of habituation within each test session. Thus, although adults offspring exposed gestationally to cocaine exhibit apparent increases in motility in certain testing situations (Heyser et al., submitted), these animals do not appear to be hyperactive in the open field.
[Supported by NIDA Grants R01 DA04478 and K02 DA00140]

62.8

GESTATIONAL EXPOSURE TO COCAINE DECREASES SENSITIVITY TO THE REWARDING PROPERTIES OF COCAINE IN INFANT RAT PUPS. C.J. Heyser, G.A. Goodwin, C.A. Moody, and L.P. Spear. Center for Development Psychobiology, Dept. of Psychology, SUNY, Binghamton, NY 13902-6000.

Previous work from our laboratory has shown that adult offspring exposed gestationally to cocaine did not exhibit a cocaine-induced conditioned place preference (CPP), findings that could reflect either a learning deficit or a decrease in the reinforcing properties of cocaine (Heyser, Miller, Spear, and Spear, submitted). In order to further characterize the effects of gestational exposure on the later reward efficacy of cocaine, offspring derived from Sprague Dawley dams that received daily subcutaneous injections of 40 mg/kg/3cc cocaine hydrochloride (C40) from Gestational Day 8-20, nutritional control dams receiving daily saline injections (NC), and nontreated control dams (LC) were tested for cocaine-induced odor preference early in life. On Postnatal Day 6, each pup received an intraperitoneal injection (i.p.) injection saline and was placed into a chamber scented with 1 cc of orange odor for 30 min. On Postnatal Day 7 (P7), each pup was given an i.p. injection of either 0 (saline), 2.0, 5.0, or 10.0 mg/kg cocaine HCl, and placed into a chamber scented with 1 cc of lemon odor for 30 min. On the third day (P8) a preference test for location was conducted between the two odors (Lemon vs Orange). Both the LC and NC offspring were observed to exhibit a preference for the odor paired with 2.0, 5.0, and 10.0 mg/kg cocaine when compared to saline controls. In contrast, C40 offspring exhibited a preference for the odor only when paired with 5.0 or 10.0 mg/kg cocaine. These results, combined with our previous CPP data in adult animals (Heyser, et al., submitted), provide evidence that prenatal cocaine exposure results in a decrease in the later rewarding properties of cocaine.
[Supported by NIDA Grants R01 DA04478 and K02 DA00140]

62.9

EFFECTS OF NEONATAL COCAINE (COC) EXPOSURE ON SEROTONIN (5HT) RECEPTORS IN RATS. M.A. Kosek, G.A. Ordway and R.M. Kliegman, Depts. of Pediatrics and Psychiatry, Case Western Reserve Univ., Rainbow Babies & Childrens Hosp. and MetroHealth Med. Ctr., Cleveland, Ohio 44106.

Knowledge of the long-term effects of COC on development of the brain is crucial to understanding the outcome of infants born to mothers who abused COC during pregnancy. We have studied the effect of COC on development of the brain monoamine system in a rat model. Previously, we found that exposure of neonatal rats to COC for 15 to 30 days resulted in profound reductions in central beta-1 adrenergic receptors, particularly in the hippocampus, and no changes in D1 or D2 dopamine receptors. Since COC also potently inhibits the neuronal reuptake of 5HT, its presence during development could interfere with maturation of the brain 5HT system. In this study, we measured 5HT-2 receptors in rats treated with saline or COC 15 mg/kg three times daily from birth to 15 days. The binding of 2 nM ^{3}H-ketanserin was quantitated in homogenates of frontal cortex, using 10 uM methysergide to define non-specific binding. There was no difference in the binding of ^{3}H-ketanserin in frontal cortex from saline (103±13 fmol/mg protein) or COC (100±8 fmol/mg protein) exposed rats. Based on these and previous monoamine receptor studies, neonatal COC exposure seems to alter only noradenergic receptor development.

62.10

***IN UTERO* METHAMPHETAMINE DIFFERENTIALLY REGULATES ADULT BRAIN MONOAMINE UPTAKE SITES.** A.D. WEISSMAN AND S. CALDECOTT-HAZARD. Addiction Research Center, NIDA, Baltimore, MD 21224 and Univ Southern Calif, Los Angeles, CA 90089.

Chronic amphetamine-like drugs exert a profound effect on monoamine systems in the brains of mature animals. These changes appear as deficits in neurochemical function (e.g. neurotransmitter uptake) that probably relate to the neurotoxicity of the compounds. We examined both the behavioral and biochemical consequences of prenatal methamphetamine (MA) exposure where a greater potential effect may occur as compared to adults. Female rats were chronically treated with either 2 or 10 mg/kg MA prior to conception and throughout the pregnancy. Pups were not given any additional drug after birth and were behavioral tested beginning at 30 days of age. Only the litters given 10 mg/kg showed behavioral deficits as compared to control litters (Caldecott-Hazard, *Soc Neurosci Abst*, 16, 586, 1990). A postmortem examination of regional brain serotonin (5HT,) dopamine (DA) and norepinephrine (NE) uptake sites showed a paradoxical effect of the two dose regimens. 5HT uptake was reduced in the cortex, striatum and hippocampus for the 2 mg/kg group, but was normal in most areas or increased in the hippocampus and pons of the 10 mg/kg litters. DA uptake was reduced in the striatum after both treatments, but the effect was attenuated in the striatum and also showed an increase in the pons of the 10 mg/kg group. A decrement occurred in NE uptake in the posterior cortex with both doses, but mirrored the effects in the other uptake systems with the greatest decrease at the lowest dose of MA. The novel dose-related changes in the monoamine uptake sites may indicate a regional regenerative process in the brain that is a function of MA exposure *in utero*. Monoamine levels are currently being assessed in the same animals. (Grants NINDS #K07NS00979 and USC FRIG).

62.11

PRE- AND PERINATAL EXPOSURE TO COCAINE INCREASES SENSITIVITY OF FEMALES TO LOW DOSE OF APOMORPHINE. J.H. McLean, J.Thomas, B.L.Slifer*, and P.Stevens*. Dept. of Psychology, University of New Orleans, New Orleans, LA 70148.

Preliminary investigations were conducted to determine whether the reported increase in dopamine (DA) metabolic activity in females exposed to cocaine (COC) during early development had behavioral significance. Adult male and female rats that had been exposed to COC (40 mg/kg) or a control treatment during prenatal and early postnatal development were injected with apomorphine (APO; 0, .1, or 1.0 mg/kg) and observed for its effects on rearing and sniffing. The results revealed that the low dose of APO which reportedly stimulates pre-synaptic receptors, had a significantly greater effect on sniffing in females exposed to COC than on sniffing in either females given control treatments or males exposed to COC. The findings suggest 1) that females are more vulnerable to exposure to COC during development; 2) that the enhanced activity of the DA system reported previously may be related to pre-synaptic function; and 3) that among APO modulated behaviors, sniffing is more strongly affected.

62.12

COCAINE INJECTED ICV PRODUCES PLACE PREFERENCE CONDITIONING IN THE NEONATAL RAT. S. Wang and G.A. Barr. New York State Psychiatric Institute, 622 W. 168th St., NY, NY 10032 and Dept. Psych., Hunter College, 695 Park Ave. NY, NY 10021

Conditioned place preference (CPP) in adult animals has been used to study the reinforcing properties of cocaine and other drugs. These drugs likely act on the mesolimbic dopamine system, in particular, the ventral tegmental area (VTA) and nucleus accumbens (NAcc), to produce their reinforcing properties. Similar reward mechanisms may be present in infants: rat pups show a CPP when morphine is administered peripherally or to the VTA, and cocaine facilitates intracranial self-stimulation of limbic forebrain sites. In the present experiments, the CPP paradigm was used to investigate whether cocaine would produce a conditioned preference in the neonate, and if so, at which site(s) in the CNS. Three or 4-day old pups were injected with cocaine (i.p.; i.c.v.; or directly to the NAcc or VTA) and then confined to an odor-cued environment for 30 minutes. Four to 5 hours later, they were tested for 4 trials of 2 minutes each with the conditioned odor on one side and unscented wood shavings on the other side. The pups injected peripherally with cocaine (0.0-30.0 mg/kg) showed no preference. The pups administered cocaine i.c.v. (0.0, 3.0, 10.0, 30.0, 100.0 µg) displayed a dose-dependent preference for the side paired with the drug injections, with a significant preference induced by the two highest doses. Results from cocaine microinjected directly into either VTA or NAcc at doses of 0-25.0 µg are still preliminary. These overall results indicate that the reinforcing properties of cocaine are present at early ages in rat and are mediated through CNS. The reasons why systemic administration of cocaine did not produce conditioned odor preference are not known. (Supported in part by DA-06600)

62.13

PRENATAL COCAINE EXPOSURE ALTERS AGGRESSIVE BEHAVIOR IN ADULT SPRAGUE-DAWLEY RAT OFFSPRING. J.M. Johns, L.W. Means, E.W. Bass, M.J. Means*, L.I. Zimmerman* and B.A. McMillen, Depts. of Pharmacol. and Psychol., E. Carolina Univ., Greenville, N.C., 27858.

Gravid rats received one of 3 treatments throughout pregnancy: s.c. injections b.i.d. of saline, 15 mg/kg cocaine (Coke I), or cocaine on a 2 day on, 4 day off schedule (Coke II). Saline treated dams were pair fed with Coke I dams. Male pups were cross fostered to surrogate dams within 24 hr of birth. At PND 180, one offspring from each litter was placed in co-habitation with an ovariectomized female for 2 weeks. Frequency, duration and latency of behaviors were recorded during two 15 min sessions for subjects exhibiting any of 13 social/aggressive behaviors while the female was replaced by a smaller male intruder. Subjects received a s.c. saline injection before session I and 2.0 mg/kg of gepirone, a $5HT_{1a}$ agonist, 30 min prior to session II. Five of 11 Coke I and 0/10 control offspring exhibited all 3 aggressive behaviors of circle threat, aggressive posture and fight attack ($p<0.05$, Fisher's Exact test). In session II, differences in most aggressive behaviors were no longer significant and aggressive behavior was reduced by gepirone in all groups. Prenatal cocaine treatment resulted in aggressive (or social) behavioral changes in adult rat offspring, but did not alter the behavioral response to a $5HT_{1a}$ agonist. (Supp. by grant DA 04895)

62.14

STRIATAL PATCH-MATRIX ORGANIZATION AND COCAINE-MEDIATED C-FOS INDUCTION IN RATS PRENATALLY EXPOSED TO COCAINE. A.M. Snyder-Keller and R.W. Keller, Jr. Wadsworth Ctr., New York State Dept. Health, Albany, NY 12201, and Dept. of Pharmacol. & Toxicol., Albany Medical College, Albany, NY 12208.

Previous studies have described morphological changes in the basal ganglia following chronic cocaine administration, particularly during development. Development of the patch/matrix organization of the striatum was examined in rats prenatally exposed to cocaine (20 mg/kg SC, maternal admin.) daily from gestational day 7-21. Immunocytochemical staining for tyrosine hydroxylase (TH), substance P (SP), enkephalin, and calbindin was used to reveal the patch-matrix organization at different ages. Overlapping patches of TH-immunoreactive terminals and SP-immunoreactive cells were visible during the first week of life. Normal compartmentalization was also revealed at 18 days of age, when neurons of the matrix were immunoreactive for enkephalin and calbindin. A greater density of 5-HT-immunoreactive fibers was apparent relative to controls. Acute cocaine (20 mg/kg,IP) produced widespread fos-immunoreactive neurons in the medial 2/3 of the striatum. Pretreatment with the NMDA antagonist MK-801 (1 mg/kg,IP) blocked cocaine-mediated c-fos induction in this medial region, but resulted in the appearance of dense fos immunoreactivity in the lateral region. These results were similar to those obtained in normal animals, and suggest that the basic organization of striatal neurons, and DA and glutamatergic afferents, is not altered by prenatal cocaine exposure at the dose used. [Supported by DA-06199.]

62.15

PRENATAL COCAINE INCREASES EXTRACELLULAR DOPAMINE IN DEVELOPING RATS AS MONITORED BY IN VIVO MICRODIALYSIS.

R.W. Keller, Jr., I.M. Maisonneuve, D. Nuccio*, J.N. Carlson, S.D. Glick. Dept. of Pharmacol. & Toxicol., Albany Medical Col., Albany, NY 12208

Pregnant rats were given injections of saline (SAL) or cocaine (COC, 10 mg/kg twice daily, SC) between gestational days 7-21. Offspring were examined by microdialysis to study the effects of prenatal COC on the nigrostriatal dopamine (DA) system. Awake rats were examined between postnatal days 12-60. 20-min samples were assayed for DA, DOPAC, and HVA. After 4 baseline samples, the rat was exposed to 20 min of intermittent tail pinch and monitored for 4 samples; an acute injection of COC (20 mg/kg,IP) followed and 6 samples were collected.

Basal extracellular fluid (ECF) levels of all DA markers, estimated from pre-implantation calibration of the probes, were markedly reduced in young rats (12 d) as compared with adult rats (60 d). Basal DA, as well as DOPAC and HVA, were elevated in the prenatal-COC rats examined at the earliest ages. The response to tail pinch was minimal in both groups. In absolute magnitude, the increase in ECF DA induced by acute COC was greater in prenatal-COC rats; however, expressed as percent of baseline the groups did not differ. Furthermore, the increase in ECF DA after acute COC was more prolonged in prenatal-COC rats. As the rats aged, the levels of DA and metabolites in the prenatal-COC rats approached those of the prenatal-SAL controls. By 60 days of age there were no differences in any of the DA parameters. Thus prenatal exposure to COC produces an elevation in ECF DA after birth that gradually returns to normal as the rat ages. [Supported by DA-06199]

DEVELOPMENTAL GENETIC MODELS

63.1

FURTHER CHARACTERIZATION OF THE DOPAMINERGIC DENDRITE DEFICIT IN SUBSTANTIA NIGRA PARS RETICULATA OF HETEROZYGOUS AND HOMOZYGOUS WEAVER MUTANT MICE: GOLGI, MAP2 AND SYNAPTIC CONNECTIVITY STUDIES.

L.C. Triarhou & B. Ghetti. Dept. Pathol. (Neuropathol.) & Med. Neurobiol. Pgm, Indiana Univ., Indianapolis, IN 46202.

Following our initial identification of a structural defect in dopaminergic (DA) dendrites of substantia nigra pars reticulata (SNr) of *wv/+* and *wv/wv* mice (*Brain Res. 501:* 373, 1989), we used Golgi, immunocytochemical and ultrastructural techniques to further investigate this deficit. Antibodies against microtubule-associated protein 2 (MAP2), a marker for dendritic processes in nervous tissue, disclosed, in accordance with our previous observation, a substantial loss of dendrites in both *wv/+* and *wv/wv*. In Golgi preparations, the diameter of fusiform varicosities was reduced in remaining dendrites of *wv/+* by 33.6% and in *wv/wv* by 40.7%. By pre-embedding electron microscopic immunocytochemistry, 49.8% of TyrOHase immunoreactive dendrites in SNr received synaptic input from unlabeled axon terminals in +/+ , 23.7% in *wv/+*, and 9.4% in *wv/wv*. These data (*i*) suggest that in addition to being fewer, remaining DA dendrites of *wv/+* and *wv/wv* are aberrant in structural and most likely functional terms, (*ii*) strengthen the view that the dendritic DA projection of the SN represents a subcellular target of the *wv* gene and (*iii*) may offer insight into how the mutation hinders process outgrowth and maintenance. (Supported by USPHS RO1-NS14426, R29-NS29283 and PDP, Research & Sponsored Programs, IU).

63.2

INCREASED SEROTONIN LEVELS IN THE STRIATUM OF THE WEAVER MUTANT MOUSE.

E.H. Stotz, B. Ghetti, and J.R. Simon. Indiana Univ. Sch. of Med., Indianapolis., IN 46202.

Homozygous weaver mice (wv/wv) and rats with 6-hydroxydopamine lesions in the substantia nigra (SN) exhibit a loss of dopamine (DA) cells in the SN and altered neurochemical markers in the striatum (STR). Rats lesioned as neonates develop a hyperinnervation of serotonin (5-HT) in the STR not seen in rats lesioned as adults. Since the weaver mutation appears at an early age, these mice were investigated to determine if they also have a striatal hyperinnervation of 5-HT by measuring the content of 5-HT along the rostro-caudal extent of the STR. In 14 month old mice there was a 60-100% increase in 5-HT on a pmoles/mg protein basis and a 15-20% decrease in protein content. Therefore, we investigated 5-HT content per section and found an increase in striatal content from 35-90% depending on the rostro-caudal section investigated. Thus, the increase in 5-HT content is not an artifact solely due to a decrease in protein content. The data for 8 month old mice mirror that in 14 month old mice. Preliminary data for 1,2 and 3 month old mice show trends which are qualitatively similar to those seen in the older mice. (Supported by RO1 NS14426 and PO1 NS27613)

63.3

SAGITTAL ORGANIZATION OF TYROSINE HYDROXYLASE EXPRESSION IN PURKINJE CELLS OF TOTTERING AND TOTTERING-LEANER COMPOUND HETEROZYGOUS MUTANT MICE.

J.A.Heckroth and L.C.Abbott. Indiana University School of Medicine, Terre Haute Center for Med. Ed., Terre Haute, IN, 47809 and University of Illinois, Dept. of Vet. Biosci., Urbana, IL 61801.

Recent reports have demonstrated the presence of tyrosine hydroxylase (TH) and TH-mRNA in cerebellar Purkinje cells in tottering (*tg*) and leaner (tg^{la}) mutant mice. In the present study we report that TH-immunoreactive (IR) Purkinje cells in tottering (*tg/tg*) and tottering-leaner compound heterozygotes (tg/tg^{la}) are distributed in rostrocaudal stripes through all lobules of the cerebellar vermis and hemispheres. The sagittal bands of TH-IR Purkinje cells are narrow (3-5 cells wide) in the anterior lobe, but become increasingly wide in more posterior lobules. The pattern of TH expression in *tg/tg* and tg/tg^{la} cerebella bears striking similarity to that described for the Zebrin molecule and 5'-nucleotidase in normal mice, although the mutual identity of these patterns has not yet been confirmed by direct comparisons in these mutants. While numerous TH-IR fibers and varicosities are present in the cerebellar cortex in the mutants, the axons of the TH-IR Purkinje cells are not stained, and the cerebellar and vestibular nuclei do not contain TH-IR terminals which can be morphologically identified as originating from Purkinje cells. What functional role the products of TH activity might play within *tg* and tg^{la} Purkinje cells, or at Purkinje cell targets, remains to be determined. The differential expression of a mutant gene in sagittal bands of Purkinje cells is not unique to *tg* and tg^{la}, but the potential abnormal expression of neurotransmitter phenotype in sagittal Purkinje cell subsets deserves further investigation.

63.4

MYOBLAST TRANSFER: THE FIRST SUCCESSFUL CELL THERAPY IN TREATING MAMMALIAN GENETIC DISEASES.

P.K. Law, T.G. Goodwin*, Q. Fang*, J.A. Florendo, H-J. Li*, M. Chen*, D.S. Kirby*, M.B. Deering*, J. Cornett*, T.J. Yoo, R.L. Holcomb*. Cell Therapy Research Foundation, Memphis, TN 38117.

Myoblast transfer therapy (MTT) is a cell therapy being developed for hereditary myopathies. It is a form of gene therapy that does not require the identification of the abnormal gene. Through a cell fusion process that occurs naturally during muscle development and regeneration, donor myoblasts insert full complements of normal genes into dystrophic muscle cells. Therefore, regardless of which gene is abnormal or which protein is missing, MTT has potential application for all hereditary muscle degeneration. Donor myoblasts also fuse among themselves to form normal myofibers that progressively replace the degenerative fibers. Thus, MTT replenishes lost cells and repairs degenerating cells. As such, it is the first genetic engineering procedure that has demonstrated any functional significance in treating mammalian genetic diseases.

The initial clinical trial on Duchenne muscular dystrophy (DMD) boys indicated that the myoblast-injected foot muscles produced dystrophin and showed structural and functional improvement.

Further development involves thirty DMD boys, aged 6 to 14 years, who are receiving myoblast injections in the major muscle groups of their lower limbs. Injected muscles include the gluteal extensors and flexors of the leg and the foot. Both limbs are being injected. Donor myoblasts are derived from cell culture of muscle biopsies from young males, preferably the normal father or normal brother of the subject. CsA treatment begins two days before myoblast injection and continues for three months. Three days prior to myoblast injection, and at three month intervals lasting up to nine months, the isometric dynamometry of muscle groups of the left and right lower limbs are recorded. Comparison of the muscle function before vs. after myoblast injection will determine if the procedure exerts any beneficial effect on the dystrophic muscles. (Supported by private donations)

63.5

GENETIC STUDIES OF MOTOR NEURON DEGENERATION (Mnd) IN THE MOUSE. A. Messer, J. Plummer*, P. Maskin*, J. M. Coffin* and W. N. Frankel*. Wadsworth Center for Laboratories and Research, N.Y. State Dept. of Health and Dept. of Biomed. Sci., Sch. of Public Health, SUNY, P.O. Box 509, Albany, NY 12201-0509; and Dept. of Mol. Biol., Tufts Univ. Sch. of Med., Boston, MA 02111.

The mouse mutant Motor neuron degeneration (Mnd), which arose spontaneously on a C57Bl/6 background, displays an adult-onset progressive degeneration of upper and lower motoneurons, leading to spastic paralysis and premature death. While many obligate +/Mnd mice from the original stocks showed clear neuropathology, with a similar age of onset and a milder disease course than Mnd/Mnd mice, current stocks held in a semi-sterile environment and bred over several years for a very consistent age of onset in Mnd/Mnd show greatly reduced penetrance in the heterozygous state. Work therefore focuses exclusively on Mnd/Mnd at this time.

In order to map the Mnd gene, F2 progeny from a B6 Mnd/Mnd outcross to AKR were scored for neurological symptoms, then assayed for strain and chromosome-specific polymorphisms. Close linkage (5cM) of Mnd to the endogenous xenotropic murine leukemia virus fragment Xmv-26, Ank-1 and Polb on proximal chromosome 8 has been observed.

In the course of examining the F2 mice from the AKR outcross, 8 - 12% showed a very early onset (<5 months) of disease symptoms, with much more rapid progression than B6 Mnd/Mnd mice. No such acceleration has been seen in outcrosses to 5 other strains, including C3H and BALB/c. Additional breeding experiments are consistent with the existence of a timing gene, unlinked to Mnd itself, which has an early allele on the AKR background.
(Supported by the ALS Association and NIH grant R35-CA44385.)

63.6

BIOCHEMICAL AND MORPHOLOGICAL STUDIES IN THE CARDIAC AND SKELETAL MUSCLES OF mdx-MICE (DM): RELEVANCE AS A MODEL FOR DUCHENNE MUSCULAR DYSTROPHY (DMD).
P.L. Johnson, S.K. Bhattacharya, R.K. Handa, M.P. Gupta, T.A. Adamec*, and D.R. Shanklin*. University of Tennessee, Memphis, TN 38163.

Membrane-mediated chronic cellular degeneration plays a fundamental pathogenetic role in hereditary muscular dystrophy in DMD, CHF-146 strain dystrophic hamsters (DH) and xmd-dogs. We have shown that EICA, increased efflux of cellular enzymes (ALD, CPK and LDH), relative cardiac hypertrophy, reduced adenine nucleotides (ADN) and cellular energy charge (CEC), aberrated dystrophin distribution, dystrophic histopathology with profound cellular necrosis are associated with DMD and DH. Because of reported dystrophin anomalies in mdx-mice, we studied the EICA, plasma and cellular enzyme activities, ADN and CEC, light- and electron microscopic changes in cardiac and skeletal muscles of DM, and compared with those obtained from C57BL-+/+ normal mice (NM). Although high plasma enzymes and mild dystrophic changes were noticeable in the skeletal muscle as evidenced by EICA, reduced cellular enzymes and morphology, myocardium was spared in DM. No changes in cellular enzymes in heart, nor in ADN or CEC in skeletal muscle were found in DM. Apparent functional impairments or altered morbidity/mortality are also not present in DM. **We conclude that** DM does not share any of the severely involved cardiac pathology with DMD and DH. Nor does any premature death occur in DM due to compromised cardiopulmonary function and congestive heart failure, unlike DMD and DH. It seems, therefore, DH is by far a better model for the study of DMD than DM. Supported by NIH grant AR38540.

63.7

ENHANCED SENSITIVITY TO HYPOXIA-INDUCED LOSS OF SYNAPTIC TRANSMISSION IN A MOUSE MODEL OF MUSCULAR DYSTROPHY. M.F. Mehler*, K.Z. Haas, J.A. Kessler and P.K. Stanton. Albert Einstein Coll. Med., Bronx, NY 10461.

Dystrophin is a member of a protein super-family that links the actin cytoskeleton to plasmalemmal glycoproteins. In neurons, dystrophin is restricted to specific somatodendritic regions and to cell populations that are particularly susceptible to hypoxic injury. To investigate whether genetic absence of dystrophin may predispose specific neuronal populations to hypoxic injury, we examined the effects of graded hypoxic insults on synaptic transmission in mdx mouse hippocampal slice preparations.

Extracellular excitatory postsynaptic potentials (e.p.s.p.s) were recorded at Schaffer collateral-commissural synapses in stratum radiatum of field CA1 in control and mdx slices incubated in dual interface recording chambers and simultaneously exposed to successive hypoxic insults (5,8, and 15 min) by substituting N_2 for O_2. Mdx slices showed an earlier and larger irreversible loss of synaptic transmission compared to controls. 30 min after a 5 min hypoxic insult, slices from control mice exhibited 86 ± 10.8% (mean ± S.E.M.) recovery of synaptic transmission, while mdx slices averaged only 27 ± 15.8% recovery to the same hypoxic insult (n=7, $p<0.05$, Student's t-test). This selective vulnerability was partially ameliorated by preincubation with the anticonvulsant diphenylhydantoin (20 µM, phenytoin, DPH). Untreated mdx slices displayed partial irreversible loss of e.p.s.p. slopes by 3 min, and complete loss of synaptic transmission, after 8 min of hypoxia. In contrast, DPH-treated mdx slices exhibited nearly complete preservation of e.p.s.p. slopes after 8 min of hypoxia. These data suggest that dystrophin deficiency may predispose susceptible neurons to damage from hypoxia that could contribute to the development of cognitive deficits in dystrophin-deficient (Duchenne) patients.
(supported by NIH, NIMH, the Klingenstein Fund and Health Foundation)

63.8

RFLP ANALYSIS OF THE DIFFERENTIAL SUSCEPTIBILITY OF THE SWR/J AND C57L/J MOUSE STRAINS TO A VIRALLY INDUCED DEMYELINATING DISEASE. Simone M. Nicholson and Roger W. Melvold*. Microbiology-Immunology Dept., Northwestern Univ. Med. School, Chicago, IL 60611

Theiler's murine encephalomyelitis virus (TMEV, a picornavirus) is a natural enteric pathogen of mice which, upon access to the central nervous system (CNS) of some strains of mice, can trigger a specific cell-mediated immune response leading to demyelination. Thus TMEV-induced demyelinating disease (TMEV-IDD) serves as an excellent model for human multiple sclerosis (MS).

The differential susceptibility among mouse strains to development of TMEV-IDD is influenced by multiple genetic loci. Three such loci have thus far been been identified : *H-2D*, *Tmevd-1* and *Tmevd-2*. We are analyzing the differential susceptibility of the resistant C57L/J (and closely related C57BL/6) and the susceptible SWR/J strains, to see if additional relevant loci can be identified.

Backcross analysis indicates a role for 1 locus associated with the H-2 complex (possibly *H-2D*) and 1 locus unlinked to H-2, where a single copy of the SWR/J allele appears to be sufficient for susceptibility, even in the absence of "susceptible" H-2 genes. RFLP analyses, in recombinant-inbred strains between C57L/J and SWR/J (SWXL) and in segregating backcross animals are being done to identify the relevant non-H-2 locus.

63.9

PROTEOLIPID PROTEIN GENE EXPRESSION IS REDUCED IN HYPOMYELINATED "Pt" MUTANT RABBIT. M. Tosic*, M. Dolivo*, K. Domanska-Janik*♦ and J.-M. Matthieu. Lab. de Neurochimie, CHUV, 1011 Lausanne, Switzerland; ♦ Dept. of Neurochemistry, Polish Academy of Sciences, Warsaw, Poland

Paralytic tremor (Pt) is an X-linked mutation in rabbits characterized by rapid rhythmic tremor of the body due to hypomyelination in the CNS. Few myelinated axons are surrounded by thin and abnormal myelin sheaths. The amount of myelin is reduced to about 30% to that of normal rabbits. However, the protein content of the Pt myelin is not significantly different from normal myelin. We studied the relative contribution of major myelin proteins to the myelin composition. A radioimmunoassay for myelin basic protein (MBP) showed about 30% reduction compared to controls, which corresponds to the extent of myelin reduction in the mutants. This reduction was confirmed by Western blots which showed similar reductions for other myelin proteins. Moreover, Western blots revealed a striking reduction in the concentration of the proteolipid protein (PLP). At the age of 4 weeks PLP concentration represented only about 15% of control values. This drastic reduction in PLP, the sex-linked transmission of the defect and the biochemical and morphological similarities with other PLP mutations in mice and rats, strongly indicate that the primary defect of the Pt mutant lies in an abnormal expression of the PLP gene.

63.10

EXPRESSION OF MHC CLASS II ANTIGENS AND MICROGLIAL RESPONSE IN TWITCHER MOUSE. M. Ohno, A. Komiyama and K Suzuki. Dept. of Pathology, Univ. of North Carolina, Chapel Hill, NC 27599-7525.

Expression of major histocompatibility complex class II (Ia) antigens in the nervous systems has been reported in immunological and non-immunological diseases. Recently, we have observed many Ia antigens bearing cells in the brain of adult twitcher mouse, a murine model of human globoid cell leukodystrophy (GLD). For understanding of the pathological role of Ia antigen expression in GLD, we investigated patterns of the appearances of Ia antigen bearing cells and reactive microglia. Affected and normal control mice on postnatal days (P) 10 to 48 were perfused with a 2% PLP solution. Vibratome and cryostat sections from cerebrum, cerebellum, spinal cord and peripheral nerve were immunostained with monoclonal antibodies against mouse Ia antigen or Mac-1 antigen. In affected mouse, Ia antigen bearing cells were recognized in the peripheral nerve on P 10. On P 20, numerous Ia antigen bearing cells were observed in the white matter of spinal cord, brainstem, cerebellum and cerebrum. They were most numerous around P 30 and progressively declined after P 40. Ia antigens were positive on ramified microglia, rod shaped cells and macrophage-like cells in both white and gray matters. Ia positive cells were extremely rare in control mice at any age. Mac-1 positive reactive microglia and macrophage-like cells have been already recognized on P 10 in the spinal cord. They increased in number and were observed throughout the brain. Almost all of Mac-1 positive cells were reactive microglia and macrophage-like cells on P 48 when markedly reduced Ia bearing cells were observed. These results suggest that Ia expression may be suppressed in the advanced disease condition. Additionally, the intensity of microglial response and macrophage infiltration are not necessarily corresponding with Ia antigen expression. (Supported by Grants HD-03110, NS24453 and ES01104).

63.11

ABNORMAL PRODUCION OF IL-1 BY MICROGLIA FROM TRISOMY 16 MICE. J. Yao*, J. Keri*, R. E. Taffs*, M. Oster-Grant and C. Colton. Georgetown Univ. Sch. of Med., Washington, DC , 20007 ; Johns Hopkins Univ. Sch. of Med., Baltimore, MD, 21218; and NIAID, NIH, Bethesda, MD, 20892.
The production of interleukin-1 (IL-1) was examined in cultured microglia obtained from the cereberal cortices of trisomy 16 (Ts16) mice, an animal model for Down syndrome. IL-1 activity was measured in cultured microglia supernatants using a D10 cell proliferation assay. Unstimulated Ts16 microglia secreted significantly higher levels of IL-1 as compared to normal littermate microglia. Stimulation with lipopolysaccharide (LPS) increased IL-1 secretion in both normal and Ts16 microglia. However, IL-1 levels were significantly higher from the LPS-stimulated Ts16 microglia then from the normal littermates. Because cells from Down syndrome and Ts16 mice are known to have enhanced sensitivity to alpha/beta interferon (IFN) due to the triplicated alpha/beta receptor gene, we also studied the effect of alpha/beta IFN on IL-1 release. The results show that alpha/beta IFN did not increase IL-1 secretion by Ts16 microglia, but induced IL-1 secretion in normal littermate controls. However, even with the IFN stimulation, the overall level of IL-1 secreted in the normal littermates was still less than seen in Ts16. It appears that microglia in the Down syndrome animal model are abnormal in their IL-1 production. This abnormality may have important consequences to the development of the CNS.

TRAUMA: SPINAL CORD

64.1

ALTERATIONS IN LUMBAR MOTONEURON EXCITABILITY FOLLOWING THORACIC CONTUSION COMPARED TO HEMISECTION SPINAL CORD INJURY. Floyd J. Thompson[1], Ronald Parmer[1], Paul J. Reier[1&2]. Depts. Neuroscience[1] and Neurosurgery[2], University of Florida, Gainesville, 32610.
Much interest has been focused on the basic mechanisms associated with the development of spastic hyperreflexia subsequent to spinal cord injury. We have been studying lumbar motoneuron excitability in the adult rat subsequent to contusion injury produced by weight-drop onto the exposed 8th segment of the thoracic spinal cord. Neurophysiological tests of motoneuron excitability were conducted at 6, 28, and 60 days post-injury. We have observed a progressive alteration in lumbar motoneuron excitability characterized by a loss of rate sensitive depression of the tibial monosynaptic reflex, decreased post-tetanic potentiation (PTP), decreased short latency autogenic depression (SLAD) of the monosynaptic reflex in a recovery curve at condition-test intervals < 50 msecs, and no significant alteration in Renshaw inhibition. These alterations have been progressive in onset, significant in magnitude, and enduring in duration. They have collectively pointed to a loss of inhibitory control of reflex excitability. To determine how these deficits obtained from a diffuse bilateral injury compare to those derived from a specific unilateral injury, we have compared these findings in post-contusion animals to animals with hemisection of the T_8 thoracic spinal cord. The animals with hemisection injuries demonstrated unilateral loss of rate sensitive depression, PTP, and SLAD at one month post-injury. In the animals with contusion injuries, the deficits progressed in severity between 28 days and 60 days. However, in the animals with hemisection injuries, the maximal deficits were observed at 28 days. Rate sensitive depression and the loss of PTP progressed toward normal values when tested at 60 days, with no change in SLAD. Although preliminary, our conclusions from these data suggest that contusion related deficits progress in severity whereas deficits produced by hemisection progress toward normal values.

64.2

EVALUATION OF THE CALCIUM CHANNEL ANTAGONIST NIMODIPINE ON AXONAL FUNCTION FOLLOWING EXPERIMENTAL SPINAL CORD INJURY. S.S. Haghighi, J.J. Oro*, T. Stiens*. Division of Neurosurgery, University of Missouri School of Medicine, Columbia, MO 65212.

In the present study, cortical somatosensory evoked potentials (CSEP) were recorded to evaluate whether the administration of nimodipine could improve axonal function in the cord after an acute spinal cord compression injury (SCCI). Forty rats received a 52-gm static compression injury on the cord at C7-T1 for five minutes, and were randomly allocated to one of four treatment groups. Each group was given an intravenous infusion of one of the following over two hours, commencing one hour before compression: placebo; and nimodipine at 0.5, 0.25, and 0.125 μg/kg/min (ten rats in each group).

The preinjury physiological parameters (latency, amplitude, blood pressure and heart rate) were not significantly different ($p > 0.05$) among the treatment groups. Immediately following the SCCI, 80% of the animals in the placebo group lost their evoked responses. All animals in this group regained CSEPs within 15 minutes after decompression. The rate of evoked potential loss for the nimodipine-treated animals at 0.5, 0.25 and 0.125 μg/kg was 100%, 30% and 50%, respectively. The rate of recovery of the responses after decompression was 20%, 80%, and 80% for 0.5, 0.25, and 0.125 μg/kg, respectively.

64.3

THIOPENTAL INCREASES SURVIVAL OF SPINAL CORD NEURONS AFTER PHYSICAL INJURY. G. Wang, J.H. Lucas and G.W. Gross. Center for Network Neuroscience, Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Cooling at 17°C for 2h significantly increased survival of mammalian spinal cord (SC) neurons subjected to a defined physical injury: amputation of a primary dendrite 100 μm from the soma (Lucas et al., Brain Res. 517: 354, 1990). Possible mechanisms of hypothermic (HT) protection are general reduction of cell metabolism or a more specific inhibition of neuronal electrical activity. In the present study we tested whether addition of MgCl2 (to stop synaptic activity) or thiopental (TP) increased survival of lesioned SC neurons.

Monolayer cultures (21-28 DIV) of embryonic (13-14 day) mouse SC were used for these experiments. A primary dendrite was transected from 10 neurons in each culture by a UV laser microbeam; 10 other neurons were selected to be unlesioned controls. After surgery the culture medium was replaced with medium containing 12-15 mM MgCl2 or 200 μM TP.

At 24h survival was determined by erythrosine B. Survival in the TP group was 76% ± 5.4 (S.D.) compared to 50% ± 10.2 in the control (no TP) group (independent T test, $p < 0.05$). Survival in the MgCl2 group was 37% ± 5.2 compared to 45% ± 10.4 in the control (no added MgCl2) group. Each percentage represents the mean of 5-6 observations (cultures). The TP and HT studies suggest that reduction of cell metabolism increases the probability of SC neuron survival after physical injury.

Supported by PHS grant 29683 and by the Hillcrest Foundation of Dallas, TX founded by Mrs. W.W. Caruth, Sr.

64.4

EFFECT OF METHYLPREDNISOLONE (MP) ON SURVIVAL OF SPINAL CORD (SC) NEURONS AFTER DENDROTOMY. L.J. Schaffer, J.H. Lucas and G.W. Gross. Center for Network Neuroscience, Dept. of Biol. Sciences, Univ. of North Texas, Denton, TX 76203

A recent clinical study found MP significantly enhanced neurological recovery after SC injury at dosages of 30 mg/kg body weight (Bracken et al., New Eng. J. Med. 322: 1405, 1990). This dosage produces maximum plasma concentrations of 20-30 μg/ml in animals. The purpose of this study was to determine whether MP increases the probability of survival of mammalian SC neurons subjected to a defined physical injury: amputation of a primary dendrite 100 μm from the soma.

Monolayer cultures (21-28 DIV) of dissociated embryonic (13-14 day) mouse SC were used for these experiments. A UV laser microbeam was used to transect a primary dendrite from 10 neurons in each culture; 10 other neurons served as unlesioned controls. The medium of experimental cultures contained MP at concentrations ranging from 10 - 100 μg/ml.

Neuronal survival was determined 24 h after surgery by erythrosine B. Survival in the control (no MP) group was 59%. At MP concentrations of 10, 20, 30, 60 and 100 μg/ml survival was 53%, 58%, 58%, 40% and 40% respectively. Each percentage represents the avg. survival from 3-6 experiments (cultures). These data indicate that MP does not protect SC neurons subjected to physical trauma, and that concentrations greater than 30 μg/ml may decrease the probability of survival.

Supported by PHS grant NS 29683 and the Hillcrest Foundation of Dallas, TX founded by Mrs. W.W. Caruth, Sr.

64.5

EFFECTS OF THE MACROPHAGE TOXIN SILICA ON SECONDARY DAMAGE IN SPINAL CORD INJURY A.R. Blight, Center for Paralysis Res., Purdue Univ., West Lafayette, IN 47907.

A model of spinal cord trauma in guinea pigs, using lateral compression to a set thickness, produces a delayed functional loss below the injury at 1-2 days, followed by a partial recovery over several weeks, as measured using hindlimb motor behavior, vestibulospinal reflex testing, and mapping the receptive field of the cutaneus trunci muscle reflex. The role of inflammatory events in these secondary changes, was investigated with intraperitoneal injections of silica. Eleven matched pairs of animals were injured. One of each pair was selected randomly and injected with a suspension of 1.2 g of silica dust in sterile saline, immediately after injury and surgical closure. The animals survived up to 2 months from injury, then were fixed by perfusion with glutaraldehyde. Histopathology of the lesions was quantified by line sampling of myelinated axons, and measurement of all blood vessels, in plastic sections through the center of the lesion. The secondary onset of functional loss below the lesion was delayed by 1-2 days in silica treated animals with respect to controls. The number of myelinated axons at the center of the lesion 2-3 months after injury was higher in the silica treated animals, most significantly in the dorsal quadrant of the cord. Myelin sheath thickness and axon caliber distribution were not significantly different. Hypervascularity of the lesion was significantly reduced in silica treated animals. These findings support the hypothesis that macrophage activity during the first few days after injury plays a significant role in secondary tissue damage and is responsible for a proportion of long-term neurological deficits.

64.6

POTASSIUM DEPENDENCE OF CALCIUM CHANGES IN SPINAL NEURONS IN ORGANOTYPIC CULTURE: STUDIES WITH FURA-2. M. Tymianski, C.H. Tator. University of Toronto, Ontario, Canada.

Neuronal death and dysfunction following spinal cord injury is considered to be mediated by a rise in intracellular calcium concentration ($[Ca^{2+}]_i$). The injury process also results in large rises in extracellular potassium ($[K^+]_o$). We examined the effects of a rise in $[K^+]_o$ on intracellular calcium in organotypically-cultured mouse spinal neurons. Cultures mounted in a microscope stage incubator were perfused with media supplemented with two concentrations of $[K^+]_o$ (4.5 mM and 25 mM). In some experiments, neurons were exposed to increasing concentrations of extracellular calcium ($[Ca^{2+}]_o$). Neurons were perfused for 7 minutes at each $[Ca^{2+}]_o$. At the end of each perfusion period, $[Ca^{2+}]_i$ was measured with the fluorescent calcium indicator Fura-2. At $[K^+]_o$'s = 4.5 mM, there was no increase in resting levels of $[Ca^{2+}]_i$ (80-100 nM) over a range of $[Ca^{2+}]_o$ spanning 1 mM to 10 mM. However, experiments performed on neurons at $[K^+]_o$ = 25 mM revealed a sudden rise in $[Ca^{2+}]_i$ to levels exceeding 1000 nM after 50 minutes, irrespective of the levels of $[Ca^{2+}]_o$ in the perfusate. These results indicate that there exists a significant delay between exposure to high $[K^+]_o$ and a rise in $[Ca^{2+}]_i$. This rise is unlikely to occur solely through activation of voltage-gated calcium channels by potassium-induced depolarization, as the delay implies involvement of a second-messenger system. We also show that normal neurons are not immune to secondary injury effects.

64.7

CHANGES IN RECURRENT INHIBITION IN PATIENTS WITH SPINAL CORD INJURY J.M. Shefner*, S.A. Berman, M. Sarkarati*, and R.R. Young. West Roxbury VA Medical Center, West Roxbury, MA 02132

The etiology of spasticity after spinal cord injury is unknown. One spinal circuit that may be affected involves recurrent inhibition of motor neurons via Renshaw cells. Suprasegmental influences may alter recurrent inhibition; animal studies show increased recurrent inhibition after spinal cord injury.

We studied the effects of spinal cord injury or recurrent inhibition using the conditioned H reflex technique (Pierrot-Deseilligny, E., Bussel, B. Brain Res. 1975; 88: 105-108). A submaximal tibial nerve stimulus (S1) is presented prior to a supramaximal stimulus (S2), so that action potential collision permits an H reflex (H') to be elicited in response to S2. H' amplitude is partially a function of Renshaw cell activity.

In 10 patients, H' reflexes have been found to be absent in 6 at some time after their injury, in contrast to their uniform presence in controls. Sequential studies show reduction or disappearance of H' reflexes as spasticity increases; drugs that reduce spasticity produce reappearance of the H'. In patients who did have an H' reflex, its amplitude and the range of stimulus intensities that could elicit the reflex were both reduced as compared to controls.

We conclude that recurrent inhibition via Renshaw cell activity is increased in spinal cord injury, and that measures of recurrent inhibition may correlate with clinical measures of spasticity.

64.8

VULNERABILITY OF INJURED SPINAL CORD AXONS TO HYPOXIA M.G. Fehlings, K. Sakatani, M.R. Lee and W. Young NYU Medical Center, New York NY 10016

Traumatized spinal cord axons undergo secondary injury which may in part be due to ischemia. However, it is unknown whether injured spinal axons are more vulnerable to hypoxic/ischemic injury than normal axons. Thus, we examined the effect of hypoxia on normal and injured spinal cord axons *in vitro*. A segment of dorsal column (DC) was isolated from the thoracic cord of adult rats (n=16). The compound action potentials (CAPs) activated by bipolar supramaximal stimuli were recorded at 2 points with glass microelectrodes (1-2 MΩ). The DC segment was compressed between the microelectrodes for 15 sec. with a modified aneurysm clip (closing force = 2 g). Following recordings in oxygenated Ringer's solution, the DC was rendered hypoxic by superfusion with N_2-saturated Ringer for 1-2 hrs. After hypoxia, the preparations were superfused with oxygenated Ringer for 90 minutes.

Hypoxia at 37 ° C (n=6) attenuated the CAP ($p<0.05$) recorded distal to the lesion (11.7 ± 5.9% of control), more than the CAP recorded proximally (36.8 ± 6.4 % of control). Maximal CAP recovery proximally (71.4 ± 11.1 %) exceeded ($p<0.05$) that recorded distally (38.2 ± 3.5%). At 24-27° C (n=6) hypoxia also caused greater CAP diminution ($p<0.01$) of the injured segment (16.1 ± 7.4% of control) than in the proximal portion (74.3 ± 6.2% of control). Oubain (0.1-1.0 mM; n=4) attenuated the CAP of injured axons more ($p<0.05$) than in proximal uninjured axons, mimicking the effects of hypoxia. We conclude that hypoxia affects injured axons more than uninjured axons and that inhibition of membrane bound Na^+-K^+-ATPase partially mimics hypoxia-induced axonal dysfunction.

64.9

MDL 27,531 REDUCES HYPERREFLEXIA FOLLOWING CHRONIC SPINAL TRANSECTION IN RATS. J.H. Kehne, H.J. Ketteler*, T.C. McCloskey*, Y. Senyah*, M. Dudley, A.M. Ogden*, and J.M. Kane*. Marion Merrell Dow Res. Inst., Cincinnati, OH 45215.

MDL 27,531 (4-methyl-3-methylsulfonyl-5-phenyl-4H-1,2,4-triazole), a selective antagonist of strychnine seizures (Neurosci. Abst. 15:1176, 1989), was tested for potential antispastic activity in a model of spinal cord injury in rats. Rats that had chronic spinal cord transections (T_5) displayed intermittent spontaneous hindlimb contractions ("hyperreflexia") which could be quantified with an automated apparatus. MDL 27,531 (5-40 mg/kg, i.p.), like the alpha$_2$-adrenergic agonist clonidine, decreased this hyperreflexia. Specific reflex responses produced by a rapid mechanical extension of the hindlimbs were not decreased, suggesting that the compounds did not generally disrupt reflex function. In contrast to clonidine, MDL 27,531 did not produce ataxia in normal rats, nor did it affect the cardiovascular system. MDL 27,531 had a lower side effect profile than the antispastic agents baclofen or diazepam. MDL 27,531 was inactive in a variety of in vitro receptor binding assays, but did enhance binding of the benzodiazepine antagonist ^{3}H-Ro 15-1788 in vivo without directly elevating GABA levels. MDL 27,531 is a novel compound with potential for antispastic activity and minimal side effects compared with existing drug therapies.

64.10

NEW INSIGHTS INTO THE MECHANISM OF FORMATION OF CYSTS IN THE SPINAL CORD OF RATS AFTER SPINAL CORD INJURY. G.Guizar-Sahagun*, I.Grijalva*, I. Madrazo, R.Franco-Bourland*, E.Oliva*, A.Ibarra*, H.Salgado*, A.Zepeda*, R.Barrios* and B.Ortega-Corona. Camina Research Center for Spinal Cord Regeneration, 14050 Mexico City, Mexico.

To characterize the process of formation and the anatomical features of post-traumatic cysts of the spinal cord (SC), 54 adult rats were subjected to graded SC contusion of the lower thoracic region. The injured zone and neighboring areas were studied by light and electron microscopy, from day 1 to 1 year after contusion. Three phases were characterized in the cyst formation: 1- NECROSIS, from the day 1 to 1-2 weeks, showing hemorrhage, thrombosis, axonal segmentation and inflammatory infiltration. 2- REPAIR, from 1-2 to 8-15 weeks, showing necrotic tissue reabsorption, revascularization, trabeculation, and delimitation between the damaged and the preserved tissues. 3- STABILITY, from 8-15 to 52 weeks, no further significant changes were observed. The cyst wall does not have a uniform structure or cell composition, and its thickness can be very variable, even in the same specimen. The characterization of stages of SC cyst formation is useful to plan strategies for their use as transplant receptacles.

64.11

SPINAL CORD INJURY IN RATS, USING A DISPLACEMENT CONTROLLED IMPACT DEVICE: EFFECTS OF NALMEFENE, U-50488H, AND YM-14673 D.L.Behrmann,* J.C.Bresnahan, M.S.Beattie, B.R.Shah.* The Ohio State University, Columbus, OH 43210

A reproducible spinal cord contusion (SCC) in rats is made (spinal T9) using a displacement controlled impact device (DCID). Force and displacement transducers on the impact probe allow for immediate verification of spinal cord displacement (DSPL), time course of injury (20-23 msec), and resulting force. Varying DSPL produces tight behavioral groups with consistently predictable spinal cord lesions and degrees of incomplete neurologic deficit ranging from mild to severe. This allows for assessment of positive and negative treatment effects and of pharmacologic efficacy at different levels of neurologic deficit. Neurologic recovery is evaluated using open field walking (OFW), inclined plane (IP), grid walking (GW), and footprint analysis (FA). Percentage of tissue sparing is determined at the epicenter and by volumetric analysis of the lesion.

We used the DCID (DSPL=1.10 mm) to compare the efficacy of 3 compounds previously shown to improve behavioral outcome after acute SCC in rats: the opioid antagonist Nalmefene (NLF), which has increased activity at kappa receptors, the specific kappa agonist U-50488H (U50), and the TRH analogue, YM-14673 (YM), which has no kappa receptor binding activity in vitro. These were administered i.v. bolus or by osmotic pump over 7 days, beginning 30 min. after injury: NLF (0.1 mg/kg bolus, n=8; 3.6 mg/kg pump, n=8), U50 (10 mg/kg bolus, n=8; 71.4 mg/kg pump, n=8), YM (1 mg/kg bolus, n=8), controls (sterile water, n=8). Results indicated better OFW performance for all 3 compounds given i.v. bolus. Also, YM resulted in improved inclined plane score, a greater percentage of "grid-walkers" vs "non-grid-walkers" (also supported by FA), and sparing of fibers at the lesion epicenter. The results argue against a kappa receptor mediated mechanism of injury and suggest potential use of these compounds, especially YM, for treatment of acute spinal cord injury. Supported by NIH Grant NS10165 and Training Grant NS07291.

64.12

A NEURONAL CASCADING EFFECT: TESTING A NOVEL HYPOTHESIS IN THE ENTERIC NERVOUS SYSTEM. J.Y. Jew and T.H. Williams. Dept. of Anatomy, Univ. of Iowa, Coll. of Med., Iowa City, IA 52242.

We hypothesize that a perturbation such as an obstruction-induced distention of a segment of gut is transmitted from the intestine wall to the dorsal root ganglia (DRG). That is, there is a sequential effect, whereby changes at the second (higher) level depends on changes at the first (lower) level. We focused on a sensory pathway using the DRG as our experimental model. Male Sprague-Dawley rats (250-300 g. b.w.) were anesthetized with Nembutal (40 mg/kg b.w.), and the ileum was partially obstructed by application of a plastic cuff around the terminal ileum. Approximately 3 weeks later, control and obstructed animals were reanesthetized, and the terminal ileum was injected with the fluorescent retrograde tracer Fast Blue (FB) eight days prior to sacrifice. In DRG, the most intensely labeled cells and the greatest number of labeled cells were found at T11 and T12. In T11 DRG, the mean perikaryon area of FB-labeled neurons was 224% of control. The mechanism(s) mediating the sensory ganglion responses after a perturbation of the gut wall are not known. Our observation that only the FB-labeled DRG neurons are greatly enlarged makes it unlikely that hormones, nonspecific inflammatory substances generated in the gut, or neuron growth-promoting substances conveyed to the DRG by vascular transmission, are responsible. Consideration of other factors is also required, in particular, increased neuronal firing or transport of trophic factors, such as NGF or glial maturation factor, that may act as a regulator for the neuroplastic response in DRG neurons servicing the distended segment. In conclusion, neuronal responses to the obstruction extend beyond changes within the viscus. This is the first morphological report documenting synergistic and neuroplastic consequences of a perturbation in a sensory autonomic ganglion. We hypothesize that, with variations, this concept of a cascading or sequential effect applies to sensory ganglia servicing all viscera. Supported by grant DK38123.

TRAUMA I

65.1

REGIONAL BRAIN CONCENTRATIONS OF SODIUM AND POTASSIUM AFTER EXPERIMENTAL BRAIN INJURY. M.J. Thomas, D. Breault*, B. Nolan*, D. Smith and T.K. McIntosh. CNS Injury Laboratory, Surgical Research Center, Department of Surgery, University of Connecticut Health Center, Farmington, CT 06030, USA.

Alterations in the extracellular and intracellular cation concentrations after experimental brain injury have been reported by several investigators; however, there is little known about the specific time course following brain injury. In the present study, male Sprague-Dawley rats (300-375gm) were anesthetized and subjected to a moderate (2.3-2.5atm) lateral fluid-percussion brain injury directly over the left parietal cortex. Sham-operated control (n=28) and injured animals (n=28) were sacrificed at 10min, 1, 6 and 24h post-injury, brains removed and dissected into left and right parietal cortex, left and right adjacent cortex, bilateral hippocampi and brainstem. Dissected tissue samples were weighed, extracted and analyzed for $[Na^+]$ and $[K^+]$ by flame atomic absorption spectrophotometry. At 10min post-injury, $[Na^+]$ increased in the left hippocampus ($p<0.05$). At 1h, $[Na^+]$ increased in injured and non-injured cortex and bilateral hippocampi, while $[K^+]$ decreased in the injured and left adjacent cortex ($p<0.05$); all cations remained increased through 24h ($p<0.05$). Brainstem $[Na^+]$ decreased at 6h and $[K^+]$ decreased at 1h and remained decreased through 6h ($p<0.05$). These prolonged alterations in regional cation concentrations may contribute to irreversible secondary injury associated with brain trauma. Supported, in part, by NIH NS26818, VA Merit Review Grant 74R, and a grant from the Brain Trauma Foundation.

65.2

REDUCING BRAIN EDEMA IN EXPERIMENTAL CONCUSSIVE BRAIN INJURY WITH POSTINJURY TREATMENT BY POLYSACCHARIDE-DEFEROXAMINE. M. Tangoren*, L. Levi*, A. Wolf, R. Broadwell and B. Hedlund. Div. Neurosurgery, Univ. MD, Sch. Med., Balto., 21201.

Prophylactic administration of iron chelators, such as deferoxamine, improves outcome following several types of brain injury, probably due to free radical scavenging. Our hypothesis is that similar improvement can be achieved with a deferoxamine-polysaccharide conjugate (DFO-PSC) (Biomedical Frontiers, Minn.) after moderate fluid percussive injury in the rat. Fifteen minutes after receiving a right parasagittal 2.9 atm concussive injury (LD25), surviving rats from a group of 90 received intravenous DFO-PSC (50 mg/kg) or a similar volume of vehicle. One, 2, and 5 days postinjury, brain edema was determined in multiple cortical and deep brain regions using a gravinometric technique. Thirty-two rats received the drug, and 32 received vehicle. The difference in edema was statistically significant in all brain regions after each period postinjury. The specific gravity of the right cortical areas in the drug group on day 1 were 1.0506 (.0017) vs. control of 1.0426 (.0023), p<.001; day 2: 1.0485 (.0023) vs. 1.0424 (.0023), p<.001; day 5: 1.0491 (.0023) vs. 1.056 (.0026), p<.01. These data suggest post-injury treatment with DFO-PSC dramatically reduces concussive brain edema.

65.3

U-50488H REDUCES VASCULAR PERMEABILITY AND EDEMA IN RAT SPINAL CORD INJURY. Z.X.Qu,* J.Xu, P.L.Perot* and E.L.Hogan. Depts. of Neurology and Neurosurgery, Med. Univ.of SC, Charleston, SC 29425.

Vascular injury plays a very important role in the secondary injury process of CNS trauma. It has been reported that U-50488H, a selective opioid κ receptor agonist, has neuroprotective and diuresis effects. This study reports the effect of U-50488H on vascular permeability and edema in rat contusive spinal cord injury. A sensitive fluorometric method was used for the evaluation of U-50488H on extravasation of fluorescein isothiocyanate conjugated dextran (FITC-D, a macromolecular tracer). Edema was measured by dry-wet weight method in rat contusive cord with 40g-cm trauma force.

The results showed that U-50488H, 5, 10, 20 and 40 mg/kg ip at 0.5 hr before and after trauma reduced vascular permeability expressed as a vascular injury index (VII) in a dose-dependent manner. Multiple injections of U-50488H at 0.5 hr before and 0.5, 2, 8 and 22 hr after trauma also reduced VII significantly ($p<0.01$). The same administration of U-50488H significantly diminished edema formation. The water content in spinal cord was 71.11±0.27% compared to the control 72.47±0.22% ($p<0.01$). These results suggest U-50488H has a beneficial and protective effect on vascular damage in CNS trauma. Supported by NS-11066.

65.4

LATENCY REDUCTION OF VISUAL EVOKED RESPONSE (VER) - POSSIBLE GLUTAMATERGIC EXCITATION SECONDARY TO A FOCAL BRAIN INJURY S. XU*, H.G. WAGNER, Lab. of Neuropathology and Neuroanatomical Sciences, NIH, NINDS, Bethesda, MD 20892

Following localized freezing injury to the cortex, we have observed calcium deposits at sites in addition to the injured area. These deposits suggest the presence of a secondary hyper-neuroexcitation which may be related to the neuro-excitotoxicity. We looked for physiological evidence of this neuroexcitation.

Electrodes were placed in the rat cranium over the visual cortex, one were later a cold probe was applied transcranially to the parietal cortex. VER to a stroboscopic flash were processed through a computer.

In the unlesioned rat the latency for the VER was about 44 ± 3 msec (Mean ± SD). Four to six hours after placement of the freeze, the latency had decreased to 40 ± msec (N = 13) ($P<0.01$). The latency reached its lowest values of 37 ± 2 msec 1 to 3 days after application of the lesion. There was a little change in the unlesioned side. One week later there was a partial recovery of latency (42 ± 3 msec).

Glutamate is believed to be involved in the neuronal death after brain injury. The secondary neuronal hyperexcitation may be also glutamatergic. Further support we found in the observation that the effect of cold injury on latency or VER was reduced or abolished by application of MK-801 (3 mg/Kg i.p.). And in unlesionsed rats the latency of VER also reduced after topical application of L-glutamate (1mM).

Our results suggest that there is secondary neuroexcitation of the cortex adjacent to a cold lesion and it may related to glutamate.

65.5

RISES OF EXCITATORY AMINO ACIDS TO TOXIC LEVELS UPON IMPACT INJURY TO THE RAT SPINAL CORD AND THE EFFECTS OF METHYLPREDNISOLONE ON THOSE LEVELS.

D. Liu and D.J. McAdoo Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550

Release of excitatory amino acids (EAA) has been implicated in damage arising from injury to the central nervous system. These substances are neurotoxic in vitro at concentrations exceeding normal physiological levels in extracellular fluid. We utilized microdialysis sampling and analysis by high pressure liquid chromatography to determine whether impact injury to the rat spinal cord caused extracellular levels of EAA to reach levels that are toxic in in vitro experiments. In vivo calibration of the microdialysis fiber demonstrated that glutamate rises from 21 μM before injury to ca. 200 μM following injury. Parallel results for aspartate were 7 μM to 122 μM. Other neurotransmitter amino acids also rise dramatically in response to spinal cord injury; most non-neurotransmitter amino acids rose much less. Glutamate is strongly toxic to cultured neurons at 50-100 μM,[1] and aspartate has comparable toxicity. Thus impact injury of the rat spinal cord raises EAA concentrations to toxic levels, adding to the evidence that those substances may contribute to secondary damage upon injury to the spinal cord. A recent clinical study[2] demonstrated that 30 mg/kg of methylprednisolone ameliorates the long-term deficits caused by spinal cord injury in humans. In preliminary experiments, 30 mg/kg of methylprednisolone administered to rats at the time of injury hastened the decline in excitatory amino acid levels.

[1] D.W. Choi et al., J. Neurosci., **7** (1987) 357-368.
[2] M.B. Bracken et al., New Engl. J. Med. **322** (1990) 1405-1411.

65.6

IN VITRO AND *IN VIVO* ANALYSIS OF TIRILAZAD MESYLATE (U-74006F) AS A HYDROXYL RADICAL (•OH) SCAVENGER.

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Salicylate (SA) can be used to measure •OH formation because it reacts with •OH *in vivo* and *in vitro* to form dihydroxybenzoate (DHBA). We tested the neuroprotectant U-74006F as a scavenger of •OH based on blockade of DHBA formation during CNS injury. •OH was generated *in vitro* by mixing H_2O_2 with ferrous ammonium sulfate in phosphate buffer. U-74006F was added to the mixture and then analyzed by HPLC for loss of parent and/or appearance of products. Results showed a 50% loss of U-74006F after a 1 min exposure to •OH. A reaction product was identified by LC-MS which had a mass increase of 16. In other experiments, mice were given a concussive head injury, treated with vehicle or U-74006F i.v. (10 mg/kg) followed by SA i.p. (100 mg/kg) and later sacrificed. Brain DHBA was measured by HPLC-EC and brain SA by HPLC-UV. •OH formation was expressed as a ratio of DHBA to SA. Results showed a 50% increase in •OH after concussive injury. This increase was blocked by U-74006F. We conclude that U-74006F decreases •OH formation following CNS injury possibly by a •OH scavenging mechanism.

65.7

Corticosterone's Mediation of Traumatic Brain Injury.

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Elevated corticosterone levels have been shown to exacerbate neuropathological damage produced through a variety of insults. Clinical reports indicate that following head trauma, cortisol levels are markedly elevated in patients with poor outcome. In the present experiment, adult rats were treated chronically with corticosterone acetate (15ug/ml) supplied in their drinking water. Although a mild level of injury (1.75 atm.), produced no deficits in vehicle treated animals, corticosterone treated rats displayed significant motor and cognitive deficits. The exhibited deficits did not appear to be due to serum hyperglycemia. The data demonstrate that elevated corticosterone levels potentiate deficits following traumatic brain injury. The results also suggest that elevated basal levels of corticosterone may mediate the age-related increase in vulnerability to deficits following traumatic brain injury.

Supported by a NIA dissertation study award

65.8

EVIDENCE OF CALPAIN ACTIVATION FOLLOWING MODERATE TRAUMATIC BRAIN INJURY (TBI).

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Promising new research has indicated that neurochemical alterations mediated by muscarinic and NMDA receptors comprise crucial components of trauma pathophysiology. We have investigated the possible involvement of calpain 1, an important enzymatic intermediate of adaptive and pathological neuronal processes, in these trauma-induced neurochemical alterations. In this study, we examined the immunoreactivity of microtubule-associated protein 2 (MAP2), a principal in situ substrate of calpain 1, as an index of endogenous calpain 1 activity. MAP2 immunoreactivity was examined in homogenates of hippocampal and cortical tissue obtained from naive, sham-injured or fluid percussion injured animals. Rats were injured at 2.1 atm and were sacrificed 3 hours after injury. Tissue was homogenized in the presence of specific calpain inhibitors (0.1 mM leupeptin) and chelators (1 mM EGTA) to eliminate in vitro calpain activity and MAP2 proteolysis during tissue processing. MAP2 immunoreactivity was quantitated on nitrocellulose transfers using an anti-MAP2 monoclonal antibody. Sham injury had no effect on MAP2 levels in either cortex or hippocampus. However, TBI caused a significant decrease in both hippocampal and cortical MAP2 levels. The effect appears to be region selective since the MAP2 decrease is more pronounced in hippocampus than in cortex. The observations are consistent with in situ activation of calpain 1 caused by moderate TBI, and suggest that calpain proteolysis is involved in the development of TBI-induced functional deficits. Supported by NIH NS21458.

65.9

REGIONAL DISTRIBUTION OF ENDOGENOUS IgG AND IgM RESEMBLES 72KD HEAT SHOCK PROTEIN EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY.

H. Soares, B. Osteszewska*, K. Okiyama, and T.K. McIntosh. Surg. Research Cntr, Univ CT Health Cntr, Farmington, CT 06030.

Previous fluid percussion (FP) brain injury studies in our laboratory have demonstrated consistent patterns of blood brain barrier (BBB) breakdown in cortical, hippocampal, and thalamic regions. There exists little argument concerning the damaging consequences of hemorrhage into neural tissue. However, few studies have focused upon various "protein exudates" following acute damage to the BBB. The present study examined endogenous immunoglobulins in brain following traumatic head-injury. Anesthetized adult rats were subjected to moderate (2.2-2.4 atms) FP brain injury. Animals were sacrificed at 5 min, 2 hrs, 4 hrs, and 24 hrs post-injury (n=4/group). Sham operated animals (n=4) served as controls. Anti-rat IgG and IgM immunocytochemistry demonstrated staining of both neurons and glia in regions of trauma induced BBB breakdown. The majority of positive anti-rat IgG/IgM stained cells appeared shrunken and necrotic. However, many positive cells exhibited a more Golgi-like appearance. Positive cells were observed as late as 24 hours post-injury. The pattern of anti-rat IgG/IgM staining bore striking similarities to the pattern of 72KD heat shock protein expression after brain injury (McIntosh et. al., Neurosci Abst, 1989). Cross-reactivity between rat and mouse immunoglobulins has been widely documented. Staining with anti-mouse IgG in our model exhibited the same patterns as the anti-rat IgG's. Care must be taken when utilizing anti-rodent immunoglobulins as secondary antibodies in rodent brain models involving breakdown of the BBB. These results indicate that endogenous immunoglobulins are present on (or in) neurons and glia following brain trauma. Supported, in part, by NS26818, a VA Merit Review grant 74R, and a grant from the Brain Trauma Foundation.

65.10

IN VITRO PROTECTION OF BOVINE BRAIN SUPERNATE PROTEINS BY EXOGENOUS HEAT SHOCK PROTEIN-70.

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Previous publications have demonstrated in vivo protection of CNS tissues following hyperthermia. Transient hyperthermia induces the synthesis of Heat Shock Proteins (HSP's). We have examined the in vitro effect of HSP-70 on bovine brain supernate porteins for potential protective effects.

High speed sterile supernate of bovine brain was prepared. The assay for thermo protection was carried out by heating the solution to 75° C for one hour and percipitating the coagulated proteins by centrifugation. Both bovine serum albumin (BSA) and HSP-70 were evaluated for the ability to prevent protein denaturation and percipitation following thermal stress.

Bovine HSP-70 and BSA were themselves resistant to heat denaturation. We also found that BSA was able to weakly protect the proteins in brain supernate (p=.04). HSP-70 demonstrated a stronger ability to protect brain proteins in solution (p=.001). We conclude that purified bovine HSP-70 is able to bestow thermotolerance upon a supernate of bovine brain proteins.

65.11

GM1 GANGLIOSIDE TREATMENT IN RATS WITH CORTICAL CONTUSION. R. Sutton, L. Lescaudron, X. Chen*, K. Repola* and D. Stein. Brain Research Lab., I.A.B., Rutgers University, Newark, NJ 07102.

Male rats with moderate (M-TBI) or severe (S-TBI) cortical contusion injury of the right sensorimotor cortex were treated (i.p.) with saline, GM1 (10 mg/kg) for 5 days postinjury (P-GM1), or GM1 for 3 days preinjury & 5 days postinjury (P+P-GM1). Recovery from deficits in beam-walking (BW) ability was unaffected by GM1 after S-TBI whereas BW recovery after M-TBI was worsened by P-GM1 but not P+P-GM1. The L-R forelimb wire grip time (pathologic grasp) was increased by P-GM1 and P+P-GM1 at 24 hr after M-TBI or S-TBI (compared to saline controls); deficits on this task were not present by day 8 (vs shams). A decrease in the severity of left forelimb contraflexion seen in saline controls from 24 hr to 8 days after M-TBI or S-TBI was blocked by P-GM1 and by P+P-GM1 treatment. No significant effects of treatment on additional neurological deficits (chin, tactile or visual placing, extensor rigidity, hindlimb extension) were found. Neither P-GM1 nor P+P-GM1 affected area of cortical necrosis after M-TBI or S-TBI.

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65.12

DELETERIOUS EFFECT OF ETHANOL ON THE RECOVERY OF THE SEP AFTER SPINAL CORD INJURY. H.F. Martin, S. Katz, Dept. of Physiology and W.O. Boggan, Dept. of Psychiatry, Medical University of South Carolina, Charleston, South Carolina 29425

The objective of this study was to determine the effect of blood ethanol on recovery of electrophysiological conduction following calibrated impact trauma to the spinal cord in anesthetized rats. Conduction was evaluated by Somatic Evoked Potentials (SEP's). Sub-maximal trauma doses of 8 and 12 gm-cm were chosen which resulted in moderate recovery of untreated animals after trauma, (70 and 50% respectively, compared to pre-trauma SEP).

In the treated groups blood ethanol was increased immediately prior to trauma by a continuous IV infusion for 15 - 20 minutes of a high dose (15%) or a low dose (5%) solution of ethanol in saline. Blood samples were taken at the end of the infusion period and analyzed for % blood ethanol by either gas chromatography or by enzymatic colorimetric assay. In a previous study, the 15% solution produced a concentration of 173.8 mg% in blood, and 7.81 and 9.42 mg/gm tissue in brain and spinal cord respectively. High dose pretreatment decreased the % recovery of the SEP over a 5 hour post-trauma monitoring period. The average recovery was 50% in the 8 gm-cm group and 30% in the 12 gm-cm group. Thus, in the presence of intoxicating levels of blood ethanol, the alteration of the SEP at the 8 gm-cm trauma dose closely resembled the change seen in untreated animals following a 12 gm-cm trauma dose. In low dose pretreated groups of animals, SEP recovery was close to that observed in untreated animals suggesting a dose dependent effect of ethanol treatment.

These experiments document the deleterious effects of blood ethanol on subsequent physiological recovery from neural trauma. Subsequent experiments should be directed at further defining the mechanism by which ethanol exerts this effect in order to suggest avenues of therapy and/or prevention in intoxicated individuals with spinal cord injury resulting from accidental trauma.

65.13

ALCOHOL POTENTIATES BRAIN EDEMA FORMATION AFTER HEAD TRAUMA. M. Morehead, S. Murphy and H. Goldman. Department of Pharmacology, Wayne State University, Detroit, MI 48201.

In our rat model of closed-head, moderate concussion we found that acute ethanol (EtOH) intoxication exacerbated brain injury. EtOH (2.4 g/kg) in 20 ml normal saline (≈5% solution) was administered intraperitoneally to male Wistar rats (330-430 g) 10 min prior to a fixed-head mechanical impact. In one set of experiments chronic epidural cannulas were cemented into the skull and intracranial pressure (ICP) was monitored for up to 7 days post-trauma. In intoxicated (blood alcohol 150 mg/dl) impacted animals, ICP rose to 23 ± 2 (SEM) mmHg at 1 hr compared to 3.8 ± 0.3 mmHg for non-impacted, non-intoxicated controls; 7.6 ± 1 mmHg for animals impacted only and 8.5 ± 2.5 mmHg for animals receiving EtOH only. By 24 hr, ICP rose to 33.6 ± 5 mmHg in the trauma + EtOH group compared to 23.6 ± 8.1 in impacted only animals and <10 mmHg in control animals or those receiving EtOH only. By 30 hr post-trauma, ICP peaked in both groups at 36.0 ± 2.4 and 31.0 ± 3.1 mmHg and fell to normal levels by 6 days. In another set of experiments regional brain permeability-capillary surface area products (rPS) were examined at 2 hr post trauma. During this time interval, rPS increased in 6 of 16 regions, including the cerebellum, hypothalamus and frontal cortex after combined exposure to both EtOH + trauma, compared to 4 of 16 regions clustered in the rostral forebrain after simple head injury. These initial experiments suggest that EtOH intoxication signficantly potentiates the edema formation and brain injury that follows closed-head moderate concussion. These differences also suggest that the time available for treatment of brain edema may be much shorter when EtOH intoxication complicates head injury.

65.14

HYPOTHERMIA BLUNTS ACETYLCHOLINE INCREASE IN CSF IN TRAUMATICALLY BRAIN INJURED RATS. B.G. Lyeth, J.Y. Jiang, S.E. Robinson,[1], H. Goo[1], L.W. Jenkins, and R.L. Hayes. Division of Neurosurgery & [1]Department of Pharmacology and Toxicology, Virginia Commonwealth University Richmond, Virginia 23298.

Excessive activation of muscarinic acetylcholine (ACh) receptors significantly contributes to the pathophysiological consequences of traumatic brain injury (TBI)(Lyeth et al., 1988). Moderate hypothermia (30°C) significantly reduces mortality, and motor deficits associated with TBI in the rat (Clifton et al., 1991). In order to determine whether the protective effects of hypothermia involve a decreased release of ACh, we examined the effects of moderate hypothermia (30°C) on levels of ACh and choline (Ch) in CSF following moderate (2.2 ATM) fluid percussion TBI in rats. Rats were anesthetized, intubated, and ventilated with 2% isoflurane in 70% N_2O/30% O_2 prior to TBI. Three groups were examined: sham TBI at 37°C, TBI at 37°C and TBI at 30°C. An intracisternal CSF sample was withdrawn 5 minutes after TBI. ACh and Ch were analyzed by mass fragmentography. ACh concentrations were significantly higher in 37°C TBI rats (0.21 ±.04 nM/ml) compared to shams (0.11 ±.01 nM/ml) (ANOVA, Tukey $p<.05$). In contrast, no significant changes in ACh were observed between 30°C TBI rats (0.14 ±.02 nM/ml) and shams. Ch concentrations were significantly higher in both 30°C (16.4 ±1.3 nM/ml) and 37°C TBI rats (11.6 ±1.1 nM/ml) compared to shams (3.7 ±0.2 nM/ml) (ANOVA, Tukey $p<.05$). These results are consistent with the role of excessive muscarinic cholinergic neurotransmitter-receptor interactions in TBI pathophysiology. These results suggest that the neuroprotective effects of moderate hypothermia in TBI may be related to the inhibition of excessive release of ACh associated with injury. Supported by NIDRR # H133B80029; NIH # NS 21458, NS 21587, NS 24413, NS 19950.

65.15

A SECOND CONCUSSIVE INSULT FOLLOWING A FLUID PERCUSSION INJURY RESULTS IN INCREASED EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) AND THE DEVELOPMENT OF MORPHOLOGICAL DAMAGE. H.Badie*,D.A.Hovda, and D.P.Becker. Div. Neurosurg., UCLA Sch. Med., Los Angeles, CA 90024-6901

Evidence now exists that following a fluid percussion (F-P) injury the brain becomes vulnerable to a second insult. To determine the morphological consequences of a second F-P injury during this period of vulnerability 25 rats were studied. Animals received two lateral F-P injures (3.5-4.0 atm) spaced at either 1 or 2 hours, allowed to survive for 10 days and were then processed for GFAP immunohistochemistry and cresyl violet histology. Both sham and single insults were also studied for comparisons. As sampled over the cerebral cortex (in 1.04 mm^2 increments) the number of cells expressing GFAP was 2.9 , 2.4 and 3.5 times that of normal intact animals for single, 1 hr and 2 hr double injury groups. Quantitative histological analysis revealed that there was no cell loss in animals sustaining a single injury, however in the double insult groups, there was evidence of contusions at the gray-white matter junction. Cell counts using nissl stained sections indicated that, in sham animals, cells <35um^2 (glia) constituted only 31% of the population, however, in the double insult animals this increased up to 54% being the most evident in those animals who received their injuries only 1 hr apart. These results indicate that following a F-P injury, a second (concussive) insult increases the expression of GFAP, the development of gliosis and the formation of contusions. (UPHS RO1 NS27544).

66.1

ADAPTATION OF THE FLUID PERCUSSION DEVICE REDUCES THE FORMATION OF CONTUSIONS IN A MODEL OF TRAUMATIC BRAIN INJURY. S.Thomas*, A.Yoshino,D.A.Hovda, and D.P.Becker. Div. Neurosurg., UCLA Sch. Med., Los Angeles, CA 90024-6901

The fluid percussion (F-P) apparatus has been used to produce experimental traumatic brain injury with animals positioned at the tip of the apparatus. This type of injury has often resulted in contusions. We have adapted the F-P model by interconnecting a 20 cm, rigid polyethylene tube between the animal and the apparatus. Animals injured in this fashion typically do not exhibit morphological damage (concussion). The current study describes the physical characteristics of the pressure wave, at various levels of atmospheric pressure (atm), at the tip of the apparatus compared to those seen at the end of the extension tube. In addition, physiological and anatomical variables were measured in 20 rats who sustained an injury under these two conditions at low (3 atm) and high (5 atm) injury levels. The results indicated that at any given level of atm, the pulse at the tip of the apparatus, consisted of a single wave form lasting 20-23 msec. At the end of the tube the pulse exhibited an initial positive wave which was of the same amplitude and duration as that generated at the tip, however it was followed by an oscillating wave 4-5 times smaller that the first (2-3 peaks, 20 msec ea). In the animal studies, when the F-P was applied directly: 1) blood pressure (BP) = 40-57 mm Hg, 2) apnea time= 15-18 sec,3)unconsciousness time= 94-294 sec. In contrast, with the extension tube 1) BP = no change, 2) apnea time= 0-1.25 sec, 3) unconsciousness time = 2-12 sec. Only animals connected directly to the apparatus exhibited contusions. The results indicate that with the extension tube the injury produced by F-P results in a concussive (diffuse) injury as oppose a contusion (localized) injury.(UPHS RO1 NS27544).

66.2

THE EFFECT OF FLUID RESUSCITATION ON BRAIN TRAUMA-POTENTIATED LACTIC ACIDOSIS IN HEMORRHAGIC SHOCK. X-Q. Yuan, C. E. Wade, G. Hanson and W. G. Rodkey. Letterman Army Institute of Research, Presidio of San Francisco, CA 94129, U.S.A.

To study the effect of fluid resuscitation on the response of blood lactate to hemorrhagic shock combined with traumatic brain injury, thirty anesthetized rats were randomly assigned to four groups: hemorrhagic shock (13.5 ml/kg/10 min, n=7) (H), hemorrhagic shock following brain trauma (fluid percussion model, 2.9 atm, 25 msec, n=7) (TH), hemorrhagic shock treated with lactated Ringer's solution (3 times shed blood volume, n=8) (HR), hemorrhagic shock following brain trauma treated with lactated Ringer's solution (n=8) (THR). Group H showed no significant changes in blood pH, HCO_3 and base excess (BE_b) after hemorrhage. In Group TH, blood pH and BE_b were significantly reduced after the hemorrhage, while in Group HR, the values of both variables were significantly raised after resuscitation. Acid-base status showed no significant difference between Groups THR and H. Group H showed a significant increase in blood lactate level after the hemorrhage. Group TH had greater elevations in blood lactate levels with a significantly higher value over Group H at 70 min (26.8±2.9 vs 18.5±1.9). However, no significant differences in blood lactate levels were found between Groups H vs HR or THR. Both brain trauma and fluid replacement increased blood pyruvate levels with marked reduction in the ratio of lactate to pyruvate levels. Our data indicate that traumatic brain injury potentiates arterial blood lactate accumulation and metabolic acidosis after hemorrhage, and infusion of lactated Ringer's solution can relieve these disturbances.

66.3

LOW FREQUENCY VARIATIONS IN HUMAN INTRACRANIAL PRESSURE AND JUGULAR VENOUS OXYGEN SATURATION

C. F. Contant, Jr*., J. D. Crouch*, C. S. Robertson*, and R. G. Grossman. Dept of Neurosurgery, Baylor College of Medicine, Houston, TX 77030

To determine if the slow variations in brain metabolism observed in animal models can be detected in indirect measurements of cerebral blood flow in humans, we have examined intracranial pressure(ICP), jugular venous oxygen saturation (SJVO2) and arterial pressure(AP) data from 10 patients.

The patients were treated at the Ben Taub General Hospital. All were comatose (GCS $\leq$ 8) and had ICP, SJVO2 and AP catheters. Informed consent was obtained from next-of-kin. Data were collected at the bedside by a computerized monitoring system. A discrete Fast Fourier Transform (FFT) was applied to 307.2 seconds of data collected at six minute intervals. From the FFT the frequency spectrum was produced, and the portion of the spectrum from 0.00 to 0.30 Hz was plotted as a function of time.

Peaks due to respiratory variation were seen in the ICP and AP arrays, though not in the SJVO2 array. Very low frequency variation [0.0083 to 0.033 Hz (.2 to 2 cycles per minute)] was detected in all three arrays. Low frequency variations (0.033 to 0.10 Hz) that did not demonstrate a dominant frequency were characterized by changes in the spectral edges.

In five of the ten patients there was at least one period where there was a dominant wave occurring in the frequencies between 0.04 to 0.10 Hz (2.4 to 6.0 cycles per minute) and observed in all three arrays. The AP and ICP arrays were often similar, though at no time did the AP array include peaks that were not present in the ICP waves as well. We hypothesize that the variations of SJVO2 result from low frequency changes in the cerebral blood flow, and that these variations are related to the metabolic and vascular variations observed in animals.

66.4

MAGNETIC RESONANCE IMAGING OF INTRACEREBRAL BLOOD IN THE RABBIT. K.H. Taber, Magnetic Resonance Center, Baylor College of Medicine, Houston, TX 77030

The evolution in appearance of experimental intracerebral hemorrhage in the anesthetized rabbit was examined during the hyperacute stage (0-4 hr). Both T2 weighted spin echo (SE) and gradient echo (GE) magnetic resonance images (MRI) were acquired on a Bruker BioSpec 2.35T magnet system before and after intracerebral injection of either normal or defibrinated blood. Following imaging subjects were sacrificed by perfusion-fixation and the brain removed for correlative histology.

The signal intensity of injected arterial blood was always hypointense in GE images. Initially, the appearance was mixed on the SE images. Areas that were isointense to brain at 15 min became hypointense by 2-4 hours. Defibrinated blood was initially isointense on both GE and SE MRI. Small areas of hypointensity developed by 2-4 hours. The lesion size was always larger on the GE images than on the SE images.

The respective appearances of normal and defibrinated blood on SE and GE MRI indicates that the clotting contributes significantly to the hypointense appearance.

66.5

THREE DIMENSIONAL RECONSTRUCTION OF ASTROGLIOSIS IN THE PONS FOLLOWING UNILATERAL LESION OF THE INFERIOR OLIVE

K. Ito*, R.D. Skinner, M. Morrison-Bogorad+, E. Powell, and W.S.T. Griffin. Depts. Anatomy and Pediatrics, UAMS, Little Rock, AR 72205; +Dept. Neurology, UTSWMC, Dallas, TX 75235

Glial responses to lesions of cerebrum, using a variety of techniques,including needle insertion, electrocoagulation, irradiation, or chemical substances, have been well studied. To examine the issue of local glial responses to discreet lesions of the pons, a ventral approach, using an electrolytic technique, was taken to avoid injury of the dorsal pons and involvement of cells in locations distant from the lesion side. The lesion was confined to the pyramid and regions of the inferior olivary nuclei on the right side. Survival for 24 hours post-lesion predicted greater than 90% long term survival. The astrocytic response to the lesion was reconstructed with a Biographic image analysis system. Three dimensional reconstruction revealed that astrocytes in the pons responded in both temporal and spatial modes to this unilaterally placed electrolytic lesion. The number and morphology of astrocytes were analyzed at 2, 4, 6, 10, and 24 days post-lesion (dpl) using a specific antibody that recognizes glial fibrillary acidic protein (GFAP). Reactive astrocytes, having eccentrically placed nuclei and elongated thick processes, were detected in the pons at each day examined, with the number always greater ipsilateral to the lesion. At 2 dpl, some reactive astrocytes, but most were still immature. Reactive astrocytes were most numerous and hypertrophic at 4 dpl. At and after 6 dpl, reactive astrocytes diminished in size and number. Supported by HD 14886.

66.6

QUANTITATIVE CHANGES IN MIDBRAIN AXONAL MORPHOLOGY CORRELATES WITH DEGREE OF HEAD INJURY. M.R. Park, J.B. Schweitzer, D.H. Hilton*, J.T. Robertson*. Depts. Pathology, Anatomy & Neurobiology, and Neurosurgery, Univ. Tenn., Memphis, College of Medicine, Memphis, TN 38163.

We have used cat fluid percussion injury, a well-established model of the clinicopathologic syndrome of diffuse axonal injury (DAI), to produce and calibrate clinically reproducible head injury at mild, moderate, and severe levels. We have developed a cat coma scale, verified the EEG changes that have been reported with traumatic brain injury, and quantitatively analyzed changes in axonal morphology.

Although much of the clinical literature has focused on the retraction ball as the microscopic marker of DAI, we and others have noted that axonal varicosity short of the formation of retraction balls are a more wide-spread abnormality in head trauma. Variability within individual axonal profiles has been measured and used to quantitate the degree of axonal damage at short time periods (10 h) after injury. Both paraffin- and plastic-embedded tissue sectioned and stained with silver stains, osmium, or toluidine blue have been used to allow evaluation of longitudinally cut axons at the light-microscopic level. White matter tracts known to be implicated in the syndrome of DAI have been evaluated. These brain areas are sampled at standard levels and video images of the material captured for morphometric analysis. We have used the coefficient of variation (the standard deviation divided by the mean x 100—a measure of the dispersion of observed values around the mean) to quantitate axonal varicosity. For example, 2900 measurements of over 200 axons in the medial longitudinal fasciculus showed mean CVs of 23%, 28%, and 35% in uninjured, moderately, and severely percussed subjects, respectively. Statistical analysis of this variability in intra-axonal diameter shows a clear and significant relationship with the severity of injury. These quantitative measurements may allow a more precise definition of the anatomic locus of clinically significant lesions.

66.7

ACUTE EFFECTS OF AXOTOMY ON THE ULTRASTRUCTURE OF CULTURED NEURONS OF THE GASTROPOD MOLLUSK, *Helisoma trivolvis*. D.G.Emery. Dept of Zoology and Genetics, Iowa State University, Ames, IA 50011.

While neurons of many arthropods and annelids can withstand axotomy injury, there is little direct evidence on the vulnerability of molluscan neurons. Neurons P1 and P5, with a length of the single native axon attached, were removed from pedal ganglia of *Helisoma* and placed in cell culture. After 24 hrs in culture, by which time the axon had developed a growth cone, the axon was transected with a glass microneedle. Viability 24 hrs. later was determined by dye exclusion. Some neurons were fixed within minutes of injury and processed for transmission electron microscopy. Neuronal death after axotomy occurred at a frequency similar to that reported for mammalian neurons in culture. Injured mammalian neurons exhibit swollen mitochondria, dilated smooth ER (SER) and Golgi bodies, and disrupted microtubules, thought to be responses to ion influx through the lesion (Emery et al., 1987, Exp. Brain Res. 67:41-51). Unlike mammalian neurons, the axons of *Helisoma* neurons contain both granular ER and SER. The mitochondria are quite small (0.1-0.2μm by $\leq 1\mu$m). Within 10 minutes after axotomy dilated SER was seen in the peripheral cytoplasm of the axon stump and the perikaryon, but the mitochondria and Golgi bodies showed no effects. There also was little disruption of the microtubules in the axon stump. This may indicate a difference, compared to mammals, in the calcium homeostatic mechanisms of neurons of *Helisoma* or in the permeability of the membranes of organelles to cations. The mechanism of neuronal death after injury, in the absence of overt mitochondrial pathology, may also differ from that of mammalian neurons.

66.8

IN-VIVO LONG-TERM POTENTIATION FOLLOWING TRAUMATIC BRAIN INJURY. M.H. Ray, S.J. Goldberg, R.J. Hamm, and R.L. Hayes. Depts. of Psychology, Rehabilitation Medicine, Anatomy, and Neurosurgery, Virginia Commonwealth U., Med. Coll. of Va., Richmond, VA 23298.

We have systematically examined the effects of moderate traumatic brain injury (TBI) on the development of in-vivo long-term potentiation (LTP) in the Schaeffer collateral/CA1 pathway of the rat. These studies provide direct electrophysiological assessments of synaptic transmission in the hippocampus produced by injury. Previous research has indicated that the hippocampus, a critical area for memory, is selectively vulnerable to damage following (TBI).

In-vivo LTP development was assessed two hours following TBI. Population spikes, a measurement of summated unit spikes, were measured in the CA1 pyramidal layer in response to Schaeffer collateral stimulation. Two baseline input/output curves were obtained prior to high frequency stimulation (HFS) and repeated at 10, 30, 60, and 90 minutes following the HFS. LTP development was assessed as enhancement of population spike amplitude following HFS. LTP development was significantly suppressed in the injured group (n=8) compared to the control group (n=6) at 10 minutes ($F_{(1,12)}=5.69, p<.05$), 30 minutes ($F_{(1,12)}=10.82, p<.05$), and 60 minutes ($F_{(1,12)}=11.86, p<.05$), but not at 90 minutes following HFS.

The hippocampus is considered to be a critical brain area for memory in humans and other species. In addition, LTP is proposed to be an electrophysiological index of memory function. The findings from these experiments suggest that damage to the hippocampus may contribute to the memory deficits that accompany TBI.

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66.9

THE EFFECT OF AGING ON THE LONG-TERM DEFICITS FOLLOWING TRAUMATIC BRAIN INJURY. R. J. Hamm, D. M. Gbadebo, B. G. Lyeth, L. W. Jenkins, and R. L. Hayes. Depts. of Psychology and Neurosurgery, Medical College of Virginia, Richmond, VA 23298.

Age is one of the most important predictors of outcome following human traumatic brain injury (TBI). This study employs fluid percussion brain injury to investigate the effects of aging on long-term behavioral outcome following experimental TBI in rats. Three-month-old (n=8) and 20-month-old (n=11) rats were injured at a low level (1.7-1.8 atm) of fluid percussion brain injury. Body weight and behavioral outcome (beam balance and beam walking) were assessed for 5 days following injury. Injury did not produce significant weight loss in either age group. At the low level of injury used in this study, the 3-month-old rats did not demonstrate any significant deficits on the behavioral measures. The 20-month-old rats displayed significant beam-balance deficits on each of the 5 postinjury test days. On the beam-walking task, the 20-month-old rats had significant deficits for the first 3 postinjury days. These data demonstrate that aging is associated with increased behavioral deficits following TBI, in the absence of weight loss differences between age groups. Since the rat model of fluid percussion TBI reproduces the age-related increase in morbidity observed in cases of human TBI, the fluid percussion model should be useful for studying mechanisms responsible for the age-related increase in vulnerability to brain injury.

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66.10

METHYLPHENIDATE FACILITATES LOCOMOTOR RECOVERY AFTER UNILATERAL SENSORIMOTOR CORTEX ABLATION. A.E. Kline, M.J. Chen, D.Y. Tso-Olivas, & D.M. Feeney. Depts. of Psychology and Physiology, University of New Mexico, Albuquerque, NM 87131.

A single administration of d-amphetamine (AMPH) or its analogues phentermine and phenylpropanolamine given 24 hr after sensorimotor cortex (SMCX) ablation in the rat facilitates recovery of BW when combined with symptom-relevant experience (SRE) (CRC Critical Reviews in Neurobiol. 3(2):135-197, 1987). However, a single administration of methylphenidate (MPH) (3-15mg/kg), also an AMPH analogue, did not facilitate recovery (Neurosci. Abstr. 14:1152, 1988). Perhaps because the study employed only 3 BW trials during the period of drug action there may have been insufficient SRE for MPH to exert its effects on BW. Multiple MPH doses or increased SRE after a single dose may be required for MPH to promote recovery. At 24 hr after SMCX ablation, MPH (10 mg/kg) was given daily for 3 days using the standard BW protocol, which consists of BW trials at 1,2,3, and 6 hr post-injection. This regimen produced a significant and enduring improvement of BW ability after the third injection. A combined total of 9 BW trials were accumulated during the drug action period following the 3 doses. A second study increased SRE after a single administration of MPH by providing a BW trial every 15 min during the drug action period (1-3 hr). At 24 hr after a right SMCX ablation or sham surgery, MPH (10mg/kg) or saline was administered. A significant and enduring facilitation of BW recovery was also produced by this regimen. These studies suggest that the role of SRE during drug action is an important variable for functional recovery. Supported by U.S. Army Grant DAMD17-91-Z-1006 and DHHS 5 SO6GMO8139-17.

66.11

RECOVERY OF REFLEXES AND POSTURAL REACTIONS FOLLOWING A SEVERE TRAUMATIC BRAIN INJURY. S.J. Sullivan, J. Provost*, V. MacPherson*, Y-L. Boulanger*, M. Vanier*, E. Dutil*, A. Forget* and G. Drouin*, Concordia Univ., Hôpital du Sacré Coeur, Univ. de Montréal, Institut de réadaptation de Montréal, Montréal, Québec Canada. H3S 2J4

A severe traumatic brain injury (TBI) results in the temporary suppression of normal CNS activity immediately following the injury. We investigated the pattern of recovery of CNS activity by quantifying (using standardized protocols): the Extensor (ER) and Flexor Withdrawal (FW) reflexes of the lower limbs; Equilibrium Reactions -seated (ERS) and standing (ERST) within 1 month, and, at 3 and 6 months post-injury in 36 TBI (X GSC = 6.58) patients. Positive responses for: ER (79, 93, 95%); FW (64, 89, 92%); ERS (36, 63, 68%) and ERST (19, 48, 59%) demonstrated a hierarchical pattern in their initial recovery (<1 month). Significant (NcNemar χ^2) recovery, relative to the initial values, was observed at 6 months for all variables with FW, ERS and ERST showing meaningful ($p \leq .05$) increases by 3 months. These data indicate an early and systematic recovery of reflexes and postural reactions following a TBI. (Supported by the; SAAQ and FRSQ).

66.12

KETAMINE AND MAGNESIUM ATTENUATE MEMORY LOSS AFTER EXPERIMENTAL BRAIN INJURY. D.H. Smith, K. Okiyama and T.K. McIntosh. Surg. Research Center, Univ. of Conn. Health Center, Farmington CT 06030.

Memory dysfunction is one of the most common features following traumatic brain injury (TBI). The N-methyl-D-aspartate (NMDA) receptor has been shown to be a mediator in both memory function and secondary damage following TBI. We have previously demonstrated that antagonists acting at the NMDA receptor-associated glycine site attenuate post-TBI memory dysfunction. In the present study, we examined the ability of two non-competitive NMDA receptor antagonists ketamine (Ket) and magnesium (Mg^{++}), both individually and together, to attenuate post-TBI memory dysfunction. Male Sprague-Dawley rats (350-400g) were trained in the Morris water maze (MWM) to find a submerged platform (20 trials over 2 days) using external visual cues. 2.5 h after the final trial, animals were anesthetized (sodium pentobarbital 60mg/kg ip) and subjected to lateral fluid percussion (FP) brain injury of moderate severity (2.4 atm). Fifteen min post-injury, the animals randomly received 0.5 ml intravenous administration over 15 min of either Ket (4 mg/kg, n=9), $MgCl_2$ (35 umol, n=9), Ket and $MgCl_2$ (identical doses) or saline (n=9). All animals were tested for memory retention at 42 hours post-injury in the MWM using a video/ computer recording unit. Animals treated with either Ket or Mg^{++} showed highly significant memory preservation compared to saline treated control animals ($p<.01$). Animals treated with combined Ket and Mg^{++} also showed highly significant memory preservation ($p<.01$), however, with no apparent additive effect. These results suggest that the NMDA receptor may play a role in post-traumatic memory dysfunction and that NMDA receptor antagonists may be potentially beneficial in the treatment of this aspect of brain injury. Supported, in part, by NS26818, a VA Merit Review grant 74R and a grant from the Brain Trauma Foundation.

66.13

THE COGNITIVE ENHANCER BMY-21502 IMPROVES SPATIAL LEARNING IN BRAIN INJURED RATS. J.E. Shaw*, D.H. Smith and T.K. McIntosh. Surg. Research Center, Univ. of Conn. Health Center, Farmington CT 06030

Cognitive dysfunction is a common clinical feature of traumatic brain injury (TBI). Few drug treatments, however, have been developed to attenuate this dysfunction. We have previously shown that lateral fluid-percussion (FP) brain injury produces a profound retrograde **memory** deficit in rats. In the present study, we characterized changes in **learning** ability following FP brain injury and examined the potential modulatory effects of the cognitive enhancer, BMY-21502, on post-traumatic learning. Male Sprague-Dawley rats (340-400 g) were subjected to lateral FP brain injury of moderate severity (2.4 atm) or sham surgery (no injury). One week following brain injury, animals were trained in the Morris water maze (MWM) to find a submerged platform (20 trials over 2 days) using external visual cues. Latency (time taken to find the platform) was recorded for each trial. BMY-21502 (10mg/kg; s.c.; n=13 injured; n=11 sham) or vehicle (n=12 injured; n=10 sham) was administered 30 min prior to the start of training on both days. A highly significant impairment in learning was observed in untreated injured animals (compared to untreated sham animals, $p<0.001$). Brain injured animals treated with BMY-21502 showed a highly significant improvement in the capacity to learn compared to injured animals treated with vehicle ($p<0.005$). BMY-21502 did not produce significant changes in learning capacity in sham animals. These results suggest that a dysfunction in learning ability following TBI may be attenuated with the use of BMY-21502. Supported, in part, by Bristol-Myers Squibb and a grant from the Brain Trauma Foundation.

66.14

MUSCARINIC RECEPTOR BLOCKADE REVEALS COVERT SPATIAL MEMORY DEFICITS FOLLOWING MILD TRAUMATIC BRAIN INJURY (TBI). C.E. Dixon, T. Richardson*, W.C. Taft and R.L. Hayes. Division of Neurosurgery, University of Texas Health Science Center at Houston, Houston, TX 77030 and Division of Neurosurgery, Med. College of Va. Richmond, VA 23298.

New areas of research indicate that TBI produces receptor mediated neurochemical alterations resulting in pathological changes in neuronal function not necessarily associated with cell death (Lyeth et al, Brain Res.526:249-258,1990). Hippocampal CA1 neurons appear to be the most sensitive to these neurochemical alterations. The purpose of this study was to determine whether the cholinergic system of injured rats is chronically impaired following mild TBI. We used the Morris water maze, a spatial memory task dependent on cholinergic hippocampal function to assess cognitive performance. Rats (N=8) were placed onto a foam block that was 3 cm thick. A pneumatic impactor was used to make a direct central impact (7 meters/sec) to the exposed skull under 2% isoflurane anesthesia. Water maze testing started on Day 8 post-injury. Rats were given 4 trials per day for 5 consecutive days. For each trial, latency to find a hidden platform was timed. On Day 13, rats were injected 15 min prior to being tested with a dose of scopolamine (1 mg/kg) that did not adversely affect performance in normal rats. Results showed that this magnitude of head impact acceleration produced no overt motor or maze deficits. However, scopolamine significantly increased the latency to find the platform in the injured group, but not the uninjured group. These results suggest that even mild TBI can produce increased sensitivity to disruption of cholinergically mediated memory function. These data provide addition support for the hypothesis that TBI produces pathological alterations in receptor coupled functions. These alterations may persist even in the absence of overt behavioral deficits.
Supported by CDC 303547, NS21458.

66.15

BEHAVIORAL DEFICITS FOLLOWING CORTICAL CONTUSION IN THE RAT. T. Carbary*, P. Kraemer* and S.W. Scheff. Depts. Psychology and Anatomy & Neurobiology, Univ. Kentucky, Center on Aging, Lexington, KY 40536-0230.

Little is known about the complex response of the CNS following cortical contusion. Numerous experimental models have employed different methodologies to produce brain injury resulting in a wide spectrum of clinically relevant injury. To date no single experimental model produces a complete picture of closed head injury identical to that observed in humans. An electronic controlled pneumatic impact (ECPI) model has been developed which manifests neuropathology similar to the human condition. To further develop this ECPI animal model we compared neuropathological and behavioral results after injury.

Rats were randomly assigned to one of four different treatment groups: Impact (IP), Sham, Subdural hematoma (SDH) and Anesthesia control (AC). Prior to surgical manipulation, all animals were trained and behaviorally tested for associative memory using a Pavlovian conditioned fear task. IP animals with a contusion over the posterior portion of the cortex showed a marked deficit within 24h after the injury. Sham animals, which underwent surgical manipulations without impact and SDH animals, which were subjected to a rupture of a dorsal cortical vein creating a subdural hematoma, were not significantly different from AC animals. Behavioral deficits correlated positively with the extent of neuropathology as determined from histological evaluation of cortical damage. The ECPI model appears to be extremely sensitive to both behavioral and morphological aspects of cortical contusion.

66.16

RETENTION OF AN 8-ARM MAZE TASK OVER THREE RECOVERY INTERVALS FOLLOWING PREFRONTAL CORTEX LESIONS. W.L.Isaac, J.N.Freeman*, T.L.Long* and M.Fritts*. Dept. of Psychology, East Tenn. State Univ., Johnson City, TN 37614.

This study attempted to assess the influence of time from lesion of either medial or lateral frontal cortex on recovery of function. Gavin & Isaac (1986) and Isaac, Nonneman and Scheff (in prep.) found temporal limitations to recovery. Fifty male Long-Evans derived rats (30 days old) were used. They were acclimated to the test setting. The rats were then trained to visit each arm of the 8-arm maze once. A criterion of no more than 1 error/day for 2 consecutive days was set to define learning. Upon criterion, each rat received one of the lesions listed above. Testing resumed after 1 of 3 retention intervals: 12, 18 or 24 days and continued for 15 days on the maze. The lesions were histologically assessed. A repeated measures ANOVA shows a Lesion effect, a Blocks effect and a Lesion x Blocks interaction. A trend toward a retention interval effect exists. This differs from previous research that looked at the occipital and entorhinal cortices. Potential factors responsible for the absence of the recovery interval effect will be addressed.

66.17

NIMODIPINE-ENHANCED RELEARNING OF 8-ARM MAZE AFTER HIPPOCAMPAL LESIONS. S. Finger, C. Nelson* and Johny Bawa*. Psych. Dep't., Washington Univ., St. Louis, MO 63130.

Rats trained in an 8-arm radial maze were given electrolytic lesions of the dorsal hippocampus or sham operations. Within 24 hours of surgery, approximately half of the rats in each groups began 14 daily oral treatments with the central calcium channel blocker, nimodipine. The untreated rats that received the larger hippocampal lesions did not relearn the maze 2-1/2 times the preoperative mean. The untreated rats that received the smaller lesions showed milder deficits. Nimodipine significantly improved the maze scores of the animals with the smaller lesions, but not the scores of the rats with large hippocampal lesions or sham operations. It was unclear whether nimodipine led to the sparing of more cells in the hippocampal region, or whether spared-but-affected cells were returning to normal modes of functioning more rapidly. These findings suggest that spared hippocampus was mediating behavior, and extend our previous findings showing that nimodipine can enhance recovery of function on higher cognitive tasks after hippocampal damage.

66.18

ASTROCYTIC PROCESS REMODELLING FOLLOWING DAMAGE IN THE RAT CEREBRAL CORTEX. **K.R. Isaacs, N. Hawrylak, N. Ghia*, and W.T. Greenough. Neuroscience Program, Depts. of Psych. and Cell & Struct. Biol., Beckman Institute, Univ. of Illinois, Urbana, IL 61801.**

Astrocytes in the brain exhibit morphological changes in response to mechanical damage and degeneration. These changes can impede host-transplant integration. In this study, immunohistochemical and quantitative anatomical techniques were employed to examine the amount and preferred orientation of astrocytic processes following stab wounds in the cerebral cortex.

A 23 gauge needle was lowered 3 mm into the rat cortex in order to induce a wound. The brains were processed 48h (n=2), 96h (n=3), 10 days (n=4) and 20 days (n=4) post lesion (dpl) for GFAP immunohistochemistry. GFAP positive cells with clearly recognizable soma and processes located 150 to 350 microns distant from the edge of the wound were drawn from a Zeiss microscope interfaced to an IBM computer. Control cells were drawn in the contralateral hemisphere. The process field was divided in half through the center of the soma parallel to the needle track. The hemifield oriented towards the wound and that oriented away from the wound were analyzed separately.

The percent of process length was significantly greater in the hemifield proximal to the wound compared to 1/2 of the total for control cells at both 10 and 20 dpl ($p < .01$). At 48h and 96h post lesion the trend was similar but did not reach statistical significance. Within cells at the wound site the hemifield towards the wound had more astrocytic process than that away from the wound at 96h, 10dpl and 20 dpl ($p< .05$). The overall pattern of data suggests an initial general process hypertrophy of astrocytes in the wound zone, followed by retraction of processes oriented away from the wound. Supported by ONR N00014-89-J1556

66.19

TEMPORAL EXPRESSION OF GFAP IMMUNOHISTOCHEMICAL REACTIVITY IN THE SUPERIOR COLLICULUS OF ADULT RATS FOLLOWING MONOCULAR ENUCLEATION. C. Collins, N. Hawrylak and W.T. Greenough. Beckman Inst., Neurosci. Prog., Dept. Psych. and Cell & Struct. Biol., Univ. of Illinois, Urbana 61801.

Age related changes in astrocytic responses to damage or degeneration in the nervous system may play an important role in plastic phenomena. Reactive gliosis is characterized by hypertrophy of astrocytic perikarya and processes, an increase in the number of intracellular filaments and increased immunoreactivity of glial fibrillary acidic protein (GFAP). Light microscopy was utilized to study the the temporal expression of the astrocytic response to enucleation in the superior colliculus (SC) of young and middle age adult rats. Animals in the two age groups, 70-90 days and 13-17 months were unilaterally enucleated and allowed to survive 1, 2, 3, 5, 7 or 9 days. Animals were perfused intracardially with 4% paraformaldehyde and processed for GFAP immunohistochemistry.

In both age groups, at one day post-enucleation, astrocytic labelling is detectable in both colliculi. In the colliculus contralateral to the enucleation a dense GFAP reactivity was observed throughout the mediolateral and dorsoventral regions, whereas the response in the ipsilateral superior colliculus appears to be distributed in patches. A similar, somewhat less intense patchy pattern of immunoreactivity was observed in non-lesioned animals. The initial response in both age groups is noticeably concentrated in the more lateral regions of the colliculi. More detailed studies of differences in the lateral geniculate nucleus (LGN) are currently in progress.

Comparison of the age related astrocytic response demonstrates that older rats reach a maximal response (day 3) in the contralateral colliculus before young adult rats (day 5). Supported by NIA AG10154.

EPILEPSY: BASIC MECHANISMS I

67.1

NEUROTROPHIC FACTORS ACCUMULATE IN THE RAT HIPPOCAMPUS FOLLOWING KAINATE-INDUCED SEIZURES. D.H. Lowenstein[1], S. Seren[2], M. Manthorpe[3] and F.M. Longo[1], Dept. of Neurology, UCSF, San Francisco, CA(1), FIDIA Res. Lab, Padua, Italy (2), and School of Med., UCSD, La Jolla, CA(3).

Synaptic reorganization occurs in the hippocampus following various forms of seizure activity and may play a role in epileptogenesis. To characterize further the molecular basis of these changes, we measured neurotrophic activity in individual rat hippocampi harvested 1hr, 12hrs, or 7d following IP injection of 12mg/kg kainic acid (KA) and compared this to controls receiving IP saline. Serial dilutions of hippocampal extract were added to cultures of either embryologic day 8 chick dorsal root ganglia or ciliary ganglia neurons, and neuronal survival at 24hrs was measured by a colorimetric assay in which viable neurons convert a tetrazolium derivative to a blue formazan product. The extract dilution supporting 50% of the maximum NGF or CNTF-induced neuronal survival was used to quantitate the trophic units (TU)/ml of trophic activity. Hippocampi from 1hr and 12hr KA animals had no significant difference in neurotrophic activity compared to controls. However, 7d samples had a 2.9-fold increase in NGF-like activity compared to controls (57 ± 18 TU/ml, n=8 vs. 20 ± 10 TU/ml, n=4, mean ± S.D.) and a 2.3 fold increase in CNTF-like activity (101 ± 16 TU/ml, n=8 vs. 45 ± 4 TU/ml, n=4).

These results suggest that: i) one or more neurotrophic factors accumulate in the rat hippocampus following KA-induced seizures, ii) since NGF does not support ciliary neurons in this assay, trophic factor(s) other than or in addition to NGF are present, and iii) accumulation of substantial amounts of these factors occurs after a delay of at least 12 hrs.

Supported by NS 01424 (DHL) and United Cerebral Palsy (FML)

67.2

LOCAL GENERATION OF BURST DISCHARGES IN DENTATE GRANULE CELLS ASSOCIATED WITH NMDA MEDIATED TRANSMISSION AND MOSSY FIBER SPROUTING *G.Golarai, T.Sutula Neurosci. Training Prog., Univ. of Wisconsin, Madison, WI 53792*

Kindling induces long-lasting enhancement of the NMDA component of the EPSP and mossy fiber (MF) sprouting in rat dentate granule cells (DGC). To investigate the role of these alterations in generation of epileptic activity in dentate gyrus (DG), intra- & extracellular recordings were obtained in DG of slices from naive and kindled rats. In both groups perforant path (PP) and hilar (H) shocks evoked an EPSP and a single spike. With block of $GABA_a$ inhibition in kindled DGCs, PP and H shocks evoked APV sensitive population burst discharges (37/38). Normal DGCs did not burst with $GABA_a$ blockade (0/6), but developed APV sensitive population bursts when the NMDA component of the EPSP was enhanced in 0 Mg^{+2}, which reversed to normal upon return to normal Mg^{+2} medium (9/9). After enhancement of the NMDA component of the EPSP, with block of $GABA_a$ inhibition in normal Mg^{+2}, PP and H shocks evoked population bursts in the normal DGCs. Population bursts in normal and kindled slices were recorded in DG isolated from entorhinal cortex and hippocampus. Local circuitry in the normal DG is therefore sufficient to generate population bursts if the NMDA component of the EPSP has been enhanced. If kindling-induced MF sprouting establishes recurrent excitatory circuits, this could provide additional local excitatory drive and contribute to the generation of epileptic activity. It appears that a complex interaction of enhanced excitatory drive, diminished inhibition, and circuit reorganization plays a role in generation of epileptic activity in the DG.

67.3

FACTORS INFLUENCING GRANULE CELL COLLATERAL SPROUTING IN ORGANOTYPIC CULTURES OF MOUSE HIPPOCAMPUS. B.W. Coltman*, E. Earley*, L. Alworth* and C. F. Ide. Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

Collateral sprouting of dentate granule cell axons correlates with the development of epileptiform neural activity in rodents. Organotypic slice culture of rodent hippocampal tissue is a model system for the controlled study of sprouting in vitro. Organotypic rollertube cultures were prepared from hippocampal slices derived from postnatal day 7 (P7) mice. Timm's staining was utilized to assay the degree of collateral sprouting by granule cell axons. Little sprouting was present one week post-culture; by two weeks, considerable sprouting was apparent; and at three weeks, intense sprouting obscured the dentate granule cell layer. A septal to temporal gradient in the degree of sprouting was observed in a study including 8 hippocampi (59 viable cultures). Eighty-two percent of cultures from the septal region of the hippocampus showed heavy to medium sprouting compared to 71% from mid-hippocampal regions and 43% from temporal hippocampal regions . Further studies are underway which use co-cultures to assay the influence of afferent innervation on collateral sprouting of dentate granule cells.

67.4

HIPPOCAMPAL MOSSY FIBER OUTGROWTH IN A MUTANT WITH INHERITED SPIKE WAVE SEIZURES *X. Qiao, R.S. Chafetz* and J.L. Noebels.* Developmental Neurogenetics Laboratory, Section of Neurophysiology, Department of Neurology and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Zinc-containing mossy fibers in the hippocampus were examined in two inherited spike-wave seizure mutants, stargazer (*stg*, Chr 15) and tottering (*tg*, Chr 8), using Timm's sulfide silver and selenium staining methods. We found increased staining in the molecular and granule cell body layers of the dentate gyrus in both adult *stg* and *tg* temporal hippocampus. The increase was far more striking in *stg* than in *tg*, corresponding to the two-fold greater seizure frequency in the *stg* mutant. Optical density measurements of staining in control (+/+) and *stg* showed no quantitative difference in the hilar region, but a mean 48% increase in the mutant granule cell body and molecular layers. In addition, we noticed an unusual pattern of diffuse fiber staining within the CA3 pyramidal cell body layer. Dicarbocyanine (DiI) fluorescent dye labeling of the mossy fiber system at the hilus demonstrated the presence of individual mossy fibers in both the molecular layer of the dentate gyrus and the CA3 intrapyramidal layer in *stg*, consistent with the abnormal zinc staining pattern. A developmental study with paired *stg/stg* and +/+ mice (n= 3-5 pairs) at 5 different ages (2, 4, 8, 16 and 24 weeks) indicates that epileptic discharges begin no sooner than postnatal days 17-18, whereas the abnormal axonal outgrowth does not appear before 2 months of age. These and other data suggest that inherited 6 sec^{-1} spike-wave discharges, like other more severe convulsive patterns of seizure activity, are sufficient to cause abnormal axon reorganization in hippocampus.

67.5

HIPPOCAMPAL CA3 PYRAMIDAL CELLS OF THE EPILEPTIC MUTANT MOUSE *STARGAZER* DISPLAY A DISTINCTIVE GENE-LINKED HYPEREXCITABILITY. *U. Namgung* and J.L. Noebels.* Developmental Neurogenetics Laboratory, Department of Neurology and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Hippocampal network excitability was examined *in vitro* in a single locus mutant mouse with generalized epilepsy, the stargazer(*stg/stg*, Chr15), using extracellular recordings of spontaneous interictal burst discharges in CA3 pyramidal cells activated by convulsants. Burst discharges in both control(+/+) and *stg/stg* genotypes appeared 5-10 min after elevating $[K^+]_o$ to 10 mM. Mean burst durations did not significantly differ between +/+ and *stg/stg* hippocampal slices exposed to bath applied convulsants, but mean frequency was significantly higher in *stg/stg* slices than in +/+ slices [46% increase in 10 mM $[K^+]_o$ (.35±.02 [+/+, n=109] vs .51±.03 Hz [*stg*, n=78] P<.01), 33% increase in 10 mM $[K^+]_o$+10 μM 4-AP (.52±.05 [n=20] vs .69±.04 Hz [n=16]; P<.05) and 85% increase in 10 mM $[K^+]_o$+10 μM PTX solutions (.27±.01 [n=10] vs .50±.08 [n=12]; P<.05)]. Addition of 10 μM 4-AP to 3 mM $[K^+]_o$ solutions caused a 163% increase in burst rate for *stg/stg* slices (.08±.02 [n=7] vs .21±.05 Hz [n=6]; P<.05), but adding 10 μM PTX to 3 mM $[K^+]_o$ solutions did not produce epileptic bursts in either genotype. These results demonstrate a clear network excitability defect in the isolated *stg* hippocampus that alters convulsant-activated burst frequency but not burst duration, a pattern exactly opposite to that observed in the tottering mutant(*tg/tg*, Chr 8)(Noebels and Rutecki, 1990, Brain Res. 524, 225). Since generalized spike-wave seizures in *stg* are identical to those seen in the hypernoradrenergic *tg* mutant, these data suggest that the two mutations initiate generalized epilepsy by altering two distinct neuronal excitability control mechanisms.

67.6

EVIDENCE FOR ENHANCED $GABA_B$-MEDIATED INHIBITION IN HIPPOCAMPAL SLICES FROM LETHARGIC MICE, A GENETIC MODEL OF ABSENCE EPILEPSY. S. Clark, R. A. Morrisett, W. A. Wilson, D. A. Hosford. Depts. of Pharmacology and Medicine (Neurology), Duke University & the Veterans Administration Medical Centers, Durham, NC 27705

The **lethargic** mouse is a genetic model of absence epilepsy which exhibits 6 Hz spike-wave discharges that are blocked by ethosuximide and by the $GABA_B$ antagonist CGP 35348 (Hosford et al., Soc. Neurosci., Abst. 17, 1991 & Am. Epilepsy Soc., 1991). Since it has been hypothesized that $GABA_B$ currents may play a role in the generation of bursts during absence seizures (Crunelli, TINS 14, 1991), we investigated the strength of $GABA_B$-mediated inhibition of synaptic potentials in brain slices from **lethargic** and age-matched control mice.

We utilized paired (200 ms) stimulus protocols and the 6,7-dinitroquinoxaline-2,3-dione/picrotoxin (DNQX/PTX) model (10 μM each) to pharmacologically isolate NMDA receptor-mediated population synaptic potentials as an indirect measure of post-synaptic $GABA_B$-mediated responses (Morrisett et al., Neuroscience, 1991). At 200 ms, the second stimuli occurs at the peak of the $GABA_B$ IPSP. The resulting responses were recorded in str. radiatum and in str. pyramidale of area CA1 of the hippocampus. In slices from **lethargic** mice, $GABA_B$-mediated inhibition of the NMDA EPSP was enhanced and was maximal at 200 ms. In control mice, this paired pulse inhibition of the NMDA EPSP was not readily apparent.

We conclude that enhanced $GABA_B$-mediated responses exist in the hippocampus of a genetic mouse model of absence seizures.

67.7

THE LETHARGIC MOUSE: A GENETIC MODEL OF ABSENCE EPILEPSY. D.A. Hosford, S. Clark, W.A. Wilson, V. MacMillan* & Z. Cao*. Epilepsy Research Lab, Duke & V.A. Med. Ctrs., Durham, NC 27705.

The **lethargic** mouse, a mutant with a single-gene defect, expresses spontaneous seizures and ataxic gait (Dickie, Mouse News Lett., 1964). We characterized the behavioral, electrographic and anticonvulsant profiles of seizures in this model.

Using stocks from Jackson Labs we colonized congenically wild mice (+/+: all progeny of C57Bl/6JEi females X C3H/HeSnJ males) and **lethargic** mice (lh/lh: 25% of progeny of lh/+ X lh/+). Bipolar recording electrodes were stereotaxically implanted bilaterally into 7-week mice (n = 32). Over 80% of lh/lh but no +/+ or lh/+ mice exhibited bilaterally synchronous bursts (mean duration 1.4 sec; range 0.4 - 3.0 sec) of 6 Hz spike-wave discharges with a mean frequency of 127/hr (range 43 - 239/hr). Bursts were accompanied by behavioral arrest but no clonic activity, similar to early results of J. Noebels (Adv. Neurol. 44, 1986). Ethosuximide blocked seizures in a dose-dependent manner that was maximal (99%) at 400 mg/kg i.p. (n = 6 mice; mean serum level = 47 μg/ml by HPLC). Phenytoin had no effect on seizure frequency (n = 6 mice) even at 40 mg/kg i.p. (mean serum level = 21 μg/ml). Taken together, these data strongly argue that seizures in **lethargic** mice resemble those of absence epilepsy.

Hypotheses that $GABA_B$ receptors contribute to burst-firing in absence epilepsy (Crunelli, TINS 14, 1991) led us to test a $GABA_B$ antagonist on seizure frequency. In preliminary studies, CGP 35348 (100 mg/kg i.p.) totally suppressed seizures for more than 60 min after a brief period of torpor. These findings, similar to results from rat models of absence epilepsy (O.C. Snead III, personal communication), suggest that $GABA_B$ receptors contribute to seizures in **lethargic** mice. The enhanced $GABA_B$ drive observed in **lethargic** brain slices strengthens this possibility (S. Clark, Soc. Neurosci. Abst. 17, 1991). The **lethargic** mouse is a powerful model in which to examine pathologic mechanisms of absence epilepsy.

67.8

C-FOS PROTEIN-LIKE IMMUNOREACTIVITY IS DEPENDENT ON AGE AND METHOD OF SEIZURE INDUCTION. F.E. Jensen, I.R. Firkusny*, and G.D. Mower. Dept. of Neurology, Children's Hosp. and Harvard Med. Sch., Boston, MA 02115.

Administration of the convulsant, pentylenetetrazol (PTZ), results in widespread c-fos protein-like immunoreactivity in the adult rat. We compared fos immunoreactivity in adult rats to 10 d.o. (P10) rats at 2 and 4 hours after PTZ induced generalized convulsions (GC). At both ages, the amygdala, hypothalamus, and pyriform cortex appeared most sensitive to seizures, staining earlier and after less severe seizures than dentate gyrus (DG) and hippocampus (HI). Fos immunoreactivity occurred earlier in adults (2hrs) than in the P10 rats (4hrs). The distribution of immunoreactive cells was generally similar between the ages, except that cortical staining was present in layer II in the adults and layer VI of the P10 rats.

The response to another convulsant, flurothyl, was tested at 2, 4, and 6 hours after seizures (GC) in the two age groups. At both ages, the distribution was similar to that induced by PTZ. However, the time course was shorter than PTZ in the adults, with staining present at 2 hours and absent by 4 and 6 hours. For the P10 rats, staining was most prominent at 2 hours, but persisted in layer VI of the cortex at both 4 and 6 hours.

Because we have previously shown that acute global hypoxia (3%O_2) results in seizures in young rats (P10) but not in adults, we examined fos immunoreactivity at 2, 4 and 6 hours after hypoxia induced seizures in P10 rats. The distribution and time course was different from both PTZ and flurothyl. No immunoreactivity was present until 4 hours after hypoxia, and the DG, HI, and layer II of cortex were highly stained, with only moderate staining of the amygdala and pyriform cortex. These data suggest that there are differences in the pathways activated by PTZ, flurothyl, and hypoxia induced seizures, and that these are also dependent on age.

67.9

TRIAZOLOBENZODIAZEPINE-BASED ANTAGONISM OF PLATELET-ACTIVATING FACTOR AND INDUCTION OF *fos* EXPRESSION IN HUMAN SH-SY5Y NEUROBLASTOMA CELLS. **J.P. Doucet,*[1,2] V.L. Marcheselli,[1] & N.G. Bazan.[1,2] [1]LSU Eye Center & Neuroscience Center; [2]Department of Biochemistry and Molecular Biology, LSU Medical Ctr., New Orleans, LA 70112.**

The anxiolytic triazolobenzodiazepines alprazolam and triazolam are potent antagonists of platelet-activating factor (PAF) activity (*Science* 226:1454,1984). Two triazolobenzodiazepine-based PAF antagonists, BN50726 and BN50739, promote metabolic recovery of postischemic canine brain (*J Neurochem* 56:311,1991). We previously showed that BN50726 and BN50739 displace PAF from high-affinity, intracellular binding sites in rat brain (*J Biol Chem* 260:9140,1990). The related antagonist BN50730 has the highest affinity for these sites (*Trans Am Soc Neurochem* 22:187,1991), and BN50730 decreases hippocampal and cortical *fos* expression evoked by a single electroconvulsive shock (*Soc Neurosci Abs* 16:629,1990), suggesting that PAF may mediate the primary genomic response to seizure. In SH-SY5Y cells, PAF stimulation of the *fos* promoter region requires the Ca^{2+}/cAMP-response element (*J Neurosci Res* 24:558,1989), and the corresponding induction of *fos* expression is abolished by equimolar concentrations of the ginkgolide PAF antagonist BN52021. Here we show that, in serum-deprived SH-SY5Y culture, a 30-min, 1 μM-pretreatment with either triazolam or BN50730 is insufficient to suppress equimolar PAF-evoked *fos* expression. In addition, induction of *fos* by Ca^{2+}-dependent, K^+ depolarization of SH-SY5Y cells is likewise resistant to triazolobenzodiazepine-based PAF antagonism. These results are consistent with the insufficiency of triazolobenzodiazepines to antagonize PAF-evoked Ca^{2+} influx in neural hybrid cells (*Science* 240:1792,1988) and suggest exclusivity of triazolobenzodiazepine-based PAF antagonism and expression of intracellular PAF-binding sites for mature, nonproliferating neuronal cells. (Supported by NINDS NS23002).

67.10

STIMULATION OF HIPPOCAMPAL CYCLOPHILIN EXPRESSION FOLLOWING LIMBIC SEIZURES. **G.L. Yount, C.M. Gall[1] and J.D. White, Div. Endocrinology, Dept. Medicine and Dept. Neurobiology and Behavior, SUNY, Stony Brook, NY 11794 and [1] Dept. Anatomy and Neurobiology, Univ. Cal., Irvine, CA 92717**

Previous studies have shown that generalized limbic seizures, induced by unilateral focal electrolytic lesion of the hippocampal dentate gyrus hilus, lead to dramatically increased expression of neuropeptide (enkephalin, dynorphin, neuropeptide Y), transcriptional regulatory protein (c-*fos*, c-*jun*, NGFIA) and growth factor (NGF, BDNF) mRNAs by dentate gyrus granule cells. In the present study, the influence of hilus lesion (HL)-induced seizures on the abundance of mRNA coding for cyclophilin in rat hippocampus was analyzed. Cyclophilin (peptidyl- prolyl *cis-trans* isomerase) is found in almost every cell in the body. In the rat brain, cyclophilin mRNA is expressed at comparatively high levels in the cerebral cortex and hippocampus. By nuclease protection analysis a significant increase in cyclophilin mRNA content was observed in the dentate gyrus following HL-induced seizures. The increase began 6 hours post-HL, reached a maximum (2.5-fold) at 12 hours post-HL and returned to control values by 48 hours post-HL. Cyclophilin mRNA levels remain stable in the cerebral cortex throughout the same time course. Thus, increased cyclophilin expression is a component of the complex genomic response of hippocampal neurons to recurrent seizure and may be required for the sequelae of neurochemical events induced in these cells.

(ADAMHA MH00801, NIH NS26748)

67.11

C-FOS INDUCTION AND AUDIOGENIC SEIZURES: MAPPING CELLS INVOLVED IN BOTH INDUCTIVE AND SEIZURE-PRECIPITATING EVENTS. **M.G. Pierson and A.M. Snyder-Keller**. Wadsworth Center for Labs and Research, New York State Dept. Health, Albany, NY 12201.

Rats normally resistant to audiogenic seizures can be made susceptible by exposing them to an intense noise during a critical period during development. We are studying c-fos induction within cells of auditory nuclei in relation to both the inductive noise exposure and the audiogenic seizures that occur in response to subsequent noise exposure. Four hr after noise exposure at 14 days, fos-immunoreactive cells were present in a discrete zone within the central nucleus of the inferior colliculus (IC). At longer intervals after noise exposure, numerous fos-immunoreactive cells were seen in dorsal and external cortices of the IC. These two patterns of c-fos induction could be diffentially altered by pretreating animals with the NMDA antagonist MK-801, which promotes the development of audiogenic seizure susceptibility. Audiogenic seizures that occur in response to a subsequent noise exposure resulted in dense fos immunoreactivity in the IC as well as afferent and efferent auditory nuclei. By restricting either the inductive or the seizure-precipitating noise exposure to one ear, we have been able to demonstrate that widespread c-fos induction occurs only in previously "sensitized" lobes of the IC. The pattern and extent of fos immunoreactivity correlated well with observed seizure behaviors. Fos immunocytochemistry offers a simple anatomical approach towards identifying the sites of epileptogenesis in this model of developmental epilepsy. Supported by the Epilepsy Foundation of America.

67.12

EFFECT OF MK801 ON DISTRIBUTION OF FOS IMMUNOREACTIVITY FOLLOWING STIMULATION OF THE ANGULAR BUNDLE. L. M. GRIMES, C. L. MITCHELL AND M. I. BARNES*. LMIN/NIEHS/NIH, Research Triangle Park, NC 27709

Continuous stimulation of the angular bundle (CSAB) for 45 min. uniformly produces status epilepticus in rats. We previously reported that such stimulation results in widespread induction of fos immunoreactivity (Mitchell et al. Neurosci. Abs., 16:348, 1990). The purpose of the present study was to determine the effect of MK801 on convulsive behavior and expression of fos under CSAB for 45 min. In animals given saline followed in 1 hr. by 45 min. CSAB, fos was expressed in widespread areas of the brain. MK801, 1 mg/kg, given 1 hr. prior to CSAB prevented the status epilepticus associated with 45 min. of CSAB. Also, fos was expressed in several limbic areas not expressed in the saline plus CSAB group. However, whereas fos was expressed in the saline plus CSAB group in the striatum and brain regions interconnected with it, these regions did not express fos in the MK801 plus CSAB group. These findings suggest that status epilepticus is associated with expression of fos in striatum and its interconnected areas. In addition, those areas known to be most sensitive to cell death following status epilepticus were seen to express fos immunoreactivity in the MK801 plus CSAB group but not the saline plus CSAB group.

67.13

MATURATIONAL DIFFERENCES IN GENE EXPRESSION OF GABA-A α1 RECEPTOR SUBUNIT IN RAT SUBSTANTIA NIGRA. E.F. Sperber, D.E. Pellegrini-Giampietro, L.K. Friedman, R.S. Zukin and S.L. Moshé, Departments of Neurology, Neuroscience and Pediatrics, Albert Einstein College of Medicine, NYC, NY.

Actions of GABA are mediated predominantly through the GABA-A receptor, a member of the large family of ion-channel forming receptors. Functional studies in oocytes and transfected cell lines indicate that the native GABA-A receptor is a heteroligomer comprised of α, β, γ, δ or ε subunits. GABA receptors in the substantia nigra (SN) are implicated in the control of seizures. Effects of GABA-A agonists infused in the rat SN are age-specific; in 15-day old pups, GABA-A agonists are proconvulsant whereas in adults they are anticonvulsant. The present study was undertaken to determine whether a developmental regulation in the gene expression of the GABA-A α1 subunit in the SN may play a role in the age-specific effects of GABA-A agonists on seizures. *In situ* hybridization was used to measure the GABA-A α1 subunit mRNA in 15-day old and adult rats. Nigral sections were hybridized with a ^{35}S-labelled riboprobe directed against the α1 subunit mRNA. Resolution at the cellular level was obtained by use of emulsion-dipped sections. In the adult SN, α1 mRNA was expressed at high levels in the pars reticulata and at low levels in the pars compacta. A similar pars reticulata/pars compacta pattern was observed in the pup SN, but there was a decrease in grains/neuron ratio. These results suggest that ontogenetic alterations in the nigral GABA-A α1 subunit may contribute to developmental differences in seizure suppression.

67.14

The immunocytochemical pattern of heat shock protein 72kDa induction following seizures induced from pre-piriform cortex. Shinichi Shimosaka*, Yuen So, Roger P. Simon, Department of Neurology, UCSF.

To study the vulnerability of neuronal regions to seizures emanating from deep pre-piriform cortex , we performed microinjections of bicuculline (294-1764 pMOL, 0.3 µl per injection) into the area tempestus (AT) of 14 rats. Cell injury was assessed after 72 hours in two ways: 1) immunocytochemical localization of a monoclonal antibody to the non-constituative 72kDa heat shock protein (hsp72) as a marker of stress, 2) cells failing to exclude an acid dye (acid fuchsin) as an indicator of neuronal death. AT bicuculline injection resulted in 10-86 min of sustained epileptiform EEG discharges. Hsp72 induction was seen only in animals with greater than 20 minutes of electrographic status epilepticus.

Hsp72 was first observed in the ipsilateral amygdaloid complex and the ipsilateral dorsal medial thalamus. With greater than 60 min of status, bilateral amygdaloid and thalamic staining for hsp72 occurred. Acid fuchsin staining was seen in these same cell groups, with frank necrosis occurring in the piriform cortex adjacent to the amygdala. Four control animals received bicuculline infusions into the subarachnoid space resulting in a different pattern of hsp72 expression occurring after 3-10 min. of sustained epileptiform discharges: parietal cortex, hippocampus, dorsal medial and ventral thalamic nuclei.

These results suggest that the dorsal amygdala and dorsal medial thalamus have selective functional interconnections with deep pre-piriform cortex and that neuronal death occurs at these regions from electrographic status epilepticus of greater than 20-60 minute duration emanating from AT.

67.15

ABNORMALITIES IN BRAIN SEROTONIN UPTAKE AND STEADY STATE CONCENTRATION IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR). M.A. Statnick, J.W. Dailey, P.C. Jobe, and R.A. Browning, Dept. of Physiology, Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901; and Dept. of Basic Sciences, Univ. of Illinois Sch. of Med., Peoria, IL 61656.

Neurochemical deficiencies in brain serotonin (5-HT) and norepinephrine (NE) play a role in determining seizure predisposition of the GEPR. Previous studies have shown a parallel decrement in NE steady state levels, high affinity NE uptake, and dopamine ß-hydroxylase activity in the GEPR suggesting a reduction in the NE terminal fields in the GEPR. To further evaluate the nature of the 5-HT deficit in GEPRs, regional steady state concentration and high affinity uptake were measured by reverse phase HPLC in age matched, male, severe seizure GEPRs (GEPR-9s) and seizure-resistant controls (SRCs). Regional brain 5-HT levels were found to be significantly reduced in the GEPR-9 cortex, caudate nucleus, amygdala, septum, hippocampus, and corpora quadrigemina when compared to SRCs, while the hypothalamus displayed a nonsignificant (P=0.06) reduction. No differences in 5-HT content between GEPR-9s and SCRs were observed in the thalamus, midbrain reticular formation, cerebellum, or pons medulla. 5-Hydroxyindoleacetic acid (5-HIAA) was significantly reduced in the cortex of GEPR-9s, but not in any of the other regions examined. Fluoxetine-sensitive high affinity uptake of ^{3}H-5-HT into crude synaptosomes was reduced in the GEPR-9 hippocampus. However, no difference in 5-HT uptake was observed in the amygdala, hypothalamus, thalamus , midbrain reticular formation, or corpora quadrigemina between GEPR-9s and SRCs. These findings contrast with the parallel decrement in presynaptic NE markers in GEPR-9s, and suggest that the mechanism(s) responsible for the reduced 5-HT levels in GEPR-9s may be different from that responsible for the NE deficit.

67.16

AMINO ACIDS IN SUBSTANTIA NIGRA: INTRACEREBRAL MICRODIALYSIS IN GENETICALLY EPILEPSY-PRONE RATS. **M.C. Doretto, R. Burger, N.Garcia-Cairasco and P.C. Jobe. Univ. of Illinois College of Medicine at Peoria and Faculty of Medicine of Ribeirao Preto,SP, Brazil.**

Previous studies have shown that GABA transmission plays an important role in audiogenic seizure regulation. Excitatory amino acids may also participate in seizure regulation as evidenced by the fact that bilateral microinjections of NMDA receptor antagonists in substantia nigra (SN) block or reduce seizure severity in the severe seizure genetically epilepsy-prone rats (GEPR-9s). Accordingly, we undertook a microdialysis study of K^+ stimulated release of amino acids in the SN of GEPR-9s and non-epileptic controls. A 1 mm U-shaped probe was inserted into the SN of awake and freely moving rats (seven GEPR-9s and four non-epileptic controls), and used to perfuse a 100mM K^+ solution for 2h. Four 30uL samples were collected prior to high K^+ stimulation (basal release), during 100mM K^+ perfusion, and post-K^+ infusion. After pre-column derivatization with phenylisothiocyanate, levels of aspartic (ASP) and glutamic (GLU) acids, glycine (GLY), taurine (TAU) and GABA were measured by reversed phase high performance liquid chromatography. Two hours after the initiation of high K^+ infusion, the percent increases relative to basal were, for non-epileptic controls, 35%, 74%, 68%, 847% and 283% respectively for ASP, GLU, GLY, TAU and GABA. Corresponding increases for GEPR-9s were 14%, 10%, 41%, 505% and 123% respectively. GABA release in GEPR-9s was significantly less than in non-epileptic controls ($p<0.05$) throughout the K^+ stimulation period. The amino acid release for ASP, GLU, GLY and TAU was not statistically different between GEPR-9s and non-epileptic controls. These results may suggest that GABAergic mechanisms and associated nigral efferents play a role in the seizure susceptibility observed in GEPR-9s.

67.17

NORADRENERGIC REGULATION OF FOREBRAIN AND BRAINSTEM SEIZURES IN NON-EPILEPTIC AND GENETICALLY EPILEPSY-PRONE RATS (GEPRs).

P.K. Mishra, A.F. Bettendorf*, R.L. Burger*, J.W. Dailey, M.K. Eldadah, C. Wang, R.A. Browning and P.C. Jobe. Univ. of Illinois College of Medicine at Peoria and SIU School of Medicine, Carbondale, IL.

The role of norepinephrine in regulating brainstem seizures has been well documented. These seizures are characterized by running/bouncing clonus, and tonic extensor convulsions. Such evidence comes partially from studies with genetic models of epilepsy which are characterized by innate noradrenergic deficits and from selective lesioning of noradrenergic neurons and/or pathways. The present study was conducted to evaluate whether forebrain seizures, characterized by facial and forelimb clonus, are regulated by norepinephrine. Accordingly, one hundred female Sprague-Dawley rats (49-55 day old) and sixty moderate seizure genetically epilepsy-prone rats (GEPR-3s) were either injected with 50 mg/kg ip of DSP-4 (N-(2-chloroethyl)-N-2-bromobenzylamine, Sigma) or vehicle. Three weeks later, these animals were tested for facial and forelimb clonus threshold via corneal electrode stimulation. Three days after the initial corneal electroshock, these animals were tested for brainstem seizure intensity using supramaximal electroshock (150 mA) delivered through ear clip electrodes. Two days after the supramaximal electroshock, animals were sacrificed and the extent of norepinephrine depletion was assessed in 9 brain areas. Widespread norepinephrine deficits were detected in the brain areas innervated by locus ceruleus in the DSP-4 treated animals. Also, the facial and forelimb clonus thresholds (convulsive current$_{50}$) in these animals were significantly reduced below control (vehicle) values as indicated by the Litchfield and Wilcoxon test. Brainstem seizures were facilitated as indicated by increased hindlimb extension-flexion ratios in the drug-treated animals. These observations provide additional support for the noradrenergic hypothesis of seizure regulation of brainstem and forebrain seizures. The evidence also strengthens the concept that the innate noradrenergic deficits, localized both in rostral and caudal portion of the GEPR brain contribute to seizure predisposition. Supported in part by NIH grant # NS22672.

67.18

MONOAMINERGIC AND AUDITORY INDICES IN NON-AUDIOGENIC SEIZURE (AGS) SUSCEPTIBLE GENETICALLY EPILEPSY-PRONE RATS (GEPRS).

C.E.Reigel, K.D.Bell*, M.E.Randall, C.L.Faingold. Dept. of Pharmacol., Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430, Dept. Basic Sci., Univ. of Ill. Col. of Med. at Peoria, Peoria, IL 61656 and Dept. Pharmacol., South. Ill. Univ. Sch. of Med., Springfield, IL 62708

The GEPR was developed from Sprague-Dawley (SD) rats through selective breeding for AGS susceptibility. Nearly all of the members of the current GEPR-9 colony exhibit full tonic extensor convulsions when sound stimulated, reflecting over 25 generations of inbreeding for this trait. The highly consistent AGS responsiveness of the GEPR has allowed pathophysiological studies of the etiology of epilepsy. AGS susceptibility in the GEPR has been characterized by and is believed to be mediated through widespread deficits in central noradrenergic and serotonergic function as well as peripheral hearing deficits.

In a similar manner, those rare GEPR-9s who are not AGS susceptible represent a unique opprtunity to study the pathophysiology of epilepsy. Over a 6 month period, 7 non-AGS susceptible GEPR-9s were identified during normal colony AGS screening. AGS susceptible littermates and non-AGS susceptible SD rats served as 2 control groups. Both AGS susceptible and non-AGS susceptible GEPR-9s were determined to possess peripheral hearing impairment as compared to SD rats. Regional monoamine content was determined in all groups. Both AGS susceptible and non-AGS susceptible littermates were found to possess characteristic regional deficits in norepinephrine (NE) and serotonin as compared to SD rats with one notetable exception. Non-AGS susceptible GEPR-9s did not possess a deficit in midbrain NE content. These results suggest a deficiency in NE at the level of the midbrain is critical for the expression of AGS susceptibility in the GEPR.

67.19

EXCITATORY AMINO ACIDS: GABA IMBALANCE IN GENETICALLY EPILEPSY PRONE BALB/c MICE IS PYRIDOXINE REVERSIBLE. S. Dolina, J. Peeling, G. Sutherland, N. Pillay, S. Zalcman, A. Greenberg. University of Manitoba, Winnipeg, MB R3E 0V9

Neurotransmitter amino acid levels were determined by ^{1}H-NMR spectroscopy in the brain tissue of selectively bred epilepsy-prone (EP) and epilepsy-resistant (ER) substrains of BALB/c mice. Higher levels of excitatory amino acids (EAA), glutamate and aspartate, and higher ratio of EEA/GABA were observed in the cerebellum, hippocampus, cortex and brain stem of EP compared to ER animals. The EAA/GABA imbalance was corrected by pyridoxine (100 mg/L in drinking water) given to EP mice prenatally and throughout their lifespan, and was found to be region- and sex-specific. The combination of lower GABA level and EAA excess produced the severe glutamate/Gaba imbalance in the hippocampus and cerebellum in EP (9.67 ± 0.36) vs ER (7.61 ± 0.13) females. These indices in both regions were reversed in pyridoxine-treated EP females. Pyridoxone treatment of EP males increased GABA and led to a decrease in EAA/GABA ratio in the cerebellum. The GABA deficiency which produced a higher glutamate/GABA ratio in the cortex of EP females (8.65 ± 0.07) compared with ER (6.6 ± 0.43) was not correctable by pyridoxine. In contrast, EAA levels and EAA/GABA ratios in the cortex and brain stem of EP males were pyridoxine reversible. Pyridoxine-dependent enzyme(s) of the glutamate system are currently under study in the EP animals. (Supported by the R.J. Reynolds Corp.)

EPILEPSY: ANIMAL MODELS I

68.1

NEUROPROTECTION FROM EXCITOTOXIC DAMAGE IN A RAT MODEL OF STATUS EPILEPTICUS. L.P Penix*, F. Mansouri*, A.M. Morin and C.G. Wasterlain. Epilepsy and Neurology Research Labs., VAMC, Sepulveda, CA and Brain Research Institute, UCLA.

The rat model of Status Epilepticus (SE) described by Sloviter produces a pattern of hippocampal damage similar to that observed in human patients with Temporal Lobe Epilepsy and SE. In this model, there is a loss of recurrent inhibition in response to paired pulses given 40msec apart at stimulation frequencies greater than 1 Hz. The loss of recurrent inhibition is a result of death of hilar interneurons. We have used this model of 24 hour stimulation of the perforant path in rats to test the ability of NMDA and non-NMDA antagonists to prevent loss of neurons and recurrent inhibition. After 24 hours of intermittent 20 Hz stimulation of the perforant path, untreated animals had 52.9 ± 15.8 % reduction of hilar interneurons on the side of stimulation compared to the unstimulated side and 100.9 ± 4.9 % loss of recurrent inhibition at the 4 Hz stimulation rate. Animals treated with a constant infusion of 120 µg/kg/hr of the non-competitive NMDA antagonist MK-801 had 20.7 ± 28.3 % reduction of hilar interneurons with 53.4 ± 44.3 % loss of recurrent inhibition. Preliminary results on two animals given a constant infusion of the non-NMDA antagonist NBQX (1 mg/kg/hr) showed no protection against loss of recurrent inhibition but did show only 19.4 ± 7.9 % loss of hilar interneurons. These data suggest a selective protection of subpopulations of interneurons by NMDA and non-NMDA antagonists, with the NMDA blocker protecting interneurons involved in paired pulse inhibition.

68.2

THE INFLUENCE OF NEONATAL HYPOXIA ON ELECTROCONVULSIVE SHOCK SEIZURES IN RATS. C.T. Lombroso,[1] C.D. Applegate,[2] F.E. Jensen,[1] J.L. Burchfiel,[2] I. Firkusny[1] and G.M. Samoriski.[2] The Children's Hospital,[1] Boston, MA 02115 and Univer. of Rochester Sch. of Med.,[2] Rochester, NY 14642.

Neonatal hypoxia, but not anoxia, produces an acute ictal event in immature rats (F.E. Jensen, et al., Ann. Neurol.,v. 29,1991), and results in an increased susceptibility to pentyleneterazol- (PTZ) and fluorothyl-induced seizures in adulthood. In this study we examined the effects of neonatal hypoxia on susceptibility to corneal electroconvulsive shock (ECS) stimulation as adults.

At post natal day 10, male, Long Evans hooded rats were exposed to a 3% O2 environment until heart rate decreased to 30% of basal. This procedure is acutely epileptogenic in rats of this age. Rats were tested for susceptibility to ECS-induced seizures at 60 days of age. ECS stimulation was delivered via corneal electrodes using a Grass S-48 stimulator (150 V, low impedance output) and consisted of a 400 ms train of 15 ms square waves at 400 Hz.

No differences in ECS-induced seizure manifestations were observed between hypoxic and non-hypoxic littermate controls. Thus, neonatal hypoxia increases susceptibility to generalized seizures in the PTZ and fluorothyl models but not in the ECS model. Results indicate the importance of evaluating the effects of neonatal hypoxic or other insults using multiple seizure models. We are currently assessing the effects of neonatal hypoxia on susceptibility to kindled seizures.

68.3

DEVELOPMENT OF VOLTAGE SENSITIVE CALCIUM CHANNEL TYPES OF AUDIOGENIC SEIZURE MICE. M.L. Smart, M.S. Esplin, C.L. Mouritsen, D.M. Woodbury, M.J. Litzinger Depts. of Pediatrics, Physiology, and Biology, Univ. of Utah, SLC, UT.

Development of voltage sensitive calcium channel (VSCC) types mark a critical period in Swiss Webster mouse neurodevelopment (Grover et.al., 1990, Litzinger et.al., 1990). Omega conotoxin (w-CgTx), believed to mark the presynaptic N type VSCC and PN-200, a 1,4 - Dihydropyridine, believed to mark the L type VSCC, show a rapid increase in binding on postnatal days 10 to 15. This time period corresponds to a "critical period" noted by Himwich, 1962, which signal the activation of electrocortical maturation.

Susceptibility of the DBA/2J mice to seizure is greatest at juvenile ages (days 16-21) and dissipates thereafter. This study compared the development of VSCC types in these audiogenic seizure mice. W-CgTx binding was done in whole brain from DBA/2J mice and shows a gradual increase between postnatal day 0 and day 7, and then plateaus between day 8 and day 16 (Esplin et.al., 1991, Litzinger et.al., 1991). Preliminary binding data with PN200 in the same frozen whole brain samples show a gradual increase between day 3 and day 13. These results suggest a developmental difference between VSCC types in DBA/2J mice. Perhaps this developmental difference underlies the basic mechanism of audiogenic seizures. This work was sponsored by NICHD K08 00886-02.

68.4

SODIUM VALPROATE EFFECT ON ETHANOL WITHDRAWAL IS MEDIATED BY ENDOGENOUS ETHANOL. F. Poldrugo, I. Bykov* and S. Ostrovsky*. Alcohol Research Group, Univ. of Trieste, 34100 Italy; Inst. of Biochemistry, BSSR Acad. Sci., Grodno 230009 USSR

Sodium valproate (SV) is known to be useful in the treatment of the ethanol withdrawal syndrome (EWS) in humans, but its mechanism of action is unclear.

The drug interpheres with the enzymes involved in catabolism of gamma-hydroxy-butyric acid (GHB), which in turn is competitively inhibited by ethanol (E) since both substances are degradated by alcohol dehydrogenase (ADH). A possible effect of SV in the treatment of EWS is thus via a change in E catabolism.

Experiments were performed in Sprague-Dewley rats receiving SV in acute or chronic doses (100-400 mg/kg), and in rats in EWS after chronic E intoxication. Endogenous ethanol (EE) was measured by gas-liquid chromatography.

Both acute and chronic administration of SV increased the EE level in animal brain, plasma and liver and the use of SV prolonged the time of E elimination during EWS.

The results suggest that the suppressive SV effect on EWS in animal and humans may be mediated through a direct effect on EE concentrations in brain and an indirect effect on E catabolism in the liver because of a relationship with the metabolism of GHB.

68.5

KINDLING-LIKE EFFECTS OF A PENICILLIN-INDUCED AMYGDALINE EPILEPTIC FOCUS DURING 23 H RECORDINGS IN FREELY MOVING CATS. A. Fernández-Guardiola, A.Martínez*, R.Fernández-Mas* R. Gutiérrez. Inst. Mex. Psiquiatría, México 14370, D.F.

The topical application of penicillin to the amygdala induces focal discharges[1]. The amygdaline penicillin focus is a useful model of "massed kindling". Two cats were implanted with a 16-channel cortical isometric matrix. Two were implanted for conventional sleep recordings and 4 had electrodes in both amygdalae and frontal cortices. All of them had a cannula implanted into left amygdala for penicillin delivery (50-100 IU). EEG mapping showed an early ipsilateral frontal propagation followed by a contralateral frontal activation. These progressive EEG manifestations were concomitant to behavioral changes resembling kindling stages within 5 h. Twenty four hour sleep recordings, starting at 8:30 AM, one hour before penicillin delivery, revealed dose-dependent REM latency augmentation, though the percentage of SWS and REM was unaffected. Amygdaline spiking was noticeably facilitated during SWS. As PGO activity appeared during REM, amygdaline and interictal cortical spiking were diminished or even suppressed. The consolidation of amygdaline penicillin epileptogenesis requires time for the involvement of distant structures responsible for the kindling-like behavioral manifestations since interictal spiking is present in areas distant from the primary focus.

1. Gloor et al., EEG clin. Neurophysiol. 43: 79-94,1977.

68.6

Voltage Sensitive Dye Maps Differential Patterns of Seizure Spread. **D.S. Sacks and R.M. Dasheiff. Dept. of Veteran Affairs, VAMC, University Dr., Pgh, Pa, 15240 and Univ. of Pittsburgh Epilepsy Center.**

It is our hypothesis that unique spatial and temporal patterns of polarization throughout the brain reflect the underlying neural circuits activated by different seizures. The voltage sensitive dye diO-C(2)-5 and computerized image analysis was used to histologically map the in vivo electrical activity of these neural circuits during drug induced seizures in awake animals (Soc. Neurosci. Abst., 16: 1335, 1990). The rats received one of four treatments prior to dye injection: 1) i.p. Kainic Acid (KA) 12 mg/kg in saline; 2) i.p. saline; 3) intrajugular bicuculline (BIC) 0.25 mg/kg in DMSO; or 4) intrajugular DMSO. EEG activity was recorded via hippocampal and subdural electrodes and the dye injection timed to coincide with seizure activity.

The present study indicates that both agents produced depolarization relative to their specific controls. However, each drug produced qualitatively different patterns of neural activity. In our technique, dye concentration reflects the relative depolarization of neural tissues in such a way that lower dye concentration indicates greater depolarization. The dye concentration in the KA animals was Thalamus > Limbic > Brainstem > Cortex while the BIC animals showed a different pattern of Cortex > Limbic> Thalamus > Brainstem. These results demonstrate that the seizure circuits are activated differentially, in keeping with work using other techniques. Further spatial and temporal analysis will provide insight into seizure pathophysiology.

68.7

IDENTIFICATION OF A HYDROPHOBIC DOMAIN ON VOLTAGE-GATED SODIUM CHANNELS LABELLED BY ^{3}H-PHENYTOIN. J. Francis, L. Spero and W. M. Burnham. Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Electrophysiological studies suggest that phenytoin (PHT) limits repetitive neuronal firing via selective inhibition of voltage-gated sodium flux. Pharmacological studies, however, have failed to reveal a significant biochemical action of the drug within the therapeutic concentration range (1-10 μM). We previously reported the existence of a specific and saturable ^{3}H-PHT binding site with a K_d of approximately 5 μM. We here report that the ^{3}H-PHT binding site is a hydrophobic domain on the voltage-gated sodium channel, related to, but distinct from, the alkaloid toxin binding domain labelled by ^{3}H-batrachotoxinin A 20-α-benzoate (^{3}H-BTX-B). Specific ^{3}H-PHT binding in neocortical synaptoneurosomes at 22°C reveals a single population of sites with an K_d of 1.7 μM and a B_{max} of 5.5 pmol/mg protein. This specific ^{3}H-PHT binding is inhibited by ligands specific for the hydrophobic neurotoxin site 2 on the sodium channel, with a rank order of inhibition similar to that previously reported for inhibition of the binding of ^{3}H-BTX-B. Scatchard analysis indicates these interactions to be allosteric and non-competitive. Other anticonvulsant ligands which compete with the ^{3}H-PHT site include mephenytoin, ethotoin, phenobarbital and carbamazepine. These interactions are also allosteric in nature and occur at supra-therapeutic concentrations. It is concluded that the ^{3}H-PHT binding site lies on the voltage-gated sodium channel. Since the K_d for binding to this site falls within phenytoin's clinical concentration, it is proposed that phenytoin exerts its anticonvulsant effects by binding to this specific channel domain.

68.8

ARE THERE C-14, 2-DEOXYGLUCOSE CORRELATES OF THE EPILEPTOGENESIS PRODUCED BY COBALT CORTICAL IMPLANTS? R.M. Cooper and G. Van Ostrand*. Behavioural Neuroscience Research Group, Dept. of Psychology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

The 2-DG autoradiographic technique, which permits assessment of functional activity throughout the brain, could be used to determine how well anti-convulsants target ictal sites and to answer other questions of interest to epileptologists. Our findings with cobalt rod (.5 x 1.0 mm) implants in rat cortex suggest that correlates do exist: (1) Both cobalt and copper are toxic to tissue, but only cobalt is epileptogenic and only cobalt produced a ring of elevated 2-DG uptake around the implant site. (2) Cortical elevation in glucose metabolism peaked at 9 d after implant and disappeared around 21 d, mirroring the reported EEG and behavioral seizure time course for large cobalt implants. (3) The literature also indicates that secondary foci, presumably a reflection of retrograde and anterograde axonal transport of cobalt ions, may occur. In this connection we observed 2-DG "hot spots" in the LGN of rats with area 17 cobalt implants. (4) Finally, cobalt-induced elevations in 2-DG uptake were erased or attenuated by sodium pentobarbital anesthesia suggesting that the abnormal activity is affected by an anticonvulsant. Our findings point to a useful 2-DG marker of the excessive neuronal activity associated with brain cobalt implants.

68.9

ISOGUVACINE SUPPRESSION INDUCES LONG-LASTING EPILEPTOGENIC CHANGES IN THE RAT. **S. Brailowsky and T. Montiel*. Instituto de Fisiología Celular, U.N.A.M., México 04510 D.F., Mexico.**

Since the original description of an epileptogenic withdrawal syndrome induce by cessation of chronic (7 days) intracortical GABA infusion in photosensitive baboons, the phenomenon has been confirmed in non-epileptic baboons and rats and both in cortical and limbic structures. This "GABA-withdrawal syndrome (GWS)" represents a new model of partial status epilepticus.

We have also found that baclofen, a $GABA_B$ receptor agonist, induces paroxysmal activity at the cortical injection site. In this report, we have induced a GWS-like phenomenon through semi-acute (2 hs) intracortical infusion of isoguvacine, a $GABA_A$ receptor agonist. The behavioural and EEG effects of this agent are more pronounced than those of GABA itself during infusion suggesting an accumulation at the injection site. Cessation of drug infusion induces an epileptogenic process comparable to the GWS both in latency, duration and morphology. We conclude that the GWS is a $GABA_A$ receptor-dependent phenomenon.

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68.10

CORTICAL AND SUBCORTICAL PROPAGATION OF AN AMYGDALINE PENICILLIN EPILEPTIC FOCUS IN "ACUTE" AND FREELY MOVING CATS. R.Gutiérrez, A. Martínez*, R. Fernández-Mas*, A. Fernández Guardiola. Inst. Mex. Psiquiatría, México 14370, D.F.

In order to explore whether an amygdaline chemical epileptic focus follows the same cortical propagation pattern than the electrical kindling, we carried out subcortical recordings and cortical mapping in cats with an amygdaline penicillin focus. Fifteen cats were used: 11 for "acute" and 4 for "chronic" experiments. Penicillin (50-5000 IU) was delivered through a cannula implanted in the left amygdala. Six animals were prepared as encéphale isolé and 5 were anaesthetized with uretane. Two "acute" and 2 chronic cats were epidurally implanted with a 16-channel isometric matrix (10-20 international) for monopolar EEG recordings and subsequent brain mapping. The interhemispheric transfer was conducted mainly through stria terminalis (ST) and pericommissural fibers (PCF) and in a lesser extent by corpus callosum. Spikes appeared also in Habenula and Septum. The shortest latencies were evident in ST, PCF and ipsilateral prefrontal cortex. The AM spiking projection was more evident in the insular and ectosylvian posterior cortices, bilaterally in the frontal regions and an activation of the contralateral temporal lobe appeared towards the end of the AM spiking activity. The electrical and the behavioral manifestations were asymmetrical throughout the experiment. We conclude that the induction of a penicillin amygdaline focus is a model of "massed kidling". The secondary epileptogenic site is in the prefrontal cortices.

68.11

CYTOCHEMICAL DETERMINATION OF ALUMINUM IN MOTOR CORTEX OF RECIPIENT CATS IMPLANTED WITH ALUMINA CREAM EPILEPTOGENIC FOCUS. A. Feria-Velasco, I. De la Rosa*, M. Velasco* and F. Velasco. Exptl. Path. Div., Unidad Invest. Biomed. Occte., I.M.S.S.; Dept. Morphology, Univ. Autónoma de Guadalajara; Guadalajara, Jal., and Unidad Invest. Biomed. Centro Méd. Nal., I.M.S.S. México, D.F.

Focal motor epilepsy is induced in cats by intracranial injection of alumina cream (AC), obtaining a constant model with a latent, convulsive and remission stages. Progressive reduction in thickness and cellularity in motor cortex (MCx) adjacent to AC deposit is seen with preferential loss of inhibitory GABAergic neurons. In this work, the role of free aluminum in the events sequence of the model was explored. For this, the AC focus of implanted cats obtained at latent, convulsive and remission stages were transplanted beneath the MCx of intact recipient cats. Electrocorticographic (ECG) recordings and motor behavior were analized in donnors and recipients, up to 100 days after AC implantation. A semiquantitative study of aluminum in MCx adjacent to the epileptogenic focus was done by means of aurine tricarboxylic acid stain and Bielschowsky technique, and by a qualitative X-ray spectrometric study (EDAX) for aluminum in a scanning electron microscope. A progressive reduction in amount of non-phagocyted alumina gel at the center of epileptogenic foci, and reduction of aluminum identified by the histochemical techniques and EDAX study, were seen in recipient cats when implanted with foci obtained from donnors at latent, convulsive, and remission stages, respectively. Data correlate well with ECG recordings in recipient cats, in which a classical pattern was seen when latent stage-focus was implanted; moderate number of abnormal spikes with ocassional convulsions were seen when a convulsive stage-focus was implanted; and few abnormal spikes with no convulsions were seen when a remission stage-focus was implanted. It is concluded that the amount of GABAergic interneurons destroyed in the MCx adjacent to AC deposits is proportionally related to the amount of free aluminum diffusing from the AC epileptogenic focus.

68.12

BRAINSTEM RETICULAR FORMATION UNIT ACTIVITY DURING ETHANOL INTOXICATION AND WITHDRAWAL ASSOCIATED WITH AUDIOGENIC SEIZURES. **A.Riaz and C.L.Faingold. Dept. Pharmacology, Southern IL Univ. School of Medicine, Springfield, IL 62794.**

Ethanol withdrawal (ETX) in normal rats induces susceptibility to audiogenic seizures (AGS). Previous studies support an important role of the brainstem reticular formation (RF) in AGS. The present study examined effects of ethanol treatment (ET+) and ETX on single neuron responses using microwire electrodes implanted into the pontine RF. Ethanol was given intragastrically (9-15g/kg/day) for 4 days. Neuronal responses to AGS-inducing stimuli (12kHz tone bursts) were recorded in awake rats before ET+ (control), during ET+ and during ETX. The mean evoked activity during ET+ was significantly decreased from control and significantly increased in ETX at all intensities. The degree of response "habituation", occurring with increasing repetition rates was measured as % of firing at 1/2sec. Before ET+, mean firing was decreased from 1/2sec response rate to 74.5±7% (at 1/sec) and to 49.5±5.3% (at 2/sec). During ET+, habituation was intensified to 49.3±11.5% (mean at 1/sec) and to 30.5±10% (at 2/sec). During ETX, mean habituation was decreased at 1/sec (79.5±9.2%) and at 2/sec (85.4±13.8%), indicating that RF neuronal habituation is augmented during ET+ and reduced during ETX. During ET+ spontaneous firing of RF neurons was decreased (10.8±4% of control) but markedly increased during ETX (229±67.3%). Ethanol affects the actions of GABA and excitant amino acids (EAAs). Microinjection of GABA agonists or EAA antagonists into the RF blocks AGS during ETX. During ET+, increased GABAergic and decreased EAA transmission may mediate reduced RF firing. ETX may lead to hyperexcitability due to rebound changes in amino acid transmission. Thus, increased RF excitation and reduced inhibition may play a role in AGS susceptibility during ETX. (NIH NS 21281)

68.13

AUDIOGENIC SEIZURE SEVERITY IN THYROID-DEFICIENT RATS INCREASES AFTER NOREPINEPHRINE DEPLETION. **D.L. Patrick Paiva, C. Wang, R.A. Browning and C.L. Faingold. Depts. Pharmacol., Physiol. Southern IL. Univ. School of Medicine, Springfield, Carbondale, IL 62794.**

Neonatally thyroid deficient (THX) rats and genetically epilepsy-prone rats (GEPR-9s) both display audiogenic seizures (AGS) and significant hearing deficits. GEPR-9s are transiently thyroid deficient during development. THX rats exhibit submaximal AGS with wild running and clonus, while GEPR-9s display maximal AGS with tonic hindlimb extension (TLE). Brain norepinephrine (NE) deficits are an important determinant of AGS severity in GEPR substrains. This study examined regional brain NE levels and AGS severity in THX rats before and after depletion of NE using N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4, 50 mg/kg, ip). Thyroid hormone levels in normal neonatal rats were depleted using propylthiouracil (0.0075% postnatal day 0-19). AGS severity was evaluated before and after DSP-4 according to the scale (0-9) of Jobe using a bell (122 dB SPL, for 60 sec or until AGS onset). One week following AGS testing the brains of THX rats were removed and NE levels were subsequently evaluated using HPLC. THX rats exhibit a median AGS score of 2, and NE levels were not different from normal rats. Two weeks following DSP-4, AGS severity scores were significantly increased (median = 5) with 25% of THX rats (N=12) exhibiting TLE. THX rats displayed significantly decreased NE (but not dopamine) levels in all brain areas examined 3 weeks following DSP-4. THX rats exhibited a smaller degree of NE depletion, and AGS severity (median score = 3) was also reduced 3 months after DSP-4 treatment, suggesting recovery from both effects occurs in parallel. Thus, NE deficits are not observed in AGS-susceptible THX rats, but depletion of NE increases AGS severity in THX rats, further supporting the critical role of brain NE in determining AGS severity. (NIH NS 21281)

68.14

CHRONIC SEIZURES IN JUVENILE WISTAR-FURTH RATS FOLLOWING SYSTEMIC ADMINISTRATION OF KAINIC ACID. G.T. Golden, P.F. Reyes, G.G. Smith* and T.N. Ferraro. DVAMC, Coatesville, PA 19320 and Thomas Jefferson Univ., Phila., PA 19107.

Wistar-Furth (WF) rats are exceptionally sensitive to the convulsant effect of systemic kainic acid (KA) (Neuroreport,2,141-144,1991). Sixteen male juvenile (36-37 days old) WF rats were given KA 10 mg/kg, sc and observed for seizure activity. Seven age-matched male WF rats served as controls. Animals had food and water intakes and body weights recorded for seven days prior to KA and for 100 days after. Animals were placed in an activity monitor each week. Mortality (usually 80% at this age and dose) was minimized by administering 10cc lactated ringer's solution sc, six hours after KA and for the first five days post-KA and by providing cookie mash and sucrose-powdered rat chow until body weight stabilized. One animal died in status 3h19min post-KA, 15 animals survived both the acute and chronic phase. Over the next 100 days 53% (8/15) of surviving animals were observed having multiple spontaneous behavioral seizures. Spontaneous seizures were first observed at 12 days post-KA and could be observed in some animals until termination of the experiment at 100 days post-KA (137 days old). Spontaneous seizures consisted of staring, facial and abdominal myclonic jerks, running-bouncing seizures, and major clonic seizures ranged from 15 sec to 90 sec in duration. Results indicate that KA treated juvenile rats show chronic spontaneous,recurrent seizure activity as adults. Supported by VA funds & NIA grant #5301AG0814802.

68.15

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHANGES IN HIPPOCAMPUS AND PREPIRIFORM CORTEX OF WISTAR-FURTH RATS WITH CHRONIC KAINIC ACID INDUCED SEIZURES. P.F. Reyes, G.T. Golden, G.G. Smith* and T.N. Ferraro. Neurology and Pharmacology, Thomas Jefferson Univ., Phila., PA and DVAMC, Coatesville, PA 19320.

Sixteen adult male Wistar-Furth rats with kainic acid (KA)-induced seizures, and seven normal age-and sex-matched controls were utilized in this study. Seizures were induced by injection of KA (10mg/kg, sc) between 36-37 days of age. All KA animals demonstrated behavioral seizures and status epilepticus during the four hr observation period following KA. More than half of the KA treated animals were observed having multiple spontaneous behavioral seizures over the next 100 days. Seizures appeared spontaneous since they could not be elicited by visual, auditory, or tactile stimulation. Animals were sacrificed 136-137 days of age, and selected brain sections were prepared and stained with H&E, LFB-CV, and silver impregnation and reacted with antibodies to GFAP. The KA group showed varying degrees of neuronal loss, reactive gliosis, and astrocytic Rosenthal fiber formation in the CA1 and CA3 segments of the hippocampus, and neuronal loss in the prepiriform cortex. The presence of observed seizures correlated directly with the severity of hippocampal alterations; Rosenthal fibers were the best indicator of neuro-glial damage in the hippocampus. Supported by NIA grant #5301AG0814802 and VA funds.

68.16

HYPERREACTIVITY IN RATS WITH SEIZURE-INDUCED BRAIN DAMAGE: ROLE OF NUCLEUS ACCUMBENS. S L. Hartgraves, M. S Tristan*, M. L Williams*, S. A. Miller, and M. R. Murphy, University of Texas Health Sciences Center at San Antonio, Texas, 78284; Armstrong Laboratory, Brooks AFB, Texas, 78235.

Organophosphate (OP)-induced seizures in rats result in hyperreactivity to physical stimuli and an exaggerated locomotor response to stimulant drugs such as d-amphetamine(d-Amph) and caffeine(Caff). Extensive damage to the limbic system is present in these animals, and may underlie this heightened sensitivity to stimulants. The objective of this study is to assess this hyperreactivity by measuring drug-induced locomotor activity, before and after 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens (NAS). HPLC will be used to measure catecholamines in the NAS at study completion.

Rats exhibiting seizures and weight loss following injections of the highly toxic OP soman, were screened for responses to 1.5 mg/kg d-Amph and 20 mg/kg Caff. Those showing 2-3 fold increases in locomotor activity (compared to controls), as well as control animals, underwent additional 6-OHDA/sham lesions of the NAS, to examine its role in stimulant-induced exaggerated responses.

Animals with dopamine depletions in the NAS greater than 65% showed reduced responses to both d-Amph and Caff. This information is examined in light of reports showing different neural substrates for d-Amph and Caff-induced locomotor activation (Swerdlow et al., 1986).

68.17

A FLEXIBLE PERFORATED MICROELECTRODE ARRAY FOR EXTENDED NEURAL RECORDINGS. B.C. Wheeler, S.A. Boppart* and C.S. Wallace, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA 61801.

A flexible and perforated 32-element planar microelectrode array has been fabricated for measuring evoked potentials in brain slices or from any brain or ganglion surface. Electrodes are spaced 200 μm apart in a 4 x 8 array and are sandwiched between layers of insulating polyimide. The polyimide was etched by wet chemical and reactive ion etchants with the results compared. In addition to etching holes through the polyimide to expose recording sites 15 μm in diameter, perforations surrounding the electrodes were etched through both layers. The perforations allow increased circulation of artificial cerebrospinal fluid to the recording surface of the tissue and, hence, increased viability. Evoked potentials were recorded from rat hippocampal slices to demonstrate the functionality of the 32 simultaneously recording, spatially distributed electrodes. Individual spikes and EPSPs were recorded over time to determine the slice viability. Comparisons between perforated and nonperforated arrays showed an average increase of 10 hr to the viability of the slice while using the perforated array. The flexible nature of the array allows shaping to contoured tissue surfaces while still permitting adequate circulation of fluids by way of the perforations.

68.18

MECHANISM OF GAMMA-BUTYROLACTONE(GBL)-INDUCED ACTIVATION OF TYROSINE HYDROXYLASE (TH) IN DOPAMINERGIC NERVE TERMINALS. S.M. Lasley and M.C. Green*. Dept. of Basic Sci., U. Illinois Coll. of Med., Peoria, IL 61656

GBL is metabolized in vivo to gamma-hydroxybutyrate (GHB) and GBL-treated rats have been proposed as a model of generalized absence seizures. GBL derivatives have been shown to be active at the $GABA_A$ receptor-Cl^- channel, but the parent drug has also been employed to test the autoreceptor activity of dopamine (DA) agonists in vivo by virtue of its blockade of DA neuronal impulse flow and elevation of synaptic TH activity. Male Long-Evans hooded rats were utilized at 85-100 days of age to examine the time course and activation of TH by GBL employing multiple approaches. Conversion of ^{3}H-tyrosine by TH isolated from caudate-putamen of rats receiving 750 mg/kg i.p. GBL reached maximal values by 30 min post-injection and remained at this level until 90 min. In combination with a decarboxylase inhibitor, GBL produced a 2.3-fold increase in striatal L-DOPA content at this dose and time after administration. However, direct measurement of the increase in striatal enzyme activity in GBL-treated rats by the tritium release method observed no more than a 60-70% activation. Kinetic analyses of the change in TH activity as a function of 6-MPH_4 concentration employing nonlinear regression found that the GBL-induced activation was due primarily to a doubling of the maximal velocity of the enzyme form with high affinity for the cofactor. In contrast, a 1.0 mg/kg i.p. dose of haloperidol caused a 2.4-fold increase in V_{max} of the high affinity form 2 hr after administration. These results suggest that the GBL effects on DA neurons may be a significant behavioral component of the GHB model of generalized absence seizures. (Supported by ES04359 and the Children's Miracle Network)

68.19

FACILITATION OF SENSORY EVOKED POTENTIALS: CHANGES WITH KINDLING OF HIPPOCAMPUS AND AMYGDALA IN GUINEA PIGS. Arnold Lidsky, NYS Basic Research, Staten Island, NY 10314

Kindling as a model of epileptogenesis and neural plasticity, extensively studied in the rat, cat and rabbit emphasizes EEG afterdischarges(AD) and generalized seizure development. These observations are here extended to guinea pigs receiving daily kindling for up to 150 days in either dorsal or ventral hippocampus, the amygdala, or parietal cortex, with serial testing of visual (VEP) and auditory (AEP) evoked potentials at 14 and 30 day intervals. Changes in EP morphology persisted for at least 2 months following termination of kindling. ADs were longest for hippocampal animals and shortest for cortical controls(C), with progressive prolongations in all groups. VEP components occurring between 40 and 150 msec after light flashes were both faster and larger in limbic-kindled animals within 30 days and reached plateaus at 60-90 days of kindling, while earlier components showed no significant changes. C animals showed paradoxical prolongation of the N3 component of the VEP. AEP latencies decreased in all kindled animals with amplitudes remaining constant in hippocampal-, and decreasing in both cortical and amygdala-kindled guinea pigs. Thus focal kindling, rarely manifesting in tonic-clonic convulsions in these hippocampal animals, altered specific remote sensory conduction in guinea pigs, with longterm consequences including hypersensitivity to peripheral sensory input.

68.20

CHOLINERGIC STIMULATION OF THE VENTROPOSTERIOR THALAMIC NUCLEUS INDUCES LIMBIC SEIZURES: BEHAVIORAL, ECoG, AND METABOLIC CORRELATES S. Mraovitch*, Y. Calando*, P.J. Goadsby+, J. Seylaz*. * *Laboratoire de Recherches Cérébrovasculaires, CNRS UA 641, Université Paris VII, Paris (France). + Department of Neurology, The Prince Henry Hospital, Sydney, Australia.*

It has recently been demonstrated that cessation of chronic unilateral infusion of GABA into the somatomotor cortex in rats induces focal epileptic spikes ("GABA withdrawal syndrome"; GWS) and marked increase in local cerebral glucose utilization (lCGU) in the ipsilateral ventroposterior thalamic nucleus (VP). Since the VP is a site of termination of cholinergic afferents originating in the upper brain stem we sought to determine whether neurons within the VP were the origin of the thalamo-cortical activation which is thought to trigger the GWS epileptic manifestation.

Wistar rats (300-350g) were used in the experiments. Carbachol (CCh, 10μg in 100nl) was stereotaxically injected unilaterally into the VP via a glass micropipette to determine behavioral and ECoG changes. lCGU was studied by quantitative 2-DG analysis in anesthetized (alpha-chloralose), paralyzed (d-tubocurarine) and artificially ventilated rats.

CCh injection into the VP (n=7) caused behavioral alterations (wet dog shakes) and the ECoG (n=15) bilateral bursts of paroxysmal discharge with a latency of 25-30min. LCGU was markedly increased in CCh-injected rats (n=4) in ipsilateral dorsal (+259±39%), postero-ventral CA3 (+226%±13%) and CA3 (+247±9%)hippocampal fields, the lateral septum (+122±47%;Mann-Whitney test, $p<0.05$).

We conclude that activation of the VP cholinergic receptors induced behavioral, ECoG and marked metabolic alteration in the hippocampus and the lateral septum, known to be associated with limbic seizures. Further research

70

SYMPOSIUM. THE BASAL GANGLIA IN THE '90'S:WEDDING THE NEURAL SYSTEM TO CELLULAR AND MOLECULAR MECHANISMS. C. Gerfen, NIMH (Chairperson); R. Albin, Univ. Michigan; J.Mink, Washington Univ.; P. Bolam*, Oxford Univ.; C. Wilson, and J. Surmeier, Univ. Tennessee, Memphis.

The basal ganglia presents a considerable challenge to relating neuroanatomical organization to behavioral function. This symposium will present clinical, electrophysiological, neuroanatomical and molecular studies that together offer a new view of basal ganglia function. The synthesis of these studies suggest that the basal ganglia transforms excitatory inputs from the cerebral cortex into a delicately balanced opposition in the activity of the two major outputs systems of the striatum. The mechanisms of this balanced opposition will be discussed at the systems level, as well as at the cellular and molecular levels. **Roger Albin** will introduce the concept of the balanced opposition of striatal output systems as this mechanism relates to the spectrum of clinical disorders associated with basal ganglia disease. **John Mink** will describe single unit recording studies of internal globus pallidus activity in relationship to voluntary limb movements of monkeys. **Paul Bolam** will describe ultrastructural studies of the synaptic organization of the inputs to neurons that are recorded from by Mink, to provide a morphologic basis to the pattern of physiologic activity. **Charles Wilson** will describe intracellular neurophysiological studies of the mechanisms of integration of corticostriatal inputs by the major output neuron of the striatum, the medium spiny neuron. **Jim Surmeier** will describe application of patch-clamp techniques to acutely dissociated striatal neurons to study the modulation by acetylcholine muscarinic and dopamine receptors of potassium and sodium conductances. **C. Gerfen** will describe in situ hybridization histochemical studies that demonstrate the differential modulation through D_1 and D_2 dopamine receptors of striatonigral and striatopallidal output neurons.

71

SYMPOSIUM: REGULATION OF ION CHANNELS. L.K. Kaczmarek, Yale U (Chairperson); R.A. Frizzell*, Univ. of Alabama; K-W Yau, Johns Hopkins Univ.; R. MacKinnon*, Harvard Univ.; B. Ganetzky, Univ. of Wisconsin.

In the last four years, the molecular identity of a variety of ion channels has been revealed. Studies with these channels are beginning to identify discrete regions of the proteins that form part of the ion pore itself. Other regions and components of the channel proteins allow their activity to be regulated by protein kinases or cyclic nucleotides. Moreover, new types of ion channels are being discovered, based on the existence of conserved regions that are required for ion channel function and selectivity. The symposium will use examples of channels found in both neuronal and neuronal cells to illustate these approaches.

The first part of the symposium will discuss how ion channel are regulated by protein kinases and cyclic nucleotides. These presentations will use two examples; i) chloride channels in epithelial cells, where a genetic defect in the response to protein kinases has led to the identification of a regulatory protein (R.A.Frizell) and ii) new insights into the function of cation channels that are gated by changes in cyclic nucleotides (K.-W. Yau). The second part of the symposium will focus on potassium channels. It will describe experiments showing that one short segment of the Shaker family of channels determines drug sensitivities and plays a major role in ion permeation (R.MacKinnon). The final presentation (B. Ganetzky) will show how this knowledge combined with genetic studies, is bringing about the identification of new classes of potassium channels.

VISUAL CORTEX: STRIATE CORTEX

73.1

AN *IN VIVO* WHOLE-CELL PATCH STUDY OF THE LINEARITY OF IPSP-EPSP INTERACTIONS IN CAT VISUAL CORTEX D. Ferster and B. Jagadeesh Department of Neurobiology, Northwestern University, Evanston, IL 60208.

Postsynaptic inhibition operates by two distinct mechanisms, hyperpolarization and shunting of excitatory postsynaptic currents (EPSCs). The predominant inhibitory mechanism determines the arithmetic operation—addition or multiplication—that synapses perform during neuronal computations (*PNAS* 80, 2799). Hyperpolarizing IPSPs interact linearly with EPSPs; the positive and negative synaptic currents sum to produce a net change in membrane potential. Shunting synapses interact nonlinearly with EPSPs; the shunting-induced increase in membrane conductance reduces the size of EPSPs by a constant fraction. We have searched for shunting inhibition by measuring the effect of visual stimuli on the size of small, electrically-evoked test EPSPs in cortical neurons.

Intracellular current-clamp records were obtained from neurons in anesthetized adult cats with the whole-cell patch method (*J. Neurosci. Methods* 30:203). A bar of light was passed across each cell's receptive field at the same time that a monosynaptic test EPSP was generated by electrical stimulation of the lateral geniculate nucleus. Decreases in test-EPSP size were evoked by stimuli of the preferred orientation, but the decreases were not caused by shunting. They occurred only during periods of visually-evoked depolarization, and were presumably caused by reduction in the driving force on EPSCs that accompanied the depolarization. Visually-evoked hyperpolarizing IPSPs were accompanied by a slight increase in test-EPSP size. Stimuli of the null orientation caused no significant change in test-EPSP size. We conclude that for inhibition evoked by the simple natural stimuli employed here, the predominant inhibitory mechanism is hyperpolarization, rather than shunting.

73.2

VISUALLY-EVOKED OSCILLATIONS OF MEMBRANE POTENTIAL IN NEURONS OF CAT AREA 17. †B. Jagadeesh, †D. Ferster and *C. Gray. †Neurobiology Dept., Northwestern University, Evanston, IL 60208 and *The Salk Institute, La Jolla, CA 92186.

Extracellularly-recorded single unit and field potential activity in visual cortex exhibit 30-60 Hz oscillatory responses to specific visual stimulation (*PNAS* 86:1698). Intracellular records obtained *in vivo* from neurons in cat area 17 using the whole-cell patch technique (*J. Neurosci. Methods* 30:203) showed oscillations of neuronal membrane potentials similar to previously observed extracellular oscillations.

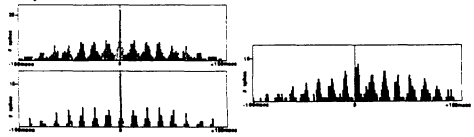
Intracellular oscillations resemble those seen extracellularly in the following properties: 1) The membrane potential, like the local field potential, oscillates at frequencies between 30 and 60 Hz in response to optimal visual stimuli, with amplitudes of up to 10 mV; 2) The oscillations are smaller in layer 4 simple cells, which receive monosynaptic excitation from the LGN, than in complex cells with polysynaptic geniculate input. 3) Oscillations with the greatest amplitude are evoked by stimuli of the preferred orientation of the cell, as defined by slow EPSP responses; 4) The exact frequency of the oscillation is variable from moment to moment. 5) The onset latency, frequency and duration and phase of the oscillations vary slightly from trial to trial. 6) The average frequency of the oscillations varies little with stimulus orientation. 7) Spike activity is synchronized with the depolarizing phase of the oscillation in membrane potential.

The oscillations do not arise solely from neuronal electrical properties. In some cells, depolarizing current pulses did evoke oscillatory responses, but not of the frequencies or amplitudes characteristic of visually-evoked oscillations, suggesting that in these neurons, membrane potential oscillations reflect rhythmic changes in synaptic input.

73.3

VISUALLY-EVOKED OSCILLATIONS IN MONKEY STRIATE CORTEX. M.S.Livingstone Dept. Neurobiology Harvard Med. School, Boston MA 02115.

Two- and three-electrode recordings from 11 recording sessions from halothane-anesthetized squirrel monkeys were analyzed for interneuronal correlations in firing and for oscillatory firing patterns, as described in the cat by Gray and Singer (PNAS 86:1698, 1989). Many neurons did show oscillatory firing, but only in response to visual stimuli. The oscillations were correlated between cells in the same orientation column, within and between layers, but not between cells of different orientation selectivity. The oscillations were faster than was observed in the cat--50 to 90 Hz. Some of the cells showed oscillation frequencies near 60 Hz, but this does not appear to be an artifact of the refresh rate of our TV monitor, because these cells showed the same oscillation frequencies to a constant light source. The periods of oscillatory firing lasted only 40 to 100 msec even when the entire visual response was much longer. More than half the cells in layers 4Cα and 4B showed striking oscillatory firing at some time during most responses, and about 20% of the cells in layers 2/3 showed occasional clear oscillations.



(Left) autocorrelograms from two cells recorded simultaneously in deep layer 3 (top) and layer 2. The crosscorrelogram (right) shows that the oscillations in the two cells were correlated, with the layer 2 cell lagging the layer 3 cell by 4 msec.

Supported by the McDonnell-Pew Program and the Office of Naval Research.

73.4

ON OSCILLATING NEURONAL RESPONSES IN MONKEY VISUAL CORTEX. M.P. Young[1], K. Tanaka[2], S.Yamane[3]. 1: University Laboratory of Physiology, Parks Rd., Oxford, OX1 3PT, U.K. 2: Frontier Research Program, RIKEN, Saitama, Japan. 3: Division of Neuroscience, ETL, Tsukuba, Japan.

We recorded multi-unit activity (MUA) and local field potentials (LFP) in areas V1 and MT, and MUA from the inferotemporal cortex (IT) of monkeys (Macaca fuscata). Recordings in all areas were made under conditions of anaesthesia as close as possible to those in studies of oscillating responses in the cat (e.g. Gray et al 1989, Nature 338:334). In addition, we recorded MUA in the IT of behaving monkeys while the monkeys performed a face discrimination task (see Yamane et al 1988, Exp. Brain Res. 73:209).

In areas V1 and MT, LFP power spectra showed broad band increases (1-100Hz) in amplitude on stimulation by swept optimally-oriented light bars, and not a shift in power from low to mid-frequency, as has been reported in the cat. MUA autocorrelograms (ACGs), classified by fitting Gabor functions, showed oscillations mainly in the alpha range. MUA ACGs from IT in the anaesthetised monkey showed no oscillations, when stimulated by images previously determined to be effective. MUA ACGs from IT in the behaving monkey showed only one oscillating response, with a frequency of 44Hz. The very low incidence in the monkey of oscillating responses in the 30-70Hz range (1 in 424 recordings) suggests that such oscillations are unlikely to serve a function in the monkey, and that there may be a species difference between monkey and cat in the dynamics of neural activity in the visual cortex.

We used a method very similar to that of Engel et al (1990) (Eur. J. Neuro. 2:588) to classify ACGs as oscillating. We found that this and related methods may have led to Type I error by failing to take account of badness of fit between Gabor functions and their ACGs, and by Gabor functions 'ringing' in response to short phasic phenomena which could be consistent with activity in other than the oscillatory domain. In the present data, these problems, if uncorrected, would have led to overestimation of the incidence of oscillation by between 54% and 100%.

73.5

CHARACTERISTICS OF TEMPORAL FREQUENCY TUNING OF NEURONS IN MACAQUE V1

M.J. Hawken, R.M. Shapley, D.H. Grosof, Center for Neural Science, New York University, 6 Washington Pl., New York, NY 10003.

We have measured the temporal frequency tuning of neurons in different layers of Macaque V1 using both chromatic and achromatic sine wave grating stimuli under mid-photopic conditions. In these experiments we were particularly concerned with the measurements of the full range of frequency response and with measurement of integration time. These measurements were made in order to compare the transformation that occurs between the LGN and cells in different layers of the striate cortex. Unlike the transformation that occurs between cat LGN and cortex where there seems to be significant attenuation of high temporal frequency, in monkey V1 a number of cells show responses to temporal frequencies up to 40 to 50 hz. The integration time, derived from the slope of the response phase versus temporal frequency, was as short as 40 msec in some cortical cells. This value is close to that measured in Macaque LGN neurons.

Supported by NIH grants EY-8300, EY-1472, and an NRSA.

73.6

ANOMALOUS RESPONSES IN V1 TO ANOMALOUS CONTOURS?

D.H. Grosof, R.M. Shapley & M.J. Hawken, Center for Neural Science, New York University, New York, NY 10003.

Von der Heydt & Peterhans (1989) investigated the responses of monkey V1 neurons to abutting line gratings which were drifted parallel to the grating bars. They asked whether V1 responses signaled the orientation of the perceived anomalous ("illusory") contour on the boundary between two abutting gratings, or the orientation of the inducing bars in the gratings. V1 neurons always responded more when the bars were at the cell's preferred orientation. Thus, they concluded that V1 cells are not directly involved in the signalling of anomalous contours.

Testing orientation-selective neurons in monkey V1, we have recorded responses to drifting patterns made up of an abutting pair of sine gratings offset in spatial phase but equal in frequency, contrast and color. A range of speeds, colors and spatial frequencies was used. Responses were averaged, synchronized to the passage of the texture boundary across the receptive field. When the bars making up the patches are perpendicular to the cell's preferred orientation, and the drift direction is parallel to the bars, only a weak response is expected according to most receptive field models. But we found that some cells respond more vigorously to the boundary of the patch--an anomalous contour--than one would expect from their responses to full-field temporally modulated gratings.

Support: NEI EY-1472 (RS), EY-8300 (MH), and a NRSA (DG).

73.7

RESPONSES OF SINGLE UNITS IN MACAQUE V1 TO MOVING PATTERNS.

Vincent P. Ferrera and John H. R. Maunsell

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Several lines of psychophysical and physiological evidence suggest that motion processing is broken down into two stages: initial sampling by linear "component" direction selective units, followed by integration across different directions by "pattern" motion units. Movshon and his collaborators have obtained physiological evidence that the component stage is carried out in area V1, and that pattern motion processing occurs in area MT. However, their analysis leaves a significant proportion of the cells unclassed as either component or pattern direction selective. In order to examine potential contributions of these unclassed neurons, we recorded from 48 single units in area V1 of alert macaque monkeys trained to fixate a small spot while stimuli were presented at eccentric locations in the visual field. Direction tuning curves were obtained using cosine gratings and patterns that consisted of the sum of two gratings with different orientations ("plaids.") We confirmed the result that most cells in V1 are component direction selective, although a small number are clearly pattern direction selective. Furthermore, about one-fifth of the cells have good orientation tuning for gratings and plaids, but their tuning curves for plaids are inconsistent with either the component or pattern prediction, which puts them in the unclassed category. The difference in response of these cells to the two different stimuli may be due to the operation of oriented inhibitory mechanisms whose orientation preference is orthogonal to that of the cell's excitatory drive. We have constructed a model which demonstrates that units with such inhibition could be used in a network which computes pattern motion. We conclude that many neurons in V1 carry signals which may contribute to pattern motion processing.

Supported by NIH Fellowship NS08658-02 to V.P.F. and ONR Grant N00014-90-J-1070

73.8

REVERSIBLE INACTIVATION OF FUNCTIONALLY CHARACTERIZED SITES IN CAT VISUAL CORTEX: EFFECTS ON ORIENTATION TUNING AND DIRECTIONAL SELECTIVITY.

J. M. Crook, Z. F. Kisvárday and U. T. Eysel. Dept. of Neurophysiology, Faculty of Medicine, Ruhr University of Bochum, W-4630 Bochum, Germany.

Iontophoresis of GABA was used to reversibly inactivate small sites of defined orientation and directional specificity at a horizontal distance of some 500–700μm from single cells recorded in area 18 of cat visual cortex, and the effects on orientation and directional selectivity studied. Inactivation of sites where the preferred orientations differed by 45° or more from that of a recorded cell (cross orientations) caused substantial broadening of orientation tuning in some two-thirds of cells. Broadening of tuning always reflected an increase in response to non-optimal orientations, but typically occurred in the absence of a marked increase in response to the optimum. Application of GABA at sites where the preferred orientations were within 22.5° of that of a recorded cell (iso orientations) rarely affected orientation tuning, but often modified directional specificity due to an increase in response to the direction of motion preferred by cells at the inactivation sites; the result was either an increase or a decrease in directional selectivity depending on the relative direction preference at the recording and inactivation sites. All GABA-induced effects had a short time course and were reversible. The results provide evidence for a contribution of cross-orientation inhibition to orientation tuning and of iso-orientation inhibition to directional selectivity.

73.9

PATHWAYS IN THE VISUAL CORTEX REVEALED BY RECEPTIVE FIELD MAPPING OF CORRELATED DISCHARGE.

G.M. Ghose*, I. Ohzawa, and R.D. Freeman. Biophysics and Neurobiology Groups, University of California, Berkeley, CA, 94720.

Cross correlation analysis of the discharge activity from two neurons has been used to infer neural interactions in a large number of preparations. One limitation in interpreting correlated activity is that it can arise as a result of a single shared common input or because of the temporal superposition of many different neural interactions.

In order to identify pathways leading to pairs of neurons within the cat's visual cortex, we constructed receptive field maps of correlated discharge. Correlated discharges that originate from different pathways are expected to have different latencies or spatial profiles reflecting the underlying inputs. Extracellular activity from pairs of cells was recorded during the presentation of random sequences of small bright and dark bars flashed in and around the two receptive fields. Reverse correlation of spike pairs is used to reveal the spatiotemporal stimulus selectivity of correlated discharge.

For most of the cell pairs studied, multiple features are observed in the spatiotemporal response maps of correlated discharge. These "receptive fields" of correlated discharge can be classified on the basis of their latencies. Single regions whose peak precedes the response peak of each member cell by about 20-50 ms. are associated with nearly synchronous correlated discharge. Response peaks which follow the responses of member cells by 50-90 ms. include cases of asynchronous discharge, with interspike intervals between the two neurons as large as 100 ms. Unlike the early responses, more than half of these late responses are elicited by stimuli presented outside of the classical receptive fields of the two cells.

These results suggest a two stage sequence of stimulus-evoked discharge in the visual cortex. Synchronous geniculate inputs invoke primary cortical responses. Secondary responses, often consisting of asynchronous discharge between cortical neurons, reflect intracortical and extra-receptive field feedback. (EY01175)

73.10

V1 RESPONSES TO TWO-SURFACE TRANSPARENT AND NON-TRANSPARENT MOTION.

Ning Qian, Richard Andersen and Edward Adelson, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

It is well known that a pattern composed of two sets of random dots moving across each other produces a perception of motion transparency while a counterphase grating consist of two identical sine wave gratings drifting in opposite directions does not. We recorded from the directional selective V1 cells of a behaving macaque monkey using these two types of two-surface patterns together with their corresponding one-surface patterns (i. e., a single moving random dot or sine wave pattern). We first compared a cell's response to a one-surface pattern moving in its preferred direction with its response to the corresponding two-surface pattern having one component moving in the preferred direction and the other in the antipreferred direction. This allowed us to determine the amount of antipreferred direction inhibition. We then compared this inhibitory effect between the random dot patterns and the counterphase gratings. We found that most cells showed similar amount of inhibition among these two types of patterns. Furthermore, for the majority of the cells this inhibitory effect was weak. This result suggests that cells appear to detect the component moving in their preferred directions regardless of whether the two-surface pattern is perceptually transparent or not. Thus the perception of transparent motion must be processed at a stage beyond V1. We have also generated some other two-surface patterns that are perceptually either transparent (e. g., randomly spaced line patterns) or non-transparent (e. g., equally spaced line and paired random dot patterns). Using Adelson-Bergen model of motion detection, we found through computer simulations that the presence or the absence of motion transparency in these patterns can be predicted by the amount of locally unbalanced motion energies in opposite directions and that V1 cells are rather similar to the unidirectional motion energy detectors. We are currently testing whether MT neurons respond differently to transparent and non-transparent patterns.

73.11

A MODEL OF DIRECTION-SELECTIVITY IN VISUAL CORTEX WITH MASSIVE EXCITATORY FEEDBACK. H.H. Suarez, C. Koch & R.J. Douglas. CNS, Caltech, Pasadena, and Department of Physiology, University of Cape Town Medical School, South Africa.

The model consists of two populations of cortical neurons. One population is excitatory and represents the pyramidal neurons, and the other is inhibitory and represents smooth cells. Each cell has two compartments: the soma, and a dendritic cylinder. The pyramidal somata contain seven ionic currents that obey Hodgkin-Huxley- like dynamics, including calcium-dependent adaptation. The synaptic connections within this network are consistent with the canonical microcircuit (Douglas and Martin, 1990): the major part of the excitatory input to cortical cells is derived from other cortical cells, and only a minor part (20 %) is derived from geniculate input. Visual input from the geniculate is simulated by a detailed model of X-cells (Victor, 1987). Inhibition is supplied only by intracortical neurons. In the null direction, the weak geniculate excitation is vetoed by simultaneous cortical inhibition. However, in the preferred direction, cortical inhibition is delayed and so the weak geniculate input is amplified by the excitatory cortico-cortical connections (Douglas and Martin, 1991). In this model, single cells show only small increases in input conductance (< 30 % relative to control) in the null direction. These changes are consistent with *in vivo* intracellular recordings (Douglas et al., 1988). Despite the strong excitatory feedback connections, the neural responses are graded with stimulus contrast because adaptation restricts the discharge of the pyramidal cells.

73.12

STEREOSCOPIC RESPONSES IN MACAQUE VISUAL CORTEX: EFFECTS OF LUMINANCE AND COLOR. G.F.Poggio and C.E.Connor, The Bard Laboratories, Dept. of Neuroscience, Johns Hopkins University, Baltimore, MD 21205.

Depth perception is degraded for chromatic stimuli under conditions of isoluminance. To evaluate the neural mechanisms underlying the phenomenon, we have analyzed the response of disparity-selective neurons in V1, V2 and V3 complex of the visual cortex of the alert rhesus monkey performing a fixation/detection task, during presentation of behaviorally irrelevant dynamic random-dot stereopatterns (RDS) of different contrast but constant average luminance, and of solid-figure stereograms (SFS). Typically, the stereo response was unaffected by luminance contrast of chromatic SFS (e.g. red bar on green background), whereas the response to monochromatic SFS (e.g. red on red) was predictably smaller the lower the luminance contrast. The response to red/green RDS, on the other hand, was commonly reduced at lower contrasts, though only seldom abolished at or near isoluminance. For some neurons, the magnitude of the stereo response was uniformly strong at all contrast conditions in which one of the two colors of the RDS was brighter (usually green > red), and it became significantly smaller when the contrast reversed. Finally, a few neurons gave equal responses to chromatic RDS irrespective of the contrast between red and green components. No relations were observed between types of disparity selectivity (tuned or near/far) and contrast effects. The results indicate that both brightness and chromatic signals may contribute to the magnitude of the stereo response, and have different interactive effects depending on the spatial configuration of the visual pattern. (Grant EY02966).

FORMATION AND SPECIFICITY OF SYNAPSES I

74.1

THE ROLES OF THE SYNAPTIC BASAL LAMINA AND OF INNERVATION IN DIRECTING THE ACCUMULATION OF A SYNAPTIC MOLECULE, mAb 3B6 Antigen, IN REGENERATING SKELETAL MUSCLES. S. Rotshenker, H. Sugarman and A. Dunaevsky-Hutt. Dept. of Anatomy, Hebrew Univ. Med. Sch. Jerusalem, Israel.

MAb 3B6 antigen, a molecule concentrated at endplate / junctional regions and myotendinous junctions in innervated muscles, appears in denervated muscles in restricted perijunctional areas that are continuous with and centered on endplates. In 86% to 90% of denervated regenererating myofibres, mAb 3B6 antigen was preferentially localised at former junctional and perijunctional regions. In innervated muscles, this colocalization was 97% to 99%. A peri-junctional distribution of the antigen was present in 85.9% of denervated regenerating myofibres compared to 50.5% of innervated ones. MAb 3B6 antigen was detected in the cytoplasm of most denervated regenerating myofibres but in none of the innervated ones. These results indicate that the basal lamina directs the preferential accumulation of mAb 3B6 antigen at original synaptic sites whereas innervation down regulates its overall production and presence in perijunctional regions.

74.2

AGRIN SYNTHESIZED BY MOTOR NEURONS INDUCES MYOTUBES TO AGGREGATE AChRs AT NEUROMUSCULAR JUNCTIONS IN CULTURE. Noreen E. Reist, Michael J. Werle, and U.J. McMahan. Dept of Neurobiology, Stanford University, Stanford, CA 94305.

During development, motor neurons provide molecules that induce myotubes to aggregate acetylcholine receptors (AChRs) at developing neuromuscular junctions. Several lines of evidence have led to the hypothesis that the inductive molecule is agrin, a protein that causes cultured myotubes to form aggregates of AChRs. We have generated anti-agrin sera that inhibit the aggregating activity of agrin on cultured myotubes. These sera stain the neuromuscular junctions in chick but not rat muscles, indicating that they recognize agrin in a species specific manner. To test the ability of the anti-agrin antibodies to inhibit motor neuron induced AChR aggregation, we cocultured chick motor neurons with chick myotubes or chick motor neurons with rat myotubes in the presence of the anti-agrin IgGs. In both types of culture the number of AChR aggregates on the myotubes at and near neuromuscular contacts was markedly lower than in similar cultures grown in presence of preimmune or normal IgGs. On the other hand, the anti-agrin IgG had no effect on the number of AChR aggregates induced on chick myotubes by rat motor neurons. These experiments provide the most direct evidence to date that agrin synthesized by motor neurons mediates the formation of AChR aggregates at developing neuromuscular junctions.

74.3

cDNA THAT ENCODES AGRIN. K.W.K. Tsim, M.A. Ruegg, G. Escher, S. Kröger and U.J. McMahan. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

We have for the first time isolated a cDNA that encodes a protein which, based on sequence homology, antigenicity, and activity is agrin, a basal lamina molecule that induces cultured myotubes to form on their surface aggregates of acetylcholine receptors (AChRs). The full-length agrin cDNA is 8,045 nucleotides long and contains an open reading frame encoding a protein of 1,917 amino acids with a calculated M_r of 206,695. The deduced protein has four potential N-glycosylation sites and several repeated regions containing motifs that are similar to those in other proteins: 8 cysteine-rich repeats similar to repeats in laminin and s-laminin, 6 cysteine-rich repeats similar to EGF-repeats and 9 cysteine-rich repeats similar to the active domain of the Kazal family of protease inhibitors. Expression studies reveal that agrin can be distinguished from agrin-related proteins in the CNS by its having an 11 amino acid stretch absent from agrin-related proteins and that this stretch is required for AChR-aggregating activity. Polymerase chain reaction studies on RNA from the marine ray CNS reveal that this stretch is conserved in agrin across species, as expected. Similar studies show that fractions of chick spinal cord enriched in motor neurons are enriched in agrin transcripts while fractions enriched in non-motor neurons are enriched in the transcripts of agrin-related proteins. Altogether, these findings support the hypothesis that agrin is synthesized by motor neurons and mediates the motor neuron and basal lamina-induced aggregation of AChRs at the neuromuscular junction in skeletal muscle.

74.4

AGRIN IS A MEMBER OF A GROUP OF MOLECULES HAVING HIGH STRUCTURAL SIMILARITY BUT DIFFERING IN FUNCTION. M.A. Ruegg, K.W.K. Tsim, S.E. Horton*, S. Kröger, and U.J. McMahan. Dept. of Neurobiology, Stanford University, Stanford, CA 94305

We used a 4.7 kb cDNA encoding the region of chicken agrin that is active in aggregating acetylcholine receptors (AChRs) on cultured myotubes to isolate from chicken libraries two homologous cDNAs that encode agrin-related proteins (ARPs). The cDNA for one of the proteins (ARP-1) was from a brain library (E13) and the cDNA for the other (ARP-2) was from a muscle library (E13); both were nearly the same length as the agrin cDNA. The coding regions of the cDNAs for the ARPs were identical to that of agrin except that a 33 base pair (bp) stretch was missing from that of ARP-1 and both the 33 bp and a 12 bp stretch were absent from that for ARP-2. Transfecting the ARP cDNAs into COS cells resulted in the synthesis of proteins recognized by anti-agrin antibodies but inactive in the AChR- aggregation assay on myotubes. Thus, the proteins encoded by the ARP cDNAs were greater than 99% identical to agrin in their amino acid composition but they lacked agrin's activity. Transfection of hybrids between the agrin cDNA and ARP cDNAs showed that the lack of activity in ARPs was owing specifically to the absence of the inserts; accordingly, both the 33 and the 12 bp inserts are required for agrin's AChR aggregating activity. Northern blot analysis indicates that the full length message for the ARPs is similar to that of agrin. Southern blot analysis on genomic DNA revealed that agrin and the ARP transcripts are derived from a single copy gene. Altogether, our findings demonstrate for the first time that agrin is a member of a protein family in which one (agrin) is capable of aggregating AChR on cultured muscle cells and some (ARPs) are not and that agrin and ARPs are generated by alternative mRNA splicing.

74.5

FOCALLY APPLIED AGRIN INDUCES THE FORMATION OF ACHR AGGREGATES AT SITES OF AGRIN-MYOTUBE INTERACTION.
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Several lines of evidence have led to the hypothesis that agrin mediates the motoneuron- and basal lamina (BL)-induced aggregation of AChRs in developing and regenerating skeletal muscles. The hypothesis predicts that the aggregates form at or near the site where agrin interacts with the myofiber. Accordingly we devised a way to apply agrin focally to cultured myotubes and determined the distance between the site of application and the induced AChR aggregates. COS-7 cells, transfected with a construct of a partial agrin cDNA that encodes the active region, were seeded at low density onto a sheet of BL isolated from the chick retina. Agrin secreted by the COS cells adhered tightly to the BL in the vicinity of the cells. Myoblasts seeded onto the BL sheets after removal of the COS cells formed myotubes some of which were directly apposed to the agrin patches. By differential staining of the agrin patches and AChR aggregates we observed that agrin-induced AChR aggregates were coextensive with the agrin patches, even where the patches were only 5μm in diameter. We conclude that agrin induces the formation of AChR aggregates at or very near the site where it interacts with cultured myotubes. By transfecting a number of different cDNA constructs, we identified a stretch of approximately 100 amino acids that is required for binding the secreted agrin to the BL sheets.

74.6

TRANSCRIPTS RECOGNIZED BY PROBES FOR AGRIN/AGRIN-RELATED mRNA IN MOTOR NEURONS ARE DEVELOPMENTALLY REGULATED. G. Escher, K. Tsim. S. Horton*, & U.J. McMahan. Department of Neurobiology, Stanford CA 94305-5401.

RNA probes that recognize transcripts for both agrin and agrin-related proteins strongly label by *in situ* hybridizations motor neurons and certain nuclei, laminae and ganglia throughout the nervous system. As part of a study aimed at determining the functional role of these proteins and how they are regulated, we used *in situ* hybridization methods to examine in chick lumbosacral motor neurons the level of expression of the transcripts at different times during development. The earliest day that elevated levels of agrin/agrin-related transcripts were detected was E5, the day at which the motor neurons are beginning to form functional neuromuscular junctions. From E7 to E13, when there is an exponential increase in the formation of neuromuscular contacts, levels of agrin/agrin-related mRNA became markedly increased. By one month after hatching, when neuromuscular junction formation is nearly complete, the level of transcripts is greatly reduced, but above background. The correlation between increased levels of agrin/agrin-related transcripts and the period of neuromuscular synapse formation is consistent with the hypothesis that agrin mediates the motor neuron-induced aggregation of AChRs on myofibers at the developing neuromuscular junction.

74.7

EXTRACTS ENRICHED IN BASAL LAMINA PROTEINS INDUCE MOTOR NEURONS TO FORM PRESYNAPTIC SPECIALIZATIONS IN THEIR NEURITES. M.J. Werle, Z.O. Obeso*, and U.J. McMahan. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305

Molecules stably bound to synaptic basal lamina at neuromuscular junctions direct the formation of presynaptic specializations in regenerating axon terminals. The specializations include enlargements (varicosities) of the terminal which have a high concentration of synaptic vesicles and active zones. We have found that basal lamina fractions of the synapse-rich electric organ of *Torpedo* induce motor neurons to form varicosities, having high concentrations of vesicles, in their neurites. The active molecules migrate on gel filtration columns along with standards having a molecular mass of 15-30 kDa. Treatment of the extracts with trypsin or heat destroys the activity indicating that the active molecules are proteinaceous. Monoclonal antibodies that immunoprecipitate the activity recognize molecules highly concentrated at the neuromuscular junctions of *Torpedo* and frog as well as the innervated surface of the *Torpedo* electric organ. Altogether, our findings raise the possibility that the active protein(s) mediate(s) the basal lamina-induced formation of some, perhaps all, of the presynaptic apparatus in regenerating axon terminals and that it is the counter part of agrin, the protein thought to mediate the basal lamina induced formation of postsynaptic apparatus in regenerating muscles.

74.8

NEURONALLY DERIVED AGRIN-LIKE MOLECULES APPEAR EARLY AT EMBRYONIC FROG NERVE-MUSCLE SYNAPSES IN CULTURE. M.W. Cohen and E.W. Godfrey. Dept. of Physiology, McGill Univ., Montreal, Que. and Dept. of Cellular Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI.

If agrin or a closely related molecule is the neural agent which causes aggregation of acetylcholine receptors (AChRs) during nerve-muscle synaptogenesis, agrin-like molecules derived from the neuron should be present at the synapse from the onset of AChR aggregation. This prediction was tested in cultures of spinal cord (SC) and myotomal muscle (M) cells derived from embryos of Xenopus laevis (X) and Rana pipiens (R). Using anti-agrin antibodies which are reactive in both species, we observed immunostain at AChR aggregates along all four combinations of nerve-muscle contact, and even at the smallest (<0.5 μm) and most distal aggregates along early (<8 hr) SC_X-M_X contacts. In contrast, there was little if any stain at AChR aggregates on non-innervated M_X and M_R cells in the same cultures. Furthermore, an anti-agrin antibody which is reactive in R but not in X stained sites of aggregated AChRs along SC_R-M_R and SC_R-M_X contacts but not along SC_X-M_X and SC_X-M_R contacts. These results provide the most direct evidence to date that neuronally derived agrin-like molecules are present at embryonic nerve-muscle synapses from the onset of AChR aggregation.

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74.9

CHARACTERIZATION OF A PUTATIVE AGRIN RECEPTOR FROM TORPEDO ELECTRIC ORGAN J. R. Fallon, J-Y. Ma* and M. A. Nastuk. Worcester Fdn. Exper. Biol., Shrewsbury, MA. 01545

Agrin is a component of the synaptic basal lamina that is likely to play a central role in orchestrating postsynaptic differentiation at the neuromuscular junction. However, little is known about how agrin interacts with the cell surface, or of the nature of its membrane receptor. Here we have used membrane preparations from the synapse-rich *Torpedo* electric organ (TEO) to characterize the cell surface receptor for agrin. TEO membrane fractions bind soluble agrin as judged by immunofluorescence microscopy, RIA, and absorption of bioactive agrin from solution. Binding measured by all three assays requires calcium and is abolished by pretreatment of the membranes with trypsin. Agrin binds selectively to TEO membrane fractions enriched in synaptic components: membranes from non-synaptic regions of electric organ or from *Torpedo* liver show low or undetectable levels of agrin binding. Agrin binding to TEO membranes is unaffected by removal of peripheral membrane proteins by alkaline treatment. The putative agrin receptor from TEO membranes can be solubilized in non-ionic detergent with retention of calcium sensitive binding, and can be separated from the AChR by gel filtration chromatography. We are currently using affinity methods to identify and purify this receptor. The high affinity, saturable, and calcium dependent agrin binding of this integral membrane protein, coupled with its localization to synaptic membranes, strongly suggest that the molecule characterized here represents the cell surface receptor that mediates agrin-induced AChR clustering. Support by ACS, March of Dimes and HD 23924.

75.1

DUAL REGULATION OF C-FOS TRANSCRIPTION BY CALCIUM AND CYCLIC AMP IN PRIMARY CULTURES OF CORTICOSTRIATAL NEURONS. F.M. Vaccarino, H.N. Le*, E.J. Nestler. Dept. of Psychiatry, Yale University, New Haven, CT 06508.

NMDA receptor agonists increased the level of c-fos mRNA in neuronal monolayers from rat cerebral cortex and striatum; this increase appeared to be dependent on a transmembrane calcium influx and on the activation of protein kinase C (PKC). Activators of adenylate cyclase, such as vasoactive intestinal peptide (VIP) and dopamine D_1 receptor agonists, also increased c-fos mRNA; this action was mimicked by forskolin and by lipophilic analogs of cAMP and was mostly independent of extracellular calcium. To define the DNA elements responsible for the calcium/PKC- and the cAMP-regulated c-fos transcriptional activation, we performed transient transfection experiments of c-fos promoter constructs. Primary cultures of corticostriatal neurons were transfected with chimeric constructs carrying deletion mutants of the c-fos promoter fused to the bacterial gene for chloramphenicol acetyltransferase (CAT). A plasmid carrying the β-galactosidase gene was co-transfected to provide an internal control for transfection efficiency. Different varieties of neurons stained for β-galactosidase by histochemistry, while glial cells were unstained. The induction of CAT by different c-fos promoter constructs after cell stimulation by agents specific for the calcium/PKC or for the cAMP second messenger pathways will be discussed.

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75.2

INTERACTION OF CYCLIC AMP AND PHOSPHOINOSITIDE SECOND MESSENGER SYSTEMS. B.W. Rigatti, M. Smith*, J.J. Mann. Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

The interaction of second messenger systems in neurons is a critical aspect of cellular function under normal conditions or pathological states. We therefore studied the effects of phytohemagglutinin (PHA) and beta-adrenergic receptor stimulation on the cyclic AMP (cAMP) and the phosphoinositide (PI) second messenger systems in lymphocytes obtained from normal human venous blood.

Phytohemagglutinin (PHA) stimulation of PI turnover was 5.07 ± 2.7 times unstimulated controls ($p < 0.05$). The beta-adrenergic receptor agonist, isoproterenol (ISO) alone, did not alter PI turnover in comparison to the control. The combination of PHA and ISO stimulated PI turnover 3.54 ± 2.04 times unstimulated controls. The addition of ISO did not depress PI turnover to PHA compared to the PI response to PHA alone ($p > 0.20$).

PHA did not alter cAMP levels (8.8 ± 2.2 pmol/10^6 cells) in comparison with unstimulated controls (9.0 ± 1.6 pmol/10^6 cells). ISO alone stimulated cAMP formation 2.5 times over both PHA treated cells and controls, producing levels of 22.1 ± 2.7 pmol/10^6 cells. The combination of PHA and ISO stimulated cAMP levels to 2.7 times those of the PHA treated cells or controls, producing levels of 23.8 ± 2.7 pmol/10^6 cells.

These experiments suggest that the elevation of cyclic AMP levels by the beta-adrenergic receptor agonist, ISO, did not inhibit PHA activation of PI turnover. Similarly, PHA activation of PI turnover failed to alter beta-adrenergic generation of cyclic AMP. We conclude that in the lymphocyte there is no inhibitory effect on PI turnover by the cAMP second messenger system, and that the PI second messenger system has no effect on the cyclic AMP system. Future studies should examine neuron and other cell systems to determine where similar constraints on second messenger cross-talk are present. This work was supported by MH40695.

75.3

MONITORING INTRACELLULAR CYCLIC NUCLEOTIDES IN LIVING NEURONS WITH DIRECTLY CYCLIC NUCLEOTIDE-GATED CHANNELS FROM OLFACTORY RECEPTOR CELLS. R.H. Kramer, Ctr. for Neuro. and Behav., Columbia Univ., New York, NY 10032

Vertebrate olfactory receptor cells contain cation channels that are directly activated by cAMP and cGMP. Here it is shown that these channels can be used as cyclic nucleotide detectors in other cell types. The receptive portion of dissociated catfish olfactory receptor cells (dendrite and cilia) was sucked into a patch pipette (~1 uM tip diam.). After forming a gigaohm seal, the cell was passed through the air/water interface, disintegrating the soma and exposing an extensive inside-out "patch" containing hundreds of channels. Calibration of cyclic nucleotide sensitivity revealed identical $K_{1/2}$'s of activation for cAMP and cGMP (about 2 uM), with a detectable current appearaing at concentrations as low as 0.1 uM. The cyclic nucleotide-gated current did not desensitize, and was unaffected by cAMP-dependent protein kinase, GTP-γ-S, IP_3, and internal Ca ions (at -50 mV). Hence the channels are reliable selective indicators of cyclic nucleotides.

After calibration, a patch pipette containing the channels was inserted into an intact living neuron in the subesophageal ganglion of the land snail Helix. A microeletrode in the neuron was used to monitor and control voltage. Application of 20 uM 5-HT, which modulates ionic currents and broadens action potentials, elicited an inward current in the detector patch, equivalent to increasing cyclic nucleotide concentration to 1 uM. Activation and recovery of the detector current was rapid (<10 sec). Repeated 5-HT applications resulted in a diminished neuronal response and a diminished detector current, indicating that desensitization occurs at the level of cyclic nucleotide concentration.

75.4

OLFACTORY RECEPTOR NEURONS EXPRESS COMPONENTS OF TWO DISTINCT SECOND MESSENGER SYSTEMS : $INSP_3$ AND THE G-PROTEIN Golf. A.M. Cunningham,* G.V. Ronnett, P.B. Manis, R.R. Reed and S.H. Snyder. Dept of Molecular Biology and Genetics and Dept of Neuroscience, The Howard Hughes Medical Institute, The Johns Hopkins University Sch. of Med., Baltimore, MD 21205.

Odorant-induced signal transduction occurs in the specialized cilia of olfactory sensory neurons. Many lines of evidence indicate this process is mediated at least in part by a G-protein coupled cascade which utilizes cAMP as an intracellular second messenger. The recent description of a multigene family encoding olfactory specific seven transmembrane domain proteins, putative odorant receptors, strengthens this hypothesis [Buck & Axel, 1991]. Recently, $InsP_3$ has been shown to dynamically increase in cilia preparations in response to some odorants [Boekhoff et. al., 1990] raising the possibility that the two pathways may function, perhaps interactively, in odorant detection.

Utilizing the previously described technique for primary culture of neonatal rat olfactory neurons [Ronnett et. al., 1991] we varied the culture conditions to enrich for a particular neuronal class based on phenotype - designated ORN-1. These small bipolar cells were easily distinguishable morphologically from other neuronal types present. PCR analysis of the cultures indicated mRNAs for Golf and the olfactory cyclic nucleotide-activated channel were present in low abundance. In contrast, $InsP_3$ receptor and NGFR mRNAs were abundant. These findings were confirmed by Northern analysis. Immunocytochemistry revealed that ORN-1 cells expressed Golf, OMP and other proteins known to be enriched in olfactory neurons but were negative for GFAP and other non-neuronal markers. ORN-1 cells were also immunoreactive for synaptophysin, N-CAM and $InsP_3$ receptor. Preliminary electrophysiological studies showed the cells generated action potentials in response to depolarizing current pulses and had appropriate inward and outward currents. Double labeling immunofluorescence confirmed that all ORN-1 cells identified in culture expressed both Golf and $InsP_3$ receptor. Similar analysis of adult olfactory neuroepithelium by confocal microscopy showed Golf and $InsP_3$ receptor colocalized to the cilia, although $InsP_3$ receptor had a less restricted distribution than Golf, being also present in the dendritic process of the neuron. Binding studies on cilia preparations confirmed specific $InsP_3$ binding activity. Colocalization of the two components, $InsP_3$ receptor and Golf, in the same sensory neuron presents intriguing opportunities for interaction of the two signaling pathways, including the possibility that some olfactory receptors directly activate both cascades.

75.5

SECOND MESSENGER SYSTEMS ACTIVATED BY ODORANTS IN PRIMARY CULTURES OF OLFACTORY RECEPTOR NEURONS. G.V. Ronnett, D.J. Parfitt, L.D. Hester and S.H. Snyder. Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205.

We have previously described a method for primary culture of rat neonatal olfactory receptor neurons in which odorants elicit rapid, transient elevations in intracellular cAMP levels and adenylyl cyclase (AC) activity (Ronnett et.al., 88:2366-2369, 1991). The degree of elevation is both odorant and concentration dependent, showing activity for some odorants at sub-nanomolar concentrations. This odorant sensitive AC activation is entirely calcium-dependent, with maximal activation between 10-100 μM free calcium. Odorants cause an even more rapid increase in production of inositol phosphates. To investigate a possible inter-dependence of these two signalling pathways, the ability of odorants to induce calcium flux in cultured cells as well as the ability of calmodulin inhibitors to prevent cyclase activation were studied. The complex interactions suggested by these experimental results may have implications for other receptor systems.

75.6

SECOND MESSENGER RESPONSES OF THE CHICK RETINAL MELATONIN RECEPTOR. J.T. Laitinen[1] and J.M. Saavedra[2]. 1) Dept Physiol, Univ Kuopio, SF-70211 Kuopio, Finland and 2) Sect Pharmacol, Lab Clin Sci, NIMH, Bethesda, MD 20892.

We have recently localized the chick retinal melatonin (MT) receptor to the inner plexiform layer (Brain Res. 1990,528,349). This site shows GTP modulation of agonist binding and thus resembles the mammalian MT receptor. The present studies were aimed to clarify MT signal transduction pathways in the chick retina.

In the presence of 1 mM IBMX, forskolin (FSK) caused a 50-fold increase in cAMP accumulation (EC_{50} 10 μM). At higher doses, FSK also increased cGMP levels. MT had modest inhibitory effects on basal and FSK-stimulated cAMP levels, as well as on basal cGMP levels. In our hands, cholinergic stimulation elicited a 3-fold increase in retinal IP_1 accumulation. MT had no effect on retinal phosphoinositide metabolism. In chick retinal membranes, MT failed to affect Na^+/K^+-ATPase activity suggesting no direct interaction with this pump. However, a modest inhibition of ouabain sensitive Rb^+-uptake was observed.

We conclude that multiple second messenger systems could be affected by MT receptor activation in the chick retina.

75.7

PHOSPHORYLCHOLINE-STIMULATED PROLACTIN RELEASE: A NOVEL SECOND MESSENGER SYSTEM. *W.D. Jarvis[1] and R.M. MacLeod, Departments of Neuroscience[1] and Medicine, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908.*

Receptor-activated catalysis of choline-linked phospholipids occurs in both transformed and normal cell types, including anterior pituitary cells, through multiple enzymatic routes. Most notable are phospholipase C-mediated cleavages of choline-linked phosphatides and sphingomyelins to yield a complex spectrum of diglycerides and ceramides. These lipid messengers are widely known to act as both positive and negative effectors of various isoforms of protein kinase C; more obscure, however, is the biological relevance of phosphorylcholine (*P*Cho), the obligatory co-product of these reactions.

We have evaluated the potential effector activity of *P*Cho in modulating prolactin release. Primary cultures of normal female rat anterior pituitary cells were incubated with *P*Cho at varying concentrations (0.001-10 μM) for varying intervals (15-120 min) in serum-free RPMI-1640 medium. Exposure to *P*Cho for 30 min resulted in significant ($p<0.01$) increases (226%) in prolactin release, but was without effect on growth hormone release; free choline and pyrophosphate were inefective in modifying prolactin release, as were free and phosphorylated headgroup compounds of other phospholipid species. The response to *P*Cho proved to be concentration-related, exhibiting an EC_{50} of approximately 720 nM, and was abolished by co-exposure to dopamine (500 nM) or somatostatin (250 nM). A similar ability of *P*Cho to elicite hormone release was also demonstrated in transformed pituitary cells such as the GH_3 mammosomatotrophic cell line and the dopamine-sensitive MMQ subclone of the 7315a lactotrophic tumor cells. We conclude from these observations that *P*Cho acts as a cellular effector molecule and participates in receptor-activated prolactin release from normal and transformed pituitary cells. Supported by NIH grant CA-07535.

75.8

CORTICOSTERONE DEPENDENCE AND TIME COURSE FOR RESPONSIVENESS OF MEDIAL PREOPTIC AREA (mPOA) NEURONS TO PGE_2. G.E. Resch. Univ. of Missouri-Kansas City, MO 64108.

Corticosterone (B) restores cold tolerance in adrenalectomized (Adx) rats following whole body hormone replacement. A previous report from this laboratory demonstrated restoration of cold tolerance following replacement of compound B in the mPOA at doses that were not effective when administered subcutaneously. Adx rats exposed to -4°C for up to 2 hrs. showed a 3.2 ± 0.4°C (mean ± SEM) fall in Tc. PGE_2 and B were microinjected into awake unrestrained female Sprague Dawley rats (200-250 gm) using a 29 ga injector inserted through a guide tube implanted into the mPOA 7.5 mm below bregma and 0.5 mm lateral to the midline. All injections were unilateral. Tc was monitored with a YSI telethermometer. PGE_2 microinjection into previously identified mPOA PGE_2 sensitive sites elicits a rise in colonic temperature (Tc) in the intact rat but not in the Adx rat. Replacement of B in the mPOA at low doses (100 ng 2X/d) for five days restored the Tc responses to PGE_2. Replacement of B into PGE_2 sensitive sites of the mPOA for 2 hrs., 2 days, or four days had no effect on cold tolerance or PGE_2 elicited responses. Restoration of cold tolerance and PGE_2 responsiveness in Adx rats occurred between days 4 and 6 of hormone replacement. This time course is similar to the restoration of cold tolerance after whole body B replacement. Replacement of B unilaterally in ADX rats restored cold tolerance and Tc response after PGE_2 microinjection to 84% and 82% of normal, respectively. The data indicate that cold tolerance involves neuronal responsiveness to PGE_2 and further that B effects on cold tolerance are at least partially accounted for by B effects on PGE_2 responsiveness of mPOA neurons.

75.9

FREE ARACHIDONIC ACID LEVELS CONTROL GLUTAMATE UPTAKE RATE IN NEURONS AND ASTROCYTES. A.Volterra, D.Trotti*, P.Cassutti*[1], C.Tromba* & G.Racagni. Ctr. Neuropharmacology & Inst. Pharmacol. Sci., Univ. Milan, [1] Farmitalia Carlo Erba, Milan, Italy.

High-affinity glutamate (GLUT) uptake systems on neuronal terminals and astrocytes control GLUT clearance and synaptic availability. Arachidonic acid (AA) is released at GLUT synapses upon NMDA receptors stimulation. Using both synaptosomes and cultured astrocytes from rat cerebral cortex, we find that AA (1-50 μM) reduces GLUT uptake rate up to 50% in a rapid and reversible manner. Poly-unsaturated fatty acids, but not 12-lipoxygenase metabolites mimick AA effect. Potent inhibition is seen also with the PLA_2 activator melittin (0.2 μg\ml) and the AA reacylation blocker thimerosal (100 μM). In prelabelled astrocytes the two agents at these concentrations increase basal ^{3}H-AA extracellular accumulation by 1-2 fold. Albumin, a free fatty acid-binding protein, enhances basal GLUT uptake and prevents inhibition by melittin, thimerosal and AA. These data indicate that neuronal and glial GLUT transport systems are highly sensitive to changes in free AA levels. Therefore, uptake inhibition seems a relevant mechanism underlying AA-induced potentiation of GLUT synapses.

75.10

SYNAPTIC MEMBRANE G-PROTEINS ARE COMPLEXED WITH TUBULIN *IN SITU*. Kun Yan and Mark M. Rasenick. Department of Physiology and Biophysics and the Committee on Neuroscience, University of Illinois College of Medicine P.O. Box 6998 Chicago, IL 60680

Adenylyl cyclase in membranes prepared from C6 glioma cells is activated by GppNHp, an hydrolysis-resistant GTP analog. In saponin-permeable C6 cells, where adenylyl cyclase is coupled tightly to the β adrenergic receptor, GppNHp is without effect in the absence of a β receptor agonist. Despite this, dimeric tubulin liganded with GppNHp, bypasses the receptor to stimulate adenylyl cyclase. Experiments using the photoactivatible GTP analog, azidoanilido GTP (AAGTP) suggest that the mechanism for this involves direct guanine nucleotide transfer from tubulin to the stimulatory G-protein (G_s). Similar experiments with cerebral cortex membranes show tubulin-GppNHp-mediated inhibition of adenylyl cyclase and the transfer of nucleotide from tubulin to Giα. Although both cerebral cortex membrane and C6 glioma membrane have Gs, only the former has Gi1. Since tubulin binds to G_s and G_{i1} with a higher affinity than to G_o, G_t, G_{i2}, and G_{i3}, the lack of G_{i1} in C6 membrane is a likely explanation for the stimulation of adenylyl cyclase by tubulin in C6 cells. In the cerebral cortex synaptic membrane, quantitative immunoblotting suggests that 6% of the total protein (integral and peripheral) is tubulin. Co-immunoprecipitation studies indicate that nearly 90% of the synaptic membrane Gs exists in preformed complexes with membrane tubulin. This is true for only about 8% of the total Gi1. This tight association of tubulin-G_s in the cerebral cortex membrane renders tubulin added to synaptic membranes free to interact with Gi1, ultimately inhibiting adenylyl cyclase. Given the preformed Gs-tubulin complexes in the membrane, it is plausible that tubulin is involved in the stimulation of neural adenylyl cyclase, especially in response to neurotransmitters which are coupled, primarily, to other second messenger systems.

75.11

CATALYTIC SUBUNITS OF *APLYSIA* NEURONAL cAMP-DEPENDENT KINASE WITH NOVEL AMINO TERMINI. S. Beushausen*, E. Lee*, B. Walker* and H. Bayley. Worcester Foundation for Experimental Biology, Shrewsbury MA, 01545. Lab of Neurobiology, NIH, Bethesda, MD, 20892.

cAMP-dependent protein kinase (PKA) is involved in synaptic plasticity in *Aplysia californica*. Two forms of PKA catalytic (C) subunits generated by mutually exclusive use of two internal exon cassettes (A1 and A2) have been demonstrated in *Aplysia* neurons. Two additional subunits with alternative N-termini, N1 and N2, are derived from two exons and spliced in combination with either of the internal cassettes generating four distinct C isoforms. The N1 terminus is homologous to termini previously sequenced in several species and includes a signal for myristylation. The N2 terminus is 21 amino acid residues longer than N1 and has homology with the non myristylated yeast C-subunit, TPK1. Nuclease protection experiments suggest that exon N1 is transcribed from a contiguous promoter region with multiple initiation sites. Exon N2 is upstream from N1 indicating that N2 transcripts arise via alternate promoter use. Quantitative nuclease protection experiments suggest that promoter choice and alternative splicing of the internal cassettes are independent of one another. Transcripts derived from N1 and N2 are of about equal abundance in the nervous system, while A1 containing transcripts are about three times as abundant as those containing A2. N2 polypeptides, enriched from Aplysia extracts, autophosphorylate and can phosphorylate the synthetic peptide substrate, kemptide. These data support the view that subtleties in *Aplysia* neuronal PKA activity arises from a complex array of regulatory and catalytic subunits that generate multiple holoenzyme forms with different substrate specificities, regulation and subcellular localization.

75.12

CHARACTERIZATION OF 12-LIPOXYGENASE IN APLYSIA NERVOUS TISSUE. A.Thekkuveettil*, D.J.Steel, M.Abe*, J.H.Schwartz and S.J.Feinmark. Depts. of Pharmacology, Pathology, Center for Neurobiology and Behavior, and Howard Hughes Medical Institute, Columbia University, NY, NY, 10037

Studies on Aplysia neurons show that 12-lipoxygenase (LO) metabolites modulate ion channel function. The synthesis and significance of these products in vertebrate nervous systems is the subject of current debate. Stereochemical analysis of the Aplysia 12-LO product shows that the isomer formed is 12(S)-HETE, confirming that the process is enzymatic. We are currently localizing and purifying Aplysia 12-LO. In contrast to mammalian LO, which are primarily soluble, most of the Aplysia 12-LO activity is particulate. Like mammalian leukocyte 12-LO, the invertebrate enzyme apparently loses activity during the reaction, possibly because of enzyme inactivation during catalysis. Our results indicate that the spectrum of arachidonic acid metabolites in neurons and the sheath around ganglia are quantitatively different and differentially affected by Ca^{2+}. The sheath serves as a neurohemal organ, receiving and distributing hormones. It is tempting to speculate that 12-LO metabolites may regulate these processes.

76.1

OPTIC AXONS ARE ATTRACTED BY WOUNDS IN ORGAN CULTURED EMBRYONIC CHICK EYES. W. Halfter. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

In order to study the response of growing axons to lesions in the developing nervous system, a wound healing paradigm was devised in organ-cultured embryonic chick eyes. The eyes, consisting of retina and lens, were incubated in culture medium after removing pieces from the E4 to E7 retinal tissue (wound diameter between 100-200μm). Within minutes after lesioning, the inner limiting membrane sealed the wound edges, and after a further 10 hrs, the wound area was reduced to 50% of its original size. The wound was completely closed after 24 hrs in culture. Histological studies showed that the wound closure was not accomplished by cell migration or cell proliferation, but most likely by a contraction of extracellular matrix fibrils of the vitreous body that connected the wound margins. Staining of the specimens with silver, vital dyes or axon-specific antibodies showed that axons from an area of 300 μm around the wound were headed toward the center of the closing wound. The axons left the optic fiber layer at the wound center and formed a neuroma at the ventricular side of the retina, showing a pattern and concentration of nerve fibers similar to that seen at the optic nerve head, the natural convergence point for axons in the retina. The studies suggest that either the mechanics of wound closure or factors secreted at the wound margins attract the growing retinal axons. The navigation of retinal axons toward the wound site in a pattern similar to that seen at the optic disc suggests that the same or a similar axon guiding mechanism operates in both cases.

76.2

A MIDLINE NEURONAL POPULATION IS PRESENT AT THE MAMMALIAN OPTIC CHIASM DURING EARLY RETINAL GANGLION CELL AXON INGROWTH. L. Feng* & D. W. Sretavan, Lab. of Neurobiology, Rockefeller University., New York, NY. 10021

Specific retinal ganglion cell (RGC) axon routing at the mammalian optic chiasm is established during embryonic development as axons from nasal retina project specifically into the contralateral optic tract while a population of RGCs in temporal retina send axons ipsilaterally.

Examination of the neuroepithelial region of the future optic chiasm prior to arrival of retinal axons revealed a population of MAP-2 immunoreactive cells with neuronal-like arborization patterns. These neurons were distributed on the ventral surface of the brain as a striking inverted V-shaped band pointing anteriorly. Individual neurons had multiple processes some of which pointed towards the midline while others ran along the approximate course of the future optic tract. The first retinal axons entering the chiasm grew in close association with the processes of these neurons to turn into one or the other optic tract but did not penetrate posteriorly through this neuronal population. Both ingrowing retinal axons and processes of chiasm neurons at this stage expressed the cell surface molecule L1. With arrival of later retinal axons, the inverted "V" shape pattern was lost and the chiasm neurons were instead distributed as a flattened band straddling the midline with retinal axons crossing the midline directly anterior to them.

The early presence of these neurons prior to retinal axon arrival, their strategic location at the chiasm and the growth of the earliest retinal axons among their processes raise the possibility that these midline neurons influence the navigation of retinal axons at this pathway branch point during embryonic development.

76.3

AXONAL PLASTICITY IN CROSS-FACIAL NERVE GRAFTING J.K. Terzis, M.D., M. Hamilton, and M. Slater
EVMS, MRC, Norfolk, VA. 23507

Facial paralysis is an extremely debilitating disorder. This project seeks to elucidate the mechanisms underlining the regenerating fibers which mediate the success or failure of CFNG.

Work to date has yielded the following results: The establishment of a strong experimental CFNG model in the rat. We have developed a two Stage Cross-Facial procedure in the rat similar to that used in humans. The Saphenous n. is used as the graft, tunneled across the face, and coapted to the donor (L) facial n.(Stage I). Three months later (Stage II) the distal coaptations are made on the contralateral (R) side: Group B; non-connectivity, Group C; correct targets (facial), Group D; foreign target connectivity. The normal anatomy will serve as the control group (A). Three months after stage II, animals are photographed, in vivo nerve stimulations are performed, and all relevant muscles and the graft are harvested for end-plate and axon counts.

Quantitation of end-plate and axon counts have proven that facial and non-facial target muscles became reinnervated. Evaluation of the morphological and electrical data in this model will provide a scientific rationale to guide CFNG procedures.

76.4

THE FIRST PATHWAY OF THE RAT NEOCORTEX IS CONSTRUCTED BY THE THALAMOCORTICAL AND CORTICOTHALAMIC PIONEER AXONS. R. Erzurumlu and S. Jhaveri, M.I.T., Cambridge, MA, 02139.

We have studied the emergence of corticofugal and corticopetal projections in fetal rats. Various combinations of tracers DiI and DiA were placed in the neocortex, dorsal thalamus and midbrain tegmentum, of embryonic (E14-19) rats.

The first axonal profiles seen in the neocortical mantle on E14 are those of preplate (PP) neurons. These axons are located superficially and do not appear to be directed towards the future site of the internal capsule. On E15, cortical plate (CP) is first seen encouched in the ventrolateral PP splitting it into a superficial marginal zone (MZ) and subplate (SP). Concurrently a group of pioneer axons, tipped with large, forking growth cones cascade ventrally over the dorsal striatum. These axons arise from immature, pyramidal-shaped neurons situated in the lower border of the CP, with their apical dendrites branching in the MZ. Corticofugal neurons accumulate along a lateromedial gradient, closely following the progression of the CP in this dimension. None of these cells are back-labeled from the dorsal thalamus on E15. Tracer placements in the dorsal thalamus reveal a second front of pioneer axons ascending towards their corticofugal counterparts. Double labeling experiments show that complex growth cones of both pioneer fronts embrace in the striatum. On E16 the two pioneer systems pass one another and head towards their final targets. Within the intermediate zone of the neocortex, thalamocortical axons fasciculate along a lateromedial gradient just beneath corticofugal axons. At this age, tegmental pioneer axons are situated at the base of the striatum. On E17 thalamic and cortical projection cells can be back-labeled from their respective target zones. This is also the time when tegmental axons are first seen in the developing neocortex. We conclude that: 1) The CP emerges prior to arrival of corticopetal projections; 2) both corticothalamic and thalamocortical axons bridge the pathway between the thalamus and neocortex; 3) The first afferent system to arrive in the neocortex is from the dorsal thalamus. Supported by NIH Grant NS 27678.

76.5

MONOCLONAL ANTIBODY DELINEATES A SUBSET OF OPTIC NERVE FIBERS IN XENOPUS. S. Hoskins, G. Kirchbaumer*, and D. Eastzer*, Biology Dept., City College of CUNY, New York NY 10031.

We are using a monoclonal antibody approach to define molecules whose distribution might differentially mark subsets of retinal ganglion cells or their axons, and perhaps play a role in guiding axons to proper CNS targets.

Gro1 was generated in mice tolerized over a 4 day neonatal period with membrane preparations from thalamus of premetamorphic tadpoles. Beginning at postnatal day 16, the mice were immunized with membrane prepared from ipsilateral thalamus and optic tract of one-eyed frogs or metamorphosing tadpoles. Fusions were carried out by standard methods during the tenth postnatal week, and supernatants screened on methanol fixed sections of fresh-frozen brain. Gro1 staining is visualized using HRP-coupled secondary antibodies.

Gro1 staining is found on optic, olfactory and vomeronasal nerves, beginning by premetamorphic stage 50 and persisting postmetamorphically. In the central retina, gro1 immunoreactivity is found in a small number of ganglion cells interspersed with unstained cells. Stained cells are not seen in peripheral retina. Near the eye, gro1 positive fibers are seen throughout the optic nerve. Near the brain, immunopositive fibers lie primarily ventrally and at the periphery of the nerve. In the optic chiasm, gro1 positive fibers are found ventrally. In the brain, gro1 positive fibers innervate both thalamic and tectal targets, but thalamic innervation is quite sparse.

Gro1 appears to define an epitope shared by a subset of ganglion cells whose axons segregate in the tadpole optic nerve and chiasm. The retinal locations of gro1 positive ganglion cells and the staining patterns in brain suggest that gro1 expression may correlate with CNS destination.

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76.6

ALTERED AXONAL TRAJECTORIES IN *FAS* AND *NAC* MUTANTS OF *DROSOPHILA MELANOGASTER*. K.E. Whitlock and J. Palka, Dept. of Zoology, University of Washington, Seattle, WA 98195.

The wing of *Drosophila* has several classes of readily identifiable sensory receptors. The neurons associated with these receptors arise in the periphery and grow centrally to make connections in the central nervous system (CNS). The morphology of their central terminations was studied by staining the axons with Di I (Molecular Probes) and light reacting with DAB to yield permanent preparations. The preparations were then drawn with a camera lucida and analyzed for tract pattern and degree of branching.

There are several distinct axonal tracts and areas of branching in the CNS of wild type flies to which specific sensory receptors on the wing project. Several of the *fasciclin (fas)* mutants as well as the *neurally altered carbohydrate (nac)* mutant display altered axonal trajectories in the CNS when compared to wild-type flies and controls with similar genetic backgrounds. One phenotype seen in both *fas II* mutants and *fas I; fas III* double mutants is extra branching within the normal tract pattern. A second phenotype observed is the reduction or complete loss of one of the tracts apparently due to the axons shifting to a neighboring tract. This was seen in *fas III* and in the most extreme form in *nac* flies. The finding that *fas* and *nac* mutants display altered axonal trajectories in the CNS implies that the cell surface molecules encoded or affected by these genes are important in the regulation of pathway choice.

Mutants were further analyzed by labelling developing neurons in the periphery using anti-HRP. Both misrouting of normally present axons and the appearance of ectopic neurons in the wing were observed.

Finally, the behavior of the mutants was studied using a phototaxis test to assess sensory-motor function on a gross level. Several of the *fasciclin* mutants and the *nac* mutant display aberrant phototaxis, but the cause of this altered behavior could not be assigned to any observed structural defect. This research was supported by a NIH Traineeship (KEW) and NINDS Research Grant R01-NS07778 (JP).

76.7

SOMITE REMOVAL DISRUPTS THE DISTRIBUTION OF TENASCIN AND THE PATTERN OF PERIPHERAL NERVE PROJECTIONS. J. W. Yip and Y. P. L. Yip*. Dept. of Physiology, University of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

The relationship between the hexabrachion extracellular matrix molecule tenascin and the outgrowth of peripheral nerves was examined in normal and somite-removed chick embryos using double immunofluorescent staining. Cryostat sections of normal and experimental embryos at different stages of development were stained with EC/8 antibody against intermediate filaments in neuronal processes and with a polyclonal antibody against tenascin. In the normal embryo, the outgrowth of peripheral nerves follows a tenascin rich pathway restricted to the anterior half of the somite. Indeed, spinal nerves at the brachial levels follow a tenascin rich girdle into the limb. With time, tenascin disappears along neuronal pathways and is found only to surround peripheral nerves.

To examine whether tenascin along nerve pathways is produced by somitic cells, and whether the somite is involved in the growth and guidance of peripheral nerves, segments of newly formed somites or the segmental plate of Stages 13-14 (48-53 h) embryos were unilaterally removed. Somite removal disrupts the segmental organization of peripheral nerves. Moreover, somite removal causes a reduction of tenascin along and surrounding peripheral nerve pathways, suggesting that tenascin is produced, at least in part, by somitic cells. In addition, preliminary results indicate that segmentally specific patterns of sympathetic preganglionic projections are altered in somite-removed embryos.

These results indicate that somites are important in the organization of peripheral nerves and that tenascin may be involved in this process.

76.8

AN INCREASE IN INTERNAL FREE CALCIUM CONCENTRATION COINCIDES WITH TWO DIFFERENT GROWTH CONE BEHAVIORS. Stephen J. Moorman and Richard I. Hume, Department of Biology, The University of Michigan, Ann Arbor, Michigan 48109.

In vitro, sympathetic preganglionic growth cones fasciculate on motor neuron neurites, collapse after contact with the cell bodies and neurites of dorsal root ganglion neurons (DRG), and grow across the cell bodies and neurites of sympathetic ganglion neurons (SYM). All of these cell types might potentially be encountered by a growing preganglionic axon *in vivo*. These cell-type specific responses suggest that contact mediated recognition might be sufficient for growth to and interaction with appropriate targets.

We have performed experiments aimed at elucidating the cellular mechanisms that underlie these growth cone behaviors. We have monitored internal free calcium concentration ($[Ca_i]$) in growth cones and neurites, using FURA-2, during the cell-specific interactions. In our standard culture conditions, preganglionic growth cones and neurites that had grown out of explants showed spontaneous, low level fluctuations in $[Ca_i]$. These low level fluctuations were independent of growth cone contact with any other neurons. Following contact with the neurites of DRG, preganglionic growth cones showed rapid 5-6 fold increases in $[Ca_i]$ followed by oscillations of $[Ca_i]$ between resting level and the maximum level seen upon initial contact. These $[Ca_i]$ oscillations continued for several minutes, and by 4-6 minutes after contact the growth cone collapsed. Following contact with the neurites of SYM, the preganglionic growth cones also showed a transient 5-6 fold increase in $[Ca_i]$. However, within a few seconds the $[Ca_i]$ returned to baseline. No persistent large excursions from baseline $[Ca_i]$ were detected as the growth cone continued to grow. Although both crossing and collapse are accompanied by an initial increase in $[Ca_i]$, it remains possible that different temporal patterns of change in $[Ca_i]$ may underlie specific growth cone behaviors. (Supported by the American Paralysis Assoc. and N.I.H.)

76.9

EXPRESSION OF SV-40 T-ANTIGEN IN GNRH NEURONS INHIBITS ORGANIZATION OF TERMINALS IN THE MEDIAN EMINENCE IN TRANSGENIC MICE. R.I. Weiner, K.K. Thind, J.J. Windle[1]*, P.L. Mellon[1]* and P.C. Goldsmith. Reproductive Endocrinology Center, Univ. Calif., San Francisco, CA 94143 and [1]Salk Institute, La Jolla, CA 92037.

Transgenic mice were made which express a hybrid gene consisting of 2 kb of the GnRH promoter/enhancer fused to the coding region of the potent transforming oncogene, SV40 T antigen (Tag). All adult mice expressing the transgene were infertile and had infantile gonads and accessory sex organs. The anterior pituitary of these animals immunostained weakly for LH compared to normal controls. Although cell bodies and processes of GnRH neurons were immunostained in the preoptic area, no terminals were observed in the external layer of the median eminence (ELME). This lack of organization at the neurohemal junction occurred exclusively for GnRH endings, since both the tuberoinfundibular dopaminergic and vasopressin neurosecretory systems showed their characteristic organization. In a developmental study, animals were sacrificed on embryonic days (E) 12-20. We confirmed earlier work in controls showing fusiform GnRH neurons migrating towards the cribiform plate along the nervus terminalis prior to E16. In transgenic mice neurons were rounded, but still were capable of migrating into the brain. By E16 GnRH neurons were moving ventrocaudally into the preoptic area, where both immunopositive cell bodies and neuronal processes were observed. GnRH cell lines (GT1) derived from a tumor in these transgenic mice send out extensive neurites which form numerous contacts with processes and perikarya of adjacent cells. We conclude that GnRH-Tag transgenic mice are infertile due to a specific developmental defect preventing extension and organization of GnRH axonal endings in the ELME, thereby preventing GnRH from reaching the anterior pituitary. Supported by NIH grants HD08924 (R.W.) and HD20377 (P.M.).

76.10

A DYNAMIC VIEW OF NEURULATION AND NEURAL CREST MIGRATION IN NORMAL CHICK EMBRYOS USING 16 MM TIME-LAPSE HIGH-DEFINITION PHOTOMICROSCOPY. T. Jaskoll, G. Greenberg* and M. Melnick*. University of Southern California School of Dentistry, Los Angeles, CA 90089.

The dynamic process of neural tube formation and neural crest migration in live, unstained cultured White Leghorn avian embryos, H.H. stages 8-11, was investigated by time-lapse cinematography using a new prototype high-definition light microscope which greatly enhances resolution, sharpness and depth of field. These studies provide previously unobserved details of neural apposition and fusion. During development neural tube closure in the trunk region markedly differs from that observed in the head. The cephalic neural folds slowly elevate, then rapidly touch and appose. They gradually "zip-up" in the anterior and posterior direction. In the trunk region where the neuroepithelium bulges adjacent to the somites, the edges of the folds pulsate and forcefully make contact at these bulging regions; the intersomitic epithelia retract, remain open and then slowly close. Between the open folds only a few bridging cells were seen, probably representing the sites of initial cell adhesion that had been stretched during epithelial retraction. Through focusing into the embryos revealed a meshwork of synchronously pulsating neural crest cells below the epithelial surface. The in vivo crest cell migration pattern was investigated. Selected embryos were fixed in paraformaldehyde, stained with antibodies to cell adhesion molecules (N-CAM) and processed for whole mounts. N-CAM was observed on the leading edges of the open neuroepithelia; the loss of N-CAM was associated with tube apposition. Supported by NIH DE07006.

ISCHEMIA I

77.1

NEUROPROTECTIVE EFFECTS OF GM1 GANGLIOSIDE TREATMENT AFTER FOCAL CEREBRAL ISCHEMIA IN RATS. S. Mazzari, A. Lazzaro *, M.S. Seren, T. Koga*, N. Schiavo*, R. Zanoni*, and A. Leon. Fidia Research Labs, 35031 Abano Terme, Italy.

Systemic GM1 treatment exerts beneficial effects in different models of cerebral ischemia (Karpiak S.E., Mahadik S.P., Wakade C.G. CRC Crit. Rev. Neurobiol. 5, 221, 1990). We now report GM1 effects following MCAo in rats according to Tamura A. et al. (J. Cereb. Blood Flow Metab. 1, 53, 1981). GM1 was administered (30mg/kg) immediately after MCAo (i.v.) and then once a day (i.m.). As shown in table I, 7 days after MCAo, striatal alterations such as decrease of choline acetyltransferase (ChAT) activity, dopamine (DA) content (percentage reduction versus contralateral), and brain infarcted area were reduced by GM1 treatment.

Table I.

	ChAT activity	[DA]	infarcted area
Saline (n=8)	-49.7±5.2	-76.6±2.2	32.8±2.9
GM1 (n=8)	-35.6±3.5**	-60.3±3.5**	23.1±3.7*

** p<0.01 *p<0.05

These findings provide further evidence that GM1 treatment, likely via its RADA action (Manev H. et al. Faseb J. 4, 2789, 1990), results in lasting neuroprotection after cerebral ischemia.

77.2

ISCHEMIA, HYPOGLYCEMIA AND KINDLING INCREASE BRAIN LEVELS OF mRNAs FOR NEUROTROPHIC FACTORS

J. Bengzon[1]*, Z. Kokaia[1]*, P. Ernfors[2], M.L. Smith[3]*, R. Ekman[4], B.K. Siesjö[3], H. Persson[2] and O. Lindvall[1]*

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Expression of mRNA for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and hippocampus-derived neurotrophic factor/neurotrophin-3 (HDNF/NT-3) was studied using in situ hybridization after 2 and 10 min forebrain ischemia induced by bilateral carotid artery occlusion plus a lowering of blood pressure, after 1 and 30 min of insulin-induced hypoglycemia and after hippocampal kindling. In situ hybridization was performed using alpha-S35-d(ATP)3'-end labeled 50-mer oligonucleotide probes.

Two min forebrain ischemia induced marked increases in BDNF and NGF mRNA in dentate gyrus granule cells. The increase was detectable 30 min after the insult and maximal levels were seen after 2 hrs. At 2 hrs there was also an increase in BDNF mRNA in the piriform cortex. At 24 hrs NGF and BDNF mRNAs had essentially returned to baseline. One min hypoglycemia induced similar patterns of increased expression of BDNF and NGF mRNAs as those seen after 2 min ischemia. Less pronounced increases were observed after 10 min ischemia and 30 min hypoglycemia. Two hrs after focal kindled seizures, increases in BDNF and NGF mRNA were confined to the dentate gyrus. Generalized seizures gave rise to increases in more widespread regions including the hippocampal CA1-CA3 region, amygdala, and the piriform and parietal cortices. HDNF/NT-3 mRNA was not affected in either of the three experimental paradigms. The increased mRNAs for neurotrophins and the presumed increases in the factors themselves could possibly play a role in protecting neurons from seizure-, ischemia- and hypoglycemia-induced damage.

77.3

DIFFERENTIAL REGULATION OF AMPA RECEPTOR SPLICE VARIANTS IN THE CA1 REGION FOLLOWING ISCHEMIA. H. Monyer (1), B. Nellgård *(2), P.H. Seeburg (1) and T. Wieloch (2). (1) Center for Molecular Biology Heidelberg, F.R.G., and (2) Lab. for Experimental Brain Res. Lund, Sweden.

Recent molecular cloning studies have shown that four related high affinity AMPA receptors GluR-A, -B, -C, and -D exist in two versions (Flip and Flop) which arise from alternative splicing. A difference of only a few amino acids (9 to 11) preceding the predicted fourth transmembrane region imparts distinct properties on AMPA- and glutamate-elicited currents. The cell specificity of these alternatively spliced mRNAs is particularly evident in the hippocampus: both variants are expressed in the CA1 pyramidal cells whereas in the CA3 area there is a conspicuous lack of Flop mRNAs. The CA1 region is particularly vulnerable to cerebral ischemia and the development of neuronal necrosis can be mitigated by AMPA receptor blockade. Thus the question arises whether ischemia may produce a switch in channel composition.

In this study we analyzed the Flip/Flop expression pattern in the hippocampus following ischemia. Reversible cerebral ischemia of 10 minutes duration in a rat model was induced by bilateral common artery occlusion with combined hypotension. An upregulation of Flop mRNAs with a concomitant downregulation of the Flip variants can be documented in the CA1 region one hour following a transient ischemic episode with no noticeable changes in the CA3 region at this time point. There is no evidence for changes of several other control mRNAs (map2, $GABA_A$ receptor $\alpha 2$ subunit) which are also expressed in this brain area. 24 hours following ischemia both Flip and Flop mRNAs are downregulated in the CA1 pyramidal neurons.

77.4

NEURONAL DEATH INDUCED BY ENERGY DEPLETION PROCEED INDEPENDENTLY OF CYTOSOLIC CALCIUM CONCENTRATION M. Nedergaard* and M. Khavata Dept. of Neurology and Neuroscience, Cornell Univ. Med. College, 1300 York Avenue, New York, NY 10021

Cytosolic calcium ($[Ca^{++}]_i$) increases during neuronal death. However, it is unclear whether this antecedent increase in $[Ca^{++}]_i$ is necessary for the initiation or maintenance of those processes leading to death, or rather follows cellular injury. We examined the temporal relationship of calcium influx to neuronal death following lethal energy depletion in vitro. Cultures were prepared from E16 rat forebrain; after 14 days in vitro, $[Ca^{++}]_i$ was measured in single neurons loaded with Fluo-3, using a laser scan confocal microscope (MRC-600, BioRad). Neuronal death was defined by propidium iodide (PI) entry. Dual-emission imaging with an excitation of 414 nm permitted simultaneous detection of $[Ca^{++}]_i$ (emission 540 nm) and PI uptake (emission 620). Superfusion with both the glycolytic inhibitor iodoacetate (0.2 mM) and the electron transport inhibitor rotenone (10 μM), resulted in ATP content falling to undetectable levels in less than 5 min. Superfusion of iodoacetate and rotenone induced a slow steady increase of $[Ca^{++}]_i$ among neurons, with a 5-fold increase in $[Ca^{++}]_i$ occurring within 5 min (n=11). This increase in $[Ca^{++}]_i$ did not occur when calcium (3 mM) was exchanged for 50 mM EGTA (n=6), a treatment which resulted in $[Ca^{++}]_i$ levels remaining at or below resting values during energy depletion. Nevertheless, PI uptake occurred within 44±12 min in calcium-free media, analogous to the 47±11 min latency noted among neurons incubated in 3 mM $CaCl_2$. Population studies measuring the release of intracellular LDH similarly failed the to reveal any dependence of cellular viability upon the extracellular calcium levels. Thus, cultured embryonic neurons die within 30-90 minutes following energy depletion, by a process which can proceed independently of $[Ca^{++}]_i$.

77.5

EFFECTS OF LOW CALCIUM AND NMDA ANTAGONIST ON ATP DURING "ISCHEMIA" IN THE RAT HIPPOCAMPAL SLICE. D. Lobner and P. Lipton. Dept. of Physiol., Univ. of Wisconsin, Madison, WI 53706.

Exposure to 0 Ca buffer or 1mM ketamine allows recovery of synaptic transmission measured 60 minutes after a 5 minute period of "ischemia" (anoxia plus 0 glucose). The effects of these treatments on ATP levels in the CA1 region during ischemia were studied.

ATP (% of pre-ischemia normal)

	Pre-ischemia	Ischemia 1'	Ischemia 2.5'	Ischemia 5'	% recovery of population spike
Normal	100±4	92±3	46±4	13±1	0
20' 0 Ca	116±11	47±8	16±3	17±4	93±11
TTX	98±3	89±3	67±2	29±2	
20' 0Ca/TTX	93±3	81±3	55±3	16±1	
Ketamine	92±4	81±7	55±3	14±3	100±8

1) ATP falls far more rapidly in the 0 Ca buffer. This effect is greatly attenuated by TTX (tetrodotoxin), suggesting it is due to enhancement of ischemic sodium entry by the 0 Ca buffer.

2) Transmission recovers fully after 0 Ca ischemia although ATP falls more quickly than it does in normal buffer. Thus, during ischemia, reducing intracellular calcium is more important than maintaining ATP levels for protecting against transmission damage.

3) The NMDA anatagonst ketamine also allows full recovery of synaptic transmission. It has no effect on ATP fall but has been shown to decrease calcium influx during ischemia.

4) Further investigation will include studying whether maintaining ATP during 0 Ca provides protection against longer ischemic periods.

77.6

RELATIONSHIP OF PRESENCE OF THE CALCIUM-BINDING PROTEIN PARVALBUMIN IN GABAERGIC INTERNEURONS AND THEIR SURVIVAL AFTER TRANSIENT GLOBAL ISCHEMIA. C. Nitsch* (SPON: European Neuroscience Association) Section of Neuroanatomy, Anatomy Institute of the University, CH-4056, Basel, Switzerland.

Transient global ischemia in gerbils (Meriones unguiculatus) causes delayed neuronal cell death in the hippocampus thought to be triggered by a glutamate-receptor mediated lethal calcium influx. The degeneration involves the pyramidal neurons of the CA1 sector, but spares local GABAergic interneurons. The hypothesis that the calcium-binding protein parvalbumin (PV) might protect a subpopulation of GABAergic neurons (Nitsch et al, Neurosci. Lett. 103: 263, 1989) was challenged by Freund et al (Exp. Brain Res. 83: 55, 1990) in a rat model. Therefore, a quantitative study was performed in male gerbils subjected to bilateral occlusion of the common carotid arteries for 7 min under halothane anesthesia. Serial sagittal vibratome sections were stained for PV- (Celio et al, Cell Calcium 9: 81, 1988) and for GABA-immunoreactivity, and collected in 3 groups (controls, short survival for up to 14 days, and long survival up to 77 days). Three representative sections were analyzed per animal. Both, in the short and long survival group there was a significant increase in the number of PV-containing neurons in the CA1 sector, whereas no change was found in CA3 sector and dentate gyrus. We conclude that in gerbils PV-containing GABAergic neurons resist ischemia-induced nerve cell death and that in vulnerable areas they even increase PV-synthesis resulting in visualization of PV in neurons where it is below detection limit in controls. However, PV is not a prerequisite for neuronal survival: the GABAergic neurons in stratum lacunosum-moleculare which do not contain PV are also resistant against ischemia induced nerve cell death.

77.7

ASTROCYTIC CELL DEATH SECONDARY TO COMBINED GLUCOSE-OXYGEN DEPRIVATION IS DEPENDENT ON EXTRACELLULAR CALCIUM. SE Haun*, EJ Murphy*, CM Bates*, and LA Horrocks. Depts. of Pediatrics and Medical Biochemistry, The Ohio State University, Columbus, OH 43210.

Extracellular calcium has been proposed to play a major role in the pathophysiology of cerebral ischemia. To test this hypothesis, we studied the effect of removing extracellular calcium on the rate of cell death in primary cultures of rat cortical astrocytes exposed to combined glucose-oxygen deprivation ("ischemia"). "Ischemia" was created by placing cell cultures in an anaerobic incubator and replacing media with glucose-free buffer that had been equilibrated with 85% N_2, 10% H_2, and 5% CO_2. Cell death was quantitated by lactate dehydrogenase (LDH) efflux. Standard buffers (+ Ca^{++}) contained 1.8 mM calcium whereas calcium-free buffers (- Ca^{++}) contained no added calcium and 25 μM EGTA. Control buffers contained 30 mM glucose. Four groups (n=6 each group) were studied: 1) Ischemia + Ca^{++}, 2) Ischemia - Ca^{++}, 3) Control + Ca^{++}, and 4) Control - Ca^{++}. Aliquots of buffer were removed for LDH measurement after 12 and 24 hours of exposure to experimental conditions. LDH efflux (expressed as percent of total LDH) was 2 to 3 fold higher in the Ischemia + Ca^{++} group (58.1 ± 2.4 vs. 19.1 ± 1.1 at 12 hr; 80.2 ± 1.5 vs. 30.3 ± 2.5 at 24 hr; mean ± SEM) as compared to the Ischemia - Ca^{++} group. The Control - Ca^{++} group had much higher LDH efflux (6.3 ± 0.5 vs. 0.0 ± 0.0 at 12 hr; 14.6 ± 0.8 vs. 0.9 ± 0.3 at 24 hr; mean ± SEM) than the Control + Ca^{++} group. This study supports a role for extracellular calcium in the pathophysiology of cerebral ischemia.

77.8

AGE-DEPENDENT SENSITIVITY TO ANOXIA: NEWBORN RAT HIPPOCAMPAL NEURONS HAVE A LONGER LATENCY AND SMALLER Ca_i^{2+} RISE THAN ADULT. J. E. Friedman and G. G. Haddad. Sec. of Respiratory Medicine, Dept. Pediatrics, Yale Univ. Sch. of Med., New Haven, CT 06510

In vivo and in vitro studies have shown that hypoxia-induced synaptic release of glutamate with the subsequent opening of NMDA channels and entry of Na^+ and Ca^{2+} are responsible for neuronal degeneration. Since in these models the direct effect of hypoxia cannot be distinguished from that of glutamate, we investigated the effect of O_2 deprivation on freshly dissociated rat hippocampal CA1 neurons. We monitored changes in free Ca_i^{2+} using Flo-3 and a Laser Scanning Confocal Microscope. Anoxia was achieved by saturating the perfusing buffer with N_2 + 1-2mM $Na_2S_2O_4$, an oxygen scavenger. PO_2 reached 0 within 30 sec of switching perfusate. These studies were performed in neonatal (1-8d) and adult (≥22d) rats. The experimental protocol consisted of (1) baseline (10 min, oxygenated Krebs buffer), (2) anoxia (10 min) and (3) reoxygenation. A significant rise in intracellular free Ca^{2+}, as demonstrated by increased fluorescence, was observed in all cases. The percent maximum fluorescence change for adults was 40.4±7.2 (n=9), while that of neonates was 23.3±8 (n=6). The latency in onset of the rise in calcium was 1.9±0.3 min for adults (n=15) and 13.2±1.3 min for neonates (n=5). $CoCl_2$ (1mM) added to both the oxygenated and nitrogenated buffers reduced the baseline fluorescence by approximately 40% but failed to halt the observed rise in Ca_i^{2+}, albeit the amplitudes were reduced: 26.5±5.7 (n=4) for adults and 7±2.8 (n=4) for neonates. When extracellular calcium was replaced with cobalt, adult neurons exhibited a 7.7% rise. Latency was not affected. We conclude that in direct response to anoxia (a) the rise in Ca_i^{2+} is mostly of extracellular origin and (b) the pattern and magnitude of change in Ca_i^{2+} in adults is different from that of neonates.

77.9

CALCIUM INDEPENDENCY OF ANOXIA-INDUCED ^{3}(H) ASPARTATE RELEASE IN HIPPOCAMPAL SLICES. L.Annunziato, S.Amoroso*, S.L.Sensi* and G.F. Di Renzo*. Section of Pharmacology, Department. of Human Communication Sciences, 2nd School of Medicine, University of Naples, Via S. Pansini 5, 80131 Naples, ITALY.

When energy production is impaired in the brain, as a consequence of anoxia, hypoglicaemia or ischemia, a massive release of glutamate and aspartate occurs in the extracellular space. Evidence has been provided that this presynaptic release of excitatory amino acids is involved in post-anoxic cell death. At the present time the mechanism by wich glutamate and aspartate are released during anoxia, is not clearly identified. In the present study we investigated the mechanisms involved in presynaptic release of aspartate induced by anoxia in hippocampal slices. Ischemic conditions have been mimicked (1) by replacing the normal O_2-equilibrated medium by N_2 equilibrated (95% N_2-5% CO_2) glucose free solution or (2) by using oligomycin + 2-deoxyglucose in a glucose free medium. Both these treatment increased aspartate release. This presynaptic release induced by anoxia was completely independent from extracellular calcium since neither Ca^{++} chelation by EGTA, nor inorganic calcium entry blocker (2mM La^{+++}, 300 µM gadolinium) and organic calcium entry blockers (10 µM verapamil, 10 µM nimodipine) prevented anoxia-induced aspartate release. These results suggest that anoxia-induced aspartate release might be due to the reversal of the Na^+-cotransport system of aspartate, as a consequence of the decay in Na^+-electrochemical gradient due to the decreased activity of the Na^+-K^+ ATPase.

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77.10

PREINCUBATION WITH CREATINE PREVENTS ANOXIC DAMAGE IN RAT HIPPOCAMPAL SLICES AND AUGMENTS THE EFFECTS OF NMDA RECEPTOR ANTAGONISTS. A. J. Carter and R. E. Müller*, Dept. of Pharmacology, Boehringer Ingelheim, 6507 Ingelheim, Germany.

We have investigated the relationship between energy metabolism, anoxic damage and NMDA-receptor antagonism in vitro. Anoxic damage was assessed by measuring protein synthesis in rat hippocampal slices with [^{14}C]-lysine. The levels of energy metabolites - phosphocreatine (PCr), creatine, AMP, ADP and ATP - were measured by ion-exchange HPLC. Protein synthesis and the levels of PCr and ATP were reduced after 20 min anoxia. Preincubation with creatine dose-dependently (0.03 - 3 mmol/L) increased the levels of PCr and prevented the impairment of protein synthesis. Creatine did not influence baseline rates of protein synthesis under normoxic conditions or the levels of ATP. Incubation with ß-guanidinobutyric acid, a synthetic analogue of creatine which cannot be phosphorylated, did not prevent anoxia-induced impairment of protein synthesis and did not enhance the levels of PCr or ATP. Incubation with an NMDA antagonist also did not significantly prevent the impairment of protein synthesis. Incubation with a combination of both creatine and an NMDA antagonist provided complete protection. These results indicate that energy depletion is a major factor which is responsible for anoxic damage in this model.

77.11

CYCLOHEXIMIDE ATTENUATES ISCHEMIC DAMAGE TO CA1 HIPPOCAMPAL NEURONS IN RATS T. Mima*, I. Halaby, D. Levy, and W. Pulsinelli. Dept. of Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021

Protein synthesis inhibitors have been reported to reduce selective ischemic necrosis of CA1 hippocampal neurons (Shigeno et al. Neurosci Let, 1990; Goto et al. Brain Res, 1990). To test whether this neuroprotective response is based on the degree of protein synthesis inhibition or other mechanisms peculiar to the drugs, we examined the effects of several protein synthesis inhibitors including actinomycin-D. Male Wistar rats were subjected to 10 minutes of 4-vessel occlusion ischemia (Pulsinelli W, Duffy T. J Neurochem, 1983). Sublethal doses of each inhibitor were injected subcutaneously either 1 hr before or immediately after ischemia. The animals were perfusion-fixed 5 days later. The grade of ischemic injury to hippocampus was quantified blindly on a scale of 0-3 (0 = normal brain, 3 = more than 75% of CA1 neurons damaged).

	Dose (mg/kg)	Mean Grade CA1 Damage: Vehicle (n=6)	Pre-Rx (n=6)	Post-Rx (n=6)
Cycloheximide	2.5	2.9±0.2	1.9±0.7*	2.8±0.3
Anisomycin	5.0	2.4±0.8	2.5±0.7	2.7±0.7
Actinomycin-D	0.2	2.3±0.8	2.8±0.3	3.0±0.0

The selected dose of these drugs inhibits protein synthesis for at least 4 hours. However, only pre-ischemic treatment with cycloheximide significantly reduced CA1 damage (*$p < 0.001$). The results suggest that inhibition of "specific proteins" might differ with each inhibitor or that cycloheximide may be neuroprotective via some other mechanism.

77.12

CALPAIN AND CALPASTATIN IN CEREBRAL ISCHEMIA. K. Ostwald, P. Andiné*, H. Hagberg*, E. Nilsson* and J-O. Karlsson*, Institute of Neurobiology, PO Box 33031, 400 33 Göteborg, Sweden.

In one project we studied the effects of in vitro ischemia on the total levels of calpain and calpastatin and their distribution between the membrane and the cytosolic fraction. We found that incubation of brain homogenates at different pH resulted in a transfer of calpains from the cytoplasmic compartment to the membranes when pH was lowered from 6 to 5. Incubations of bisected brains in saline at 39°C for 1,5 h resulted in decreased µ-calpain activity in the cytosol and increased hydrophobicity of the remaining cytosolic m-calpain activity.

Our second project involved a model for transient cerebral ischemia in 7-day postnatal rats (unilateral occlusion of the common carotid artery combined with hypoxia, 7.7 % for 2 h). The arterial occlusion per se does not affect the cerebral blood flow whereas the hypoxia inflicts ischemic damage in widespread regions of the hemisphere on the side of the ligature, leaving the other hemisphere virtually unaffected. Compared with control animals, we found a bilateral 20% decrease in Ca-activated proteolysis in the cytosolic fraction after a two hour ischemic attack followed by 30 min of reperfusion. After 20 hours of reperfusion the level of Ca-activated proteolysis was practically restored. We also found a decrease in calpastatin activity in the membrane fraction of the ischemic hemisphere after one hour of reperfusion. This might indicate an increase in proteolytic activity in the membrane fraction during ischemia.

VISUAL SYSTEM: RETINA

78.1

IMMUNOHISTOCHEMICAL STAINING OF THE DEVELOPING TELEOST RETINA: IS GABA A PIONEERING SUBSTANCE FOR RODS? M.M. Hagedorn and R.D. Fernald*, Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403 and *Department of Psychology, Stanford University, Stanford, CA 94305

Vertebrate retinal development consists of sequential production of specific cell phenotypes, beginning with cone photoreceptors and concluding with rods. We have analyzed cell proliferation and neurogenesis in the developing teleost retina In particular, we have analyzed the late addition of rod photoreceptors using immunostaining.

Cones can be distinguished at day 4, followed shortly by putative rod precursors at day 4.5 (Hagedorn and Fernald, 1991). Approximately 12-20 hrs later, these late added cells first show reactivity to opsin and by Day 6 rod photoreceptors in the central retina have a mature morphology. Vimentin- positive radial fibers are widely distributed throughout the embryonic retina from Day 4-Day 6 and on Day 7, the first vimentin-positive Müller cells were observed.

Concomitant with rod neurogenesis is the production of GABA by the horizontal cells. Horizontal cells completely cover the embryonic retina in a grid-like pattern by Day 5, therefore differentiation of the horizontal cells is not coupled with GABA production. In adults, only horizontal cells close to the margin produce GABA. In other vertebrate retinas, GABA activity in horizontal cells appears early in embryogenesis and then disappears (Redburn, 1987) and is considered a pioneering or tropic substance. In H. burtoni, the GABAergic activity of the horizontal cells appears at the onset of rod neurogenesis and is maintained only in the margin of the adult retina. GABA may play some role in stimulating the genesis of rod photoreceptors.

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78.2

DEVELOPMENT OF TYROSINE HYDROXYLASE- IMMUNOREACTIVE CELLS IN THE RODENT RETINA. D.K. Wu* and C.L. Cepko. Department of Genetics, Harvard Medical School, Boston, MA 02115.

The appearance and development of tyrosine hydroxylase immunoreactive (THIR) cells were studied in whole mounts of developing rat and mouse retinas. Previous studies have shown that THIR cells appear around postnatal day 2. Using a more sensitive method of staining, THIR cells were first detected around embryonic day 19 at the periperal temporal margin of the rat retinas. By postnatal day 5, TH positive cells were found across the entire retina. The 'early' THIR cells are small unipolar neurons with large nuclei and very little cytoplasm. Presumably, they are the class II THIR cells. Class I THIR cells which have larger cell bodies and often exhibit two primary dendrities appear between postnatal day 5 and day 7. However, with the same staining method, the earliest THIR cells in mouse retinas were detected at postnatal day 4. The lack of THIR cells at earlier developmental ages supports the hypothesis that the class II THIR are not present in the mouse retina.

78.3

MECHANISMS MEDIATING CAT RETINAL GANGLION CELL DEVELOPMENT. S.J. Ault, K.G. Thompson, Y. Zhou, A.G. Leventhal
Anat. Dept. U. of Utah Med. Sch., Salt Lake City, UT 84132

We examined the morphologies and distributions of α cells in regions of mature cat retina selectively depleted of β cells due to unilateral visual cortex lesions at birth. As a result of this procedure, α cells close to the area centralis in β cell-poor regions grew to be abnormally large; this size increase was greater in temporal than in nasal retina. In contrast, in more peripheral regions depleted of β cells, α cells were smaller than normal. In fact, the normal central to peripheral α cell size gradient was absent in the β cell-poor hemiretinae even though α cells retained their normal central to peripheral density gradient and were distributed normally in a 'mosaic-like pattern'. It may be that central and peripheral α cells are affected differently because the number of afferents which are made available as a result of β cell loss is sufficient in central but not peripheral retina to compensate for the loss of axon collaterals to the thalamus. Similar mechanisms may explain the differences observed between nasal and temporal retina since, following cortical ablation, nasal α cells, which project to layers A and C of the LGNd, should lose more axon collaterals than do temporal ones, which project only to layer A_1. We suggest that 1) ganglion cell size depends upon intraretinal interactions which are not class specific since the elimination of one class of cell affects the size of another, 2) trophic support from target nuclei via axon collaterals may also be involved, and 3) the development of the normal territorial distribution of α cells is class specific since it develops whether or not β cells are present.

78.4

RETINAL GANGLION CELLS WITHIN THE PRIMATE FOVEA. Kirk G. Thompson, Steven J. Ault, Audie G. Leventhal, Anat. Dept., Univ. of Utah Sch. of Med., Salt Lake City, UT 84132

HRP labeled retinal ganglion cells within and around the foveal pit were studied in new world (*Saimiri sciureus*) and old world (*Macaca fascicularis*) monkeys. Most cells within the foveal pit resembled peripheral B type (midget) ganglion cells; their cell body and dendritic field areas were, respectively, 4 and 15 times larger than those of cells in the foveal slope. Around the edge of the foveal pit, the cell body and dendritic field areas of the cells were, respectively, 2 and 4 times larger than those of cells in the foveal slope. The primary dendrites of ganglion cells in the foveal pit arborized in the IPL within the foveal pit and were on average three times longer than those of cells in the foveal slope. These long primary dendrites were randomly oriented with respect to the center of the foveal pit. In contrast, the primary dendrites of ganglion cells around the edge of the foveal pit encircled the foveal pit and arborized in the IPL at the inner edge of the foveal slope. More peripherally, the proximal dendrites of retinal ganglion cells tended to be oriented toward the center of the foveal pit. In all areas studied, the initial segment of the ganglion cell's axon strongly tended to be on the opposite side of the cell body from its proximal dendrite. Our results suggest that retinal ganglion cell morphology is in large part determined by extrinsic factors such as the density of neighboring cells and the position and density of their afferents. The coverage of the foveal pit by the dendritic fields of retinal ganglion cells may provide the anatomical basis for the clinical phenomenon of macular sparing.

78.5

EXPRESSION OF AN AXONAL GROWTH ASSOCIATED PROTEIN, GAP-43, IN DEVELOPING RETINA. L.S. Honig, D.T. Hess, J.H.P. Skene and C.J. Shatz. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

To study molecular correlates of retinal development in the cat, particularly at those stages when activity-dependent retinogeniculate segregation occurs, we have examined the pattern of expression of GAP-43, an axonally transported growth-associated phosphoprotein. In situ hybridization (ISH) using antisense riboprobe for GAP-43 mRNA at E28 reveals high signal in the forming ganglion cell layer distributed in a central-to-peripheral gradient. Between E43-E57, signal remains high, but is present throughout the retinal ganglion cell (RGC) layer. From E57 to postnatal day P27, signal in the RGC layer decreases overall, but high signal persists in scattered large cells, presumably alpha cells based on their size, distribution and late maturation. This same population of 18-30 um diameter cells stains with monoclonal antibody [9-1E12: D.J. Schreyer] to GAP-43. At early fetal ages, there is little signal in other retinal layers, but at E57 and later, clear ISH signal is also present in the inner nuclear layer. This signal may be in amacrine cell bodies, hence correlating with the prominent immunostaining observed for GAP-43 within the inner plexiform layer - the terminating fiber zone of amacrine cell processes. Thus, transcription of GAP-43 mRNA is an early feature of postmitotic retinal neurons, and its expression is consistent with the idea that both ganglion and amacrine cells use GAP-43 in the process of axonal growth, and that amacrine cells may continue to express GAP-43 postnatally. Supported by Dana Fellowship (LSH), NIH EY06012 (DTH), EY07397 (JHPS), and NSF BNS8919508 (CJS) grants.

78.6

DEVELOPMENT OF MEMBRANE CURRENTS IN DISSOCIATED RETINAL GANGLION CELLS OF THE CAT. I.Skaliora, L.C.Liets*, R.P.Scobey and L.M.Chalupa. Depts of Psychology and Neurology, Neurobiology Graduate Group and Center for Neurobiology, University of California, Davis, CA 95616.

We are investigating the development of membrane excitability in retinal ganglion cells (RGCs) during the period of axonal pathfinding and the establishment of synapses at central targets. Isolated neurons are obtained after enzymatic treatment of the retinae and are kept in suspension at 10°C for up to three days after eye removal. RGCs are identified by retrograde labeling with rhodamine beads previously injected into the optic tracts and subcortical targets. Voltage-clamp recordings are obtained with the whole-cell patch clamp recording technique. Membrane currents are determined during a series of depolarizing voltage steps from a holding potential of -70mV. Peak inward current amplitudes, normalized for differences in membrane area, are found to increase with fœtal age. The Na current is pharmacologically isolated for study of the channel kinetics. Results to date indicate that the increase in inward current is primarily due to an increase in the number of Na channels rather than a change in channel properties. The development of specific membrane currents in relation to the onset of impulse activity is being assessed.

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78.7

SPONTANEOUS CORRELATED BURSTING ACTIVITY OF RETINAL GANGLION CELLS IS UNIQUE TO THE PERIOD OF AXONAL SEGREGATION IN THE LGN. R.O.L. Wong, M. Meister* and C.J. Shatz. Dept. Neurobiology, Stanford Univ., Stanford, CA 94305.

In the ferret visual system, the axonal inputs of retinal ganglion cells segregate into eye-specific layers in the lateral geniculate nucleus (LGN) between postnatal days P3 and P21. Using a multielectrode array to simultaneously record action potentials from many neurons in the neonatal ferret retina, we recently reported spontaneous waves of activity travelling across the ganglion cell layer, resulting in locally correlated bursts of action potentials in neighbouring cells. The developmental time course of this activity pattern has now been determined more accurately. From P4 to P22, neurons fired rhythmic bursts, 2-8 secs in duration. The interburst intervals were 40-90s for P4-15 but decreased to 15-30s by P22. The waves propogated at about 110 μm/s throughout this period. By P30, the wave-like activity disappeared; most cells fired in irregular, asynchronous bursts. Cells in the adult retina fired continuously in darkness, at rates of 2-30 Hz. To establish the identity of the bursting cells, single cells were recorded *in vitro* before being intracellularly dye-filled to reveal their dendritic morphology. Both alpha- and beta-like ganglion cells, known to project to the LGN, fired in regular bursts up to P23 and developed non-rhythmic activity by P30. Thus, the periodic wave-like activity pattern of ganglion cells appears to be unique to the period of axonal segregation and is likely to facilitate this process.

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78.8

ALDEHYDE DEHYDROGENASE IN THE DORSAL RETINA. U.C. Dräger, P. McCaffery and P. Tempst*. Departments of Neurobiology and Genetics, Harvard Medical School, Boston, MA 02115.

In a search for factors in positional determination of the retina we compared proteins from different parts of embryonic mouse retinas by subcellular fractionation and high-resolution protein gel analysis. The cytosolic fractions contained a 53kD protein that was much more abundant in dorsal than ventral retina. Nitrocellulose blots of this protein from 150 dorsal and ventral retina halves were digested with trypsin, and microsequencing of the resulting peptide fragments by Edman degradation identified it as an aldehyde dehydrogenase (AHD). Sections of embryonic mice labeled with AHD antisera showed the first expression of the enzyme early on embryonic day 9 (E9) in the dorsal part of the eye vesicle and in the adjacent surface ectoderm. The labeled surface ectoderm gives rise to the lens and the cornea, which continued to be brightly labeled up to adulthood. In the neural retina of all ages, from eye-cup stage to adult, AHD expression was very high in a dorsal zone comprising about one-third of retinal area, and levels in ventral retina were much lower. In addition, axons and their growth cones emerging from ganglion cells in the dorsal retina were brightly labeled during the axon outgrowth phase, which lasts from E12.5 up to a few days after birth. While in the embryo the enzyme was expressed in undifferentiated stem cells and in neurons, in the adult mouse retina it was mainly present in Müller glial cells, indicating that it is the spatial localization rather than the cell type which matters for the expression of the enzyme.

(Supported by EY 01938)

78.9

THE DORSAL RETINA CONTAINS AN ALDEHYDE DEHYDROGENASE THAT CONVERTS RETINALDEHYDE TO RETINOIC ACID. P. McCaffery, M.-O. Lee*, G. Lara*, N. E. Sladek* and U.C. Dräger. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115, and Dept. of Pharmacology, University of Minnesota Medical School, Minneapolis, MN 55455.

A 53kD protein present in much higher amounts in dorsal than ventral embryonic mouse retina was identified as an aldehyde dehydrogenase (AHD). AHD's represent a group of enzymes which have in common the capacity of NAD-dependent dehydrogenation of aldehydes to carboxylic acids, but which differ in subcellular localizations, and physical and catalytic properties. The AHD from embryonic retina was found to be a cytosolic isoform with preference for aliphatic substrates and basic isoelectric point. In these characteristics it resembled AHD-2, the isozyme that accounts for over 90% of NAD-dependent capacity for retinoic acid synthesis in adult mouse liver. Comparison of embryonic AHD with AHD-2 purified from adult liver by isoelectric focussing gel analysis confirmed their identity. Homogenates of embryonic retinas showed NAD-dependent retinoic acid synthesis from retinaldehyde as detected by high-pressure liquid chromatography. In this *in vitro* test the synthetic capacity for retinoic acid in dorsal retina was about 15 times higher than in ventral retina. Whether AHD-2 is responsible for retinoic acid synthesis in the embryonic eye *in vivo*, and what fraction it contributes to the total retinoic acid content, remains to be determined.

(Supported by EY 01938 and CA 21737)

78.10

DEGENERATIVE AND REGENERATIVE RESPONSES OF AXOTOMIZED RABBIT RETINAL GANGLION CELLS. E. Famiglietti, S. Sharpe* and G. Thurlow*. Dept. Anat. and Lions' Sight Ctr., Univ. Calgary, Calgary, AB, Canada T2N 4N1.

Retinal ganglion cells (GCs), like other central mammalian neurons, exhibit abortive attempts at regeneration following axonal damage. We are developing a model of GC regeneration in rabbit retina, supported initially by peripheral nerve grafting (PNG) directly through the sclera and retina, in order to provide an established source of trophic factors, as well as a more favorable environment for axonal regeneration. The range of rabbit GC types are especially well characterized, and we may thus be able to determine specifically which of the rabbit's well-characterized GC types can be rescued with normal inputs intact. GC survival at 2 months is up to 30% at the retinal perimeter and less centrally. Of the central surviving GCs, a few hundred cells, distributed mostly near the PNG, are larger than normal, hyperchromatic (c+) and some are lobulated in contour. Some very large, round, hypochromatic (c-) GCs exhibit several features of "chromatolysis". Without optic nerve crush, GC loss in the segment of retina containing GCs axotomized by the PNG insertion is still massive, leaving less than 100 cells at 4 months. Most are very large (1,000 μm^2 or more) and c+. At 6 and 9 months reduced silver staining reveals similar cells with axons entering the graft. Most have at least some normal dendrites and a typical axon, but many also exhibit 1 or 2 convoluted processes branching in the inner and occasionally the outer plexiform layers. A few completely normal cells are present at 9 months, mainly in peripheral retina.

78.11

CHARACTERIZATION OF A NOVEL CNS PROTEOGLYCAN: DISTRIBUTION IN THE ADULT RAT RETINA AND OPTIC NERVE. D.J. Bidanset, R.C. Williams and E.E. Geisert, Jr. Department of Cell Biology, Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, Alabama 35294.

A novel CNS-specific glycoprotein containing sulfated polylactosamine side chains is identified by a monoclonal antibody TED15. The antibody recognizes a carbohydrate epitope that is removed from the core protein by keratanase digestion. The relative molecular weight of the glycoprotein is not altered following heparinase, heparitinase or chondrotinase ABC digestions. By immunoblot analysis, the TED15 antigen is differentially distributed in the CNS, being present in the optic nerve but absent from the retina. In the optic nerve the TED15 antigen appears to be associated with astrocytes, while in the retina no labeling of astrocytes or Muller cells is observed. The TED15 antigen is found in the CNS of monkey, cat, ferret, rat and chicken, but is absent in gold fish, frog and medicinal leech. The tissue and species distribution of the TED15 antigen indicates that it has a unique function in the maturation of mammalian and avian CNS, and suggests that it may play a role in the lack of long tract axonal regeneration. Supported by The Spinal Cord Society, The Whitehall Foundation Inc., and PHS grant NS23613.

78.12

A NEW VISUAL SYSTEM MUTATION: ACHIASMATIC DOGS WITH CONGENITAL NYSTAGMUS. Robert W. Williams, Preston E. Garraghty, and Dan Goldowitz University of Tennessee, College of Medicine, Memphis, TN and Vanderbilt University, Nashville, TN.

A mutation has been identified in a family of black Belgian sheepdogs in which the entire retinal projection extends back into the ipsilateral optic tract. Tight inbreeding over three generations indicates that the mutation is an autosomal recessive with complete penetrance. The pattern of misrouting of optic axons is opposite that seen in albinos and other *c* locus mutants in which an excess of fibers cross at the chiasm. All achiasmatic mutants have a persistent horizontal nystagmus. The pendular eye movements are conjugate and are associated with head tilt and head oscillation. This behavioral complex is remarkably similar to that seen in some human albinos, and in both cases is probably related to disparities among sensory and motor maps.

The eye and fundus of mutants are normal, but in several cases, the optic papilla is abnormally small. With the exception of the chiasm, necropsy cases and MRI scans do not reveal any obvious central abnormalities. The callosum is intact and the hypothalamus and 3rd ventricle appear normal. Although we have not yet studied the lateral geniculate nucleus, we predict marked abnormalities in lamination.

The achiasmatic mutation may prove valuable as an animal model for human congenital nystagmus. It may also prove useful for developmental studies of axonal pathfinding in mammalian CNS and for studies of the generation, integration, and plasticity of sensory and motor maps.

We thank Drs. John Cummings, Alexander de Lahunta, John Hathcock, and Susan Tieman for their generous help in getting this projected started. We especially thank Mary Young for her extraordinary efforts in breeding these dogs. Supported by NEI R01-6627.

PEPTIDES: RECEPTORS I

79.1

MOLECULAR CLONING AND CHARACTERIZATION OF THE GENES CODING FOR THE RECEPTORS FOR HUMAN GASTRIN-RreleasING PEPTIDE (GRP) AND NEUROMEDIN B (NMB). E. Giladi[1], M. Daly*[2], S.L. Naylor*[2], and E.R. Spindel*[1]. [1]Div of Neurosci, Oregon Rgl Primate Res Ctr, Beaverton, OR 97006, [2]Dept of Cellular and Structural Biol, U Texas Health Sciences Ctr, San Antonio, TX 78284

Bombesin-like peptides are potent growth factors, neurotransmitters and paracrine hormones. GRP and NMB are the two characterized mammalian bombesin-like peptides and pharmacologically distinct receptors for these peptides exist. Previously we have reported the cloning of the mouse GRP receptor by means of expression in *Xenopus* oocytes and the cloning of a rat NMB receptor from an esophageal cDNA library. Sequence analysis revealed these receptors belonged to the 7-membrane spanning domain, G protein-linked receptor superfamily. Now to facilitate the study of the physiologic and pathologic roles of bombesin-like peptides in human systems, we have cloned the genes encoding their receptors. The human genes were isolated by screening an EMBL3 library with the respective rodent receptor cDNAs. Sequence analysis showed high homology between the rodent and human forms. Unlike the adrenergic receptors, introns separate in part the hydrophobic membrane spanning domains. Somatic cell hybrids were screened by polymerase chain reaction to localize the chromosomes encoding these genes. The GRP receptor was localized to the X chromosome between p11 and q11 while the NMB receptor was localized to chromosome 6 between q21 and q ter. Southern blot analysis indicated that both these genes are single copy genes. Northern blot analysis showed highest levels of NMB receptor mRNA levels in esophagus, duodenum and antrum. Whether NMB, like GRP is localized only in neurons in the GI tract remains to be determined. Analysis of the promoter regions of the genes will allow determination of how these genes are regulated.

79.2

CHIMERIC BOMBESIN PEPTIDE RECEPTORS: ANALYSIS OF DOMAINS INVOLVED IN HIGH AFFINITY LIGAND BINDING. Zahra Fathi, Hagit Shapira, and James Battey. Lab. of Neurochemistry, NINDS, NIH Bethesda, MD 20892.

The bombesin-like peptides gastrin-releasing peptide (GRP) and neuromedin-B (NMB) are important in regulating a number of biological activities including secretion, smooth muscle contraction, and modulation of neuronal activity. The bioactivities associated with these peptides are mediated by high-affinity binding to receptors, GRP-R and NMB-R, present on the surface of many target cells. cDNA clones encoding GRP-R and NMB-R have been isolated from mouse swiss 3T3 fibroblasts and rat esophogous repectively. The two receptors show 56% amino acid identity, and encode seven putative transmembrane domains characteristic of the G-protein coupled receptor superfamily. Ligand binding studies have shown that while both receptors bind both ligands, GRP-R has higher affinity for the GRP ligand in contrast to NMB-R which prefers NMB. In this study a series of chimeric GRP-R and NMB-R have been constructed. These constructs were first assayed for function in oocytes and then stably transfected into BALB 3T3 fibroblasts. Ligand displacement studies using bombesin receptor agonists and antagonists have been used to examine domains involved in high affinity ligand binding.

79.3

CHARACTERIZATION AND FUNCTIONAL EXPRESSION OF A HUMAN SUBSTANCE P RECEPTOR cDNA. Y. Takeda, K.B. Chou, J. Takeda, B.S. Sachais and J.E. Krause. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Substance P (SP) brings about its diverse biological actions by activating a G-protein coupled receptor, which in turn results in the stimulation of particular effector systems. Recently, the rat SP receptor was cloned and molecularly characterized, and was shown to be a member of the G-protein coupled receptor superfamily. In this work we have also molecularly characterized the human SP receptor. RNA was isolated from the IM-9 immunoblast cell line, and a partial receptor fragment was generated by the polymerase chain reaction (PCR). The 5' and 3' extents of the coding region were determined by genomic cloning and sequence analysis, and a full coding region containing cDNA was generated by PCR. The human SP receptor displayed greater than 94% identity to the rat receptor. The human SP receptor cDNA was inserted into the vector, pM[2], in which cDNA expression is controlled by the Harvey murine sarcoma virus LTR. Receptor expression was characterized in transiently transfected COS-7 cells using a filtration assay with ^{125}I-Tyr^{-1}-SP as ligand. ^{125}I-Tyr^{-1}-SP specifically bound to transfected cells with high affinity (Kd=0.24 nM), at a level of 151,000 sites/cell. The potency of tachykinin agonists on the displacement of ^{125}I-Tyr^{-1}-SP was as follows: SP > physalaemin > SP methyl ester > neuropeptide γ > neurokinin A = neuropeptide K = neurokinin B > senktide; this is consistent with the binding site being an NK-1 type tachykinin receptor subtype. SP stimulation of transfected cells resulted in the transient production of IP_3 as measured by a radioreceptor assay. These results demonstrate that we have cloned and functionally expressed the human SP receptor.

79.4

REGULATION OF SUBSTANCE P RECEPTOR mRNA EXPRESSION BY cAMP AND GLUCOCORTICOIDS IN HUMAN AND RAT CELL LINES. J.E. Krause, J. Takeda, J.D. Cremins and R. Raddatz. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We have examined the effects of various hormones and second messenger systems on substance P receptor (SPR) mRNA expression in model human astrocyte (U373 MG) and rat glial (LRM55) cell lines. These cell contain NK-1 type tachykinin ligand binding sites based on pharmacological criteria, and thus were appropriate to examine the mechanisms regulating SPR mRNA expression. Cell lines were treated with pharmacological agents, and total RNA was isolated and SPR mRNA levels were assessed using nuclease protection methods. ß-Actin mRNA levels served as a control. Both cell lines express authentic SPR mRNA which is detectable as a ~4.0 kb species on Northern blots or as 677 or 588 base pair protected species in protection assays. In both cell lines, the stimulation of intracellular cAMP levels by treatment with forskolin, dibutyrl cAMP and/or isobutylmethylxanthine resulted in a time and concentration dependent 300 to 400% increase in SPR mRNA levels, with a lag time of 1 hr for onset. Phorbol ester, cGMP and increased intracellular Ca^{++} levels were without effect. These cAMP effects may be exerted via a cAMP response element present in the 5' flanking region of the SPR gene (Hershey et al., JBC 266:4366, 1991). A variety of steroid hormones were also examined; cortisol selectively decreased SPR mRNA levels in a time and concentration dependent manner with 40% and 70% reductions observed after two and twenty hours of treatment, respectively. Estradiol, progesterone and testosterone were without effects. These studies suggest that SPR mRNA regulation can occur both as a result of changes in intracellular second messengers and in the hormonal milieu.

79.5

GR82334, A PHYSALAEMIN ANALOGUE WHICH IS A SELECTIVE NK-1 RECEPTOR ANTAGONIST. R.M.Hagan*, I.J.M. Beresford*, S.J.Ireland*, C.C. Jordan* and P. Ward*. Department of Neuropharmacology, Glaxo Group Research, Ware SG12 0DP, U.K.

GR82334 {[D-Pro^9[spiro-γ-lactam]Leu^{10},Trp^{11}]-PHY(1-11)} is an analogue of the non-mammalian tachykinin physalaemin (PHY) containing the (S)-spirolactam bicyclic conformational constraint which confers antagonist activity at NK-1 receptors (see Ward et al., J. Med. Chem., 33, 1848-1851, 1990). GR82334 behaved as a reversible competitive antagonist in vitro at NK-1 receptors in guinea-pig ileum, guinea-pig trachea and U-373MG human astrocytoma cells (pK_B values 7.64, 7.23, 7.55 respectively) but had no agonist or antagonist activity at NK-2 or NK-3 receptors at concentrations up to 10μM. In the neonatal rat hemisected spinal cord preparation, GR82334 antagonised ventral root depolarisation-induced by the NK-1 agonist, substance P methylester (SPOMe). However, GR82334 exhibited reduced potency (approximately 10-fold) in this preparation compared to other NK-1 receptors, suggesting NK-1 receptor heterogeneity. In anaesthetised rats, GR82334 (0.1-2.5 μmol/kg i.v.) inhibited plasma protein extravasation in skin evoked by an NK-1 agonist, δAva[L-Pro^9,N-MeLeu^{10}]SP(7-11) (GR73632), or by electrical stimulation of the fibularis nerve. In mice, GR82334 (1 nmol) given intrathecally, was able to antagonise the hind-limb scratching response induced by concurrent administration of SPOMe, causing a 41-fold rightward shift in the dose-response curve to SPOMe. In conclusion, GR82334 is a potent antagonist at NK-1 receptors in peripheral and CNS tissues both in vitro and in vivo.

79.6

ELECTROPHYSIOLOGICAL DEMONSTRATION OF NK_3 RECEPTORS IN THE RAT SUBSTANTIA NIGRA PARS COMPACTA *IN VITRO*.
K.D.KEEGAN*, G.N.WOODRUFF AND R.D.PINNOCK. PARKE-DAVIS RESEARCH UNIT,ADDENBROOKES HOSPITAL SITE, HILLS ROAD, CAMBRIDGE, CB2 2QJ, U.K.

Electrophysiological studies show a weak action of substance P (SP) on dopamine (DA) neurons of the substantia nigra (SN) *in vitro*. In this study, we have used specific ligands for the different tachykinin receptors to characterise the receptor type involved in the tachykinin response in the SN,pars compacta. 350µm slices of rat brain were cut and recordings were made by conventional electrophysiological techniques. Senktide (1-1000µM) excited 13 of 49 DA neurones in the SN in a dose dependent manner (ED_{50} 41.2±9.9 nM n=5). Senktide responses were prolonged (10 to 30 minutes before recovery) and there was a marked desensitization of the response to high doses (1µM) of senktide. The NK1 receptor agonist [Sar^9,Met(O_2)^{11}]SP1-11 and the NK2 receptor agonist [βAla^8]NKA4-10 were inactive on senktide sensitive neurones (n=6). The majority (95%) of senktide sensitive neurones were also sensitive to neurotensin. The results in this study demonstrate that a subpopulation of neurotensin sensitive dopamine neurons in the substantia nigra are sensitive to the NK3 receptor agonist senktide. The inactivity of the NK1 and NK2 receptor agonists suggests that only NK3 receptors are present on these neurones.

79.7

STRUCTURE-ACTIVITY STUDIES OF TRUNCATED NEUROPEPTIDE Y ANALOGUES IN BRAIN AND PERIPHERAL TISSUES. J.C. Martel[1], H. Satoh[1*], Y. Dumont[1], A. Cadieux[2*], M. T-Benchekroun[2*], A. Fournier[3], S. St-Pierre[3] and R. Quirion[1]. (1) Douglas Hosp. Res. Ctr., McGill Univ., Montréal, Canada, H4H 1R3, (2) Dept. Pharmacol., Sherbrooke Univ., Sherbrooke, Canada J1K 2R1, (3) INRS-Santé, Pointe-Claire, Canada, H3R 1G5.

Truncated neuropeptide Y analogues of the [1-4-aca-18-36]-NPY type, were tested for their ability to inhibit rat vas deferens contraction, to induce the contraction of the rat descending colon and to inhibit [^{125}I]PYY binding in rat forebrain membranes. Substitutions of the tyrosine (Tyr) residue in position 1 by either phenylalanine (Phe), cyclohexylalanine (Cha), D-Tyr, N-Me-Tyr, Tyr-O-Me, Tyr-O-Et, 1p-nitro-Phe, p-Iodo-Phe, p-Chloro-Phe, and 3-5-Bromo-Phe produced analogues having two-three times lower potencies than pNPY in the rat vas deferens (EC_{50}: 50-150 nM). These same analogues were only slightly weaker than pNPY in the rat forebrain binding assay. However, in the rat descending colon, most behaved as weak agonists (EC_{50}: 1-2μM); the analogues Tyr-O-Et, p-Iodo-Phe and 3-5-Bromo-Phe being completly inactive. Taken together, these results indicate that the tyrosine residue in position 1 is more critical for receptor activation in the rat colon than in the rat vas deferens and CNS binding assay. In addition, analogues substituted in position 1 by either Tyr-O-Et, p-Iodo-Phe and 3-5-Bromo-Phe may prove useful to differentiate NPY receptor classes in various peripheral tissues. Supported by the MRCC.

79.8

QUANTITATIVE LOCALIZATION OF NEUROPEPTIDE Y Y1 RECEPTORS IN RAT BRAIN AS REVEALED USING THE SELECTIVE AGONIST [LEU^{31},PRO^{34}]-NPY. Y. Dumont[1], A. Fournier[2], S. St-Pierre[2] and R. Quirion[1]. (1) Douglas Hospital Res. Ctr, McGill University, Montréal, Québec, Canada, H4H 1R3 (2) INRS-Santé, Pointe-Claire, Québec, Canada, H3R 1G5.

We have investigated the distribution of the neuropeptide Y Y1 receptor subtype in various areas of rat brain using receptor autoradiography and membrane preparations and [^{125}I]PYY (25pM) as radioligand. Various competitors including pNPY, PYY, NPY_{2-36}, NPY_{13-36} and [Leu^{31},Pro^{34}]-NPY displaced specific [^{125}I]PYY binding sites in various regions with different affinity and Hill coefficients below unity. This suggests the heterogeneity of [^{125}I]PYY labelled sites. The selective Y1 receptor agonist [Leu^{31},Pro^{34}]-NPY was found to be specially useful to discriminate between Y1 and non-Y1 sites having markedly greater affinity (>1000) for the first class of sites. Competition data revealed that 60-65 % of the overall population of NPY binding sites was of the Y1 type in cortical areas while they represented only 20-30 % in the cerebellum and olfactory bulb. Very low proportions (10-20 %) of Y1 sites were detected in the hippocampus and striatum. The nature of non-Y1 sites remains to be established but may include more than one class of receptors. Supported by the MRCC.

79.9

NEUROPEPTIDE Y RECEPTOR-MEDIATED RESPONSES IN DIFFERENTIATED PC12 CELLS. D.A. DiMaggio, J.M. Farah, Jr. and T.C. Westfall. Dept. of Pharmacol. Physiol. Sci., Saint Louis Univ. Sch. of Med., St. Louis, MO 63104 and G.D. Searle & Co., Chesterfield, MO 63198.

The presence of neuropeptide Y (NPY)-selective receptor subtypes (NPY1 and NPY2) has been demonstrated on the basis of differences in pharmacologic potency and binding of C-terminal fragments at sites within sympathetic neuroeffector junctions. In the present study, we have used PC12 rat pheochromocytoma cells, which synthesize NPY and express Y1 receptors, in order to study cellular aspects of NPY function concomitant with catecholamine (CA) neurotransmission. PC12 cells were treated with factors which induce cellular differentiation for 5-7 d. In dexamethasone (dex)-treated PC12 cells, the Y1 selective agonist (leu^{31}, pro^{34})-NPY (LP-NPY), but not NPY 13-36, was shown to inhibit forskolin-evoked cAMP in a dose-dependent and PTX-reversible manner. LP-NPY was shown to inhibit binding of NPY 1-36 to intact dex-treated cells, whereas C-terminal fragments of NPY were without effect. In contrast, in PC12 cells differentiated with nerve growth factor (NGF), both LP-NPY and NPY 13-36 were shown to inhibit evoked cAMP in a PTX-sensitive manner, with additive effects. Inhibition of evoked cAMP by NPY 13-36 was attenuated following withdrawal of NGF 24 h prior to experiments. Additionally, both LP-NPY and 13-36 were shown to inhibit binding of NPY 1-36 to intact, NGF-treated PC12 cells. Interestingly, in both NGF- and dex-treated PC12 cells, LP-NPY was shown to enhance nicotine- and K^+-evoked CA release, while long C-terminal fragments of NPY were shown to inhibit evoked CA release in NGF-treated cells. The data provided by these sympathoadrenomedullary models suggest that NGF treatment of PC12 cells induces expression of Y2 receptors, and that modulation of CA release by NPY may not be mediated through cAMP.

79.10

A POTENT NEW SYNTHETIC ANALOG OF VASOPRESSIN WITH RELATIVE AGONIST SPECIFICITY FOR THE PITUITARY

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The physiological actions of vasopressin (AVP) are mediated by two well-characterized classes of receptors, referred to as V_1 (vasoconstrictor) and V_2 (antidiuretic). AVP is also a potent secretagogue of corticotropin (ACTH) at the pituitary (pit), and the receptor that mediates this action is not as well characterized. In many respects it resembles the classic V_1 receptor; this includes the observation that all synthetic analogs with full agonist activity at the pit have been vascular V_1 agonists as well, even if the vascular activity is less. The aim of this study was to synthesize a prototypical pit vasopressin agonist, specifically one with no vascular agonist activity.

The analog deamino-D-3-(3'-pyridyl)-Ala^2, AVP was synthesized by solid phase methods and assessed for biological activity by conventional bioassays. It is a weak (1/381 as AVP) agonist at V_2 receptors in the antidiuretic bioassay. It is an antagonist of the vasoconstrictor response to AVP in the V_1 bioassay (pA_2=6.22, and no measurable agonist activity). Despite its antagonist activity at vascular V_1 receptors, the analog is a full, relatively potent agonist at pit AVP receptors. Its potency stimulating ACTH secretion by ovine pit cells *in vitro* is 1/34 that of AVP, and it is similarly potent in sheep *in vivo*. These data indicate that analogs of AVP can be synthesized with relative agonist specificity for the pit, and thus add further evidence that the pit receptor is distinct from the V_1 vasoconstrictor receptor. Supported by the NH & MRC of Australia and the Polish Scientific Research Committee.

79.11

THE VIP ANTAGONIST [D-Phe^2]-VIP INHIBITS PACAP SIGNAL TRANSDUCTION AT TYPE II BUT NOT TYPE I RECEPTORS. B.D. Shivers[1], T.J. Görcs*[1], D. H. Coy*[2], A. Arimura[1] and P.E. Gottschall*[1]. [1]U.S.-Japan Biomedical Research Laboratories, Tulane University Hebert Center, Belle Chasse, LA 70037 and [2]Peptide Research Labs, Department of Medicine, Tulane Univiversity Medical Center, New Orleans, LA 70112.

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) belongs to the vasoactive intestinal polypeptide (VIP)/glucagon/secretin family of proteins, and stimulates adenylate cyclase in cultured rat pituitary cells, neurons and astrocytes. Two related peptides of 38 or 27 residues, PACAP38 and PACAP27, arise from the PACAP precursor and bind to two, distinct high affinity sites whose abundance varies across tissues. While only the PACAPs bind with high affinity to the first site (Type I), the PACAPs and VIP bind with similiar affinities to a second site (Type II) that may represent the VIP receptor. To determine if VIP antagonists distinguish between Type I and II receptors, we assayed binding in rat tissues enriched in Type I (brain) and Type II (liver) binding sites. Type I binding sites (k_a = 0.64 nM) were measured in 6 brain regions: B_{max} hypothalamus > hippocampus > septum > brainstem > cerebellum > cortex. Hypothalamic binding capacity (38 pmol/mg protein) was 3-fold greater than that measured in cortex. The order of potency for 4 VIP antagonists in displacing 200 pM[^{125}I]PACAP27 from Type II binding sites in liver was: [D-Phe^2]-VIP > [4-Cl-D-Phe^6, Leu^{17}]-VIP >> [Ac-Tyr^1, D-Phe^2]-GRF (1-29 Amide) = [D-Phe^2, Nle^{27}]-GRF (1-29 Amide). In the hypothalamus, 2 µM [D-Phe^2]-VIP displaced only 7% of [^{125}I]PACAP27 but displaced 99.9% of [^{125}I]PACAP27 in liver. In liver, unlabeled PACAP27 was 10-fold more potent than VIP which, in turn, was 10-fold more potent than [D-Phe^2]-VIP in displacing [^{125}I]PACAP27 from the predominant Type II site. Our results show that [D-Phe^2]-VIP distinguishes between these two binding sites and, hence, is useful for elucidating the roles of PACAP and VIP in the brain and peripheral tissues. (Supported by NIH AM-09094 and Takeda Chemical Co. to A.A. and DK-30167 to D.H.C. [4-Cl-D-Phe^6, Leu^{17}]-VIP was the gift of J. Rivier of the Salk Institute in La Jolla, CA.)

79.12

UREIDOACETAMIDES: POTENT AND SELECTIVE CHOLECYSTOKININ B RECEPTOR ANTAGONISTS IN THE RODENT BRAIN. A. Doble, P. Bertrand, A. Böhme, M. Reibaud, J.D. Bourzat, M. Capet, C. Guyon, F. Manfré, C. Cotrel, J.C. Blanchard. Rhône-Poulenc Rorer, CRVA, 13, quai Jules Guesde, 94400 Vitry-sur-Seine, France.

The octapeptide cholecystokinin (CCK) is one of the most abundant neuropeptides in the mammalian brain, where it is thought to be involved in feeding behaviour and in anxiety. Two subtypes of CCK receptors, CCK_A and CCK_B, have been identified in the central nervous system. This report describes a novel family of CCK_B antagonists, the ureidoacetamides. In radioligand binding experiments with [^{3}H]-CCK_{8S} and membrane preparations from guinea-pig cortex, these compounds show affinity for this receptor subtype in the low nanomolar range, the most potent having a K_i of 0.6 nM. Nanomolar affinity was also observed for gastrin receptors in the rat stomach. Their affinities for CCK_A receptors in guinea-pig pancreas are higher than 10^{-6} M. No activity was observed at a wide variety of other neurotransmitter receptors. Ureidoacetamides, at low concentrations (10 - 50 nM), antagonize the excitatory effect of CCK_{8S} on rat hippocampal neurons in vitro, measured using extracellular recording techniques. CCK_A-mediated responses in the guinea-pig ileum were not inhibited by these CCK_B antagonists. Ureidoacetamides may thus be useful in exploring the role of CCK in the central nervous system.

DRUGS OF ABUSE--COCAINE: TRANSPORTERS AND TOXINS

80.1

[^{125}I]RTI-55: A HIGH AFFINITY LIGAND FOR THE DOPAMINE TRANSPORTER. J.W. Boja, A. Patel*, F.I. Carroll*#, M.A. Rahman*#, A. Philip*#, A.H. Lewin*#, R. Vaughan*, T.A. Kopajtic* and M.J. Kuhar. Molecular Pharmacology Laboratory, NIDA Addiction Res Ctr, P.O. Box 5180, Baltimore, MD 21224. #Research Triangle Institute, Research Triangle Park, NC 27709.

The iodinated cocaine analog RTI-55 (3β-(4-iodophenyl)-tropane-2-carboxylic acid methyl ester) has nanomolar affinity for the dopamine transporter. Both saturation and kinetic studies in rat striatal membranes indicated that [^{125}I]RTI-55 bound to both a high and low affinity binding site with affinities of approximately 50 times that previously reported for [^{3}H]cocaine. Similar results were observed with solubilized dog striatal membrane fractions. Competition studies with various monoamine uptake inhibitors and various receptor binding agents, indicated [^{125}I]RTI-55 had a pharmacological profile in the rat striatum consistent with that of the dopamine transporter. However, competition studies in the frontal cortex and pons-medulla indicated that [^{125}I]RTI-55 also may bind to the serotonin transporter in these regions. These results suggest that [^{125}I]RTI-55 is the most potent ligand for the dopamine transporter thus far discovered. Its high specific activity and lack of quenching makes it a superior ligand for autoradiography. It also binds with high affinity in vivo and therefore has application in PET and SPECT scanning.

80.2

A COCAINE ANALOG AND A GBR ANALOG BOTH LABEL THE SAME PROTEIN IN RAT STRIATAL MEMBRANES. A. Patel*, J.W. Boja, J. Lever*#, R. Lew, R. Simantov*, F.I. Carroll*+, A.H. Lewin*+, A. Philip*+, Y. Gao*+, R. Vaughan* and M.J. Kuhar. NIDA/ARC, Baltimore, M.D. 21224. #Johns Hopkins University, Baltimore, M.D., +Research Triangle Inst., Research Triangle Park, NC 27709.

Several GBR analogs have been used as photoaffinity probes to identify the dopamine transporter/cocaine receptor in brain. Because of the evidence that cocaine is a noncompetitve inhibitor of 3H-GBR 12935 (Berger et al, Eur J Pharmacol. 176(1990) 251-252), we examined whether or not cocaine binds to the same protein as GBR 12935 and the other GBR photoaffinity probes. In parallel experiments, both [^{125}I]DEEP and [^{125}I]RTI-82 (3β(p-chlorophenyl) tropane-2β-carboxylic acid, 4'-azido-3'iodophenyl ethyl ester) were used to photoaffinity label a site identified as the dopamine transporter/cocaine receptor according to its pharmacological properties. The molecular weights of the labeled proteins were examined by SDS-PAGE and autoradiography. Both probes were incorporated into a broad band of identical mobilities, suggesting a glycoprotein of approx 80,000 Da. Thus, it appears that the GBR anlogs bind to the same protein as cocaine does. Work is in progress to discern if both labels reside in the same peptide fragments upon enzymatic digestion.

80.3

COCAINE RECEPTOR PROBES IN HUMAN AND NONHUMAN PRIMATE BRAIN: IN VITRO CHARACTERIZATION AND IN VIVO IMAGING. B.K. Madras, M.A. Fahey*, M.J. Kaufman, R.D.Spealman, J. Schumacher*, O.Isacson, A.L. Brownell*, G.L. Brownell* and D.R. Elmaleh*. Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772-9102, Massachusetts General Hospital, Boston, MA 02114.

The phenyltropane analogs of cocaine, CPT (WIN 35,065-2) and CFT (WIN 35,428), are high affinity ligands for cocaine recognition sites on the dopamine transporter. In membranes of human caudate-putamen and nucleus accumbens, the pharmacological specificity of [^{3}H]CFT binding sites was similar to that reported in cynomolgus monkey caudate-putamen. The rank order of potency of these drugs, CFT, GBR 12909 > WIN 35,065-2 > (-)-cocaine > bupropion, also corresponded to their rank order of potency for inhibiting dopamine uptake. The in vivo distribution of tracer doses of [^{11}C]CPT and [^{11}C]CFT was monitored in cynomolgus monkey. High resolution positron emission tomography (PCR-I) revealed high uptake of [^{11}C]CFT and [^{11}C]CPT in the caudate-putamen with striatal:cerebellar ratios > 2. These studies suggest that CFT and CPT or other phenyltropane analogs may be suitable for development of imaging probes for monitoring presynaptic dopamine nerve terminals in Parkinson's disease and other neurodegenerative disorders. Supported by grants from the Parkinson's Disease Foundation, and USPHS grants DA06303, DA00499, RR00168, MH14275, DA00088.

80.4

COMPARATIVE PET STUDIES OF THE BINDING OF CARBON-11 LABELED COCAETHYLENE AND COCAINE IN BABOON BRAIN. J.S. Fowler, N.D. Volkow , R.R. MacGregor*, S.L. Dewey , S.J. Gatley*, J. Logan*, D. Schlyer* and A.P. Wolf*. Brookhaven National Laboratory, Upton, NY, 11973.

Cocaethylene (CE), the ethyl homolog of cocaine, is a metabolite of cocaine (COC) which is present in blood and neurological specimens of individuals co-administering cocaine and alcohol (Hearn et al, J. Neurochem. 56: 698, 1991 and Jatlow et al, Life Sci. 48: 1787, 1991). CE has potency similar to cocaine at the dopamine reuptake site causing speculation that its presence may play a role in cocaine abuse and toxicity. We have compared the uptake and clearance of [^{11}C]COC and CE (5-8 mCi, 4-6 μg) in regions of interest (ROI) of baboon brain over a 50 min period along with the amount of unchanged tracer in arterial plasma (CP) from which the steady state distribution volumes (DV,(cc/g)) were calculated as the slope of a plot of $\int_0^T$ ROI(t)/ROI(T) vs $\int_0^T$ Cp(t)dt/ROI(T) (Logan et al, J. Cereb. Blood Flow Metab. 10: 740, 1990). Absolute uptake and clearance of the two compounds in striatum were indistinguishable. Debenzoylation of CE in plasma was slower than COC as determined by in vitro incubation of [^{11}C]CE and COC with plasma. The ratio of the plasma integral of CE:COC at 30 min was 0.98 for one baboon and 1.2 for the other. Plasma free fractions were also similar. DVs for the two baboons were 7.2 and 7.9 for CE and 6.7 and 7.7 for COC in the striatum and 4.6 and 5.2 for CE and 4.1 and 4.5 for COC in the cerebellum. These studies demonstrate a striking similarity in the initial uptake and clearance of CE and COC in baboon brain. Supported by DOE/OHER and NIDA.

80.5

RADIATION INACTIVATION STUDIES OF THE DOPAMINE TRANSPORTER PROTEIN. S. P. Berger, E. S. Kempner* and S. M. Paul. NIH, Bethesda, MD 20892.

Recently we reported a complex interaction of cocaine with the dopamine transporter protein inasmuch as cocaine was a non competitive inhibitor of [3H]GBR-12935 binding to the transporter. Madras and colleagues have likewise reported that dopamine reuptake inhibitors structurally unrelated to cocaine bind to a "subpopulation" of the two sites labeled by [3H]2 beta-carabomethoxy-3 beta-(4-fluorophenyl)tropane (CFT, also designated WIN 35,428, a cocaine analogue). To further explore the question of whether the molecular mechanism of cocaine's interaction with the dopamine transporter differs from other dopamine reuptake inhibitors we have utilized a technique called radiation inactivation to determine the molecular weight of protein(s) important to the binding of dopamine reuptake inhibitors to the transporter protein. From the amount of radiation needed to inactivate binding of tritiated dopamine reuptake inhibitors molecular weights of involved proteins can be calculated based on the following assumptions. According to "target" theory inactivation is an "all or none event" i.e., one radiation "hit" results in destruction of the target protein and larger targets are more likely to be "hit" by a given amount of radiation. As the amount of radiation increases the amount of functional activity (binding to the dopamine transporter) decreases in an exponential fashion enabling one to calculate a molecular weight target size. Data will be presented comparing the target sizes obtained with [3H]CFT to [3H]mazindol and [3H]BTCP (a PCP analogue that binds with high affinity to the dopamine transporter protein). Preliminary data to be discussed suggests a higher target size for [3H]CFT.

80.6

QUANTITATIVE IN VITRO AUTORADIOGRAPHY OF COCAINE BINDING SITES IN RAT AND HUMAN BRAIN. N. Volkow, J. Fowler, R. Hitzemann, A. Wolf*, K. Dillon* and #A. Biegon, Brookahven National laboratory, Upton, NY 11973 and #Lawrence Berkeley Laboratory, Univ. of California, Berkeley, CA 94720.

The neuroanatomical distribution and pharmacological identity of cocaine binding sites were examined by quantitative autoradiography of tritiated cocaine (N.E.N, 32Ci/mmole) in sections of rat and huma brain obtained postmortem. Using 10nM tritiated cocaine with or without excess (100μM) unlabeled cocaine to assess non-specific binding, we found the optimal conditions for autoradiography were a 30min incubation at room temperature in phosphate buffered saline (PBS) followed by 2X1 min washes in ice cold PBS. Sections were apposed to tritium sensitive film for 8 to 16 weeks alongside with tritium standards. A video-camera based computerized image analysis system was used to quantitate the autoradiograms. The highest density of binding sites in the human brain was found in the caudate and putamen. Moderate to low levels of displaceable binding were found in the anterior and medial thalamic nuclei, the hippocampus and the cortex. Binding in basal ganglia was fully displaceable by the dopamine uptake blockers GBR12909 and nomifensine. Binding in the hippocampus was displaceable by the serotonin uptake inhibitors fluoxetine and imipramine. High density of binding was also observed in the rat dorsal raphe nucleus, which was fully displaceable by fluoxetine but not by desipramine, a norepinephrine uptake blocker. These data suggetst that cocaine binds to dopaminergic as well as serotonergic uptake sites in human and rat brain.

80.7

EVIDENCE FOR THE INVOLVEMENT OF THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR IN THE EFFECTS OF COCAINE. Y. Itzhak, I. Stein and D.C. Mash. Depts. of Biochemistry & Molecular Biology, Pharmacology and Neurology, University of Miami School of Medicine, Miami, FL 33101.

Seizures and lethal-convulsions in human cocaine users are now recognized as concomitant with cocaine-related death. The present study was undertaken in order to investigate a possible association of the NMDA type of the glutamate receptors in the toxic effects of cocaine. *In-vivo* experiments indicated that the potent noncompetitive and selective NMDA receptor antagonist, MK-801, abolishes completely the convulsive effect of cocaine in mice and also reduced the mortality by 50-60% as compared to control cocaine-treated mice. In a series of *in-vitro* experiments, the binding of the competitive and selective NMDA receptor antagonists, [^{3}H]CPP and [^{3}H]CGP-39653, to brain membranes derived from rats treated with cocaine (40mg/kg/day for 7 days) or saline was examined. Results indicated approximately 50% decrease in the number of CPP and CGP-39653 binding sites in brain membranes from cocaine-treated rats as compared to saline-treated rats. The protective effect of MK-801 on cocaine-induced convulsions and the down-regulation of the NMDA receptor following repeated exposure to cocaine, strongly support the hypothesis that the glutamatergic system has a prominent role in cocaine toxicities.

Supported by BRSG SO7 RR-O5363 Division of Research Resources, NIH, and DA06227 from NIDA.

80.8

NEW EVIDENCE LINKING PCP NEUROTOXICITY IN RATS WITH PCP PSYCHOSIS IN HUMANS JW Olney, MA Sesma, J Labruyere*, MT Price Washington Univ., St. Louis MO 63110 & Univ. of MO-St. Louis, MO 63121.

In rodents NMDA antagonists, including phencyclidine (PCP), ketamine and MK-801, induce pathomorphological changes in cingulate-retrosplenial (C-RS) neurons consisting of vacuole formation, mitochondrial dissolution and an abnormal expression of heat shock protein (HSP). These changes are readily prevented by co-administration of muscarinic cholinergic antagonists. In humans, it is known that PCP is a psychotogen and that humans anesthetized with ketamine frequently manifest psychotic symptoms which can be prevented or diminished by co-administration of benzodiazepines (e.g. diazepam). We have now found that diazepam, and other GABAmimetics (e.g. pentobarbital), prevent all pathomorphological effects of NMDA antagonists, including the expression of HSP (see Sesma et al., NS Abstr., '91). This establishes a link between the neurotoxic (rat) and psychotomimetic (human) properties of NMDA antagonists, and suggests that the circuitry involved may include NMDA receptors which, by activating inhibitory GABAergic interneurons, modulate the release of acetylcholine from axonal endings on C-RS neurons. Blockade of the NMDA receptor would inactivate the GABAergic interneuron and disinhibit acetylcholine release, causing cholinergic hyperstimulation of the C-RS neuron (as the proximate cause of injury). Psychotic symptom formation would follow as a psychological reflection of the dysfunctional state induced in neural networks integrally associated with the injured C-RS neurons. Our findings provide neuroscience for the first time with morphological correlates of psychotic symptom formation, including identification of specific transmitter systems and specific CNS neurons involved. In addition, our data suggest that anticholinergic and GABAmimetic drugs (alone or in combination) can prevent *both* the neurotoxic and psychotomimetic side effects of PCP, ketamine or other NMDA antagonists, thereby improving their safety as neuroprotective drugs. Supported by grants to M.A.S. (NSF 861 8448 & Univ. MO Weldon Spring Fund and Research Leave Award) and to J.W.O (RSA MH38894, DA05072, DA53568, AG05681).

80.9

EFFECTS OF 2,4,5-TRIHYDROXYMETHAMPHETAMINE ON THE CENTRAL SEROTONERGIC AND DOPAMINERGIC SYSTEMS. M. Johnson, J.W. Gibb, G.R. Hanson, R.L. Foltz* and H.-K. Lim*. Dept. Pharmacology and Toxicology, and Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112.

2-Hydroxy-4,5-methylenedioxymethamphetamine (2-OH-MDMA) is a metabolite of 3,4-methylenedioxymethamphetamine (MDMA) in the rat brain. We predicted that 2-OH-MDMA can be dealkylated to produce 2,4,5-trihydroxymethamphetamine (THM). The effects of THM on the central serotonergic and dopaminergic systems were examined in order to determine whether THM accounts for MDMA-induced neurotoxicity. Male Sprague-Dawley rats (180-250 g) were injected i.c.v. with 50 µg, 100 µg or 200 µg THM and killed 5 days later. Transmitter concentrations and in vitro tryptophan hydroxylase (TPH) activity were measured with HPLC-EC. Tyrosine hydroxylase (TH) activity was determined with a radioisotopic method. THM reduced TPH activity in a dose-dependent manner reaching 50%, 70 % and 10 % of control in the cortex, neostriatum and hippocampus, respectively, after a 200-µg dose. The decreases in serotonin concentration were less in the side contralateral to the THM injection. THM (50 µg, 100 µg or 200 µg) increased TPH activity from 123% to 144% of control in the dorsal raphe; in the median raphe, only the 50-µg dose increased TPH activity (132% of control). THM reduced neostriatal TH activity to 21% of control but had no effect in the nigra. These results suggest that THM is a potent neurotoxin which destroys dopaminergic and serotonergic nerve terminals.(Supported by USPHS grants DA 00869, DA 04222 and DA 05860)

80.10

METHAMPHETAMINE (MA) INDUCES DOPAMINE (DA) DEPLETIONS AND 6-HYDROXYDOPAMINE (6-OHDA) FORMATION IN RATS INJECTED 4 TIMES IN A 3 HR PERIOD. L.S. Seiden and G. Vosmer. Univ. of Chicago, Pharm/Phys. Sci., Chicago, IL 60637.

Male Long-Evans hooded rats were injected (4 x) with 5,10 or 20 mg/kg of MA (1 inj/hr). Following the 4th injection, the rats were either sacrificed immediately or at .5, 1, 2 hrs or 14 days after the last injection. 6-OHDA was found in the posterior striatum in rats receiving 10 mg/kg and sacrificed immediately or 2 hrs after the 4th injection. These results are consistent with a prior report (Seiden & Vosmer, 1984) in which 6-OHDA was detected after a single injection of MA (100 mg/kg). In the present study the proportion of rats exhibiting MA-induced 6-OHDA formation was greater and the levels higher than in the original study, possibly due to differences in the rat strain, route of administration, or dissection of the striatum. As in the original study, rats sacrificed 2 weeks after the last injection showed depletions of DA and 5-hydroxytryptamine (5HT) in the striatum and 5HT in hippocampus. MA induced the formation of 6-OHDA in both hooded rats and Sprague-Dawley rats. Both strains, irrespective of injection regimen, showed similar neurotoxicity in that both DA and 5HT were depleted 2 and 8 weeks after the last MA injection. We examined the ability of MK-801, a competitive antagonist at the n-methyl-d-aspartate receptor, to block the formation of 6-OHDA because MK-801 can block MA-induced neurotoxicity (Sonsalla et al., Science, 1989). The results of this experiment suggested that MK-801 blocked the MA engendered formation of 6-OHDA. These results also suggest that 6-OHDA is involved in the neurotoxicity of MA, but calcium channel blockade by MK-801 may be involved as well. This research is supported by RSA #10562 (L. Seiden); NIDA #DA-00250.

80.11

STUDIES ON N-MONOMETHYL-1-(4-METHOXYPHENYL)-2-AMINOPROPANE, AN ANALOGUE OF METHAMPHETAMINE. T.D. Steele*, A. Martello and G.A. Ricaurte. Dept. of Neurology, Johns Hopkins School of Medicine, Baltimore, MD 21224.

N-Monomethyl-1-(4-methoxyphenyl)-2-aminopropane (PMMA) is a ring-substituted methamphetamine analogue that has surfaced in the illicit drug market. Behavioral studies in animals indicate that while PMMA produces significant central effects, it does not appear to be a simple amphetamine-like stimulant (Glennon et al.. Pharm. Bioch. Beh. 31:9-13). This study assessed the neurotoxic potential of PMMA, and compared it to that of 1-(4-methoxyphenyl)-2-aminopropane (PMA) and 3,4-methylenedioxymethamphetamine (MDMA). Rats were injected with PMMA (20, 40 and 80 mg/kg; s.c.) twice daily for 4 days. One week later, they were examined for their content of brain monoamines. PMMA produced a dose-dependent depletion of 5-HT, with the highest dose reducing hippocampal 5-HT by approximately 50%. Comparison to PMA and MDMA revealed that PMMA and PMA produced comparable 5-HT-depleting effects, but that these were not as pronounced as those induced by MDMA. Striatal DA was not affected by any of the compounds. These findings suggest that PMMA, like PMA and MDMA, possesses 5-HT neurotoxic activity. Further, they suggest that para substitution may be an important determinant of the selective 5-HT neurotoxic action of certain amphetamine analogues.

80.12

METHAMPHETAMINE CYTOTOXICITY STUDIED IN CULTURED RAT VENTRAL MIDBRAIN NEURONS. J.F. Cubells, D. Sulzer and S. Rayport. Dept. Psychiatry, Ctr. Neurobiol. & Behav., Columbia Univ.; Dept. Neuropathology, NYS Psychiatric Institute, NY 10032.

Methamphetamine (METH) and other psychostimulants are known to exert cytotoxic effects on dopaminergic neurons. To investigate subcellular mechanisms of cytotoxicity, we used postnatal ventral midbrain cultures that contain at least 10% dopaminergic neurons. To exclude confounding excitotoxic effects, 500 µM kynurenate was included with METH. We assessed the viability of neurons after incubation in 100 µM METH for 5 days using casein-AM/ethidium homodimer staining (Live/Dead Assay®, Molecular Probes). There was no significant neuronal loss; however, we found vacuolar structures in the cytoplasm of METH-treated neurons, similar to those observed previously with 3,4-methylenedioxy-amphetamine (Won *et al.*, Soc. Neurosci. Abstr., 16:304, 1990). We took advantage of the selective cytoplasmic staining resulting from the cleavage of casein-AM by cytoplasmic esterases to membrane-impermeable casein; vacuolar structures either did not stain or stained lightly, suggesting that they derive from membrane-delimited organelles. To examine the possibility that the vacuoles arise from endocytic structures, we incubated cultures with fluorescent latex microspheres (1 µL/mL; Luma-Fluor); both neurons and glia showed extensive punctate labelling. We never observed vacuolar structures containing microspheres, regardless of whether cultures were labelled prior to or during METH exposure. On that basis, vacuolar structures are unlikely to be endosomes or lysosomes. To assess the possibility that the vacuoles originate from mitochondria, we will present results using the mitochondrial dye 4-Di-2-ASP and a monoclonal antibody (a generous gift of A. Miranda, Dept. Neurol., Columbia Univ.) to cytochrome *c* oxidase complex subunit IV, a marker of the inner mitochondrial membrane.

INGESTIVE BEHAVIOR: MOLECULAR

81.1

GALANIN GENE EXPRESSION IN THE HYPOTHALAMUS OF LEAN AND OBESE ZUCKER RATS AT DIFFERENT AGES AND AFTER FOOD DEPRIVATION. M. Jhanwar-Uniyal, S.C. Chua, Jr.* and S.F. Leibowitz. The Rockefeller University, New York, N.Y. 10021.

The neuropeptide galanin (GAL) induces food intake, specifically of fat, when injected into the paraventricular nucleus (PVN). It also has an inhibitory effect on the release of insulin and corticosterone. The present investigation examined whether GAL gene expression is altered in the hypothalamus of genetically controlled obese rats, which have abnormal endocrine balance, lipid metabolism, eating behavior and body weight. GAL mRNA levels in the hypothalamus of Zucker lean and obese rats were measured at different ages (11, 24 and 40 wks) and under satiated versus food deprivation (FD) conditions.

Quantitative Northern blot analysis of the hypothalamus, in addition to circulating hormone measurements, indicate that: a) GAL mRNA in the obese rats, relative to their lean littermates, is significantly lower ($P<0.05$) at puberty (11 wks), no different at 24 wks, and reliably higher at 40 wks ($P<0.05$); b) levels of hypothalamic GAL mRNA increase significantly after 48 hrs food deprivation, although only in obese rats at 11 ($P<0.05$) and 24 ($P<0.05$) wks of age and not at 40 wks of age; c) GAL mRNA levels are positively correlated with body weight and BMI in the lean and obese rats at 11 and 24 wks of age; and d) Zucker obese rats exhibit decreased circulating levels of aldosterone, in addition to an increase in insulin and glycerol levels, insulin resistance, body weight and BMI. These results reveal disturbances in hypothalamic GAL gene expression which may be associated with the physiological and endocrine changes observed in Zucker obese rats.

81.2

CENTRAL INSULIN ADMINISTRATION INHIBITS INCREASED HYPOTHALAMIC EXPRESSION OF NEUROPEPTIDE Y mRNA INDUCED BY FASTING. MW Schwartz, AJ Sipols‡, DP Figlewicz‡, SC Woods‡, SE Kahn*, D Porte, Jr.*, G Sanacora§, JD White§ and DG Baskin† Depts of Medicine, †Biological Structure, and ‡Psychology, Seattle V.A. Medical Center, Univ. of Washington, Seattle, WA, and §Dept. of Medicine, S.U.N.Y., Stoneybrook, NY

Hypothalamic insulin administration suppresses food intake, whereas neuropeptide Y (NPY) has the opposite effect. Since fasting lowers circulating insulin levels and increases NPY synthesis in the hypothalamic arcuate nucleus (ARC), insulin could act to inhibit ARC NPY gene transcription. To test this hypothesis, we quantified the effect of 72 hr of food deprivation on expression of preproNPY mRNA in the ARC of male Long-Evans rats receiving 3rd ventricular injections (5 µl/12 hr) of either saline vehicle (n=5) or insulin (160 ng; n=4), compared to vehicle-treated, free feeding controls (n=6). Coronal sections of rat brain (six 15 µm sections per animal) were analyzed by *in situ* hybridization using an oligonucleotide probe complementary to preproNPY mRNA. Hybridization was quantified by computerized image analysis (hybridization area x density; mean ±SE) of resultant autoradiographs. Fasting in vehicle-treated rats increased ARC levels of preproNPY mRNA to 178 ± 20% those of fed controls ($p<0.05$ by ANOVA). In contrast, ARC levels of preproNPY mRNA in fasted, insulin-treated animals were unchanged from those of fed controls (99 ± 14%), significantly lower than vehicle-treated, fasted animals ($p<0.05$). Thus, central insulin administration blocked the effect of fasting on expression of mRNA for NPY in the ARC. Conclusion: Reduced circulating insulin levels may be the mechanism by which fasting stimulates ARC NPY gene expression. Inhibition of ARC NPY gene expression may be the physiological basis for the central action of insulin on food intake and body weight regulation.

81.3

ACTIVITY-INDUCED ANOREXIA DOES NOT AFFECT THE EXPRESSION OF HYPOTHALAMIC NEUROPEPTIDE mRNAs. J. Licinio, M.-L. Wong, P. W. Gold* and J. Glowa. Clin. Neuroendocrinology Branch, Bldg 10/3S231, NIMH, Bethesda, MD 20892.

Hypothalamic neuropeptides, such as CCK, CRH, GAL, NPY, and POMC, play an important role in the acute regulation of ingestive behavior and food intake. The levels of those peptides have been found to be abnormal in human eating disorders, suggesting that they may contribute to the pathophysiology of those disorders. In this study we examined the effects of chronic activity-induced anorexia in male Sprague-Dewley rats on the levels of neuropeptide gene expression, measured by *in situ* hybridization histochemistry.

PEPTIDE	LOCATION	CONTROL	ACTIVITY	
CCK	PVN	50123 ± 348	50301 ± 322	NS
	Hippocampus	18196 ± 633	17586 ± 692	NS
	Basal Ganglia	18397 ± 831	17711 ± 928	NS
CRH	PVN	40088 ± 1349	39266 ± 801	NS
GAL	ARC	37198 ± 534	37282 ± 254	NS
NPY	ARC	45007 ± 1740	44636 ± 1179	NS
POMC	PVN	41229 ± 442	42113 ± 946	NS
	ARC	42956 ± 785	44402 ± 1338	NS

Disintegrations/min/mg wet weight of tissue, mean ± SEM

We found that in a behavioral paradigm resulting in profound changes in energy expenditure, appetitive behavior, and body weight in male rats, there were no long-term alterations in the levels of expression of hypothalamic neuropeptide mRNAs.

81.4

INCREASED C-FOS IMMUNOREACTIVITY IN THE ARCUATE NUCLEUS OF FASTED RATS. D. G. Baskin and M. W. Schwartz. Depts. Medicine and Biological Structure, Univ. Washington, Seattle, WA 98195, and V. A. Medical Center, Seattle, WA 98108

The adaptive response to food deprivation involves modulation of the expression of genes encoding neuropeptides in the hypothalamic arcuate nucleus (AN). Since nuclear transcription factors such *c-fos* may regulate neuropeptide gene transcription, we hypothesized that fasting would activate expression of *c-fos* in AN neurons. To test this hypothesis, male rats were fasted for 48 hrs. with water ad lib (N = 2). Controls received food as usual (N = 3). Brains were perfused with 4% paraformaldehyde in PB for 20 min, removed and immediately put in cold 25% sucrose buffer overnight. Coronal cryostat sections (25 μm) were immunostained free floating (ABC peroxidase) with *fos* polyclonal antibody (M. Iadarola, NIH). Numbers of immunoreactive cells in the AN sections were counted bilaterally by computer image analysis (MCID) and expressed as positive cells per AN section side. *Fos* immunoreactivity was observed in perikaryal nuclei of AN neurons in both fed and fasted groups. However, the number of neurons with *fos*-like immunoreactive nuclei was doubled ($p<0.05$, t-test) in the fasted (240 ± 38) compared to fed rats (119 ± 28). The results indicate that fasting stimulates expression of *c-fos* in AN neurons. This response may mediate the changes in neuropeptide gene expression which occur in the AN during fasting.

81.5

INCREASED HYPOTHALAMIC CORTICOTROPIN-RELEASING FACTOR (CRF) mRNA IN SHORT-TERM STARVATION BUT NOT SHORT-TERM RESTRAINT STRESS. R. Briones-Urbina*, T. Hattori and S.R. George. Dept. Pharmacology, Univ. of Toronto, Toronto, Ont. CANADA M5S 1A8.

Centrally administered CRF produces behaviors similar to those caused by stress, including suppression of feeding. CRF peptide levels and mRNA are also increased following multiple stressors. However, differences in the response to starvation stress and other physical stressors has not been established. We studied hypothalamic CRF mRNA levels following short-term sustained starvation and short term restraint stress in adult male Sprague Dawley rats. Animals were housed in environmental rooms with 12h light-dark cycles and handled daily at the onset of dark. On the test day, at the onset of dark, groups of animals were: 1) handled as usual 'control'; 2) 'starved' for 4 hrs; 3) 'stressed' for 10 min every hr for 4 hrs; 4) 'starved-stressed' as per 2) and 3). All animals were sacrificed at the end of the 4 hours and their tissues rapidly frozen. Northern blotting analysis (total hypothalamic RNA) was performed using a 48 base synthetic ^{32}P-labelled oligonucleotide complementary to amino acids 22-37 of CRF. A 1.4 Kb band compatible with CRF mRNA was seen, but no differences in intensity were detected among the groups. In situ hybridization used the same probe labelled with ^{35}S. Equally increased levels of mRNA were seen in the paraventricular nucleus of the hypothalamus (PVN) in the "starved" and "starved-stressed" groups, but not in the "stressed" group as compared to controls.

We report increased levels of CRF mRNA in the PVN of rats following 4 hrs of starvation at the onset of dark (time of maximal feeding) with no changes in rats that underwent intermittent restraint stress for the same 4 hours. It is suggested that different mechanisms may drive the peptide responses to these two types of stressors.

81.6

EXPRESSION OF *FOS* IN THE RAT BRAIN FOLLOWING SYSTEMIC INJECTIONS OF CHOLECYSTOKININ. D.Y. Chen, Y. Gu, M.F. Gonzalez, and J.A. Deutsch*, Department of Psychology, UCSD, La Jolla, CA 92093

The expression of *fos*, the protein produced by the c-*fos* proto-oncogene, was mapped in the brain of rats following intraperitoneal injections of cholecystokinin (10 μg/kg, Bachem) or control injections of normal saline. Male Sprague-Dawley rats were tested 15-hours-fasted. Two hours after the injections the rats were perfused and their brains extracted and sectioned using a vibrating microtome. The sections were reacted for *fos* immunocytochemistry using commercially available *fos* antibody (Microbiological Associates) and ABC reagents (Vector Lab). Following injections of CCK *fos* expression was observed in neurons of the solitary nucleus, extending from the commissural part to about 1mm rostral to this level. More *fos*-positive cells were found at the commissural level than in the more rostral aspects of the nucleus where fos induction was present. However, the intensity of the *fos* staining was stronger in the anterior region. Intense *fos* expression was also found bilaterally in the parvocellular region of the paraventricular nucleus of the experimental animals.

These findings suggest that the decreases in food intake induced by exogenous CCK exert their actions on the areas of the solitary nucleus and the paraventricular nucleus that exhibited *fos* expression. Since the commissural solitary nucleus receives sensory information from the gastrointestinal tract via the vagus nerve, the present data support previous research concerning the functions of this site in eating regulation. On the other hand, we also have found that the same structures that express *fos* in response to CCK also express *fos* in response to lithium chloride injections at doses that produce reductions in eating comparable to those induced by CCK.

81.7

C-FOS-LIKE IMMUNOREACTIVITY IN CAUDAL HINDBRAIN FOLLOWING FEEDING SUPPRESSIVE INTRAINTESTINAL NUTRIENT INFUSIONS OR EXOGENOUS CCK-8 INJECTION. R.C. Ritter and J.T. Maundu*. Department of VCAPP, Washington State University, Pullman, WA 99164

Exogenous CCK and intestinal infusions of some nutrients suppress food intake via an action on capsaicin-sensitive, vagal sensory neurons. To evaluate the distribution of hindbrain neurons activated by intestinal nutrients or exogenous CCK, we used a polyclonal, affinity purified antibody (Oncogene Science) against the 4 to 17 residues of human fos to identify c-fos-like immunoreactive neurons in the nucleus of the solitary tract (NST) and area postrema (AP) following CCK-8 injection or intestinal nutrient infusions. All infusions were isotonic, pH 7.4. Exogenous CCK-8 (2 or 4ug/kg), oleate (0.065 or 0.13 kcal.ml), maltose and maltotriose (0.13 or 0.26kcal/ml) all caused similar patterns of c-fos expression in the NST and AP. Neither control injections nor intraintestinal saline infusion induced c-fos. Furthermore, intraintestinal trypsin inhibitor, which elevated circulating CCK to levels comparable to those produced by exogenous CCK-8 also did not induce c-fos. We also examined the effect of CCK injection and oleate infusion on c-fos expression in the caudal hindbrains of capsaicin-pretreated rats. Expression of c-fos immunoreactivity in response to either treatment was attenuated or abolished following capsaicin-pretreatment. These results indicate 1) that both intestinal nutrient infusions and exogenous CCK activate neurons in the primary sensory vagal projections of the hindbrain, 2) that hindbrain c-fos induction by intestinal nutrients probably is not due to direct action of circulating CCK on the NST or AP and 3) that induction of NST and AP c-fos immunoreactivity by at least some nutrients depends on stimulation of capsaicin-sensitive neurons. Supported by NINDS Grant NS20561.

81.8

EXPRESSION OF C-FOS PROTEIN IS INDUCED IN SPECIFIC BRAIN NEURONS BY METABOLIC INHIBITORS THAT INCREASE FOOD INTAKE. S. Ritter, N.Y. Calingasan, T.T. Dinh and J.S. Taylor*. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

To further elucidate neural pathways important for glucoprivic and lipoprivic feeding, we examined the distribution of c-fos protein in rat brain after 2-deoxy-d-glucose (2DG)- and mercaptoacetate (MA)-induced inhibition of glucose and fatty acid metabolism, respectively. 2DG (200 and 600 mg/kg), MA (600, 800 and 1200 umol/kg) and vehicle solutions were infused remotely in the absence of food 1-4 hr prior to sacrifice. MA increased c-fos immunoreactivity markedly in the area postrema/nucleus of the solitary tract (AP/NTS), dorsal motor nucleus of X (DMV), lateral parabrachial area (LPB) and central nucleus of the amygdala (CNA) and slightly in the locus coeruleus (LC). Staining was greatly attenuated or abolished by abdominal vagotomy. 2DG increased immunoreactivity in the AP/NTS, DMV, LPB, LC and paraventricular nucleus (PVN) and the staining was not significantly altered by vagotomy. Results are consistent with lesion data suggesting (1) that 2DG and MA stimulate feeding by enlistment of different neural substrates, (2) that the vagus nerve, LPB and CNA, but not the PVN, participate in MA-induced feeding and (3) that 2DG feeding is nonvagal but requires neurons in the AP/NTS region. Supported by PHS #RO1 DK40498.

81.9

EXPRESSION OF *FOS* IN THE RAT BRAIN FOLLOWING SYSTEMIC INJECTIONS OF LITHIUM CHLORIDE. Y. Gu, M.F. Gonzalez, J.A. Deutsch* and D.Y. Chen, Department of Psychology, UCSD, La Jolla, CA 92093

The expression of *fos*, the protein produced by the c-*fos* proto-oncogene, was mapped in the brain of rats following intraperitoneal injections of lithium chloride (LiCl) (3 and 0.65%) or control isotonic injections of saline (4.14 and 0.9%). Male Sprague-Dawley rats were used as subjects. These doses of LiCl have been shown to have and emetic effect and to produce conditioned taste aversion learning. Two hours after the injections the rats were perfused and their brains extracted and sectioned using a vibrating microtome. The sections were reacted for *fos* immunocytochemistry using commercially available *fos* antibody (Microbiological Associates) and ABC reagents (Vector Lab). Following injections of 3% LiCl *fos* expression was observed in nucleus of the solitary tract, the supraoptic and paraventricular hypothalamic nuclei, and the basolateral nucleus of the amygdala. Injections of 0.65% LiCl also induced *fos* in the same structures, but the intensity of expression and the number of positive neurons were significantly less in all of these structures, specially in the supraoptic nucleus and the basolateral amygdala. Normal saline did not induce *fos* expression in any region. However, hypertonic 4.14% saline induced *fos* in all the structures except the basolateral amygdala, but in a much weaker fashion.

These results, including the expression of *fos* in a dose-related fashion following LiCl injections, suggest that *fos* immunocytochemical procedures can be used to map some of the structures that are stimulated by the emetic effects of LiCl, and possibly other emetic agents. The mechanisms involved in the induction of *fos* by LiCl remain to be elucidated.

81.10

HYPOTHALAMIC NEUROPEPTIDE Y GENE EXPRESSION: ANALYSIS IN LEAN AND OBESE ZUCKER RATS IN RELATION TO AGE AND NUTRITIONAL STATE.
S.F. Leibowitz, M. Jhanwar-Uniyal and S.C. Chua, Jr.*. The Rockefeller University, New York, N.Y. 10021.

Hypothalamic injection of neuropeptide Y (NPY) stimulates food intake, particularly of carbohydrate, and also releases insulin and corticosterone. Moreover, NPY-like immunoreactivity in hypothalamic nuclei is found to vary in association with the diurnal cycle and nutritional state, while NPY gene expression is altered in genetically controlled obese mice and rats with abnormal neuroendocrine function. To examine further a potential role for this peptide in the development or maintenance of obesity, the present study examined the levels of NPY mRNA in the hypothalamus of Zucker lean and obese rats, at different ages (11, 24, and 40 wks) and under satiated versus food-deprived (FD) conditions.

Quantitative Northern blot analysis of the hypothalamus, in addition to measurements of circulating hormones, indicate that: a) hypothalamic NPY mRNA levels in the obese rats, relative to their lean littermates, are normal at the time of puberty (11 wks) but are significantly higher at maturity (24 and 40 wks); b) levels of NPY mRNA, in both lean and obese rats, increase similarly after 48 hrs food deprivation in 11- and 24-wk-old rats, but at 40 wks of age are resistant to change after deprivation; c) Zucker obese rats at all ages exhibit decreased circulating levels of aldosterone, in addition to increased insulin and glycerol levels, insulin resistance, body weight and BMI. These findings, revealing abnormal neurochemical patterns primarily after puberty, indicate that hypothalamic NPY neurons may be involved in the maintenance, but possibly not the development, of obesity in these genetically controlled animals.

81.11

COEXISTENCE OF INCREASE IN NEUROPEPTIDE Y (NPY) WITH THE BEGINNING OF HYPERPHAGIA IN THE OBESE ZUCKER RAT. B. Beck, A. Burlet, R. Bazin*, J.P. Nicolas, C. Burlet. INSERM U.308, Mécanismes de Régulation du Comportement Alimentaire. 54000 NANCY and *INSERM U.177. PARIS (France)

Neuropeptide Y (NPY) has been detected in the central nervous system and particularly in the hypothalamus. Numerous cell bodies have been located in the arcuate nucleus. When centrally injected, it strongly stimulates food intake and we have previously shown that the adult obese Zucker rat with a well-established hyperphagia is characterized by higher NPY concentrations in different hypothalamic areas involved in the regulation of food intake. We therefore hypothesized that NPY might play a role in the development of obesity and hyperphagia. To confirm this hypothesis, we measured NPY in several microdissected brain areas of 16 lean (L) and 15 obese (O) suckling Zucker pups (16 days old) and a few days after weaning (30 days old rats) when hyperphagia takes place. The results were analyzed by two way ANOVA and revealed a significant effect of age ($p<0.001$) in the arcuate nucleus - median eminence (ARCME), paraventricular nucleus (PVN) and lateral hypothalamus (LH) and a significant effect of genotype ($p<0.01$) in the ARCME. Between 16 and 30 days of age NPY increased by 58% (L) and 100% (O) in the ARCME, by 134% (L) and 124% (O) in the NPV and 49% (L) and 74% (O) in the LH. NPY in the ARCME which was not different between lean and obese rats at 16 days significantly increased at 30 days in the obese rat (60.6 ± 3.1 (O) vs 49.5 ± 2.3 (L) ng/mg protein ; $p<0.01$). These results showed therefore that NPY levels are increased in the obese Zucker rat in an area where it is synthesized. This increase in NPY occured concomitantly with the increase of food intake.They support our hypothesis of a role of NPY in the development of hyperphagia in the obese Zucker rat.

PSYCHOTHERAPEUTIC DRUGS

82.1

EFFECT OF THE 5-HT_{1A} AGONIST FLESINOXAN ON THE 5-HT SYSTEM: ELECTROPHYSIOLOGICAL STUDIES IN THE RAT CNS. C. de Montigny, C. Ortemann* and P. Blier. Neurobiological Psychiatry Unit, McGill University, Department of Psychiatry, Montreal, Quebec, Canada H3A 1A1

Flesinoxan (FLE) is a high-affinity (pKi = 8.8) and selective 5-HT_{1A} ligand. The present *in vivo* electrophysiological studies were undertaken to determine the effect of FLE on the 5-HT system in the rat brain.

A two-day treatment with FLE (5 mg/kg/24 h, s.c.- delivered by an osmotic minipump) reduced markedly the firing activity of dorsal raphe 5-HT neurons. There was a partial recovery after 7 days, and a complete one after 14 days of treatment. At this point in time, there was a 3-fold shift to the right of the dose-response curve of the effect of intravenous LSD on 5-HT neuron firing activity, indicating a desensitization of somatodendritic 5-HT autoreceptors.

The agonistic activity of FLE, applied by microiontophoresis onto dorsal hippocampus pyramidal neurons, was found to be 15 times greater than that of gepirone, another 5-HT_{1A} agonist. Furthermore, both FLE and gepirone applied concurrently with 5-HT, readily blocked its effect on the same neurons, indicating that FLE, as gepirone, acts as a partial agonist at postsynaptic 5-HT_{1A} receptors in this region of the rat forebrain.

A 14-day treatment with FLE (5 mg/kg/24 h, s.c.) did not modify the responsiveness of dorsal hippocampus pyramidal neurons to microiontophoretic applications of 5-HT or FLE, nor to endogenous 5-HT released by the electrical stimulation of the ascending 5-HT pathway.

The present results indicate that FLE is a potent agonist of both somatodendritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors and that, at the latter, it acts as a partial agonist. The fact that its long-term administration induces a desensitization of somatodendritic, but not of postsynaptic, 5-HT_{1A} receptors would predict that it might be an effective anxiolytic/antidepressant in humans.

82.2

EFFECT OF REPEATED ELECTROCONVULSIVE SHOCK TREATMENT ON THE FUNCTION OF SEROTONIN NEURONS. P. Blier and C. Bouchard*. Department of Psychiatry, McGill University, Montreal, Canada H3A 3A1.

The present studies were undertaken to assess the effect of repeated electroconvulsive shocks (ECS) on the sensitivity of somatodendritic 5-HT_{1A} autoreceptors in the rat dorsal raphe *in vivo*, using microiontophoresis, and on the function of 5-HT terminals in the guinea pig hypothalamus *in vitro*, using superfusion of slices preloaded with [^{3}H]5-HT. Sprague-Dawley rats and guinea pigs received, under halothane anesthesia, six ECS (150 V, 10 ms, 50 Hz for 1 s) over two weeks and the experiments were carried out two days later. In a first series, a reduced hypothermic response to the 5-HT_{1A} agonist 8-OH-DPAT (0.5 mg/kg, s.c.) was observed in ECS-treated rats, as previously reported by other investigators. In a second series, the firing activity of rat 5-HT neurons and their responsiveness to microiontophoretically-applied 5-HT and 8-OH-DPAT was unaltered following ECS. In a third series, the electrically-evoked release of [^{3}H]5-HT (20 mA, 2 ms, 3 Hz for 2 min) from guinea pig hypothalamic slices was not altered following ECS. In addition, the attenuating effect of the 5-HT_{1D} autoreceptor agonist 5-carboxyamidotryptamine, and the enhancing effect of the 5-HT_{1D} autoreceptor antagonist methiothepin as well as that of the 5-HT_3 agonist 2-methyl-5-HT on [^{3}H]5-HT evoked release were unchanged in slices of ECS-treated guinea pigs.

These results indicate that, following repeated ECS, 1) the sensitivity of somatodendritic 5-HT_{1A} autoreceptors in the rat dorsal raphe is normal, 2) terminal 5-HT_{1D} autoreceptors in the guinea pig hypothalamus are normosensitive and still tonically activated, and 3) the capacity of 5-HT_3 receptors to enhance 5-HT release is not affected. In conclusion, repeated ECS does not alter the function of 5-HT neurons and, consequently, the hypothermia induced by systemic 8-OH-DPAT is unlikely to result from the activation of somatodendritic 5-HT_{1A} autoreceptors on dorsal raphe 5-HT neurons.

82.3

EEG SPECTRAL ANALYSIS OF VARIOUS HIGH AFFINITY SIGMA LIGANDS IN RATS. F.C. Tortella and L. Robles, Neuropharmacol. Br., Walter Reed Army Inst. Res., Washington, DC 20307.

The present study attempted to assess the in vivo pharmacology of high affinity sigma ligands by evaluating changes in EEG function in freely-moving rats. Using the technique of computer-derived EEG spectral analysis we have previously reported differential EEG responses for the sigma/PCP ligand (+)-SKF10047 (a dual peak EEG spectra) and the sigma/DM ligand dextro-methorphan (a slowing of the spectral pattern) (Tortella & Robles, Neurosci. Abst., 1989). Subsequently, a series of high affinity sigma ligands have been studied. In adult SD rats the iv administration of (+)-3-PPP (5 mg/kg) produces an EEG response characterized by a distinct increase in spectral power restricted to the 6-7 Hz frequency band. In contrast, 2.5 mg/kg DTG or haloperidol (HAL) produces similar EEG responses measured as a distinct increase in spectral power focused about the 2.5-3.5 Hz frequency bands. Thus, generation of EEG spectral fingerprints reveals an *in vivo* differentiation among sigma compounds which 1) supports the possiblity of multiple sigma recognition sites and 2) questions the heuristic classification of HAL as a sigma antagonist.

82.4

CLOZAPINE POTENTLY INHIBITS SHAM FED SUCROSE: D_4 RECEPTORS MAY BE NECESSARY FOR SWEET TASTE REWARD IN THE RAT.

L.H. Schneider, J.H. Dokko*, J.D.Davis# J. Gibbs and G.P. Smith, Cornell University Medical College, Dept. of Psychiatry, Bourne Lab, White Plains, NY 10605 and #Dept. of Psychology, University of Illinois, Chicago, IL 60680.

PURPOSE: The antipsychotic efficacy of clozapine (CLOZ), an 'atypical' neuroleptic, may be due to its potent blockade of the newly characterized D_4 dopamine (DA) receptor [Van Tol et al., Nature, 350:610 (1991)]. We wanted to determine whether CLOZ would reduce the rewarding potency of sham fed sucrose at doses without motor effects, as do selective D_1 or D_2 receptor antagonists and haloperidol [Schneider, Annals, NYAS, 575:307 (1989)].

METHODS: Effects of CLOZ on intake of 40% (1.2M) sucrose and rate of licking during 30 min of sham feeding were determined in 11 male, Sprague-Dawley rats after 4.75h food deprivation. CLOZ or vehicle (0.1M citric acid) was administered ip at -20 min.

RESULTS: CLOZ ID_{50} for intake was determined for each rat and ranged from 1 to 6 mg/kg (see Table).

CLOZAPINE (mg/kg)	0	1	1.5	2	3	5	6
Intake (ml/30 min)	31.2 (11)	17.5 (2)	12.5 (1)	17.0 (4)	14.5 (2)	14.0 (1)	15.5 (1)

CONCLUSIONS: The potent inhibitory effect of CLOZ (ID_{50}) on the sham fed intake of sucrose is consistent with the hypothesis that D_4 receptors are necessary for the central rewarding effect of the orosensory stimuli of sucrose.
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82.5

EFFECT OF TRIMIPRAMINE STEREOISOMERS ON ^{45}Ca UPTAKE IN SYNAPTOSOMES FROM RAT FRONTAL CORTEX. G. Beauchamp*, P.-A. Lavoie and R. Elie*. Département de pharmacologie, Université de Montréal, Montréal (Québec), Canada H3C 3J7.

Depolarization-induced and Na^+-Ca^{2+} exchange-induced uptake of ^{45}Ca were studied in synaptic terminals of rat frontal cortex, a limbic system brain region. Net K^+-induced ^{45}Ca uptake, a specific index of voltage-dependent calcium channel function, was the difference between uptake (after 10 sec) in high-K^+ choline and low-K^+ choline media: in vitro, racemic trimipramine (T) inhibited this process with an IC_{50} of 57 µM, and (+)-T (IC_{50} = 18 µm) was more potent ($p<0.05$) than (-)-T (IC_{50} = 91 µM). At 2 µM and 20 µM, T inhibited ^{45}Ca uptake by 16% and by 38% respectively; the steady-state brain concentrations of T in patients could fall within this range, and cause such a degree of calcium channel inhibition. The possible role of calcium channel inhibition in the therapeutic effect of T is strengthened by the parallel order of potency of T stereoisomers in the clinic (La Presse Médicale, 69, 1425, 1961) and in calcium channel inhibition. Net Na^+-Ca^{2+} exchange-induced ^{45}Ca uptake was the difference between uptake (after 10 sec) in low-K^+ choline and low-K^+ sodium media: (+)-T (IC_{50}= 159 µM) and (-)-T (IC_{50}= 153 µM) did not differ in inhibitory potency, and no effect on Na^+-Ca^{2+} exchange would be expected at therapeutic steady-state brain T concentrations.

82.6

DEXAMETHASONE-STIMULATED BRAIN ORNITHINE DECARBOXYLASE ACTIVITY IS PREVENTED AFTER LONG BUT NOT SHORT-TERM LITHIUM CHLORIDE TREATMENT. G.M. Gilad, V.H. Gilad, R. Li* and R.J. Wyatt. NPB, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032.

Ornithine decarboxylase (ODC), the enzyme catalyzing the formation of putrescine in the first step of polyamine biosynthesis, is known to increase after various brain traumas, stressful stimuli, and glucocorticoid hormone treatments. In peripheral tissues hormone-stimulated ODC activity can be prevented by acute lithium treatment. Here we report that, unlike peripheral tissues, hormone (dexamethasone 3-5 mg/kg, i.p.)-stimulated ODC activity in rat hippocampus could be prevented by daily lithium injections (LiCl 106 mg/kg, s.c.) for 14 d, but not by a single LiCl injection (212 mg/kg, s.c. or i.p.) given 24 h prior to, together with, or after hormone treatment. The data indicate that the effect of lithium on hormone-induced ODC response in the brain is both concentration and time dependent, and implicate polyamines in the mechanism of lithium action.

82.7

CLOZAPINE INCREASES C-FOS EXPRESSION IN LIMBIC BUT NOT BASAL GANGLIA NUCLEI: COMPARISON WITH HALOPERIDOL AND SELECTIVE D1 AND D2 RECEPTOR ANTAGONISTS. G.S. Robertson and H.C. Fibiger. Division of Neurological Sciences, Dept. of Psychiatry, University of British Columbia, Vancouver, Canada V6T 1Z3.

The mechanisms by which the atypical neuroleptic, clozapine, produces its unique therapeutic effects in the treatment of schizophrenia without having the motoric side effects characteristic of typical antipsychotics remain unclear. Recently, it has been reported that expression of the proto-oncogene c-fos, which encodes a 55-kilodalton phosphoprotein, Fos, is increased in the striatum by haloperidol. Hence, we have examined the effects of clozapine, haloperidol and selective D1 (SCH 23390) and D2 (raclopride) antagonists on Fos immunoreactivity in the forebrain. Haloperidol (0.5, 1 mg/kg) and raclopride (1, 2 mg/kg) increased Fos immuoreactivity in neurons of the striatum (STR) and nucleus accumbens (NAc). In contrast, clozapine (10, 20 mg/kg) increased Fos immunoreactivity in the NAc, medial prefrontal cortex and septum but not the STR. SCH 23390 (0.5, 1 mg/kg) had no effect on Fos immunoreactivity in the prefrontal cortex and septum while it reduced Fos immunoreactivity in the striatum and NAc. Destruction of mesencephalic dopaminergic neurons with 6-hydroxydopamine abolished the increase in Fos expression in the STR and NAc produced by haloperidol and raclopride, and also blocked the clozapine-induced increase in the NAc. These results suggest that haloperidol increases Fos expression in the STR and NAc by blocking D2 dopamine receptors and that a selective action on c-fos expression in the limbic system may contribute to clozapine's unique therapeutic profile.

82.8

EFFECT OF TREATMENT WITH ECS AND DESIPRAMINE ON $5HT_{1A}$ RECEPTORS IN RAT BRAIN. GN Pandey, SC Pandey,* L Isaac, JM Davis. Illinois State Psychiatric Institute, Chicago, Illinois 60612

It has been reported that 8-OH-DPAT-induced hypothermia is attenuated by chronic ECS or desipramine (DMI) treatment, thus suggesting down-regulation of $5HT_{1A}$ receptors in brain. We determined the effects of both acute and chronic ECS or DMI administration on $5HT_{1A}$ receptors in rat cortex and hippocampus brain region. Rats were administered ECS (75 mA for 0.2 seconds) or DMI (10 mg/kg IP) once daily for 14 days for chronic and 1 day for acute studies. Rats were sacrificed after 24 hours of the last treatment for chronic studies and 1 hour after treatment for acute studies. $5HT_{1A}$ receptors were measured by binding techniques using ^3H-8-OH-DPAT as the radioligand. We observed that chronic but not acute ECS or DMI treatment caused significant decrease in the B_{max} of ^3H-8-OH-DPAT binding in rat cortex, where $5HT_{1A}$ receptors are presumably located pre- and postsynaptically, but had no significant effect on ^3H-8-OH-DPAT binding in hippocampus, where the $5HT_{1A}$ receptors are presumably located postsynaptically. Thus, our results indicate that both chronic ECS and DMI cause down regulation of presynaptic $5HT_{1A}$ receptors but do not alter postsynaptic $5HT_{1A}$ receptors. No effects of acute or chronic treatment were observed on K_d of $[^3H]$-8-OH-DPAT binding to $5HT_{1A}$ receptors either in cortex or hippocampus. These results thus suggest that changes in presynaptic $5HT_{1A}$ receptors caused by antidepressants may be related to their mechanism of action.

82.9

A SINGLE DOSE OF REDUCED HALOPERIDOL (RHAL) PRODUCES A POTENT AND LONG LASTING INHIBITION OF [^{3}H]DEXTROMETHORPHAN (DM) AND SIGMA LIGANDS BINDING TO MOUSE AND GUINEA PIG BRAIN. J. M. Musacchio and M. Klein*. Dept. Pharmacology, N.Y.U. Medical Center, New York, NY 10016.

The administration of haloperidol (HAL) inhibits the binding of (+)-[^{3}H]SKF-10047 to the haloperidol-sensitive sites in mouse brain. This effect requires the repeated administration of high doses of HAL and is not produced by one day treatment (Itzhak & Alerhand, FASEB J. 3:1668, 1989). This has been confirmed by several laboratories and uniformly attributed to "receptor" down regulation. Thus, HAL has been labeled a "sigma receptor agonist".

The possibility that the DM_1/σ_1 site might not be a neurotransmitter receptor (Life Sci. 48:543, 1991) and other considerations, lead us to test the effects of RHAL. Administration of 1.9 mg/kg of HAL for 7 days to C57BL/6 mice produced no effect, whereas RHAL inhibited the binding of [^{3}H]DM and (+)-[^{3}H]3-PPP by 25 and 38% respectively. Two days after a single dose of RHAL binding was inhibited by 15 and 30% respectively.

We found that guinea pigs were much more sensitive than mice: a single s.c. injection of 0.1 mg/kg of RHAL produced a marked inhibition of binding to the DM_1/σ_1 site, which was easily detectable even 10 days after the injection. Thus, RHAL could be or produce a neurotoxic metabolite that might explain tardive dyskinesia and other side effects of chronic neuroleptic administration. Supported in part by USPHS grants MH-29591, NS-23926 and Research Scientist Award MH-17785.

82.10

SKF38393 OR PILOCARPINE INJECTED INTO VENTROLATERAL STRIATUM ELICIT TD-LIKE BUT NOT PRIMED DYSTONIA-LIKE ORAL MOVEMENTS IN RATS ADMINISTERED CHRONIC HALOPERIDOL. G. D. Ellison and U. Liminga*. Department of Psychology, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024

Oral movements (OMs) in rats administered continuous haloperidol (HAL) gradually develop smooth, small OMs with a peak energy at 1-3 HZ, the same energy spectrum reported for tardive dyskinesia (TD) in humans, whereas rats given HAL in weekly, large injections develop a "primed dystonia" syndrome characterized by large OMs with steep onset slopes and peak energy at 4-6 HZ.

In order to study the location of the altered pattern generator underlying these effects, rats pretreated with HAL using these two drug regimens for at least 9 months were implanted with bilateral cannula in ventrolateral striatum (VLS) and substantia nigra (SN). It was found that microinjections of SKF38393 or pilocarpine into VLS led to increased OMs which had an exaggeration of the characteristic OM form of rats pretreated with continuous HAL, but this was not true of those pretreated with intermittent HAL. In contrast, bilateral bucuculline into SN did not alter OM form in either group.

These findings indicate that while these two syndromes following chronic HAL may have different neurochemical substrates, both can be altered by injections of a D1 dopamine or a cholinergic agonist into VLS.

ALZHEIMER'S DISEASE: GENETICS AND GROWTH FACTORS

83.1

HIGH RESOLUTION DETECTION OF POLYMORPHISMS IN SPECIFIC GENES AND ITS APPLICATION TO NEUROLOGICAL DISEASE. S.E. Poduslo. Dept. of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

In the past, polymorphisms have been detected by hybridization of DNA probes to genomic DNA (Southern hybridizations). This approach can find moderately frequent polymorphisms. We have developed a more sensitive technique that allows detection of single base changes in genes (Poduslo, Dean, Kolch and O'Brien, "Detecting high-resolution polymorphisms in human coding loci by combining PCR and single strand conformation polymorphism (SSCP) analysis," *Am. J. Human Genetics*, in press, 1991). Sequences of interest are amplified and simultaneously radio-labeled by the polymerase chain reaction. The samples are then denatured and subjected to electrophoresis for SSCP analysis. In addition, if restriction enzymes that cut frequently (with four base pair recognition sequences) are used in digests of the amplified DNA, multiple genetic variations can be resolved after electrophoresis. Alternatively digested non-radio-labeled samples can also be resolved after electrophoresis. Using this strategy we have detected a Hae III polymorphism in the c-kit proto-oncogene (4q11-21), which is linked to other chromosome 4p markers. We found a 2 bp deletion in the insulin-like growth factor I receptor gene located on 15q25-26. We have also found a Hae III polymorphism in proteolipid protein (xq21.3-q22) that we are characterizing. Thus, these techniques are sufficiently sensitive to detect single base changes and allow the placement of DNA segments or genes on the linkage maps of human chromosomes. Such an approach will facilitate the identification of candidate genes involved in human disease (e.g., Alzheimer's Disease). (Supported by the Alzheimer's Institute and the South Plains Foundation)

83.2

IMMEDIATE-EARLY GENE EXPRESSION IN ALZHEIMER'S DISEASE. H.Endo[1], J.M.Stephens[2], P.H.Pekala[2], G.A.Higgins[1], S.Kittur[1]. [1]Mol. Neurobiol., NIA/NIH, Baltimore, MD, 21224; [2]Dept of Biochem., East Carolina Univ., Greenville, NC, 27858.

We have studied immediate early-gene expression of c-*fos*, c-*jun* and *jun*-B in Alzheimer' s disease (AD). The purpose of the present study was to determine if any of these immediate early-genes were activated in AD when compared to control brains. RNA was isolated from 5 AD and 4 control individualis with no neurological disorder. The postmortem interval ranged from one and a half hour to twelve hours. Northern blot analysis was performed and the intensities of the bands was analysed by laser densitometry. Transcript size were as follows; 2.2 kb for c-*fos*, 3.2kb and 2.7kb for c-*jun* and 2.1kb for *jun*-B. C-*fos* mRNA was decreased by 37% in AD brain ($p=0.0576$) compared to control brain. C-*jun* mRNA was decreased by 41% but this finding was not statistically significant. *Jun*-B mRNA was significantly decreased by 55% in AD brain ($p<0.01$). This immediate early-gene expression seems to be involved in long-term adaptative responses and in cell plasticity processes that may play a role in AD.

83.3

COLLABORATIVE LINKAGE ANALYSIS OF CHROMOSOMES 19 AND 21 LOCI IN FAMILIAL ALZHEIMER DISEASE. M.A. Pericak-Vance*[1], J.L. Haines*[2], P.H. St. George-Hyslop*[3], J. Bebout*[1], C. Haynes*[1], R. Tanzi*[2], L. Yamaoka*[1], J.F. Gusella*[2], A.D. Roses[1]. [1]Neurology Div., Duke Univ. Med. Ctr., Durham, NC 27710; [2]Molecular Neurogenetics Lab., Mass. Gen. Hosp., Boston, MA 02129; and [3]Tanz Neuroscience Institute, Univ. Toronto, Canada M5S 1A8.

Familial Alzheimer disease (FAD) is a debilitating disorder. The exact genetic etiology remains unknown. FAD loci on chromosome 21 (Ch 21) and chromosome 19 (Ch 19) have been suggested. Two-point analyses on 59 FAD families were performed for markers APP, D21S1/D21S11, D21S59, D21S13, D21S16,D19S13, BCL3, ATP1A3, and APOCII. Results summed over all 59 families excluded close linkage to all markers; only D21S1/D21S11 (+1.26 @ 30 cM), and D21S13/D21S16 (+0.88 @ 30 cM) had lod scores over 0.50. Multipoint analysis of Ch 21 gave a peak location score of +2.30 near D21S1/D21S11; a similar analysis of Ch 19 was negative. Dividing families into early and late onset groups, lod scores above 0.5 were found for D21S1/D21S11 (+3.05 @ 15 cM), D21S13/D21S16 (+0.74 @ 30 cM) (early onset), and ATP1A3 (+0.69 @ 15 cM) (late onset). Affecteds only analysis of ATP1A3-D19S13 multipoint gave a peak location score of +4.4 (near ATP1A3) in the late onset families. Updated analyses, including CA repeat polymorphism data, are in progress.

83.4

CO-EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND LOW AFFINITY NGF-RECEPTOR ($p75^{NGFR}$) GENES IN HUMAN BASAL FOREBRAIN NEURONS: ALTERATIONS IN ALZHEIMER'S DISEASE. C. E. Byrne[1], H. S. Phillips[2], E. J. Mufson[3] and G. A. Higgins[1]. [1]Mol. Neurobiol., NIA/NIH, Baltimore, MD 21224; [2]Dept. Dev. Biol., Genentech, S. San Francisco, CA 94080; [3] Rush Medical Ctr., Chicago, IL 60612.

NGF, BDNF and NT-3 bind the low affinity NGF-R ($p75^{NGFR}$), but additional factors, such as the *trk* family of proto-oncogenes may also be required for high affinity neurotrophin signal transduction. High resolution *in situ* hybridization was used to determine the distribution of neurotrophins and their receptor elements in the human brain, including a comparison of BDNF, $p75^{NGFR}$ and *trk* mRNAs between tissue from normal aged individuals and Alzheimer's disease (AD) patients. In the normal aged human CNS, BDNF mRNA is widely expressed in cortical regions. In AD, we confirmed that BDNF mRNA was abolished within hippocampal (Phillips *et al, submitted*) and entorhinal neurons. BDNF and $p75^{NGFR}$ mRNA levels were both reduced in the nucleus basalis in AD. These results indicate that BDNF and $p75^{NGFR}$ mRNAs are co-localized within neurons of the nucleus basalis in the human, and suggest that expression of these molecules may be coordinately down-regulated in AD.

83.5

VASCULAR ENDOTHELIAL GROWTH FACTOR: A HEPARIN BINDING GROWTH FACTOR WITH A DIFFERENT DISTRIBUTION THAN BASIC FIBROBLAST GROWTH FACTOR IN NORMAL BRAIN AND ALZHEIMER'S DISEASE.

Corona RJ, Chorsky R*, Baird A*, Gonzalez AM*, Patel L* and Stopa EG. Department of Pathology, SUNY Health Science Center, Syracuse, New York and Department of Molecular and Cellular Growth Biology, The Whittier Institute, LaJolla, California. Vascular endothelial growth factor (VEGF) is a specific and potent mitogen for vascular endothelial cells, that has been recently isolated from conditioned media of bovine pituitary follicular stellate cells. Like bFGF, VEGF also has heparin-binding properties. In previous studies, we have shown that basic fibroblast growth factor (bFGF) is increased in Alzheimer's Disease (AD) and appears to be associated with the heparan-sulfate like proteoglycans bound to B/A4 amyloid (Biochem. Biophys. Res. Commun. 171,690-696, 1990). In the present study, we examined VEGF in 4% paraformaldehyde fixed samples of prefrontal cortex and hippocampus from control (n=6) and Alzheimer brains (n=7) in order to assess the possibility that VEGF might be a brain growth factor. A specific polyclonal antibody (AB-61) directed against the amino terminus of VEGF revealed widespread VEGF-like immunoreactivity within neurons and their processes in both prefrontal cortex and hippocampus. Unlike bFGF, only weak VEGF-like immunoreactivity was seen in astrocytes. In the AD cases studied, there was no obvious association with the B/A4 amyloid containing neuritic plaques or neurofibrillary tangles. Our observations suggest that VEGF may be a novel brain growth factor. The different distribution of VEGF-like immunoreactivity relative to bFGF suggests that factors other than passive binding to the heparan-sulfate like proteoglycans may be important in the development of the heparin-binding growth factor alterations observed in Alzheimer disease. Supported by AG09301, NS 28121.

83.6

IMMUNOHISTOCHEMICAL STUDY OF FIBROBLAST GROWTH FACTOR RECEPTOR IN BRAINS OF THE RAT AND HUMAN.

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The immunocyochemical localization of presumed receptors for fibroblast growth factor (FGF-R) was studied in mammalian brain using an antibody to the 136-152 amino acid sequence. The synthetic haptenic antigen was conjugated to carrier poly-*L*-glutamate with water soluble carbodiimide and injected into rabbits. The best antiserum was able to detect 7.8 ng of the haptenic peptide, but did not react even with 2.0 µg of poly-*L*-glutamate or glutamate. Immunohistochemical staining in rat brain revealed a positive reaction mainly on neuronal cell surfaces, and less intensely on surfaces of glial cells and blood capillaries. Brain regions rich in the positive structures were the hippocampus, hypothalamus, substantia nigra and cerebral cortex. Since we have previously shown that acidic FGF is abnormally expressed in reactive astroglia surrounding senile plaques of Alzheimer diseases (*Dementia*, in press), we examined for the expression of FGF-R in human brain. In cerebral cortices of both Alzheimer cases and age-matched controls, immunopositive structures were observed mainly in neuronal cells of deeper cortical layers and in a few capillaries. The positive neuronal staining, however, appeared to be decreased in both density and intensity in Alzheimer cases as compared with controls.

The present study indicates that FGF-R may be down-regulated in cortical neurons with a concomitant hyper-production of FGF in reactive astrocytes surrounding cortical senile plaques.

83.7

LOSS OF NGF RECEPTOR IN ALZHEIMER'S DISEASE: AN AUTORADIOGRAPHIC STUDY. O. Strada[1*], E.C. Hirsch[1], F. Javoy-Agid[1], S. Lehéricy[1*], M. Ruberg[1*], J.J.Hauw[2*] and Y. Agid[1]. (1) INSERM U289 and (2) Laboratoire de Neuropathologie R. Escourolle, Hopital de la Salpêtrière, 75013 Paris, France.

Basal forebrain cholinergic neurons, known to be trophically affected by NGF, degenerate in Alzheimer's disease (AD). NGF binding has so far not been analyzed pharmacologically in the human forebrain, however. We analyzed the expression of the high affinity NGF receptor, by autoradiography using 125I-NGF 2.5S in the caudate nucleus, putamen, ventral striatum, substantia innominata and nucleus tegmenti pedunculopontinus in 6 Alzheimer patients and 5 controls. This was compared to acetylcholinesterase (AChE) activity in the same areas.

In control brains, high levels of 125I-NGF 2.5S binding were observed in the basal forebrain and striatum (from 0.32 to 0.49 fmoles/mg of tissue equivalent). No specific binding was observed in the nucleus tegmenti pedunculopontinus. Saturation study revealed a 25-40 pM Kd value in the striatum and basal forebrain. NGF binding sites were heterogeneously distributed in the striatum with patches of low NGF receptor density corresponding to the acetylcholinesterase poor striosomes, surrounded by a NGF receptor richer matrix (30%; p>0.05). In Alzheimer brains, a large decrease of NGF receptor density was observed in the caudate nucleus, putamen, ventral striatum and substantia innominata. These results indicate a selective vulnerability of neurons expressing NGF receptor in AD. Interestingly the decrease of AChE activity was less severe than the loss of NGF receptors.

83.8

ALZHEIMER'S AND NORMAL BRAIN CONTAIN mRNA FOR COMPLEMENT COMPONENTS C1qB, C3 and C4. M. Lampert-Etchells*, S.A. Johnson, C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089.

In Alzheimer's disease (AD) brain, immunohistochemistry demonstrates complement components associated with plaques, tangles, and dystrophic neurites. The presence of these components suggests activation of the classical pathway. (McGeer, et al.1989. Can. J. of Neurol. Sci. 16:516). A previous study in this laboratory identified a complement lysis inhibitor mRNA elevated in AD hippocampus (May, P.C. et al. Neuron, 5:831-839). In order to understand the origin and possible role of these gene products in the Alzheimer's disease process we analyzed complement specific mRNA gene expression in the brain by Northern and *In situ* hybridization techniques.

By Northern analysis using cRNA probes from coding regions, we detect 1.0kb C1qB RNA, 5.6kb C3 RNA and 5.5 kb C4 RNA in Alzheimer's and normal frontal cortex. *In situ* analysis of Alzheimer disease tissue from frontal cortex demonstrates increased hybridization of C1q and C4 probes in the outer layers of the cortex. Extensive plaques and tangles are seen in these same layers by Bielschowski silverstaining. Microscopically, clusters of grains are seen over large neuron-like cells and also over small cells in the cortex as well as the hippocampus. Ongoing studies address cell types expressing complement and the location of complement mRNA expressing cells in relation to Alzheimer's pathology.Supported by Alzheimer's Association IIRG-88-069 and Lead award AG-7909 to C.E. Finch.

83.9

COMPLEMENT mRNA UP-REGULATION DURING RESPONSE TO DEAFFERENTING LESIONS. S. A. Johnson, G. M. Pasinetti, M. Lampert - Etchells, N. Laping, C. Zarow and C. E. Finch. Andrus Ctr. and Dept. of Biol. Sci. Univ. So. Cal., Los Angeles, CA 90089

Activated complement (C) components are selectively associated with senile plaques, dystrophic neurites and tangled neurons in AD cortex and hippocampus (McGeer et al., Can. J. Neurol. Sci. 1989; 16, 516). This and other data on brain C derives from immunohistochemical studies; C mRNA has not been documented in the adult brain.The C system, a cytotoxic arm of the immune system, responds to bacteria and malignant cells, as well as to membrane fragments, aggregated protein and other abnormal structures. Cytotoxic mechanisms include depositing large transmembrane pores, the membrane attack complex, while promoting anaphylaxis and chemoattraction of phagocytic cells. We used northern blot and in situ hybridization to examine expression of various C mRNAs in AD and in rat brain lesion models of AD. C expression in AD is presented in a companion abstract (Lampert-Etchells et al.). We find elevated C1q b-subunit and C4 mRNAs in hippocampus after perforant path transection and in striatum after frontal cortex ablation. The time course of C mRNA increase is similar to GFAP and SGP-2, astrocyte mRNAs that increase during synaptic reorganization after afferent lesion. In situ hybridization with C anti-sense probes after decortication shows specific signal initially in the wound cavity (18 hrs). At 3d intense signal is seen only in striatum, the target of the ablated cortical neurons, where synaptic reorganization occurs for several days post-lesion. Combined C in situ hybridization and immunocytochemistry with OX-42, a microglial specific antibody, shows numerous C expressing microglia in the striatum. Further experiments on the role of C in synaptic plasticity are in progress. Supported by a LEAD award (AG-7909) to CE Finch.

83.10

DETECTION OF COMPLEMENT mRNAs IN HUMAN BRAIN BY NORTHERN HYBRIDIZATION ANALYSIS AND POLYMERASE CHAIN REACTION. D.G. Walker and P.L. McGeer. Kinsmen Lab., Dept of Psychiatry, Univ. of B. C., Vancouver, B.C., Canada, V6T 1W5.

Complement proteins associated with the classical pathway have been detected immunohistochemically in Alzheimer disease (AD) brain tissue (McGeer et al, Can J Neurol Sci 16:516, 1989). To determine whether complement proteins can be locally synthesized, we examined temporal cortex mRNAs for appropriate transcripts. C3 and C4 mRNAs were detected by Northern hybridization in all brains tested. These included 5 AD cases, 4 cases without neurological disease, and 1 case of carcinoma with brain metastases. C1q, C3, and C4 mRNAs were detected in the same extracts by the reverse transcription polymerase chain reaction (PCR) technique. Intron-spanning sequences for probe synthesis were selected from the Genbank data base so that amplification of genomic DNA could be excluded. Semi-quantitative PCR analysis of complement mRNA levels of C3 and C4 were made by measuring ^{32}P dCTP incorporation into amplified material at a stage where amplification was occurring at an exponential rate. There was a 4.27 fold increase in expression of C3 mRNA (p=.053) and a 9.33 fold increase in expression of C4 mRNA (p=.026) by ANOVA analysis. C9 mRNA was detected only variably by PCR. Since cells of monocytic origin can synthesize some complement proteins, microglia are a possible source for production in brain.

84.1

EFFERENT NEURONS OBSERVE THE COMPARTMENTAL ARCHITECTURE OF THE SUPERIOR COLLICULUS. R.-B. Illing* (SPON: European Neuroscience Association), Unit for Morphological Brain Research, Univ.-HNO-Klinik, W-7800 Freiburg, Germany.

The superior colliculus (SC) shares a compartmentalized architecture with several other brain structures. The intermediate collicular layers are characterized by several sets of neurochemically defined compartmental domains. Various collicular afferents terminate in a clustered pattern such that some sets of terminal clusters lie in register with certain neurochemically defined patterns, while other pairings assume a complementary or non-matching relationship.

To determine if collicular efferents originate from neurons having a distinct spatial position with respect to compartmental domains, axonal tracing with fluorochromes (Fluorogold or Fast Blue) was combined with intracellular Lucifer Yellow injections and acetylthiocholine histochemistry to visualize efferent cells and acetylcholinesterase (AChE)-rich compartments in single sections of the rat SC. Descending collicular neurons of the intermediate layers backfilled from the predorsal bundle were situated mostly outside AChE-rich patches but tended to reside at the edges of the AChE-rich domain; particularly small perikarya were occasionally clustered inside the patches. Cell bodies with ascending axons to the intralaminar thalamic nuclei had a preference for the space outside AChE-rich patches. The dendrites of many efferent cells ran straigth into AChE-rich neuropil, but others apparently selected their input from domain border regions.

I propose that the compartmental architecture of the SC provides a matrix to which not only afferents but also major collicular efferents are specifically related. A subpopulation of efferent neurons appears to be situated in strategic position at domain borders, with the geometry of their dendritic trees coordinated with the heterogeneous environment.

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84.2

THE DISTRIBUTION OF GLYCINERGIC BRAIN STEM NEURONS PROJECTING TO THE SPINAL CORD. A RETROGRADE TRACING STUDY USING ^{3}H-GLYCINE. J.C. Holstege*, C.M.H. Bongers*, H. Goedknegt*, W. Taal* and M. Godschalk, Dept of Anatomy, Erasmus University Medical School, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Recently we found a glycinergic projection from the ventro-medial lower brainstem to spinal motoneuronal cell groups by combining anterograde transport of WGA-HRP with glycine immunocytochemistry at the ultrastructural level in rat (J.C. Holstege and C.M.H. Bongers, Eur. J. Neurosci. Suppl. 3 (1990) p. 96). In order to verify this projection and to determine whether additional projections were present, the (presumed transmitter-specific) retrograde transport of ^{3}H-glycine from the spinal cord was used.

In several rats, 20-60 µCi. of ^{3}H-glycine was injected in the lower cervical spinal cord under deep pentobarbital anesthesia. After 2 days survival, the rats were re-anesthetized and perfused transcardially with 250 ml. saline followed by 750 ml. of 3% guteraldehyde and 1% paraformaldehyde in phosphate buffer. The brainstems were removed and frozen sections were cut, mounted on glass slides and processed for light microscopic autoradiography by dipping in liquid emulsion using 6 weeks exposure.

Preliminary results showed retrogradely labeled neurons in four areas: 1) medial of the trigeminal nucleus at the level of the caudal half of the inferior olive mainly on the ipsilateral side, 2) large cells in the nucleus giganto-cellularis mostly on the contralateral side, with labeled axons of large diameter immediately crossing to the MLF on the ipsilateral side of the injection, 3) weakly labeled neurons in the ventro-medial reticular formation immediately dorsal and, to a limited extent, rostral of the inferior olive and 4) neurons bilaterally in the medial vestibular nucleus. In addition to the retrogradely labeled cells, anterogradely labeled terminals were also seen, especially in the medial part of the caudal brainstem.

It may be concluded that the retrograde transport technique with ^{3}H-glycine is "glycine-specific" since retrogradely labeled cells were found only in the areas mentioned above, while other cell groups known to project to the spinal cord (e.g. the lateral vestibular nucleus and the cell groups in the dorso-lateral pons) were not labeled. The anterograde labeling, on the other hand, may be aspecific.

84.3

PROJECTIONS OF VESTIBULOSPINAL AXONS TRAVELLING OUTSIDE THE VENTRAL FUNICULI IN THE CAT. A. Donevan, J. MacDonald*, P.K. Rose, Department of Physiology, Queen's University, Canada, K7L 3N6.

Recent studies using the anterograde tracer PHA-L have shown that the medial and descending vestibular nuclei project to the upper cervical spinal cord via multiple funicular pathways including the lateral funiculi (LF), dorsolateral funiculi (DLF) and the dorsal columns (DC). (Donevan et al., J. Comp. Neurol. 302: 1-14, 1990). These pathways were not included in classical descriptions of vestibulospinal tracts. In the present study we have used serial reconstructions of single collaterals stained with PHA-L to map the termination zones of the "new" vestibulospinal pathways.

The terminal field of each collateral was closely related to the funicular location of its parent axon. For example, most collaterals arising from either ipsilateral or contralateral LF axons projected in a narrow band across lamina VII or dorsal lamina VIII, usually as far medial as the central canal. All contralateral DLF axon collaterals had terminations in lamina IV, while some projected ventrally to lamina V or dorsally to laminae III and II. The small number of DC axon collaterals examined had a characteristic widespread termination crossing multiple laminae, as dorsal as lamina II and as ventral as lamina VIII. The notable absence of a significant number of boutons in the ventral horn from any of the collaterals indicates that these vestibulospinal axons do not play a direct role in the control of head movement. Rather, they appear to be involved in the modulation of somatosensory processing or the transmission of segmental reflexes in the upper cervical spinal cord.

(supported by MDAC, and MRC)

84.4

THE ROLE OF Gp Ia AND CUTANEOUS AFFERENTS IN ACTIVATING THE LUMBOSACRAL MOTOR CENTRAL PATTERN GENERATOR. J.W. Commissiong. NIH-NINDS-CNB, Bldg.10/5N214, Bethesda, MD. 20892.

Seven (PN7) and 14 (PN14) day old rats were spinalized at T6 under ether anesthesia. The PN7, but not the PN14 group recovered co-ordinated stepping function in the hindlimbs. At 16-18 of age, or later, a laminectomy was done in the unanesthetized, intercollicular decerebrate rat, and recordings were made from L2-L6 ventral nerve filaments, while stimulating Gp Ia afferents in the L4 dorsal root. In the unoperated control (UOC), PN7 and PN14 groups, the highest percentage of motoneurons was activated in the L4 segment. In the PN7 group, unlike the UOC and PN14 groups, a high percentage of motoneurons was activated in the L3 and L5, as well as in the L2 and L6 segments. The widest divergence of cutaneous afferents within the L2-L6 segments also occurred in the PN7 group. Functional recovery in the PN7 group may be related to a wide distribution of Gp Ia and cutaneous afferents within the lumbosacral motor central pattern generator. The mechanism by which peripheral, afferent signals to the generator, initiates patterned discharges in motoneurons, and causes coordinated stepping, is still unclear.

84.5

DIAZEPAM IN MEDULLARY RETICULAR FORMATION REDUCES EVOKED EMG RESPONSES IN BACK AND NECK MUSCLES; PROGESTERONE ENHANCES THE EFFECT. S. Schwartz-Giblin, A. Robbins and D.W. Pfaff, Rockefeller University, N.Y., N.Y. 10021.

Contraction of the deep back muscle, lateral longissimus (LL) contributes to the vertebral extension of lordosis during reproductive behavior in the female rat. Under urethane anesthesia (1.4g/kg), sites in the nucleus gigantocellularis (Gi) which receive inputs from the midbrain central gray (Robbins et al., Exp Brain Res, 1990), activate LL EMG and dorsal neck EMG with 1/sec trains (300 msec long) of biphasic pulses at 250 Hz. The test stimulus consists of 20 trains; repeated tests are at intervals of at least 10 min. In ovariectomized rats, a 32 gauge cannula containing 20-80 ng crystalline diazepam (1:100 diazepam:dextrose) is inserted through a guide cannula to which a bipolar stimulating electrode is attached. The electrically-evoked EMG response in either LL or neck is reduced at 5, 15, 30 and 60 min after diazepam in the reticular formation compared to the mean of 3 predrug controls and to the effect of a dextrose cannula (post hoc Tukey, p=.01). With dextrose, the response is reduced at 60 min. Progesterone (1mg, i.p.) 1 hr after local diazepam further reduces EMG responses at 15 min (p=.05) but has no effect when given after local dextrose. Drug and hormonal modulation occurred when the current (≤60 uA) was 1.2-1.5 x threshold. Large EMG units recruited last by reticulospinal fibers are the first to be inhibited when diazepam is put into Gi. Given that progesterone facilitates lordosis, it is important that the potential for progesterone to decrease medullary reticulospinal drive on axial muscles is state dependent; it depends upon $GABA_A$ receptor modulation by diazepam in Gi.

84.6

BACK MUSCLE EMG RESPONSES EVOKED BY CUTANEOUS FLANK NERVES IN RATS: EFFECTS OF SPINAL TRANSECTION, ANESTHESIA AND STEROID HORMONES. D.A. Holtzman, D.W. Pfaff and S. Schwartz-Giblin, Rockefeller University, N.Y., N.Y. 10021.

This study examines EMG responses in the lateral longissimus muscle to cutaneous flank nerve stimulation in ovariectomized (OVX) rats. When anesthetized with a low surgical dose of urethane (1.0 g/kg) 24 h after complete spinal transections (between T1 and T2), 15 of 19 OVX rats were capable of producing the same early (11-30 ms) and late (50-120 ms) responses to ipsilateral stimulation as CNS-intact rats. However, at this anesthetic level, only 6 of 18 transected rats had early and late flank nerve-evoked responses following contralateral stimulation, whereas all CNS-intact rats showed these responses ($p < .05$). It appears that descending information **facilitates** back muscle responses to **contralateral** cutaneous inputs. As early as 3 min after 1 mg (i.v.) of progesterone, the EMG response to unilateral flank nerve stimulation is decreased in CNS-intact, OVX rats (13 of 17) with 1.4 g of urethane/kg [Schwartz-Giblin and Pfaff (1990) J. Neurophysiol.]. This effect was not seen with the lower anesthetic dose in 4 of 4 CNS-intact or in 5 of 7 transected rats in the present study. No effect of estrogen (pretreatment or acute) was found in transected rats. Depression of unilateral cutaneous reflexes by progesterone appears to be state-dependent, consistent with studies by others which show reduced excitability of multisynaptic reflex pathways by anesthesia and potentiation of $GABA_A$ synapses by progesterone.

84.7

INITIAL STUDIES ON THE MODIFICATION OF SPINAL STRETCH REFLEXES IN SPINAL CORD INJURED PATIENTS. R.L. Segal and S.L. Wolf. Div. Phys. Ther., Depart. Rehab. Med., Atlanta, GA 30322.

This abstract reports the findings from studies aimed at reducing hyperactive biceps spinal stretch reflexes (SSR) in spinal cord injured (SCI) patients using operant conditioning. Ten subjects (2 have withdrawn) have participated or are participating in the study. Subjects were randomly assigned to control (N=5) or treatment (N=5) groups. For both groups the first 6 sessions (baseline) and last 4 sessions (follow-up) involve no operant conditioning. The middle 24 sessions involve operant conditioning for the treatment group, while the sessions are the same as baseline and follow-up for the control group. Of the three subjects who have completed treatment, one subject did well (SSR decreased by almost 60%). A second subject was also doing well until the 10th treatment session when he contracted a urinary tract infection. Interestingly, this subject modulated the SSR downward within a session, but not below baseline levels. A third subject showed no ability to modulate the SSR. Two subjects have finished as control subjects and demonstrated relatively stable SSR's.
Supported by the AMERICAN PARALYSIS ASSOCIATION.

84.8

BILATERAL MAUTHNER LESIONS CAUSE INCREASES IN EMG MEASURES OF ESCAPE RESPONSES. M. B. Foreman and R. C. Eaton. Center for Neuroscience, University of Colorado, Boulder, CO 80309-0334.

In earlier work, we showed that electromyographic (EMG) recordings from the trunk musculature can account for kinematic parameters of the Mauthner (M-) initiated escape response of goldfish. A long term goal is to look for EMG parameters specifically associated with the firing of the M-cell. In this work we compare EMG recordings from animals in which one or both M-cells were electrolytically lesioned with normal and sham-lesioned animals. We here focus on an unexpected finding: four principal measures of the EMG size increased from 200 - 271% during escapes in fish without M-cells. The variability of these EMG parameters also increased. In contrast, these parameters changed from only 0 - 38% for non-M responses in fish with one M-cell remaining. Thus, we suspect that *bilateral* M-cell lesions cause damage to ancillary networks, possibly involving PHP cells, that may be much less affected by *unilateral* lesions. When intact, these networks appear to actively suppress non-essential motor output during escape responses. As in previous studies there was a significant increase in response latency associated with M-cell loss. Interestingly, despite the large increase in EMGs, there were no significant differences in other kinematic parameters associated with lesions of the M-cells. [Supported by NIH grant NS22621].

84.9

AN IMPORTANT AREA FOR RESPIRATORY RHYTHMOGENESIS IN THE MEDULLA OF THE FROG RANA CATESBEIANA: AN IN VITRO STUDY USING THE HEMISECTED BRAINSTEM. HA McLean; SF Perry*; N Kogo & JE Remmers. Dept. of Medical Physiology. University of Calgary. Calgary, AB. Canada. T2N 4N1.

In the isolated brainstem of the frog, respiratory-related motor output is recorded from the rootlets of the cranial nerves V, IX, X and XII using suction electrodes. Two kinds of periodic activity are present: one related to lung ventilation and one related to buccal ventilation. Midsagittal section of the brainstem left the motor output associated with lung ventilation unchanged but abolished that associated with buccal ventilation. The hemisected brainstem responsed to changes in superfusate pH by increasing the burst frequency of the motor ouput in a linear fashion with decreasing pH values from 8.6 to 7.6.

We have attempted to locate an area important for generating or modulating the motor output associated with lung ventilation. Microinjections (10 nl) of glutamate (100 and 10 mM) and GABA (100 mM) into a discrete area in the medulla resulted in respective increases and decreases in the frequency of the respiratory motor output. Microinjections of these neurotransmitters into other areas of the medulla, rarely influenced the motor output. The area yielding the largest number of positive responses was marked by injection of rhodamine labeled microspheres. Histological analysis revealed that this area lies 200 - 500 μm below the ventral surface near the exit of the VI cranial nerve, and appears to be in the reticular formation just caudal to the level of the IX motor nucleus. Supported by AHFMR.

84.10

MAP OF BRAINSTEM NEURONS ACTIVATED BY CHEMICAL STIMULATION IN THE RAT PERIAQUEDUCTAL GRAY AS REVEALED BY THE EXPRESSION OF THE C-FOS PROTEIN. *J.Sandkühler & T.Herdegen* II. Physiolgisches Institut, Universität Heidelberg, Germany.

Activation of efferents of the midbrain periaqueductal gray (PAG) may produce a wide variety of behavioral, somatosensory and vegetative responses. Here we have used the expression of the proto-oncogene *c-fos* encoded FOS protein as a cellular marker of brainstem neurons which are activated by microinjections of bicuculline ($GABA_A$ receptor antagonist, 200 pmol in 50 nl) into PAG sites of pentobarbital anesthetized rats. The animals were perfused 90 min after the injections and the nuclear FOS protein was detected by immunocytochemistry (FOS-IR, avidin biotin method, primary antibody at 1:6000) in free floating 50 μm frontal sections through the brainstem.

After bicuculline injections into dorsal sites of the rostral PAG, FOS-IR was elevated above levels in sham treated (NaCl injected) animals *bilaterally* in the: -dorsal part of the rostral PAG, -throughout the caudal PAG, -commissural part of the solitary tract ncl., -rostral ventrolateral medulla, -medial vestibular ncl., -ncl. locus coeruleus, -rostral ventromedial medulla, -superior colliculus, -periventricular gray, -medial hypothalamus, -supraoptic ncl., -suprachiasmatic ncl. and -zona incerta. Preliminary results suggest that the injections into ventrolateral sites of the PAG produce a clearly different pattern of FOS-IR within the PAG and the brainstem.

These results provide a direct, functional substrate for the diversity of output functions of the PAG overlapping with known anatomical efferent connections. The putative role of proto-oncogenes such as *c-fos* for longterm adaptive changes of neuronal function suggest that efferents of the PAG may be involved in the plasticity of brainstem neurons. SUPPORTED BY A GRANT FROM THE DEUTSCHE FORSCHUNGSGEMEINSCHAFT (SA 435).

TRANSMITTERS IN INVERTEBRATES: COELENTERATES, ANNELIDS, ARTHROPODS

85.1

NEUROPEPTIDES IN COELENTERATES: STRUCTURE, LOCALISATION, BIOSYNTHESIS AND ACTION. C.J.P. Grimmelikhuijzen[1], D. Darmer[1*], C. Schmutzler[1*], H.-P. Nothacker[1*], K. Carstensen[1*], R.K. Reinscheid[1*], K.L. Rinehart[2*] and I.D. McFarlane[3*]. [1]Center for Molecular Neurobiology, Martinistr. 52, 2000 Hamburg 20, FRG, [2]School of Chemical Sciences, University of Illinois, 1209 W. California St., Urbana, IL 61801, [3]Department of Applied Biology, University of Hull, Hull HU6 7RX, UK.

Coelenterates have the simplest nervous system in the animal kingdom and were probably the first animal group that evolved a nervous system. Until recently, the nature of neurotransmitter substances in coelenterates has remained unknown. By means of several radioimmunoassays, we have now isolated 13 novel neuropeptides from sea anemones and 2 from hydromedusae. These peptides are all related and contain the C-terminus Lys-X-NH_2 or Arg-X-NH_2, where X represents Ala, Asn, Ile, Phe, Pro or Trp. Three peptides contain an N-terminal L-3-phenyllactyl residue which protects against degradation by nonspecific aminopeptidases. The neuropeptides are located in different sets of neurons in sea anemones. Each neuropeptide has a characteristic action. The precursor protein for the sea anemone neuropeptide Antho-RFamide (<Glu-Gly-Arg-Phe-NH_2) has been cloned. In *Calliactis parasitica* it harbors 19 copies of immature Antho-RFamide (Gln-Gly-Arg-Phe-Gly) together with 7 other, novel, putative neuropeptide sequences. The precursor of *Anthopleura elegantissima* contains 15 copies of Antho-RFamide and 18 other, putative neuropeptides.

C.J.P. Grimmelikhuijzen, K.L. Rinehart, E. Jacob, D. Graff, R.K. Reinscheid, H.-P. Nothacker and A.L. Staley (1990) Proc. Natl. Acad. Sci. USA, 87, 5410-5414.

D. Darmer, C. Schmutzler, D. Diekhoff and C.J.P. Grimmelikhuijzen (1991) Proc. Natl. Acad. Sci. USA, 88, 2555-2559.

85.2

THERMAL AND CHEMICAL INPUTS DIFFERENTIALLY AFFECT SEROTONIN NEURONS THAT EXPRESS FEEDING BY THE MEDICINAL LEECH. J.R. Groome* and C.M. Lent. Dept. Biology, Utah State Univ. Logan UT 84322-5305.

Hirudo medicinalis ingest mammalian blood. The physiological components of this behavior are activated by serotonin-containing neurons, and we have found that either thermal or chemical stimulation of the prostomial lip synaptically excites serotonergic Retzius cells (RZ) throughout the CNS. Thermal effects have a more rapid onset and chemical stimulation evokes long-lasting excitation of RZ. Atropine (1μM) inhibits the thermal excitation of RZ (82%) and 1mM kynurenic acid decreases the chemical excitation by 85%. These data suggest that acetylcholine is the transmitter released from neurons in the thermosensory pathway, while glutamate mediates chemical excitation of RZ. Leech ingestive behavior depletes ganglionic serotonin by 28% (Lent *et al. J. Comp. Physiol. A* 168:191-200, 1991), and these two sensory pathways differentially affect central and peripheral serotonin stores. Thermal stimulation depletes 20-25% of the serotonin from the CNS, but does not deplete it from body wall. Chemical stimulation does not deplete central serotonin, but reduces the levels of this monoamine in the body wall by 28%. These data suggest distinct physiological and biochemical roles for thermal and chemical inputs to RZ during the expression of stereotyped ingestive behavior.

85.3

CARBACHOL AND SEROTONIN-ENHANCED RUBIDIUM UPTAKE RATE IN LEECH GLIA. M.C. Foster, C.M. Castiglia*, and A.J. Saubermann, Dept. of Anesthesiology, SUNY at Stony Brook, Stony Brook, NY 11794-8480.

Glia have receptors for carbachol (CARB) and serotonin (SER). Functions of these neurotransmitters (NT) in glial cells are not clear. It has been suggested that they may modify extracellular K clearance. The extent to which CARB and SER alter K metabolism in leech CNS was studied by electron microprobe analysis. Rb was substituted for K in order to study its uptake into neurons and glia. Leech (*Hirudo Medicinalis*) ganglia were perfused 60 s in 4 mM Rb Ringers' with and without CARB or SER, then frozen, cryosectioned, and analyzed for water content and concentrations of K, Rb, Na, Mg, P, Cl, and Ca in neurons and glia. In neurons, SER caused a small, dose-dependent increase in Rb uptake; CARB had little effect. In glia, both SER and CARB caused a dose-dependent increase in Rb uptake; Rb (mmol/Kg dry wt) was 28±2 (control); with 5, 50, and 500 µM NT, Rb was 36±3, 53±3, and 70±3 (CARB), and 52±4, 63±3, and 89±11 (SER). These results suggest that SER and CARB may help regulate K metabolism. (Supported by NIH NS21455.)

85.4

THE STEROID HORMONE, 20-HYDROXYECDYSONE, CONTROLS GANGLIONIC FUSION DURING DEVELOPMENT OF THE MOTH, *MANDUCA SEXTA*. T.M. Amos and K.A. Mesce. Graduate Program in Neuroscience and Dept. of Entomology, Univ. of Minnesota, St. Paul, MN 55108.

Manduca sexta is a holometabolous insect whose adult pterothoracic ganglion is formed when the larval meso- and metathoracic ganglia fuse with the first and second abdominal ganglia. The first gross morphological evidence of ganglionic fusion is apparent during the first few hours after pupal ecdysis. The pterothoracic ganglion is recognizable in its adult form 7 days later. As the various ganglia begin to fuse, the level of 20-hydroxyecdysone (20-HE) also slowly rises for the third and final time. We investigated whether the changing levels of 20-HE that occur shortly before and after pupal ecdysis regulate pterothoracic ganglion formation.

Normally there are two relatively small peaks of 20-HE that occur prior to pupation, and a third rise that begins at pupal ecdysis. In order to manipulate 20-HE levels, animals were ligated below the prothoracic segment. This isolated the various ganglia from the prothoracic glands, the only known source of ecdysone (20-HE precursor). Animals ligated after the first peak of 20-HE did not form pupal cuticles and showed no evidence of ganglionic fusion. Animals ligated after the first 20-HE peak and subsequently infused with physiological levels of 20-HE, mimicking the second peak, formed perfect pupal abdomens. No signs, however, of ganglionic fusion were evident. When animals, which had been induced to form a pupal cuticle, were again infused with 20-HE to mimic the post-pupation rise, the ability to form the pterothoracic ganglion was restored. This is the first study to demonstrate that all three fluctuations in 20-HE, during the larval to pupal transition, are required for the initiation of a developmental process.

85.5

THE MECHANISM OF AN EXCITABILITY CHANGE INDUCED BY MUSCARINIC RECEPTORS IN AN IDENTIFIED INSECT MOTONEURON. B. A. Trimmer and J. C. Weeks. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

In larval *Manduca sexta*, the identified motoneuron, PPR, receives direct cholinergic input from proleg mechanosensory neurons. In addition to eliciting fast unitary EPSPs via nicotinic receptors, stimulation of these afferents can evoke slow, long-lasting (5-20 s) depolarizations (sEPSPs) of muscarinic pharmacology. Both the sEPSP, and muscarinic agonists such as oxotremorine-M (oxo-M), lower the spike threshold of PPR. To investigate the mechanism of this change in excitability we used a single electrode voltage clamp to monitor currents evoked by muscarinic agents. The main current activated by oxo-M is slow and long-lasting, allowing us characterize its voltage dependence using slowly ascending (40 s) voltage ramps applied at the soma. This inward current is largest near PPR's normal resting potential (-50 mV), and declines with depolarization or hyperpolarization. The current is TTX-insensitive but is reversibly abolished when all external Na is replaced with N-methyl-D-glucamine. These findings suggest that a major component of the muscarinic current is carried by Na but is independent of the voltage-gated Na channel. To help analyze the regulation of this current, we injected various agents into the soma of PPR through the voltage clamp electrode. Injection of GTPγS causes a prolongation of PPR's response to oxo-M and a persistent activation of the muscarinic current, effects that are consistent with the action of a G-protein. These data suggest that the excitability of PPR can be regulated by muscarinic modulation of a Na-dependent inward current via a G-protein.

Supported by the Whitehall Foundation, NIH and NSF.

85.6

MOLECULAR ANALYSIS OF *FMRFamide* GENE EXPRESSION IN *DROSOPHILA*. L. E. Schneider and P. H. Taghert. Dept. of Anatomy and Neurobiology, Washington University Medical School, Saint Louis, Mo 63110.

The *Drosophila FMRFamide* gene encodes a single detectable transcript that is expressed in a small, stereotyped pattern of ~100 central neurons. To study the molecular mechanisms that underlie this restricted pattern of expression we have generated a P element construct containing the *FMRFamide* gene fused to a ß-galactosidase reporter gene. The construct contains 8 kilobases of the *FMRFamide* gene: 5 kilobases of upstream sequence, the short untranslated first exon, the entire intron, and the first 70 base pairs of the open reading frame. We have isolated two independent transformed lines with this construct. At all stages of development, the pattern of expression in animals that are homozygous for the construct is nearly identical to the expression of the endogenous gene. This suggests that the regulatory elements that are necessary for the appropriate pattern of *FMRFamide* gene expression are included in this construct. To determine whether expression in individual groups of neurons is directed by distinct DNA elements or, conversely, whether all neurons require the same regulatory elements, we are generating additional constructs that delete the intron or portions of the upstream sequence. These constructs will allow a more precise definition of the regions of the *FMRFamide* gene that direct expression to the appropriate subset of neurons. This work was supported by NIH grant #NS21749

85.7

A GENETIC ANALYSIS OF THE *FMRFamide* GENE REGION IN *DROSOPHILA*. M.A. O'Brien, M. Roberts*, and P.H. Taghert. Dept. of Anatomy and Neurobiology, Washington University Med. School, St. Louis, MO 63110.

We are using *Drosophila* molecular genetics to investigate the functions of modulatory neuropeptides. The gene of interest encodes a complex precursor protein with many diverse FMRFamide-related sequences. We are in the process of creating mutations in the gene in order to investigate the development and physiology of animals that lack its expression. Deletions were created in the 46C region, the cytological locus for the *FMRFamide* gene, using X-rays. One of these deficiency stocks, Df(2R)X3, has a visible deletion extending from 46C-E that includes the *FMRFamide* gene. Using cosmid and phage clones of surrounding genomic regions, the proximal breakpoint of the Df(2R)X3 deletion was mapped by Southern blot analysis to ~20kb upstream of the gene. Another deficiency stock, Df(2R)X1, does not delete the gene and its proximal breakpoint was mapped to within ~15kb downstream of the *FMRFamide* gene. Together, these 2 deletion stocks define a small ~40kb genomic interval that includes the *FMRFamide* gene; they were used to screen for point mutations. Because the phenotype of a *FMRFamide* gene mutation cannot be predicted, we began by screening for lethal phenotypes among ~1300 EMS mutagenized chromosomes. Seventeen lethal alleles mapping to the Df(2R)X3 were obtained with EMS mutagenesis. Five of these map to the small ~40kb interval defined by X3 and X1 breakpoints. Complementation analyses revealed that these 5 lethal mutations segregate into 2 complementation groups; therefore, at least 2 genes with lethal mutant phenotypes are likely to reside in this defined interval. We are in the process of determining if either is the *FMRFamide* gene. To account for the possibility that a *FMRFamide* gene mutant does not have a lethal phenotype, we are also mobilizing a P element from a position ~25kb upstream from the *FMRFamide* gene and screening for insertions into the gene as well as new deletions. These molecular genetic approaches should provide a novel means of studying neuropeptide function *in vivo*. This work was supported by NIH Grant # NS08460 and #NS21749.

85.8

PROCTOLIN MODULATION OF MUSCLE EXCITABILITY IN THE LARVAL HOUSEFLY IS MIMICKED BY PHORBOL ESTERS. L.D. Acevedo, D.N. Mbungu* and M.E. Adams. Entomology Dept, Univ of California, Riverside, CA 92521.

The neuropeptide proctolin is a cotransmitter for one of the motor neurons innervating the longitudinal ventrolateral muscles (6A and 7A) of the larval housefly, *Musca domestica*. Repetitive motor neuron activity or direct proctolin application leads to the appearance of calcium action potentials in normally inexcitable muscle targets, suggesting that the peptide modulates a voltage-sensitive calcium current (Duce and Adams, *Soc. Neurosci. Abstr.* 15:25). We find that the proctolin-activated muscle action potential is enhanced by bath application of 0.5-1 µM Bay K 8644 and is inhibited by 5 nM nifedipine; both effects are reversible. Thus, these insect muscle calcium channels are pharmacologically similar to vertebrate L-type channels.

We have studied the mechanism by which proctolin activates the muscle action potential by examining activators and inhibitors of different second messenger pathways. The phorbol ester PMA (phorbol 12-myristate 13-acetate) evoked an action potential similar to that elicited by proctolin, and which was blocked by nifedipine. The response to PMA was reversible and the muscle did not respond to the inactive 4α analog of PMA. Staurosporine, an inhibitor of protein kinases A and C, suppressed both the spontaneous and proctolin-induced action potentials. Membrane permeable analogs of cAMP and cGMP, and IBMX, did not evoke action potentials. Our data suggest that protein kinase C is involved in the generation of the proctolin-induced potential, which results from a dihydropyridine-sensitive calcium current.

Supported by a UC President's Fellowship to LDA and NIH NS-24472 to MEA.

85.9

COMPLETE PROCESSING OF A SYNTHETIC PROHORMONE OF AKH I RECONSTITUTED IN VIVO. R.C. Rayne* and M. O'Shea. Neurosciences Interdisciplinary Res. Centre, University of Sussex, Brighton, Sussex BN1 9QG, U.K.

We have chemically synthesized the complete prohormone of AKH I and developed an in vitro assay in which all in vivo post-translational events in precursor processing are reconstituted. AKH I is an amidated 10 residue insect hormone synthesized by the neuroendocrine cells of the glandular corpus cardiacum (CC). The AKH I prohormone consists of one copy of AKH I, a gly-lys-arg processing site and a 28 residue C-terminal peptide. The prohormone first forms a homodimer precursor which is then processed into two AKH I molecules and a dimer of the 28 residue C-terminal peptide. The C-terminal peptide contains a processing site (arg-lys) which is unused. To study processing of the synthetic precursor in vitro, extracts from the CC were incubated with synthetic and biosynthetically radiolabelled precursor. The in vitro system generates sequentially AKH-gly-lys-arg, AKH-gly-lys, AKH-gly and finally the bioactive form, AKH-NH_2. It may therefore allow us to isolate and characterise all enzymes involved in in vivo processing of AKH I precursor. Also, structural studies (NMR and X-ray crystallography) of the synthetic prohormone are in progress in order better to understand the influence of precursor structure on processing.

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85.10

THE ACTIONS OF L-GLUTAMIC ACID ON MOTONEURONS OF THE LOBSTER CARDIAC GANGLION. H. Hashemzadeh-Gargari. and J. Freschi. Neurology Dept., Emory University, Atlanta, GA 30322.

Although in the mammalian nervous system, glutamate is exclusively an excitatory neurotransmitter, in invertebrates it may be either excitatory or inhibitory. For example, in the crustacean stomatogastric ganglion, glutamate may cause either a Na^+-dependent excitatory response or inhibitory responses mediated by Cl^- or K^+ conductances. We studied the effects of glutamate on lobster cardiac ganglion neurons voltage-clamped with two microelectrodes. We found that glutamate caused a depolarization and increase in bursting of the motoneurons (follower cells), and under voltage-clamp this effect was mediated by a dose-dependent increase in conductance leading to an inward current. The response to L-glutamate was mimicked by quisqualic acid and L-aspartate (quisqualate > glutamate > aspartate). Kainic acid, N-methyl-D-aspartate, glutamate diethyl ester, and D-glutamate were not effective. The antagonist kynurenic acid, over a concentration range of 0.1 to 1 mM, inhibited the response. The response was not modified by spermine (polyamine site) nor by glycine. The reversal potential of the glutamate-evoked current was between -20 and 0 mV, and the response was blocked in Na^+-free saline. We conclude that these neurons contain glutamate receptors, similar to mammalian non-NMDA receptors, that activate a cation current, carried by K^+ and Na^+. The physiological response appears identical to that produced at muscle glutamate receptors of crustaceans and insects (Supported by NIH NS-22628).

85.11

MULTIPLE RECEPTORS MEDIATE THE MODULATORY EFFECTS OF SEROTONERGIC NEURONS IN A SMALL NEURAL NETWORK. B. Zhang and R. M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The gastro-pyloric receptor (GPR) cells are a set of cholinergic/serotonergic mechanosensory neurons that modulate the activity of neural networks in the crab stomatogastric ganglion (STG). Stimulation of these cells evokes a variety of slow modulatory responses in different STG neurons that are mimicked by exogenously applied 5-HT; these responses include tonic inhibition, tonic excitation, induction of rhythmic bursting and induction of bistable plateau properties.

We used pharmacological antagonists to show that these four classes of modulatory response are mediated by at least four distinct 5-HT receptors in the STG. GPR- and 5-HT-induced inhibition of the pyloric constrictor (PY) cells was selectively antagonized by gramine. Tonic excitation of the lateral pyloric (LP) cell was blocked by cinanserin. Induction of rhythmic bursting in anterior burster (AB) cell and the electrically-coupled pyloric dilator (PD) cells was antagonized by cinanserin and atropine at concentrations that do not block muscarinic cholinergic receptors in the STG. Induction of plateau potentials in the dorsal gastric (DG) cell was not blocked by any of the drugs we tested. These results show that a modulatory sensory neuron can use 5-HT to evoke multiple modulatory responses via multiple 5-HT receptors. They also provide further evidence that the GPR cells use 5-HT as a neurotransmitter. Supported by NIH #NS 17323.

85.12

FMRFAMIDE-RELATED PEPTIDES FROM CRAYFISH PERICARDIAL ORGANS. A. J. Mercier, I. Orchard, M. Skerrett and V. TeBrugge. Dept. of Biol. Sci., Brock University, St. Catharines, Ont., L2S 3A1, and Dept. of Zoology, University of Toronto, Toronto, Ont., M5S 1A1.

The pericardial organs of crayfish contain high amounts of FMRFamide-like immunoreactivity (FLI), suggesting a hormonal role for FMRFamide-related peptides (Mercier, Orchard & TeBrugge, J. exp. Biol. 156: 519-538, 1991). The present study was undertaken to determine the amino acid sequences of such peptides. Extracts of pericardial organs from Procambarus clarkii were fractionated on two sequential reversed-phase HPLC columns (a C18 column, followed by a Phenyl column), using 0.1 % TFA and gradients of acetonitrile. The peak fraction from the phenyl column (containing the highest amount of FLI) was found to contain two peptides with the sequences NRNFLRF-NH_2 and DRNFLRF-NH_2. We have named these peptides "NF_1" and "DF_2", respectively, due to their similarities with identified peptides from lobster PO's, peptides "F_1" (TNRNFLRF-NH_2) and "F_2" (SDRNFLRF-NH_2) (Trimmer et al, J. Comp. Neurol. 266: 16-26). Synthetic forms of peptides NF_1 and DF_2 increased the rate and amplitude of contraction of isolated crayfish hearts, as did the peak fraction from the phenyl column. DF_2 increased the size of EPSP's in phasic extensor muscles; effects of NF1 on this preparation are being studied. The results support a neurohormonal role for FMRFamide-related peptides in crayfish.

Supported by NSERC Canada.

REGULATION OF RESPIRATION AND AUTONOMIC FUNCTIONS

86.1

INTRACELLULAR AND EXTRACELLULAR RECORDINGS OF POSTERIOR HYPOTHALAMIC NEURONS IN VITRO: RESPONSES TO HYPOXIA AND HYPERCAPNIA. **G.H. Dillon and T.G. Waldrop. Dept. of Physiology & Biophysics, Univ. of Illinois, Urbana, IL 61801.**

Several studies have suggested posterior hypothalamic (PH) neurons modulate cardiorespiratory responses to stimulation of central and/or peripheral chemoreceptors. The present studies were conducted to examine responses of PH neurons *in vitro* to hypercapnia and hypoxia. 400 μm slices were taken from 150-250g, male Sprague-Dawley rats. Slices were placed in a recording chamber and perfused with nutrient media equilibrated with 95% O_2/5% CO_2. Extracellular and intracellular (whole-cell patch) recordings were obtained and responses to hypercapnia (7% CO_2/93% O_2) and hypoxia (5% CO_2/95% N_2 or 10% O_2/5% CO_2/85% N_2) were examined. EXTRACELLULAR RESULTS: 31% of PH neurons studied were excited by elevated CO_2. 84% of PH neurons were stimulated by hypoxia (0% or 10% O_2); the response to hypoxia was shown to be dose-dependent. Only 13% of PH neurons were stimulated by both hypoxia and hypercapnia. In addition, stimulation in response to hypoxia or hypercapnia was observed during synaptic blockade. INTRACELLULAR RESULTS: Input resistance of studied cells was 173±17.4 MΩ. Neurons studied in response to hypoxia had a resting V_m of -51.8±4.1 mV. 67% of these cells depolarized and increased their discharge during the hypoxic bout; hyperpolarization and a decrease in activity followed the stimulation. PH neurons studied in response to elevated CO_2 had a resting V_m of -49.8±3.8 mV. Hypercapnia increased the firing rate of 42% of those cells studied in the whole-cell patch configuration. Results of these studies suggest: 1) PH neurons modulate responses to both hypercapnia and hypoxia, but via separate subpopulations of cells, 2) the posterior hypothalamus may be a site of central hypercapnic and hypoxic chemoreception (Supported by NIH 38726; American Heart Association).

86.2

VAGAL INPUT TO THE VENTRAL RESPIRATORY GROUP (VRG) OF THE RAT. F. Hayashi*, S.K. Coles, R. Behnia*, and D.R. McCrimmon. Depts. of Physiology & Anesthesia, Northwestern University, Chicago, IL 60611.

Neuronal candidates for mediating the Breuer-Hering reflex prolongation of expiration (E), were identified in the rostral VRG of anesthetized, paralyzed, and vagotomized rats (n=12). Intracellular recording of VRG neurons was used to identify synaptic potentials (PSPs) elicited by low intensity stimulation (.5-100 Hz, .05-.1 ms, 5-20 μA) of the vagus nerves. Antidromic stimulation of the vagus and superior laryngeal nerves and of the spinal cord was used to identify axonal projections of recorded neurons. Sixteen non-antidromically activated E neurons were recorded and divided into two groups: 1) those which rapidly depolarized in early E (E-Dec, n =12) and 2) those exhibiting progressive depolarization during E (E-Aug, n=4). EPSPs were observed in 4 E-Dec neurons (latency = 2.2-4.0 ms) and IPSPs in 1 E-Dec neuron (latency = 5.2 ms). PSPs were not observed in E-Aug neurons. During tetanic stimulation E-Dec neurons depolarized and discharged tonically. In contrast, E-Aug neurons ceased firing. Twenty-five non-antidromically activated inspiratory (I) neurons were recorded and divided into three groups: 1) those which progressively depolarized throughout I (I-Aug, n=18), 2) those which rapidly depolarized during early I (I-Dec, n=5), and 3) those which depolarized during late I (n=2). EPSPs (latency of activation=2.2 and 2.6 ms) were seen in two I-Aug neurons, but only one IPSP (latency=5.4 ms) was observed. All I neurons decreased their discharge rate during tetanic vagal stimulation. These results are consistent with the hypothesis that vagal afferents paucisynaptically excite a subset of E-Dec neurons in the VRG that, in turn, inhibit I neurons, thereby delaying the onset of the next inspiratory cycle.

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86.3

ACETYLCHOLINE (ACh) RELEASE IN THE MEDIAL PONTINE RETICULAR FORMATION (mPRF) PREDICTS RESPIRATORY FREQUENCY. R. Lydic, H.A. Baghdoyan, and Z.Lorinc, Dept. of Anesthesia, Penn State Univ., College of Medicine, Hershey Pa 17033.

Studies using *in vivo* microdialysis recently suggested that respiratory frequency during the carbachol-induced REM sleep-like state (DCarb) was accompanied by enhanced ACh release (*FASEB J. 5*:A734, 1991). We now report that levels of ACh release in the mPRF predict respiratory frequency during DCarb and during quiet wakefulness (W). To date, we have studied 4 intact, unanesthetized cats instrumented for polygraphic and respiratory recordings and trained to sleep in the laboratory. A microdialysis probe (2mm tip, 20kDa molecular cutoff) was placed in the mPRF for collecting dialysate samples during wakefulness and during the DCarb state produced by microinjecting carbachol (4.0μg/0.25μl) into the contralateral mPRF. Dialysis samples were analyzed for ACh using HPLC and a BAS electrochemical detector (LC-4B). A statistically significant increase ($p<0.0001$) was observed in ACh release (mean pmoles $\pm$S.D.) during DCarb (0.71 $\pm$0.29) as compared to W (0.42 $\pm$0.08). Respiratory frequency in breaths/min declined to an average of 30 during DCarb from 38 in W. Regression analyses revealed that levels of ACh release accounted for most of the variance in respiratory frequency during both DCarb (r^2=0.97) and W (r^2=0.91). Thus, state-dependent changes in respiratory frequency can be expressed as a function of endogenous ACh release in non-respiratory regions of the mPRF known to regulate REM sleep.

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86.4

EVIDENCE FOR A ROLE FOR TONIC RELEASE OF AN EXCITATORY AMINO ACID AT THE CAUDAL-SUBRETROFACIAL AREA IN THE CONTROL OF VENTILATION. J.E. McManigle*, W.H. Panico,* S. Pineo,* A.M.T. Da Silva*, K.L. Dretchen and R.A. Gillis*. Georgetown Univ., Washington, D.C. and Uniformed Services Univ. of Health Sci., Bethesda, MD.

Neurons in the caudal-subretrofacial area (cSRFA) of the ventrolateral medulla of the cat exert a major role in the control of ventilation (Neurosci. Abstr. 16: 298, 1990). Microinjection of excitatory amino acids (EAA) receptor agonists into the cSRFA produces decreases in respiratory activity. For example, using concentrations of 50 and 100 mM of L-glutamate and microinjecting 20 nl/side results in apnea. Nearly identical effects are noted with microinjection of N-methyl-D-aspartate (NMDA) (0.78 mM) and (RS)-alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA) (0.05 mM), agonists for the NMDA and non-NMDA receptors, respectively. To determine whether tonic release of an EAA occurs at the cSRFA and exerts control over ventilation, we evaluated the effects of dihydrokainate, an inhibitor of glutamate and aspartate reuptake (J. Neurochem. 32: 121, 1979). Dihydrokainate (10 to 100 ng/20 nl) microinjected bilaterally into the cSRFA (located approximately 3 mm rostral to obex, 4 mm lateral to midline and 1.5 mm below the ventral surface) of chloralose-anesthetized cats while monitoring tidal volume (V_t), respiratory rate (f), blood pressure (BP) and heart rate (HR), produced significant reductions in V_t (20.2$\pm$2.8 ml to 14.5$\pm$1.9 ml, $p<0.05$) (N=6). This occurred without significant changes in f, BP and HR. Pretreatment with a specific antagonist of non-NMDA receptors, namely, 6-cyano-7-nitroquinoxaline -2, 3 - dione (CNQX), counteracted the respiratory depressant effect of dihydrokainate. These results suggest that tonic release of an EAA occurs at the cSRFA and exerts an inhibitory effect on ventilation that is mediated through activation of a non-NMDA EAA receptor.

86.5

CARBONIC ANHYDRASE IN BRAINSTEM RESPIRATORY CHEMOSENSITIVE REGIONS. Y. Pan, K. Nauss*, D.G. Bernard, R.M. Douglas*, and C.O. Trouth. Dept. of Physiol. & Biophysics, Coll. of Med., Howard Univ., Washington, D.C. 20059.

Regions of respiratory chemosensitivity that respond to cerebrospinal fluid (CSF) pH changes and inspired carbon dioxide have been described on the ventral medullary surface (VMS). The present investigation was undertaken to determine the presence and distribution of carbonic anhydrase (CA) in the rat brainstem as studied by histochemical and immunocytochemical methods. Histochemical staining of soluble and membrane-bound CA was found to be more intense in nerve fibers than in neuronal cell bodies. The intensity increased with age from newborn to adult. Immunocytochemical staining of soluble CA was intense in the cytoplasm of neurons and glia especially in the endothelium of blood vessels and within the marginal glia. In the adult rat, CA is ubiquitous throughout the medulla with greatest intensity being at the VMS and in the dorsal medulla in and around the Nucleus Tractus Solitarius and Cuneate Nucleus. In the neonate there was a paucity of clearly stained neuronal and glial elements. **SUPPORT:- NIH-NIGMS Grant # 806GM08016 AND NIH Grant PH 55-T 32 GM-07800.**

86.6

PROSTAGLANDIN H-SYNTHASE (PGHS) IMMUNOREACTIVITY AND ENZYME ACTIVITY IN THE LATE GESTATION OVINE BRAIN. J.L. Norton, S. L. Adamson*, A. D. Bocking*, T. Zakar*, D. Olson* and V. K. M. Han*. MRC Group in Fetal and Neonatal Health and Development, The Lawson Res. Inst., London, and Mt. Sinai Hosp., Toronto, ON, Canada.

PGHS is the rate limiting enzyme in prostaglandin (PG) biosynthesis. PGs may play a role in the regulation of breathing *in utero*. To determine if PGs are synthesized in the developing brain, we have used immunocytochemistry (ICC) to identify PGHS immunoreactive (IR) neurons, and a specific enzyme assay to measure PGHS activity in different brain regions. ICC was performed on Bouin's fixed tissue sections from fetal ovine brains (120-130 d gestation, n=3), using avidin-biotin-peroxidase technique with specific antiserum against ovine PGHS. PGHS enzyme activity was determined in microsomes from different brain regions by measuring the PGE_2 production in the presence of 10 mM arachidonate. PGHS-IR neurons and fibers were observed in the gracile and cuneate nuclei, dorsal vagal nucleus and raphe obscurus of the medulla, reticular formation of the pons, superior colliculi of the midbrain, lateral hypothalamus, dentate gyrus and CA3 regions of the hippocampus, and widely distributed in the gray matter of different regions of the cerebral cortex. PGHS enzyme activity (pg PGE_2/mg prot/4 min) in different brain regions were as follows: cortex (frontal) 1967.8, medulla 474.4, pons 191.4, superior colliculus 3101.5, cerebellum 108.7, and hypothalamus 601.8. PGHS enzyme activity of the different regions correlate well with the number of PGHS-IR neurons in each region. These findings indicate that functional PGHS-IR neurons are present in specific regions of the late gestation ovine brain, and suggest a role for locally synthesized PGs in the regulation of fetal breathing.

86.7

MUSCLE SYMPATHETIC NERVE AND HEMODYNAMIC RESPONSES DURING HYPOVENTILATION INDUCED HYPERCAPNIA IN HUMANS

David E. Anderson*, Virend K. Somers*, Mary P. Clary*, Christine A. Sinkey*, Erling A. Anderson, NIA, Baltimore MD; and U of Iowa, Iowa City IA

Respiratory inhibition and increased arterial pressure (AP) have been reported during preavoidance stress in dogs and during daily activities in humans. Hypoventilation increases arterial carbon dioxide (pCO_2) which could stimulate chemoreceptors and increase muscle sympathetic nerve activity (SNA) and AP. We sought to determine whether increased AP during hypoventilation induced hypercapnia is associated with increased SNA.

We measured AP, heart rate, oxygen saturation, end tidal CO_2, forearm vascular resistance, (FVR, plethysmography), SNA (microneurography) and central venous pressure (CVP) in 6 sitting subjects. Measurements were taken during: 1) baseline; 2) hypoventilation (6-8 breaths/min; normal tidal volume) and 3) recovery (8 min each; data mean $\pm$SE).

	Control	Hypoventilation	Recovery
pCO_2	34 ± 0.1	42 ± 3.0*	34 ± 1.0
MAP (mmHg)	94 ± 6	105 ± 7*	97 ± 6
CVP (mmHg)	0.1 ± 1.9	6.0 ± 1.3*	2.2 ± 1.7
HR (bpm)	70 ± 2	67 ± 2*	70 ± 4
SNA (units)	622 ± 175	682 ± 195	658 ± 203
FVR (units)	43 ± 8	39 ± 9	42 ± 8

Hypoventilation increased pCO_2 and caused marked increases in AP and CVP without vasoconstriction in the forearm. Despite increased AP and CVP, SNA did not decrease. Thus, hypoventilation observed during daily activity promotes increased AP which 1) fails to produce the expected baroreflex inhibition of SNA and 2) is not mediated by sympathetic vasoconstrictor activity to skeletal muscle.

86.8

CENTRAL CHEMOSENSORY CONTROL OF TONIC CARDIO-RESPIRATORY AND MOTOR RESPONSES IN A PRIMATE MODEL OF BRAIN INJURY. R.M. Millis*, D.G. Bernard, C.O. Trouth, and D.H. Wood*. Dept. of Physiol. & Biophysics, & Neurol., Coll. of Med., Howard University, Washington, D.C. 20059.

Hypoxic brain injury was produced in primates by slow infusion of physiological saline into an epidural balloon catheter (1cc/min). Mean arterial blood and airway pressure and intracranial pressure (ICP) were measured continuously in spontaneously breathing baboons (3-6kg) anesthetized with ketamine hydrochloride. Arterial blood gas tensions were measured by respiratory mass spectrometry. Cerebral perfusion pressure (CPP) was calculated. Results showed that ICP> 30cm H_2O was associated with decreased CPP, simultaneous, periodic vasopressor response, hyperventilation, and muscle rigidity in four of ten animals studied. Administration of sodium bicarbonate intra-arterially reversed the muscle rigidity and periodic responses, and increased arterial plasma bicarbonate levels. Periodic vasopressor, ventilatory and motor responses may be mediated by central chemosensory reflexes, and rapidly reversed by sodium bicarbonate. **SUPPORT: MBRS 206 GM 08016.**

86.9

Immunocytochemical identification of catecholaminergic neurons in the vasopressor areas of the medulla oblongata in the swine. S. D. Wang, R. H. Lin*, H. T. Horng*, J. C. Liu*, J. S. Kuo* and C. Y. Chai*. Dept. of Biology and Anatomy, National Defense Medical Center, Dept. of Medical Res., Taichung Veterans General Hospital, Institute of Biomedical Sciences, Academia Sinica, Taiwan, R.O.C.

The aim of this study is using immunocytochemical techniques to determine the anatomical relationships of catecholamine neurons with our previously defined vasopressor areas in the dorsal (DM) and ventrolateral (VLM) regions of medulla oblongata and also the region in between, the parvocellular reticular nucleus (PVC) of Swine. The immunoperoxidase and immunofluorescence are used to identify the distribution of immunoreactive neurons, nerve fibers and terminal processes of catecholaminergic nature in the above regions. The distribution of catecholamine-synthesizing enzyme i.e., dopamine-beta-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) are examined in the brain sections of the medulla oblongata. The results show that noradrenergic (DBH-positive and PNMT-negative) and adrenergic (DBH-positive and PNMT-positive) neurons exist in DM, VLM and PVC coincided with the areas functionally active for pressor responses. This suggests that in swine the DM,VLM and PVC contain a mixed population of catecholaminergic neurons which may contribute to the vasopressor responses on chemical and electrical stimulation.

86.10

IMMUNOHISTOCHEMICAL STUDY OF NEUROPEPTIDE Y IN THE BULBOSPINAL TRACT. C.J.Tseng,H.C.Lin*, S.D.Wang and C.S.Tung*. Departments of Pharmacology, Physiology and Anatomy, National Defense Medical Center, Taipei,Taiwan, R.O.C.

Neuropeptide Y (NPY), a 36 amino acid polypeptide, has been shown to be a co-transmitter with adrenaline and noradrenaline in many sympathetic nerves. Our previous studies suggested that NPY may be crucial in cardiovascular regulation. In the present study, we proposed to establish an immunohistochemical techniques to stain the Tyrosine Hydroxylase (TH), Dopamine ß Hydroxylase (DBH), Phenylethanol-N-Methyl-Transferase (PNMT) and NPY in neurons or nerve terminals of the rat brainstem and spinal cord. By using these techniques, we also determined the effects of C_1 area lesions on NPY nerve terminals in the intermediolateral column (IML). Our results demonstrated that many catecholamine- and NPY-containing neurons exist in medulla, such as the nucleus tractus solitarii, area postrema, A_1 and C_1 areas. Their terminals are also found in the IML. After unilateral microinjection of 6-hydroxydopamine (6-OHDA) into the C_1 area, the catecholamine- and NPY-containing neurons were disappeared and their terminals diminished in the IML. ON the other hand, following administration of 6-OHDA intrathecally, the catecholamine- and NPY-containing neurons and terminals extinguished or decreased both in the brainstem and spinal cord. These results indicate that most of the NPY and catecholamine immunoreactivity found in the IML were projected from the rostral ventrolateral medulla, and they may hold an important functional role in the bulbospinal pathway to regulate the cardiovascular function.

86.11

CENTRAL OPIOIDERGIC MECHANISMS IN THE ROSTRAL VENTROLATERAL MEDULLA CONTRIBUTE TO THE HYPOTENSIVE RESPONSE OF PROPRANOLOL IN THE SPONTANEOUSLY HYPERTENSIVE RAT. R. L. Tackett and L. R. Portis. Department of Pharmacology and Toxicology, Cardiovascular Pharmacodynamics Laboratory, College of Pharmacy, University of Georgia, Athens, GA 30602.

Previous experiments in our laboratory have demonstrated that the hypotensive response to centrally administered propranolol is associated with an increase in central norepinephrine and beta endorphin levels. These findings, thus, suggest an interaction between catecholaminergic and opioidergic systems in the antihypertensive action of propranolol. The rostral ventrolateral medulla (RVLM) represents a possible neuroanatomical site for the interaction of catecholaminergic and opioidergic neuronal systems in the regulation of cardiovascular responses. The present study was designed to determine if opioidergic mechanisms in RVLM are involved in the hypotensive actions of propranolol. Male spontaneously hypertensive rats were anesthetized with pentobarbital, ventilated artificially and instrumented for recording arterial pressure and heart rate. Bilateral microinjection of propranolol (0.25 nmole) in RVLM resulted in a significant decrease in arterial pressure of 46% at 45 min. Bilateral injection of naloxone (20 nmole) into RVLM did not alter blood pressure or heart rate. However, naloxone pretreatment significantly attenuated the hypotensive response produced by microinjection of propranolol into RVLM. These data support the involvement of RVLM opioidergic systems in the hypotensive actions of propranolol.

86.12

LASER-DOPPLER FLOWMETRY MEASUREMENT OF SYMPATHETIC CUTANEOUS VASODILATION. M.C. Koss and M. Kawarai*. Univ. of Okla. Hlth. Sci. Ctr., Okla. City, OK 73190.

This study was undertaken to determine if laser-Doppler flowmetry could effectively measure neurally evoked sympathetic cutaneous vasodilation in anesthetized cats after pretreatment with anti-adrenergic drugs: 1) acute quanethidine administration, 2) chronic monoamine depletion and 3) α-adrenoceptor blockade with prazosin and yohimbine. We also addressed the question of a casual relationship between neurally evoked vasodilator and sudomotor responses. Guanethidine produced a depression of basal cutaneous blood flow whereas α-adrenoceptor blockade did not. In all groups, preganglionic sympathetic nerve stimulation produced intensity-dependent increases of digital skin blood flow along with sympathetic-cholinergic sudomotor responses. Vasodilator responses were not altered by i.v. propranolol or atropine, however, evoked sudomotor responses were blocked by atropine. Low doses of hexamethonium (1.5 mg/kg, i.v.) selectively abolished cutaneous vasodilation but not concomitantly evoked sudomotor responses. These results demonstrate that cutaneous digital vasodilation can be measured in cats following removal of either pre- or postjunctional vasoconstrictor mechanisms. Neither muscarinic nor ß-adrenoceptor mechanisms appear to be involved. Cutaneous vasodilation does not appear to be a consequence of concomitant sudomotor activation. (Supported by KAO Corp.)

CELL DIFFERENTIATION AND MIGRATION I

87.1

N-ACETYLGLUCOSAMINE (NAGA) PRODUCTION BY DEVELOPING WHITE MATTER ASTROCYTES IN THE FETAL RABBIT. T. Bass[1], P. Gurtner*[2], and F.J. Liuzzi[3]. Depts. of Pediatrics[1], Neurosurgery[2], and Anatomy & Neurobiology[3], Eastern Virginia Medical School, Norfolk, VA 23507.

Developing CNS tracts are thought to be compartmentalized by extracellular matrix proteins (ECMP) produced by astrocytes. NAGA, a major constituent of many of these ECMP has a high binding affinity for various plant lectins. We used the tomato lectin, *Lycopersicon esculmentum* (LE) to identify NAGA producing astrocytes and to document developmental patterns of appearance in the fetal rabbit.

New Zealand White rabbit pups were sacrificed after delivery by caesarean section at 22 to 32 days gestation. Other pups were allowed to deliver vaginally and were sacrificed at 2 and 4 postnatal days. All brains were fixed in Zamboni's fixative and paraffin embedded sections were incubated with biotinylated LE which was detected with peroxidase conjugated streptavidin and aminoethylcarbazole chromagen.

NAGA positive astrocytes were found in and between developing white matter tracts and were concentrated at their margins. The numbers of NAGA positive astrocytes increased with increasing gestational age. This study suggests a developmental regulation of ECMP production by astrocytes which may in turn influence white matter development.

Supported by Children's Health Foundation, Children's Hospital of the King's Daughters, Norfolk, VA

87.2

SCHWANN CELLS FAIL TO REMYELINATE AXONS FOLLOWING INJURY IN CULTURE IN DIFFERENTIATION SUPPORTING MEDIUM. C. Fernandez-Valle, P.M. Wood, and M.B. Bunge. The Miami Project, Univ. of Miami Sch. of Med., Miami, FL. 33136.

Ascorbate dependent acquisition of basal lamina (BL) on the Schwann cell (SC) is required for initial myelin formation in vitro. To better understand this linkage we asked whether SCs could use previously deposited BL to form myelin after injury. Well-myelinated axon fascicles in rat dorsal root ganglion neuron-SC cultures were crushed to cause myelin breakdown and reformation. Axons were injured on one side of the culture; the opposite side served as a control. Cultures were maintained for 3-5 weeks after crush (sufficient for initial myelin formation) in: 1) myelinating (M) medium containing ascorbate, 2) M medium lacking ascorbate, and 3) M medium lacking ascorbate but with protease inhibitors (PIs) to minimize BL loss. Sudan black staining was used to visualize myelin; electron microscopy was performed to determine axon size, presence of BL, and myelin thickness. We were surprised to find that SCs did not appear to myelinate axons following injury, even in M medium in which they could have formed new BL; in fact, BL was lost and did not reform on the injured side, in contrast to the uninjured side where BL persisted. BL was lost after injury in medium lacking ascorbate as well and PIs did not prevent this BL loss. A time course study of myelin disappearance showed an initial rapid degradation into myelin ovoids (1-5 days) but many SCs contained myelin debris 3 weeks after injury. In sum, conditions favoring SC BL formation (and thus myelination) are not present after injury in these cultures of purified populations of neurons and SCs. Possibly the slow clearance of myelin debris limited the SC's ability to reform BL. (Supported by NIH 09923 and The Miami Project).

87.3

Exogenous Purified Basal Lamina Components Cause Abnormal Schwann Cell Ensheathment of Sympathetic Neurites in Culture.
Y. J. Obremski and M. B. Bunge The Miami Project, University of Miami School of Medicine, Miami, FL 33136

We have used a tissue culture system of superior cervical ganglion neurons (SCGNs) and Schwann cells (SCs) to model development of unmyelinated nerve. SCs in SCGN+SC cultures do not completely differentiate; SC basal lamina (BL) deposition is patchy and neurite ensheathment is poor. Previous experiments have shown that a diluted extract of BL, Matrigel, improves these two parameters. Could this effect be due to a specific component of Matrigel? SCGN+SC cultures were supplemented with one of three BL components (known to be in Matrigel), laminin, type IV collagen, or heparan sulfate proteoglycan, or with all three. Surprisingly, all four different additions, like Matrigel, improved the two parameters we examined; more BL was deposited on SCs and ensheathment was increased.

However, these cultures do not resemble normal nerve. The SCs appear hypertrophic with numerous processes. Some SC ensheathment is exaggerated; multiple wraps of SC cytoplasm may be seen on a single neurite, or two processes may envelop a neurite and then proceed beyond it for some distance. Other processes do not ensheathe neurites, but instead run parallel to them. Our earlier work had shown that the addition of fibroblasts (Fbs) to SCGN+SC cultures most closely models unmyelinated nerve; we had hypothesized that Fbs might contribute one of the three molecules tested to SC BL. We now suggest that while this might be the case, Fbs must also contribute another factor that promotes normal SC function. (Supp. by NIH 09923 & The Miami Project. Gifts of molecules from A. Charonis, E. Tsilibary, J. Hassell)

87.4

DIFFERENTIATION IN THE SKATE RETINA.
WJ Brunken, MD Murphy,* TK Waters* & DD Hunter[1] Boston College and Tufts University[1] Boston Ma

The retina develops along a stereotyped path with a reasonably consistent pattern from species to species. Specifically, ganglion and amacrine cells develop first, followed by neurons of the outer retina including photoreceptors. Retinal progenitors are multipotent and maintain their remarkable plasticity until their last mitosis. Environmental and cell-cell interactions are likely to be critical in guiding the ultimate fate of progenitors. We have identified previously one such factor, s-laminin in the outer retina; here we focus on other factors, transmitters, which may be important in cell induction. Retinal development extends over 4 months in the skate, making it a good system for the study of cell induction.

First, we used BrDU to obtain birthdates of retinal neurons. Neurogenesis begins in the central-most region of the retina and continues as a wave to the retinal margin which lags behind by several weeks. Ganglion and amacrine cells are born first followed closely by photoreceptors and then horizontal and bipolar cells.

Interestingly, unlike other species, there is a long lag in the differentiation of postmitotic cells in this retina. We have used several markers of differentiation: 5HT, TH, GAD, rhodopsin, protein kinase C and GFAP immunoreactivities. Rods are born in stage 3 but do not make rhodopsin until late stage 5 (4 weeks later); of the transmitter markers 5HT appears first in early stage 4; TH in late stage 4, and GAD in late stage 5. Bipolar genesis is late (stage 5) and correlates with the end of ganglion cell death. Maturation of bipolar cells (PKC +) does not occur until stage 6 (hatching). GFAP positive Müller cells are the last to appear. The correlation of bipolar cell birth with the end of ganglion cell death and the early appearence of the biogenic amine transmitters suggests a trophic effect on retinal development while the coincidence of GAD at the end of synaptogenesis suggests that it may be involved in the termination of growth.
Supported by EY 6776 WJB & Boston College Undergraduate Research Program

87.5

LAMININ ISOFORMS IN THE NEURAL RETINA: POTENTIAL MODULATORS OF CELLULAR DIFFERENTIATION.
D.D. Hunter, A. Torio*, R. Libby*[1], and W.J. Brunken[1]. Neuroscience Program, Tufts Univ. Sch. of Med., Boston, MA 02111 and [1]Biology Dept., Boston College, Chestnut Hill, MA 02167.

The development of the neural retina follows a stereotyped time course that begins with an undifferentiated neuroepithelium populated by multipotential progenitor cells and ends with a highly differentiated tissue containing diverse cell types. The identities of the factors that guide this differentiation have remained elusive; a likely location for such factors, however, is the extracellular space in which retinal cells develop. We have shown that the extracellular matrix component s-laminin is present in the neural retina, that s-laminin expression parallels the differentiation of rod photoreceptors, and that photoreceptors specifically interact with s-laminin *in vitro*. In marked contrast, laminin (A/B1/B2) is absent from s-laminin-rich areas of the neural retina. These data suggest that s-laminin acts in a manner quite unlike laminin (A/B1/B2) during the development of the neural retina.

We have now begun to address the role of s-laminin in the neural retina more directly. First, we have implemented an *in vitro* system to ask whether the interaction of precursor cells with s-laminin induces a photoreceptor lineage. When precursor cells are exposed to an s-laminin-rich matrix, many begin to express the photopigment rhodopsin, suggesting that s-laminin may participate in this lineage. Second, we have used *in situ* hybridization histochemistry to ascertain the cellular source of s-laminin in the neural retina, both in adulthood and during development, in order to more precisely define the role of s-laminin in the acquisition and maintenance of an adult phenotype. Finally, we have used s-laminin affinity chromatography to isolate a putative s-laminin receptor. Together, these results provide support for our hypothesis that s-laminin is a component of the process that induces undifferentiated precursor cells to follow a rod photoreceptor lineage.

Supported by the Pew Memorial Trusts (DDH) and EY06776 (WJB).

87.6

NEURONAL MIGRATION ON LAMININ IN VITRO APPEARS TO DEPEND ON NEURITE OUTGROWTH.
S. Liang*, J. M. Tew, Jr. and K. A. Crutcher, Department of Neurosurgery, Univ. of Cincinnati, Cincinnati, OH 45267.

Neuronal migration is an important morphogenetic event in the development of the nervous system. Available evidence indicates that migration in vivo depends on neuronal/glial cell interactions (Rakic, 1972; Gasser and Hatten, 1990) and extracellular matrix factors such as laminin (Liesi, 1985). We found that laminin, when used as a substrate to culture chick embryonic sympathetic neurons, stimulates migration whereas more adhesive substrates, i.e., poly-lysine and poly-ornithine, do not. Time-lapse videomicroscopy demonstrates that neuronal migration on laminin correlates with neurite extension. Translocation of neuronal perikarya occurs in the direction of previously extended neurites or advancing growth cones. Such movement is often accompanied by retraction of trailing neurites. The net result of migration often is the aggregation of perikarya. Although anti-laminin antibodies result in a reduced rate of migration, the primary effect appears to be due to retraction of neurites. In addition, inhibition of neurite extension, through addition of 1,2 dioctanoyl-sn-glycerol (Weeks et al., 1990), delays migration for several hours until neurite formation is resumed. These results indicate that migration of primary neurons in vitro is supported by laminin and does not require interaction with other cells. The apparent dependence of neuronal motility on neurite extension in vitro suggests that directional migration may normally depend on neurite guidance in vivo. Furthermore, the fact that such motility in culture results in aggregation of neuronal perikarya may reflect a mechanism by which ganglion formation occurs in vivo.

87.7

epi-1 MUTATIONS CAUSE DEFECTS IN BASAL LAMINAE, AXON OUTGROWTH, AND TISSUE DEVELOPMENT IN C. ELEGANS.
D.H. Hall and E.M. Hedgecock*. Albert Einstein College of Medicine, Bronx, N.Y. 10461 and Johns Hopkins University, Baltimore, M.D. 21218.

Five mutant alleles of the gene epi-1 were isolated in the nematode C. elegans on the basis of their disruption of gonadogenesis, due to improper epithelialization of the uterus. Molecular and genetic data suggest that epi-1 may encode a laminin A chain (J. Yochem and I. Greenwald, pers. comm.). The primary defect in epi-1 appears to involve loss of integrity in the basal laminae. Electron microscopy of epi-1(rh165) animals shows that many laminae break down into loose fragments, form multiple stacked sheets, or condense into gobs of extracellular material. In many areas, tissues adhere to neighboring tissues abnormally, for the lack of a basal lamina to keep them separated. The pharynx is surrounded by a distinctly thicker basal lamina which remains intact in epi-1. The pharynx is still well formed, resulting in a well fed "monster" with many somatic cell types disoriented and/or malformed. Germ cell development is primitive in rh165, while somatic cells achieve adult fates. Milder epi-1 alleles are fertile, but rh165 is sterile. Nerve cords and the nerve ring show local mispositions, but most neurites remain in organized fascicles. Synaptic contacts are still common within the nerve ring, but are reduced in number elsewhere as many axons fail to reach appropriate targets. In C. elegans, another laminin gene, unc-6, is required for guidance of axons along the dorsal/ventral axis (Hedgecock et al., Neuron, 2: 65-80, 1990; Ishii, Wadsworth, Stern, Culotti & Hedgecock, pers. comm.). No visible disruptions of the basal laminae are noted in unc-6. The defects in axon guidance in epi-1 do not appear to be global, although local errors in dorsal/ventral guidance and cell position are noted.

87.8

ADHESION MOLECULES AND THE MIGRATION OF LHRH NEURONS IN THE OLFACTORY NERVE DURING DEVELOPMENT.
R. B. Norgren, Jr. and R. Brackenbury. Depts. of Psychiatry and Anatomy and Cell Biology, Univ. Cincinnati Coll. Med., Ohio 45267

Immunocytochemical results suggest that luteinizing hormone-releasing hormone (LHRH) neurons migrate from the olfactory epithelium to the olfactory nerve in several species of mammals [Schwanzel-Fukuda, 1990] and in chicks [Norgren, 1991]. As the migrating LHRH neurons appear to be restricted to the medial half of the olfactory nerve in chicks, we were interested in whether adhesion molecules define the migratory path of the LHRH neurons. Thus far, we have examined the distribution of N-CAM, N-cadherin and fibronectin in the olfactory system of the developing chick. Intense immunostaining for N-CAM was observed in the olfactory epithelium and in an early outgrowth of olfactory nerve of an E4 chick. In addition, a band of anti-N-CAM immunostaining was observed in the most superficial portion of the telencephalon; however, this staining was less intense than that observed in the adjacent olfactory nerve. Although these results indicate high levels of N-CAM immunostaining in portions of the olfactory system, the staining does not appear to be limited to regions where LHRH neurons are observed. Although only a few LHRH neurons are found in the olfactory epithelium of an E4 chick, the entire olfactory epithelium expresses high levels of N-CAM. Similarly, N-cadherin staining was observed in the olfactory nerve, but did not appear to be restricted to the medial half of the nerve. Since N-CAM and N-cadherin staining were found in both the medial and lateral halves of the the developing chick olfactory nerve, it seems unlikely that these cell adhesion molecules play a decisive role in the migration of LHRH neurons within the medial half of olfactory nerve although they may play a permissive role. Fibronectin staining was absent in the olfactory epithelium and nerve, but was observed in the surrounding mesenchyme indicating that this extracellular matrix molecule is probably not directly involved in the migration of LHRH neurons. We are currently examining additional cell adhesion and extracellular matrix molecules in the developing chick olfactory system. This work was supported by BRSG S07 RR05408-29 (RBN).

87.9

POLYSIALIC ACID ON NEURONAL CELL ADHESION MOLECULES INHIBIT ADHESION AND MIGRATION.
A. Harel, J. Silver[1], U. Rutishauser[2] and D. Roufa. Gliatech, Inc. Beachwood, OH 44122 and [1]Dept. of Neurosciences & [2]Genetics, Case Western Reserve University, Cleveland, OH 44106.

Traumatic injury to adult rat brain results in the formation of a glial-fibroblastic scar. Similar injury to newborn rat brain does not lead to a fibroblast-containing scar (Rudge et al., 1989 Exp. Neurol. 103:1). Brain neuronal cell adhesion molecule (NCAM) undergoes developmentally regulated changes in its polysialic acid (PSA) content. Embryonic brain NCAM contains three-fold more PSA than adult brain NCAM (Rothbard et al., 1982 JBC 257:11064). We examined the effect of NCAM's PSA content on neuronal, glial and fibroblast adhesion and migration. Alternating stripes of laminin (LN) and high PSA NCAM (NCAM-H) or low PSA NCAM (NCAM-L) were blotted onto nitrocellulose coated tissue culture dishes. A cell suspension of either meningeal fibroblasts or astrocytes was added and examined for adhesion to the substratum and migration. Both fibroblasts and astrocytes attached to LN or NCAM-L but not to NCAM-H. Cells did not migrate onto NCAM-H containing stripes for at least 6 days. The effect was dose dependent and fibroblasts were more sensitive than astrocytes to NCAM-H. Treatment of NCAM-H coated plates with neuraminidase resulted in removal of the inhibition. These results demonstrate that the extent of NCAM polysialylation affects cell migration and thus may contribute to the difference in scar formation between adult and embryonic brain.

87.10

LAMININ AND MEROSIN PROMOTE NEURONAL MIGRATION, BUT ARE ANTI-ADHESIVE A. L. Calof[1] and A. D. Lander[2] [1]Dept. of Biology, University of Iowa, Iowa City, IA 52242 and [2]Dept. of Brain and Cognitive Sciences, Mass. Institute of Technology, Cambridge, MA 02139.

We have used cultures of a proliferative neuroepithelium, the olfactory epithelium (OE) of the embryonic mouse, as a model system for studying how molecules of the extracellular matrix (ECM) regulate migration, motility and adhesion of central nervous system neurons and their progenitors. Neuronal cells of the OE, which undergo extensive migration during normal development were shown to be highly migratory in culture as well. Migration of OE neuronal cells was found to depend strongly on substratum-bound ECM molecules, being specifically stimulated and guided by laminin (or the laminin-related molecule merosin) in preference to fibronectin, type I collagen, or type IV collagen. Motility of OE neuronal cells, examined by time lapse video microscopy, was high on laminin-containing substrata, but negligible on fibronectin substrata. Interestingly, quantitative assays of adhesion of OE neuronal cells to substrata treated with different ECM molecules demonstrated no correlation, either positive or negative, between migratory preferences of these cells and the strength of cell-substratum adhesion. Furthermore, experiments in which cell-substratum adhesion was measured on substrata containing combinations of ECM proteins uncovered the finding that laminin and merosin are *anti*-adhesive for OE neuronal cells, i.e. cause these cells to adhere poorly to substrata that would otherwise be strongly adhesive. Evidence was obtained that this anti-adhesive effect is due to a direct cellular response to laminin, which alters the way in which neuronal cells respond to adhesive molecules, rather than to an interaction of laminin with other ECM molecules. In further support of this idea, laminin was found to inhibit the formation of focal contacts by OE neuronal cells. Experiments to identify the site(s) on laminin responsible for stimulating motility and mediating anti-adhesivity are in progress. *Supported by a grant from the Whitaker Health Sciences Fund.*

87.11

NEURAL CREST-DERIVED CELLS DO NOT ACQUIRE A NERVE-RELATED LAMININ RECEPTOR UNTIL AFTER THEY MIGRATE INTO THE GUT. V. M. Tennyson, M. J. Howard, H. D. Pomeranz, T. P. Rothman, and M. D. Gershon. Dept. Anat. & Cell Biol., Columbia Univ., P & S, N.Y., NY, 10032.

The enteric nervous system is formed by derivatives of the neural crest. These cells are unable to colonize the terminal gut of the lethal spotted (*ls/ls*) mouse. An excess of laminin accumulates in the mesenchyme of the presumptive aganglionic *ls/ls* bowel; therefore, we have proposed that crest-derived cells in the gut may cease migrating and extend neurites when they encounter laminin. The current experiments were carried out as a partial test of this hypothesis. EM immunocytochemistry revealed that abnormal accumulations of amorphous laminin-immunoreactive material ("puffs") are present in the outer gut mesenchyme of the presumptive aganglionic *ls/ls* bowel by day E14 and enlarge by day E16. In both avian and murine bowel, crest-derived cells within the gut acquire the immunoreactivity of a 110/120 kDa laminin-binding protein (present on the surfaces of ganglia and nerves) that they do not express at earlier stages of migration. Laminin-binding protein immunoreactivity appeared to be expressed by developing neurons just before neurofilament immunoreactivity could be detected. In contrast to controls, no cells become laminin-binding protein-immunoreactive in the terminal colon of the *ls/ls* mouse. Laminin-binding protein was found by EM immunocytochemistry to be a surface protein, which is present on crest-derived cells, and is preferentially located at sites where these cells make contact with puffs of laminin. Results are consistent with the hypothesis that aquisition of a neural laminin receptor by crest-derived cells enables these cells to extend neurites in response to laminin. The absence of this receptor on crest-derived cells prior to their arrival at their site of terminal differentiation may enable them to migrate through laminin-containing regions of the embryonic mesenchyme without responding prematurely as neurons. Supported by NIH grants HD 17736, NS 15547, and HD 21032.

87.12

AN IN VITRO MODEL OF OLFACTORY RECEPTOR NEURON DEGENERATION AND DIFFERENTIATION. S.K. Pixley. Dept. Anat. & Cell Biol., Univ. of Cincinnati Coll. Med., Cincinnati, OH 45267.

Previous work in vivo shows that if connection with the olfactory bulb is lost, olfactory receptor neurons (ORNs) degenerate, then are replaced by division and differentiation of neuronal stem cells. Differentiation is marked by expression of the olfactory marker protein (OMP), detected by immunostaining.

We show that, in dissociated cell cultures of newborn Spraque-Dawley rat nasal mucosal tissues, OMP+ neurons were present at 1-3 days after plating, but not at 4, 10 and 15 days post-plating. Non-OMP+ neurons were present throughout. If nasal cells were plated onto non-neuronal cells from the olfactory bulb, OMP+ cells were present at 1-3 days and absent at 4 and 10 days post-plating. Suprisingly, at 15 days post-plating, numerous OMP+ cells appeared in the co-cultures. Also present at 15 days were sustentacular cells (SUS-1+) and dark basal cells (keratin+). OMP+ neurons had a typical ORN morphology. Immunostained cells were found in large, multi-cellular clumps that often had fluid-filled cavities. We are investigating bulb-specificity and whether neurogenesis is involved. This in vitro system models in situ neuronal differentiation. {Grant support: Amer. Paralysis Assoc. #PB1-8803-1; NIH P01-DC00347-06}.

87.13

DEVELOPMENT & ULTRASTRUCTURE OF PURKINJE NEURONS IN DISSOCIATED CELL CULTURES. **M.E. Dunn, K. Schilling, J.I. Morgan, & E. Mugnaini. Neuromorphology Lab, U.Conn, Storrs, CT 06269 & Neuroscience Dept., R.I.M.B., Nutley , NJ 07110.**

Purkinje neurons (PCs) grown in culture undergo plastic changes when grown under conditions known to inhibit electrical activity. The present study was conducted to elucidate the fine structure and synaptic organization of PCs developing in electrically active cultures. Cerebellar cells obtained from E15 mouse embryos were grown in culture for up to 3 weeks before they were fixed and embedded for electron microscopy. Identification of PCs was obtained with antisera to L7, Calbindin, PEP-19, and Parvalbumin. PCs were distinctly immunostained at 1 week, but obtained their most characteristic morphological features at 2 and 3 weeks. At 2 weeks, PC bodies were uniquely covered with somatic spines which contained microfilaments and formed synaptic junctions with boutons. At 3 weeks, spines were mostly present on terminal branches of large dendrites. A large dendritic stem often gave rise to the axonal process after ceasing to emanate spines. This neurite formed boutons with a dense axoplasm and pleomorphic vesicles. The large cell bodies contained an organelle rich cytoplasm, including an extensive hypolemmal cisterna. Subsurface cisterns with a narrow lumen were not present in PCs at 3 weeks, although they were found in other large neurons provided with numerous axosomatic synapses and interpreted as deep nuclear neurons. Stellate and granule neurons were also identified in these cultures by immunocytochemical and morphological criteria. In conclusion, most of the ultrastructural features of PCs and other cerebellar neurons in vivo are reproduced in dissociated cultures despite the absence of a layered organization.

Supported by NIH grant NS-09904 & DFG grant SCHI271/2-2.

87.14

MIGRATORY MECHANISMS OF GRAFTED FETAL ASTROCYTES. J.J. Bernstein, G. Tadvalkar and W. Goldberg. Lab. CNS Inj. Regen., DVA Med. Ctr. Wash., DC 20422 & Depts. Neurosurg. Physiol. George Wash. Univ. Sch. Med. Wash.,DC 20037.

Fetal astrocytes grafted into lesioned adult host spinal cord migrate centimeters from the site of implantation. Fourteen day gestation purified, cultured rat spinal cord fetal astrocytes (E14) were incubated with rhodamine labeled microbeads, grafted in aspiration pockets in the fasciculus gracilis (FG) of the third cervical spinal segment (C3) of adult rats and followed for 7 days. The cells migrated approximately 5.2 mm/7 days and were observed rostrally in C1FG. The fetal astrocytes also migrated caudally, laterally and ventrally into the FG, fasciculus cuneatus, and the dorsal, lateral and ventral horns of the spinal cord. In an in vitro model, 10^5 cultured E14 fetal spinal cord astrocytes were seeded on wafers of hydrated collagen I. The fetal astrocytes migrated through the wafer and digested the collagen I fibrils. Immunohistochemistry revealed that the astrocytes contained plasminogen activators (tissue, tPA, and urokinase, uPA) which result in thrombolysis and cell digestion. The fetal astrocytes also contained guanidinobenzoatase (GB), a serine protease, associated with tumor metastasis and migration. GB cleaves the cells attachment to fibronectin and releases the cell from the substrate to allow for migration. These characteristics of fetal astrocytes were part of the molecular mechanisms for migration of these cells during development and after neural grafting into mature CNS.

Supported by DVA and NIH, NCI 48956 (JJB).

88.1

GNRH CELLS IN THE HYPOGONADAL MOUSE FORM FUNCTIONAL CONNECTIONS DESPITE THEIR BIOSYNTHETIC DEFICIENCY. I. Livne, M. J. Gibson, and A. J. Silverman. Dept. of Medicine, Mt. Sinai Sch. of Med., New York, N. Y. 10029 & Dept. of Anat. & Cell Biol., Columbia Univ., New York, N. Y. 10032

The hypogonadal (hpg) mouse has an undeveloped reproductive tract due to a truncated GnRH gene, resulting in failure to synthesize GnRH. Cells in the OVLT region of the hpg brain have low levels of GnRH mRNA but are devoid of the neuropeptide. The normal distribution of GnRH transcribing cells suggests that in spite of their failure to synthesize the peptide, GnRH cells migrate properly to their residence areas in the CNS. To elucidate the extent of differentiation of the mutant GnRH cells we have performed an in situ hybridization study in mice which were given injections of the fluorescent tracer Fluoro-Gold (FG). Only cells terminating on fenestrated capillaries, such as those of the hypophyseal portal system in the median eminance (ME), can capture and concentrate systemically delivered FG. We have therefore hypothesized that the finding of FG in cells containing GnRH mRNA, will indicate that GnRH cells in the hpg brain are capable of innervating their proper target. The experimental procedure for detection of GnRH mRNA and FG in the same cell was performed as follows: Normal and hpg adult males were given 3 i. p. injections of 40 mg/Kg FG and perfused. Sections through the septal-POA-AHA extent were processed for standard in situ hybridization with [^{35}S] labeled oligonucleotide complementary to the GnRH coding region. The slides were analyzed to identify GnRH transcribing cells which accumulate FG. In brain sections from 2 hpg males 54.5% and 64% of the GnRH transcribing cells were double labeled with FG, while in 1 normal brain 61.2% were double labeled. We have demonstrated that GnRH transcribing cells in the hpg brain concentrate FG. This finding suggests tnat mutant GnRH cells are capable of elaborating functional connections with their major target, the ME, in the absence of their principal secretory product. NS 20335

88.2

NORADRENERGIC INNERVATION OF THE REGENERATED RAT CEREBELLUM AFTER POSTNATAL X-IRRADIATION. P.E. Kunkler*, and W.J. Anderson. Department of Life Sciences, Indiana State University, Terre Haute, IN. 47809

In previous studies, our laboratory indicated that fractionated x-irradiation in rat pups begun at postnatal day 1 (PN1) through PN5 resulted in an alteration in the number and distribution of noradrenergic fibers throughout the molecular layer and the regenerated external granular layer (EGL). The present study was performed to determine the timing sequence of the alterations and to identify which if any cell types were the target of the noradrenergic fibers. Long Evans hooded rats were bred, and at birth were assigned to six conditions: x-irradiated at postnatal day 1 with 1 to 5 daily doses (150r each) and control animals for each age group. Rat pups were sacrificed at daily intervals after x-irradiation and at 10, 21 and 30 days of age. All animals were prepared for either routine histology or immunohistochemical staining. In the x-irradiated groups, sacrificed at 24 hr after the last treatment, numerous noradrenergic fibers were present throughout the regenerated EGL. At 10 days of age the expanding molecular layer had numerous fibers within its deep portion with some continuing up to the EGL. The fibers of the higher x-irradiated animals (5X) tend to run in a longitudinal plane within the developing cerebellum, while control and low dose x-irradiation fibers projected radially in the molecular layer in the sagittal plane. The fiber projections were reminiscent of the Bergmann glia radial projections. At longer survival times the fibers were associated with ectopic cells within the molecular layer. Occasional ectopic cell nest were seen with a dense plexus of noradrenergic processes scattered within. This suggest that the noradrenergic fibers may play a role in the formation of ectopic cells and that x-irradiation may alter the directional orientation of the these fibers.

88.3

VOLTAGE-DEPENDENT CALCIUM CHANNELS (VDCCs) AFFECT NEURON MIGRATION: A LASER MICROSCOPIC STUDY IN CEREBELLAR SLICE PREPARATIONS. H. Komuro and P. Rakic, Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510

Molecular and cellular mechanisms underlying neuronal migration are not well understood. In the present study we have examined the effects of various blockers of voltage-sensitive channels on the movement of postmitotic cells in brain slice preparations. Slices 400 to 800 µm in thickness were dissected from the cerebellum of 7-14-day-old mice, stained for 30 min in 25 µg/ml solution of carbocyanin dye (DiI) and maintained in a CO_2 incubator for 2 hrs in MEM. The migration of postmitotic cells was followed for several additional hours using a laser scanning confocal microscope and positions of their leading tips recorded every 10 min on a attached color video printer. In the control slices, granule cells migrated along Bergmann glial fibers at the rate of 10-20 µm/h over a distance of up to 100 µm, some of them reaching Purkinje cell layer within 6 hours, which is comparable to the rate *in vivo*. However, the addition of L or N type blockers of VDCCs to the slice medium (e.g. ω-conotoxin or Cd^{++}) suspended migration. In contrast, blocking of L subtype of VDCCs by nifedipine or a sodium channel by tetrodotoxin did not affect the movement, suggesting that N type of VDCC's may be essential for proper neuronal migration.

Supported by NIH grant NS22807

88.4

CELL-SPECIFIC SORTING IN THE SUBVENTRICULAR AND INTERMEDIATE ZONES OF THE EMBRYONIC RAT NEOCORTEX. S.A. Bayer and J. Altman. Indiana-Purdue University, Indianapolis, IN 46205 and Purdue University, West Lafayette, IN 47907.

The embryonic rat neocortex was studied at daily intervals after the dams were given a single injection of tritiated thymidine (5 µCi/gram body wt.). Within 24 hrs after injection, bands of heavily labeled cells are seen in the intermediate zone (superior bands, sb) and in the subventricular zone (inferior bands, ib). Three of the bands are postulated to be composed of cortical neurons because their appearance and disappearance can be correlated with the known timetables of neurogenesis of layers VI-II. Layer VI neurons sojourn in the early-appearing sb1 just below the cortical plate, layer V neurons appear next in sb2 in the lower intermediate zone, then layers IV, III, and II neurons sojourn successively in ib1 in the lower subventricular zone. Two bands, sb3 and ib2 cannot be linked to any neuronal population and are postulated to contain glia. These data suggest that neurons destined for cortical layers VI-II, *interrupt their migration* to the cortical plate to accumulate in cell-specific bands, or sojourn zones. The subventricular and intermediate zones may be considered as "staging areas" for different classes of neocortical neurons.

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88.5

EVIDENCE FOR RAPID MIGRATION OF NEWLY BORN CELLS IN THE ADULT RAT DENTATE GYRUS. H.A. Cameron, E. Gould, D.C. Daniels* and B.S. McEwen Lab. of Neuroendocrinology, Rockefeller Univ. N.Y., N.Y. 10021

Unlike the majority of neuronal populations which undergo neurogenesis during a discrete developmental period, granule cells continue to be added to the rat dentate gyrus for at least 1 year postnatally (Kaplan, M.S. and Bell, D.H. 1984. J. Neurosci. 4:1429-41). Although the birth of new granule cells in the adult dentate gyrus has been well established, it is presently unknown whether these cells undergo local cell division or migrate shortly after birth. Adult (6 mos) female rats were injected with ^{3}H-thymidine (5.0 µCi/gm body wt.) and perfused either 1, 6, or 24 hours after injection. The tissue was processed for autoradiography and counterstained with cresyl violet. Light microscopic examination of ^{3}H-thymidine labelled tissue from each time point revealed labelled cells of varying morphologies; some cells appeared to be glia (irregularly shaped small cell bodies) whereas others showed characteristics of granule cells (round or oval medium cell bodies). With time, the proportion of ^{3}H-thymidine labelled cells in the hilus steadily diminished while that of ^{3}H-thymidine labelled cells in the granule cell layer increased. These results provide evidence that newly born cells in the dentate gyrus migrate from the hilus to the granule cell layer within 24 hours of DNA synthesis. Combined immunohistochemical and autoradiographic studies to determine when these newly born cells express neuronal/glial specific markers (glial fibrillary acidic protein,vimentin / neuron specific enolase,neurofilaments) are ongoing.

(Supported by NS 08804 , MH 41256)

88.6

ANTI-ASTROTACTIN ANTIBODIES DISRUPT THE CYTOSKELETAL ORGANIZATION OF MIGRATING GRANULE NEURONS. G. Fishell and M.E. Hatten. Dept. of Pathology and Center for Neurobiology, College of Physicians and Surgeons, Columbia University, 10032.

During CNS development, neurons migrate from proliferative zones to specific cortical laminae along radial glial processes. *In vitro* studies indicate that migrating neurons form a specialized adhesion site, 'the interstitial junction', between the neuronal cell soma and the glial fiber. The neural glycoprotein astrotactin provides one neural component of these specialized neuron-glia adhesions. The observation that fine cytoskeletal elements project into the 'interstitial junction' suggested that astrotactin may provide a link between the site of neuron-glial adhesion and the cytoskeleton of migrating neurons (Gregory et al, 1988). To examine this possibility, neurons were treated with 1.0 mg/ml of anti-astrotactin antibodies and examined by correlated video and electron microscopy. For comparison, in separate experiments neurons were treated with 1.0 mg/ml anti-NCAM antibodies. Video microscopy provided evidence that, in the presence of anti-astrotactin antibodies, neurons ceased migration and adopted a posture characteristic of stationary neurons. Correlated electron microscopic analysis revealed a disorganization of cytoskeletal elements into tangles of filaments in the area rostral to the neuronal nucleus. In contrast, neurons treated with antibodies against NCAM were indistinguishable from untreated cultures. These observations raise the possibility that astrotactin, as well as mediating adhesion to the glia fiber on the cell surface, provides a linkage to the neuronal cytoskeleton on the cytoplasmic side.

88.7

ROLE OF THE CYTOSKELETON IN GLIAL-GUIDED NEURONAL MIGRATION. R.J. Rivas,*G. Fishell, and M.E. Hatten. Department of Pathology and Center for Neurobiology and Behavior, Columbia University, New York, NY 10032.

Glial-guided neuronal migration is a critical step in the development of cortical regions of the mammalian brain, ushering young neurons from proliferative zones to the positions where they establish synaptic connections. To study cytoskeletal involvement in neuronal migration, migrating granule neurons in microcultures prepared from early postnatal cerebellum were analyzed by confocal microscopy. Optical sectioning (0.6 um steps) through the neuronal cell body and leading process of fixed neurons with migration "profiles" shows a morphology highly polarized in the direction of migration. Indirect immunofluorescence with tubulin antibody revealed a cagelike network of microtubules which are concentrated in the rostral portion of the cell body and extend into the leading process. Mitochondrial (DiOC6(3)) and Golgi staining (rhodamine wheat germ agglutinin) showed that these microtubule-associated organelles are also concentrated in the rostral portion of the cell body. Staining for actin with rhodamine phalloidin revealed bright fluorescence which appeared associated with the cell body plasma membrane. Current experiments are directed towards understanding the relationship of the neuron-glial adhesion molecule, astrotactin, to the cytoskeleton.

88.8

MIGRATION OF THORACIC PREGANGLIONIC NEURONS IN THE CHICK AND RAT. C.J. Forehand and E.B. Ezerman. Dept. of Anat. and Neurobiol., Univ. of Vermont, Burlington, VT 05405.

Motor neurons project axons through the ventral root while their somata are migrating from the ventricular zone to the motor columns (Farel & Bemelmans, Neurosci. Lett. 18:133, '80). Rat and chick thoracic preganglionic axons also project axons peripherally while their somata are migrating; we have taken advantage of this phenomenon to study the migration of these neurons in the spinal cord.

An *in vitro* labeling paradigm was utilized to study the migration of thoracic preganglionic neurons in 4-10 day (D4-D10) chicken embryos and 13-17 day (E13-E17) rat embryos. Neurons were retrogradely labeled from the sympathetic chain with fluorescently-tagged dextran amines in living *in vitro* preparations of the thoracic body wall, spinal column, and sympathetic chain.

Preganglionic neurons that project to the chain at early time points (D41/2-5 in the chick, E131/2-15 in the rat) appear to arise from the ventral aspect of the ventricular zone and migrate first laterally and then dorsally. In the chick, this lateral migration is extremely limited; the dorsal migration begins just as the neurons leave the ventricular zone. In the rat, these early neurons migrate first to the lateral edge of the cord, and then dorsally. Preganglionic neurons that project to the chain at later time points (D6-7 in the chick, E16-17 in the rat) appear to arise from a more dorsal region of the ventricular zone and migrate laterally at their appropriate dorsoventral level. At D4 in the chick and E13 in the rat, there is no indication of a ramus projecting to the presumptive area of the sympathetic chain. Also at these times, none of the neurons retrogradely labeled from the ventral root are found outside of the lateral motor column. Thus the dorsal migration of these cells may be signalled by the periphery once the axons enter the chain.

88.9

PATTERNS OF SYMPATHETIC PREGANGLIONIC NEURONAL MIGRATION IN SPINAL CORD OF EMBRYONIC RATS. J.A. Markham and J.E. Vaughn. Div. of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

Preganglionic neurons were retrogradely labeled by microinjections of HRP into the sympathetic chain ganglia of rats on embryonic days 13-16 (E13-16). Observations at E13½ indicated that preganglionic and somatic motor neurons were initially intermixed in the primitive motor column of the ventral thoracic spinal cord. However, by E14, preganglionic motor neurons were segregated dorsolaterally from somatic motor cells. On E15, the two subclasses of motor cells were located in separate columns with the preganglionic cells being positioned more dorsally than the somatic motor neurons. In addition, by E15, the alignment of the dorsal-most preganglionic cells had changed from a dorsoventral to a predominantly mediolateral orientation. By E16, the preganglionic autonomic motor column was located well within the intermediate gray matter, and a number of its constituent cells were found in increasingly more medial positions. These results, in conjunction with previous observations, suggest that the migration of preganglionic neurons in the embryonic rat spinal cord can be divided into three distinct phases: a primary movement from the ventricular zone to form a single primitive motor column together with somatic motor neurons; a secondary dorsal translocation of the preganglionic neurons resulting in the formation of separate autonomic and somatic motor columns; and a tertiary medial movement of certain preganglionic cells giving rise to the medially-located autonomic nuclei. Spatiotemporal considerations suggest that different migratory pathways may be utilized during these phases. Examples of substrates that may form the different routes are radial glia, early forming fiber pathways and medially-directed dendrites of other preganglionic neurons (see companion presentation by Phelps *et al.*) *Supported by grants NS25784 and NS18858 from NINDS.*

88.10

SUBSTRATES UNDERLYING THE MIGRATORY PATTERNS OF SYMPATHETIC PREGANGLIONIC NEURONS IN EMBRYONIC RAT SPINAL CORD. P.E. Phelps, R.P. Barber, and J.E. Vaughn. Div. of Neurosciences, Beckman Research Inst. of the City of Hope, Duarte, CA 91010.

Previous results indicate that precursors of preganglionic neurons follow a circuitous route in achieving their final spinal cord locations. For certain preganglionic cells, this can involve both a secondary dorsal and a tertiary medial translocation (see Markham and Vaughn, companion presentation). Since the substrates these cell use for their translocations are not known, we initiated the following two studies. To investigate possible dorsal migration pathways, ChAT-immunocytochemical preparations of thoracic spinal cord were double-labeled with TAG-1/SNAP (from M.Yamamoto) to examine relations between preganglionic neurons and axons of early-forming association cells. On E14, TAG-1 positive fibers were detected just dorsal to the primitive motor column, and by E15.5, such elements were directly apposed to the lateral border of preganglionic neurons, suggesting that they could provide a dorsal migration route. To initiate studies of possible medial migration pathways, ChAT-immunocytochemical preparations of upper lumbar cord were selected because medially-located preganglionic cells, or central autonomic neurons, are most numerous at this spinal level. These studies revealed that the tertiary medial translocation of preganglionic neurons began by E15.5, and that many of these neurons are located medially by E18. Since some preganglionic neurons appear to migrate medially, they probably utilize a radially-oriented migratory pathway. Current investigations are focused on whether radial glial cells or, alternately, transverse dendritic bundles of early-forming lateral preganglionic neurons might provide such a substrate for the medial translocation of the neurons that come to reside in the central autonomic region. *Supported by NS25784 and NS18858.*

88.11

PREGANGLIONIC NEURONS EXHIBIT NORMAL MIGRATION PATTERNS IN CULTURED SLICES OF EMBRYONIC RAT SPINAL CORD. R.P. Barber, J.A. Markham, P.E. Phelps and J.E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The use of organotypic cultures of CNS slices as a tool for studying developmental phenomena has gained prominence with the development of better culturing techniques and materials. Using a modification of this preparation, we have shown that spinal cord slices from embryonic day 14 (E14) rats grown *in vitro* on a rocker table contain neurons that express ChAT, GAD or neurofilaments for 3 wk in culture. It is known that at E14 *in vivo* there is only a single primitive column of mixed motor neurons (autonomic and somatic) in the ventral thoracic spinal cord. By E15, there is a dorsal translocation of autonomic neurons into the intermediate gray matter, and by E17, the separation of autonomic and somatic neurons is complete. This is followed by a medial translocation of some preganglionic neurons to form the medially-located autonomic nuclei. To determine if these translocations occur *in vitro*, the ventral roots and autonomic chain ganglia of E14 embryos were injected with a fluorescent tracer before sectioning and explanting the spinal cord slices. After 72 h in culture, there was a substantial dorsal translocation of cells into the intermediolateral cell column, as well as a medial migration of some preganglionic neurons. Thus, the migration of preganglionic motor neurons appears to occur *in vitro* in much the same pattern as it does *in vivo*. These preliminary results pave the way for future experimental studies of migratory pathways *in vitro* to explore the substrates that are necessary for these translocations to occur. *Supported by grants NS25784 and NS18858 from NINDS and funds from the Sadie and Norman Lee Endowment.*

88.12

A GOLGI STUDY OF DEVELOPING GLIAL CELLS IN THE RABBIT LUMBAR SPINAL CORD. S.A. Scoville*[1], D.E. Scott[2] and F.J. Liuzzi[3] Departments of Neurosurgery[1,2,3] and Anatomy and Neurobiology[2,3] Eastern Virginia Med. Sch., Norfolk, Va 23501

Spinal cords from embryonic rabbits ranging from E22-E31, early post-natal and adults were processed using a modified rapid Golgi procedure of Del Rio Hortega. The development of one glial type was traced from E22, at which time its soma was either bordering on, or oriented towards the central canal with one primary radial process extending toward the pial surface. This cell type appeared to migrate away from the central canal and by E26 some of these cells appeared to transform within the developing gray matter into stellate cells without radial processes. These cells exhibited increasing complexity and attained an adult-like morphology by PN 2. Other developing glial cells have been identified and characterized. These include white matter astrocytes, both stellate and radial, as well as tanycytes. In addition, the developmental relationships of glial cells with the vasculature and myelination were examined.

This work was supported in part by research funds from the Department of Neurosurgery, EVMS.

88.13

INHIBITION OF NEURAL CREST CELL MIGRATION BY THE MESENCHYME OF PRESUMPTIVE AGANGLIONIC SEGMENTS OF MUTANT GUT T. P. Rothman and M. D. Gershon. Dept. Anat. & Cell Biol., Columbia U., P & S, NY, NY 10032.

The terminal bowel of the lethal spotted (*ls/ls*) mouse becomes aganglionic because the presumptive aganglionic tissue is not colonized by viable neural or glial precursors. Crest-derived cells of *ls/ls* animals are able to colonize control segments of bowel *in vitro*, but no source of crest-derived cells is able to colonize the last 2 mm of *ls/ls* colon. Collagen type IV, laminin, and hyaluronic acid accumulate in the mesenchyme of the abnormal *ls/ls* gut. The current experiments were carried out to test 2 hypotheses: (i) that crest-derived cells enter the presumptive aganglionic *ls/ls* bowel, but die: (ii) that crest-derived cells fail to colonize abnormal *ls/ls* gut because pathways that lead these cells from the crest to bowel are defective. Crest-derived cells leave segments of gut and re-migrate in host embryos when bowel that has already been colonized is back-transplanted into a crest migration pathway. Segments of duodenum or terminal colon were dissected from control or *ls/ls* embryos (E13-14) and back-grafted between the neural tube and somites of quail embryos (E2; adjacent to somites 8-24). Host cells were distinguished from mouse cells by demonstrating the quail nuclear marker and NC-1 immunoreactivity was used as a crest marker. No murine cells migrated out of presumptive aganglionic *ls/ls* gut to reach neural structures in host embryos. Quail crest-derived cells entered control segments of bowel. In contrast, the grafts of presumptive aganglionic *ls/ls* tissue could not be colonized by crest-derived cells of host origin, and often appeared to obstruct their migration. It is concluded that crest-derived cells cannot be liberated by back-transplantation from presumptive aganglionic *ls/ls* bowel, and that this tissue cannot be colonized by crest-derived cells, even when it is placed in a crest cell migration pathway. The results indicate that aganglionosis arises in *ls/ls* mice because an inherent abnormality of non-neuronal elements of the bowel wall inhibit migration of crest-derived cells. Supported by HD 21032, HD 20470, and NS15547.

88.14

MYOTOMAL CELLS PLAY A ROLE IN GUIDING THE MIGRATION OF THE LATERAL LINE PRIMORDIUM IN ZEBRAFISH. W.K. Metcalfe and J.T. Graveline*. Institute of Neuroscience, U. of Oregon, Eugene, OR 97403.

We are interested in learning how cells migrate along precise pathways during embryonic development. We have chosen the developing lateral line of zebrafish to study this question because it is a simple system in which a dramatic cell migration takes place within the skin of the embryo. The sensory organs of the zebrafish midbody lateral line are derived from a primordium that migrates along a stereotyped pathway within the skin. The pattern of the lateral line sensory organs, as well as the path of the peripheral sensory nerve, are determined by the path of migration of this primordium, but what guides the primordium?

We observe that the lateral line primordium fails to migrate along the trunk of embryos homozygous for the *spadetail* mutation. In these embryos, trunk myotomes are malformed, suggesting that myotomal patterning may play a role in directing the migration of the primordial cells. To test this idea, we caused focal disruptions of a few trunk myotomes prior to the onset of primordium migration either by heat shock during myotome formation, or by laser ablation of early myotomal cells. Both of these manipulations resulted in local disruptions of the path of migration of the primordium. Thus, although the primordium migrates within the skin, its migration pathway appears to be dependent upon some feature of the underlying myotomes. Supported by NIH grant NS27122.

PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING II

89.1

EFFECT OF SERUM OR INTEGRIN RECEPTOR BLOCKADE ON NEURITIC OUTGROWTH FROM ADULT AND EMBRYONIC MOUSE RETINAL EXPLANTS ON LAMININ. C.A. Bates and R.L. Meyer. Developmental Biology Center, UC Irvine, Irvine, CA 92717.

Laminin is an excellent substrate for the outgrowth of adult rodent optic neurites in culture, but it has been reported that laminin is ineffective in promoting neuritic outgrowth from embryonic retinal explants. One possibility is that serum in the media interferes with neurite extension on laminin in the embryonic retinal explant. To test this, we cultured adult and embryonic (E15) mouse retinal explants on laminin coated coverslips in serum containing and serum free media. Adult explants showed similar levels of neuritic outgrowth in serum and serum free media. In contrast, the E15 explants grew extensive haloes of neurites within 24 hours in serum free media and showed little or no neuritic outgrowth in media with serum. We then looked for differences in the laminin receptors in adult and E15 explants. Explants were cultured in media containing GP140 (C. Damsky), an antibody which blocks the laminin binding integrin B1/B3 subunit. GP140 had little effect on the outgrowth of neurites from adult explants. In contrast, it completely blocked the extensive neuritic outgrowth from E15 explants for at least 5 days in culture. This demonstrates that embryonic optic neurites do respond positively to laminin in the absence of serum and suggests that the positive response of adult neurites to laminin may involve receptors other than the B1/B3 integrin subunits. Supported by NS26750 (RLM) & HD07029 (CAB).

89.2

MATURATION OF EMBRYONIC AVIAN RETINAL GANGLION CELLS *IN VITRO*. N. Morissette* and S. Carbonetto. Center for Research in Neuroscience, Montreal General Hospital Research Institute and McGill University, Montreal, Quebec, Canada H3G 1A4.

Integrins are a superfamily of adhesive molecules several of which mediate neurite growth *in vitro* and nerve regeneration *in vivo*. Integrin function in chick retinal ganglion cells (RGCs) is down-regulated between embryonic days 6 and 12. To help study this regulation, we undertook to establish an *in vitro* system in which changes in integrin function parallel those *in vivo*. Primary cultures of neural retinal cells were grown on laminin substrates and neurite outgrowth of RGCs was assessed. Secondary cultures were prepared by replating primary cultures on laminin substrates. Preliminary data indicate that early (5-7 days) embryonic chick RGCs will lose their laminin integrin function following maturation in primary retinal cultures and that their neurite outgrowth in secondary retinal cultures is similar to that of RGCs allowed to mature to the same absolute age *in ovo*. Similar to RGCs in primary cultures, chick RGCs which mature *in vitro* remain capable of extensive neurite outgrowth on rat astrocytes suggesting that non-integrin receptor activities on their surfaces (e.g. N-cadherin) are intact. This avian retinal system could prove to be useful in studies of integrin regulation that would be difficult to accomplish *in vivo*.

89.3

Molecular Cloning of the Rat Integrin alpha1- Subunit: A Receptor for Laminin and Collagen. M.I.Ignatius1, T.H.Large1, M.Houde2 I.W.Tawil2, A.Barton1,S.Carbonetto2, and L.F.Reichardt1, 1Dept. of Physiology and HHMI, UCSF, San Francisco, CA 94143; 2Neurosciences Unit, McGill University, Montreal General Hospital; 1650 Cedar Avenue, Montreal, Quebec Canada H3G 1A4

Integrin heterodimers mediate a variety of adhesive interactions, including neuronal attatchment to and process outgrowth on laminin. We report here the cloning and primary sequence of an Mr200k integrin alpha subunit that associates with the integrin beta 1 subunit to form a receptor for both laminin and collagen. Similarities in ligand-binding specificity, Mr, N-terminal and internal sequence make this a strong candidate for the rat homologue of the human VLA-1 integrin alpha subunit. The full length rat alpha 1 cDNAs encode a protein containing a putative signal sequence and a mature polypeptide of 1152 amino acids, with extracellular, transmembrane and cytoplasmic domains. Several structural features are conserved with other integrin alpha chains, including (a) a sequence motif repeated seven times in the N-terminal half; (b) potential divalent cation binding sites in repeats 5, 6 and 7, and (c) alignment of at least 15 of 23 cysteine residues. This rat alpha 1 sequence also contains a 200 amino acid I domain, inserted between repeats 2 and 3, that is homologous to I domains found in the same position of other integrin a subunits (alpha 2, Mac-1, LFA-1, GP150). The rat integrin alpha 1 subunit has several unique features, though, including a 38 residue insert between two Ca2+/Mg2+ binding domains that contains 4 cysteines and a potentially novel protease cleavage site, that can account for unique functions of this integrin.

89.4

LOCALIZATION AND CHARACTERIZATION OF 110 KD LAMININ BINDING PROTEIN IMMUNOREACTIVITY IN ADULT AND LESIONED BRAIN. M. Jucker, H.K. Kleinman, L.C. Walker, P. Bialobok*, L.R. Williams, D.K. Ingram. Gerontology Res. Ctr., NIA, NIH, Baltimore, MD 21224; NIDR, NIH, Bethesda, MD 20892; Neuropathol. Lab, J. Hopkins U., Baltimore, MD 21205; Fisons Corp., Rochester, NY 14624; Upjohn Co., Kalamazoo, MI 49001.

A 110 kD laminin-binding protein (110 kD LBP) isolated from newborn mouse brain extract has been identified. This protein recognizes a neurite-outgrowth promoting 19-amino acid synthetic peptide (PA22-2) derived from the laminin A chain (Kleinman et al., submitted). Antibody to 110 kD LBP strongly immunostains fibers and distinct cell populations in adult rat forebrain, in particular cortical pyramidal neurons with apical dendrites and hippocampal mossy fibers (Jucker et al., *Brain Res.*, in press). Similar immunoreactivity was found in newborn rats, adult mice, and adult nonhuman primates. Preliminary ultrastructural analysis of rat cortical pyramidal neurons revealed intracellular staining. Dual immunolabelling demonstrated colocalization of laminin-like (monoclonal 2E8 anti-laminin, gift of E. Engvall) and 110 kD LBP-like immunoreactivity in cortical pyramidal neurons. Western blot analysis of Triton soluble rat brain extract under reducing conditions revealed bands at 110 and 100 kD. However, using the pellet fraction, antibody recognized a dominant 140-150 kD band. Fimbria-fornix transection, stab wound and ischemia in rat induced intense 110 kD LBP-like immunostain of reactive glial cells. Ongoing time-course analysis revealed immunopositive reactive glial cells as early as 2 days after injury which persisted as long as 64 days. In rats subjected to transient ischemia by means of 4-vessel occlusion, the 110 kD LBP-positive glial cells in early postischemic periods served as a predictor for regions with permanent neuron loss. Distinct localization of the immunostain in adult and lesioned forebrain suggests that 110 kD LBP-like molecules might have an important function in brain and may be involved in the CNS response to injury.

89.5

INTERACTIONS OF SYMPATHETIC NEURONS WITH LAMININ DOMAINS. L. C. Plantefaber and A. D. Lander, Dept. of Brain and Cognitive Science, MIT, Cambridge, MA 02139

The interactions of neurons with laminin (LN) are believed to be mediated by multiple LN domains and to utilize several types of receptors. To assess the roles of LN domains, we are taking the following approaches: We are comparing the adhesive and neurite outgrowth activities of LN with merosin (MN), a LN variant that contains an M-chain in place of the LN A-chain. We are also testing the functions of isolated domains using proteolytic fragments of LN (E1', E3, E4, E8), a recombinant fragment containing the C-terminal 863-amino acid globular domain (G), and two 16-amino acid peptides, one containing the IKVAV A-chain sequence (present in E8, and proposed to represent a binding site), and the other representing the homologous region of the MN M-chain. The results indicate that MN supports greater adhesion and neurite outgrowth by chick sympathetic neurons than LN. In contrast, MN and LN were found to be equally adhesive for a fibroblast line, suggesting that binding sites recognized by neurons in the M-chain may differ functionally from those in the A-chain. Although only one LN fragment (E8) supported neurite outgrowth, both E8 and G supported neuronal adhesion, with LN>G>E8. Only weak adhesion to E1', E3 and E4 was observed. Adsorption of the synthetic LN- and MN-derived peptides to the substratum also promoted neuronal adhesion: However, addition of these peptides to the culture medium failed to block, at concentrations up to 0.5 mM, adhesion to LN or MN. In fact, the LN-derived peptide increased adhesion to E8 in a dose-dependent manner. Addition of this soluble peptide to culture medium did not, however, increase neuronal adhesion to albumin- or vitronectin-treated substrata. These data support the view that multiple sites, especially within the E8 and G domains, mediate the neuronal response to LN. (Supported by the Muscular Dystrophy Association)

89.6

DIFFERENTIAL EFFECTS OF LAMININ AND MEROSIN ON INTEGRIN-MEDIATED CNS NEURITE GROWTH AND REGENERATION. J. Cohen* Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London, SE1 9RT. (SPON: Brain Research Association).

Laminin (LN) is unique amongst extracellular matrix molecules in its ability to promote extensive neurite outgrowth by neurons in vitro. In the case of embryonic chick and rat retinal ganglion cells (RGC), the ability to extend neurites on LN is lost during development at the time when, in vivo, RGC axons encounter their target. LN exists in several isoforms, the products of a multi-gene family. One of these, merosin (M) is a 300kD A-chain homologue present in vivo in only a few sites, including peripheral nerve endoneurium and muscle basal lamina, consistent with a role in axon growth. As a culture substratum for RGC, merosin-containing LN (M-LN) not only duplicates the neurite promoting effects of A-chain containing LN (A-LN) but also is able to support the survival of, and induce neurite outgrowth from, older RGC that fail to grow on A-LN. Antibody blocking studies show that at all embryonic ages chick RGC neurite outgrowth on both A- and M-LN is mediated by neuronal $\alpha\beta 1$ integrin heterodimers. These findings suggest that RGC use different $\alpha\beta 1$ dimers in interactions with A-LN and M-LN, and that M-LN, but not A-LN, supports RGC regeneration.

89.7

MEROSIN BUT NOT LAMININ IS EXPRESSED IN THE MAMMALIAN CNS. E. Engvall*, D. Earwicker*, M. Manthorpe, S. Varon, T. Hagg, La Jolla Cancer Research Foundation and Department of Biology, University of California San Diego, La Jolla, CA.

Laminin and merosin, two members of the laminin family, possess each two 200 kD light chains (B_1 and B_2) and one 400 kD heavy chain (A or M). Both have potent in vitro neurite-promoting activity. In adult rat and rabbit brains, immunostaining with chain-specific monoclonal antibodies revealed laminin (A chain) only in association with blood vessels while merosin (M chain) was seen associated with neuronal fibers in a number of brain regions. B_1 and B_2 chain staining was seen in association with blood vessels and in almost all neuronal cell bodies. The expression of mRNA for merosin and laminin chains in different CNS regions was also determined. Adult rats (Sprague-Dawley) and rabbits (New Zealand White) were perfused transcardially with ice-cold PBS, their brains dissected out and selected regions were immediately frozen on dry ice and stored at -70°C. mRNA was prepared and used in reverse transcriptase-polymerase chain reaction to amplify conserved regions of the M and A chain RNA. Essentially no A chain mRNA was detectable in any of the CNS regions but M chain mRNA could be detected in almost all brain regions. In conclusion, merosin is the major laminin-like molecule of the adult mammalian CNS as it is in the adult PNS. Support: DHHS grants DK 30051 and NS 16349.

89.8

IDENTIFICATION OF A NEURONAL THROMBOSPONDIN RECEPTOR. Michael F. DeFreitas, Kurt Gehlsen*^ and Louis F. Reichardt, Department of Physiology and HHMI, University of California, SanFrancisco 94143 and ^ La Jolla Institute for Experimental Medicine, La Jolla CA 92037.

Thrombospondin (TS) is an extracellular matrix (ECM) protein found throughout the developing nervous system, particularly in the spinal cord, dorsal root ganglia, and in the cerebellum (O'Shea and Dixit, (1988) JCB 107: 2737). TS has been shown to be important in cerebellar granule cell migration (O'Shea et. al. (1990) JCB 110: 1275) and to support attachment and neurite outgrowth from embryonic chick retinal neurons (Neugebauer et.al. (1991) Neuron 6: 345). This neurite outgrowth is inhibited by antibodies to the integrin beta1 subunit.

To understand the basis of neuronal interaction with TS, we are using primary neurons, neuronal cell lines, and function-blocking antibodies to determine which receptors are involved. Our data show that for primary neurons and a neuronal cell line, adhesive interactions with TS are partially inhibited by antibodies to the integrin beta1 subunit. This implies that at least one of the receptors utilized by neurons to bind TS is an integrin heterodimer. Integrins are a large family of heterodimers which mediate interactions with ECM proteins. We are currently using function-blocking antibodies to the integrin alpha subunits that associate with beta1 to identify the specific alpha/beta heterodimer(s) which interact with TS.

89.9

NEURONAL RESPONSE TO MICROFILAMENT DISRUPTION VARIES ACCORDING TO ADHESION MOLECULE SUBSTRATE. A. Abosch and C. Lagenaur. NACS Dept., Univ. of Pittsburgh School of Medicine Pgh, PA 15261.

Microfilaments, which form a major component of the cytoskeleton, are especially prevalent in the motile regions of growth cones, and are believed important in neurite outgrowth, particularly in extension of lamellipodia and filopodia. Different cell adhesion molecules, including L1, P84, NCAM, and laminin (LN), have been shown to promote neurite outgrowth from CNS neurons. These various adhesion molecules do not necessarily engage the same intracellular mechanisms to achieve this similar outcome (Bixby et al., 1991). This study assessed the contribution of microfilaments to the process of neurite outgrowth evoked by several adhesion molecules. Dissociated cell cultures of P5 murine cerebellum showed exuberant neurite growth on the substrates L1, P84, and LN. Cortical cells from P0 mice grew well on L1, P84, and NCAM. Most cells on L1 extended two neurites, oriented 180° apart. Fewer cells emitted only one process. Somata were fusiform (length/width=2). Addition of cytochalasin B (CB) to these cultures caused disruption of phalloidin-stained microfilaments, and a reduction to 1 in number of neurites extended by most cells. CB-treated neurites were curved and significantly longer than control. Somata were round (length/width=1). Cells grown on P84 extended curved neurites. Upon addition of CB, cells continued to extend neurites, but in a more tightly curved fashion. A number of cells were noted in CB-treated cultures bearing highly arborized neurites. These cells stained with tetanus toxin, and were not seen in control cultures. On NCAM, addition of CB blocked neurite extension, but did not interfere with attachment of cells to substrate. On LN, CB caused a marked inhibition of neurite outgrowth initially, with eventual production of thicker neurites. These findings provide striking evidence of the differences in intracellular response subsequent to the binding of different cell adhesion molecules.

89.10

EFFECT OF DIFFERENT ADHESION MOLECULES AS SUBSTRATES FOR OUTGROWTH OF MAP2 AND NEUROFILAMENT POSITIVE PROCESSES FROM CULTURED CORTICAL NEURONS. W.-W. Chung, J.S. Lund, and C. Lagenaur, Department of Neurobiology, Anatomy and Cell Science, Center for Neuroscience, University of Pittsburgh, PA 15261.

In many differentiated CNS neurons, the dendrites express MAP2 (microtubule-associated-protein 2) while the axon expresses neurofilament (NF) (Pennypacker et al., Exp. Neurol. 111:25-35). In these studies we investigated the expression of these molecules in the first processes to grow out from the dissociated neocortical neurons of newborn mice. We used polyclonal antibody to MAP2 (gift from I. Fisher) and mouse monoclonal antibody (RT97) to NF. The cells were grown in a 1:1 combination of N1 medium (Bottenstein et al., Exp. Cell Res. 125:183-190) and BME with Earles salt and 10% horse serum. The substrates for growth were purified L1, N-CAM, and P84 molecules; polylysine was used as an inert control substrate. The growth of MAP2 positive processes was similar on all substrates; and they were the first processes to grow out. By 6 hours some processes also expressed NF in addition to MAP2. All cultures showed some processes having only NF by 40 hours, and by 3 days the majority of NF positive processes lacked MAP2. Most of the cells that grew on L1 and N-CAM were neurons, while many more glial cells attached with the neurons on P84 and polylysine. At 17-22 hours cultures on L1 showed more and longer NF positive processes than cultures grown on N-CAM; cultures on P84 and polylysine showed fewer, shorter NF positive processes. Our conclusion is that all these neuronal cell adhesion molecules allowed differentiation of axon and dendrites; L1 and N-CAM encouraged the rate of axonal growth relative to dendritic growth in comparison to P84 and polylysine. (Supported by EY05282 and NS25543)

89.11

STRUCTURE-FUNCTION RELATIONS OF THE NEURAL RECOGNITION MOLECULES L1 AND N-CAM. F. Appel*, T. Frei*, F. von Bohlen und Halbach*, J. Holm*, D. Barthels†*, W. Wille†, J.-F. Conscience* and M. Schachner. Dept. Neurobiology, Swiss Federal Institute of Technology, Zürich, Switzerland, and †Institute of Genetics, University of Cologne, Cologne, Germany.

L1 and N-CAM have been implicated in several types of neural cell interactions, such as neuron-neuron adhesion, neurite extension and fasciculation, neuronal cell migration, and triggering of second messenger systems. Both molecules belong to the L2/HNK-1 carbohydrate and immunoglobulin families and express fibronectin type III repeats. To identify structural domains which may be involved in distinct functions, we have generated molecular fragments by recombinant DNA technology and antibodies directed against these, and have used them in *in vitro* assays designed to probe for the different functions. Both the immunoglobulin-like domains and the fibronectin type III repeats of L1 and N-CAM mediate cerebellar neuron adhesion and neurite outgrowth in substrate-bound form. When added in soluble form to cerebellar explant cultures, immunoglobulin-like domain I of N-CAM decreases neurite fasciculation and granule cell migration, whereas domains III and IV increase neurite fasciculation. The other immunoglobulin-like domains and the fibronectin type III repeats do not induce morphological changes in these explant cultures. Thus, the domains influence cellular behaviour differently depending on whether they are offered as substrate or as soluble competitors. Furthermore, antibodies against individual epitopes induce different effects in substrate-bound or soluble form. Thus, a monoclonal antibody reacting with the fibronectin type III repeats of L1 inhibits neurite outgrowth on substrate-bound L1, while it enhances neurite outgrowth when substrate-bound. That signal transduction mechanisms are involved in evoking the different cellular reponses is indicated, for instance, by the observation that immunoglobulin-like domains, but not the fibronectin-type III repeats of N-CAM, stimulate phosphatidyl-inositol turnover in cerebellar neurons. Our observations indicate that L1 and N-CAM are composed of "cassettes" of functional domains that subserve distinct cellular functions.

89.12

L1/NgCAM SUBSTRATE ENHANCES NEURITE OUTGROWTH FROM MESENCEPHALIC TYROSINE HYDROXYLASE-POSITIVE NEURONS IN CULTURE. M.Poltorak, K.Shimoda and W.J.Freed. NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032.

The neuron-glia cell adhesion molecule (L1/NgCAM) is involved in neuronal development and regeneration. L1/NgCAM is implicated in the molecular basis of neuronal cell migration as well as in axonal elongation during formation of major fiber tracts. We have evaluated neurite outgrowth from dopaminergic (tyrosine hydroxylase-positive) neurons grown *in vitro* on different substrates. Cultures of ventral mesencephalon from rat embryos (E13) were plated on plastic dishes coated with the following substrates: L1/NgCAM, L2/HNK1 and MAG antigens from mouse brains, laminin, fibronectin, poly-l-lysine, DRG peptide and plastic alone. After 3, 4 and 6 days *in vitro*, the cultures were stained using an antibody against tyrosine hydroxylase (TH), and the length of TH-positive neurites was measured by computer assisted image analysis in a double blind fashion. After 3 and 4 days in culture the TH positive neurites were significant longer when grown on L1/NgCAM as compared to all other substrates studied. At 6 days laminin and fibronectin as well as L1/NgCAM showed positive effects on neurite outgrowth as compared to the poly-l-lysine coating. These data indicate that L1/NgCAM may be involved in differentiation or axonal elongation from substantia nigra dopaminergic neurons.

89.13

DIFFERENTIAL EXPRESSION OF ADHESION MOLECULES ON SUBPOPULATIONS OF SENSORY NEURON GROWTH CONES. M.G. Honig. Dept. of Anat. and Neurobiology, Univ. of Tennessee-Memphis, Memphis, TN 38163.

We are interested in understanding how, during development in the chick hindlimb, some sensory neurons grow along cutaneous nerves to the skin while other sensory neurons grow with motoneurons to innervate muscle. As one approach to this issue, we have begun to characterize the expression of adhesion molecules on the growth cones of cutaneous afferents as compared to muscle afferents. To do this, we use a tissue culture system since, in contrast to sectioned material, this allows us to visualize immunofluorescent labelling on individual growth cones. The expression of NCAM and L1 was assessed with antibodies described previously by Landmesser, Rutishauser and colleagues (Dev. Biol. 130: 645; Neuron 4: 655). In DRG explant cultures, we found a fairly wide range in the level of expression of NCAM and L1 on different growth cones. Moreover, the levels of expression of NCAM and L1 appeared to be correlated. In some experiments, we made explant cultures from embryos in which either cutaneous or muscle afferents had been retrogradely labelled with dil, and examined the expression of adhesion molecules on the dil-labelled growth cones that extended out of these explants. We found that cutaneous growth cones tended to express lower levels of L1, of NCAM, and in particular, of the heavily sialylated form of NCAM, than did growth cones of muscle afferents. Thus, cutaneous and muscle afferents appear to differ in the expression of various adhesion molecules and this may be related to how they interact with other axons during outgrowth into the limb. (Supported by NS-26386)

89.14

AN EARLY STEP IN THE GENERATION OF SENSORY AFFERENT PRESYNAPTIC PROJECTIONS IN THE CNS IS MEDIATED BY A MANNOSE-SPECIFIC RECOGNITION. J. Song*, S.R. Heisey and B. Zipser. Dept. of Physiol., Mich. State Univ., E. Lansing, MI 48824.

Leech peripheral sensory afferents express the mannose-containing Lan3-2 epitope on their cell surface. Previously, we showed that a mannose-specific recognition involving the Lan3-2 epitope is essential for the normal generation of sensory afferent presynaptic projections. Here, we further examine details of sensory afferent development under normal and experimentally perturbed conditions. Normally, sensory afferents originate in a precise temporal order in six bilaterally arrayed sensilla in the skin of embryonic midbody segments. The third sensilla afferents differentiate first and project as fasciculated axons over a 100 μm distance into the CNS. Upon entering the CNS, sensory afferents then suddenly defasciculate and amorphously spread through a 15 μm diameter of ipsilateral neuropile. Subsequently via an undetermined molecular mechanism, sensory afferents regroup into a highly structured pattern of presynaptic projections. We found that inhibiting the mannose-specific recognition using either Lan3-2 Fab fragments or mannose-BSA does not affect sensilla structure and peripheral fasciculation. In contrast in the CNS, sensory afferents stop defasciculating and therefore no longer amorphously permeate the neuropile; instead, they continue to elongate in densely fasciculated tracts. Thus, the transition from a structured peripheral axon tract to a structured CNS pattern of presynaptic projections involves a necessary intermediate state of amorphous sensory afferent growth depending on mannose-specific recognition.

89.15

CARBOHYDRATE EPITOPES DELINEATE FUNCTIONAL ORGANIZATION IN THE LEECH SENSORY AFFERENT SYSTEM. K. Zipser* and B. Zipser. Dept. of Brain and Cognitive Sciences, MIT, Cambridge MA. 02139 and Dept. Physiology, Michigan State Univ., East Lansing, MI 48824.

Leech peripheral afferents convey information of several sensory modalities which is integrated into leech feeding behavior. Sensory afferents can be identified by three different galactose-containing epitopes. We investigated how the distribution of these three carbohydrate epitopes on subsets of sensory afferents is correlated with the functional organization of the sensory afferent system. Using antibody labeling, we found that lip afferents, which transduce chemical and heat stimuli, are divisible into two subsets by two different carbohydrate epitopes. In contrast, the gut afferents (presumptive stretch detectors) lack the lip-associated epitopes and express a third carbohydrate epitope, not found on lip afferents.

Each sensory afferent subset has a distinct CNS projection pattern. We found that the two subsets of sensory afferents, which express the lip-associated carbohydrate epitopes, have overlapping presynaptic projections. In contrast, sensory afferents which express the gut-associated carbohydrate epitope have a segregated presynaptic projection. If the different presynaptic projections input into different sensory processing networks, then the carbohydrate identity of sensory afferent subsets could serve to delineate functional pathways. Another epitope common to all sensory afferents, the mannose-containing Lan3-2 epitope, mediates an early step in the generation of sensory afferent presynaptic projections. It remains to be seen if the galactose-containing epitopes found on subsets of sensory afferents mediate subsequent steps in the generation of their CNS projections.

89.16

LECTIN BINDING AFFECTS NEURITE OUTGROWTH AND FASCICULATION IN SENSORY GANGLIA EXPLANTS. Y.She* S.Mehta*, L.Hsu. Dept.of Biology, Seton Hall Univ., S.Orange, NJ 07079; Dept.of Chemistry, Rutgers Univ. Piscataway, NJ 08854.

Both initiation of axonal elongation and subsequent neurite fasciculation are mediated by the adhesive properties of glycoproteins on the neuronal cell surface and the extracellular matrix. To identify the specific glycoconjugates involved in neuritogenesis, we have surveyed the effects of numerous lectins on neurite outgrowth from sensory ganglia explants. Lectins were applied onto collagen-coated surfaces or incorporated into growth medium with or without the neurite-promoting phorbol ester 12-0-tetradecanoylphorbol-13-acetate (TPA). Various effects were elicited by the presence of lectins. Triticum vulgaris (WGA) was found to significantly promote neurite outgrowth. Mannose-binding lectins such as Lens culinaris (LCA) or Pisum sativum (PSA) affected fasciculation by causing a marked shortening and thickening of neurite bundles. Another lectin, Ricinus communis(RCA), did not promote ganglia development and caused explants to detach from the underlying surface. (Supported by NS 21262 and Research Council of SHU)

89.17

LOCALIZATION OF HIPPOCAMPAL NEURONS TO CHEMICALLY MODIFIED SILICON NITRIDE SURFACES. J.M. Corey[1], B.C. Wheeler[1], and G.J. Brewer[2]. [1]Neuroscience Program, University of Illinois, Urbana, IL 61801 and [2]Southern Illinois University School of Medicine, Springfield, IL 62794.

Synaptic specificity of CNS neurons would be more easily studied *in vitro* by limiting the number of cells and connections formed by localized somae and processes. We previously localized hippocampal neuronal somae to specific locations on coverslips using differential adhesion. Regions of polylysine were ablated from coated coverslips by exposure to a UV laser (100 mW/cm^2; 193 nm) through a quartz mask with a pattern of square grid networks. As many as 94 % of the neurons complied to one of the networks.

More recent work, adapted from Kleinfeld et. al (J Neurosci, 8 (1988): 4108), has allowed us to chemically modify the surfaces of coverslips and electrode arrays to effect cell localization. As an insulator, silicon nitride was deposited on cover slips and electrode arrays by a plasma-enhanced chemical vapor deposition process. Photoresist was used to establish a pattern to which the cells would adhere, and hydrophobic 16-carbon alkane chains were bound to the remaining surface by reflux in 5% hexadecyltrichlorosilane solution. The photoresist was removed, leaving a pattern of silicon nitride to which was bound hydrophilic diaminoalkyltrimethoxysilane. Embryonic rat hippocampal cells were dissociated and grown in serum-free media on these surfaces. Compliance to the amino-silane pattern was above 95%. This method of patterning has several advantages over the polylysine method described above. First, the hydrophilic pattern with hydrophobic surround constitutes a greater difference in adhesivity, resulting in better restriction of processes. Secondly, the cell patterning step can be done with a conventional mask aligner using a UV lamp (i.e., without the expensive laser). Third, the resulting pattern has greater shelf life than the one to two day period of patterned polylysine. This ability to localize a few cells to specific locations on electrode arrays should permit recording from neuronal networks with simple, visible connections.

89.18

THE MAGNITUDE OF SUBSTRATE ELECTRICAL CHARGE MEDIATES THE DEGREE OF NEUROBLASTOMA DIFFERENTIATION. S.A. Makohliso*, R. F. Valentini, R. Bellamkonda*, P. Biancani and P. Aebischer, Brown University, Providence, RI 02912.

Positively charged polymer substrates elicit greater neurite outgrowth from mouse neuroblastoma (Nb2a) cells than negatively charged or uncharged substrates. The structural and adhesive properties and levels of cell attachment are similar for all materials, suggesting that trapped charges are exerting the effect. The present study investigated a possible relationship between the amount of trapped charges and the degree of Nb2a differentiation observed. Fluorinated ethylenepropylene (FEP) films were fabricated into positively charged electrets using a corona charging procedure. This process injects charge into the polymer bulk without modifying the surface properties. FEP films with projected surface voltages of 500, 1000 and 3000 V were prepared. Detailed analysis revealed that all films retained their original surface chemistry and morphology. Charge levels were monitored throughout the study with a non-contacting electrostatic voltmeter. Charged and uncharged substrates were plated with Nb2a cells in serum-containing media. Differentiation of attached cells was assessed 24, 48, 72, 96 hr after plating, by quantifying the number of cells whose neurite lengths equaled or exceeded the cell's diameter. Differentiation on the various substrates was lowest on uncharged FEP, peaked on the 1000 V substrates and decreased on 3000 V FEP. Five hundred and 3000 V FEP displayed similar levels of outgrowth. All charged substrates displayed neurites of similar length, which were longer than those on uncharged FEP. Attachment levels were similar for all charged and uncharged materials. These results suggest a relationship between the extent of neuroblastoma differentiation and the magnitude of projected surface charge.

89.19

PATTERNED NEUROBLASTOMA ATTACHMENT AND OUTGROWTH ON COVALENTLY MODIFIED/CHARGE INJECTED SUBSTRATES. R.F. Valentini, J. Ranieri*, T. Vargo*[1], J. Gardella*[1], P. Aebischer. ABC Section, Brown University, [1]Dept. of Chem., SUNY at Buffalo.

Fluorinated ethylenepropylene copolymer (FEP) with embedded positive charges enhances neurite outgrowth as compared to negatively charged or uncharged FEP. In the present study, FEP surfaces were refunctionalized with hydroxyl (OH) or amine (NH_2) groups to determine how these negatively (O^-) and positively (NH_3^+) charged (at physiologic pH) surface groups influence outgrowth in the presence or absence of embedded charges. Covalent addition of OH groups was achieved in a flow-through glow discharge chamber using methanol/hydrogen vapor. A phototooled nickel mask restricted modification to 25 adjacent strips (300 x 1000 μm) spaced 500 μm apart. NH_2 groups were bound by reacting the OH-rich regions with aminopropyltriethoxysilane. Covalent monolayer modification was verified using surface-sensitive spectrophotometric techniques. FEP was charged to a potential of +1500 V with a corona poling triode array, a technique which does not alter surface chemistry. Mouse neuroblastoma (Nb2a) cells were plated onto covalently modified FEP in 10% FCS/DMEM. Nb2a cells attached preferentially, and at similar densities, to OH and NH_2 modified strips. Nb2a cells featured a flatter morphology on NH_2 versus OH groups. The level of Nb2a differentiation on electrically charged FEP was greater than that on uncharged FEP. Neurites rarely extended into unmodified FEP regions. Neurites from occasional cells that attached on unmodified regions were longer than those on OH or NH_2 strips. These results suggest that enhanced differentiation is due primarily to positive charges embedded below the surface and that charged surface groups exert a greater influence on cell attachment and morphology.

89.20

TISSUE PLASMINOGEN ACTIVATOR MESSENGER RNA IN THE DEVELOPING MOUSE CEREBELLUM AND IN NG105-15 CELLS. G.C. Friedman* and N.W. Seeds. Neuroscience Prog., Dept. Biochem/B/Genetics Univ. of Colorado HSC, Denver, CO 80262.

Tissue plasminogen activator (tPA) has been implicated in neurite outgrowth and cell migration in the developing nervous system. NG108-15 neuroblastoma cells induced to undergo neurite outgrowth with 1 mM dB-cAMP showed a 2.5 fold increase in tPA mRNA levels as compared to morphologically undifferentiated uninduced cells. Northern blot analysis of total RNA from developing cerebella revealed that tPA mRNA levels are extremely low at embryonic day 17. tPA mRNA levels increased 4-fold by postnatal day 1 and maintained this level thru day 7; these levels progressively decreased with age into adulthood, where they were 40% of the P1 levels. A major species of mRNA was seen at approximately 2.8kb and a minor species, most evident in E17 and P1 cerebella, was seen at 3.4kb. The nature of this higher Mr species is under investigation. *In situ* hybridization was performed on whole brain sections. The cerebellum was the only region that showed significant binding with an tPA anti-sense riboprobe. In the P14 cerebellum specific binding was seen in the molecular layer just superficial to the internal granular layer. The intensity of binding decreased with development, in parallel with the northern blot results. These findings indicate that tPA mRNA levels are highest at the time of active cell proliferation, migration and differentiation in the nervous system.

89.21

MODIFICATIONS OF MOLECULES ON THE SURFACE OF MERKEL CELLS AND SUBSEQUENT EFFECTS ON NEURONAL INTERACTIONS. Weslia P. Hynicka* and Randall N. Pittman. Department of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA. 19104

Merkel cells in the rat buccal pad epidermis are the natural targets for a subpopulation of trigeminal ganglion sensory neurons. Merkel cells contain NGF and are thought to provide trophic support for sensory neurons. Sensory neurons and sympathetic neurons from the superior cervical ganglion innervate the rat buccal pad at about the same time in development; however,the sympathetic neurons innervate blood vessels and the sensory neurons innervate Merkel cells in the epidermis. In culture, sensory neurons grow onto Merkel cells and branch extensively following contact; however, sympathetic neurons will not grow onto Merkel cells in culture and growth cones often collapse and retract following contact with Merkel cells. The molecular mechanisms underlying the selective contact or avoidance of Merkel cells in culture by the two types of neurons is being studied by modifying molecules on the surface of the Merkel cells. Preliminary results indicate that modification of o-linked sugars on the Merkel cell surface decreases interactions of sensory neurons with Merkel cells but does not change the interactions of sympathetic neurons. Treatment of the Merkel cells with very low concentrations of trypsin increases the number of contacts made by sympathetic neurons but does not alter the number of contacts made by sensory neurons.Studies are underway to characterize components on Merkel cells that are involved in the contact or avoidance phenomena observed with sensory and sympathetic neurons. Supported by the Whitaker Foundation.

89.22

INVESTIGATIONS INTO THE ROLE OF TISSUE TYPE PLASMINOGEN ACTIVATOR IN CEREBELLAR GRANULE NEURON MIGRATION. J.H. Ware and R.N. Pittman. David Mahoney Institute of Neurological Sciences and Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

We are investigating the role of proteases in the development of the nervous system. Some published data are consistant with the hypothesis that tissue-type plasminogen activator (tPA) plays a role in the migration of cerebellar granule neurons, while other data are inconsistant with this hypothesis. In an effort to clarify what role, if any, tPA plays in this event, we are using ^{3}H-thymidine to pulse-label cerebellar slice explants, which contain prollferating granule neuron precursors. The explants are then cultured for 72 hours in serum free or serum containing medium augmented with various protease inhibitors or antibodies. Autoradiographic visualization of the labelled neurons is being used to determine the effects of inhibiting proteases on granule cell migration.

A second approach being used to clarify the role of tPA in neural development is to characterize tPA's expression as a function of cerebellar development. While it is clear that cultured cerebellar granule neurons can secrete tPA and it will bind with high affinity to their surfaces, it has not been shown that granule neurons manufacture tPA *in vivo*. We are investigating this possibility by performing *in situ* hybridization histochemistry on sections of postnatal day 7-17 rat brains, using a riboprobe transcribed from a rat tPA cDNA. Supported by NS22663.

89.23

DEVELOPMENTAL REGULATION OF RAT UROKINASE PLASMINOGEN ACTIVATOR IN THE CENTRAL NERVOUS SYSTEM. Anne Marie L. Inglis, Angela J. DiBenedetto*, and Randall N. Pittman, Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Growth cones of cultured rat sensory and sympathetic neurons release urokinase plasminogen activator (uPA). In vitro, some of this released uPA binds locally to the bottom surface of the neuron in discrete patches next to the substrate. Inhibition of uPA activity increases neurite outgrowth about 2-fold. This effect appears to be direct rather than through inhibition of plasmin generation. The available evidence suggests a role for uPA in the development of the nervous system, and experiments in the lab are designed in an effort to elucidate this role. We have initiated studies examining the developmental regulation of both uPA activity and mRNA levels in the central nervous system of the rat. In the cortex, uPA activity increases to a peak at postnatal day 15 and then declines, reaching a steady-state during adulthood. We have cloned a rat uPA cDNA and are presently sequencing it. Northern blot analysis is being used to determine the developmental profile of uPA mRNA. In addition, the rat uPA cDNA is being cloned into a mammalian expression vector and will be used to generate recombinant rat uPA for functional and biochemical studies. Supported by NS22663.

89.24

NEURITE OUTGROWTH INDUCED BY A SPECIFIC PROTEASE INHIBITOR (Z-LEU-LEU-LEU-AL)IN PC12 CELLS:CHARACTERIZATION AND PURIFICATION OF THE TARGET MOLECULE. Y.SAITO*[1],S.TSUBUKI[1]*, H.ITO[1]*,T.HAMA[2] AND S.KAWASHIMA*. Dept.Enzyme Biochem,Tokyo Metropol.Inst.Gerontol.Tokyo 173.[1] Dept.Chem,Aoyama-Gakuin Univ,Tokyo 157.[2] Dept.Neurosci,Mitsubishi-Kasei Life Sci.Inst.Tokyo 194, Japan.

Our previous study have shown that, among many protease inhibitors examined, only a leupeptin analogue, Ac-Leu-Leu-Nle-al(ALLNal) initiates neurite outgrowth in PC12h cells. This inhibitor also increases the cellular level of both AchE and TH. The neuritogenesis induced by the inhibitor is different in some aspects from that induced by NGF,dbcAMP,bFGF and high K^+. These findings provide evidence that the protease inhibitor and other known neurotrophic factors elicit neurite initiation by different mechanisms and suggest the existence of a novel molecule which modulates neurite initiation in PC12h cells. Recently we reported that benzyloxycarbonyl (Z)-Leu-Leu-Leu-al has 50-fold stronger potencies to initiate neurite outgrowth than that of ALLNal. To determine the target molecule, Leu-Leu-Leu-al was immobilized and used as a ligand for affinity chromatography. Proteins of 33,35 and 180K were isolated specifically from the cytoplasmic fraction of PC12h cells and the conditioned medium contained none of these proteins at all. 33K and 35K were eluted with citrate buffer (pH3), while 180K was isolated with 6M urea (pH7). 2D-electrophoresis showed that 33K and 35K were acidic proteins, and amino acid analysis by pico-tag revealed that 33K had similality to 35K. The same proteins were identified in the brains of postnatal 1day rat, but the amounts started to decrease from the brains of postnatal 14 day rat. This suggests that these proteins are involved in some process in brain development such as neuritogenesis. Further analysis of these proteins will provide unique insights into the regulation mechanism of neurite initiation and outgrowth.

PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING III

90.1

A ROLE FOR OLIGODENDROCYTES IN THE STABILIZATION OF OPTIC NERVE AXON NUMBERS. R.J. Colello*, J. Kapfhammer, and M.E. Schwab, Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland

The stabilization of axon numbers in the rat visual system occurs at a time when differentiating oligodendrocytes appear and begin myelination. As this glial cell type expresses the neurite growth inhibitors NI-35 and NI-250 upon differentiation (Caroni and Schwab, 1989), we examined whether oligodendrocytes may have a role in stabilizing this fiber projection.

To test this hypothesis, oligodendrocyte development was suppressed in the retinofugal pathway of rats by X-irradiating the optic nerves at P0, P2, and P4, a time when oligodendrocyte precursors proliferate. EM and immunohistochemical analysis of irradiated optic nerves at P15 showed that the absence of oligodendrocytes and myelin was complete. Optic fiber numbers were then determined for normal (myelinated) nerves and X-irradiated (unmyelinated) nerves. In some instances, an intravitreal injection of FGF was given to the pups at P10 in order to enhance neurite growth and sprouting.

Axon counts showed that the total fiber number in the optic nerve of irradiated animals was up to 30% higher than that of normals. Further, counts at three locations along the length of the irradiated optic nerves showed fluctuations of up to 27%, while axon numbers in normals fluctuated at most 8%.

These results indicate that, in the absence of oligodendrocytes and myelin, optic fibers are able to form sprouts. This suggests that oligodendrocytes have a role in preventing sprouting and stabilizing the number of fibers in a pathway.

90.2

GROWTH OF ADULT RAT RETINAL AXONS ON GOLDFISH OLIGODENDROCYTE-LIKE CELLS *IN VITRO* M.BASTMEYER[1], M.BÄHR[2] and C.A.O. STUERMER[1]; [1]Faculty of Biol., Univ. Konstanz; [2]MPI Dev. Biol., Tübingen, Germany.

Cross species *in vitro* asays were used to test the response of adult mammalian retinal ganglion cell (RGC) axons to oligodendrocyte-like cells of fish. Rat oligodendrocytes inhibit the regrowth of neurites (Schwab and Caroni, J Neurosci 8, 1988). They also cause collapse of fish RGC growth cones (Bastmeyer et al, J Neurosci 11, 1991). Fish oligodendrocyte-like cells, however, were found to support the growth of regenerating fish RGCs (Bastmeyer et al, Soc Neurosci Abstr 16, 1990).

Here we asked whether RGCs of adult rats would regenerate when cocultured with goldfish oligodendrocyte-like cells. We cocultured adult rat retinal explants (7 d after optic nerve crush) and glial cells from regenerating goldfish optic nerve/tract at a compromise temperature of 28°C. Oligodendrocyte-like cells were identified with the monoclonal antibody 6D2 against fish myelin proteins (Jeserich and Rauen, Glia 3, 1990).

Regenerating rat RGC axons grew in high densitity on the surface of goldfish oligodendrocyte-like cells after 9-12 d *in vitro*, but failed to grow on oligodendrocyte-free regions. The vigorous and lengthy growth of regenerating rat RGC axons on fish oligodendrocyte-like cells at 28°C is comparable to that seen on mammalian Schwann cells at 37°C.

Thus, fish optic nerve/tract oligodendrocyte-like cells have growth promoting properties which support not only the growth of fish RGC axons but also the growth of axotomized adult rat RGC axons.

90.3

SUBSTRATES FOR REGENERATING GOLDFISH RETINAL AXONS *IN VIVO* GABY STROBEL AND CLAUDIA A.O. STUERMER; Faculty of Biology, Univ. Konstanz, Germany.

In contrast to mammals, retinal ganglion cell (RGC) axons in fish regenerate successfully. Fish CNS myelin and optic nerve oligodendrocyte-like cells, offered as substrates to RGCs in culture, proved to be growth permissive and not inhibitory as in mammals (Bastmeyer et al., J. Neurosci. 11, 1991). This study aims at identifying elements of the fish optic nerve used as substrates by regenerating goldfish RGC growth cones *in vivo*.

Regenerating RGC axons were labeled by intraretinal applications of HRP, 6 d after optic nerve transection. Individual HRP labeled growth cones were identified in DAB reacted semithin sections and serially ultrathin sectioned. HRP labeled growth cones were seen in close association with myelin debris and glial cells. These cells appeared, by ultrastructural criteria, to be immature oligodendrocytes or microglia. None of the growth cones examined was found to contact astrocytes or the basal lamina ensheathing the optic nerve fascicles.

Thus *in vivo*, regenerating fish RGC growth cones may grow on myelin and immature oligodendrocytes or microglia of the injured fish optic nerve.

90.4

THE EXPRESSION OF BASEMENT MEMBRANE MOLECULES ON CULTURED SCHWANN CELLS AND THEIR ROLE IN NEURITE OUTGROWTH. B. Seilheimer and W.D. Matthew, Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

We have analyzed the expression of Schwann cell (SC) basement membrane (BM) antigens and have investigated the ability of several purified monoclonal antibodies (mabs) to perturb neurite outgrowth (NO) onto cultured SCs. Dissociated dorsal root ganglia (DRG) from P1 rats were Percoll-fractionated in order to obtain pure populations of SCs and neurons. These studies analyzed the expression of antigens in three different cultures: (1) pure SCs; (2) pure neurons; and (3) pure N/SC co-cultures. The antibodies tested resulted from tolerizing mice to adult spinal cord and then immunizing with sciatic nerve.

Immunohistochemistry revealed the distinct pattern typical of SCBM staining in vivo and in vitro. Purified SCs also expressed the antigens on their surface but at low levels. Pure neuronal cultures were negative. Hence, contact with neurites promotes the accumulation of these antigens on Schwann cell surfaces—a characteristic trait of BM proteins. To test antibodies for their ability to perturb neurite growth onto established SC monolayers, the mabs were added after the neurons had attached (3-4 hrs). After 48 hours the cultures were processed for neurofilament staining to assess NO. One mab, PC1A6, was found to reduce NO dramatically. Furthermore, neuronal cell bodies and SCs tended to aggregate, indicating that this antibody interfered with substrate attachment. This data supports the fact that BM molecules are important in axon growth and, in addition, may play a role in SC adhesion and/or migration.

90.5

THY-1 SELECTIVITY INHIBITS NEURITE OUTGROWTH ON MATURE ASTROCYTES R.J. Morris, M-C. Tiveron*, B. Pliego-Rivero*, E. Barboni* and A.M. Gormley*, Lab of Neurobiology, NIMR, Mill Hill, London NW7, UK.

Thy-1, the smallest member of the immunoglobulin superfamily, is totally excluded from growing axons during normal development. However, it is a major and ubiquitous component of the surface of mature neurons, and could contribute to the fate of axons attempting to regenerate in adult nervous tissue. To determine the effect of Thy-1 on axonal growth, we have turned to neural cell lines which are Thy-1-negative, and for which neurite outgrowth is, by molecular criteria, axonal in character. Clones stably expressing Thy-1 (obtained by transfection using beta-actin promoter) show a severe inhibition of process outgrowth on a monolayer of astrocytes, but not on other cellular substrates (including embryonic glia and Schwann cells). Analysis of the site on Thy-1 interacting with astrocytes, and that responsible for mediating a transmembrane signal which suppresses process outgrowth, will be presentèd. We suggest that the interaction of Thy-1 on the surface of mature neurons with astrocytes contributes to a down regulation of axonal growth in an astrocytic environment.

90.6

CONCENTRATION-DEPENDENT EFFECTS OF ACTIVATORS OF PROTEIN KINASE C ON GLIAL-STIMULATED NEURITE OUTGROWTH. J. Qian, and P. Levitt, Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA. 19129.

Protein kinase C (PKC), one branch of the signal transduction cascade of the phosphatidylinositol system, has been shown to play multifunctional physiological roles. In present study, the role of protein kinase C in regulating the stimulating effect of glial cells on neurite outgrowth was investigated using an *in vitro* system, in which neurons derived from the embryonic rat spinal cord or hippocampus were grown for two days in media conditioned by different populations of CNS astroglia. Neurite outgrowth was examined by immunocytochemistry using an antibody against the high molecular weight neurofilament subunit (anti-NF-H), an axonal marker, and an antibody against microtubule-associated protein 2 (anti-MAP2), a dendritic marker. Two PKC activators,12-O-tetradecanoyl-phorbol-13-acetate (TPA), a phorbol ester, and retinoic acid, a vitamin A analog, enhanced the outgrowth of axons when added into glial-conditioned neuronal cultures at low concentrations (nM levels), while at high concentrations (μM levels), inhibited the glial effect on neurite outgrowth. The addition of sphingonine, a potent PKC inhibitor, into these cultures also resulted in inhibition of neurite outgrowth. These results suggest that glial stimulation of axonal growth can be modified by manipulating the PKC second messenger systems. Supported by NIMH grant MH45507 and NIH grant NS24707.

90.7

ASTROGLIA DURING GROWTH AND DIFFERENTIATION OF NERVE TERMINALS IN CULTURE. A. J. Waclawik, H.M. Sobkowicz and B. K. August*, Dept. of Neurology, Univ. of Wisconsin, Madison, WI 53706

Interactions between neuronal growth cones and astroglia were studied in organotypic cultures of the fetal (15 dg) mouse spinal cord. Primary 24-48 hr outgrowth consists of bundles of nerve fibers extending radially whose growth cones are lead by flat translucent glial cells. Ultrastructurally, these cells cover the collagen substrate, carrying nerve fibers and growth cones atop. Secondary outgrowth proceeds slowly as a band of tissue, within which nerve fibers, intermingled with glial cells, grow both radially and circumferentially. Ultrastructurally, this outgrowth is stratified. The myriad neuronal growth cones are restricted to the outermost surfaces open to the feeding solution or substrate. In the central part, however, presynaptic and synaptic terminals, inextricably associated with astrocytic processes, prevail. With time, multiple glial lamellae, (each $c\alpha$ 60 nm thick) interconnected by tight junctions, form an elaborate glial barrier which encases all neuronal structures except neuronal growth cones, which become almost extinct. In the explant--within the intricate network of nerve cells and their fibers interspersed with glial cells--all growth ceases. The 48-hour labelling with ^{3}H thymidine, between 7-9 DIV, revealed labelled glial cells restricted almost exclusively to the rim of the outgrowth zone. Our results suggest that neuronal growth cones may mitogenically stimulate astroglial cells during active neuronal growth. The young astrocytes may consist of two populations: 1) the cells that enhance and lead neuronal growth (radial glia?) and 2) the cells that induce the differentiation of presynaptic terminals. (NIH grant NINCDS R01 DC00517)

90.8

INCREASED DENSITY OF PITUICYTES AND MICROGLIA IN THE NEUROHYPOPHYSIS DURING AXONAL DEGENERATION AND SPROUTING. J. A. Watt and C. M. Paden. Dept. of Biology, Montana State University, Bozeman, MT 59717.

In a previous study we described compensatory collateral sprouting of intact magnocellular neurosecretory efferents following partial denervation of the neural lobe (NL) in rats (*Exper. Neurol.* **111**; 9-24, '91). Ultrastructural analysis revealed a transient increase in the volume of NL occupied by glial elements with a peak at 5 days post surgery (PS). We report here the initial results from experiments designed to measure the contribution of pituicytes (astrocytes) versus microglia to this increase and to determine whether it is the result of glial hypertrophy and/or hyperplasia. Glial cell numbers were determined by counting the total number of pituicyte and microglial nuclei in plastic embedded coronal sections of NL stained with toluidine blue at 5,10,30 and 90 days PS. Cell counts of the pituicyte population revealed a 30% increase in density over control values at 5 days PS. The density of pituicytes remained elevated by 25% at 10 days PS and 23% at 30 days PS, but then decreased to a value 18% below that of intact controls by 90 days PS. Counts of the microglia population indicated increases in density of 34% over control values at 5 days PS, 29% at 10 days PS, 22% at 30 days PS and 39% at 90 days PS. Thus both glial types appear to respond rapidly to partial denervation of the NL, and this response is sustained during the initial period of axonal sprouting. In addition, these data suggest that a secondary microglial response occurs between 30 and 90 days PS. An investigation utilizing bromodeoxyuridine incorporation is currently underway to determine the extent to which glial hyperplasia contributes to these increases in cell density.

FORMATION AND SPECIFICITY OF SYNAPSES II

91.1

Immunocytochemical study of synaptophysin on synapse formation between dissociated fetal cerebral cortical cells. Masumi Ichikawa[1], Junko Kimura-Kuroda*[2], Kotaro Yasui*[2], Yoichiro Kuroda[3]. Depts. of [1]Anat. and Embryol., [2]Micorbiol., [3]Neurochem. Tokyo Metropolitan Institute for Neurosciences, Fuchu, Tokyo 183, Japan.

Synaptophysin localizing on synaptic vesicle membrane has been suggested to be a good marker for synapse. Synapse formation was examined immunocytochemically in the primary culture of dissociated cortical cells using anti-synaptophysin antibody. Dissociated cerebral cortical cells of fetal rats (14 days) were cultured *in vitro* (7-104 days). Synaptophysin immunoreactive (SP-IR) spots were observed in more than 14 days culture with fluorescent microscope. EM observation showed that the SP-IR spots represent the presynaptic terminals. Time-course of distribution of the SP-IR spots was examined. The spots could not be observed at 7 days *in vitro* (DIV). At 14 DIV, the SP-IR spots were recognized on cultured cells. Then, the density of the spots increased with DIV. At 42 and 104 DIV, the spots distributed densely on the cultured cells. These immuno-cytochemical studies suggest that the dissociated fetal cerebral cortical cells make considerable number of synapses at 14 DIV and that the synaptic density increases with day. At 42 and 104 DIV, synapses are formed densely on the cultured cells.

91.2

TRANSJUNCTIONAL ADHESION COMPLEXES (TACs) AND THE GENERATION OF SYNAPTIC SPECIFICITY. W.B.Pope, S.A.Enam, T.H-C.Tsui, & W.L.Klein. Northwestern University Institute for Neuroscience, Evanston, IL 60208.

Initial contact between potential synaptic partners is often mediated by neuronal filopodia. In vitro, filopodia form junctions that are maintained during high levels of filopodial motility. These junctions can lead to the formation of structurally identifiable synapses. Whole-mount electron micrographs have revealed that filopodia contact is mediated by transjunctional adhesion complexes (TACs) made up of extracellular microfibrils. Some of these extracellular microfibrils are associated with the cytoskeleton, and they can link the cytoskeletons of contacting neurons. This may account for the ability of filopodial contacts to withstand motile stress and enable filopodia to form nascent synaptic junctions. The molecular components of TACs are highly variable from one contact to the next. So far, three components of TACs have been identified. They are heparan sulfate proteoglycan (HSPG), purpurin, (both adhesive molecules) and AD2. All of these molecules are components of chick retinal adherons. AD2 is particularly interesting since it is nervous tissue specific and because it is present only during development. Furthermore, in fixed cultured retinal neurons AD2 expression is punctate yet evenly distributed throughout soma, neurites, growth cones, and filopodia. If cells are labeled and then fixed, however, AD2 becomes clustered in growth cones and points of neuritic contact. This suggests that AD2 is free to diffuse throughout most of the membrane excepting points of contact with the external micromilieu. Thus interaction between AD2 and the external environment may affect the regulation of AD2. Since the formation of TACs may be an initial event in synaptogenesis, further identification and characterization of adheron components present in extracellular microfibrils should give insights into the generation of synaptic specificity.

91.3

NERVE-MUSCLE COMMUNICATION DURING SYNAPTOGENESIS. I. Chow and S.H. Young. Dept. of Biology, The American University, Washington, DC 20016 and Jerry Lewis Neuromuscular Research Center.,UCLA Sch. of Medicine,Los Angeles, CA 90024.

The formation of a functional neuromuscular junction requires proper interaction between the pre- and post-synaptic components. Besides the influence of secreted soluble factors and electrical activity, direct communication between the cytoplasms of nerve and muscle cells from chick (Fischbach,Dev.Biol. 28,407,1972) and Xenopus (Allen & Warner, Neuron 6,101,1991) kept in culture has been described.

In this study, embryonic Xenopus muscle cells were manipulated into contact with the soma of neurons in 1- and 2-day old cultures. Lucifer Yellow or current pulses were injected into one of the cells. Passage of dye or current into the other cell indicating the presence of gap junctions was found in 26% of the cell pairs. These junctions appeared sporadically and only a small number of channels seemed to be open at a time. Chemical synapses were also detected in several of these cell pairs by recording miniature endplate potentials in muscle cells.

These results show that during development, direct cytoplasmic communication can occur, allowing the exchange of material between the two cells which may contribute to formation and stabilization of the synapse. Yet this coupling occurs at levels low enough to prevent "short circuiting" of the chemical transmission. (supported by grants from NIH).

91.4

SELF RECOGNITION INHIBITS GAP JUNCTION FORMATION: REGULATION BY CYTOPLASMIC CONTINUITY. P.B.Guthrie, R.E.Lee*, V.Rehder, M.F.Schmidt, and S.B.Kater. Dept. Anatomy & Neurobiology, Colorado State University, Ft. Collins, CO 80523.

Identified cultured neurons from the snail *Helisoma* show a strong correlation between electrical coupling and freeze-fracture plaques (hexagonal arrays of intramembraneous particles assummed to be connexins, or hemi-junctions). Electical coupling is always seen between pairs of neurons with actively growing neurites at the time of neurite overlap; plaques are always seen in fractures which pass between the overlapping neurites. Experimental measurement of electrical coupling between two neurons before and after cutting a subset of overlapping neurites suggests that electrical coupling is also distributed uniformly between the overlapping neurites. In contrast, electrical coupling is never seen when one of the neurons has stopped growing before neurites overlap; moreover, no plaques are observed in these cases.

Interestingly, plaques are never seen between neurites from a single neuron, even though the neurites overlapped during outgrowth. A form of self-recognition appears to be inhibiting the formation of gap junctions between sibling neurites.

Direct experimental evidence against self recognition of plasma membrane was obtained using isolated growth cones from a single cultured neuron. Such isolated growth cones will survive and grow for several days. In addition, new growth cones are generated at the severed neurite tips, which advance and often overlap the isolated growth cones from the same neuron. Experiments using the calcium indicator fura-2 show that microelectrode stimulation of the cell body results in elevated calcium levels in the stimulated neuron and in the previously isolated growth cones now physically contacted by newly formed growth cones from the stimulated neuron. No calcium rise was observed in isolated growth cones that showed no overlap. Electrophysiological measurements using two patch electrodes also showed significant electrical coupling between isolated but overlapped parts of the same neuron. The inhibition of electrical coupling between sibling neurites apparently depends on cytoplasmic continuity between the neurites, and is not inherent in the molecular composition of the membrane.

91.5

REGENERATION AND SYNAPSE FORMATION BY IDENTIFIED INTERNEURONS OF HELISOMA. I.S. Roger*, N.I. Syed, R.L. Ridgway, A.G.M. Bulloch, and K. Lukowiak. Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, T2N 4N1, Canada.

The nervous system of the snail *Helisoma trivolvis* has been used extensively for studies of neuronal regeneration. Most of these studies, however, have been focused on neurons of the buccal ganglia. Following axotomy, these buccal neurons not only exhibit neurite outgrowth, but also reinnervate peripheral targets. In the present study, we used an identified network of interneurons (L.Pe.D.1, R.Pe.D.1, V.D.4) and follower cells located in the central ring ganglia. We tested the ability of these neurons to regenerate and establish appropriate synaptic contacts following axotomy.

Central ring ganglia were isolated from adult snails. All three neurons were axotomized by crushing the connectives between the left pleural and parietal ganglia and between the right pleural and parietal ganglia. These ganglia were then incubated in defined medium for 4 days. Following incubation, intracellular staining with Lucifer yellow revealed extensive neurite outgrowth of all three cells. Simultaneous intracellular recordings demonstrated the re-establishment of appropriate chemical synaptic connections. However, some new connections were observed not only after crush of the connectives, but also in control preparations in which no crushes were made. These results demonstrate the ability of adult *Helisoma* neurons to establish both appropriate and new synaptic connections following regeneration. Our in vitro model system therefore provides an opportunity to study the mechanisms underlying the specificity of synapse formation. Supported by MRC (Canada) and the Canadian Centre of Excellence in Neural Regeneration and Functional Recovery.

91.6

TERM1: A MOLECULAR LABEL FOR THE GROWTH CONES AND DEVELOPING SYNAPSES OF AN IDENTIFIED INTERNEURON. H. Reichert and T. Meier*. Dept. of Zoology, University of Geneva, CH-1211 Geneva, Switzerland.

The chemoaffinity theory (Sperry, 1963) postulates neuron-specific molecular labels, which are aquired by individual neurons and facilitate cellular recongnition between the growth cones and the targets of the labeled cells during synaptogenesis. To date, neuron-specific molecular labels of this type have not yet been found.

Here we report the discovery and biochemical characterization of a candidate molecule of this type, called TERM1 for terminal recognition molecule 1. This cell-specific molecular label is expressed on the growth cones and developing synaptic terminals of one pair of identified descending interneurons in the embryonic nervous system of the grasshopper. TERM1 is first synthesized in the labeled neurons at the 40% stage of embryogenesis. During axonogenesis, TERM1 becomes incorporated into the axonal growth cones of these neurons. Immunocytochemical studies show that the recognized epitope of TERM1 is located on the outer cell surface of the growth cones. In the subsequent synaptogenesis phase, the TERM1 label becomes concentrated on the membrane of the presynaptic terminals of these neurons. Postembryonically the expression of TERM1 disappears. The TERM1 molecular label is not expressed in any other of the millions of neurons in the nervous system of the developing embryo.

These findings suggest a molecular labeling system that is remarkably specific for individual neurons and may be important for establishing precise synaptic connectivity. (Supported by the Swiss NSF).

91.7

ECTOPIC MUSCLES AND THEIR INNERVATION IN *OCTOPOD*, A HOMEOTIC MUTATION OF THE MOTH, *MANDUCA SEXTA*. C.I. Miles and R. Booker. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

Little is known about the role of innervation during the development and specification of insect muscle. We have addressed this issue by exploiting the homeotic mutation of *Manduca*, *Octopod* (*Octo*). Larval *Octo* animals have a pair of ectopic thoracic legs on the first abdominal segment. In addition to this ectotermal effect, *Octo* also induces ectopic mesodermal structures. Up to 4 ectopic muscles, often recognizable as thoracic coxal muscles, can occur in each ectopic leg. We have examined the innervation pattern of the ectopic muscles, using cobalt backfills and neurophysiological recordings. All of the muscles appear to be innervated by an abdominal motoneuron which normally innervates the ventralmost lateral external body-wall muscle, (VLE). In *Octo* animals it innervates the ectopic muscles as well as the VLE. We studied the developmental changes in these muscles at the larval-pupal transformation, using sectioned and stained material. Both sets of muscles degenerate at the larval-pupal transition. The ectopic muscles begin degenerating on the day after the onset of metamorphosis (day W1) and are completely degenerated 3 days later. This time course is similar to that of the thoracic and abdominal proleg muscles (Kent & Levine, 1988; Weeks & Truman, 1985). The VLE does not begin degenerating until two days after the ectopic muscles (day W3), along with other body-wall muscles. The ectopic leg muscles thus degenerate with other proleg muscles, while the VLE degenerates with the body-wall muscles.

These results demonstrate that the *Octo* mutation affects both ectoderm and mesoderm, inducing ectopic muscles as well as epidermal leg structures. The ectopic muscles are innervated by a motoneuron that also innervates an abdominal body-wall muscle. Despite their common innervation, the two muscle types follow independent time-courses for degeneration, indicating that muscles respond independently to developmental signals at metamorphosis, rather than being triggered to do so by the motoneuron that innervates them. References: Kent & Levine (1988) J Comp Neurol 276:30-43. Weeks & Truman (1985) J Neurosci 5:2290-2300.

91.8

GROWTH CONE CHOICES OF DROSOPHILA MOTONEURONS IN RESPONSE TO MISMATCHED TARGETS. A. Chiba & H. Keshishian. Dept. of Biology, Yale Univ., New Haven, CT 06511

Individual motoneurons innervate specific bodywall muscles in the *Drosophila* embryo. RP3 innervates muscles 6 and 7, and RP1 innervates the adjacent muscle 13. We have designed complementary mismatches between the RP growth cones and their targets and have examined the morphology of dye-filled RP endings. The *rhomboid* mutation, which affects formation of specific muscles, deletes muscle 7. When observed at late stage 16, RP3 either innervates only muscle 6 (80%) or no muscle (20%). RP1 innervates muscle 13 normally. These results indicate that synaptic specificity is conserved after the deletion of a single muscle. We are also examining effects of muscle deletions on synaptic specificity using both the *numb* mutation and laser ablation in wild-type embryos. Conversely, an extra muscle 13 can be added when wild-type embryos are heat-shocked. RP1 innervates both muscle 13s, but RP3's innervation pattern remains intact. In all mismatch conditions examined, the innervation patterns of other neighboring muscles (e.g. muscle 12), as revealed by anti-peroxidase labeling, appear intact. These results are consistent with the idea that individual postsynaptic cells (muscles) bear unique labels to which presynaptic (motoneuronal) growth cones respond during synaptic target recognition.

91.9

FASCICLIN III EXPRESSION DURING SYNAPTIC TARGET RECOGNITION IN DROSOPHILA. T. N. Chang, A. Chiba, D. Jay[§] & H. Keshishian. Dept. Biol., Yale Univ., New Haven, CT 06511 and [§]Dept. Cell. & Dev. Biol., Harvard Univ., Cambridge, MA 02138

Fasciclin III (fas III) is a cell surface glycoprotein proposed to function as a homophilic adhesion molecule. We find that it is expressed both by a subset of motoneurons and on specific ventral muscle fibers during *Drosophila* embryogenesis. Fas III is expressed by motoneurons RP1, RP3, and RP4, which innervate three ventral muscle fibers via the SNb nerve branch. Expression on their growth cones persists until synapses have differentiated. Fas III is also expressed on the surface of muscle fibers 6 and 7, at the site where the synapse of RP3 is made. Expression on the target surface is first detected at stage 15, when the initial contacts occur, but disappears by stage 17, after synapse formation. We are using genetic and cellular approaches to assess the role of fas III during synaptic target recognition. First, we are examining a mutant which lack fas III but has apparently normal CNS and musculature, by dye filling RP growth cones. Also we are using chromophore-assisted laser inactivation in wild-type embryos to functionally inactivate fas III at specific times during development. This technique allows us to focally denature the fas III antigen and closely associated proteins without causing cell damage.

91.10

CELLULAR AND DEVELOPMENTAL CHARACTERIZATION OF TWO ENHANCER TRAP LINES WITH EXPRESSION IN SPECIFIC EMBRYONIC *DROSOPHILA* MUSCLES. E. W. Harkins and H. Keshishian. Biology Department, Yale University, New Haven, CT 06511.

The embryonic *Drosophila* neuromuscular system is stereotypic in muscle pattern, neuronal ending location and morphology, connectivity, and cotransmitter expression. We are using Enhancer Trap mutagenesis to address the hypothesis that molecules specific to subsets of muscles fibers help establish aspects of this stereotypy either during muscle pattern formation or during growth cone recognition events. We have isolated several mutants with muscle expression of the reporter gene (βgal) during embryogenesis. We have further characterized two lines that express βgal in a very small subset of fibers. Line 3068 has bodywall expression in a pair of ventral muscles (#17 and 28) and one pleural transverse muscle (#24), as well as in several sensory cells. In addition, it is expressed in 3-4 cells per segment in the CNS. Expression is first observed in non-muscle cells at embryonic stage 12, before myoblast fusion has begun. The mutant is recessive lethal and maps to 56F-57A by *in situ* hybridization to polytene chromosomes. Line 4624 is expressed in one ventral muscle fiber (#17) in segments A2-A7, and in another single fiber each in A1 and A8. Expression is segmentally repeated in a pair of mesectodermal cells, but is strongest in 3 anterior segments. There is also expression in trachea and in the gut. A developmental analysis of this line is in progress. The mutation is viable, and maps to the right arm of the third chromosome.

91.11

AN EARLY MARKER FOR ADULT MUSCLE PRECURSORS IN *DROSOPHILA*. P.K. Rivlin, R.S. Edgecomb, C. Ghetti* and A.M. Schneiderman. Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

As part of an ongoing study investigating the development of the neuromuscular system in *Drosophila*, we are following the fate of the adepithelial cells (AC) of imaginal discs, the presumptive precursors of adult muscle (Poodry & H. Schneiderman, 1970, Roux's Archiv, **166**:1). One powerful tool for producing cell-specific markers is P-element mediated enhancer detection. Transposants which show β-galactosidase staining in embryonic mesodermal derivatives (Bellen et al., 1989, Genes Dev **3**:8288) were obtained from the Hughes P-element Drosophila Stock Center (Bloomington IN) and evaluated as potential markers for AC. The β-gal-staining pattern in whole mount preparations of third instar leg, wing and haltere discs was analyzed. Of six lines evaluated only one, WG1054 ("A79.1M2," Bellen et al. 1989) showed staining of AC. The β-gal-staining pattern in WG1054 discs resembles that seen in discs stained with peanut agglutinin (PNA) (Rivlin et al., 1990, Soc Neurosci Abstr **16**:177). β-gal-positive AC are spindle shaped and border the basal surface of the disc epithelium. In leg discs, these cells are distributed throughout the basal lumen. The β-gal-staining pattern in the wing and haltere discs, however, includes cells not previously seen in PNA-stained discs. In wing discs, they reside within a posterior region fated to give rise to pleural plates, cuticular structures ventral to the wing (Bryant, 1975, J Exp Zool, **193**:49). Their shape and position suggests that these cells are also AC. Fewer β-gal-positive cells are present in haltere discs; they have a distribution resembling that of wing discs. Although WG1054 shows no staining of adult muscle, we will use this marker to study the ontogeny of adepithelial cells and to trace their migration out of discs towards their target sites. (NSF BNS90-09833, NIH 5-T32-NS07303, NIH 5-T32-GM07469)

91.12

EXTRAGENIC SUPPRESSORS OF *UNC-55*: A GENE INVOLVED IN NEURONAL DIFFERENTIATION IN *C. ELEGANS*. Marion Clein* and W. W. Walthall. Dept. of Biology, Georgia State University, Atlanta, GA. 30302-4010

The VD and DD motoneurons (mns) of the roundworm *C. elegans* share very similar genetic programs; as a result they use the same transmitter, GABA, and they are morphologically indistinguishable. They differ in their lineal histories and their target specificities. Mutations in the gene, *unc-55*, specifically affect the VD mns by altering their pattern of synapses so that they become identical to the DD mns. The phenotype exhibited by homozygous *unc-55* mutants is to coil with the ventral side innermost when attempting to move backward, forward movement is essentially normal. Genetic strategies combined with an analysis of locomotory behavior have been employed to aid in the identification of mutations in genes that interact with *unc-55*. A maze was designed to aid in this search. The maze, whose structure was inspired by the benzene ring, is effective in enriching for noncoiler phenotypes. Typically 95 to 100% of wild-type animals navigate the maze and find the food in a 15 to 20 hour period. However, due to a number of blind alleys, 5% of *unc-55* animals are successful. The maze was used to select noncoiler animals from the F2 generation of mutagenized *unc-55* strains. Two recessive, X-linked suppressors have been obtained. When separated from *unc-55* both exhibit difficulties in moving backward but neither are coilers. Genetic and cellular analys es of these alleles are being pursued. Supported by the NSF (BNS-8911523A01).

91.13

SPECIFICITY OF SYNAPTOGENESIS DURING REGENERATION OF AN IDENTIFIED NEUROMUSCULAR JUNCTION IN *HELISOMA*. M. J. Zoran, J. L. Rotter* and P. G. Haydon. Dept. of Zoology and Genetics, Iowa State Univ., Ames, IA 50011.

Novel chemical connections form between neurons not normally connected in the buccal ganglia of *Helisoma* following axotomy (Haydon and Kater, 1988; J. Neurobiol. 19, 636). Buccal neuron 19 (B19), which normally innervates the supralateral radular tensor (SLT) muscle of the buccal feeding apparatus, is restricted in its formation of chemical connections during regeneration *in vivo* and in cell culture. Neuron B19 never forms novel chemical connections with neuronal targets. However, recent cell culture studies have demonstrated that B19 is capable of forming appropriate neuromuscular connections in cell culture (Zoran et al., 1990; Dev. Biol. 138, 202). The possibility that neuron B19 might form inappropriate chemical connections with novel muscle targets remains. Therefore, the objectives of the present study were to identify other appropriate and inappropriate muscle targets of this motoneuron and to describe the specificity of synaptogenesis during regeneration of neuron B19.

Electrophysiological studies of neruomuscular preparations demonstrated normal connections between neuron B19 and its SLT muscle target. In similar recordings, no evidence of connectivity was detected between B19 and buccal retractor (BR), anterior jugalis (AJ), posterior jugalis (PJ) and odontophore (ODT) muscles. *In vivo* regeneration experiments were performed surgically by crushing the heterobuccal and ventrobuccal nerves which contain the axons of neuron 19. During regeneration, B19 exhibited highly stereotyped pathfinding ability. Dye fills revealed that the sprouting neurites of B19 were discriminate in their selection of appropriate pathways and extended within discrete tracts in the buccal nerves. Stimulation of neuron B19 at this time of regeneration evoked excitatory junctional potentials (EJPs) in the SLT muscle fibers and elicited SLT muscle contractions. No inappropriate neuromuscular connections were detected between B19 and other buccal muscles. Cell culture studies are in progress to determine whether B19 can form novel neuromuscular connections.

92.1

INCREASE OF c-FOS AND RAS ONCOPROTEINS IN THE DENERVATED NEUROPIL OF THE RAT DENTATE GYRUS. L.L. Phillips and E.T. Belardo. Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298.

When the entorhinal cortical (EC) input to the rat dentate gyrus is destroyed, the process of sprouting and synaptogenesis begins within the denervated neuropil. The present study used immunohistochemistry to determine whether there is an increase in c-fos and ras within the denervated neuropil of the dentate gyrus during terminal growth and synapse formation. Rats were perfused with paraformaldehyde fixative at 1, 6 and 30 days after unilateral lesion of the EC, and brain sections were incubated with antibodies to either c-fos or ras oncoprotein. At 1 day postlesion light microscopic analysis showed a moderate increase in both c-fos and ras proteins over the denervated zone. However, at 6 days postlesion we observed a marked increase in denervated zone labeling for each oncoprotein relative to intact contralateral and naive controls. The c-fos label over the denervated molecular layer was generally uniform, with interspersed punctate reaction product. Neuroglia contained the highest levels of c-fos oncoprotein. Granule cell nuclei were reduced in c-fos labeling at 1 and 6 days postlesion when compared to naive controls. By 30 days postlesion increased neuropil c-fos labeling was not visible and granule cell c-fos label was predominantly nuclear, as seen in naive controls. Ras oncoprotein localization at 6 days postlesion was also uniform over the denervated zone, with intermittent punctate labeling. As with c-fos, this selective label was not seen at 30 days postlesion. No cell nuclei labeling was observed with antibodies to ras protein. Additional method and antibody controls showed labeling to be specific for each oncoprotein. These results show that both c-fos and ras are increased within the denervated neuropil of the dentate gyrus during sprouting and synapse formation, supporting oncoprotein mediated changes in gene expression and membrane signal transduction during synaptogenesis. Supported by NIH grant NS27225 to L.P.

92.2

A NOVEL METHOD FOR FORMATION OF NEURAL NETWORKS IN CULTURE. A.Kawana, H.P.C.Robinson*, K.Torimitsu and Y.Jimbo*. NTT Basic Res. Labs. Musashino, Tokyo, 180 Japan

Culture systems of vertebrate neurons provide a useful method for studying the physiology of neurons and neural circuits in a simplified , controlled enviroment. However, networks formed by conventional methods are too complex to allow meaningful investigation of their physiology. Here, we describe a novel method for the construction of patterned neural circuits in cell culture, in which both the position of neurons and the direction of their neurite outgrowth are controlled.

A silica glass substrate with 150 X 150 μm wells connected by 50 μm wide microgrooves of 10 μm depth and 200 μm length was employed. A metal mask with 150 μm diameter holes at corresponding positions to the substrate wells was laid over the substrate. A suspension of dissociated rat hippocampal neurons was plated over the metal mask and incubated for 30 min. Then, 2 ml of culture medium was added and the metal mask was removed. The cell density in the wells was controlled by varying the density of the suspension. After 2 or 3 days, neurons within the same well form synaptic connections, following which the neurites grow out from the wells along the microgrooves to form inter-well synaptic connections. The capability of thus imposing spatial and temporal order on the process of synaptic wiring should facilitate studies of the functioning of cultured neural circuits. Monitoring of neural activity in this culture system by optical methods and by electrodes embedded in the substrate will be discussed.

92.3

ACTIVITY-DEPENDENT SYNAPSE ELIMINATION AT THE VERTEBRATE NMJ *IN VITRO*. P.G. Nelson, C. Yu*, R.D. Fields and S.C. Fitzgerald*. Lab. of Developmental Neurobiology, NICHD, NIH, Bldg. 36, Room 2A21, Bethesda, MD 20892.

Mouse myotubes in the center compartment of "Campenot" chambers were innervated by axons from sympathetic ganglion neurons in two bilaterally symmetrical side compartments, or from ventral spinal cord neurons. Axons from each side can be stimulated for several days through extracellular electrodes. The efficacy of synaptic connections was monitored by observing contractions of myotubes in response to stimulating neurons from either side compartment, and by repeated intracellular recordings of synaptic potentials.

Functional cholinergic connections develop and are stable for several weeks in culture. Following 3-5 days stimulation at 30 Hz for 2 sec. at 10 sec intervals, 30% - 50% of the synaptic connections were eliminated ($p << 0.001$, chi-square, $n = 520$ muscle fibers). By Hebbian postulates the stimulated afferents in unilaterally stimulated cultures should be selectively retained. However, the probability of elimination was equal for stimulated and unstimulated inputs in unilaterally stimulated cultures (innervated by either ventral spinal cord or superior cervical ganglion neurons). Thus, elimination at this nmj is an activity-dependent process, but it does not follow Hebbian rules of synaptic plasticity.

92.4

CHRONIC NMDA RECEPTOR BLOCKADE PREVENTS THE FORMATION OF SPINOCEREBELLAR STRIPES. J.M. Alisky and D.L. Tolbert Department of Anatomy and Neurobiology and Surgery(Neurosurgery), St. Louis University School of Medicine St. Louis MO 63104.

During the period of 3 to 7 postnatal days (PNDs), developing spinocerebellar (SpCb) projections in rats segregate into 5 sagittally oriented stripes in the anterior lobe (Nunes and Sotelo, '85). To test the hypothesis that NMDA receptor activation is required for segregation of SpCb afferents, NMDA receptors were chronically blocked with the competitive antagonist aminophosphovaleric acid (APV). Elvax implants containing 0.1 M APV were placed on the surface of the anterior lobe of the cerebellum at 3 PND. SpCb projections were labeled with injections of WGA-HRP into the thoracic-lumbar junction of the spinal cord, and rats were sacrificed at 16, 19, 21 and 23 PNDs. In transverse sections of control cerebella, labeled mossy fiber terminals formed five sharply defined sagittally oriented stripes with no interstripe label. In APV treated rats, incipient stripes were present but they were not sharply delineated and many terminals were present in interstripe regions. Desegregation of stripes occurred predominantly in the apical portions of lobules II-IV, closest to the APV-elvax implant. Deeper in the lobules there was a transition zone of moderate segregation, and in the most basal portions of anterior lobules, there were normal stripes. These findings suggest that NMDA receptor activation is necessary for the establishment of spinocerebellar stripes. Supported by NIH Grant NS20227.

92.5

KINASE MANIPULATIONS DISRUPT ACTIVITY-DRIVEN RETINOTOPIC SHARPENING IN GOLDFISH TECTUM. **John T. Schmidt, Dept. Biol. Sci., SUNY-Albany, NY 12222**

Regenerating optic axons form side branches and synapses over a wide area, and retinotopically appropriate synapses are stabilized through the activation of NMDA receptors that detect the coincident activity of neighboring ganglion cells making synapses onto the same tectal cell. Calcium entering through NMDA receptor channels triggers LTP by activating C-kinase and Cam kinase, and these experiments test whether kinase activation is necessary for synaptic stabilization during sharpening. Intraocular (IO) or intracranial (IC) injections of the phorbol TPA (50μM x 2μl in Ringers, ~1μM after dilution), which activates and down regulates C-kinase, were made every other day during the sensitive period (19-35days postcrush), and the projections formed were recorded. IO-TPA prevented sharpening and resulted in multiunit receptive fields that averaged about 26° vs 11° in Ringer controls. IC-TPA also prevented sharpening (29° multiunit fields), but did not unsharpen the mature projection on the opposite tectum. IC injection of kinase inhibitors H7 or sphingosine (4μl x 10mM, 200μM after dilution) prevented sharpening (27 and 30° multiunit fields). HRP staining of these arbors shows abnormalities in branching. Thus active formation of retinotopic order (vs maintenance--Cline and Const.-Paton, 1990) is sensitive to kinase inhibitors. In retinal explant cultures TPA treatment decreases the rate of elongation of growing axons and increases the probability of retraction. This is important since an inability to grow could explain the failure to disrupt preexisting eye-specific stripes. During sharpening there is an increased capacity for LTP triggered by NMDA receptors, and these experiments support the idea of a parallel dependence of LTP and sharpening on kinase activation. (Supported by NIH grant EY03736).

92.6

ELECTRICAL ACTIVITY REGULATES THE EXPRESSION OF SYNAPSE-SPECIFIC GENES DURING NEURONAL DEVELOPMENT.

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During synaptogenesis, cell-cell interactions between neurons and their targets are thought to induce the expression of specific sets of genes. Our goals are to determine the nature of these interactions and to study the molecular mechanisms involved in this process of gene regulation.

We have constructed 2 cDNA libraries from chick retina mRNA extracted at embryonic day 9 (E9) and E15 (i.e. before and after the onset of synaptogenesis) and we have then performed a subtractive hybridization, using the E9 PCR-amplified library as a driver, to isolate cDNAs present at E15 but not at E9. Several clones corresponding to mRNAs developmentally regulated during synaptogenesis were isolated. One such clone was found to encode SNAP-25, a nerve terminal protein expressed in subtypes of synapses. Early lesion of the optic tecta had no effect on the expression of SNAP-25 by retinal ganglion cells. In contrast, blockade of electrical activity with intraocular injections of TTX strongly reduced SNAP-25 expression. Similarly, the expression of the presynaptic protein synaptophysin was strongly reduced following the TTX injection, whereas β-tubulin expression was unaffected. These data suggest that electrical activity regulates the expression of proteins involved in synapse structure and function.

92.7

FACILITATION AT THE NEUROMUSCULAR JUNCTIONS OF ALLOTRANSPLANTED NEURONS IN THE CRAYFISH. K. M. Krause and S. J. Velez. Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

The superficial flexor muscle system of the crayfish Procambarus clarkii has been used to analyze the factors that affect neurons as they make precise connections with their target cells. We had previously reported that the isolated ganglion that contained the somas of these cells could be succesfully transplanted from one crayfish to another: the neurons survived, grew and formed new synaptic contacts with the denervated target muscle; as revealed by junction potential (jp's) sizes, the contacts assumed normal characteristics by eight weeks (Krause et. al., Soc. Neurosci. Abstr. 16: 489, 1990). We followed the progress of regeneration of these allotransplanted neurons using microelectrodes to test for jp's and suction electrodes to record and stimulate the nerve. As criterion for the maturation of the contacts, we measured the facilitation properties of the synapses (jp size at 10 Hz/ jp size at 1 Hz). We found that by ten weeks the entire muscle field becomes innervated by more than one axon. Nerve stimulation has revealed that the newly established contacts do not facilitate, which suggests that these connections are not maturing normally. The lack of development of normal facilitation characteristics might be the result of the reduced level of spontaneous activity found in the isolated ganglion.

92.8

DEVELOPMENT OF TWITCH/TETANUS RATIOS DURING SYNAPSE ELIMINATION IN THE RABBIT SOLEUS MUSCLE. K. S. Cramer and D. C. Van Essen, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

The study of motor unit development and diversity is essential for understanding motor function at the muscular level. Our experimental model, the rabbit soleus muscle, is polyinnervated at birth and eliminates excess synapses over the first two postnatal weeks. It contains both fast and slow motor units, which, even at early ages, are known to be distinguishable physiologically by their twitch rise times. We examine here fast and slow motor unit development using a different measure, the twitch/tetanus ratio (TTR). We find that TTRs change during synapse elimination, and that these changes are different for fast and slow motor units.

In the 4-5 day rabbit soleus, the mean TTR is .24 ± .01 (s.e.m.) for fast units and .29 ± .01 for slow units. At this age the slow units have a significantly higher TTR than fast units ($p < .01$). By 13-15 days, however, we find that fast motor units have significantly higher ratios than slow motor units, with means at .35 ± .02 and .21 ± .01, respectively ($p < .0001$). A similar distribution has been described for 17-18 day rat soleus (Thompson and Astrow, *Soc. Neurosci. Abstr.* 16: 331, 1990). Thus, during synapse elimination, fast motor unit TTRs increase while slow motor unit TTRs decrease.

Previous studies have suggested that the ratio of mean motor unit tensions at polyinnervated versus singly innervated ages gives a useful estimate of the overall degree of polyinnervation for fast and slow muscle fibers at the early age. However, since TTRs change with age, this ratio yields conflicting estimates depending on whether the estimates are based on twitch vs tetanic tension. Consequently, more direct methods will be needed in order to obtain reliable measurements of polyinnervation by fiber type.

92.9

HORMONAL REGULATION OF MOTOR UNIT SIZE AND SYNAPTIC STRENGTH DURING SYNAPSE ELIMINATION. C.L. Jordan, P. Pawson, A.P. Arnold, & A.D. Grinnell, Psychology and Physiology Departments, UCLA, Los Angeles CA 90024.

Our previous anatomical studies have implied that androgen treatment of juvenile male rats prevents some synapse elimination in the levator ani (LA) muscle of rats. The present study confirms this implication physiologically by demonstrating that such androgen prevents some of the ontogenetic decline in motor unit size in this muscle. The size of LA motor units was estimated in androgen-treated and control rats at 7-28 days after birth by measuring the twitch tension of individual motor units relative to the twitch tension of the whole muscle. Synaptic strength was also evaluated by measuring the ratio of tetanic and twitch tensions produced by individual motor units relative to the ratio of the whole muscle. The results indicate: 1) between 7 and 21 days, the size of LA motor units declines from 10.8 to 4.2%, and androgen treatment prevents some of this decline (10.8 to 6.9%); 2) between 7 and 14 days, the tetanic/twitch ratio drops from about 4 to 1, indicating that nearly 75% of all LA synapses are below threshold at 7 days, but virtually all synapses are above threshold by day 14; 3) androgen does not alter the tetanic/twitch ratio of motor units at any age examined. Thus, androgen prevents the normal decline in the size of motor units without altering the apparent strength of synapses. Supported by NS08686 and HD15021.

92.10

THE NUMBERS OF PHOTORECEPTOR SYNAPSES IN THE FLY'S OPTIC LAMINA REVEAL DAY/NIGHT (CIRCADIAN?) CHANGES. Elżbieta Pyza* and I.A.Meinertzhagen. Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The receptor cells of the arthropod compound eye exhibit many circadian rhythms, most notably in the turnover of their photoreceptive membranes; do such rhythms also exist in their output region, the synaptic terminals? To address this question we have examined the frequencies of synaptic contacts in the first visual neuropile, or lamina, of the optic lobe in the fly *Musca domestica*. Two synapses have been compared: the afferent tetrad synapses formed upon two interneurons, L1 and L2, and their reciprocal feedback counterparts from L2, formed back upon receptor terminals; both were measured from the frequencies of presynaptic ribbon profiles in single EM sections.

In the L2 feedback synapses there is a remarkable change in frequency during the normal day/night cycle, with 80% more synaptic contacts in the night. Since this difference could be a function either of light or of a circadian rhythm, flies previously entrained to a reversed light/dark cycle were then reared in constant darkness for three days. The frequencies were also found to be higher in the fly's subjective night, which suggests a strong endogenous influence upon synaptic frequency. Compared with the values in constant darkness, there was no significant difference when the flies received 1hr of light (in either their subjective night or in their subjective day), relative unimportance of light exposure.

In the afferent tetrad synapses similarly there is a day/night change in frequency, but in that case the frequency is 20% *lower* in the night, and the relationship between the effects of light and possible circadian rhythms is more subtle. The functional significance of the changes in either of these synapses is not clear.

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92.11

COLD STRESS CAUSES A REVERSIBLE LOSS OF PHOTORECEPTOR SYNAPSES IN THE FLY'S OPTIC LOBE. J.H. Brandstätter and I.A. Meinertzhagen. Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, CANADA B3H 4J1.

Synaptic contacts turn over in the adult nervous system and this must entail both their spontaneous formation and loss. Both the formation and loss of contacts occur in the first neuropile, or lamina, of the optic lobe in the adult fly, amongst the population of afferent tetrad synapses of the photoreceptor terminals. The rates of formation and loss are hard to establish however from changes in net frequencies. We have found that cold stress causes the rapid loss of synaptic junctions, a loss which is reversible, and have started to use the reversible effects of cold as a tool to perform a stop-flow analysis of synaptic turnover. Adult (2-3d) flies, *Musca domestica*, reared at 23°C were exposed to 0°C for periods up to 12hrs. Following this, the frequency of tetrad synapses, measured from the frequencies of the single-EM section profiles of presynaptic ribbons in > 250 terminals in 3 experimental animals, decreased by 20-30% after 12hrs, whereas 2hrs of cold produced no decrease. The effect is reversible; 24hrs after the flies had returned to 23°C the value had increased, returning to normal control values (n=170, 2 flies). This increase is highly significant. We hope with this method to resolve the rate of synapse formation during warm recovery, as the first step to dissecting the dynamics of synapse turnover.

Supplementing synaptic changes we also find that, following cold exposure, the frequency of the profiles of the receptor terminal mitochondria decreases and their shape elongates; the frequency of the profiles of capitate projections, glial invaginations of enigmatic function into receptor terminals, also decreases. These changes too are reversible, but unlike the synaptic changes they are not completely restored to control values by 24hrs at 23°C.

Supported by NIH grant EY-03592 and the Canadian Centre of Excellence in Neural Regeneration and Functional Recovery.

92.12

REGULATION OF PARASYMPATHETIC NERVE DENSITY BY SYMPATHETIC INNERVATION DURING DEVELOPMENT. Peter G. Smith and Chad E. Sharp*. Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66103

The influence of sympathetic innervation upon development of parasympathetic nerve density was examined in the rat tarsal muscle, a smooth muscle target where both autonomic components are accessible for surgical manipulation. In preliminary studies, the specificity of acetylcholinesterase (AChE) histochemistry for parasympathetic nerves was determined. Ipsilateral superior cervical ganglionectomy 7 days prior to sacrifice resulted in a 39% decrease in AChE nerve density, indicating that sympathetic fibers contain this enzyme. Ipsilateral superior cervical ganglionectomy concurrent with ipsilateral pterygopalatine ganglion excision resulted in an essentially complete loss of AChE-positive fibers after 7 days, indicating that non-sympathetic AChE-positive fibers represent parasympathetic nerves originating in the pterygopalatine ganglion. The influence of sympathetic innervation on parasympathetic nerve density was investigated by bilaterally excising the superior cervical ganglia on postnatal day 5 and quantitating AChE-positive nerve density at 4 months of age. Tarsal muscles with persistent sympathetic denervation, as confirmed by absence of catecholamine histofluorescence, displayed a 46% reduction in AChE-positive nerve density as compared to acutely sympathectomized controls. It is concluded that sympathetic nerves act by direct or indirect means to enhance parasympathetic nerve proliferation within this target. Supported by NS23502.

92.13

RELATIONSHIP OF SYNAPSE DENSITY TO GROWTH IN A CRUSTACEAN MUSCLE. S.E. Huestis*, J.P. Walrond* and C.K. Govind. *Dept. Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523 and Life Sciences Division, University of Toronto, 1265 Military Trail, Scarborough, Ontario, M1C 1A4 Canada.

Growth-related changes in synapse number and distribution were investigated for the single inhibitory and excitatory motor neurons innervating lobster (*Homarus americanus*) Distal Accessory Flexor Muscles (DAFM). Both the excitor and inhibitor release more neurotransmitter onto the most distal muscle fibers than onto the most proximal muscle fibers. To regulate differences in the amount of transmitter released, the inhibitor forms more synapses distally than proximally whereas the excitator forms similar numbers of synapses in the two regions with larger active zones on the distal fiber bundle. In the present study, inhibitory axon terminals were visualized with an antibody against the neurotransmitter, γ-aminobutyric acid (GABA), linked to horseradish peroxidase or a fluor. Axon terminal varicosities, the sites where most of the inhibitory synapses are located, were counted on individual muscle fibers. Varicosity number was normalized to muscle fiber surface area to measure the synapse density. During growth, the total varicosity number and muscle surface area increases, indicating that the inhibitor makes additional synaptic contacts to accommodate the increased muscle size. Therefore, synapse density in sexually immature animals (50-420 gm) remains constant. These studies suggest that the inhibitor forms new synapses to match the increased surface area of the muscle. After sexual maturity the muscle fiber area outpaces synapse addition. Although the relationship of excitatory synapse number to growth is unknown, the size of the excitatory active zone increases with growth on the distal muscle fiber. However, active zone size remains constant in the excitatory synapses on the proximal bundle as well as in all of the inhibitory synapses. The mechanisms used to regulate differences in the amount of transmitter released regionally may be similar to those used during growth.

92.14

SYNAPTOGENESIS IN IDENTIFIED SPIKING LOCAL INTERNEURONES OF THE LOCUST SCHISTOCERCA GREGARIA. Leitch, B., Laurent, G.,& Shepherd, D.* University of Cambridge, UK.

The development of synapses on identified spiking local interneurones in the thoracic ganglia of embryonic locusts was examined using intracellular horseradish peroxidase (HRP) injection and electron-microscopy. In adult locusts, spiking interneurones of the midline group receive direct inputs onto a ventral field of branches from leg hair afferents and in turn makes output synapses from a dorsal field of branches directly upon leg motor neurones, nonspiking local interneurones and intersegmental interneurones. The aim of this study was to examine the development of these connections. Formation of the major branches occurs during 55-70% embryogenesis and is followed at 70-80% by an elaboration of this branching. Subsequently, there is a period of maturation during which the number and length of the branches are pruned to produce the adult pattern. Mature synapses are first evident on the interneurones at 70-75% development coincident with the time of arrival of the majority of afferents into the CNS. At this stage most of the ventral synapses are outputs. By 85-90% development output synapses still predominate but, the ratio of outputs to inputs is reduced from 15:2 to 4:1. Dorsal branches have predominantly output synapses from 70% embryogenesis, onwards. The work was supported by a SERC (UK) Rolling Grant awarded to Prof. M. Burrows.

92.15

DIFFERENTIAL PROLIFERATION OF AXOSOMATIC SYNAPSES IN THE NEONATAL RAT CILIARY GANGLION G.N. Robertson and P.C. Jackson Department of Anatomy, Dalhousie University, Halifax, N.S., Canada B3H 4H7

During the development of autonomic ganglia the general pattern of connections changes such that neurons in the adult receive fewer inputs, yet more synapses, than those of the neonate. Physiological evidence suggests that some initial inputs are withdrawn, while the remainder consolidate their innervation through continued synaptogenesis. To determine the effect of this change upon synaptic populations, we examined the synapses between preganglionic neurons and cell bodies in the ciliary ganglion. Each preganglionic axon makes synapses with the cell body (Forehand, 1987, J. Neurosci. 7:3274). The number, structure and location of axosomatic synapses in the rat ciliary ganglion was examined at one, three and eight weeks of age. The number of synapses increased approximately four-fold between one and three weeks and changed little thereafter. We found four distinct types of synapse; one type completely accounted for the increase in total number.

In the neonate the synapses tended to be uniformly distributed over the cell body. By three weeks of age however, five times as many synapses were found under the perinuclear glial lamella (glial "cap"); elsewhere on the cell body the glial lamella was attenuated (glial "cover") and invested far fewer synapses. The frequency of synapse types within the total population was similar in both cap and cover regions. Thus not only is the glial cap a preferred region for synaptogenesis in early postnatal development, there is a differential proliferation of one specific type of synapse.

92.16

ISTHMOTECTAL SYNAPSES IN DEVELOPING XENOPUS FROGS. S.B. Udin, M.D. Fisher*, & J.J. Norden. Dept. of Physiol., SUNY, Buffalo, NY 14214 and Dept. of Cell Biol., Vanderbilt Medical School, Nashville, TN 37232 .

During the development of binocular maps in *Xenopus* tectum, axons which relay input from the ipsilateral eye via the nucleus isthmi undergo a prolonged period of shifting connections concomitant with the dorsofrontal shift of the eyes. Isthmotectal axons arborize densely in the rostral region but also extend sparser branches into the caudal zone, which is occupied by retinotectal inputs with receptive fields in the monocular zone of the visual field. Establishment of matching binocular maps entails activity-dependent stabilization of isthmotectal axons which exhibit firing patterns correlated with those of nearby retinotectal axons. Synaptic communication is hypothesized to be essential for this process. We therefore have used electron microscopy to investigate whether isthmotectal axons make morphologically identifiable synapses during development and whether such synapses occur throughout the tectum.

HRP was injected into the left nucleus isthmi in order to fill isthmic inputs to the right tectal lobe. We found that all regions of the tectum contain morphologically identifiable synapses, along with many growth cones and structures that may be immature synapses. As in the adult, the synapses contain round, clear vesicles, have asymmetric specializations, and terminate on structures which appear to be dendrites. These results indicate that synaptically-mediated interactions may promote stabilization of appropriate isthmotectal connections and withdrawal from inappropriate regions of the tectum during development.

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92.17

DISTRIBUTION OF DORSAL ROOT AFFERENT BOUTONS ON MOTOR NEURONS IN DEVELOPING MAMMALIAN SPINAL CORD. W.D. Snider, Li Zhang, S. Yusoof, A. Konstantinidou. Dept. of Neurology, Washington Univ. Med. School, St. Louis, Mo. 63110

The interactions between afferent axons and their specific target cells during development have not been well characterized. In order to address this issue in mammals we have utilized the simple system of monosynaptic contacts between group I muscle afferents and spinal motor neurons in the rat. We have labelled dorsal root afferents with DiI and spinal motor neurons with DiA. We show that dorsal root afferent axons enter the spinal cord at E15 in fasciculated bundles. Spatially separate bundles in the dorsal horn converge in the intermediate zone, then fan out to innervate various motor pools. In general, spatial relationships apparent in the intermediate zone are maintained on the way to the motor pools. Dorsal root axons destined for axial pools grow directly to their appropriate motor pool without the benefit of guidance by dendrites. Those destined for limb motor pools pass through a region densely populated by motor neuron dendrites. Whether contact with these dendrites is important in the development of appropriate specificity is not yet clear.

Starting at E17 dorsal root axons give off branches with increasing intensity through the first postnatal week. A striking feature of these branches is that they are invariably directed towards rather than away from motor neuron somata. Thus even though axons are in close proximity to dendrites which project for long distances dorsally or medially, the afferents grow towards the somata rather than growing distally along these dendrites. Higher order branching and bouton formation occurs almost exclusively in the area of the motor neuron somata and proximal dendrites, at least through PN5.

We conclude that dorsal root afferents have their initial interactions with the somata and proximal dendrites of their target motor neurons. This occurs despite the fact that motor neurons have elaborated extensive and lengthy dendrites prior to the arrival of dorsal root afferents. Whether this pattern is typical of other spinal afferent systems is currently under investigation.

92.18

PERIPHERAL INFLUENCE ON MUSCLE AFFERENTS AND MOTONEURONS. P. Wenner, C. Lance-Jones and E. Frank. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

How are neurons specified during development to make their appropriate connections? Previously we have found that the central projections of muscle spindle afferents are influenced by the location of their peripheral axons; thoracic sensory neurons forced to supply a limb make novel but functionally appropriate connections with limb motoneurons. In this study we specifically ask if muscle sensory neurons are influenced to make central connections with motoneurons directly by the muscle they supply; similarly, are motoneurons also influenced by their target muscle?

In order to answer these questions we created chicks which had a double compliment of dorsal muscle in the thigh. The ventral half of the limb bud is replaced by a dorsal half from a similarly staged embryo (st. 17-19), (Lance-Jones, 1986). Motoneurons and muscle afferents which would normally have innervated ventral muscle now innervate donor dorsal muscle; in the same animal dorsal motoneurons and muscle afferents project to their normal host dorsal muscles. Do dorsal motoneurons in these embryos receive direct input from afferents that would normally have been antagonistic, but are now supplying dorsal muscle? Furthermore, do ventral motoneurons receive direct input from normal dorsal afferents?

Muscle sensory input to lumbosacral motoneurons was assessed using intracellular recordings from isolated spinal cords. In normal embryos ventral motoneurons do not receive direct input from dorsal afferents, and dorsal motoneurons do not receive direct input from ventral afferents. In dorsal-dorsal duplications, normal dorsal motoneurons now do receive direct input from muscle afferents supplying dorsal muscle but traveling with ventral motoneurons, suggesting that muscle afferents recognize their specific motoneuron partners based on the peripheral muscle they supply. In the two best preparations to date, ventral motoneurons innervating dorsal muscle received strong direct input from normal dorsal afferents, suggesting that motoneurons are also influenced by their target muscle. (Supported by NSF to EF).

92.19

CHANGES IN PHENOTYPIC PLASTICITY OF SENSORY NEURONS DURING DEVELOPMENT. S.C. Mears, S. Donahue*, and E. Frank, Depts. of Neurobiology, Anatomy, & Cell Science and *Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

During development in tadpoles, populations of sensory neurons are influenced by their peripheral projections to make appropriate synaptic connections within the spinal cord. If a thoracic nerve is rerouted peripherally and joined to the adjacent brachial nerve, then sensory neurons in the thoracic ganglion, which in adult frogs contains no muscle spindle afferents, are made to innervate limb muscles. When this manipulation is performed in young tadpoles, the central axons of the rerouted afferents project into ventral laminae of the dorsal gray matter where they make synaptic contacts with limb motoneurons, something that thoracic sensory neurons never do in normal frogs. We find that this plasticity is lost as development proceeds. If the same manipulation is made at late tadpole stages, rerouted thoracic afferents do not form these ventral spinal projections. What factors underlie this loss of developmental potential?

One possibility was that mature sensory neurons lose their ability to innervate muscle spindles and arborize within ventral spinal laminae, even ones that are "committed" to being spindle afferents. We tested this idea by transplanting lumbosacral sensory ganglia, which do contain muscle afferents, to the brachial level in juvenile frogs, well after the period of developmental plasticity has ended. Peripherally, these transplanted ganglia formed stretch-sensitive endings in limb muscles and centrally they projected deeply into ventral laminae of the dorsal horn, just as normal spindle afferents do. Thus mature muscle afferents are still able to reform appropriate peripheral and central projections.

Alternatively, there may be some change either in the environment surrounding sensory neurons or in the neurons themselves that makes further phenotypic specification unlikely. For example, during this developmental period, white matter tracts in the spinal cord become refractory to further axon growth, and sensory neurons have stopped proliferating and are undergoing naturally occurring cell death. Current experiments are designed to discriminate between the effects of developmental age on the sensory neurons directly and on their environment by heterochronic transplantation of late stage thoracic ganglia into young tadpoles.

Supported by NSF to EF.

92.20

SYNAPSE FORMATION BETWEEN DORSAL ROOT GANGLION AFFERENTS AND SPINAL CORD MOTONEURONS *IN VITRO*. K. Sharma* and E. Frank. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh, Pittsburgh, PA 15261.

During development sensory neurons establish highly specific sets of synaptic connections with neurons in the spinal cord. For example, muscle spindle afferents make monosynaptic connections with motoneurons whereas cutaneous afferents do not, even though both cell types use glutamate as a transmitter. To begin to understand the mechanisms responsible for this specificity, we have sought to establish an *in vitro* system where the formation of specific connections can be studied in more detail than is possible *in vivo*.

Sensory and motor neurons from chick embryos are grown in a 3-compartment chamber system ("Campenot chamber") as originally adapted for sensory and spinal neuron culture by Nelson *et al* (1989). Motoneurons retrogradely labeled *in ovo* with dil are plated on a layer of E6 chick spinal astrocytes in the central compartment and small DRG explants are placed adjacent to the chamber walls in the two side compartments. An important modification of earlier methods is the use of low melting point agarose under the Teflon chamber walls and in the two side compartments to prevent bulk flow and cell migration between compartments.

Processes from sensory neurons begin to appear in the central compartment after 2 days and these neurites cover the entire compartment (2 mm wide) by 4 days. Early neurite growth in this culture system results in the development of synaptic connections within 5 days, a time at which retrogradely labelled motoneurons can still be identified. The extensive neurite growth also results in strong mono- and poly-synaptic connections with definitive motoneurons. By culturing different classes of sensory neurons in the 2 side chambers it should be possible to determine if these different classes have different synaptic affinities for motoneurons.

Supported by NIH NS24373

92.21

THE DISTRIBUTION OF AFFERENT AXONS AND THE SYNAPTIC FORMATION IN THE SINGLE AND COMBINED CULTURES OF POSTNATAL RAT CEREBRUM. T. SHIRAI*, T. SHIGA AND K. KITAO*, Dept. of Anat., Yamagata Univ. Sch. of Med., Yamagata 990-23, Japan.

We studied the pathway of afferent axons and the synaptic formation in the single and combined cultured cerebral explants to examine the role of afferent axons on the formation of synaptic organization. We cultured a slice of cerebrum alone and that of cerebrum with that of cerebrum, thalamus, cerebellum or spinal cord from rat at one day after birth in a CO_2 incubator for 21 days, and observed cerebral explants as follows: 1) We labeled cerebral afferent axons derived from neurons in the explants of another cerebrum, thalamus, cerebellum or spinal cord with DiI. These DiI labeled axons showed many varicosities and were distributed in the cerebral explants with several branchings. 2) We observed the cerebral explants with the electron microscope. Axo-somatic, axo-dendritic and axo-dendrospinous synapses were found in the single and combined cultures of newborn cerebrum. These results indicate that the cerebrum of newborn rat may form the synaptic organization of the developed cerebral cortex in the organotypic culture.

FORMATION AND SPECIFICITY OF SYNAPSES IV

93.1

SUBCELLULAR LOCALIZATION AND BIOCHEMICAL CHARACTERIZATION OF A NEUROMUSCULAR JUNCTION-SPECIFIC ANTIGEN. S. H. Astrow and W. J. Thompson. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Last year we reported the identification of a subsarcolemmal, innervation-dependent component of the rat neuromuscular junction using a monoclonal antibody designated 3G2 (mAb 3G2). We have now examined in greater detail the localization of the antigen recognized by mAb 3G2 at adult junctions and within developing muscle fibers. At adult junctions, mAb 3G2 immunoreactivity lies beneath the acetylcholine-rich synaptic gutters, but is also present in sarcoplasmic protrusions located between, or at the edges of, the synaptic gutters. Deeper within fibers, mAb 3G2 immunoreactivity surrounds sole plate nuclei and enters the Z-lines. Overall, the pattern is similar to that reported by Sealock *et al.* (*Synapse*, 3:315) for the distribution of desmin. However, mAb 3G2 immunoreactivity is different from that for desmin in several respects: mAb 3G2 labels perijunctional Z-lines only (whereas an anti-desmin mAb reacts with both junctional and extrajunctional Z-lines, as expected), the expression of desmin is not substantially altered in denervated muscle fibers, and pre-adsorption of anti-desmin mAbs with an intermediate filament-enriched preparation removes anti-desmin immunoreactivity, whereas a similar pre-adsorption of mAb 3G2 is ineffective. In neonatal muscle fibers, mAb 3G2 immunoreactivity is found at myotendinous junctions where it forms a reticular network surrounding developing myofibrils. Differential extraction of muscle proteins, followed by Western blotting suggest that mAb 3G2 immunoreactivity is associated with a highly insoluble fraction of neonatal muscle.

93.2

INFLUENCE OF BASIC FIBROBLAST GROWTH FACTOR ON ACETYLCHOLINE RECEPTORS IN CULTURED MUSCLE CELLS. Zhengshan Dai* and H.B.Peng. Dept. of Cell Biology and Anatomy and Curr. in Neurobiology, Univ. of North Carolina, Chapel Hill, NC 27599.

The acetylcholine receptors (AChRs) in cultured *Xenopus* muscle cells consist of a high-conductance type and a low-conductance type. The low-conductance channels undergo a remarkable decrease in burst duration during development (*Leonard et al., Science 226:55,'84; Rohrbough et al., J.Physiol. 425:245,'90*). Recently we showed that basic fibroblast growth factor (bFGF), when locally applied, can mimic the effect of nerve in inducing a postsynaptic-type development. In this study, we examined whether this growth factor can influence the kinetics of AChRs. Patch clamp method was employed to record single AChR channel currents elicited by 200nM ACh from cultured *Xenopus* muscle cells. In control cultures, we observed a 3-fold decrease in burst duration of low-conductance channels between the first and second day in culture. In contrast, cultures treated with 1μg/ml bFGF at an early time (stage 23) showed only a 1.5-fold decrease in burst duration during the same period. Histogram analyses showed that the low-conductance channels were composed of a fast and a slow component and the decrease in burst duration is due to a shift in the population from the slow to the fast. bFGF appeared to slow down this shift by prolonging the lifetime of the slow channels. In older cells (stage 40) that already expressed predominantly short channels, bFGF treatment had no effect on the burst duration. These data suggest that bFGF may enhance the stability of low-conductance AChRs with long burst duration. This effect seems to parallel the stabilization of junctional AChRs at the innervated endplate. Thus, bFGF, or a related polypeptide growth factor, may mediate this and other innervation-induced changes in the postsynaptic membrane. (Supported by NIH grant NS23583 and the Muscular Dystrophy Association)

93.3

REGULATION OF POSTNATAL CHANGES IN SUBUNIT COMPOSITION OF ACETYLCHOLINE RECEPTORS AT NEUROMUSCULAR JUNCTIONS OF NORMAL AND MED MICE. C. Gary Reiness and Peter M. Colowit*
Dept. of Biology, Pomona College, Claremont, CA 91711

To study whether muscle activity regulates subunit composition of ACh receptors (AChRs) at developing neuromuscular junctions (nmjs), we stained random cryostat sections of biceps brachii muscles from normal mice and mice with motor endplate disease (med) with an antiserum specific for γ (embryonic) subunits of AChR (Gu and Hall, Neuron, 1:117 (1988)) and with rhodaminated α -bungarotoxin to reveal all AChR clusters at nmjs.

In 3-6 d med or normal mice about half the nmjs stain for the γ subunit (84/173 med; 62/133 normal). By 10-12 d few nmjs contain the γ subunit (2/225 med; 0/157 normal). Thus AChRs at nmjs of both med and normal mice switch from embryonic to adult forms postnatally. Shortly after the onset of inactivity in the med mice (10-12d), there is a return of detectable γ subunits (15d, 12/112 nmjs; 18-20 d, 87/315 nmjs; >20 d, 26/122 nmjs). Since older med muscles are inactive but innervated, this finding shows that inactivity alone is sufficient to cause synthesis and insertion of embryonic AChRs. In normal mice there was also a transient reappearance of embryonic AChR at a small but significant fraction of nmjs at the end of the third postnatal week (15d, 0/77 nmjs; 18-20d, 19/504 nmjs; >20 d, 0/63 nmjs). We suggest that this may be caused by a brief period of inactivity in some fibers, endplates or regions of endplates in normal developing muscles.

93.4

AN ACETYLCHOLINE RECEPTOR INDUCING ACTIVITY INCREASES THE NUMBER OF NA^+ CHANNELS AND ACHRs IN CHICK CULTURED MYOTUBES AND MYOBLASTS. G. Corfas and G.D. Fischbach. Dept. of Neurobiology, Harvard Medical School, Boston, MA.

ARIA, an AChR inducing activity purified from chick brain, promotes an increase in synthesis and insertion of AChRs in cultured myotubes. It has been proposed that ARIA may play a role in the formation of the neuromuscular junction. To determine whether ARIA increases the expression of other proteins concentrated at the neuromuscular junction, we have analyzed the effect of ARIA on voltage-sensitive Na^+ channels (which *in vivo* are concentrated at the endplate) in cultured myotubes. ARIA treatments that increased the total number of AChRs (as measured by ^{125}I-α-bungarotoxin (BTX)) by a factor of 2.4 in multinucleated myotubes, also increased ^{3}H-saxitoxin binding by a factor of 2. The increase in saxitoxin binding was not accompanied by changes in the single channel properties (mean open-time, conductance and voltage-dependence of activation) as measured in cell-attached patches. The number of channels/patch was low and variable, so differences in the number of Na^+ channels were not obvious. However, changes in the number of Na^+ channels could be measured reliably by voltage-clamp in whole-cell recordings of mononucleated myoblasts. Therefore, we first documented that ARIA did increase the rate of insertion (synthesis) of AChRs in myoblasts. Increases in ^{125}I-BTX binding of 1.5 - 2 fold were reliably detected after 48hs of exposure to ARIA by γ counting and autoradiography. The effect could not be explained by changes in cell size. Whole-cell Na^+ currents were also increased by 1.5 fold after 48hs of ARIA treatment. No change in voltage dependence of I_{Na} was evident. We conclude that ARIA increases the number of Na^+ channels in myotubes and myoblasts, and that the increase is comparable to the increase in the number of AChRs.

93.5

TYROSINE PHOSPHORYLATION AT ACETYLCHOLINE RECEPTOR CLUSTERS INDUCED BY A VARIETY OF STIMULI IN CULTURED *XENOPUS* MUSCLE CELLS. L.P. Baker and H.B. Peng. Curr. in Neurobiology & Dept. of Cell Biology and Anatomy, Univ. of North Carolina, Chapel Hill, NC 27599.

Our recent work has demonstrated the effectiveness of basic fibroblast growth factor (bFGF)-coated beads in inducing acetylcholine receptor (AchR) clustering, in cultured *Xenopus* muscle cells (*Neuron 6:237, 1991*). A tyrphostin tyrosine kinase inhibitor blocks the formation of clusters by these beads, indicating the involvement of a specific receptor tyrosine kinase. Using two monoclonal antibodies against phosphotyrosine (PY20 and 4G10), we found by immunocytochemistry the distinct accumulation of phosphotyrosine at AchR clusters induced by a variety of stimuli, including nerve, beads, culture substrate ("hot spots"), and electric-field. External crosslinking of AchRs with biotinylated alpha-bungarotoxin followed by fluorescently conjugated avidin results in the formation of visible "microclusters" which do not exhibit this phosphotyrosine labeling. Using beads as temporally and spatially controllable stimuli, an early phosphotyrosine accumulation is observed in the absence of detectable rhodamine-alpha-bungarotoxin labeled AchRs at 10% of bead-muscle contacts within 15 minutes of bead application. AchR clustering becomes visible and colocalized with phosphotyrosine at 20% of bead-muscle contacts within 30 minutes. This colocalization increases in number as well as fluorescence intensity to reach a plateau level within 24 hours. These results suggest (1) AchRs in the diffuse or unclustered state are not tyrosine phosphorylated, (2) tyrosine phosphorylation is induced by the application of clustering stimuli and is a feature of AchR clusters in general, and (3) the accumulation of tyrosine phosphorylated protein(s) other than or in addition to the AchR may play a critical role in the initial formation of AchR clusters. (Supported by NIH grant NS23583 and the Muscular Dystrophy Association)

93.6

AGRIN BINDING TO NEURONS OF THE EMBRYONIC CHICK SPINAL CORD. M.A. Nastuk and J.R. Fallon, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Agrin plays a well-established role in the clustering of AChRs on cultured myotubes, and is thought to direct postsynaptic differentiation at neuromuscular junctions. Recent evidence indicates that the muscle cell surface agrin receptor is distinct from the AChR, and co-aggregates with it during agrin-induced clustering. It is not known whether agrin also serves to direct the differentiation of neuronal synapses. As a first step toward investigating this question, we have asked whether neurons express agrin binding sites. Dissociated embryonic (ED 9-10) spinal cord cells were incubated briefly with *Torpedo* agrin. The distribution of bound agrin was assessed with immunofluorescence microscopy. Neurons were identified based on their morphology and positive staining for synaptic vesicle protein. A subpopulation of the neurons were found to display agrin binding sites on their surfaces. The distribution of agrin binding sites is punctate, resembling that seen on myotubes. The majority of agrin binding is calcium dependent: staining is markedly reduced when extracellular calcium is chelated from the incubation solutions with EGTA. To learn whether the neuronal agrin binding sites can redistribute in response to prolonged agrin exposure, we incubated cultures with agrin for 14-16hr at 37°C. A subset of the stimulated neurons exhibit ligand-induced aggregation of agrin binding sites. These results suggest that a population of spinal cord neurons expresses an agrin receptor, and raise the possibility that agrin participates in directing synaptic differentation in the central nervous system. HD 23924, the ACS and the March of Dimes.

93.7

COMPLEX EXTRACELLULAR MATRIX INCREASES VESICLE DENSITY IN SYNAPTIC PROFILES OF CULTURED SYMPATHETIC NEURONS. M. Loegering*, D. Higgins†, and M.I. Johnson. Univ. of Ariz. Col. of Med., Tucson, AZ 85724 and SUNY†, Buffalo, NY 14222.

Extracellular matrix plays an important role in the development and regeneration of neuromuscular junctions. This study explores its role in the development of synaptic varicosities by autonomic neurons. Embryonic rat superior cervical ganglion neurons were dissociated, plated onto a polylysine substratum and maintained in serum-free media. Non-neuronal cells were eliminated by treatment with antimitotic agents. Control cultures remained on defined media; experimental cultures received 50 μg/ml of Matrigel (Collaborative Research, Inc.) in serum-free media. After three weeks in vitro the cultures were fixed with 2% gluteraldehyde and processed for electron microscopy. Varicosities photographed randomly at high power on the electron microscope were compared. Control boutons had 40.6 ± 6.2 vesicles per μm^2 (mean ± S.E.M.) vs 109.5 ± 16.5 vesicles per μm^2 in the Matrigel treated cultures ($p < 0.005$). Quantification of the size of the profiles indicates that control varicosities are larger than those treated with Matrigel, suggesting that one mechanism by which vesicle density is increased may be induction of vesicle formation from the nerve terminal membrane. Preliminary studies also indicate that there is a clustering of varicosities on and near the soma of neurons treated with Matrigel [supported by grants NIH-NS15070 (MIJ), and NSF-BNS8909373 (DH)].

94.1

Activity Dependent Changes In Neurotrophin Expression In Rat Hippocampal Slices. S.L. Patterson[1], L.M. Grover[1,2], M. Bothwell[1], and P.A. Schwartzkroin[1,3]. Dept. Physiology & Biophysics[1], Dept. Psychology[2] and Dept. Neurological Surgery[3], University of Washington, Seattle, WA 98195.

Hippocampal neurons are known to be a major CNS site of expression for the neurotrophins - nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophin 3 (NT3). However, relatively little is known about the factors modulating their expression. The expression of NGF mRNA in hippocampal neurons is dramatically enhanced by experimentally induced seizure in rats (Gall and Isackson, 1989) and it is reasonable to suppose that neurotrophin expression may also be modulated by other, more "physiological" activity. The hippocampal slice preparation offers unique advantages for studying the role of specific neuronal activity and neuromodulatory substances in the regulation of neurotrophin expression. We have established that appropriate patterns of neurotrophin expression are observed when *in situ* hybridization is performed upon hippocampal slices after several hours in the slice chamber. Experiments are currently in progress to determine if the induction of long term potentiation (LTP) in the CA1 pyramidal cell layer results in changes in neurotrophin levels. Preliminary results suggest that there are activity dependent changes in levels of neurotrophin expression in rat hippocampal slices.

94.2

LIMBIC SEIZURES INDUCE BIPHASIC INCREASES IN HIPPOCAMPAL NGF mRNA EXPRESSION. J.C. Lauterborn, P.J. Isackson, and C. M. Gall, Department of Anatomy & Neurobiology, University of California, Irvine, CA 92717.

Work in our laboratories has established that physiological activity can induce an increase in nerve growth factor (NGF) mRNA in rat hippocampal and neocortical neurons. In particular, following seizures NGF mRNA levels increase rapidly in hippocampus, and several hours later in neocortex. In the present study, in situ hybridization and S1 nuclease protection assays were used to provide a detailed time course of seizure-induced changes in hippocampal NGF mRNA expression.

Adult male Sprague-Dawley rats received a unilateral electrolytic lesion of the dentate gyrus hilus. Such hilus lesions (HLs) induce EEG seizure discharges in hippocampus and behavioral seizures which recur from 2 to 12 hrs post-HL. Rats used exhibited at least 2 stage 4 seizures. Perfusion-fixed tissue sections from HL and paired control rats were processed for in situ hybridization with a ^{35}S-cRNA to the sequence coding for rat NGF. Levels of NGF mRNA were found to increase in two distinct phases in the dentate gyrus granule cells. Hybridization to NGF mRNA increased rapidly to maximal levels, at 600-1300% control values, by 3-6 hrs post-HL. By 12 hrs post-HL, the density of NGF cRNA hybridization dropped to 58% of control levels. This was followed by a second increase which reached 500% of control values by 24 hrs. Thus, the second increase in NGF mRNA develops after the termination of seizures and coincides with NGF mRNA increases in neocortex. By 48-96 hrs post-HL, hybridization declined to control levels. S1 nuclease protection assays corroborated these findings. These data demonstrate that recurrent HL-seizures cause a biphasic increase in hippocampal NGF mRNA content. Moreover, the temporal relationship of these increases to seizure activity suggests the first phase is directly induced by epileptiform activity whereas the second may be indirectly induced by processes set in motion during the seizure episode. Supported by NS26748 & AG00538.

94.3

SEIZURES DECREASE NT3 mRNA LEVELS IN HIPPOCAMPAL AND CORTICAL NEURONS. C.M. Gall, R.T. Bonk, and P.J. Isackson. Dept. of Anatomy and Neurobiology, Univ. of Calif., Irvine, CA 92717.

Seizure activity stimulates rapid increases in the expression of mRNAs for NGF and BDNF in hippocampal and cortical neurons suggesting that activity-dependent regulation is an intrinsic property of the NGF-family of neurotrophic factors. In the present study, the influence of recurrent limbic seizures on neurotrophin 3 (NT3) mRNA expression in rat forebrain was examined using in situ hybridization and S1 nuclease protection analyses and compared to the effects on the NGF and BDNF mRNAs.

Experimental rats received a unilateral electrolytic lesion in dentate gyrus hilus. This treatment induces recurrent, bilateral EEG seizures in hippocampus and behavioral seizures from 2 to 12 hrs postlesion. Perfusion fixed tissue sections through forebrain of hilus lesion (HL) and paired control rats were processed for in situ hybridization with ^{35}S-labeled rat NT3 cRNA. In control rats, hybridiza-tion labeled neurons of hippocampal stratum granulosum and CA2 stratum pyramidale, caudal olfactory cortex, amygdala, and caudolateral neocortex. In HL rats, hybridization was slightly increased in stratum granulosum by 3 hrs postlesion but by 12-48 hrs post-HL had declined to ~10% control values in the granule cells and was markedly reduced in neocortex and amygdala. By 6 days post-HL, hybridization had increased in str. granulosum but remained below control values. In contrast to these effects, hybridization within CA2 stratum pyramidale was only slightly reduced at 12 and 24 hrs postlesion. S1 nuclease protection assays corroborate these findings. These data demonstrate that in contrast to NGF and BDNF, NT3 mRNA expression is reduced by seizure activity. Moreover, the marked differences in the pattern and direction of change in levels of mRNAs for NGF, BDNF, and NT3 in the dentate gyrus granule cells of HL rats provide evidence that these 3 mRNAs are differentially regulated by activity within individual forebrain neurons. Supported by NS26748 & AG00538.

94.4

NGF, bFGF and IGFs PROTECT RAT HIPPOCAMPAL AND HUMAN CORTICAL NEURONS AGAINST CALCIUM-MEDIATED HYPOGLYCEMIC DAMAGE. B. Cheng and M. P. Mattson, Sanders-Brown Center on Aging and Dept. of Anatomy & Neurobiology, Univ. of Kentucky, Lexington, KY 40536.

Neuronal growth factors have received considerable attention because of their potential for preventing or repairing brain injury, but the kinds of insults they might normally protect against and their cellular mechanisms of action are unclear. Central neurons are particularly vulnerable to hypoglycemia that occurs in ischemia or with insulin overdose. Glucose deprivation caused neuronal damage and death in human cerebral cortical and rat hippocampal cell cultures. The hypoglycemic damage was dependent upon calcium influx. Nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) (10 ng/ml) each prevented hypoglycemia-induced neuronal damage when administered before or immediately after glucose deprivation. NGF also afforded protection when administered up to 12 hours following the onset of hypoglycemia. Similar protective effects were afforded by insulin-like growth factors (IGF-1 and IGF-2; 100 ng/ml), while epidermal growth factor was ineffective. NGF and bFGF prevented the elevation in intraneuronal calcium levels that mediated hypoglycemic damage. The glutamate receptor antagonists APV and DGG reduced hypoglycemic neuronal damage, while the calcium channel blockers nifedipine and nimodipine were ineffective. We conclude that growth factors can stabilize neuronal calcium homeostasis and thereby protect against environmental insults. The data also indicate that NGF acts on a broad array of central neurons including non-cholinergic neurons. (Alzheimer's Association and ILSI support).

94.5

THE INCREASE OF HIPPOCAMPAL NGF mRNA ASSOCIATED WITH BICUCULLINE CONVULSION* IS ABOLISHED IN ADRENALECTOMIZED RATS. F.Y. Sun, E. Costa, M. Fabrazzo, and L. Mocchetti. FGIN and [1]Dept. of Anatomy and Cell Biology, Georgetown Univ., Sch. of Med., Washington, DC 20007.

It has been reported that NGF gene expression in the brain can be enhanced after seizure, a condition postulated to be associated with impaired GABAergic function, suggesting that GABA might regulate NGF gene expression. NGF biosynthesis also can be increased by adrenal steroids. We tested whether increase of NGF gene expression that follows behavioral seizure culd be the result of high blood steroid levels. Bicuculline (0.4 mg/kg, i.v.), a selective $GABA_A$ receptor antagonist, was used to induce seizure in sham-operated and adrenalectomized rats. Northern Blot hybridization analysis was used to measure the contents of NGF mRNA in the hippocampus. In sham-operated rats, bicuculline induced a two-three fold increase in NGF mRNA content one hour after the injection. This effect was time dependent since it was still present 6 hr but not 9 hr after the injection. Since bicuculline increases plasma corticosterone levels, we tested whether bicuculline could increase NGF mRNA in adrenolectomized rats. In these animals, although bicuculline could still induce seizures, it failed to change NGF mRNA content. These results suggest that steroids, released from the adrenal medulla during seizures, might be the stimulus responsible for the increase of hippocampal NGF mRNA content. It remains to be established whether the increase of NGF mRNA is due to an induction of gene transcription.

94.6

EPILEPTOGENIC KAINATE LESIONS OF HIPPOCAMPUS INDUCE THE EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR (NGFR). J.E. Franck, D.L. Roberts*, S. Patterson and M. Bothwell. Depts. of Neurological Surgery (J.E.F) and Physiology & Biophysics, Univ. of WA, Seattle, WA 98195.

Intraventricular kainic acid (KA) lesions hippocampal subfield CA3. Granule cells are not damaged and their mossy fibers remain in place in CA3 despite the loss of their targets; they do not form a substantial recurrent pathway into the dentate molecular layer. This lesion induces limbic seizures and is considered a model of temporal lobe epileptogenesis.

Hippocampal cell damage and seizures are associated with aberrant plasticity in remaining neurons. Trophic factors and their receptors may play a role in such plasticity. While NGFR is not expressed in intrinsic elements of normal adult hippocampus, seizures induce NGF in dentate granule cells. The present study examined whether NGFR is also induced following epileptogenic cell damage.

Subfield CA3 was lesioned bilaterally in adult rats by intraventricular KA (.5μg/μl saline/side) and the hippocampus was immunocytochemically stained for NGFR (monoclonal IgG 192) three days to one month later. Intense NGFR immunoreactivity was present in the region of lesioned CA3 normally innervated by dentate mossy fibers at all post-lesion latencies. Much staining appeared to be associated with glial processes. While granule cells were not immunoreactive, fibers with mossy terminal-like expansions also stained for NGFR.

Seizures induce the expression of NGF in dentate granule cells. The present finding - that NGFR is expressed in the lesioned terminal field of dentate mossy fibers - suggests that NGF-containing granule cells interact with elements in the neuropil which make NGFR. This interaction may maintain mossy fibers in place despite target loss or, in other models, may assist in redirecting mossy fiber axons to form recurrent collaterals.

Supported by NIH-NINDS grant NS25155 (J.E.F.)

94.7

NEUROTROPHIC FACTOR FAMILY IMMUNOREACTIVITY IS PRESENT IN DEVELOPING MAMMALIAN CEREBRAL CORTEX. K.L. Allendoerfer and C.J. Shatz, Dept. of Neurobiology, Stanford University, Stanford CA 94305

Messenger RNA for nerve growth factor (NGF), and for the related neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), is known to be present in the developing cerebral cortex of mammals. Recent work indicates that unique cell types within the developing cortex may be responsive to this family of molecules. The early-generated and transient subplate (SP) neurons, for example, express the low-affinity NGF receptor (l-NGFR), and may require NGF or a related molecule for their survival and differentiation. We have used a polyclonal antibody which recognizes NGF, BDNF, and NT-3 (kindly provided by Dr. K. Nikolics, Genentech, Inc.; Knusel et al. 1991, PNAS 88, p. 961) to determine localization of this family of molecules within the cerebral cortex of ferrets between P1, when l-NGFR expression in the SP is high, and P10, by which time it has disappeared. At P1, the SP neurons stain darkly with the anti-neurotrophin antibody; the underlying white matter, ventricular zone (VZ), and cortical plate (CP) do not stain. At P10, the SP neurons retain their staining, and in addition, the VZ and the pyramidal neurons of cortical layer 5 are intensely immunoreactive. Outside the cerebral wall, the septal neurons of the basal forebrain also stain at both these ages. Injections of [125I]-NGF into the cortex or ventricle label the septal neurons as early as P1, indicating that the antibody recognizes cells which retrogradely transport NGF. However, the SP, VZ, and layer 5 neurons do not take up or retrogradely transport [125I]-NGF, suggesting that they may be responsive to a different member of the family. All of the cortical and septal immunoreactivity can be blocked by incubating the sections with primary antibody plus excess NGF, indicating the specificity of the antibody for the NGF family. Thus, the pattern of staining suggests that diverse cell types within the developing cerebral cortex synthesize or respond to members of the neurotrophin family, and is consistent with the idea that these molecules may play an important role in the development of CNS structures. [Supported by EY02858, the Alzheimer's and Related Disorders Association (CJS), and NSO7158 (KLA)]

94.8

A BASIS FOR POSSIBLE AUTOCRINE AND SYNERGISTIC MECHANISMS UNDERLYING ESTROGEN AND NEUROTROPHIC FACTOR EFFECTS ON NEURONAL DEVELOPMENT AND SURVIVAL. R. C. Miranda[1], F. Sohrabji[1], R. B. Hochberg[2]*, N. J. MacLusky[3], and C. D. Toran-Allerand[1]. Dept. Anat. & Cell Biol.[1], Columbia Univ. P&S, NY, NY 10032, Dept. Ob.&Gyn.[2], Yale Univ. Sch. of Med., New Haven, CT 06510 and Div. Reprod. Sci.[3], Univ Toronto, Toronto, Canada, MG1L4.

Development and survival of CNS neurons are dependent on the expression of a variety of endogenous neurotrophic factors. In the developing rodent brain, several distinct groups of cells such as the neurons of the cortical subplate, septum, nuclei of the diagonal band, hippocampus and the large cholinergic neurons of the striatum and substantia innominata express the mRNAs and proteins for estrogen and nerve growth factor (NGF) receptors. Using non-isotopic in situ hybridization histochemistry, we have found that these same neuronal subsets also co-express the mRNAs for NGF and brain-derived neurotrophic factor (BDNF), suggesting the potential for developmentally-critical autocrine and synergistic mechanisms that may insure survival of these neurons prior to and concurrent with neural differentiation and innervation. In contrast, NGF and BDNF appeared differentially expressed in many other regions such as the developing hypothalamus and cortical layers II-V. These findings suggest a basis for a putative autocrine mechanism which may be critical not only for neuronal development and survival, but also for aging and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. [Supported by NIH grants HD08364 and AG08099 (DT-A); and CA3799 (RBH); and grants from AHAF; NSF BNS 87-00400 and Research Scientist Award MH00192 (DT-A)]

94.9

ESTROGEN (ER) AND NERVE GROWTH FACTOR (NGFR) RECEPTOR mRNA REGULATION IN PERIPHERAL NEURONAL TARGETS OF NGF. F. Sohrabji[1], R. C. Miranda[1], L.A.Greene[2] and C. D. Toran-Allerand[1]. Depts Anat. & Cell Biol.[1] and Path[2], Columbia Univ, P&S, New York, NY 10032.

Recent work in this laboratory has shown that ER and NGFR co-localize in several regions of the developing and adult rodent brain. We hypothesized that estrogen sensitivity may be a universal feature of neural NGF targets. The present experiments studied dorsal root ganglion (DRG) neurons and PC12 cells, prototypical peripheral targets of NGF not previously known to respond to estrogen. DRGs were obtained from proestrus and ovariectomized (ovx) adult female rats and hybridized with digoxigenin-labeled oligodeoxynucleotides for ER and NGFR mRNAs. NGFR and ER mRNAs appeared restricted to neurons. Although both large and small DRG neurons expressed high levels of ER mRNA, there were striking differences in the pattern of hybridization between the proestrus and ovx conditions. While hybridization intensity was heterogeneous during proestrus (high estrogen), estrogen deficiency (ovariectomy) resulted in a uniformly intense staining. In striking contrast, NGFR mRNA expression appeared upregulated by estrogen, with a dramatic reduction in staining intensity in the ovx female. PC12 cells cultured with/without NGF were also studied for ER mRNA expression. While a small proportion of NGF-deprived (undifferentiated) PC12 cells expressed ER mRNA, virtually all PC12 cells exposed to NGF exhibited intense ER mRNA hybridization signal. These findings suggest that estrogen sensitivity may be a property of central and peripheral NGF target neurons. Additionally, while co-localization of NGF and estrogen receptor systems suggest a possible interactive role for these substances, the present data suggest one mechanism for this interaction: that estrogen (DRG) or NGF (PC12) may influence neuronal responsiveness to the other ligand by regulating receptor expression for that ligand. [NIH grants HD08364 & AG08099 (DT-A); NS 16036 (LAG); NSF BNS 87-00400, AHAF and Research Scientist Award MH00192 (DT-A)]

94.10

INTERACTIONS OF ESTROGEN AND NERVE GROWTH FACTOR (NGF) ON ESTROGEN RECEPTOR mRNA EXPRESSION IN EXPLANTS OF THE SEPTUM/DIAGONAL BAND. C. D. Toran-Allerand, R. C. Miranda and F. Sohrabji, Dept. Anat. & Cell Biol., Columbia Univ. P&S, New York, NY 10032.

We have shown that estrogen binding sites co-localize with NGF receptor mRNA and/or protein in developing and adult rodent brain regions such as the septum, nuclei of the diagonal band, cerebral cortex, striatum and nucleus basalis. In order to determine whether or not NGF and estrogen may interact in neurons where their receptors co-localize, we studied their effects in septal explants of the newborn mouse, an important target of these ligands during development and in the adult. Preliminary studies with non-isotopic in situ hybridization, using digoxigenin-labelled oligodeoxynucleotides, demonstrated that both estrogen and NGF receptor mRNAs were expressed in cultured septal neurons after 21 days in vitro. Concurrent exposure to estrogen and NGF appeared to increase the number of cells expressing estrogen receptor mRNA and to enhance the intensity of the hybridization signal, as compared to either ligand added alone or not at all. Whether or not the observed response results from an enhancement of neuronal survival and/or an increase in gene expression is unclear at this time. However, this interaction may represent synergism of estrogen and peptide growth factors, as has been shown extra-neurally in estrogen target regions or, perhaps, "permissive" effects of NGF on estrogen action, a mechanism that may also underlie growth factor effects. [Supported by grants HD08364 and AG08099 (NIH); AHAF; NSF BNS 87-00400 and Research Scientist Award MH00192 (DT-A)].

94.11

LONG TERM SURVIVAL OF SEPTAL CHOLINERGIC NEURONS AFTER LESIONS THAT DEPLETE THE HIPPOCAMPUS OF CELLS PRODUCING NGF OR BDNF mRNA. M.V. Sofroniew, J.D. Cooper*,C.N.Svendsen*,P.Crossman*, N.Y. Ip, R.M.Lindsay, F.Zafra*, E. Castrén, H. Thoenen, D. Lindholm*, Dept. of Anatomy, University of Cambridge, UK; Regeneron Pharmaceuticals, Tarrytown, NY, USA; Dept. of Neurochem., Max-Planck for Psychiatry, Martinsried, FRG

Most medial septal cholinergic neurons die following proximal axotomy of their projection to the hippocampus (Hp) in adult rats; in contrast, these neurons do not die for up to 120 days after excitotoxic ablation of nearly all Hp neurons (Science 247:338). We have now studied growth factor mRNA production in the Hp remnant, and the viability of septal cholinergic neurons in rats with long survival times after unilateral ablation of Hp neurons with NMDA (9-15 injection sites). By 28 days after complete Hp lesions, mRNA for NGF and BDNF and NT-3 were reduced to virtually undetectable levels in the Hp remnant, and did not recover for over 365 days, the longest times studied. In the ipsilateral septum of animals with equivalent lesions, cholinergic neurons detected by ChAT or NGFr immunohistochemistry did not decline appreciably in number for up to 500 days, but atrophied in the form of shrinkage and loss of staining intensity. These findings indicate that long term production of NGF and BDNF is not maintained by non-neuronal cells after neuronal cell death in the Hp. The findings suggest that 1) NGF and BDNF are not required for long term survival of septal cholinergic neurons in adult animals but are involved in the maintanace of structural and chemical phenotype; and 2) retrograde cell death after axotomy may not be due solely to loss of target-derived neurotrophic support.

94.12

AGED RATS DOWN-REGULATE IMMUNOREACTIVE ChAT BUT NOT NGFr IN SEPTAL CHOLINERGIC NEURONS AFTER LOSS OF HIPPOCAMPAL TARGET NEURONS J.D. Cooper*, C.N. Svendsen*, S. J. Stevens*, K.J. Baker* and M.V. Sofroniew, SPON: Brain Research Association. Department of Anatomy, University of Cambridge, England.

In young adult rats, unilateral excitotoxic lesions that ablate the hippocampus (Hp) of nearly all neurons, and reduce both NGF and BDNF mRNAs to undetecable levels, lead to the atrophy but not death of afferent septal cholinergic neurons (Sofroniew et al. this volume). To investigate the effect of Hp ablation in aged animals, NMDA or vehicle was injected unilaterally into the Hp in 9 sites in rats either 4-6 or 24-30 months of age. After survival times of 50-110 days, NMDA lesions reduced the cross-sectional area of the Hp by 60-90%. In aged rats with Hp lesions, a significant decline of 30.6 $\pm$ 2.9% occured in the number of ChAT neurons in the septum ipsilateral to the Hp lesion as compared with the unlesioned side, and sham operated control animals. In contrast, the number of NGFr neurons in neighboring sections from the same portions of the septum showed no significant decline. In young adult rats with Hp lesions, no reduction in the number of ChAT or NGFr septal neurons was observed. Since nearly all NGFr-positive neurons in the normal septum are cholinergic, these findings suggest that the loss of ChAT staining observed in aged rats represents a down regulation of enzyme expression rather than cell death. We conclude that septal cholinergic neurons also do not die in response to loss of target neurons in aged rats, but that these neurons do respond with a down-regulation of chemical phenotype, and thus appear to react more severely to loss of trophic support than their counterparts in young adult animals.

94.13

NGF WITHDRAWAL RESULTS IN ATROPHY BUT NOT CELL DEATH OF MATURE CHOLINERGIC NEURONS IN LONG TERM CULTURES UNDER INVERTED COVERSLIPS C.N. Svendsen*, K. Staley*, E. D. Bird and M.V. Sofroniew. Dept. of Anatomy, University of Cambridge, U.K.

The effects of inverting coverslips after plating (Brewer and Cotman; Brain Res. 494: 65), in the presence or absence of nerve growth factor (NGF) were studied in dissociated basal forebrain septal or dorsal root ganglion (DRG) neurons in cell culture. Cultures of the septal region at medium cell density showed significantly enhanced neuronal survival, reduced glial proliferation and increased long term neuronal survival under inverted coverslips, independent of the presence or absence of NGF. In contrast, DRG neurons were unaffected by inversion of coverslips and did not survive in the absence of NGF. We also studied the effects of NGF and NGF-withdrawal on cholinergic neurons identified by staining for AChE or ChAT in septal cultures grown under inverted coverslips. NGF (100 ng/ml) given at the time of plating or at 7, 9 or 14 days in vitro (div) increased the number of cholinergic neurons seen at 28 div. NGF antibodies (1%, kindly donated by Dr. E. Johnson) added to cultures at 14 div which had been exposed to NGF since plating caused, by 28 div, a significant 42% reduction in cholinergic cell size and a reduction in fiber staining, but no reduction in cholinergic cell number, as compared with sister wells not exposed to NGF antibodies. These findings suggest that protection against cell death provided by inverting the coverslips is independent of NGF and that NGF may regulate cholinergic and structural phenotype without being required for the survival of mature cholinergic neurons.

94.14

Multiple signals mediate hypoglossal motoneuron response to axonal injury. R.C. Hayes, R.G. Wiley, D. Greeson*, L. Moix*, R. Brady and D.M. Armstrong Fidia Georgetown Institute for the Neurosciences, Georgetown University, Washington, D.C. 20007, DVAMC, Nashville, TN, 37212.

Unilateral transection or crushing of the rat hypoglossal (XII) nerve results in an increase in NGF receptor (NGFr) mRNA levels in hypoglossal motoneurons ipsilateral to the lesion in a manner that paralleled the appearance of the protein as assayed by immunocytochemical techniques. Although the duration of the response following nerve crush was shorter lasting than that observed following transection of the nerve, the intensity of the response was greater in the crush paradigm versus that observed following transection. This robust response was most obvious four days following crushing of the nerve and appeared to be the result of an increased recruitment of motoneurons expressing NGFr mRNA. These data indicate that nerve crush is more effective in eliciting NGFr mRNA expression than transection of the nerve.

In a second study, we sought to determine if these motoneuron responses were due to loss of a factor supplied to perikarya by axonal transport. Axonal transport was blocked by local application of vincristine (VCr) to one hypoglossal nerve. After seven days, VCr produced ipsilateral loss of immunohistochemical staining for choline acetyltransferase (ChAT) without inducing NGFr staining. Tongue injections of WGA prior to sacrifice confirmed ipsilateral block of axonal transport. Combining VCr with nerve transection gave the same results. Mild trauma to the nerve without drug application elicited NGFr staining with little effect on ChAT and WGA transport. We interpret these findings to indicate that there are two separate signals responsible for the changes seen after axotomy. Failure of axonal transport causes loss of ChAT but not induction of NGFr. Whereas, local nerve damage results in the induction of NGFr.

94.15

192-IgG-SAPORIN IMMUNOTOXIN: PRELIMINARY BIOCHEMICAL CHARACTERIZATIONS. A. A. Book*[1], R. G. Wiley[3], and J. B. Schweitzer[1,2]. Department of Anatomy and Neurobiology[1] & Department of Pathology[2], University of Tennessee, Memphis 38163 & Lab of Experimental Neurology[3], DVAMC, Nashville, TN 37212.

A monoclonal antibody directed against the rat nerve growth factor receptor, 192-IgG, is specifically and bilaterally accumulated by the cholinergic basal forebrain (CBF) system following intraventricular injection. We have synthesized an immunotoxin composed of 192-IgG chemically linked to the ribosome inactivating protein saporin and then administered it intraventricularly to rats in an effort to produce a model of CBF deficit. 192-IgG was disulfide coupled to saporin (Inland) using SPDP. The 192-IgG-saporin conjugate was purified by ion exchange and affinity column chromatography using CM-52 (Whatman) and MAPS-II (Bio-Rad). 192-IgG-saporin was injected into the right lateral ventricles (8 μg total dose) of 8 adult rats, whereas normal saline was injected into 3 control rats. At two weeks survival, immunohistochemistry (IHC) showed a modest decrease in choline acetyltransferase (ChAT)-positive neurons in the medial septal nucleus and a major loss of ChAT-positive neurons in the nucleus of the diagonal band of Broca and in the nucleus basalis magnocellularis. The breakdown of 192-IgG-saporin over time, as shown by SDS-PAGE, may explain the sparing of some CBF neurons. Radioenzymatic ChAT assays of brain homogenates showed significant decreases in ChAT activity in the terminal fields of CBF neurons that correlated with the IHC impression of neuronal loss in animals given 192-IgG-saporin. ChAT activity in CBF terminal fields of experimental animals (expressed as percentages of the average control values) was as follows: cortex (32% ± 12%); hippocampus (48.3% ± 16.5%); olfactory bulbs (42.7% ± 16.6%). This immunotoxin appears to be quite effective in vivo against CBF neurons as judged by both biochemical and IHC methods. (Supported by NINDS NS01230.)

94.16

IMMUNOLESIONING: SELECTIVE DESTRUCTION OF CENTRAL AND PERIPHERAL NEURONS *IN VIVO* USING AN IMMUNOTOXIN TO THE RAT NGF RECEPTOR. R.G. Wiley, T.N. Oeltmann* & D.A. Lappi*. Lab of Experimental Neurology, DVAMC, Nashville, TN 37212 & The Whittier Institute, La Jolla, CA.

In the present study, we sought to determine if an immunotoxin to the nerve growth factor receptor (NGFr), could selectively destroy neurons bearing that receptor *in vivo*. 192 IgG, a mouse monoclonal antibody to the rat NGFr, was disulfide coupled to one of 3 ribosome inactivating proteins (saporin, ricin A chain, barley RIP) using SPDP. 192 IgG-saporin was far more active *in vivo* than either of the other constructs. Systemic injection of 4 μg of 192 IgG-saporin destroyed > 1/3 of neurons in the superior cervical ganglia (SCG), and 20 μg destroyed >95%. 350-fold larger doses of 192 IgG-ricin A or 192 IgG-barley RIP only destroyed scattered SCG neurons after systemic injections. Intraventricular injection of 4 μg of 192 IgG-saporin destroyed all of the neurons in the medial septum and nucleus of the diagonal band that stain for choline acetyltransferase (ChAT) and NGFr. Scattered ChAT- and NGFr-positive neurons survived in the nucleus basalis and numerous cerebellar Purkinje cells were destroyed after these doses of 192 IgG-saporin. Intraventricular injections of 40-fold larger doses of 192 IgG-ricin A or 192 IgG-barley RIP produced no detectable loss of ChAT+/NGFr+ cells. We conclude that 192 IgG-saporin has extraordinary potential as a selective lesioning tool. (Supported by DVAMC, Nashville, TN.)

94.17

CHRONIC RECOMBINANT HUMAN NGF TREATMENT INCREASES HIPPOCAMPAL CHOLINERGIC FUNCTION FOLLOWING FIMBRIAL LESIONS: COMPARISON BETWEEN ENDOGENOUS ACETYLCHOLINE AND NEWLY-SYNTHESIZED [$^2H_4$]ACETYLCHOLINE POOLS. E. O Junard, F. Hefti, D. J. Jenden[1], and P.A. Lapchak. Andrus Gerontology Ctr., USC & [1]Dept. Pharmacol., UCLA, Los Angeles, CA.

Recent studies have shown that chronic NGF treatment attenuates lesion-induced measures of hippocampal cholinergic function when measuring labeled pools of acetylcholine (ACh). The present study determined the effects of chronic recombinant human NGF (rhNGF) treatment (1 μg every second day for 3 weeks) on hippocampal ACh synthesis and release in vitro following unilateral partial fimbrial lesions. In this study we compared the effects of rhNGF treatment on both endogenous and labeled pools of ACh. Partial fimbrial transections reduced the hippocampal content of endogenous ACh by 47% and basal and evoked release of endogenous ACh by 32% and 46%, respectively. These lesions also reduced the synthesis of [$^2H_4$]ACh from [$^2H_4$]choline by 56%, and basal and evoked release of [$^2H_4$]ACh by 41% and 63%, respectively. Chronic rhNGF treatment elevated the hippocampal content of endogenous ACh in tissue slices of the lesioned side to 111% of control levels and the synthesis of [$^2H_4$]ACh to 119% of control levels. Chronic rhNGF treatment also increased basal and evoked release of endogenous ACh by 21% and 31%, respectively, and [$^2H_4$]ACh release by 29% and 51%, respectively. This study shows that rhNGF treatment effectively increases presynaptic hippocampal cholinergic function and that the effects of rhNGF treatment are reflected by endogenous and newly-synthesized ACh pools (Supported by HFSP).

94.18

CHRONIC NGF PRETREATMENT POTENTIATES KAINIC ACID- INDUCED INCREASES OF c-fos mRNA IN THE HIPPOCAMPAL FORMATION OF THE ADULT RAT. F. Hefti, G. Tocco[1], G. Pasinetti, M. Dugich-Djordjevic and P.A. Lapchak. Andrus Gerontology Ctr. and [1]Dept. Biol. Sci, USC, Los Angeles, CA, 90089.

Recent studies have shown that systemic administration of kainic acid (KA) results in increased levels of growth factor mRNA (BDNF, NGF) and immediate early genes (c-fos mRNA) in the hippocampal formation. Furthermore, the KA-induced elevation of c-fos mRNA appears to precede the loss of hippocampal cells. The present study determined whether pretreatment with recombinant human NGF (rhNGF) affects KA-induced elevations of c-fos mRNA in brain. Adult rats were administered either rhNGF (1 μg, every second day for 3 weeks) or cytochrome c (cc) prior to treatment with KA (10 mg/kg, s.c.) or saline. Animals were sacrificed 4 hours following KA-induced seizure activity or saline injections. In situ hybridization was carried out on brain sections using a riboprobe specific for c-fos mRNA. In cc-treated control animals, KA administration increased the levels of c-fos mRNA hybridization in the hippocampal formation compared to saline-injected controls. However, in animals chronically pretreated with rhNGF, the KA-induced increase of c-fos mRNA hybridization was significantly potentiated (by 40-50%) compared to that observed in KA injected cc-treated animals. In the absence of KA treatment, rhNGF treatment did not affect the expression of c-fos mRNA in the hippocampal formation. The present study shows that NGF pretreatment modulates excitatory amino acid responses in the hippocampal formation (Supported by HFSP).

94.19

RECOMBINANT HUMAN NGF AMELIORATE MEMORY IMPAIRMENT IN RATS WITH BASAL FOREBRAIN LESIONS. K.Kato, E.Fujiwara*, M.Iwane* and M.Suno. Biol. and Biotech. Res. Lab., R & D Div. Takeda Chem. Ind. Ltd., Yodogawa-ku, Osaka 532, Japan.

Bilateral lesions of basal forebrain (BF) neurons in rats were produced by heating at 70°C for 60 sec with a radiofrequency lesion generator. Two weeks after the operation, learning and/or memory was examined in an one-trial passive avoidance task and Morris' water maze task done for 3 consecutive days. The rat with BF lesions showed severe learning and/or memory impairment in both tasks, and the activity of choline acetyltransferase (CAT) was reduced by 10% in the frontal region of the cerebral cortex. When recombinant human nerve growth factor (rhNGF; 5 μg/2.5 μl) was injected into the right lateral ventricle of the rat with BF lesions every 3-4 days for 2 weeks, it was found that the learning impairment was ameliorated in Morris' water maze task: on the 2nd day of training the median escape latency in NGF-treated rats was 60 sec which was significantly shorter than that (120 sec) in control rats with BF lesions. In animals receiving NGF injections, CAT activity in the frontal cortex was 30% higher than that in control animals with BF lesions, and similar increases in CAT activity were observed in the parietal, occipital and temporal cortex in which CAT activity had not been affected by BF lesions. These results suggest that rhNGF activates the cholinergic system in the cerebral cortex in rats with BF lesions, and this may result in the improvement of the learning behavior which is impaired in these animals.

94.20

THE ROLE OF NGF AND INSULAR CORTEX GRAFTS IN THE RECOVERY OF INHIBITORY AVOIDANCE LEARNING. M. Escobar, M. Hiriart, A.L. Piña and F. Bermúdez-Rattoni. Instituto de Fisiología Celular, UNAM, P.O. Box 70-600, México, D.F.

We recently showed that fetal brain grafts produced a significant recovery in the ability of insular cortex (IC) lesioned rats to learn a conditioned taste aversion (CTA) task. The expression of AChE and the acetylcholine release are correlated with the recovery in the ability to learn CTA. In the present work we evaluate the role of NGF in the recovery of learning induced by IC grafts in the inhibitory avoidance task. Four groups of male rats received bilateral lesions in the IC and one group remained as unoperated control. Ten days later two of the lesioned groups received homotopic fetal IC grafts combinated with gelfoam implants impregnated of NGF (IC-NGF grafts) (20 ug/ml) or vehicle , respectively. Another group, received only homotopic fetal IC grafts, and the last group remained as a lesioned control. All groups were trained 15 days later in the IA paradigm. We found that IC-NGF grafts promoted recovery in the ability to learn the IA task assessed by the response latency. The remaining groups did not show any recovery. The Histochemical analysis showed the presence of NGF receptors and many AChE labeled fibres and somas in the IC-NGF grafts. These results support that IC-NGF grafts play an important role in the behavioral recovery of IC lesioned rats.

GROWTH FACTORS AND TROPHIC AGENTS II

95.1

RAT BRAIN CONTAINS MESSENGER RNAs FOR EGF-RECEPTOR AND TGF-ALPHA BUT NOT FOR EGF. M. Kaser*, J. Lakshmanan, K. Huff and D.A. Fisher*. Harbor-UCLA Medical Center, Perinatal Research Laboratories, Torrance, CA 90509.

Evidence for the existence of EGF-receptor immunoreactivity (Neurosci. 22:5279, 1987), TGF-alpha immunoreactivity (Soc. Neurosci. 413.25, 1990) and preproTGF-alpha mRNA (J. Neurosci. 8:1901, 1988) in rat brain have been previously reported. However, evidence for the presence of EGF immunoreactivity in rat brain remains controversial (Neurosci. Lett. 63:290, 1986 and J. Biol. Chem. 264:10447, 1989). In the present investigation, we examined brain tissue for the presence of mRNAs for EGF-receptor, TGF-alpha and EGF using specific probes for rat. Poly (A)+ RNA isolated from adult rat whole brain and brain regions (cortex, cerebellum, midbrain and brain stem) were examined by Northern blot analysis. Whole brain contained 10, 5.6 and 4.8 kb bands when probed with the EGF-receptor probe and TGF-alpha mRNA was characterized by a 4.5 kb. No band was visible on the autoradiogram when probed with EGF cDNA. Both EGF-receptor and TGF-alpha mRNAs also were detectable in cerebral cortex, cerebellum and brain stem. Our results indicate that the TGF-alpha precursor or processed TGF-alpha could be the ligand for the EGF-receptor in brain.

95.2

FURTHER CHARACTERIZATION OF A MUSCLE-DERIVED NEURITE-PROMOTING FACTOR. T.H. Oh, G.J. Markelonis, S.L. Hobbs* and S.J. Jeong. Dept Anatomy, Univ Maryland Sch Med., Baltimore, MD 21201

Survival of spinal motor neurons during embryonic development requires muscle-derived trophic factors. We have isolated a non-laminin protein from muscle which stimulates neurite outgrowth from embryonic neurons in culture. The protein also promotes the survival and growth of embryonic spinal motor neurons in culture. This trophic protein has been purified by gel-filtration chromatography followed by ion-exchange chromatography and analytical gel electrophoresis. The biologically active protein migrates as a doublet with molecular weights of 53 and 57 kDa on sodium dodecyl sulfate-gel electrophoresis. Both gel bands were excised, electro-eluted and tested for their biological activity on cultured DRG neurons. The 53 kDa protein showed virtually all of the trophic effects as compared to all other electroeluted proteins. The 53 kDa protein was digested with trypsin and the resulting peptides were fractionated by size using reverse-phase HPLC. Five peptides, ranging in length from 7 to 21 amino acid residues, were sequenced. Comparison of these peptide sequences to those stored in Gen Bank indicated no direct sequence identity to any other sequenced growth factor or muscle protein. Thus, the 53 kDa protein appears to be an undescribed neurite-promoting factor. (Supported by NIH grant NS 15013).

95.3

IDENTIFICATION OF A COLLAGEN POTENTIATED NEURITE PROMOTING FACTORS. D.E. Coyle. Department of Anesthesia, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267-0531.

Non-neural cells have been shown to release factors that influence the morphology, survival and neurite outgrowth of peripheral and central neural cells. From this work, various neurotrophic factors (NTFs) and neurite promoting factors (NPFs) have been reported. This laboratory has initially identified two NPFs isolated from the C6 glial cell line. The C6 cells are capable of producing NPF activity under growth conditions in the presence and absence of serum. The NPFs appear to be distinct from nerve growth factor, since their activities are not blocked by the presence of anti-NGF antibodies sufficient to inhibit neurite outgrowth caused by 50 ug/ml NGF. These factors are capable of inducing neurite outgrowth from PC12 cells on various substratum (plastic, laminin, collagen), but are potentiated only by the presence of collagen. Neurite promoting activity is found in the molecular weight (MW) ranges of 30-50 kDa and 454-1,000 kDa. The low MW NPF is more resistant to acid and basic pHs and is only produced when C6 cells are grown in the presence of serum. The high MW NPF is produced under both growth conditions, however, serum-free growth conditions result in a 3.5 fold increased production of this factor. Experiments, using dissociative and denaturation conditions, results in the loss of neurite promoting activity of the high MW NPF. The high MW NPF is not bound by Q-Sepharose (pH 8.0), but, does exhibit heparin binding. Based on MW, the low NPF appears to be similar to glial derived nexin reported to be produced by C6 cells,however, the high MW NPF appears to be a novel NPF whose neurite promoting activity is potentiated by collagen and is distinct from NGF and nexin.

95.4

MDF, A MUSCLE FACTOR, PRODUCES PARTIAL MOTOR RECOVERY IN 6-HYDROXYDOPAMINE LESIONED RATS BY INCREASING TYROSINE HYDROXYLASE ACTIVITY AND CATECHOL LEVELS. B.K. Jin, J.S. Schneider, X.Y. Du and L. Iacovitti, Dept. of Neurology, Hahnemann University, Philadelphia, Pa. 19102

We have previously demonstrated that treatment with a 10,000-fold purified muscle-derived factor termed MDF will partially restore motor symmetry in rats with a 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal system. Concomitantly, the number of fibers immunoreactive for the catecholamine enzyme, tyrosine hydroxylase (TH) is dramatically elevated in the MDF-treated striatum. In the present study, we examined whether these changes 1) are accompanied by biochemical changes in TH and its catechol products and 2) are long-lasting effects of MDF. 6-OHDA was unilaterally injected into the rat substantia nigra, producing partial denervation of the ipsilateral striatum. Lesions were verified by monitoring rotational behavior after amphetamine challenge (5mg/kg). Four weeks after the lesion, either partially-purified MDF or PBS+BSA (control) was infused into the damaged striatum via an indwelling canula connected to a miniosmotic pump. Test solutions were delivered 1ul/hr for 6-14 days. At sacrifice, residual pump solutions were removed and bioassayed in culture to verify their biological activity. Following MDF treatment, rats showed a significant reduction in rotational asymmetry (48%-93%) and TH activity in the damaged striatum was 5-fold higher than in PBS controls. Moreover, endogenous dopa, undetectable in intact or PBS-treated striatum, reached 13.6 µg/gm protein in the lesioned, MDF-treated striatum. Similarly, when dopamine was measured in the lesioned striatum and expressed as a % of the intact striatum, levels were increased from 5% in PBS controls to 21% in MDF-treated rats. In some rats, MDF treatment was terminated after partial motor recovery. In this case, improved motor function gradually declined, but did not fully revert to pretreatment levels for 60 days. These findings suggest that MDF increases the biochemical function of lesion spared dopamine neurons by inducing TH activity. It is likely that the consequent increase in endogenous dopa and dopamine levels contributes to the long-lasting motor recovery of lesioned rats treated with MDF. Supported by NIH NS2404-04 and the Ben Franklin Foundation.

95.5

TAURINE SYNTHESIS IN DEVELOPING CAT AND MOUSE CEREBELLUM *IN VIVO* AND *IN VITRO*. E. Trenkner, A. Gargano, P. Scala* and J.A. Sturman*, Inst. for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Taurine is essential for the development and survival of mammalian cells, in particular the cerebellum of the cat and weaver mutant mouse. Taurine biosynthesis is high in rodents but low in cats and primates which need taurine in their diet. Cysteinesulfinic acid decarboxylase (CSAD) is the rate limiting step. This study measured CSAD activity in the developing cerebellum of normal and weaver mice *in vivo* and *in vitro* as well as from kittens raised on taurine controlled diets. We found that in mouse CSAD activity is developmentally regulated. Activity declines until P14, the time when the blood brain barrier is established, and then increases to mature levels. There were no significant differences in activity between normal and weaver cerebellum indicating that the taurine deficiency in weaver cerebellum is not due to reduced CSAD activity. CSAD activity was higher *in vitro* than in tissue and activity did not increase during the culture period. In P5 and P7 cat cerebellum, on the other hand, CSAD activity was low under normal and taurine deficient conditions. However, when cultured for 3 days, there was a significant induction of activity, the amount depending on developmental stage and diet. After 3 days in culture, activity induced was greater in cerebellum from normal kittens than from taurine-deficient kittens. These results suggest that taurine-synthesis in cat is suppressed *in vivo*, or might be regulated in a yet unknown way.

Supported by NSF grant BNS-8910218 and NIH grant HD-16634.

95.6

THE MECHANISMS UNDERLYING TAURINE'S CAPACITY TO ENHANCE AXONAL FORMATION AND POLYAMINE SYNTHESIS IN VITRO. C.L. Lu, G. Yorke and F.J. Rolsen. Dept. of Anatomical Sciences and Neurobiology, Univ. of Louisville, Sch. of Med., Louisville, KY 40292.

The amino acid taurine is distributed widely in mammalian tissues and functions as a neurotransmitter. We and others have reported taurine's neurotrophic action in vitro. Although its underlying mechanism is unknown, studies suggest that it may be calcium dependent. To examine this possibility, we employed the Neuro-2a neuroblastomal cell model. With this cell line, we have shown previously that taurine increased neurite formation and ornithine decarboxylase (ODC) activity in a dose dependent manner. We now report a plateau of maximal neuritogenesis after 24 hr exposure to 4.0 mM taurine. In contrast, a 3-fold increase in ODC activities was obtained after 5 hr exposure to 1.0 mM taurine. Media supplemented with a minimum of 3.0 mM extracellular calcium prevented taurine's capacity to enhance neurite formation; whereas, increased calcium did not block taurine-induced ODC activity. Since gangliosides enhance neuritogenesis in a variety of cell culture models in a species specific, Ca^{++}-dependent fashion, we examined the interaction of taurine and gangliosides. The capacity of the 4 major gangliosides (GM1, GD1a, GD1b and GT1b) found in bovine brain to potentiate taurine was examined morphologically and biochemically. GD1a, GD1b and GT1b (150 ug/ml) enhanced taurine-mediated (1.0 mM) neurite development. These gangliosides also potentiated the effect of a subthreshold level of taurine (0.1 mM) on ODC activity. In Neuro-2a cells, it appears that neurite formation and polyamine synthesis are parallel but uncoupled events. These studies suggest that taurine potentiates neurite formation through a calcium dependent mechanism. In contrast, taurine's action on ODC activity appears calcium independent. Studies are in progress to demonstrate further the relationship between calcium and taurine's neuritogenic activity. Supported by NIH NS24524.

95.7

REGULATION OF ENKEPHALIN EXPRESSION IN DEVELOPING CHROMAFFIN CELLS AND MATURE SYMPATHETIC NEURONS BY NEURAL AND HORMONAL FACTORS. P. D. Henion and S. C. Landis, Dept of Neurosciences, Case Western Reserve Univ. Cleveland OH. 44106.

Most adrenergic cells derived from the sympathoadrenal lineage of the neural crest contain one or more neuropeptides. Although a great deal is known about the development and regulation of adrenergic properties in these cells, the regulation of peptidergic properties is poorly understood. We have examined the possible role of environmental cues in the regulation of leucine enkephalin (L-Enk) expression in developing chromaffin cells and mature sympathetic neurons. Highly enriched cultures of embryonic and neonatal chromaffin cells exposed to glucocorticoids (GLC) exhibited significantly increased L-Enk levels over controls. Cultures of neonatal chromaffin cells were exposed to high potassium to mimic the depolarizing effect of acetylcholine and to a L-Enk analog, DADLE, to mimic the effects of L-Enk which is present in a majority of cholinergic preganglionic fibers in the adrenal medulla. Depolarization stimulated L-Enk expression to levels over 4 times greater than control cultures while addition of DADLE completely blocked the depolarization-induced increase in L-Enk levels. These results, taken together with the developmental time course and pattern of L-Enk expression in chromaffin cells *in vivo* and the initiation of GLC synthesis and onset of impulse activity, suggest that GLC and preganglionic innervation regulate L-Enk expression in chromaffin cells during development. We then examined the effects of altered GLC levels and denervation on L-Enk expression in sympathetic neurons *in vivo*. Lowered GLC levels in adrenalectomized rats resulted in a dramatic decrease in ganglionic L-Enk content. Conversely, denervation of the superior cervical ganglion, like denervation of the adrenal gland, resulted in a large increase in the number of L-Enk immunoreactive cells. These results demonstrate that similar mechanisms regulate L-Enk expression in different sympathoadrenal derivatives and implicate these mechanisms in the autonomic response to stress.

95.8

EFFECTS OF D-FACTOR ON SUBSTANCE P EXPRESSION IN SYMPATHETIC NEURONS. J.A. Kessler & M. Freidin. Depts. Neurology and Neuroscience, Albert Einstein Col. Med., Bronx, NY 10461

Cytokines, a class of immunoregulators, have been shown to alter the behavior of a variety of cell types. A link between the immune and nervous systems has been demonstrated by the localization and effects of these molecules on nervous tissue. For example the cytokine, D-factor (also known as leukemia inhibitory factor), has been shown to increase cholinergic traits in cultures of rat sympathetic superior cervical ganglion (SCG) neurons (Science,1989). We have found that D-factor also regulates the expression of the neuropeptide substance P (SP) in SCG neurons. Pure neuronal cultures of the SCG do not express detectable SP peptide or preprotachykinin (PPT) mRNA. However, treatment with recombinant human D-factor (rhDF) stimulates SP expression dramatically. Further, SCG neurons grown in the presence of ganglion nonneuronal cells show a 25-fold increase in SP peptide over basal levels in response to rhDF treatment. This modulation of SP levels was also found under serum-free culture conditions in both pure neuronal and cocultures. Treatment with the glucocorticoid dexamethasone, which blocks the SP-stimulating activity of another cytokine (IL-1β) in cocultures, failed to inhibit the effects of rhDF in neuronal cultures grown with or without ganglion nonneuronal cells. By contrast, potassium-induced depolarization decreased the increase in PPT mRNA seen after rhDF exposure. In addition, rhDF decreases the levels of norepinephrine and neuropeptide Y slightly. In conclusion, the roles of the cytokine, D-factor, must be expanded to include neuronal differentiating properties as well as an immune system modulation.

95.9

REGULATION OF THE EXPRESSION OF RECEPTORS FOR NEUROTRANSMITTERS IN CULTURED SYMPATHETIC NEURONS. W.H. Ludlam*, A.B.Johnson, and J.A. Kessler. Depts. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Regulation of muscarinic and peptidergic receptor expression was studied in cultured sympathetic neurons of the neonatal rat superior cervical ganglion (SCG). Leukemia Inhibitory Factor (LIF), previously shown to stimulate levels of choline acetyltransferase activity and of substance P, was used to effect regulation of receptor expression. Exposure of SCG cultures to LIF (5 ng/ml) for 10-12 days resulted in a 75% reduction in muscarinic receptors as measured by whole cell binding of N-methyl-^{3}H-scopolamine. This effect, as shown by Scatchard analysis, was due to a reduction in total receptor number (B_{max}) rather than a decrease in receptor affinity (K_d). Concentrations as low as 1 ng/ml reduced receptor expression with a dose of 10 ng/ml having near maximal effectiveness. Reduction in receptor binding could be detected after four days of treatment with an 85% reduction after 16 days. In contrast to the decrease in muscarinic binding, Substance P receptor expression was up-regulated 75-100% in sister cultures as measured by whole cell binding of ^{125}I-labeled Tyr^8 Substance P (5 ng LIF/ml, 14-16 days tx). LIF, previously shown to be released by some populations of non-neuronal cells, could therefore play a role in the coordination of transmitter and receptor expression in the developing nervous system.

95.10

ACTH/MSH PEPTIDES ACCELERATE MUSCLE MATURATION AND FUNCTIONAL RECOVERY FOLLOWING TRAUMA OF THE DEVELOPING NERVOUS SYSTEM. L.A. Zuccarelli and F.L. Strand. Department of Biology, Center for Neural Science, New York University, New York, New York 10003.

ACTH 4-10 and α-MSH positively affect development and regeneration of nerve [1]. Here we investigate their effects on muscle maturation and motor function. 2 d old rats are subjected to sciatic nerve crush and peptide treatment (10 μg/kg/48h/10d). Walking, climbing and placing tests are performed on d 15 & 21. Pups climb a vertical mesh wall; foot failures compared to total steps are determined. Walking ability is rated on a 3 to 0 scale. In placing tests, pups are suspended by forelimbs on a platform; using their hindlimbs, they mount the platform. The time to place both hindlimbs is recorded. On d 7, 15, & 21, the extensor digitorum longus muscle is removed and incubated in 1% tetrazolium chloride solution; formazan is extracted from the muscle with acetone and analyzed at 435 nm to determine absorbance. Absorbance/dry muscle weight, as a measure of oxidation, is calculated. Total protein content is determined by the BCA method. ANOVA was used for statistical purposes.

Results show oxidation for the peptide-treated groups is significantly lower ($p<.05$) than the saline groups at d 7 & 15 indicating a change of muscle fiber type from slow oxidative to fast glycolytic. By d 21, no differences are observed. Preliminary results from protein content and behavioral tests show a trend toward peptide-prevention of protein loss and acceleration of functional recovery.[1] Strand et al., Prog. in Neurobio: 33, 1989.

95.11

NEUROTROPHIC ACTIVITY OF THE AMINO TERMINAL REGION OF ALBUMIN. S. Dostaler*, †P.M. Richardson, T.G. Flynn* and R.J. Riopelle. Queen's University, Kingston, Canada K7L 3N6 and †McGill University, Montreal, Canada H3G 1A4.

Neurite-promoting activity for embryonic sensory neurons has been detected in explants of tumours of patients with Von Recklinghausen Neurofibromatosis (NF-1). A small peptide factor (E-2) was purified from neurofibrosarcomas of three patients with NF-1 by extraction in the presence of protease inhibitors followed by reverse phase HPLC.

The addition of E-2 to cultures of isolated embryonic chick DRG neurons in defined medium promoted neurite outgrowth twice that of controls and 25-30% of the response observed with saturating concentrations of NGF.

Amino acid and peptide sequence analyses of the factor revealed strict homology with the amino terminal region of human serum albumin.

Synthetic derivatives reproduced the trophic effect of the tumour-derived material with the response by sensory neurons saturating at 50 pM.

Extraction and RPHPLC on C-18 and C-1 of normal human serum did not yield UV (214 nm) absorbing material with chromatographic behaviour resembling E-2.

Albumin exerts a trophic effect on neurons in culture that is mediated by a fragment of its primary sequence.

Supported by MRC Canada and the NCE Program in Neural Regeneration and Functional Recovery.

95.12

EPENDYMIN PROMOTES NEURITE GROWTH IN NEUROBLASTOMA CELL CULTURES. V.E. Shashoua, P. Nolan and B. Milinazzo. Ralph Lowell Labs, McLean Hospital, Harvard Medical School, Belmont, MA 02178

Ependymin is a brain extracellular glycoprotein that has been implicated in synaptic changes associated with the consolidation step of long term memory formation and neuronal regeneration. [Cell Molec. Neurobiol. 5:183-207 (1985); J. Physiol. Paris 83:232-239 (1989)]. Neuroblastoma (NB2a/d1) cells in culture were found to express ependymin mRNA and synthesize and release the protein into the media. Increased ependymin synthesis and its mRNA expression occurred after stimulation of neurite growth by dibutyryl cAMP (dbcAMP).

Ependymin appears to act as a trophic factor in the absence of dbcAMP. Additions of 1-10nM levels of ependymin to growth media containing fetal calf serum were found to enhance neurite "sprouting" and "extension" in culture. Dose-response and time-course studies were carried out. Enhanced sprouting (80%) and neurite extension (95%) occurred after 24 hrs in culture. The results suggest that one of the possible functions of ependymin may be as a trophic factor. Investigations of primary CNS cultures are in progress. This research was supported by an NIH grant No. NS25748 from NINCDS.

95.13

QUINUCLIDINYL BENZILATE INDUCES ACETYLCHOLINESTERASE POSITIVE FIBER INCREASES WITHIN ADULT RAT HIPPOCAMPUS. V. R. Healc and C. W. Harley. Dept. of Psychology and Basic Sciences, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9.

Prolonged blockade of nicotine receptors induces sprouting of axon collaterals at the neuromuscular junction (Holland, R.L. and Brown, M.C. Science, 207, 649-651, 1980). The present study looked at the role of muscarinic receptors in the control of collateral sprouting in the adult hippocampus. Quinuclidinyl benzilate (QNB; 40 $\mu g/2\mu l$), an irreversible muscarinic antagonist was injected unilaterally into the hippocampus of female Sprague Dawley rats. Beta hydroxy cyclodextrin (36%) served as a vehicle. Vehicle injections were made in the opposite hemisphere. Subjects were sacrificed 1, 4, 14 or 21 days after treatment. Brain sections (40 μm) were reacted for acetylcholinesterase (AChE). Increased AChE staining in the area of the injection site was visible at the 4 day interval in the QNB treated hippocampi. No increase in AChE staining was seen at the 4 day interval in vehicle treated hippocampi. Brains examined after a 1 day interval showed increased staining limited to the needle track in both vehicle and drug injected hemispheres which appeared to be a function of blood accumulation. At the 14 day interval staining increases were still present at the QNB injection site but were reduced compared to the 4 day interval. An increase in AChE staining was no longer evident at the QNB injection site at the 21 day interval.

These data suggest that irreversible blockade of muscarinic receptors can induce collateral sprouting of AChE reactive fibers in the hippocampus. We hypothesize that sprouting is rapidly induced and retraction occurs following renewed receptor activation.

95.14

THE ACTH (4-9) ANALOG, ORG 2766, BINDS PREFERENTIALLY TO NEURONS OF DORSAL ROOT GANGLION (DRG) AND SPINAL CORD (SC) CULTURES. F. Van Huizen, H.L.A. Philipsen* and J.A.D.M. Tonnaer. CNS Pharmacology R&D Labs, Organon Intl. BV., PO Box 20, 5340 BH Oss, The Netherlands.

Functional recovery after sciatic nerve crush is accelerated in ORG2766 treated rats. Furthermore, in a clinical study, ORG2766 has been shown to prevent the neurotoxic side-effects of the anti-tumour agent cisplatin.

In order to elucidate its neurotrophic mechanism of action, we studied the presence of binding sites for ORG2766 on in vitro cultured DRG and SC cells with biotinylated ORG2766 (b-ORG2766). The avidin-peroxidase-DAB technique revealed displacable b-ORG2766 binding especially on neuron-like cells with round somata and fine processes. Labeling with neurofilament (NF) antibody indicated that such b-ORG2766 binding cells were NF positive.

In primary cultures of DRG cells, we started to look for functional consequences of ORG2766 stimulation. The effect of ORG2766 on neurite outgrowth parameters in 1-day-old DRG cultures was compared with those of NGF. With NGF (100 ng/ml) a marked effect was seen on the number of neurite bearing neurons, neurite length and branching. With ORG2766 such clear effects were not found, although preliminary data indicated that some parameters, e.g. branching, might be responsive to ORG2766.

These data suggest that binding sites for ORG2766 are present on neurons in dorsal root ganglion and spinal cord cultures; these sites might be involved in mediating a neurotrophic effect on these cells.

95.15

NEUROTROPHIC PROPERTIES OF ORG 2766 IN NERVE CRUSH MODEL MAY DEPEND ON THE TYPE OF NEURAL INJURY AND SPECIFIC SENSORIMOTOR FUNCTIONS TESTED. J.A.D.M. Tonnaer, G.J.P.T. Schuijers*, H.A. Van Diepen* and A.M.L. Van Delft*. CNS Pharmacology R&D Labs, Organon Intl. B.V., 5340 BH Oss, The Netherlands.

The neurotrophic properties of the ACTH(4-9) analog Org 2766 were studied in rats after sciatic nerve crush. Org 2766 increased the density of myelinated axons reinnervating the denervated nerve, and facilitated recovery of sensorimotor function as measured by foot withdrawal after thermal stimulation of the footsole. These facilitating effects were only seen if the nerve was lesioned using forceps with grooved jaws and not with cross-hatched jaws, suggesting that the type of neural injury influences the trophic response to Org 2766. Functional recovery of the nerve was found to be dependent on the particular variable assessed. Different recovery rates were found when foot withdrawal was tested after thermal or electric-al stimulation. Recovery of toe spreading did not correlate with recovery of sensorimotor functioning. The possibility that Org 2766 preferentially affects specific sensorimotor aspects of nerve regeneration is being studied.

95.16

THE EFFECT OF INSULIN-LIKE GROWTH FACTOR-I ON THE NEUROMUSCULAR JUNCTION IN ADULT RAT SKELETAL MUSCLE. E. Yu*, K. Callison, J.M. Roberts-Lewis, and P. Grebow*. Cephalon, Inc., West Chester, PA 19380

Insulin-like growth factor-I (IGF-I) is considered to be a potential therapeutic agent that would stimulate peripheral nerve regeneration and sprouting. The goal of this study was to determine the ability of IGF-I to increase two indices of motor nerve terminal sprouting and function. Two groups of six normal adult female Sprague-Dawley rats were given daily subcutaneous injections of IGF-I (50 ug/kg) or vehicle for 14 days. The injections were applied directly over the gluteus muscle of the right leg, carefully avoiding the underlying tissue. The contralateral gluteus muscles were not treated. The day following the last dose, gluteus muscles from both legs were prepared for histochemical evaluation of neuromuscular endplate size or autoradiographic analysis of I-125 alpha-bungarotoxin binding to nicotinic acetylcholine receptors. The endplate size of the IGF-I treated gluteus muscle was found to be significantly greater than in vehicle-treated muscle. In addition, the endplate size in the IGF-I treated contralateral gluteus muscle was significantly greater than in the vehicle treated contralateral muscle. Acetylcholine receptor binding was also significantly increased in the IGF-I treated muscle. These results indicate that administration of IGF-I to normal adult rats increases both the size of the endplate and density of acetylcholine receptor binding at the neuromuscular junction, responses which are consistent with previous reports of enhanced sprouting in response to IGF-I treatment. Taken together, these results indicate that IGF-I may have therapeutic benefits in certain neuromuscular disease states.

95.17

EFFECTS OF TESTOSTERONE ON LEVELS OF RIBOSOMAL RNA (rRNA) IN HYPOGLOSSAL NEURONS FOLLOWING NERVE INJURY. W.H.A.Yu[1], R.B.Gibbs[2] and D.W.Pfaff[2]. [1]Dept. of Cell Biol. & Anat. Sci., City Univ. of NY Med. Sch., NY, NY 10031, and [2]Lab. of Neurobiol. & Behavior, Rockefeller Univ., NY, NY 10021.

Testosterone (T) has been shown to accelerate axonal regeneration and reduce neuronal cell loss in motor nuclei of cranial nerves following nerve injury. Mechanisms by which T exerts neurotrophic effects are not yet known. In the present study, effects of T on relative levels of rRNA in axotomized (AX) hypoglossal neurons (HNs) were examined. Young adult female rats underwent unilateral transection of the hypoglossal nerve under anesthesia. Following axotomy, one-half of the animals received s.c. implants of T while remaining animals received sham implants as controls. At 1h, 1d, 7d and 14d after surgery, animals were perfused with 4% paraformaldehyde in PBS. In situ hybridization and radioautographic procedures were performed on Paraplast embedded sections using a ^{3}H-cRNA probe. Somal area, number of grains/cell (G/C) and grains/unit area (G/A) were measured for all HNs in which nucleoli could be identified. T produced a significant increase in cell size and in relative levels of rRNA as measured by G/C and G/A. T also produced a greater increase in rRNA in AX HNs within 1h after axotomy than was observed in controls. These data are consistent with the hypothesis that the ability for T to accelerate axonal regeneration is related to an acceleration and amplification of rRNA accumulation following injury.

95.18

EXPRESSION OF IGF BINDING PROTEIN 5 IN SPECIFIC REGIONS OF THE RAT. FETAL AND ADULT NEURAL AND NEUROENDOCRINE SYSTEMS. B. Green*, R. Streck, T. Wood, P. Rotwein, and J.E. Pintar. Dept. Anat and Cell Biol., Columbia P&S, NY, NY 10032 and Dept. Metabolism, Washington U., St. Louis, MO. 63110.

Insulin-like growth factors (IGFs) are mitogenic peptides that circulate throughout the body in the adult and are synthesized during early stages of mammalian embryogenesis. A variety of indirect evidence has suggested that members of the IGF system, especially IGF-1, function as trophic molecules in the brain. The activity of IGFs is, in part, mediated by members of a family of at least six IGF binding proteins (IGFBPs). One newly cloned IGFBP, provisionally termed here IGFBP-5, was initially identified as an IGFBP that dramatically increases in abundance during muscle differentiation in vitro (JBC 264:13810). We have here used in situ hybridization to demonstrate that IGFBP5 is expressed not only in the fetal rat muscle progenitors but also in discrete locations of the developing and adult nervous and neuroendocrine systems. Further, the expression pattern is distinct from that of IGFBP2. For example, in the adult animal, BP5, but not BP2, is expressed in the anterior lobe of the pituitary gland. Further, the first demonstration of biochemical heterogeneity of posterior lobe pituicytes is shown by the distinct expression patterns for BP2 and BP5 in this tissue. In the embryo, BP5 expression in the notochord and floor plate of the neural tube is present at least as early as E11; at this stage, expression is also noted in at least some muscle progenitors located in the dermomyotome. BP5 continues to be expressed in the neural tube through E18 in regions related to the ependymal zone including the basal plate and possibly in distinct neuronal clusters that are presently being evaluated in detail. Further, the olfactory bulb at E18 expresses high levels of BP5, which suggests that BP5 may modulate effects of IGF-1 locally produced in this region. Taken together, these data are consistent with distinct roles for IGFBP-5 in different regions of the nervous system. Supported by NS21970.

95.19

A THIOL COMPOUND, 2-MERCAPTOETHANOL, PROMOTES SURVIVAL OF CULTURED CORTICAL, HIPPOCAMPAL AND OLFACTORY EPITHELIAL NEURONS. R.J. Grill and S.K. Pixley. Dept. of Anat. and Cell Biol., Univ. of Cincinnati, Cincinnati, OH 45267.

Olfactory receptor neurons (ORNs), dissociated from newborn, Spraque-Dawley rat olfactory mucosal tissues, were maintained in primary monolayer cultures for 4 days. The survival-promoting effect of 2-mercaptoethanol (2-ME) was seen previously in both serum-containing and serum-free medium. A role for thiol compounds in this neuroprotective phenomenon was confirmed when another thiol, 2-mercaptoethylamine (2-MEA) exerted a similar survival-enhancing effect.

The olfactory neurons are not unique in showing enhancement of survival in response to a thiol compound. Cortical neurons in both serum-containing and serum-free medium showed greater survival with 2-ME. Hippocampal neurons in serum-free medium were also dependent on 2-ME. These data show that several types of neurons respond to a novel class of neuron survival-enhancing factors, thiol compounds. {Supported by Amer. Paralysis Assoc. Grant PB1-8803-1, and NIH PO1-DC00347-06}.

95.20

THIOL-DEPENDENT SURVIVAL AND NEURITE OUTGROWTH OF SEPTAL NEURONS IN CULTURE. Y. Arakawa, K. Isahara and S. Tachibana. Tsukuba Res. Labs., Eisai Co., Tsukuba-shi 300-26, Japan.

Neurons in the CNS are highly susceptible to peroxide radicals generated, e.g., after ischemia, and which cause peroxidation of membrane phospholipids and perturbation of Ca^{2+} homeostasis, finally leading to cell death. A question arose as to whether these mechanisms are also involved in cell death in culture. To test this, we estimated the protective effects of thiol compounds. Fetal rat septal neurons cultured in Dulbecco's Modified Eagle Medium (D-MEM) which does not contain systeine died rapidly within 24 h, while the addition of cysteine to the medium remarkably improved the survival (EC_{50}, 40μM). This result agrees with our previous observation that Leibovitz L-15 medium which contains 1mM cysteine is superior to other culture media such as D-MEM. We have also discovered previously the neurite-promoting activity of phospholipids for septal neurons (Y. Arakawa et al., *J. Neurochem., in press*). Now we have found that this activity is also dependent on cysteine, but requires higher concentrations (more than 0.25mM). Other thiol compounds, such as glutathione and coenzyme A, also showed these survival and neurite-promoting effects. These results suggest that thiol compounds are essential for survival, probably protecting cells from toxic peroxides, and may activate the enzymes involved in neurite outgrowth.

95.21

ESTABLISHMENT OF MESENCEPHALIC GLIAL CELL LINES USING AN INDUCIBLE EXPRESSION SYSTEM: NEUROTROPHIC EFFECTS ON DOPAMINERGIC NEURONS. J. Engele and M.C. Bohn, Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

Our previous studies have shown that medium conditioned (CM) by mixed embryonic mesencephalic glia contains a factor that supports the survival and differentiation of dopaminergic (DA) neurons. To obtain sufficient material for identifying this factor(s), clonal cell lines were established using an inducible expression system. E14.5 rat mesencephalic glia were replated following mitogenic stimulation with aFGF. These were co-transfected with two DNA constructs: theSV40 T-antigen under control of the lac operon and a lac repressor/VP16 herpes activator fusion protein under control of the actin promoter[1].

As expected, addition of the galactose derivative, IPTG, results in a dose-dependent inhibition of cell proliferation. Several lines resembles type II astrocytes morphologically and are immunoreactive for A2B5, a marker for O2A progenitors, as well as the astrocytic marker, GFAP. Moreover, adding CM from these cell lines to serum-free low-density cultures of E14.5 mesencephalic neurons increases the number of tyrosine hydroxylase-immunoreactive neurons 2-4 fold.

This study has established immortalized mesencephalic astroglial cell lines using an inducible oncogene. These lines produce neurotrophic activity for DA neurons and, thus, provide a useful source for purification of this factor.

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[1]generously provided by M. Labow and A. Levine, Princeton Univ.

95.22

PHARMACOLOGICAL MANIPULATION OF A N. ACCUMBENS (NAcc) DERIVED TROPHIC FACTOR AND IT'S EFFECT ON MESENCEPHALIC CULTURE GROWTH. L.R. Ptak*, E.S. Lo, D.H. Lin*, C.M. Buhrfiend, and P.M. Carvey. Dept. Neurology, Rush U. Chicago, IL

We have previously shown that a striatal-derived neurotrophic factor is present in the brain and that it's production can be manipulated both pharmacologically and by selective lesioning. Using rostral mesencephalic tegmentum (RMT) cultures, we examined whether or not a similar factor was present in the NAcc of rats chronically treated with haloperidol (HAL), levodopa (LD), or amphetamine (AMP). Extracts from these animals were incubated with E-13.5 RMT cultures and number of viable cells and neurite extension was scored after 40 hours. HAL treated NAcc extracts increased while LD and AMP extracts decreased the number of viable cells as well as process length relative to cultures incubated with saline treated NAcc extracts. These data suggest that a NAcc derived trophic factor is present in the adult rat and that pharmacological manipulations may either increase (DA antagonists) or decrease (DA agonists) the expression of this soluble trophic substance. This factor may be involved in the treatment of schizophrenia or it's etiology.

95.23

EFFECTS OF CNS DRUGS ON THE PRODUCTION OF A STRIATAL-DERIVED NEUROTROPHIC FACTOR (SdNTF).
E.S. Lo, L.R. Ptak*, C.M. Buhrfiend, D.H. Lin*, H.L. Klawans*, D.K. Sierens*, and P.M. Carvey. Dept. Neurology, Rush U., Chicago, IL.

We have demonstrated that striatal, but not cerebellar extracts, exert trophic effects on cultured rostral mesencephalic tegmentum (RMT) cells. We have demonstrated that 6-OHDA lesions, Parkinsons disease (PD), as well as chronic haloperidol (HAL) treatment increases the production of SdNTF whereas chronic treatment with the DA agonists levodopa or amphetamine, reduces the production of the SdNTF. Kainic acid lesions of the striatum as well as normal aging reduce the production of the SdNTF. Chronic HAL treatment has also been shown to increase synaptic density in the striatum suggesting that the alterations in DA neuron growth seen in culture may occur in vivo as well. These data suggest that the SdNTF exists in the normal adult striatum and participates in the maintenance of the structural integrity of the nigrostriatal pathway. The inverse relationship between DA and SdNTF production suggests a homeostatic regulatory mechanism involving compensatory structural changes in response to alterations in DA following chronic drug treatment.

95.24

THE TOPOGRAPHICAL DISTRIBUTION OF GM1 ON NEURO-2A NEUROBLASTOMA CELLS USING CHOLERA TOXIN-B-COLLOIDAL GOLD.
I.H. Fentie and F.J. Roisen. Dept. of Anatomical Sciences and Neurobiology, Sch. of Med., Univ. of Louisville, Louisville, KY 40292.

Exposure of murine Neuro-2a neuroblastoma cells to exogenous ganglioside GM1 increases neuritogenesis *in vitro*. Our previous studies of the surface distribution of GM1 on these cells employed FITC-labeled cholera toxin-B. The label appeared uniformly distributed along the perikaryal and neuritic surfaces. The present ultrastructural study employed cholera toxin-B bound to colloidal gold and examined the GM1 distribution in living cells. The rate of internalization was examined with cells grown in media for 24 hr prior to exposure to cholera toxin-B-gold for 2, 5, 15 or 60 min and fixation. Substantial quantities of GM1 were internalized rapidly; within 2 min gold label was observed in vesicles. Internalized label increased with the duration of cholera toxin exposure, while the surface labelling remained fairly uniform. To determine the possible microtubule (MT) involvement in regulating the distribution of GM1, MT altering agents (Taxol for stabilization and Colcemid for disassembly) were employed. Quantitative analyses of label on the perikaryal and neuritic surfaces, as well as internally, were performed with computer-assisted measurement of perikaryal and neuritic perimeters. In all cases, more label was found on neuritic surfaces than on the perikarya. Greater amounts of label were found after Taxol treatment compared to untreated controls. The controls were more heavily labeled than cells treated with Colcemid. These studies demonstrate that during neuritogenesis the surface distribution of GM1 undergoes specific changes. Furthermore, microtubules appear to play a key role in anchoring and regulating the distribution of GM1. Supported by NIH grant NS24524.

95.25

DEVELOPMENTALLY PROGRAMMED CHANGES IN ENDOGENOUS GANGLIOSIDE LEVELS REGULATE NERVE GROWTH FACTOR-MEDIATED RESPONSES.
D.F. Chen and F.J. Roisen. Dept. of Anatomical Sciences and Neurobiology, Univ. of Louisville, Sch. of Med., Louisville, KY 40292.

Gangliosides are membrane bound glycosphingolipids, whose expression is under strict control during neuronal development. We and others have shown that affinity purified antibodies against the ganglioside GM1 block the actions of NGF. Our previous experiments have demonstrated that NGF-mediated neuritogenesis was enhanced when chick embryonic dorsal root ganglia (DRG) were exposed to exogenous GM1. The potentiation by GM1 occurred only during periods when NGF function was submaximal. To probe the effects of GM1 during *in vivo* neuronal development, two channel flow cytometry was employed to label GM1 and other ganglioside species. Cholera toxin-B-FITC was used to label GM1 and anti-A2B5 conjugated with phycoerythrin for other gangliosides. NGF-mandatory and permissive neurons were separated partially by 3 hr differential adhesion. We demonstrated that fluorescent intensity of GM1 but not other complex gangliosides mirrored neuronal responsivity to NGF in DRG. NGF-mandatory neurons exhibited 2x greater fluorescent intensity of GM1 labeling than NGF-permissive neurons. A small subpopulation of GM1-positive neurons also were labeled with anti-A2B5. Moreover, two neuronal populations could be distinguished on the basis of cell size. Large neurons were more differentiated, while small neurons were, perhaps, progenitor cells. The large neurons exhibited higher GM1 reactivity than small neurons. The GM1 levels in large but not small neurons mirrored NGF responsivity. Our results strongly suggest that changes in endogenous GM1 levels modulate neuronal responsivity to NGF. Futhermore, endogenous levels of gangliosides may also regulate developmentally programmed changes in sensitivity to neurotrophic factors. Supported by NIH NS24524.

NEURONAL DEATH I

96.1

TRANSPALNTED SYMPATHETIC NEURONS FROM OLD RAT AND MOUSE SURVIVE WELL AND OUTLIVE THE ORIGINAL DONOR.
J. Suhonen*, J. Koistinaho and A. Hervonen. Lab. or Gerontology, University of Tampere Medical School, PO Box 607, SF-33101 Tampere, Finland.

Sympathetic neurons (SN) have been widely used as model for neuronal aging. Young and adult adrenergic neurons are also viable as transplants in different locations. Transplantation technique provides a means for studying the effects of different mileus on neuronal degeneration and survival. Here we report on the survival of aged SN as autotransplants and allotransplants in the submandibular gland, adrenal cortex and in oculo. The survival of transplants from young and old donors was compared using catecholamine histofluorescence, immunohistohemistry of tyrosine hydroxylase and electronmicroscopical techniques.

The survival of the old SN is sitedependent. Best survival was observed after allotransplantation in the mouse submandibular gland known to contain high concentration of NGF. A majority of the SN of both young and old transplant survived up to three moths, while most neurons degenerated in control sites (other salivary glands, kidney, muscle). Adrenal cortex also provided a favourable micromileu which maintained a large population of the old SN.

To study, wether SN can outlive the original host, transplants from 36 months old rats were placed in the submandibular gland and in oculo of three months old recipient. In both locations, a population of SN survived additional 12 months and eventually also longer. The histochemical parameters and electron microscopy suggest that these neurons were functionally active.

We conclude that a population of aged SN maintain enough plasticity and regenerative potential to survive as transplants, even longer than the maximum life span of the strain.

96.2

EVIDENCE FOR A TROPHIC INTERACTION BETWEEN ENTORHINAL CORTEX AFFERENTS AND CELLS OF THE HIPPOCAMPUS.
D.C. Snyder, J. Cronin, and C.F. Ide. Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

Using exo utero surgery techniques, we have previously identified the primordium of the developing entorhinal cortex, and shown that prenatal lesioning of this primordium causes a loss of innervation to the CA1 region of the hippocampus in the mouse at birth. No other morphological changes were evident at this time. In this study, we have again lesioned the entorhinal cortex primordium at embryonic day (E) 16. At birth, the animals were fostered and allowed to develop to postnatal day (P) 21-34. Histologically, the animals showed a loss of granule cells in the superior blade of the dentate gyrus, as well as a loss of mossy cells from the hilus and pyramidal cells in CA3. Cell death was seen only in hippocampal regions adjacent to the lesioned cortex. In areas away from the lesion, no damage was seen among these cell types. In addition, no sprouting of granule cell axon collaterals occurred along the entire length of the hippocampus. In vitro intracellular and extracellular recordings were done on hippocampal slices prepared from experimental and naive control animals. Responses to hilar stimulation were comparable, but experimentals showed diminished responses to perforant path stimulation. Thus, entorhinal cortex afferent innervation of the hippocampus provides a trans-synaptic trophic influence on developing hippocampal cell types during postnatal development.

96.3

INTRARETINAL NEUROTROPHIC FACTORS PREVENT GANGLION CELL DEATH *IN VITRO*. R.Linden, R.G.de Araujo*[1] & R.A.Pires*, Instituto de Biofisica da UFRJ, Rio de Janeiro and [1]Instituto de Biologia da UFF, Niterói, Brazil.

Developmental neuron death among retinal ganglion cells (RGC) depends on competitive interactions within the retina. The present experiments were designed to test for the release of intraretinal trophic factors. RGC were backfilled with HRP injected into the superior colliculus of newborn rats. Then monolayers of the dissociated retinae were cultured in medium previously conditioned during 4-7 days by aggregates or explants of retinal cells from either rat pups or chick embryos. The conditioned media significantly increased the survival of RGC at 2-3 days after plating, when compared with control medium. The effect was dose-dependent. Medium conditioned by explants of rat cerebral cortex had no effect. The activity was not dialysable and was abolished by heating at 56°C for 30 minutes. Survival of RGC within the explants was negligible after 4 days *in vitro*. The results indicate that retinal cells, either intrinsic neurons or glia, release one or more proteinaceous trophic factors with an effect on the survival of developing ganglion cells. These molecules may underlie dendritic competition for survival during embryogenesis.

(Supported by CNPq, FINEP, FAPERJ)

96.4

MULTIPLE PATHWAYS FOR DEGENERATION OF DIFFERENTIATING PC12 CELLS CAUSED BY ACUTE WITHDRAWAL OF NERVE GROWTH FACTOR. S.Tanaka*, T.Koike, K.Kaji* and A.Takashima, Department of Natural Science, Saga Medical School, Nabeshima, Saga 84001, Tokyo Metropolitan Institute for Gerontology, Tokyo 113, Mitsubishi-Kasei Institute of Life Sciences, Tokyo 194, JAPAN.

Degenerative processes of PC12 cells caused by nerve growth factor (NGF) deprivation were characterized by morphological and biochemical criteria including measurements of cellular ATP content and release of the cytoplasmic enzyme, lactate dehydrogenase (LDH), into medium. PC12 cells treated with NGF (50ng/ml) for 10-14 days underwent massive degeneration characterized by disintegration of neurites, decrease in the ATP content to less than 50% , which occurred in conjunction with increased release of LDH into medium (25-35% of the total activity by 25h after deprivation). About 40% of the LDH release was suppressed by treating the cells with cycloheximide (0.3μM) or cordycepin (1-10μM), indicating that acitve cell death occurs only partially. Neurite dilation was largely prevented by treating the cells with the protease inhibitor leupeptin (100μM) or other inhibitors. In the presence of leupeptin, about 60-70% of the LDH release was blocked by cycloheximide. These degenerative processes were completely prevented by chronic depolarization with high potassium (>35mM), CPT-cAMP (>0.1mM), acidic and basic FGF (10ng/ml), but other growth factors including EGF, PDGF,TGF or cholinergic agonists were ineffective. These results demonstrate the presence of transcription -dependent and -independent mechanisms for degeneration of differentiating PC12 cells caused by acute withdrawal of NGF.

96.5

CHEMOTACTIC FACTORS ATTRACTING MACROPHAGES ARE PRODUCED BY DEGENERATING NEURONS IN THE DEVELOPING BRAIN. C.E. Milligan, T.J. Cunningham and P.Levitt. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

The specific mechanisms involved in the targeting of macrophages to the sites of neuronal cell death in the central nervous system following injury are unknown. We have previously shown that lesions to the rat visual cortex at birth result in a rapid and transient invasion of peripherally derived macrophages to the degenerating dorsal lateral geniculate nucleus (dLGN) within days. We hypothesized that a chemotactic factor originating from the degenerating neurons of the dLGN aids in the targeting of these macrophages after cortex lesions. To investigate this hypothesis, we used a blindwell chemotactic chamber system that consists of a lower well into which tissue slices containing putative chemoattractant agents are placed, and an upper well into which the macrophages are placed. The two wells are separated by a filter through which macrophages migrate in response to chemotactic signals. The migratory response of a peripherally derived population, the resident peritoneal macrophages, was assayed with tissue slices containing the dLGNs from animals with and without cortex lesions and the chemotactic peptide, n-formyl MET-LEU-PHE. The maximal response, assessed by percent macrophage migration, was to chemotactic factors produced by tissue slices containing the dLGNs from animals which received visual cortex lesions at birth. This activity appears to be concentration dependent. The results suggest that neurons, upon being injured, actively produce a diffusible factor that attracts specific groups of phagocytic cells to a precise location for subsequent resolution of cell death. This work was supported by NINDS Grant NS 16487.

96.6

BIOCHEMICAL CHARACTERIZATION OF DEGENERATION AND DEATH OF SYMPATHETIC NEURONS INDUCED BY NEUROTROPHIC FACTOR DEPRIVATION. T.L. Deckwerth and E.M. Johnson, Jr. Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

Sympathetic neurons depend upon NGF for survival during embryonic and postnatal development. Dissociated superior cervical ganglion neurons of embryonic rats cultured for 5-7 days in the presence of NGF respond to NGF withdrawal by atrophy, lysis and death within 4 days as measured by altered morphology, release of cellular protein, and loss of refractility, respectively. In order to elucidate the molecular mechanism which irreversibly commits the neurons to die after NGF deprivation, we measured several biochemical parameters in dying neurons as function of time after NGF deprivation relative to undeprived control neurons. Among the parameters examined are amino acid uptake, incorporation of amino acids into protein, cytochrome oxidase activity, and mitochondrial function assessed by reduction of the tetrazolium dye MTT. We find that different parameters change with different timecourses, suggesting a temporal sequence of degenerative events triggered by NGF deprivation. The temporal correlation of the change of these and other parameters with the timecourse of commitment to die as measured by the ability of NGF to rescue dying neurons after different times of NGF-deprivation is under investigation.

96.7

ENDONUCLEASE ACTIVITY IMPLICATED IN DEATH OF PC12 CELLS AND SYMPATHETIC NEURONS AFTER NGF DEPRIVATION. A. Batistatou, L.A. Greene*. Department of Pathology and Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

The purpose of this study has been to explore the mechanisms by which neurotrophic factors promote survival/prevent death of their responsive neurons. Primarily, we have used serum-free cultures of PC12 cells as a model system for studying the neuronal cell death which occurs after neurotrophic factor deprivation. In this experimental paradigm, NGF rescues the cells from death. We report that serum-deprived PC12 cells manifest an endonuclease activity that leads to internucleosomal cleavage of their cellular DNA. This activity is detected several hours before any morphological sign of cell degeneration or death. NGF and serum, which promote survival of the cells, inhibit the DNA-fragmentation. Aurintricarboxylic acid (ATA), a general inhibitor of nucleases *in vitro*, also suppresses the endonuclease activity and promotes long-term survival of PC12 cells in serum-free cultures. This effect appears to be independent of macromolecular synthesis. In addition, ATA promotes long-term survival of cultured sympathetic neurons after NGF-withdrawal. It is hypothesized that the activation of an endogenous endonuclease could be responsible for neuronal cell death after neurotrophic factor deprivation.

96.8

TWO DISTINCT MOLECULAR MECHANISMS MEDIATE PROGRAMMED CELL DEATH.
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Large numbers of cells die by a non-pathological process known as Programmed Cell Death (PCD). Relying on published data and our own results, we identified two distinct PCD pathways: apoptosis and autophagy. Apoptosis involves a nucleolytic mechanism which is expressed primarily in non-neuronal vertebrate cells. It is characterized by rapid degradation of chromosomal DNA, chromatin deposition along the nuclear membrane, and constitutive expression of the polyubiquitin gene. In contrast, autophagy appears to represent a proteolytic cell death pathway. It is observed in the majority of naturally dying vertebrate neurons and most if not all invertebrate cells undergoing PCD. Autophagic PCD is characterized by the retention of high molecular weight chromosomal DNA, nuclear pyknosis and dramatic increases in the levels of polyubiquitin mRNA. It is speculated that apoptosis arose late in phylogeny to rapidly destroy potentially dangerous mitotic cells. Supported by R01 grants (LMS and BAO) and an RCDA (LMS).

96.9

MOLECULAR REQUIREMENTS FOR NEURONAL DEGENERATION INDUCED BY DOMINANT MUTATIONS OF THE *MEC-4* GENE OF *CAENORHABDITIS ELEGANS*.

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Rare dominant alleles of two genes, *mec-4* and *deg-1*, produce abnormal gene products that result in the vacuolar degeneration of specific neurons in the nematode, *C. elegans*. Molecular characterization of these two genes has established that *mec-4* and *deg-1* are members of a gene family that appear to encode membrane-spanning proteins. The sequences of the dominant *mec-4* alleles revealed that all degeneration-inducing mutations occur at the same position, normally an Ala residue thought to be situated adjacent to the cell membrane. *In vitro* mutagenesis has demonstrated that any large amino acid substitution at this position can induce neuronal degeneration. The region surrounding this site has been further altered using site-directed mutagenesis and the influence of neighboring amino acids on the degeneration mechanism will be discussed.

By comparing genomic sequences of the *mec-4* genes of *C. elegans* and *C. briggsiae*, a closely related nematode, several additional exons upstream of the start of our partial *mec-4* cDNA clone have been tentatively identified. Gene fusions and antibody staining experiments designed to determine when and where the *mec-4* protein is expressed are in progress.

96.10

THE PREVENTION OF EARLY CELL DEATH IN THE DEVELOPING NEURAL TUBE OF THE CHICK EMBRYO BY JANUS GREEN B.

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Degenerating cells occur in the neural tube and floor plate of the chick embryo at stages (st. 17-20) when commissural neurons extend their axons ventrally and then across the ventral midline. Embryos were treated with Janus Green B (a dye which has been reported to prevent the programmed death in interdigital region of developing chick limb) for 6 hours during the peak period of cell death (st.18) in the brachial neural tube. The number of pyknotic cells was significantly reduced following treatment with Janus Green B. A number of growth and trophic factors are also being examined for their effect on this early form of cell death in the neural tube. Preliminary observations of axonal projections in the floor plate indicate that following treatment with Janus Green B the growth cones of commissural axons in this region (ventral midline) exhibit an altered morphology. By contrast, growth cones in the other regions along the commissural pathway, or growth cones of non- commissural neurons, appear normal. These initial results suggest that the death of cells in the pathway of elongating axons may play a role in axonal guidance.

96.11

CYCLOHEXIMIDE BLOCKS THE STEROID-MEDIATED DEATH OF PROLEG MOTONEURONS DURING THE METAMORPHOSIS OF *MANDUCA SEXTA*.

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The abdominal prolegs, which are locomotory appendages of the caterpillar, are lost during the larval-pupal molt of *Manduca*. This transformation involves the degeneration of proleg muscles, regression of the dendrites of proleg motoneurons (MNs), and the programmed death of a subset of the MNs. The regression and death of the MNs are triggered by the prepupal peak of ecdysteroids in the hemolymph. To investigate whether protein synthesis is involved in the death of proleg MNs, we injected developing insects with the protein synthesis inhibitor, cycloheximide. Cycloheximide was injected into intact insects during the prepupal peak of ecdysteroids (1 injection every 12 hr), or into abdomens that were ligated before the prepupal peak and infused with 20-hydroxyecdysone to replace the missing steroids (1 injection of cycloheximide at the start of the infusion). In both cases, cycloheximide blocked the death of proleg MNs in a dose-dependent fashion, suggesting that protein synthesis is required for their death. Higher doses of cycloheximide were required to prevent dendritic regression than were required to block MN death.

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96.12

ACTIVITY-DEPENDENT REGULATION OF RIBOSOMAL RNA

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Ultrastructural studies investigating the cellular mechanisms underlying trans-neuronal degeneration in the chick auditory system have described a rapid dissociation of ribosomes in postsynaptic neurons following cochlea removal. Dissociation of ribosomes begins by 1 hr after cochlea removal and is predictive of subsequent cell death. Reduced protein synthesis in these neurons, presumably due to the ribosomal changes, has been observed as soon as 30 min after cochlea removal. By 6 hrs after cochlea removal, deafferented neurons segregate into 2 populations: one population shows a 20% reduction in protein synthesis, while the other (~25%) shows a complete cessation of protein synthesis and complete dissociation of cytoplasmic ribosomes. These responses to cochlea removal are due to the loss of 8th nerve action potentials since they can be duplicated by blocking afferent 8th nerve activity with TTX.

We examined immunoreactivity for a ribosomal antigen in neurons of the chick cochlear nucleus following elimination of afferent activity. Posthatch (1-2 wks) and adult (68 wks) chickens were sacrificed 1-6 hrs following unilateral cochlea removal or intralabyrinthine TTX injection. Brain stem sections were immunostained using the Y10B monoclonal antibody, which has been shown to immunoprecipitate ribosomal RNA. Other posthatch chicks received an intracardiac injection of ^{3}H-leucine and were sacrificed 6 hrs after cochlea removal. Sections from the latter animals were processed for autoradiography in addition to Y10B immunoreactivity (Y10B-IR).

Posthatch chicks show a dramatic reduction in Y10B-IR in the ipsilateral cochlear nucleus as early as 1 hr following cochlea removal. Y10B-IR is also reduced, though to a lesser extent, in adult chickens following cochlea removal. Double-labeling for ^{3}H-leucine and Y10B revealed a postive correlation between protein synthetic activity and Y10B-IR. These results suggest that the regulation of neuronal protein synthesis by afferent activity is mediated by rapid changes in ribosomal RNA.

The Y10B antibody was a gift from J. Steitz and M. Lerner. Supported by PHS grant DC00393.

96.13

RAPID RIBOSOMAL DEGRADATION ASSOCIATED WITH TRANSNEURONAL CELL DEATH: EFFECTS OF PROTEIN SYNTHESIS INHIBTION.

Gwenn Garden, Mark Bothwell and Edwin W Rubel, Dept. of Physiology-Biophysics and Hearing Development Laboratory, University of Washington, Seattle, WA 98195.

Neuronal death during development, after target deprivation or growth factor deprivation, is inhibited by protein synthesis inhibitors. Deafferented neurons of the chicken primary auditory brain stem nucleus, n. magnocellularis, undergo cell death characterized by early dissociation of ribosomes from polysomes and endoplasmic reticulum, followed by marked ribosomal degradation. Ribosomal degradation, seen by electron microscopy is also an early degenerative change in many other cell types undergoing developmental or physiologically induced cell death. Changes in ribosome integrity can be detected immunocytochemically using a monoclonal antibody, Y10B, which immunoprecipitates ribosomal RNA . At early time points after cochlea removal, n. magnocellularis neurons show a marked decrease in Y10B immunoreactivity.

To determine if neuronal death in activity deprived n. magnocellularis neurons is dependent on new gene expression, we treated chicks with 250 µg of cyclohexamide intracranially just prior to activity deprivation by cochlea removal. This treatment inhibited protein synthesis by 95% for at least 3 hours. At several time points after cochlea removal, cyclohexamide treated and control animals were perfused with 4% paraformaldehyde. Brain stems were embedded in paraffin and serially sectioned. Sections were labeled with Y10B anti-ribosomal antibodies or stained with thionin. Activity deprived neurons in both control and cyclohexamide treated animals showed decreased immunoreactivity for Y10B when compared to neurons in the contralateral n. magnocellularis. Cyclohexamide treatment for the first 3 hours after cochlea removal did not decrease loss of Y10B immunoreactivity We conclude that cochlea removal effects on ribosome structure, as reflected by loss of Y10B immunoreactivity, occur in the absence of new gene expression.

Y10B antibody was a gift from M. Learner and J. Steitz. Support: Hartford Foundation Fellowship and NIH grants NS23343 and DC00393.

96.14

NEURONAL DEATH FOLLOWING DEAFFERENTATION IS BLOCKED BY DELAYED INHIBITION OF MITOCHONDRIAL PROTEIN SYNTHESIS.

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Removal of excitatory input to second-order auditory neurons (n. magnocellularis, NM) in the chick brain stem results in the eventual death of ~20% of these neurons. We have previously reported a rapid, dramatic proliferation of mitochondria in deafferented NM neurons (Hyde & Durham, Soc. Neurosci. Abstr. *15*:290, 1989). To determine whether mitochondrial proliferation is necessary for neuronal survival following cochlea removal, we used chloramphenicol, a mitochondrial protein synthesis inhibitor, to block mitochondrial proliferation after cochlea removal in 10-day-old chicks. When chloramphenicol is administered at the rate of 1000 mg/kg/day for the first 12 hours following cochlea removal, up to 80% of the deafferented neurons will die (Hyde & Durham, Soc. Neurosci. Abstr. *16*:984, 1990). To determine whether this effect was due to the *timing* of the treatment, the 12 hour chloramphenicol treatment was delayed for 6 hours *after* cochlea removal. After 5 days survival, these animals were deeply anesthetized and sacrificed. NM neuron counts, made in 10µm Nissl-stained paraffin sections, revealed no significant loss of deafferented neurons ($p>.40$). An additional set of animals surviving to 14 days following cochlea removal showed no significant neuronal loss ($p>.90$), indicating that neuronal death had been blocked rather than delayed. Vehicle-treated animals showed the expected degree of deafferentation-induced neuronal loss ($p<.05$).

Thus, mitochondrial protein synthesis appears to have opposing effects on neuronal survival following deafferentation. During the first 12 hours following deafferentation, mitochondrial protein synthesis is required for the survival of the majority of neurons. However, mitochondrial protein synthesis between 6 and 18 hours after deafferentation is necessary for the death of some deafferented neurons.

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97.1

MICROGLIA AND THE DEVELOPING OLFACTORY BULB.
A. O. Caggiano* and P. C. Brunjes. Univ. of Virginia, Charlottesville, VA 22903.

Microglia have been implicated as markers of cell death in the developing nervous system (e.g., *J. Comp. Neurol* **287**, 286). While it has been demonstrated that there are large increases in cell number within the rat olfactory bulb in the first 30 postnatal days, little is known about rates and patterns of cell death in this primary sensory relay. The fact that normal cell death does occur is suggested by a report indicating that blocking airflow through one half of the nasal cavity decreases cell survival rates in the bulb (*J. Comp. Neurol.* **289** 481). In addition, observations of both continuous olfactory receptor cell replacement and lifelong bulb neurogenesis suggest that the region might have unique needs for a stable population of the phagocytes. The present study investigated patterns of microglial staining in the maturing rat olfactory bulb. Microglia were visualized with the peroxidase labelled Isolectin B4 from Bandeiraea Simplicifolia BS-1 (*J. Neurosci. Meth.* **38**, 1683). Initial data indicate that by postnatal Day 10 there is a large, uniform population of glia resident in all bulb layers. By Day 30 many more glia are found in the bulb and a deep-to-superficial gradient of staining emerges, with highest numbers of cells found in the subependymal layer. Supported by grant DC-00338.

97.2

CELL PROLIFERATION IN THE DEVELOPING OLFACTORY NEUROEPITHELIUM OF THE CHICK. H. Martin. Northwestern Univ., Dep. of Neurobiology & Physiology, Evanston, IL 60201.

During the period in which the olfactory organ of the chick develops, nuclei of proliferating cells were labeled with 1-hr. pulses of [^{3}H]-thymidine. Quantitative analysis of autoradiograms suggests that there are two populations of proliferating cells in the olfactory neuroepithelium. Each population is distinguished by the location of its progenitor cells and the temporal dynamics of its proliferation. One population has progenitor cells located in the basal cell layer. Proliferation of these cells remain relatively constant. Regional variability in nuclei labeling is related to cell density and the thickness of the columnar epithelium. On the other hand, progenitor cells from the second population is located in the intermediate layer of the epithelium. Proliferation in this population varies with the age of the embryo. It is highest during an early stage of development and declines to zero at the stage when olfactory neurites are known to reach the telencephalon. Other studies have suggested that this second population represents the cell line of olfactory receptor neurons. This study provides additional evidence to suggest that, during development, the regulation of neurogenesis in the olfactory neuroepithelium of the chick is independent of the regulation of proliferation of basal cells.

97.3

MITRAL/TUFTED CELL AND OLFACTORY AXON NUMBERS ARE CORRELATED IN NORMAL AND PARTIALLY DEAFFERENTED *XENOPUS* TADPOLES. C.A. Byrd and G.D. Burd. Depts. of Anatomy and Molecular & Cellular Biology, University of Arizona, Tucson, AZ 85721.

Previous studies in several amphibian species reported that removal of the olfactory placode (OP) prevents the formation of the olfactory bulb (OB) (Piatt, 1951; Clairambault, 1971; and Stout & Graziadei, 1980). We have previously shown that throughout *Xenopus* larval development the total number of olfactory receptor cell (ORC) axons is correlated with the total number of cells in the mitral cell/plexiform layer (MC/PL) (Byrd & Burd, 1990, Neurosci. Abstr.). The present study examined tadpoles with one or both OPs removed before the ORC axons had reached the neural tube (NT). Quantitative analyses were performed on animals in which the OP did not regenerate or was significantly reduced. Animals with unilateral OP removal had one nasal capsule, one ON, and one OB; the other side of the telencephalon had no OB, did not fuse with the remaining OB, and appeared to contain only cells of the cortex. Both the number of ORC axons and the number of cells in the MC/PL were smaller than the total number of axons or cells in control animals, but these numbers were slightly larger than in one nerve or one OB of control animals. In a scatter plot of ORC axon number versus cell number in the MC/PL, these experimental animals fit on the same regression line that was generated in a normal development study (Byrd & Burd, 1990). Since it is possible that we removed some of the underlying NT with the OP, we also analyzed animals in which we removed part of the NT without disturbing the OP. The NT regenerated, and the ORC axon and MC/PL cell numbers were within the normal range. In conclusion, regardless of surgical treatment or larval stage, the number of cells in the MC/PL is correlated with the number of ORC axons. Supported by NIDCD #DC00446.

97.4

OLFACTORY RECEPTOR CELLS EXPRESS A UNIQUE ANTIGEN THROUGHOUT DEVELOPMENT AND INTO ADULTHOOD IN THE CLAWED FROG, *XENOPUS LAEVIS*. S.F. Matheson and G.D. Burd. Program in Neuroscience and Depts. of Anatomy and Molecular & Cellular Biology, University of Arizona, Tucson, AZ 85721.

We have characterized the distribution of the antigen recognized by a monoclonal antibody (E7) generated in our laboratory. Olfactory epithelia (OE) from *Xenopus laevis* larvae (stages 40-58) and young adults were examined using a combination of immunofluorescence and neuroanatomical techniques. In stage 58 larvae, cytoplasmic labelling was observed in dendrites, cell bodies and axons of olfactory receptor cells (ORCs) as well as in the olfactory nerve (ON), and extending into the OB. Unilateral ON transection resulted in total loss of specific labelling in the ipsilateral OE after 16 days. Furthermore, E7 immunofluorescence combined with retrograde labelling of ORCs by injection of Fluorogold into larval OB led to significant overlap in fluorescent regions of the larval OE. These results suggest that E7 recognizes an antigen expressed by ORCs in larvae. E7 immunofluorescence was observed as early as stage 40, two stages after ORC axons form synapses in the developing olfactory bulb (OB). Metamorphosis led to dramatic changes in E7 labelling; in young adult OE, E7 labelled the three different olfactory cavities in distinct patterns. Intense labelling occurred on the apical surface, presumably in the mucous layer, of the principal cavity, which may participate in air breathing. Cytoplasmic fluorescence, which was prominent in this cavity in larvae, was absent. The putative vomeronasal epithelium was diffusely labelled, while the third (presumably aqueous) region displayed a pattern similar to that seen in larvae. Further studies are examining the developmental time of appearance of the antigen and testing for its expression in other species. Supported by NIDCD DC00446 and ADCRC 82-1678.

97.5

STEROID REGULATED DEVELOPMENT IN MOTH OLFACTION.
R.G. Vogt and M.R. Lerner, Yale Med. Sch., New Haven, CT 06510.

The olfactory system is constructed in a spatially and temporally specific manner, with specific gene products (e.g. receptors) expressed in the "correct" cells at the "correct" times. The antenna of the moth *Manduca sexta* provides a model for studying the molecular signals which regulate spatial and temporal specificity of olfactory gene expression during development. Three classes of odorant binding proteins (OBP) are expressed in distinctly different and identifiable sensilla: the pheromone binding protein, PBP, and the general odorant binding proteins GOBP1 and GOBP2 (1991, *J. Neurobiol.* **22**:74). Development of the adult antenna occurs over about 18 days (Hildebrand & colleagues). Birth of sensory neurons occurs at about 10% of dev., axonal and dendritic outgrowth is complete by about 50%, and the neurons respond to odorants by 90%. Ecdysteroids, steroid hormones, are known to coordinate many events in insect development, and Schwartz & Truman (1983, *Dev. Biol.* **99**:103) had shown that preventing a normal decline of ecdysteroid late in adult development prevented the programmed death of certain muscles and neurons. Because PBP expression occurred at this same time, we cultured antennal fragments of staged animals for 20 hrs in the presence or absence of the active hormone 20-hydroxy ecdysone, followed by a 2 hr incubation in ^{35}S-methionine. PBP, GOBP1 and GOBP2, normally appearing 36 hrs before adult emergence, could by expressed at least 2 days early by prematurely lowering the ecdysteroid levels, suggesting that normal OBP expression is induced by the decline in steroid. This study supports a model in which olfactory development is temporally coordinated by external signals.

97.6

VOLTAGE-DEPENDENT CURRENTS IN DEVELOPING AND MATURE TASTE CELLS IN MUDPUPPY (*Necturus maculosus*). Alan Mackay-Sim, Sue Kinnamon, and S.D. Roper. Dept of Anat and Neurobiol, Colo St Univ, Ft Collins, CO 80523, and the Rocky Mt Taste & Smell Cntr, Denver, CO 80262.

Underlying taste perception is a remarkable plasticity in which the receptor cells are continually replaced by division and differentiation of basal stem cells. Thus, in the taste bud, there is a variety of cell types including stem cells, developing cells and mature receptor cells. In many species there is an additional basal cell type which resembles a Merkel cell. In the mudpuppy this Merkel-like cell possesses synaptic contacts with taste receptor cells and with sensory axons (Delay et al., 1988). The aim of the present study was to identify the whole-cell currents associated with the different taste cell types. In order to label only those cells whose apical process had reached the taste pore, WGA-RITC conjugate was applied to the tongue surface prior to cell isolation. Whole-cell currents were recorded in physiological saline and in the presence of TEA, TTX and Ba^{2+}. Cells were then classified according to their fluorescent label and their size. Mature receptor cells (those labelled with WGA-RITC) had voltage-dependent Na^+, K^+ and Ca^{2+} currents whereas most developing taste cells (elongate cells of similar size but without label) had only voltage-dependent K^+ currents. Some developing cells (5/14) had all 3 currents. Basal cells (small, round, unlabelled cells) were of two classes: those with voltage-dependent Na^+, K^+ and Ca^{2+} currents; and those with K^+ currents only. Preliminary experiments indicate that cells of the former class contain serotonin and are therefore probably a Merkel-like cell. The other basal cells may be stem cells or early-stage developing taste cells. These results provide evidence for a developmental sequence in which voltage-dependent K^+ channels are expressed earlier in development than Na^+ and Ca^{2+} channels. Na^+ and Ca^{2+} channels appear to be expressed at about the same time, apparently when the cell extends its apical process into the taste pore. Supported by NIH grants DC00244, DC00766(SCK), DC00374(SDR), and AG06557 (SDR).

97.7

BIRTH TIMES OF LABELLAR GUSTATORY NEURONS IN THE BLOWFLY *PHORMIA REGINA*. G.S. Pollack. Dept. of Biology, McGill University, Montreal, Quebec, Canada H3A 1B1.

Each labellar lobe contains two types of taste sensilla: ca. 65 interpseudotracheal papillae, each innervated by 3-4 neurons, and ca. 125 taste hairs, each with 5 neurons. The neurons of a sensillum, all of which are believed to derive from a common precursor, differ in sensory specificity and behavioral import. An unanswered question is how these differences are specified. I measured the birth times of neurons to determine whether their phenotypes might be correlated with, and thus perhaps determined by, birth order. This initial study focuses on the interpseudotracheal papillae.

Pupae of different ages were injected with the thymidine analog 5-bromo-2'-deoxyuridine (BRDU). Double-label immunocytochemistry, using antibodies specific for neuronal surfaces and for BRDU, made it possible to determine which neurons had incorporated BRDU and therefore had been born after the time of injection.

The papillae appear within the first day following pupation, with lateral ones born earlier than medial ones. In almost all cases, either all or none of the neurons of a single papilla were labeled with BRDU. This suggests that the neurons of a sensillum are born nearly simultaneously, and argues against birth order as a mechanism for specifying phenotype.

97.8

POSSIBLE CHEMOTOPIC ORGANIZATION OF THE CENTRAL GUSTATORY SYSTEM IN RATS DEPRIVED OF SODIUM CHLORIDE THROUGHOUT EARLY DEVELOPMENT.

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Dramatic alterations in the physiology and anatomy of both the peripheral and central components of the gustatory system have been reported in rats who have been raised on a low (0.03%) sodium chloride (NaCl) diet throughout very early development. Neurophysiological alterations include suppression of NaCl-elicited activity in the chorda tympani nerve (CT) and in cells located in the rostral NTS. Interestingly, recovery of peripheral CT activity results once rats are fed a NaCl replete (1%) diet; however, this replete diet does not induce normal function of central NTS cells. Instead, these cells show a "hyper-responsivity" to sodium stimuli. The present investigation was designed to determine whether metabolic changes in the NTS are also assocciated with the "hyper-responsivity" of these cells to sodium stimuli. Since the dorsal portion of the CT terminal field has been shown anatomically to be most affected by the dietary manipulations, it was hypothesized that NaCl repletion would result in increased metabolic activity of cells in the dorsalmost portion of the terminal field area. Indeed, compared to NaCl deprived rats (N=10), deprived rats fed the NaCl replete diet and 0.154M NaCl for 24 hours (N=9) show significant ($p < .05$) increases in succinate dehydrogenase (SDH) activity at the level of the NTS where the dorsal portion of the CT terminal field is typically located. Such results are suggestive of chemotopically segregated CT afferents and/or NTS cells in NaCl deprived rats. We are currently examining the effect of a 24 hour sucrose exposure to NaCl deprived rats to investigate the stimulus-specificity of this localized increase in SDH activity.
Supported by NIH grants DC00025 and DC00407.

97.9

AUDITORY INVASION OF THE VISUAL CORTEX IN NEONATALLY ENUCLEATED SYRIAN HAMSTERS (MESOCRICETUS AURATUS).

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The visual cortex (VC) of neonatally enucleated hamsters was studied to determine if its function or connections had changed. Structurally, the blind hamster's VC was similar to the normal hamster's VC and as expected did not respond to flashes of light. However, auditory evoked responses were recorded on the surface of the remnant primary VC suggesting that parts of the visually deprived VC received auditory input. In order to disclose the origin of this input, WGA-HRP was applied into the area that responded to auditory stimuli and that topographically corresponded to the VC of normal hamsters. This revealed that the reciprocal connection between the dorsal lateral geniculate nucleus (dLGN) and the VC was intact. In addition, both in normal and enucleated hamsters the VC received projections from layer VI of the auditory cortex, yet, the number of these projections was significantly larger in the blind animal. WGA-HRP injection into the dLGN disclosed also retrogradely labeled neurons in the inferior colliculus. It is concluded that the deprivation of the VC from its original input facilitates the "invasion" of a new sensory modality into this area.

97.10

DOPAMINERGIC MODULATION OF PREPULSE INHIBITION OF ACOUSTIC STARTLE: CONTROL VERSUS NEONATALLY DOPAMINE DEPLETED ANIMALS. S.B. Schwarzkopf, T. Mitra*, J.P. Bruno. Departments of Psychiatry and Psychology, Ohio State University, Columbus, Ohio 43210

The magnitude of the acoustic startle response (ASR) is reduced significantly when the startle stimulus (Pulse) is preceded by a much weaker signal (Prepulse). This phenomenon, termed "prepulse inhibition" (PPI), is disrupted by dopamine (DA) agonists systemically administered and focally applied to ventral striatum. Previous studies suggest a greater D2 versus D1 receptor involvement in the disruption of PPI. In this study, we examined DA modulation of PPI in control and neonatally DA depleted animals (tested as adults).

Seventy four Sprague-Dawley rats (250-450 gm) were tested. At 3 days of age, DA depleted animals (N=38) received intraventricular 6-hydroxy-DA (6OHDA) while controls (N=36) received intraventricular vehicle (ascorbic acid/NaCl). PPI was assessed twice (1 week between sessions, T1/T2). One group of animals (N=20) underwent startle testing without injection. Fifty four animals were tested after subcutaneous injections of apomorphine (APO, doses: .05, .1, .5 mg/kg) or saline (SAL) in a counterbalanced design. PPI was computed as the percentage decrease in startle amplitude caused by prepulses of 75, 80, and 85 db.

Results in control (sham operated) animals agree with previous studies showing significant dose related disruption of PPI by APO. Although DA depleted animals exhibited "typical" PPI in non-injected and SAL conditions, they demonstrated significantly greater sensitivity to the PPI disrupting effects of APO compared to control animals ($P < .05$ at APO doses of 0.1 and 0.5). Significant group differences were also noted for baseline startle measures at these APO doses. Findings indicate increased sensitivity of neonatally DA depleted animals to APO's effect on PPI and baseline startle.
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97.11

THE CRITICAL PERIOD FOR CHORDA TYMPANI TERMINAL FIELDS IN THE NTS OF SODIUM CHLORIDE DEPRIVED RATS.

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Sodium restriction on or before postconception day 8 produces a suppression in the response of the chorda tympani nerve to sodium salts, while responses to nonsodium salts remain unaffected. Placement on a sodium replete diet after postnatal-day 28 results in complete recovery of the peripheral nerve response; however, alterations in the size and distribution of the chorda tympani terminal field are permanent. Therefore, there must be a critical period, during which placement on the sodium replete diet after sodium deprivation is effective in reversing the alterations in chorda tympani terminal field. The present study was designed to determine whether this critical period occurs prenatally or postnatally. The terminal field size and distribution was measured in deprived rats placed on the sodium replete diet on the day of birth (birth-repleted) or on postnatal day 28 (28-day repleted) and control rats. For visualization of the terminal field, HRP was placed on the cut nerve and the tissue was processed with a modified TMB technique after 24-hour survival. Preliminary results indicate that the terminal field size of birth-repleted rats is intermediate in size between control and 28-day repleted rats. The distribution of the terminal field for birth-repleted rats was similar to that of 28-day repleted animals. These results indicate that the critical period for the effects of sodium deprivation on the chorda tympani terminal field is before birth. Experiments are in progress to precisely define the boundaries of the critical period.
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97.12

MATURATION OF THE MAP OF AUDITORY SPACE IN THE SUPERIOR COLLICULUS OF THE FERRET. A.J. King and S. Carlile*. Lab. of Physiology, Parks Road, Oxford OX1 3PT, U.K.

The postnatal development of the map of auditory space in the superior colliculus (SC) is influenced by both auditory and visual experience. We have studied the time course over which neural responses in this nucleus become spatially tuned and topographically organized, by examining multi-unit responses from the SC of normal ferrets at various postnatal ages. The animals were anaesthetized and placed in an anechoic room, where recordings were made throughout the rostro-caudal extent of the SC. Between postnatal day 33 (P33), the earliest age examined, and the mid-P40s, most auditory responses were very broadly tuned, often responding to azimuthal locations throughout both hemifields. The proportion of spatially-tuned responses then increased at all sound levels tested, so that by P50-51 average spatial tuning had reached adult levels. The number of responses tuned to appropriate positions in space, according to their locations in the SC and their proximity to visual receptive fields recorded in the same electrode track, also increased in the mid-P40s, and a topographic progression of auditory best positions was apparent at P48. However, some responses were still spatially ambiguous or broadly tuned at this age, and an adult-like map was not present until after P50. The developmental emergence of the this map coincides with the maturation of sound localization cues in the ferret.

97.13

POSTNATAL DEVELOPMENT OF THE SPECTRAL TRANSFER FUNCTIONS AND INTERAURAL LEVEL DIFFERENCES OF THE AUDITORY PERIPHERY OF THE FERRET. S. Carlile*. University Laboratory of Physiology, Parks Rd., Oxford OX1 3PT, U.K.
SPON: Brain Research Association.

The transformations of sound by the auditory periphery provide important monaural and binaural cues to sound location. The pattern and time-course of the development of these cues have important consequences for the emergence of auditory localization mechanisms. The spectral transfer functions (STFs) of the auditory periphery of the ferret were examined using an impulse response technique (bandwidth 0.1kHz to 30kHz) for sound locations on the auditory-visual horizon (Carlile, 1990 *J.Acoust.Soc.Am.* 88:2196-2204). By interpolating between the STFs determined for a number of azimuthal locations the horizon transfer function (HTF) is obtained. The HTF for the ipsilateral space of the adult ferret has a number of characteristic features. HTFs were obtained from ferrets at various postnatal ages from 32 days (P32) to P54. HTFs obtained around the onset of hearing were well outside the normal adult range. After P50 the HTFs were found to be adult-like. The changes in the patterns of interaural level differences, calculated over the same frequency range, also indicated the development of adult-like binaural cues by this time. This suggests that in the ferret, the normal pattern of sound localization cues develops rapidly over the three weeks from the onset of hearing.

97.14

REMOVAL OF THE RIGHT COCHLEA IN INFANT FERRETS CHANGES UNIT RESPONSE PROPERTIES IN THE LEFT INFERIOR COLLICULUS TO STIMULATION OF THE LEFT EAR. D.R. Moore, D. McAlpine* and R.L.Martin*. Univ. Lab. of Physiology, Oxford OX1 3PT, U.K.

Unilateral hearing loss in infancy produces a variety of changes in the central auditory system, including an enhancement of single neuron excitatory responses to acoustic stimulation of the normal ear. We studied the inferior colliculus (IC) of year-old ferrets that had one cochlea removed at P5, P25 or P39. Rate-level functions were obtained using best frequency tone stimulation of the intact ear and single-unit recording on the side of that ear. Relative to acutely deafened adult controls, units in all groups of experimental ferrets had lower thresholds, and more units with sustained, excitatory responses were found. Ferrets in the P5 group showed the greatest change; response properties were intermediate between control responses ipsi- and contralateral to the intact ear. Ferrets in the older experimental groups showed progressively reduced changes. No changes were found in the IC contralateral to the intact ear of one P25 deafened ferret, or between control and unlesioned adults. The sensitive period suggested by these results is coincident with that for known anatomical changes.

97.15

HEARING IN FERRETS WITH UNILATERAL COCHLEAR REMOVAL. R.L. Martin*, J.E. Hine* and D.R. Moore. Univ. Lab. of Physiology, Oxford OX1 3PT, U.K.
(SPON: Brain Research Association).

Unilateral cochlear removal in infancy has been shown to produce anatomical and physiological changes in auditory brainstem structures in a variety of species. Thresholds of single units in the inferior colliculi of adult ferrets subjected to unilateral cochlear removal at P5, for instance, are significantly lower than those of single units in the inferior colliculi of acutely deafened adult controls. We are currently examining the possibility that these changes are paralleled by changes at the behavioural level. Absolute intensity thresholds have been obtained for five pure tones ranging in frequency from 1 to 16 kHz using positive conditioning procedures and a descending method of limits technique. Thresholds for control animals with both cochleae intact are within the range previously reported for this species. To date, we have found no evidence of altered sensitivity in animals subjected to unilateral cochlear removal at P5. We are, however, yet to compare the sensitivity of P5 cochlear removed animals with that of animals subjected to unilateral cochlear removal in adulthood.

97.16

THE ONTOGENY OF AUDITORY THRESHOLDS DETERMINED WITH REFLEX MODIFICATION AUDIOMETRY. K. M. Crofton, X. Zhao* and M. E. Stanton. Neurotoxicology Division, U.S. EPA, RTP, NC 27711

The ontogeny of the acoustic startle response and reflex modification are known to arise prior to weaning in the rat at times that correlate with anatomical development of the auditory system. The present study examined the ontogeny of auditory thresholds using reflex modification of the auditory startle response. Reflex modification thresholds were determined using male and female Long Evans rats. Auditory thresholds were determined at various postnatal ages (independent age groups on postnatal days (PND) 20, 30, 40, 70, and 120) using 5- and 40-kHz tones (6-90 dB SPL, 40-msec) presented 90-msec prior to the onset of the eliciting stimulus (a 40-msec burst of white noise with 2.5 msec rise/decay). The results indicate mature thresholds levels (approximately 35 and 19 dB for 5 and 40 kHz, respectively) from PND 30 through PND 120. At PND 20 the high frequency threshold is at the adult level, but the low frequency threshold is still very high (~65 dB). These findings confirm longer ontogeny of low-frequency thresholds, and suggest that auditory thresholds derived using reflex modification may mature later compared to thresholds defined with other procedures.

97.17

RESPONSES IN INFERIOR COLLICULUS UNITS OF NEONATALLY DEAFENED CATS AFTER CHRONIC INTRACOCHLEAR ELECTRICAL STIMULATION (ICES) R. L. SNYDER*, P.A. LEAKE, S. J. REBSCHER*, Coleman-Epstein Laboratories, Univ. of California, San Francisco, CA 94143

Examination of effects of neonatal deafening and chronic ICES on single unit responses can provide a context for intrepreting the consequences of auditory deprivation and the application of cochlear prostheses in very young deaf children.

Implanted electrodes consisted of two pairs of contacts positioned at about the 8 kHz and 15 kHz locations within the cochlea. Chronic stimulation of one pair was initiated at approximately 2 months of age. Stimuli were charge-balanced, biphasic pulses 200 μsec per phase delivered continuously at 30 pps for periods of 2-6 hrs/day for 4 to 20 weeks. Responses to acute ICES of each electrode pair were recorded in acutely deafened adults; in neonatally deafened, unstimulated adults; and in chronically stimulated, neonatally deafened adults. In all three groups neurons showed responses to ICES which were comparable to those evoked acoustically. Most responded with either onset or sustained increases in discharge rates. Like acoustic responses, ICES responses showed either monotonic or nonmonotonic increases in activity. Latencies ranged from 5 to over 40 msec. Response thresholds varied systematically across the IC depending upon the cochlear region stimulated and reflecting its tonotopic organization. Responses in deafened/unstimulated and normal animals were not statistically different. However, responses in chronically stimulated animals showed systematic differences from those in the other groups: The spatial resolution of the IC central nucleus was degraded, the perstimulus response latencies were significantly shorter, the late response latencies were significantly longer, and the frequency of occurrence of inhibitory and late responses was significantly higher.

These results suggest 1) that development of the cochleotopic organization and some basic response properties in IC neurons were not affected by the absence of normal postnatal acoustic input and 2) that the limited regime of ICES employed in these studies produced significant quantitative distortions in the perstimulus response patterns of IC neurons. (Supported by NINCDS Contract NS-7-2391)

98.1

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE GLUCOSE TRANSPORTER IN NEURAL TRANSPLANTS AND IMPLANTED GLIOMA. J.M. Rosenstein, and T.W. Moody. Depts. of Anat. Neurosurg. and Biochem. The George Washington University Med. Ctr, Wash. D.C. 20037.

The erythroid or brain-type glucose transporter (GT) is an independent and highly reliable marker for cerebral microvessels with blood-brain barrier function or cells linked by tight junctions. It is also present in varying amounts throughout the neuropil exclusive of neurons. Tissues from animals bearing fetal neocortical grafts or implants of C_6 glioma cells were examined for the expression of GT following aldehyde perfusion. In fetal rat brain the antigen is localized to microvessels as early as 17 days prenatally. However, immuno-gold staining showed that many microvessels in CNS grafts as late as 3 weeks postoperative either expressed GT very weakly or not at all. At later times most graft vessels were immunostained comparably to host vessels but neuropil staining remained reduced. Extensive angiogenesis followed glioma implantation; at 3 days vascular sprouts were positive for GT. Subsequently, the neovasculature was composed about equally of positively and negatively stained vessels. Biopsy specimens of human glioma showed a similar pattern. These results indicate that the glucose transporter within CNS transplants can be reduced below the fetal state. In C_6 tumor implants over time many microvessels lack GT expression. (NS-17468).

98.2

MUSCLE ISOGRAFT AS A SITE OF ENTRY FOR CIRCULATING CELLS INTO BRAIN. S. Ishihara*, M. Sawada*, J. Kim*, L. Chang* and M Brightman. Lab. of Neurobiology, NINDS, National Institutes of Health, Bethesda, MD 20892

An isograft of skeletal muscle on rat dorsal medulla is a permanent opening in the blood-brain barrier. Is the graft site also an entry for circulating monocytes that can bring virus or enzymes into brain? A bolus of $2X10^6$ peritoneal macrophages (MØ) activated with phorbol ester and labeled with a fluorescent dye, was infused into the vertebral artery via the axillary artery. The brains, with their 5 week old grafts, were fixed, frozen and cut at 15 - 30 μm.

In rats without grafts, very few activated MØ lined vessels and none entered choroid plexus or brain. In rats with muscle grafts, non-activated MØ invaded grafts but very few entered the medulla at 2 h. Activated MØ entered both graft and medulla. The extent of MØ migration was the same at 2 and 24 h. In control rats with gel foam as a graft, a few activated MØ invaded gel and brain. Non-activated and activated MØ lined blood vessels near muscle and gel grafts. Thus, the entry route into brain from graft sites may be more from perturbed brain vessels than from muscle or gel grafts. Analysis is being made for major histocompatability and LFA1 antigens in MØ and for induction in vessels, of I-CAM 1 antigen, involved in leukocyte adhesion.

98.3

REINNERVATION OF ADULT RAT SKELETAL MUSCLE USING EMBRYONIC SPINAL CORD CELLS GRAFTED INTO THE AXOTOMIZED TIBIAL NERVE. D.E. Erb, J.H. Kim, W.H. Lee and R.P. Bunge. Depart. of Neurol. Surg., The Miami Project to Cure Paralysis, Univ. of Miami Sch. of Med., Miami, FL 33136.

Denervated skeletal muscle cannot effectively contract without connection to the brain stem or spinal cord and undergoes progressive atrophy and functional decline. Efforts to elicit muscle contraction or significantly retard muscle atrophy by direct electrical stimulation of the muscle are relatively ineffective without a nerve supply. We have employed a novel approach to re-establish innervation of the denervated gastrocnemius muscle in the adult rat. A heterogeneous neuronal population dissociated from ventral spinal cord regions of E14 rat embryos, was grafted as 10-70 μl aliquots (20,000-300,000 cells) into the distal stump of an axotomized tibial nerve. Within 3 weeks, 15 μm frozen serial sections of the injection site contained viable cells including many multipolar motor neuron-like cells with nuclei up to 48 μm in diameter. The injection site also contained neuronal processes immunoreactive with antibodies against neurofilaments. These processes coursed from the injection site through the axotomized distal nerve stump into the muscle. In some animals, reinnervation of denervated muscle by grafted cells was electrophysiologically confirmed by extracellular recordings from single muscle fibers. Stimulation of the neuronal injection site produced single muscle fiber action potentials with stimulus intensities from 100 μA to 400 μA and latencies ranging from approximately 4 to 5 msec. Compound action potentials evoked by stimulation of the injection site remained intact following injection of pancuronium bromide, whereas single motor unit activity was completely abolished. Supported by The Hollfelder Foundation.

98.4

FLUORESCENT LABELING WITH PKH-26 TO CHARACTERIZE THE INCORPORATION OF NERVE ISOGRAFTS AND ALLOGRAFTS. D. Parvin*, T.E. Trumble, & E. Cohen*. Department of Orthopaedics RK-10, University of Washington School of Medicine, Seattle, WA 98195.

Objective: To evaluate the role of host and graft cells and the physiology of graft incorporation in nerve transplantation in order to optimize nerve repair and regeneration.

Methods and Materials: 1.2 cm sciatic nerve isograft and allograft transplantation was performed using Lewis and Brown-Norway rats. In some, the grafts were labeled with PKH-26 (Zynaxis Cell Sciences, Malvern, PA), in others the host was labeled. Repaired nerves were harvested post-transplantation, and analyzed in cross-section with the Bioquant software system (R&M Biometrics Inc., Nashville, TN) as to the percent of area labeled with PKH-26 fluorescent dye.

Results: 1) Fluorescent labeled host cells were detected only in the epineurium of the nerve graft at day 3, in epineurium and perineurium by day 14 and not until day 25 in endoneurium. 2) Host cells migrated distally into the graft (95% fluorescence) from the dyed proximal host limb (85% fluorescence). 3) PKH-26 labeled graft cells migrated distally, with a 96% distribution of fluorescence in the distal host limb (unstained at transplant) compared to 76% in the proximal limb. 4) Allograft cells were destroyed both within the graft and after migrating distally into the host limb, resulting in loss of the vital dye demonstrated by a 40% difference between the peak fluorescence and the nadir in allograft compared to 20% in isografts.

Conclusions: Nerve graft incorporation occurs in 3 phases, with contributions from both host and graft elements. Optimal nerve repair and regeneration requires viable and histocompatible grafted tissue.

98.5

PERIPHERAL NERVE GRAFT REPAIR CHANGES SIZE AND HISTOCHEMICAL PROFILE OF AFFECTED MUSCLE FIBRES. H.Hashimoto*, A.Mautes* and A.C.Nacimiento. Neurosurgical Research Laboratory, Saarland University Medical School, 6650 Homburg/Saar, F.R.G.

A 10 mm gap created by excision in the peroneal nerve of the rat was microsurgically repaired by autologous grafting of an appropriate segment of radial nerve. After 14, 30 and 90 days size and histochemical profile of all extensor digitorum longus (EDL) fibers were assessed by computer-assisted quantification. Size increased significantly in the oxidative groups type I (SO) and type IIa (FOG), whereas the glycolitic type IIb (FG) decreased within 30 days and regained preoperative levels at 90 days. Type IIc, an intermediate group between IIa and IIb normally few in number, appeared at 30 days, and its size increased steadily over 90 days. Histochemical profiles showed a relative increase of type I, and decrease of type IIa and IIb fibers. Type IIc relative contribution increased proportionally with the latter decrease. This reinnervation pattern after nerve graft repair signals considerable functional remodelling of the affected motor units, and suggests a strong motoneuronal role in its determination.

98.6

ABERRENT CYTOARCHITECTURE OF GRAFTED SUPRA-CHIASMATIC NUCLEUS STILL CAN RESTORE CIRCADIAN RHYTHMICITY INDUCED BY AN IN SITU LESION G.J. Boer, H.A. Griffioen, H. Duindam and W.J. Rietveld (spon: ENA). Neth. Inst. for Brain Res., Amsterdam, NL; Lab. of Physiology, University of Leiden, NL.

Grafting of the fetal SCN has been shown to restore circadian rhythmicity in rodents subjected to lesions of this 'biological clock' nucleus. The characteristics of the surviving SCN needed to explain recovery are poorly understood. A study was initiated to correlate VP, VIP, SOM and NPY immunocytochemical staining of an E17 solid block graft with the course of recovery of drinking rhythm.

Recovery was seen in 30% of the animals and is usually visible after 2-3 weeks. Cells of the implanted fetal SCN are then still immature. VP and VIP graft/host efferents cannot be seen yet but become visible 2 weeks later. The existence of SOM efferents could not be established. The cytoarchitecture of the implanted SCN shows contiguously located clusters of VP and VIP neurons, but in contrast to in situ, SOM cells nestle outside this field. Afferent NPY fibers preferentially ramify on SOM cells. After 5 weeks maximal outgrowth is reached. All recovery animals revealed a surviving SCN of partially aberrant configuration together with efferent connections. However, these outcomes were also seen in some of the animals that did not recover.

In conclusion, grafted fetal SCN rearranged its cytoarchitectural development but it could still be functional. The results have so far not been conclusive on what aspect of the 'new' SCN is needed for recovery.

98.7

A NEW MODEL TO STUDY INTERACTION BETWEEN TRANSPLANTED FOETAL NEURONS AND THE MATURE CENTRAL NERVOUS SYSTEM: OPTHOTOPIC DORSAL ROOT GANGLION ALLOGRAFTS, C.M. Rosario*, H. Aldskogius, T. Carlstedt and R.L. Sidman. Dept. of Anatomy, Karolinska Institutet, Stockholm and Dept. of Neuropathology, Harvard Medical School, Southborough, MA 01772-9102.

Transplanted, foetal neurons are able to survive and form functional contacts in the central nervous system of mature host animals. Neuronal transplants, including dorsal root ganglia can survive and differentiate following heterotopic transplantation. However, no attempts appear to have been made to determine if foetal DRGs can survive and differentiate in an orthotopic location. In the present study we transplanted E-15 DRGs to replace excised lumbar DRGs in adult rats and analyzed survivability, phenotypic differentiation and process outgrowth from the transplants. These analyses have been made with conventional light microscopy, immunocytochemistry for CGRP, and neuroanatomical tracing methods, including retrograde tracing with single or double labels and with transganglionic tracing from the peripheral nerve to the spinal cord. The results show that transplanted DRG cells survive, differentiate and send axons peripherally into sciatic nerve and centrally through the dorsal root. Suggestive evidence for axonal ingrowth into the spinal cord has been obtained, but this is under further study with additional methods.

98.8

BIOCHEMICAL COMPARISON AND CHARACTERIZATION OF CULTURED BOVINE CHROMAFFIN CELLS AND RAT BRAIN SLICES CONTAINING BOVINE CHROMAFFIN CELL TRANSPLANTS. J. Ortega and J. Sagen. Dept. of Anat. and Cell Biol. Univ. of Il at Chicago.

Initial biochemical studies on rat brain slices containing bovine chromaffin cell transplants in the periaqueductal gray (PAG) revealed that 2-4 week old xenografts released significantly higher basal and nicotine stimulated levels of catecholamines (CA) and met-enkephalin (ME) than control brain slices. The purpose of the present study was to compare and characterize the biochemical and pharmacologic activity of isolated bovine chromaffin cells in vitro to that of transplanted bovine chromaffin cells. Bovine chromaffin cells were isolated using standard primary culturing techniques, and either transplanted into the rat PAG or plated for in vitro studies. Following a 2, 4, or 8 week period, brain slices (350 μm) containing the transplant and PAG were sectioned, placed in a chamber and superfused continuously (2.0 ml/min.) with oxygenated artificial CSF (37°C). Following a one hour stabilization period, perfusion samples before and after nicotine stimulation (60 μM) were collected and analyzed for ME and CA content, using RIA and HPLC, respectively. Studies on cultured bovine chromaffin cells consisted of the collection of basal and nicotine stimulated samples from chromaffin cells 2, 7, 14, and 21 days in culture. The results of this study reveal that cultured bovine chromaffin cells release basal and stimulated levels of both ME and CA up to 21 days in culture. Over the 21 day period the epinephrine:norepinephrine (E:NE) ratio declined from 4:1 to 2:1. This decline in epinephrine content can be attributed to the lack of steroidal influence from adrenal cortical cells. In contrast, samples collected from brain slices containing bovine chromaffin cell transplants showed no significant decrease in the E:NE ratio over an 8 week period. The results of this study indicate that the host environment can play an important role in modulating the activity of transplanted bovine chromaffin cells. The reasons for this are not completely clear, but endogenous steroidal activity could impart some influence on the biochemical activity of these xenografts. Supported in part by NIH grants NS25054 and NS28931.

98.9

ANOMALOUS CHOLINERGIC REINNERVATION OF THE INTERPEDUNCULAR NUCLEUS (IPN) BY THALAMUS TRANSPLANTS. T.C. Eckenrode*, M.Murray and F.Haun. Dept. of Anatomy & Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129

We showed previously that cell suspension transplants of embryonic habenula cells placed in neonatal or adult rats contain both Substance P (SP) and ChAT immunoreactive cells . However only peptidergic reinnervation of deafferented habenular target sub-nuclei of the IPN was seen in adult-transplanted hosts. To test specificity, we transplanted ventral thalamus cells (which do not normally project to the IPN) into adult hosts after IPN denervation. At 2 mos. post-implantation, thalamus transplants contain no SP-staining cells and the host IPN shows no peptidergic reinnervation. However the transplants do contain numerous well-differentiated ChAT-positive neurons, an unexpected result in that the cholinergic phenotype is normally seen in the thalamus only transiently, at mid-to-late gestation. Moreover ChAT staining is seen in the IPN of animals with thalamus transplants, in contrast to complete absence of ChAT staining in the IPN of lesion-only controls as well as animals with habenula transplants. These results show the adult-deafferented IPN is able to support cholinergic reinnervation from an anomalous source, but not from its normal afferents. Supported by NIH grants NS28856 (FH) and NS 16556 (MM).

98.10

HYPOTHALAMIC TRANSPLANTS CONTAINING HISTAMINERGIC NEURONS: II. ELECTROPHYSIOLOGY. Hanna Bergman*, George D. Prell[1] and Ann-Charlotte Granholm. Dept. Cell Biol., Univ. Linköping, S-581 85 Linköping, Sweden, [1]Dept. Pharmacol., Mount Sinai School of Medicine, New York, NY 10029. Histamine is a monoamine transmitter produced by neurons in the hypothalamic tuberomamillary nucleus. Extracellular electrophysiological recordings of histamine-type neurons in hypothalamic transplants in the anterior eye chamber of albino rats were performed. We found numerous spontaneously firing neurons with a mean firing rate of 2.8±0.4 Hz (n=27) and a mean spike duration of 1.0±0.1ms (n=16). Furthermore, 10 of 14 neurons responded to local application of the selective histamine autoreceptor agonist α-methyl-histamine (0.1-10 μM) with inhibitions of neuronal firing rate, and 5 of 6 cells responded to 5-200 μM histamine with inhibitions. The auto-receptor antagonist thioperamide caused excitations in 4 of 5 cells tested, at doses of 1-4 μM. The respective H_1 and H_2 antagonists dexchloropheniramine and ranitidine had no effects in any of the 3 cells tested. At present, levels of histamine and its metabolites, as well as the optimal fetal donor stage for histaminergic transplants are being examined. Taken together, these data indicate that histaminergic neurons in intraocular transplants of hypo-thalamic tissue will survive and develop many of the physiological features seen *in situ*. The project was supported by the Swedish MRC, grant #8650 and NINDS-28012. The autoreceptor compounds were generously provided by Dr. J.M. Arrang.

98.11

The Pineal Gland: Effects of Grafting Sites on Functional Recovery. Wutian Wu and David E. Scott Dept. of Anatomy & Neurobiology, Eastern Virginia Medical School, Norfolk, VA 23501.

Nighttime serum melatonin concentration increases significantly in pinealectomized rats in which pineal glands were grafted into the third cerebral ventricle. Recent studies demonstrated that there was no day-night variation in serum melatonin concentration in these animals. In the present investigation, pineal glands were grafted into several sites in pinealectomized rats, including the third cerebral ventricle, the renal capsule, the anterior chamber of the eye, and in situ (where the gland is normally found). Ten weeks after grafting, blood samples were collected twice from each rat and serum melatonin concentration was measured by RIA. Results demonstrate that nighttime serum melatonin concentration increased significantly in animals with pineal transplants in the third cerebral ventricle and the anterior chamber of the eye as compared with nongrafted pinealectomized host rats. Day-night variation in serum melatonin concentration was observed only in animals with pineal grafts in the anterior chamber of the eye. More neurites were found within the grafts in the latter site than any other graft location. These results indicate that only certain locations in the host can foster recovery of function after transplantation. Supported by National Science Foundation Grant No. BNS8709687.

98.12

INTERPRETING THE ABSENCE OF A BLOOD-BRAIN BARRIER (BBB) IN BRAIN GRAFTS. R.D. Broadwell, B.J. Baker, and W.F. Hickey*. Univ. MD Sch. Med., Baltimore, MD 21201, and Washington Univ. Sch. Med., St. Louis, MO 63110.

Although BBB vessels indigenous to CNS grafts are sustained, breakdown of the BBB in CNS grafts is attributable to: 1) rupture of interendothelial tight junctions in graft vessels by perfusion-fixation of the host; 2) damage to host vessels during graft delivery; 3) graft placement in proximity to normally permeable host vessels supplying the pial surface and circumventricular organs; 4) graft rejection vis-a-vis the host immune response. The latter is prevalent in xenogeneic grafts and in allografts associated with donor/host mismatch in major/minor histocompatibility complexes. Donor cells stained immunohistochemically for its MHC class I appear within the host spleen, suggesting a triggering of the host immune response. These cells most likely enter the general circulation through host cerebral vessels damaged during graft placement. Allografts between outbred donor and host of the same strain (e.g., Sprague Dawley or Wistar rats) likewise will reject; by 10 days post-grafting, the CNS grafts exhibit populations of immunohistochemically identifiable MHC classes I+ and II+ cells (microglia, macrophages, lymphocytes), and CD4+ (T-helper) and CD8+ (cytotoxic) lymphocytes. PC12 cell suspension grafts placed in the CNS of outbred hosts (Sprague Dawley rats) are rejected similarly. NIH Grant #NS18030.

99.1

PAIN REDUCTION BY ADRENAL MEDULLARY TRANSPLANTS IN THE SPINAL SUBARACHNOID SPACE OF TERMINAL CANCER PATIENTS. J. Sagen, A.P. Winnie*, H. Wang, T.J. Krolick*, and G.D. Pappas. Dept. of Anat and Cell Bio. and Pain Control Center, Univ. of Illinois at Chicago, Chicago, IL 60612.

Work in our laboratory over the past several years has demonstrated that the transplantation of adrenal medullary chromaffin cells into the spinal subarachnoid space can markedly reduce pain in rodents. This analgesia most likely results from the release of opioid peptides and catecholamines from the transplanted cells. The current study is an initial attempt to assess the potential for this approach to alleviate chronic pain in man. Approval for this preliminary study was obtained from the IRB of the University of Illinois. Consenting patients selected for the study were suffering from terminal cancer pain with reduced responsiveness to narcotic analgesics. Pain levels were determined using a Visual Analog pain scale prior to and at weekly intervals following the procedure. In addition, records of daily narcotic intake and activity were kept. When possible, CSF samples were collected via lumbar puncture for biochemical and cytological analysis before and at several intervals following transplantation. Of the first four patients selected for the study, three suffered from metastatic CA of the colon and one from metastatic CA of the breast. Human adrenal glands were obtained from the Regional Organ Bank of Illinois and adrenal medullary tissue was prepared for transplantation in our laboratory. Tissue was transplanted via lumbar puncture using a 14 ga Tuohy needle. Cyclosporine A was administered for two weeks beginning one day prior to the procedure. All four of the patients demonstrated progressive decreases in their pain scores over time, with a concomitant decrease in narcotic intake. Three of the patients remained pain free for over four months, with no recurrence of pain. One patient, who developed spinal cord compression secondary to metastasis was initially pain free, but the pain returned after 10 weeks. Spinal CSF samples revealed increased levels of both met-enkephalin and catecholamines for at least 4 months following the transplantation. Results of this study suggest that adrenal medullary transplants in the spinal subarachnoid space may be a promising approach for the management of chronic pain in humans. Supported in part by NIH grants NS25054 and NS28931.

99.2

DYNAMIC ASSESSMENT OF NEURAL GRAFT SURVIVAL IN THE INJURED CAT SPINAL CORD USING MAGNETIC RESONANCE IMAGING. E.D. Wirth III, D.P. Theele*, T.H. Marecl*, D.K. Anderson and P.J. Reier. Depts. of Neuroscience, Neurosurgery and Radiology, Univ. of Florida College of Medicine, Gainesville, FL 32610 and VA Medical Center, Cincinnati, Ohio.

Although previous work has demonstrated the usefulness of magnetic resonance imaging (MRI) for visualizing intraspinal transplants *in vivo*, the degree to which MRI can differentiate developing fetal neural tissue from evolving spinal cord pathology has not been investigated. Eight adult female cats received a hemisection injury at the L1 level, followed immediately by implantation of either embryonic cat spinal cord (SC) or neocortex (NCx) into the cavity. The spinal cords of two control animals were hemisected, but received no transplant. At each specified imaging interval (1, 2, 4, 8, 12, 16, 20 weeks post-grafting or post-lesion) multislice T_2-weighted (TR/TE = 2000/90 msec) and intermediate-weighted (TR/TE = 1000/30 msec) spin-echo images were obtained in the transverse and sagittal planes. Following sacrifice, low-power light microscopy was used for determination of transplant survival and correlation with MR images.

All control animals and graft recipients exhibited medium to high signal intensity on both intermediate and T_2-weighted images during the first four weeks following surgery. After 4-8 wks., the transplant site in three recipients remained slightly hyperintense on intermediate images, but became isointense with the host spinal cord on T_2 images. Histological analysis established that this appearance was indicative of graft survival. The transplant/lesion site in the controls and remaining recipients presented high signal for both sets of imaging parameters. Post-mortem specimens from this group revealed cavitation at the transplant/lesion site. We conclude that determination of transplant survival may be observed as early as four weeks post-grafting by a decrease in signal on T_2-weighted images. *Supported by American Paralysis Association #TC8704, NIH PO1 NS27511-01, NIH P41-RR-02278, PHS Award MH15737*

99.3

INCREASED VASCULARITY AND CYTOCHROME OXIDASE LEVELS IN FETAL SUSPENSION GRAFTS OF THE ADULT RAT SPINAL CORD. P.J.Horner*, Dept. Physiology, The Ohio State Univ., B.T. Stokes Dept. Physiology, The Ohio State Univ., P.J.Reier Dept. Neurosci., Univ.Florida and F.R.Sharp Dept.Neurology, VA Med Ctr, San Francisco, CA.

Anatomical and behavioral studies of graft-mediated repair in an experimental spinal cord contusion injury have indicated that fetal grafts can alter post-transplantation behavior (Stokes and Reier, 1990). Physiological factors that influence the graft microenvironment (Stokes and Reier, 1991) may play a vital role in this repair process. The present experiments examined the relationship between two such factors in 3 month transplants: metabolic capacity as assessed by cytochrome oxidase histochemistry and graft vascularity. Cytochrome oxidase experiments were conducted according to the technique of Wong-Riley (1979) and diaminobenzidine reacted horizontal spinal cord sections (20um) were analyzed via densitometry. To quantitate vascularity, cords were fixed in 4% paraformaldehyde, 3.5% glutaraldehyde in 0.1M Sorensons' PBS overnight, sectioned, treated with 2% osmium tetroxide, dehydrated and embedded in epon. Computer assisted morphometry was used to determine blood vessel number, surface fraction, area and orientation distribution. Transplants show levels of cytochrome oxidase significantly above host gray matter. Although the number of very small (<10um) vascular profiles is decreased in graft and adjacent graft/host regions, average vessel area and surface fraction are markedly increased in comparison to host gray matter. These data provide evidence of a metabolically-active transplant with a well established vascular network, characteristics which may be important in graft mediated repair.

99.4

ENHANCED ANTINOCICEPTION BY ADENOSINERGIC AGONISTS IN RATS WITH ADRENAL MEDULLARY TRANSPLANTS IN THE SPINAL SUBARACHNOID SPACE. J.L. Stark*, H. Wang, W.F. Silverman, and J. Sagen. Dept. Anat. and Cell Bio., Univ. of Illinois at Chicago, Chicago, IL 60612 and Dept. Morphol., Ben-Gurion Univ. Fac. Health Sciences, Beer-Sheva, Israel.

Our laboratory has demonstrated that the transplantation of adrenal medullary tissue into the spinal subarachnoid space of rats can induce potent analgesia. Neurochemical assays have revealed increased levels of norepinephrine and epinephrine in the spinal CSF for at least 6 months following transplantation of adrenal medullary tissue. Recent work in other laboratories has shown that there is a potent synergism between adrenergic and adenosinergic systems in the production of antinociception in the spinal cord. This may be important since spinal release of adenosine has been implicated as a neural basis for the analgesic effects of vibration in humans, and is a potential physiological means to increase antinociception following transplantation. Since the transplanted chromaffin cells release catecholamines, it should be possible to induce antinociception with subthreshold doses of adenosinergic agonists in animals with adrenal medullary transplants. To assess this, antinociceptive responses to intrathecal injections of several doses of A1/A2 adenosine agonist 5'-N- ethylcarboxamide adenosine (NECA) or A1 adenosine agonist R-phenylisopropyladenosine (R-PIA) were compared in animals with adrenal medullary and control striated muscle transplants. Results revealed a potentiation in the dose-responsiveness to NECA in adrenal medullary, but not control transplanted animals. Doses of NECA as low as 0.065 μg produced significant antinociception. This potentiation could be blocked by pretreatment with α-adrenergic antagonist, phentolamine, suggesting that catecholamines released from the transplants may mediate this effect. In contrast to NECA, adrenal medullary transplants did not alter responsiveness to R-PIA. Results of this study indicate that adrenal medullary transplants may potentiate antinociception to adenosinergic agonists via co-activation of α-adrenergic and A2 adenosine receptors, and suggest a possible means of increasing antinociception following adrenal medullary transplantation. Supported in part by NIH grants NS25054 and NS28931.

99.5

PAIN REDUCTION BY TRANSPLANTS OF POLYMER ENCAPSULATED BOVINE CHROMAFFIN CELLS IN THE RAT SPINAL SUBARACHNOID SPACE. H. Wang, P.A. Tresco, P. Aebischer, and J. Sagen. Dept. Anat. and Cell Biol., Univ. of Illinois at Chicago, Chicago, IL 60612 and Artificial Organs, Biomaterials and Cellular Technology, Brown Univ., Providence RI.

Studies over the past several years have indicated that the transplantation of adrenal medullary chromaffin cells into CNS pain modulatory regions may provide a renewable source of opioid peptides and catecholamines for the continued alleviation of pain. Donors for these studies have included both allogeneic and xenogeneic sources. Bovine adrenal glands may be an ideal donor source since chromaffin cells can be readily isolated in large amounts and the cells continue to produce high levels of opioid peptides and catecholamines. Previous findings in our laboratory have indicated that bovine chromaffin cells transplanted to the rat CNS can reduce pain. However, the survival of xenografts is limited by host immunologic responses. In order to circumvent this problem, bovine chromaffin cells were immunologically isolated by polymer encapsulation. Bovine chromaffin cell suspensions were encapsulated with an acrylic copolymer, and basal and nicotine stimulated release of catecholamines and met-enkephalin were determined. Either chromaffin cell-loaded or empty control capsules were transplanted in the rat subarachnoid space at the level of the lumbar enlargement. Baseline pain sensitivity and response to nicotine were determined using the tail flick, paw pinch, and hot plate tests. Results indicated that transplants of encapsulated chromaffin cells, but not control capsules, significantly reduced pain sensitivity following nicotinic stimulation. This analgesic response could be observed for at least 3 months following transplantation. Chromaffin cells could be identified immunocytochemically in the transplanted capsules after termination of behavioral testing. These results suggest that transplantation of encapsulated bovine chromaffin cells may be a means of alleviating pain without the complications of potential immunologic rejection. Supported in part by NIH grants NS25254 and NS28931.

99.6

ADRENAL MEDULLARY TRANSPLANTS REDUCE PAIN IN RATS WITH EXPERIMENTAL PAINFUL PERIPHERAL NEUROPATHY. A.T. Hama*, W.J. Miller*, H. Wang and J. Sagen. Dept. of Anat. and Cell Biol., Univ. of IL at Chicago, Chicago, IL 60612.

Recent work in our laboratory has demonstrated the effectiveness of adrenal medullary tissue in attenuating nocifensive responses to various noxious stimuli. Endogenous neuroactive substances such as catacholamines and opioid peptides, found abundantly in adrenal chromaffin cells, are thought to reduced nociception at the spinal level. Until recently, no satisfactory model of chronic neuropathic pain was available that would allow the examination of the effects of these compounds. Using a recently developed model of a painful peripheral neuropathy, we evaluated the effects of adrenal medullary chromaffin cells transplanted into the subarachnoid space. A peripheral neuropathy was induced by loosely tying 4 ligatures (4-0 chromic) around the right sciatic nerve. This procedure produces various pain syndromes including: allodynia, hyperesthesia and spontaneous pain. After baseline behavioral evaluation using innocuous and noxious stimuli, rats were given either adrenal medullary tissue or control striated muscle transplants. Animals were tested weekly for 12 weeks. Animals with adrenal medullary tissue transplants showed decreased allodynia to both cold plate and innocuous tactile stimuli. In addition the weight loss in control transplanted animals was attenuated by adrenal medullary transplants. Following termination of behavioral studies, the transplants, host spinal cord and sciatic nerve were analyzed morphologically using immunocytochemistry and electron microscopy. The sciatic nerve on the ligated side showed a marked decrease in large diameter primary afferents, with smaller axons relatively spared. Immunohistochemical analysis of spinal cord dorsal horn showed reduced levels of substance P and calcitonin gene-related peptide ipsilateral to the ligation at 2 weeks. The increased incidence of chromatophilic cell bodies ("dark neurons") in the dorsal horn ipsilateral to the ligation was reduced in animals with adrenal medullary transplants but not control transplants. These findings indicate that adrenal medullary transplants may be effective in reducing neuropathic pain. Aided by a grant from the Paralyzed Veterans of America Spinal Cord Research Foundation.

99.7

ANATOMICAL AND BEHAVIORAL EFFECTS OF TRANSPLANTS IN SPINAL KITTENS. D.R. Howland, B.S. Bregman, A. Tessler, and M.E. Goldberger. Department of Anatomy and Neurobiology, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Spinal cord transplants (E21-E26) survive in the transected thoracic spinal cords of newborn kittens and alter the behavioral and anatomical development of the animals. Behavior of normal kittens, of spinal kittens and of spinal + transplant kittens was studied from birth to 5 mos. In spinal kittens, a transplant accelerates the development of overground locomotion by about 6 wks. Although spinal kittens achieve quadrupedal locomotion (overground and treadmill) the performance of transplant animals is always superior in weight support, postural stability and coordination between fore and hindlimbs. HRP and immunocytochemistry (ICC) demonstrate that no descending systems grow past the lesion in spinal animals. ICC identified several host fiber systems characterized by fine axons bearing varicosities in the transplant. Descending serotoninergic and noradrenergic systems grow extensively in the transplant. These systems also re-enter the host caudal to the transplant and the serotoninergic system grows as far caudally as L6. The multiorigin Substance P and dorsal root origin Calcitonin Gene Related Peptide are found throughout the transplant and accumulate densely in many Substantia Gelatinosa-like regions in the transplant. These results indicate that embryonic spinal cord transplants support the growth of host systems and affect the locomotor development of neonatal kittens with complete spinal cord transections. (Supported by Grant NS24707).

99.8

AXON GROWTH INTO SCHWANN CELL GRAFTS PLACED IN LESIONED ADULT RAT SPINAL CORD. **C.L. Paino, C. Fernandez-Valle, and M.B. Bunge, The Miami Project, Univ. of Miami Sch. of Med., Miami, FL 33136**

Schwann cell (SC) grafts placed in lesioned adult rat spinal cord elicits axonal ingrowth (Paino and Bunge, Soc. Neurosci. Abst. 16:1282 '90). SC-only implants were prepared in vitro on collagen with either dissociated SCs (dSC) or SCs associated with basal lamina they assembled (blSC). The collagen was rolled, enclosing the SCs, and placed in spinal cord cystic cavities that were induced photochemically (Cameron et al., Exp. Neurol. 109:214-23 '90) at the T9 level in adult female Sprague-Dawley rats either 5 or 28 days earlier. No immunosuppression was used. At 14 and 28 days and 3 and 6 months after grafting, animals were perfused and analyzed histologically with silver and toluidine blue stains and electron microscopy (EM). By 14 days bundles of unmyelinated and occasional thinly myelinated axons populated dSC and blSC implants. By 28 days and thereafter, great numbers of unmyelinated and myelinated axons were present in all preparations. By contrast, acellular collagen grafts were devoid of axons. EM of blSC implants at 28 days and 3 and 6 months showed that unmyelinated axons were at least 5X more numerous than myelinated axons; myelin thickness was greater by 3 months. Longitudinal sections of the host/implant junction revealed isolated or small bundles of axons associated with elongated (SC-like) cells extending at least 1mm caudal to the graft into degenerating corticospinal tract areas. These findings indicate that SCs induce profuse axon growth and suggest that, in long term grafts, axons may leave the SC implant and extend for some distance in degenerating white matter tracts if accompanied by SCs. (Supported by The Miami Project, NIH 09923, and The Spanish Ministry of Education).

99.9

INTRASPINAL TRANSPLANTS OF FETAL RAPHE NEURONS INHIBIT SPROUTING OF CGRP-IMMUNOREACTIVE PRIMARY AFFERENTS IN THE RAT INTERMEDIOLATERAL CELL COLUMN. V.R. Holets, R. Melinek and S.M. Onifer. The Miami Project and Department of Neurological Surgery, University of Miami, Miami, FL 33136.

Collateral sprouting and reactive synaptogenesis are responses of the CNS to injury that are well documented in the hippocampus and spinal cord. The response of spinal afferents to injury has been studied with an emphasis on changes in the dorsal horn. The present study focuses on changes in the afferent input to the intermediolateral cell column (IML) of the rat following a serotonin-depleting lesion, and the effect of transplantation of fetal raphe neurons into the thoracic spinal cord at varying times post-lesion.

Adult rats received an intrathecal injection of 5,7-DHT to lesion the raphe-spinal 5-HT fibers. An increase in the density of calcitonin gene-related peptide (CGRP)-immunoreactive (IR) fibers was observed in IML at 1 and 3 mo post-lesion. One wk post-lesion some animals received an intraspinal transplant of E14 raphe neurons in cell suspension into T8-T10 segments. The fetal neurons survived and reinnervated IML at early time points post-transplant (1 mo), and spread to reinnervate the dorsal and ventral horns at later times (3, 6, and 9 mo). There was no increase in the density of CGRP-IR fibers in IML at any level of the spinal cord as compared to lesion controls. These findings suggest that the ingrowth of the 5-HT-IR fibers from the transplant inhibits the sprouting of the CGRP-IR afferents to IML. Experiments are underway to determine the pattern of outgrowth of fetal raphe neurons transplanted into the spinal cord at later time points (3 mo) post-lesion when the CGRP-IR fibers have already increased in density in IML. Supported by The Miami Project, The Daniel Heumann Fund for Spinal Cord Research, and the American Heart Association.

99.10

INFLUENCE OF FETAL SPINAL CORD TRANSPLANTS ON RECOVERY OF MOTOR FUNCTION AFTER SPINAL CORD INJURY. Ellen Kunkel-Bagden, Paul J. Reier, Hai-Ning Dai* and Barbara S. Bregman, Dept. of Anatomy and Cell Biology, Georgetown Univ., Washington, DC 20007.

Fetal spinal cord transplants placed into the damaged spinal cord of a neonate promote the recovery of locomotor function. We wished to determine whether transplants would also influence the recovery of motor function after spinal cord injury in the adult. Transplant-mediated recovery of function was assessed after spinal cord hemisection with a series of quantitative tests of reflex responses and locomotion. Adult male rats (14) were tested for reflex responses and trained to cross runways and walk on a treadmill. The rats then received a mid-thoracic "over-hemisection" (HX, N=5) followed immediately by the placement of embryonic (E14) spinal cord transplants into the lesion site (HX+TP, N=6). Three normal animals served as controls (CON). After 4 weeks the animals were again trained and tested for reflex responses and locomotor function. Footprint analysis revealed that animals with a HX had an increased base of support, but the presence of the transplant (HX+TP) decreased this toward normal (CON) values. Animals with transplants were also able to cross the runways faster and with fewer errors than the hemisected animals. Analysis of the lesion sites revealed that the transplant-mediated alterations in recovery were dependent upon good appositon of the transplant with the host spinal cord. Although the ingrowth of axons from the host CNS into the transplant in the adult is less extensive than after similar lesions at birth, this anatomical plasticity is sufficient to promote recovery of function. Supported by NIH NS27054, NS19259, NS01356 (to BSB) and NS27511 (to PJR).

99.11

PARAPLEGIA IN EXPERIMENTAL ANIMALS: IT'S ANATOMY UNDER DIFFERENT CONDITIONS OF LESION AND TRANSPLANTATION. P. Rai and G.D. Das. Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN 47907.

The role of neural transplantation in the injured spinal cord was investigated in adult rats. Central hemorrhagic lesions were made at Thoracic (T-10/T-11) and Lumbar (L-2/L-3) levels of the spinal cords, and embryonic neocortex, brainstem or spinal cord tissue was transplanted. Control animals did not receive transplants. The ventral funiculi (VF) in thoracic lesion and motor neurons (MN's) in lumbar lesion animals were either spared or damaged in both experimental and control groups. After 2-3 months, the animals were sacrified and their spinal cords were examined. Behavioral and histological data showed that animals with VF damage at T10/T-11, irrespective of the presence or type of neural transplant, were paraplegic. Similarly, animals with L-2/L-3 lesions showed paraplegia when there was MN damage, irrespective of the presence or type of neural transplant. The animals in both groups with VF or MN sparing did not show paraplegia. These results support earlier reports, emphasizing the role of spared VF in thoracic spinal lesions in spontaneous recovery from paraplegia. Sparing of MN's in lumbar spinal lesions is also essential to prevent paraplegia. Finally, embryonic neural transplants did not possess any ameliorative properties under these conditions of spinal cord trauma.

100.1

CHARACTERIZATION OF PROTEIN KINASE C ACTIVITY IN CEREBRAL MICROVESSELS OF AGED RATS. K. Morgenstern*, P. Grammas and O. Hanson-Painton*. University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

The phospholipid and Ca^{2+} dependent protein kinase, PKC, is a critical component of receptor-mediated signalling pathways. Previous studies in brain demonstrate that PKC regulation is altered in aging and dementia. In this study, we examined microvascular PKC in isolated cerebral microvessels (MVs). Partial purification of PKC from MVs, using Q-Sepharose batch adsorption and single step salt elution into microfuge tubes, resulted in an enrichment of PKC specific activity in both the cytosolic and particulate fractions by 52- and 41-fold, respectively. In addition, single step fractionation of PKC resulted in greater than 100% yields, suggesting that a PKC inhibitor was present in crude homogenates. This was supported by the finding that purified rat brain PKC activity was inhibited in a dose-dependent manner by the Q-Sepharose effluent (50% at 10 μg/ml). MV crude cytosol from aged rats showed a 56% decrease in PKC specific activity compared to control while minor differences in PKC levels were observed after fractionation on Q-Sepharose. These results demonstrate the utility of batch adsorption to Q-Sepharose for the study of PKC and PKC inhibitory activities in MVs. Furthermore, results from aged MVs suggest that alterations in PKC inhibitor levels could underlie altered PKC activity and cell responsiveness in aging. Supported by American Health Assistance Foundation and OCAST HSO-008.

100.2

BLOCKING OF IL-1 INDUCED INFLAMMATION BY MONOCLONAL ANTIBODIES TO ADHESION MOLECULES James A. Martiney, Tina M. Calderon*, Joan W. Berman*, and Celia F.Brosnan*. Albert Einstein College of Medicine, Bronx, NY 10461.

Previous results from this laboratory have shown that intraocular injection of interleukin-1b (IL-1b) in the rabbit retina induces an acute and chronic inflammatory response associated with the epiretinal vessels. Analysis of this reaction have defined the following series of events associated with the acute inflammatory episode: a mononuclear (MN) and polymorphonuclear (PMN) exudate accompanied by an increase in vascular permeability and hemorrhage that peaks between 6 and 24 hours post-intraocular challenge (PIC). No further migration of PMN or hemorrhagic events occurred after 24h PIC. A more persistent MN cellular inflammation followed this acute episode. To test the role of specific adhesion molecules in these events, monoclonal antibodies (MAbs) directed against CD18,the common beta chain of the integrin LFA-1, (R15.7, provided by R.Rothlein) and against a novel endothelial cell adhesion molecule specific for monocytes (IG9, Calderon,et.al., Circulation V82, p.III-94,1990) were employed. The results show that significant inhibition of all parameters of inflammation was achieved with both MAbs,including a reduction of the IL-1-induced increase in vascular permeability and hemorrhage. However, the pattern of reduction of cellular inflammation was different between the two MAbs. With the MAb R15.7, the most striking change was a decrease in PMN and MN extravasation whereas with MAb IG9 both intravascular and extravascular PMN and MN cell numbers were decreased. Interestingly, contralateral eye involvement due to systemic effects was also inhibited in these animals. Supported by NS11920 and 07089.

100.3

VASCULAR REMODELING ADJACENT TO BRAIN IMPLANTS OF EMPTY AND PC12 CELL-LOADED POLYMER CAPSULES. C.B. Jaeger[1], P. Aebischer[2], S.R. Winn[2], P.A. Tresco[2], L.A. Greene[3]. [1]CPR, Purdue Univ.SVM , W. Lafayette, IN 47907; [3]Artificial Organ Lab., Brown Univ., Providence, RI 02912; [3]Dept. of Pathol. Columbia Univ., New York, NY 10032.

Previous work indicated that repair of the blood-brain barrier (BBB) occurs following implantation of polymer capsules. Here we studied the fine structure of brain capillaries at the interface of polymer capsules and host brain. Capsules were prepared from polyvinyl-acrylic copolymer tubing with a molecular cut-off of 50KDa. Suspended PC12 cells were loaded into tube segments of 3- to 5 mm and the ends were closed with liquid polymer. Empty and cell-filled capsules were implanted in the brains of rats or guinea pigs. At 1/2, 1 and 2 months animals were euthanized and the ultrastructure of capillaries and host brain tissue near implants was examined. Structural changes were related to BBB function as indicated by Evans blue marker. In short-term implants, endothelial cell junctions near the capsule surfaces were leaky to serum proteins. This correlated with gaps in the junctions, and non-overlapping and open junctions. Double layered endothelial cells with interdigitating, multilayered cytoplasm was seen in newly formed vessels. Macrophages, processes of reactive astroglia, fibroblasts and collagen bundles were noted in the capsule membrane and near capillaries. Integrity of the BBB was observed at two months following implantation of empty and cell-filled capsules. Changes in capillary structures could reflect transient alterations of the CNS microenvironment. The data are relevant for evaluating the usefulness of PC12 cell-filled capsules as alternatives to neural tissue implants. Supported by grant No. NS27694 from NINDS.

100.4

EXTRACELLULAR SPACE VOLUME FRACTION IN THE REGION OF A BACTERIAL CEREBRITIS. W. Lo and D. McNeely*. Dept. of Pediatrics, Ohio State Univ. Columbus, OH 43205.

As a first step in studies which seek to modify the development of inflammatory brain edema, we sought to measure the brain extracellular space volume fraction (VFecs) in the brain surrounding a bacterial cerebritis. The VFecs was measured with an ion-selective microelectrode sensitive to tetramethylammonium chloride (TMA-ISM). Sprague-Dawley rats were intracerebrally inoculated with S. aureus; the VFecs was measured 4 days later when the blood-brain barrier is known to be permeable. The VFecs in bacterially inoculated animals was 49% +/- 7% (n=3), and in controls was 24.5% +/- 8.5% (n=4)($p<0.01$) The VFecs could be reliably measured down to 1000 microns below the cortical surface, and the regional increase in the VFecs could be mapped in a band surrounding the bacterial inoculation site.

This two-fold increase in the VFecs occurs in the cortex and demonstrates that a pronounced expansion of the VFecs can occur in gray as well as white matter in an acute inflammatory lesion.

(Supported in part by NINDS k08-NS01235)

100.5

Changes in the blood-brain barrier to macromolecules after an excitotoxic lesion in the CNS , by S. Marty*, I. Dusart and M. Peschanski, Groupe de Recherche Sce Médecine Nucléaire, CHU Henri-Mondor, 94010 Créteil cédex, France

In situ injection of an excitotoxin induces a rapid neuronal death and profound changes of glial and vascular cell populations. Among these, we have described a massive leakage of the blood-brain barrier (BBB) to macromolecules, at the same time GFAP immunostaining disappears. After a few weeks, however, GFAP+ hypertrophied astrocytes reappear and send processes that surround blood vessels. This observation led us to hypothesize that these reactive astrocytes may tend to reform a *glia limitans* eventually limiting the functional alteration of the BBB. To check this hypothesis, we have studied, in parallel, the leakage of the BBB to peroxidase and the astroglial environment of blood vessels.

Two groups of rats were sacrificed one day to one year after KA: (i) in the first group HRP was allowed to circulate for 45 minutes after iv injection. Rats were perfused with mixed aldehyde, the brain was cut on a cryostat and peroxidase was revealed histochemichally using TMB. (ii) a second group was directly perfused and brain sections were processed using standard electron microscopy techniques.

Two periods were defined : (i) As soon as twenty-four hours, and up to two months after KA, leakage of the blood-brain barrier was visible. During the same period, there was a swelling then a disappearance of astrocytic endfeet in apposition to the vascular walls. Swollen astrocytes, characterized by a pale cytoplasm and few intermediate filaments were observed during the first two weeks, sometimes exhibiting signs of cell suffering. (ii) After this period, up to one year after KA, a leakage of the BBB was no longer observed. In parallel, astrocytic processes making a basal lamina were systematically observed around endothelial cells, in most cases totally surrounding them although without apposition to the vascular wall.

The present results indicate that the evolution of the BBB for peroxidase is closely paralleled in the KA lesion by astroglial changes. The leakage of BBB occurs during retraction of endfect and recovery during rebuilding of an abnormal *glia limitans* by reactive astrocytes. (supported by MRT grant 106728).

100.6

IMPROVED BRAIN UPTAKE OF A CHEMOTHERAPEUTIC AMINO ACID WITH HIGH AFFINITY FOR THE CEREBROVASCULAR LARGE NEUTRAL AMINO ACID TRANSPORTER. Y. Takada, D. Vistica*, N. Greig*, S.I. Rapoport, and Q.R. Smith. Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20892.

The blood-brain barrier restricts the brain uptake of many hydrophilic drugs and limits their usefulness in the treatment of brain diseases (e.g., brain tumors, AIDS, Alzheimer's disease). In an attempt to improve brain delivery through facilitated transport, agents were sought with high affinity for the cerebrovascular nutrient carriers of the blood-brain barrier. One such agent that was found was D,L-2-amino-7-bis[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-naphthoic acid (NAM), an analog of the poorly penetrating anticancer drug L-melphalan. NAM, because of its increased hydrocarbon mass on the amino acid side chain, was predicted to have improved affinity for the large neutral amino acid carrier (L-system) of the blood-brain barrier. Following tritium labelling and examination of cerebral uptake in rats, NAM was shown to be taken up into brain at a ~10-30-fold greater rate than L-melphalan at tracer concentrations. Transport was BCH sensitive (>95%) and saturable with a V_{max} of ~1 nmol/min/g and a K_m of <1 μM. For comparison, the K_m of L-melphalan is ~150 μM, whereas the K_m of L-phenylalanine, the highest affinity endogenous amino acid, is ~10 μM. The primary degradation product of NAM (dechlorinated NAM) was also shown to be transported into brain by the L carrier, though with less affinity (K_m ~6 μM). The results demonstrate that rational drug modification can be used to improve drug delivery to brain via the saturable blood-brain barrier nutrient carriers.

100.7

EFFECT OF ACUTE AND CHRONIC HYPERTENSION ON THE BRAIN UPTAKE OF LIBENZAPRIL IN RATS. J-P Tang[1,*], S. Melethil,[1] F. Douglas[2,*], A. Rakhit[2,*] and G. Kochak[3,*]. [1]Schools of Pharm. & Med., Univ. of Missouri, Kansas City, MO, and Ciba-Geigy Corp., [2]Summit, NJ, and [3]Ardsley, NY.

Brain uptake of libenzapril (LZP) in Sprague-Dawley (SD) rats was essentially similar at MAP values (± SD) of 96 ± 4 (normal, n=6) and 148 ± 2 mmHg (phenylephrine induced acute hypertension, n=6). However, at a MAP of 171 (± 2) mmHg (n=8), uptake of LZP by all the 7 regions examined (medulla, olfactory lobe, colliculus, pons, diencephalon, cortex and cerebellum) was significantly higher compared to the 2 groups with lower MAPs. These results show that the effect of acute hypertension on brain permeability of LZP is observed only above a certain threshold pressure and are consistent with pressure-mediated autoregulation of cerebral blood flow.

Brain uptake in spontaneously hypertensive (SH) rats (chronic case) with a MAP of 149 ± 10 (n=8) mmHg was higher than the corresponding values in the SD group with the MAP of 148 mmHg in 6 out of the 7 regions. Since it was shown that the brain uptake of LZP was similar in SD and Wistar-Kyoto (normotensive controls for SH rats), these results show that the blood-brain barrier is more permeable to LZP in chronic hypertension. This enhancement in permeability may be related to morphological differences in the vascular endothelium. (Supported in part by Ciba-Geigy Corp.).

100.8

PROSTAGLANDIN D_2 PRODUCTION IN HUMAN CEREBROMICROVASCULAR ENDOTHELIUM. F. Bacic, S. Uematsu, R.M. McCarron and M. Spatz. LNNS, NINDS, NIH, Bethesda, MD 20892.

Prostaglandin D_2 (PGD_2), a cyclooxygenase product of arachidonic acid metabolism was detected in various tissues including brain in many animal species and man. The cellular origin of PGD_2 in human brain is still uncertain although its synthesis and metabolism are well documented. Recently we demonstrated the presence of PGD_2 among other prostaglandins in the medium obtained from cultured endothelium (EC) derived from cerebral capillaries, and small and large microvessels. Here we show that PGD_2 release from the EC can be augmented by vasoactive peptides. Endothelial cultures were established from isolated capillaries and microvessels of human brain by a modified technique of Gerhard et al. (*Brain Res. Bull.*, 1988). Confluent propagated EC were washed and preincubated with 1 ml serum-free medium (Gibco 199) for 30 min at 37°C. PGD_2 release was evaluated after 1-4 hrs of incubation with angiotensin II [Ang II (10^{-8}M-10^{-6}M)], arginine-vasopressin [AVP (10^{-8}M-10^{-6}M)] in the presence or absence of [Sar^1, Ala^8]-Ang II (10^{-5}M) or [1-(β-mercapto-β,β-cyclopentamethylene propionic acid) POMT, 10^{-6}M], respectively; by immunoassay with the use of specific PGD_2 antibodies. Ang II and AVP greatly increased PGD_2 release from the EC into the medium. AVP was a more potent inducer than Ang II. The stimulatory effect of Ang II and AVP was almost completely inhibited by their specific receptor antagonists [(Sar^1, Ala^8)-Ang II and POMT-AVP, respectively]. The inducible increase of PGD_2 was also inhibited by pretreatment with cyclooxygenase blockers (ASA, indomethacin). These findings represent the first demonstration of receptor-mediated release of PGD_2 from EC of human cerebral capillaries and microvessels induced by well known vasoconstrictive peptides.

100.9

EXPERIMENTAL EVIDENCE OF ENZYMATIC BARRIER PROTECTING BLOOD-BRAIN BARRIER FROM LEUKOTRIENE C_4 T. Baba, K. L. Black, T. Inamura* and D. P. Becker. Division of Neurosurgery and Brain Research Institute, Univ. of California, Los Angeles, CA 90024.

We previously reported that intracarotid infusion of leukotriene C_4 (LTC_4) selectively increases blood-brain barrier (BBB) permeability within ischemic tissue in the brains of middle cerebral artery (MCA)-occluded rats. To reveal the mechanism how LTC_4 selectively opens the BBB in brain ischemia, and not to normal brain, BBB permeability in the MCA-occluded rats was determined by quantitative autoradiography using ^{14}C-aminoisobutyric acid, with/without pretreatment of acivicin, an inhibitor of gamma glutamyl transpeptidase (γ-GTP). 72 hours after MCA occlusion, γ-GTP activity had disappeared in ischemic areas, LTC_4 (4 μg total dose) infused into the carotid artery ipsilateral to the MCA occlusion after 72 hrs selectively increased unidirectional transfer constant for permeability, Ki, approximately two fold within ischemic tissue. No effect on BBB permeability was seen within non-ischemic brain tissue or in ischemic tissue after only 24 hours post MCA occlusion, when γ-GTP activity still remains moderately in histochemistry. 24 hours after MCA occlusion, however, LTC_4 could increase Ki with pretreatment of acivicin. We also examined the effects of acivicin on γ-GTP histochemistry and HPLC. Acivicin almost completely blocked both the activity of γ-GTP in brain capillaries and the metabolism of LTC_4 in isolated bovine brain capillaries. We further examined binding of LTC_4 to isolated bovine brain capillaries with/without acivicin. After 10 minutes of incubation, LTC_4 binding markedly decreased in the samples without acivicin, while it remained high in those with acivicin. These findings indicate that there is "enzymatic barrier" which protects normal brain capillaries from the vasogenic effects of LTC_4.

100.10

SELECTIVE OPENING OF THE BLOOD-TUMOR BARRIER BY INTRACAROTID INFUSION OF LEUKOTRIENE C4-- DOSE RESPONSE STUDY. C.C.Chio*, K.L.Black, T.Baba. Div. of Neurosurgery, Univ. of Calif., Los Angels, Sch. of Med., Los Angels, CA 90024

Leukotriene C4 (LTC4) has been shown to selectively increase the blood-tumor barrier permeability after intracarotid infusion in RG-2 tumors in rats. In this study , four doses of LTC4 (Vehicle, 0.5 μg, 5μg and 50 μg) were infused into right internal carotid artery for 15 minutes in female Wistar rats (n=7 in each group). Rats were implanted with RG-2 tumors in the right basal ganglion 10 to 11 days before. Blood-tumor permeability was determined by quantitative autoradiography using ^{14}C aminoisobutyric acid.

The unidirectional transfer constant for permeability (Ki) within the tumor was increased two-fold in the 5 μg and 50 μg groups. No significant increase was observed after 0.5 μg infusion. B.P., Hct, body temperature, and arterial blood gas all remained within physiological range. Maximal opening of tumor-blood barrier by LTC4 in tumors may be achieved in rats with a total dose of 5 μg infusion.

100.11

MECHANISMS OF BLOOD-BRAIN BARRIER BREAKDOWN IN MULTIPLE SCLEROSIS. L. Claudio, C. S. Raine, C.F. Brosnan* Department of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.

The blood-brain barrier (BBB) is characterized by interendothelial tight junctions, few pinocytotic vesicles and high mitochondrial content in CNS capillary endothelium. We have analyzed ultrastructural and morphometric features of capillaries from brain biopsies of 8 cases of multiple sclerosis (MS). The data were expressed as the percentage of endothelial cell area occupied by the respective organelles. The values for vesicular content ranged from 0.53% in endothelium located within normal-appearing CNS parenchyma to 1.1% in endothelium located within a lesion. Mitochondrial content ranged from 10.87% in normal areas to 4.72% in lesions. Thus, these results showed an inverse correlation between transcytotic vesicles and mitochondrial content in endothelial cells. The endothelium also showed a high number of cytoplasmic projections protruding into the lumen, a feature commonly associated with active inflammation. In acute inflammatory lesions there was evidence of gaps between endothelial cells in venules. Interendothelial tight junctions in capillaries appeared normal. There were no significant changes in coated vesicle area or the in thickness of capillary basal laminae. Our previous studies on experimental autoimmune encephalomyelitis (EAE), a laboratory model of MS, have shown that clinical signs correlate with increased vesicular transport and decreased mitochondrial content in capillary endothelium. Taken together, these data suggest that vesicular transport is an important mechanism of edema formation in CNS inflammation and that a high mitochondrial content may play a major role in the maintenance of BBB function. Supported in part by NIH grants: NS 11920, NS 08952, NS 07098

100.12

INCREASED PERMEABILITY ACROSS THE BLOOD-NERVE BARRIER OF ALBUMIN GLYCATED *IN VITRO* AND *IN VIVO* FROM PATIENTS WITH DIABETIC POLYNEUROPATHY. J. F. Poduslo and G. L. Curran*. Molec Neurobiol Lab, Depts of Neuro & Biochem/Molec Biol, Mayo Clinic, Rochester, MN 55905 USA

The blood-nerve transfer of human plasma albumin (ALB) glycated with D-glucose was investigated by measuring the permeability coefficient-surface area (PS) product of the BNB to radioiodinated ALB in normal rat sciatic nerve. ALB, isolated by CM-Affi-Gel Blue chromatography, was glycated *in vitro* for 1, 3, 10, 19 and 30 weeks. Glycated ALB (gALB) was separated from ALB by boronate-affinity chromatography. The efficiency of this separation was assessed by chromatography of ALB glycated with [^{14}C]-glucose and by rechromatography of isolated ALB and gALB after radioiodination. The gALB was also shown to have a higher M_r and be completely separated from ALB after SDS-PGE in a TRIS-Borate-EDTA buffer. After 1 week of glycation, the gALB-PS was 2.2 fold greater than the ALB-PS (0.724 ± 0.063 S.D. $\times 10^{-6}$ vs. 0.328 ± 0.053 S.D. $\times 10^{-6}$ ml/g/s; $P < 0.0001$) and increased with the time of glycation to a maximum value of 16.2 fold at 30 weeks (4.656 ± 1.117 S.D. $\times 10^{-6}$ vs. 0.288 ± 0.042 S.D. $\times 10^{-6}$ ml/g/s; $P < 0.0001$). The PS of gALB isolated from patients with diabetic neuropathy was significantly increased ($P < 0.0001$) compared to the PS for ALB isolated from the same patients. It is hypothesized that the increased permeability of gALB across the BNB, as well as the observed quantitative increase in ALB, IgG, and IgM in patient sural nerve biopsies (Poduslo et al, PNAS 85:4879-4883, 1988), contributes to osmotic changes in the nerve microenvironment which may play a role in the development of the neuropathy over a prolonged period of time. (NS14304-P4)

100.13

DISCORDANT MATURATION OF THE PERINEURIAL-NERVE AND BLOOD-NERVE PERMEABILITY BARRIERS IN RATS.

N. A. Azzam, A. A. Zalewski, and R. N. Azzam*. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Minifascicles of nerve fibers are present in cables formed in silicone tubes and in the healed anastomosis site between cut nerve ends. Because blood vessels are excluded from the endoneurium of these fascicles, the axons are protected only by a perineurial permeability barrier. This situation is different from normal where the passage of agents into the endoneurial environment of the nerve fibers is regulated by two barriers, the perineurial-nerve barrier (PNB) and the endoneurial blood-nerve barrier (BNB). We have begun a series of experiments to determine how and why barriers develop. We report here the results of a developmental study of the PNB and BNB, examined with the permeability tracer horseradish peroxidase (HRP). The tracer was administered intravenously (IV) or topically and localized in sciatic nerve of rats by a histochemical reaction seen by electron microscopy (EM). No barriers were present 1-9 days postnatally, and IV or topically applied HRP flooded the endoneurium. At 15 days, IV HRP leaked into the nerve from most endoneurial vessels whereas topically applied HRP was retained outside the nerve. Some vessels continued to leak HRP at 30 days, but all were impermeable at 100 days. EM revealed that intercellular junctions were present between endothelial cells of the BNB, but not the perineurial cells of the PNB, at 3 days. Despite this difference, perineurial cells formed impermeable junctions before endothelial cells. Our results demonstrated that the PNB developed before the BNB. Further studies are needed to determine whether the same or different factors govern barrier formation in nerve.

100.14

SATURABLE TRANSPORT OF NEUTRAL AND BASIC AMINO ACIDS AT THE BLOOD-NERVE BARRIER OF THE RAT PERIPHERAL NERVE.

K.C. Wadhwani, Q.R. Smith and S.I. Rapoport. Lab of Neurosciences, NIA, NIH, Bethesda, MD 20892.

Unidirectional transport of L-leucine, L-arginine, and L-glutamic acid from blood into rat sciatic nerve were quantitatively determined, using an in-situ perfusion technique (Wadhwani et al. Am. J. Physiol. 258: R1436, 1990). The mean permeability-surface area products (PA) of ^{14}C-L-leucine for the blood-nerve barrier (BNB) was 61 ± 5 x10^{-5} ml/s.g wet wt (n = 4), and decreased by >90 % when 10 mM [L-leucine] or 10 mM [BCH] was added to the perfusion medium. Transport appeared stereospecific as 10 mM [D-leucine] produced less inhibition than 10 mM [L-leucine]. Mean PA of ^{14}C-L-arginine was 20 ± 2 x10^{-5} ml/s.g (n = 5), and fell by 50 % when perfusion medium contained 10 mM [L-arginine]. At 20 or 50 mM [L-arginine], mean PA of ^{14}C-L-arginine was 4 ± 1 x10^{-5} ml/s.g wet wt (n = 5). No significant change was produced in BNB PA of ^{14}C-L-arginine when perfusion media contained either 50 mM [BCH], 50 mM [MeAIB], or Na-free 165 mM [Tris]-buffer. Mean BNB PA of ^{14}C-L-glutamic acid was 2 ± 1 x10^{-5} ml/s.g (n = 5), and did not differ when perfusion medium contained either 10 or 50 mM [L-glutamic acid]. Our results demonstrate the presence of a carrier mediated transport system for L-leucine, and a different system for L-arginine, at the blood-nerve barrier of the rat peripheral nerve.

100.15

EFFECT OF SELECTIVE ASTROGLIAL DAMAGE ON BLOOD-BRAIN BARRIER DEVELOPMENT IN VIVO.

J.M. Krum. Dept. of Anatomy, George Washington University Medical Center, Washington, D.C. 20037.

Recent studies have suggested that astrocytes can induce certain endothelial blood-brain barrier (BBB) characteristics in vitro. In order to determine if intact perivascular astroglia are necessary for development of BBB properties in vivo, neonatal rats aged 6-21 days received systemic injections of 6-aminonicotinamide (6AN), an antimetabolite of nicotinamide which specifically affects glial cells. Several BBB functions were examined, including the BBB to endogenous (serum albumin) and exogenous (intravascularly administered HRP) protein and endothelial expression of the glucose transporter antigen (GTA). Ultrastructural examination revealed no damage to endothelial cells or neurons, although massive perivascular astroglial degeneration occurred as a result of cytotoxic edema. Endothelial GTA expression remained at normal levels for each age examined. In animals younger than two weeks postnatal, even though astroglia were significantly diminished, no defect in the BBB was observed. Protein extravasation occurred only in animals older than two weeks, and leakage progressively increased with age. The destruction of astroglial endfeet does not appear to influence the expression of endothelial GTA, but does compromise the BBB to protein with increasing age. Studies are now in progress to determine how the defect in the BBB to protein occurs and to examine the effects of astroglial destruction on other BBB characteristics in vivo. (NIH-NS-17468).

100.16

PROTEIN KINASE C MODULATES NEURAL MICROVESSEL MORPHOGENESIS.

John Laterra*, Ravi R. Indurti*, Joseph Bressler, Gary W. Goldstein*. The Kennedy Research Inst., Baltimore, MD 21205

Astrocytes ensheathe central nervous system (CNS) capillaries, regulate endothelial differentiation and induce CNS microvessel endothelial cells to organize into capillary-like tubes in vitro (Laterra et al., J. Cell. Physiol. 144:204, 1990). We examined the effect of phorbol 12-myristate 13-acetate (PMA), a protein kinase C activator, on astroglial-induced capillary-like tube formation in vitro. PMA had no effect on the proliferation of either C6 cells, a cell line of astroglial origin, or bovine retinal microvessel endothelial (BRE) cells. PMA at concentrations of 0.1, 1.0, 10.0 and 100.0 nM inhibited C6-induced capillary-like tube formation by 50, 75, 95, and 100% respectively. 4a-phorbol 12,13-di-decanoate, a PMA analogue that does not activate PKC, was without effect. These results suggest that PKC activation modulates morphogenic astroglial endothelial interaction within the central nervous system.

100.17

IMMUNOCYTOCHEMICAL IDENTIFICATION OF TIGHT JUNCTIONS BETWEEN ASTROCYTES IN VITRO AND IN VIVO.

George M. Smith and H.David Shine. Department of Neurosurgery, Baylor College of Medicine, Houston, Texas 77030.

An integral aspect of the blood-brain barrier is the development of tight junctions between specific non-neuronal cells of the central nervous system (CNS). To identify tight junctions in vitro or in vivo, we used a monoclonal antibody (ZO-1) that recognizes a 225 kd cytoplasmic protein associated with tight junctions (Stevenson et al., 1986 J. Cell Biol. 103:755). This protein displayed a continuous staining pattern around the perimeter of 80-90% of GFAP-positive astrocytes in confluent cultures. Expression of ZO-1 by astrocytes was contact dependent and only occurred in confluent monolayers. To determine if astrocyte morphology affected tight junction formation, astrocytes were grown in the presence of di-butyryl cAMP or serum-free medium. Under these conditions astrocyte morphology changed from polygonal to stellate and the number of cells that stained with anti-ZO-1 dramatically decreased. In these stellate astrocytes staining localized exclusively to the cell bodies of clustered astrocytes. To determine if astrocytes in the rat CNS possess tight junctions, frozen brain sections were double labeled with ZO-1 and GFAP antibodies. The majority of stellate astrocytes were not ZO-1-positive; however GFAP-positive ependymal cells that have a cuboidal shape and line the ventricles were ZO-1-positive. Cells previously shown to have tight junctions, such as pial fibroblasts, endothelial cells, and cells of the choroid plexus also stained positive for ZO-1. These data indicate that the formation of tight junctions between astrocytes depends on cell contact and morphology. Supported by Navy-N60014-89-J-3003 and the Kleberg Foundation.

100.18

KINETICS OF FATTY ACID DISSOCIATION FROM ALBUMIN AND TRANSPORT INTO BRAIN.

Q.R. Smith, H. Nagura*, K. Washizaki, J. DeGeorge*, P.J. Robinson, and S.I. Rapoport. Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20892.

Brain uptake of unesterified fatty acid from blood far exceeds that predicted by the free ("non protein bound") fraction, indicating that some fatty acid may be stripped off albumin as blood passes through the cerebral circulation. To examine this further, the brain uptake of unesterified [14C]palmitate was studied in the presence and absence of albumin in anesthetized rats using an in situ brain perfusion technique (Takasato et al., 1984). Results were analyzed using a model that incorporates rates of palmitate binding and release from albumin, as well as blood flow and transport across the blood-brain barrier (Robinson and Rapoport, 1986). [14C]Palmitate was found to be taken up into brain from whole rat blood or artificial plasma (2-3% albumin with ~100 µg/ml palmitate) with a blood-to-brain transfer coefficient (K_{in}) of 2-3 x 10^{-4} ml/s/g. The measured free (nonprotein-bound) fraction of palmitate in plasma equaled 0.005-0.01%, and the k_{off} from albumin was obtained from the literature (Svenson et al., 1974). To explain the observed uptake, it was calculated that a blood-brain barrier permeability-surface area product (PA) to free palmitate of at least 1 ml/s/g would be required. Evaluation of free palmitate uptake in the absence of protein, and with and without preperfusion, indicated that free palmitate was essentially completely extracted (E > 0.9) from perfusion fluid up to a flow rate of 0.5 ml/s/g (the highest flow rate examined). The calculated lower limit of PA for free palmitate was thus 1.2 ml/s/g. The results indicate that unesterified fatty acid uptake into brain can be explained with a simple model that incorporates dissociation from protein and transport into brain.

100.19

VOLUME REGULATION IN ISOLATED TURTLE CEREBELLUM UNDER HYPER- AND HYPOOSMOTIC CONDITIONS. D. Križaj, M.E. Rice & C. Nicholson. Dept. Physiology & Biophysics, New York University Medical Center, New York, NY 10016

Does isolated brain tissue behave as an osmoregulator or an osmoconformer? Turtle (*Pseudemys scripta*) cerebella were incubated (30 min.) in Ringer solutions whose osmolarity was adjusted by addition or subtraction of NaCl. The volume of the extracellular space (as the volume fraction, α) and its geometry (as tortuosity, λ) were measured with ion selective microelectrodes using the diffusion of a probe ion (TMA^+) as described previously (*J. Physiol.* 321:225, 1981). Synaptic potentials evoked by peduncular stimulation were monitored continuously.

The cerebellar tissue behaved as a perfect osmometer up to about 586 mosmoles, where $\alpha = 0.53 \pm 0.06$, an increase from its value of $0.22 + 0.06$ in the control Ringer (326 mosmoles) solution; decreasing control NaCl by half (to 256 mosmoles) decreased α to 0.12 ± 0.02. In addition, λ showed a small but consistent decrease from 1.70 ± 0.13 in controls to 1.54 ± 0.08 at 706 mosmoles. These data conformed to Archie's Law ($\lambda^2 = \alpha^{-\beta}$), with $\beta = 0.2$. Field potentials gradually diminished with increasing osmolarity and disappeared around 600 mosmoles. This effect was always reversible upon return to normal Ringer.

This study suggests that isolated cerebellar tissue does not exhibit significant short-term regulation of its extracellular volume. A possible relation between a physical parameter (α) and the geometry of the space was also demonstrated by the conformation of the data to Archie's Law. Supported by NINDS NS-13742 and NS-28642 to CN.

100.20

CISTERNAL MICRODIALYSIS TO EVALUATE EFFECTS OF FUROSEMIDE AND ETHACRYNIC ACID ON BLOOD-TO-CSF TRANSPORT OF CHLORIDE. C. Johanson, D. Palm*, M. Dyas* and N. Knuckey. Program in Neurosurgery, Dept. of Clin. Neurosciences, Brown Univer./ R.I. Hospital, Providence, RI 02902.

Microprobe dialysis in the cisterna magna (CM) can be used to assess CSF ion transport and fluid formation rate. Male Sprague-Dawley rats (200-300g) were anesthetized with ketamine/xylazine and secured in a head frame. A 2-mm BAS microprobe was inserted through a burr hole in the occipital bone, angled parallel to the occipital squama, and directed into the CM. Cl-36, injected IV after nephrectomy, was allowed to penetrate into CSF for eventual dialysis from CM. Average steady-state control values for volume of distribution (Vd, i.e., dpm/g CSF ÷ dpm/g plasma) of Cl-36 in cisternal dialysate were 111 ± 11% (n=4). The potent loop diuretics furosemide or ethacrynic acid, 50 mg/kg, significantly decreased the level of Cl-36 in CM dialysate by 34% and 32%, respectively. Acetazolamide (50 mg/kg), an inhibitor of carbonic anhydrase but not a loop diuretic, reduced the steady-state Vd of Cl-36 by 55%. In all experiments the arterial pressure was between 80 and 130 mm Hg. Because Cl transport from blood to CSF is proportional to CSF formation rate, we conclude that loop diuretics are able to substantially alter CSF flow in the rat CNS, presumably by inhibition of Na-K-Cl cotransport in the choroid plexus. Supported by funds from R.I. Hospital and by NIH grant NS 27601.

100.21

EXTRACELLULAR PATHWAYS FOR ENTRY OF SERUM PROTEINS TO THE CNS. P. Fishman, R. Broadwell and M. Sofroniew. Univ. MD, Sch.Med.,Balto., 21201, and Cambridge Univ.,Cambridge,U.K.

Extracellular pathways circumventing the blood-brain barrier (BBB) were identified in rats by immunohistochemical localization of endogenous serum proteins albumin, IgG, compliment C9, and IgM and by HRP delivered intravenously. Each of the proteins enters the CNS thru fenestrated vessels in circumventricular organs (e.g., median eminence, subfornical organ, etc.); the proteins gain extracellular access to adjacent brain parenchyma and ependymal lining of the ventricular system. Blood-borne proteins entering the subfornical organ are distributed within the white matter of the corpus callosum. An extracellular pathway also exists at the level of pial surface vessels; immunoreaction product for the serum proteins and HRP appear on the pial surface, within subarachnoid macrophages, the Virchow-Robin spaces and associated perivascular cells, and in the subpial parenchyma. Immunoreaction product for IgG is localized within punctate granules in cell bodies of hypothalamic neurosecretory neurons and motor neurons, the axons of which project outside the BBB. The findings suggest that blood-borne immune and compliment macromolecules have unrestricted extracellular access to the CNS. Widespread distribution of serum proteins within the CNS may have important implications in experimental and pathologic autoimmune dysfunction within the CNS. Supported by NIH/NINDS Grant #NS18030.

100.22

IN VIVO TRANSLOCATION AND SELECTIVE REMOVAL OF ^{125}I-TYR-MIF-1 FROM THE RAT BRAIN AFTER ICV INJECTION. **L.M. Maness, A.J. Kastin, W.A. Banks, and J.E. Zadina, Dept of Medicine and Neuroscience Training Prog, Tulane U Sch of Med, New Orleans, LA 70112; VA Med Ctr, New Orleans, LA 70146.**

The endogenous peptide Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) is known to be transported out of the CNS (Am J Physiol 259:E1,1990). Here, we further defined this process by injecting ^{125}I-Tyr-MIF-1 or ^{125}I-albumin icv and contrasting their distributions over time in brain slices by autoradiography. At 20 min, albumin could not be detected outside the ventricles (VENT), but Tyr-MIF-1 had nearly completely cleared the space and was localized in the periventricular tissue (PVT). At 120 min, albumin had moved almost completely into the PVT, but Tyr-MIF-1 was barely evident in the sections.

		ALBUMIN	TYR-MIF-1	
20 min	VENT	+++	0/+	0 = No Labeling
20 min	PVT	0	++/+++	
120 min	VENT	0/+	0	+++ = Max Labeling
120 min	PVT	++	0/+	

These results suggest that while albumin is not readily removed from brain within the tested time period, Tyr-MIF-1 moves rapidly into the PVT with subsequent removal from the CNS.
(Supported by NIMH/APA P32 MH18882 and VA).

100.23

BLOOD-TO-BRAIN TRANSPORT AND METABOLISM OF CIRCULATING VASOPRESSIN. B.V. Zlokovic, W.A. Banks[1,*], H. El Kadi[*], J. Erchegyi[1,*], J.G. McComb[*] and A.J. Kastin[1]. Department of Neurological Surgery and Division of Neurosurgery Childrens Hospital Los Angeles, Univeristy of Southern California, Los Angeles, California and Veterans Affairs Medical Center and Tulane University School of Medicine, New Orleans, Louisiana[1]

Recent in vivo experiments suggested that arginine-vasopressin (AVP) is taken up at the luminal side of the blood-brain barrier (BBB) by peptide-specific carrier and/or receptor mediated process [Biochim.Biophys.Acta (1990) 1025: 191-198]. In this study, a capillary depletion step [J.Neurochem. (1990) 54: 1882-1888] was coupled to the vascular brain perfusion (VBP) method to examine whether a significant brain distribution of circulating [^{3}H]-AVP represents either sequestration to cerebral microvessels or its transport across the BBB. Cerebrovascular permeability of [^{3}H]-AVP determined in the forebrain was almost ten times higher than for the vascular space marker [^{14}C]-sucrose. The [^{3}H]-radioactivity determined in the vascular pellet after a dextran density centrifugation of the forebrain homogenate was barely above the background level, and after 10 min of the VBP experiment was indistiguishable from [^{14}C]-sucrose. HPLC analysis of radioactivity of the postcapillary supernatant revealed time-dependent fragmentation of blood-borne neuropeptide. The percentage of intact [^{3}H]-AVP during brain perfusion progressively declined from 49% at 1 min to 11.9% at 10 min. The major detectable labeled metabolite was [^{3}H]-phenylalanine (labeled amino acid residue of [^{3}H]-AVP), while the percentage of radioactive peptide fragments increased to about 11% at 10 min. The results indicate transport of intact AVP across the BBB and its rapid in vivo metabolism in the brain with no evidence of capillary sequestration (Supported by Childrens Hospital Los Angeles and the VA).

100.24

ENDOTHELIAL CELLS EXPRESS BRAIN-TYPE GLUCOSE TRANSPORTER IN A TISSUE CULTURE MODEL OF THE HUMAN BLOOD-BRAIN BARRIER. A.A. Hurwitz*, W.T. NORTON, J.W. Berman*, and W.D. Lyman*. Dept. of Pathology, Albert Einstein Coll. of Med., Bronx, NY 10461.

Endothelial cells of the Blood-Brain Barrier (BBB) have been shown to have unique morphologic and functional characteristics. In addition, BBB endothelium expresses specific cell surface molecules. Studies have indicated that brain-type glucose transporter (GLUT-1) can be solely localized to the microvessels of the CNS. Here, we describe the expression of GLUT-1 on human fetal endothelial cells in co-culture with autologous astrocytes. Astrocytes were seeded on one side of a permeable tissue culture insert. Early passage human fetal umbilical vein endothelial cells were added to the gelatin coated, opposite side of the insert. The two cell types were cultured for up to two weeks when they were tested for glial fibrillary acidic protein(GFAP), Factor VIII, and GLUT-1 immunoreactivity. Our results show that astrocytes maintained GFAP expression and endothelial cells were factor VIII positive. In addition, only endothelial cells grown in co-culture expressed GLUT-1 and this expression appears to be contact-dependent. These findings support the use of this model as a system to examine the development of the BBB as well as to characterize BBB-specific molecules. In addition, this model may permit examination of neuropathological processes involving the BBB. Supported by USPHS grants NIH NS 07098-14, MH 47667, MH 46815.

100.25

DECREASED CHLORIDE TRANSPORT IN CHOROID PLEXUS ASSOCIATED WITH CHRONICALLY ELEVATED INTRACRANIAL PRESSURE. N.Knuckey, J. Preston*, M. Dyas*, R. Jenkins*, M. Epstein, D. Palm*, and C. Johanson. Program in Neurosurgery, Dept. of Clin. Neurosciences, Brown Univer./RI Hosp., Providence,RI 02902.

Choroid plexus (ChPl) ion transport, and CSF formation secondarily, may be down-regulated when intracranial pressure (ICP) is chronically elevated. To test this hypothesis we analyzed the transport of Cl by ChPl excised from rats subjected to ICP elevation by kaolin hydrocephalus. Adult male rats (6-8 wk), anesthetized with ketamine, underwent injection of kaolin (375 mg) into cisterna magna. Three weeks later, the ICP and ventricular volume were assessed prior to removing the lateral ChPl for transport studies. We analyzed the ability of ChPl isolated in artificial CSF to transport Cl outward from epithelial cells. The experimental findings allowed categorization into 4 groups, i.e., either controls or those with mild, moderate or severe hydrocephalus. With maximally dilated ventricles and ICP 15 cm water or greater in severe hydrocephalus, the Cl-36 efflux constant (per sec) was reduced 35% from the control value of 0.029. Moderately hydrocephalic rats with less extensive increases in ICP (5-15 cm water) showed smaller decreases in ability to transport Cl outward into CSF. Electron micrographical analysis revealed no apparent alteration in ChPl epithelial ultrastructure. The findings fit the idea of down-regulated transport, possibly as a homeostatic response to ICP rise. Supported by NIH NS 27601.

100.26

RAPID BRAIN TRANSPORT OF MANGANESE(II)-54 AT THE RAT BLOOD-BRAIN BARRIER Q. Rabin, S.I. Rapoport, and Q.R. Smith. Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Manganese (Mn) is essential for brain function, yet is neurotoxic at elevated concentrations. To evaluate the factors that regulate Mn uptake into the central nervous system, the blood-brain barrier transport of Mn was studied in rats using an in situ brain perfusion technique (Takasato et al., 1984). Both cerebral hemispheres of the pentobarbital-anesthetized rat were perfused by direct infusion of physiologic saline or artificial blood containing Mn(II)-54 and [14C]sucrose into the carotid artery. At the end of perfusion, the rat was killed and tracer concentrations were determined in perfusate and brain. Mn(II)-54 was found to be taken up into brain at a very rapid rate during perfusion with physiologic saline that did not contain plasma proteins. The blood-brain barrier transfer coefficient (K_{in}) for Mn-54 in rat parietal cerebral cortex equaled $1.02 \pm 0.14 \times 10^{-3}$ ml/s/g (6.12 ml/min/100g)(n=18) and was not appreciably affected by pre- or post-perfusion with tracer-free fluid to wash-out the cerebral vasculature. The K_{in} for brain Mn-54 uptake agreed well with previous in vivo estimates in rats, after correction for the protein-bound fraction (Murphy et al., 1991), suggesting that "free" Mn is the form that crosses the blood-brain barrier. Addition of 5 mM EDTA to the perfusate essentially abolished uptake, whereas addition of transferrin (3 mg/ml) had no effect. The results demonstrate that Mn(II)-54 is rapidly taken up into brain with a K_{in} 50-100 times that of calcium, and that it is likely the "free", nonprotein bound form of Mn that crosses the blood-brain barrier.

100.27

PURIFICATION OF A CALCIUM BINDING PROTEIN FROM CARP CEREBROSPINAL FLUID. G.D.Chang, C.C.Chen* and F.L.Huang*. Inst. of Biochem. Sci. and Dept. Zool., Natl Taiwan Univ., PO Box 23-106, Taipei 10098, Taiwan.

A calcium binding protein was purified from carp cerebrospinal fluid to apparent homogeneity. Using ammonium sulfate precipitation, Sephacryl-S200 column, Phenyl-Sepharose hydrophobic interaction column, Hydrophase HP-PEI HPLC protein column chromatography, the protein was purified with high yields. Under reduced and non-reduced SDS PAGE, it behaved as a molecule of 60 kDa. Calcium binding assay was conducted after electrophoretic transfer of the proteins from SDS polyacrylamide gel onto nitrocellulose paper (J. Biochem., 95:511-519, 1984).

Tissue distribution of this calcium binding protein was examined by a homologous radioimmunoassay, western blotting and immunocytochemical methods. It has been found that this protein is also present in serum and brain extract in much higher concentrations than in other tissue fluids (semen, ovarian fluid) or other tissue extracts.

100.28

DIFFERENTIAL EXPRESSION OF Na,K-ATPase ISOFORMS IN THE CHOROID PLEXUS AND CEREBRAL MICROVESSELS OF THE GUINEA-PIG BRAIN. A.J. Saraj*, A.A. McDonough[1,*], J.G. McComb* and B.V. Zlokovic. Departments of Neurological Surgery, Physiology and Biophysics[1], and Division of Neurosurgery, Childrens Hospital Los Angeles, University of Southern California School of Medicine, Los Angeles, California

The choroid plexus (CP) and the blood-brain barrier (BBB) play major roles in maintaining Na^+ and K^+ concentrations in the cerebrospinal fluid (CSF) and brain extracellular fluid (ECF). Na,K-ATPase is a key enzyme responsible for active transport of Na^+ and K^+ between blood, ECF and CSF. Three isoforms of Na,K-ATPase α subunit have been characterized, all found in brain. Previous cytochemical and ouabain-binding studies have demonstrated localization and relative enrichement of Na,K-ATPase in the choroid epithelium and cerebrovascular endothelium. In this study we utilized isoform specific antibodies to detect Na,K-ATPase subunits α_1 and α_2 in the choroid plexus and cerebral microvessels of the guinea-pig. Vascular brain perfusion technique [J.Neurochem. (1988) 46: 1444-51] was used to eliminate any protein source from regions other than the brain. Different brain regions (e.g., cortex, cerebelum, caudate, hippocampus, midbrain), microvessels from cerebral cortical mantles and choroid plexi from lateral ventricles were pooled from 3 to 19 guinea-pigs and prepared for immunobloting procedure as described [Am.J.Physiol. (1985) 248: C247-C251]. α_1 and α_2 were detected in all brain regions studied. Only α_2 was detected in cerebral microvessels with undetectable α_1 form, while choroid plexus showed the presence of α_1 form and a much lower signal of α_2. The data support the hypothesis that blood-CSF barrier and BBB regulate brain CSF and ECF ionic composition by different mechanisms. (Supported by Childrens Hospital Los Angeles. Antibodies to rat Na,K-ATPase subunits are provided by Dr. K. Sweadner).

REGULATION OF GENE EXPRESSION I

101.1

TISSUE SPECIFIC EXPRESSION OF THE MYELIN P2 PROTEIN GENE.

Vinodh Narayanan and Benedetta Ripepi, Departments of Pediatrics, University of Pittsburgh, and The Children's Hospital of Pittsburgh, Pittsburgh, PA.

The myelin P2 protein, a member of a family of fatty acid binding proteins, is expressed only in Schwann cells and oligodendrocytes. It is more abundant in peripheral nerves and nerve roots, than in the spinal cord and brain. What are the segments of the P2 gene that limit its expression to oligodendrocytes and Schwann cells, and account for this gradient of expression within the nervous system?

We have previously isolated and analyzed the structure of the mouse myelin P2 gene (V. Narayanan, et al, J. N.chem, in press). By removing the first exon of the P2 gene, and replacing it with the E. coli β-galactosidase gene, *lac Z* (derived from pNlacF, a gift of Dr. R. Palmiter), we have constructed two mini-genes that express the lac Z protein. The first, MP2PrLacZ, contains 2 kbp of 5'-flanking sequence fused with the lacZ gene, and the second, MP2lacZ, contains the rest of the gene as well. These mini-genes will be used to generate lines of transgenic mice, and the expression of lac Z assayed by histochemical staining with X-gal. This will allow us to localize the part(s) of the P2 gene responsible for tissue specific expression.

101.2

ASTROCYTE-SPECIFIC TRANSCRIPTION OF THE HUMAN GENE FOR GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP). F. Besnard*, K. Masood*, Y. Su* and M. Brenner. Lab. of Molec. Biol., NINDS-NIH, Bethesda, MD 20892

The gfa gene encodes glial fibrillary acidic protein (GFAP), an intermediate filament protein found almost exclusively in astroctyes. To identify regions of the human gfa gene that regulate its transcription, we have conducted cell transfection studies with reporter gene constructs. A 2.2 kb 5'-flanking fragment has been found to confer astrocyte-specific expression. Analysis of this fragment has revealed three regions critical for activity; a basal promoter extending from -40 to +40 (RNA startpoint = +1), a promoter proximal element between -132 and -70, and a promoter distal element between -1660 and -1489. Chimeric constructs indicate that cell specificity is largely controlled by the distal element. In addition, when the region between the distal and proximal elements is deleted, reporter gene activity increases about 10-fold, and the proximal element is no longer essential. These effects appear to be due to proximity of the distal element to the basal promoter, rather than to removal of a silencer region.

Both the proximal and the distal elements contain previously identified regulatory motifs as well as a novel 10 bp sequence. The functionality of several of these elements is indicated by their presence in regions showing strong DNase I footprints, and by the results of site-directed mutagenesis.

101.3

TRANSCRIPTIONAL REGULATION OF β-CGRP IN C6 GLIOMA CELLS.

Marc A. Kirschner and Susan G. Amara, Section of Molecular Neurobiology. Howard Hughes Medical Institute.333 Cedar St. Yale University School of Medicine. New Haven, CT., 06510.

α-CGRP and β-CGRP genes show distinct patterns of expression within the nervous system and are differentially regulated by a variety of second messengers. 5'-flanking region sequences of both neuropeptides are highly divergent, suggesting distinct mechanisms of transcriptional regulation. Vector constructs containing serial exonuclease III deletions of the β-CGRP promoter upstream of a luciferase reporter gene have been used in transfection experiments in C6 glioma cells to identify *cis* elements important for the high levels of endogenous β-CGRP expression seen in this cell line. Extracts of cells transfected with the full length construct containing 2.3 kb of the 5'-flanking region contain luciferase activity of the same order of magnitude as seen with an SV40 promoter. Several activator and repressor elements have been identified within the 2.3 kb upstream of the β-CGRP cap site and will be discussed. The identification of such *cis*- acting elements is an initial step towards the characterization of neuronal *trans*-acting factors important in the transcriptional regulation of β-CGRP.

101.4

DETERMINATION OF TRANSCRIPTION STARTS AND PROMOTER ACTIVITY OF THE RAT B-50 (GAP-43) GENE.

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The neuron-specific phosphoprotein B-50 (GAP-43) gene is encoded by a single copy gene. The B-50 gene includes 3 exons (Nedivi et al., *Soc. Neurosci. Abstr.*, 15:503.15, 1989; Grabczyk et al., *Eur. J. Neurosci.* 2: 822, 1990).

Comparison of the primary structure of the B-50 gene upstream of the translation start with the published sequence by Grabczyk et al., 1990, showed that two out of four genomic exon 1 clones contained a deletion varying between 2 and 58 bp of alternating A and G residues. The apparent instability of this region in E. coli may be related to the potential of homopurine residues to form unusual DNA conformations (H-DNA). Data obtained by rapid amplification of cDNA ends (RACE) suggest the presence of multiple transcriptional initiation sites upstream of -81. The most 5' ends are located upstream of -235. Some of the RACE products showed a deletion upstream of -81, in a homopurine rich area. This deletion was the result of an artifact of the used reverse transcriptase (AMV-RT).

Primer extension of poly(A)$^+$ RNA from 8 day old rat brains, using an upstream primer in exon 2 with AMV-RT and MMLV-RT, showed that AMV-RT gives rise to several shorter products than MMLV-RT, since AMV-RT stops aspecifically in purine rich sequences. Data from both RTs indicate two major transcription starts at -133 and -342. These transcription starts for cDNA synthesized with MMLV-RT were supported by PCR using nested primers.

Promoter analysis of the -1012 to -114 bp fragment upstream of the translation start, with or without the 56 bp AG deletion, in NGF differentiated PC12 cells, using luciferase as reporter gene, showed that this deletion caused a 4-fold decrease in the B-50 promoter activity compared to the intact fragment.

101.5

RETINAl DNA-BINDING PROTEINS THAT INTRERACT WITH THE OPSIN PROMOTER.

X. Yu* and C. J. Barnstable, Dept. of Ophthalmology and Visual Science, Yale University School of Medicine, 330 Cedar St., New Haven, CT 06510.

Opsin is one of the earliest genes expressed in differentiated photoreceptor cells. Its expression is regulated primarily at the transcription level. Transgenic mice studies and sequence homology between species imply two regions in its promoter to be important in opsin gene regulation. One is within 300 bp to the transcription start site, the other is about 1.5 kb upstream. Protein binding sites within these regions have been identified (Morabito, M and Barnstable C. J. (1989) Soc. Neurosci. Abstr. 15,1269.) Our results indicate that at least two proteins bind to the proximal region. One of them, Ret1, a developmentally regulated retina specific binding factor, has an apparent molecular weight of 40 kd on SDS-PAGE. However, the binding activity is eluted at about 100 kd by a gel filtration chromatography column. Another protein of 105 kd binds to a retina specific binding site 5' to the Ret1 site. The protein that binds to the distal region which is highly homologous between species also has an apparent molecular weight of 105 kd. Its binding activity is restricted to retina and some other tissues and appears not to be developmentally regulated based on gel retardation assay. Further study of these DNA-binding factors will shed light on opsin gene regulation and photoreceptor differentiation.

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101.6

ISOLATION AND CHARACTERIZATION OF THE PROMOTER REGION OF RAT TRYPTOPHAN HYDROXYLASE (TPH) GENE

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Lab. of Molec. Neurobiol., Cornell Univ. Med. Coll., The Burke Med. Res. Inst., White Plains, NY 10605.

TPH is the rate-limiting enzyme in the biosynthesis of a monoamine neurotransmitter, serotonin. Since the regulation of serotonin levels in the brain is directly related to TPH activity, understanding TPH regulation at the molecular level is an essential step toward elucidating the function of serotonin in the central nervous system. With this aim in mind, we isolated the rat TPH gene from a Charon 4A rat (Sprague-Dawley, female) genomic library. Seven TPH positive clones were isolated with a rat dorsal raphe nucleus-derived TPH cDNA probe. Southern blot analyses of the recombinant phage DNA using several different portions of the cDNA probes identified two independent clones, each containing about 14.5 kb of TPH genomic DNA insert. Further detailed restriction enzyme mapping and DNA sequence analysis of the clones revealed the 5' promoter region. In addition, a mouse genomic TPH clone was isolated from an EMBL3 mouse (C57BL, male) genomic library using the rat cDNA as probe. This 15.1 kb mouse genomic clone containing the 5' flanking region is currently being characterized to identify the upstream consensus sequences among rodents, which may be functionally important for the regulation of TPH gene expression.

To further delineate the sequence elements that are responsible for directing cell type specific and developmentally regulated transcription of the TPH gene, we are carrying out deletion analysis of the 5' flanking region of the rat TPH gene and assaying its expression in a mouse mastocytoma cell line and transgenic animals.

101.7

COLD-INDUCED ALTERATIONS IN THE BINDING OF ADRENOMEDULLARY NUCLEAR PROTEINS TO THE PROMOTER REGION OF THE TYROSINE HYDROXYLASE (TH) GENE.

L.L. Miner, S.P. Pandalai, and B. Kaplan. Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Previously, we have demonstrated that cold exposure induces a rapid trans-synaptically mediated increase in rat adrenomedullary TH gene expression. To explore the molecular genetic mechanisms mediating this adaptive response, adrenomedullary nuclear protein extracts were prepared from control and cold-stressed (5°C) rats and were incubated with fragments obtained from the 5′ flanking region of the bovine TH gene. Radiolabelled DNA-protein complexes were resolved from unbound DNA using a mobility shift assay. Two of these fragments (0 to -82 bp and -213 to -268 bp) showed cold-specific alterations in gel migration patterns. These shifts were observed within 1 hr of cold exposure and were abolished by sympathetic denervation of the gland. The -213 to -268 bp fragment contains a highly conserved AP1 consensus sequence, and the mobility shift could be eliminated by competition with synthetic AP1 sequences, but not by the SP1 consensus sequence. Combined, these data suggest that cold-induced alteration in TH gene expression are mediated by alterations in the interaction of trans-acting factors with regulatory elements present in the 5′ flanking region of the gene and implicate the AP1 transcription complex in the trans-synaptic modulation of TH gene expression in vivo.

101.8

3' ELEMENTS RESPONSIBLE FOR NEUROENDOCRINE TISSUE-SPECIFICITY IN THE HUMAN TYROSINE HYDROXYLASE GENE.

S. C. Wong*, M. Moffat*, and K. L. O'Malley. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Tyrosine hydroxylase is the rate limiting enzyme in the synthesis of catecholamines. The expression of tyrosine hydroxylase gene is restricted to dopaminergic, noradrenergic, and adrenergic cells in the central nervous system as well as sympathetic ganglia and adrenal medullary cells in the periphery. Our laboratory has previously demonstrated that the region responsible for the cell-type specific expression of the human tyrosine hydroxylase gene may be located in a 760 base pair region immediately 3' of the exon 13 (Gandelman K.-Y. *et al.*, 1990). Nucleotide sequence analysis of the 760 base pair region reveals the presence of two consensus sequences (CAXXTG) recognizable to the helix-loop-helix family of transcriptional factors. The two CAXXTG motifs are separated by 140 bases. Using transient expression assays, we showed that the up-stream CAXXTG exhibits tissue-specific enhancement irrespective of the presence of the down-stream CAXXTG and that the 100 base pair region immediately 3' of the exon 13 may contain additional elements essential for the tissue-specific expression of the human tyrosine hydroxylase gene. The involvement of the up-stream CAXXTG motif in tissue-specific expression was further demonstrated with the double stranded oligonucleotides containing the same CAXXTG sequence. These new findings support our previous report that the elements responsible for neuroendocrine tissue-specificity expression in the human tyrosine hydroxylase gene is located at the 3' end of the gene.

101.9

ANALYSIS OF E BOXES IN THE CHICK MUSCLE ACETYLCHOLINE RECEPTOR δ-SUBUNIT GENE. Xin-Min Wang*, Ying-Shuan Lee*, and Jakob Schmidt. Dept. of Biochem. & Cell Biol., State Univ. of New York at Stony Brook, Stony Brook NY 11794.

The chick muscle δ-subunit promoter contains several copies of the MyoD recognition motif CANNTG or 'E box': at -110 from the translation start site (1); at -156 (2) and -204 (3) where they are embedded in α subunit enhancer similarities; and at -238 (4) and further upstream in the silencer region 5' of -207 (Wang et al., EMBO J. 9, 783, 1990). DNAse I footprinting shows that proteins from fibroblast and myotube nuclei bind to E boxes 2 and 3. Base substitutions in these elements and at sites in close proximity (at -193/-191 and -164/-162) abolish factor binding and reduce transcriptional activity in all cells. Corruption of E box 1, on the other hand, does not impair promoter activity in muscle cells and doubles activity in fibroblasts. The MyoD family member myogenin footprints to 1 and 3, but not to 2. In cotransfection experiments myogenin overrides the silencer effect in 3T3 and 10T1/2 cells, probably by activation of the myogenetic program, and not as a consequence of direct binding to the promoter, because appropriate constructs remain inactive in cotransfected HeLa and Cos-1 cells. Nevertheless, competition experiments suggest that E boxes in the silencer region interact with factors present in fibroblasts; perhaps such interactions play a role in the suppression of the δ-subunit gene in non-muscle cells.

101.10

A Negative Retinoic Acid Response Element in the Rat Oxytocin Promoter Restricts Transcriptional Stimulation By Constitutive Transactivation Domains. **By Steven M. Lipkin, Ronald B. Emeson and Michael G. Rosenfeld.**
CMM 239, UC San Diego, La Jolla 92093

We have identified a high-affinity binding site in the rat Oxytocin promoter that mediates negative transcriptional regulation by the retinoic acid receptor. To determine whether strong, constitutive transactivation domains could stimulate gene transcription when bound to a DNA binding site that mediates transcriptional repression, we fused the Herpes Simplex Virus Protein 16 (VP16) transactivation domain to the retinoic acid receptor amino-terminus. This chimeric receptor demonstrated an enhanced ability to increase mRNA transcription when bound to promoters containing palindromic thyroid hormone/retinoic acid response elements, but surprisingly still repressed gene transcription when bound to promoters containing the negative retinoic acid response element. These results suggest that the activity of even constitutive transactivation domains can be constrained by the DNA binding sites to which they are bound.

101.11

REGULATION OF OXYTOCIN GENE TRANSCRIPTION BY ESTROGEN AND THYROID HORMONE R.A.H.Adan* H.Rigter J.P.H.Burbach* Rudolf Magnus Institute, University of Utrecht, the Netherlands; Gezondsheidsraad, the Hague.

This study is focussed on endocrine factors involved in the regulation of oxytocin (OT) gene transcription. Plasmids having a 5'-flanking region of the rat OT gene (-363/+16) cloned in front of the firefly luciferase gene were cotransfected with expression vectors for the human estrogen receptor or the rat thyroid hormone receptor in P19 EC Cells. Estrogen strongly stimulated rat OT promoter activity. Two regions of the promoter were located each conferring approximately 15-fold stimulation, between nucleotides -172 and -149 and between -148 and +16 Thyroid Hormone also stimulated OT promoter activity, but less strongly than estrogen. Responsiveness accounting for about 5-fold stimulation was located between nucleotides -172 and -148. There was no cooperativity between estrogen and thyroid hormone stimulation. The sequences of the OT promoter mediating these responses are characerized by site directed mutagenesis and by footprints with purified receptor-proteins. Thyroid hormone and estrogen regulate the OTgene

101.12

CIS ACTING ELEMENTS INVOLVED IN THE REGULATION OF VASOPRESSIN GENE EXPRESSION. K. Pardy*, R.A.H. Adan*#, D.A. Carter, J.P.H. Burbach# and D. Murphy. Neuropeptide lab, Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511 and #Rudolf Magnus Institute, University of Utrecht, 3521 GD Utrecht, The Netherlands.

Cyclic adenosine 3',5'-monophosphate (cAMP) has been implicated as a mediator of the physiological regulation of vasopressin (VP) gene expression (1). As no appropriate VP synthesising cell lines exist, we have investigated the effect of cAMP on VP gene expression using transfection into two heterologous cell lines (CV1 and P19EC). 5' upstream regions of the bovine, human and rat VP genes were linked to the reporter genes chloramphenicol acetyl transferase and luciferase and the response of reporter enzyme activity to cAMP activation was analysed for each construct following transfection into each cell line. The bovine VP promoter was found to give highest basal expression of reporter enzyme activity and also the greatest response to cAMP (2.5 fold increase). Further analysis of the sequences involved in mediating the response to cAMP was performed using 5' deletion mutants of the bovine VP gene. A cAMP responsive element has been mapped to within 179bp 5' to the start of transcription and DNase I footprint analysis has been performed on this region to identify interactions between DNA binding proteins and VP promoter sequence.

(1) Carter D.A. and Murphy D. Brain Res. 487:350-356 (1989)

101.13

PROMOTER ACTIVITY OF THE 5' FLANKING SEQUENCE OF THE MOUSE SEROTONIN 1C RECEPTOR GENE ASSAYED IN XENOPUS OOCYTES. L.J. Bloem, Y. Chen*, J. Liu*, J. Harts, and L. Yu. Dept. of Med. & Mol. Genetics, Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

The serotonin 1c (5-HT_{1C}) receptor is found in many brain regions, but is particularly enriched on the epithelial cells of the choroid plexus where it couples to phosphotidylinositol turnover. To understand more about the molecular processes that regulate the regional expression pattern of the receptor, we have isolated the 5' flanking sequence of the mouse 5-HT_{1C} receptor gene and cloned it into a vector containing the 5-HT_{1C} receptor cDNA. The plasmid DNA was injected into the nucleus of *Xenopus* oocytes. After two days of incubation, individual oocytes were tested for the presence of the 5-HT_{1C} receptor by voltage-clamp recording. Upon superfusion of serotonin-containing Ringer's solution, depolarizing membrane currents were detected in injected oocytes, suggesting that the 5-HT_{1C} receptor is functionally expressed under the promoter activity of the 5' flanking sequence. This assay system provides the sensitivity of electrophysiology recording and will be used to analyze the *cis*-acting elements in the promoter region of the receptor gene and the *trans*-acting factors that regulate the region-specific expression of the receptor gene.

101.14

CHARACTERIZATION AND EXPRESSION OF RAT RETINAL cGMP-GATED CHANNEL AND GUANYLATE CYCLASE GENES. Iqbal Ahmad and Colin J. Barnstable. Dept. of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CT 06510.

cGMP plays a pivotal role in visual transduction in rod photoreceptors. A light dependent decrease in cGMP concentration leads to closure of cation channel and restoration of cGMP levels by guanylate cyclase in the dark opens the channel. We have cloned and characterized rat rod photoreceptor cGMP-gated channel gene.The deduced aa sequence shows a strong homology to both the bovine rod channel and the rat olfactory channel. In situ hybridization studies using antisense probe have shown that cGMP-channel mRNA to be expressed in rod photoreceptors. In addition, cGMP-channel mRNA are also localized in the inner retina. This has been confirmed by PCR analyses performed on cDNA from microdissected retinal sections.The presence of cGMP-channel mRNA in medullary layers of kidney as revealed by in situ hybridization and PCR analyses confirms our earlier report of a 3.2 kb cGMP-channel mRNA in kidney. We have obtained a partial cDNA clone of guanylate cyclase from retina by PCR amplification using primers flanking a conserved region in carboxyl portion of intracellular domain of brain guanylate cyclase. Results regarding the temporal and tissue specific expression of this gene will be presented.The cGMP gated channel and guanylate cyclase may be members of family distributed throughout the nervous system or even throughout the body. (Supported by EY00785,EY05206 RPB Inc., ARVO/ALCON Fellowship).

102.1

Interactions between sigma ligands and NMDA in rat cortex and hippocampus

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Many compounds possess high affinity for the sigma binding site. Much effort has been put into attempts to differentiate agonist from antagonist actions of these compounds. Several lines of evidence have suggested an interaction between the sigma site and the NMDA receptor. We have therefore investigated these interactions by microiontophoresis both in vitro using slices of rat hippocampus (500 um) and in vivo using cells in the neocortex and hippocampus of urethane-anaesthetised animals. A majority of the neurones tested were depressed by ditolylguanidine (DTG) or 3-PPP administered by microiontophoresis. Responses to amino acids (NMDA, quisqualate) and carbachol were also inhibited reversibly on most cells. There was little evidence for any selectivity in these actions of sigma ligands. These inhibitory effects were not prevented by the iontophoresis or intraperitoneal administration of haloperidol (0.1 mg/kg). Spike height remained unaffected during application of sigma ligands, indicating that the depressant effects were not due to local anaesthetic activity.

102.2

COMPLEX ALTERATIONS IN THE KINETICS OF INHIBITORY POSTSYNAPTIC CURRENTS BY THREE VOLATILE ANESTHETICS IN CULTURED RAT HIPPOCAMPAL NEURONS. M.V. Jones*, L.A. Hornberger, H.K. Radke and N.L. Harrison, Dept. of Anesthesia and Critical Care and *Dept. of Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, Il 60637

We have studied the effects of the volatile anesthetics (VAs) enflurane (ENF), halothane (HAL), and isoflurane (ISO) on inhibitory postsynaptic currents (IPSCs) in cultured rat hippocampal neurons. Whole-cell recordings were made from pairs of neurons at 25°C, using K gluconate based intracellular solutions, under conditions that prevent polysynaptic events. Single action potentials were evoked in one neuron, and elicited transient outward IPSCs in the 'postsynaptic' neuron voltage-clamped at -40mV. IPSCs were blocked by bicuculline, and reversed between -70 and -80mV. VAs were applied via the perfusion line, and concentrations were determined by gas chromatography of samples withdrawn from the bath during each experiment. Data were filtered at 2kHz and digitized at 4kHz. IPSC decay kinetics were fit using a version of the simplex algorithm. Time constants (τ) are expressed as mean ± SEM.

IPSC decay phases were usually better fitted by two exponentials (τ_{fast} (75%)= 20±1 ms; τ_{slow} (25%) = 73±7 ms) than by one. In the presence of VAs, the relative contribution of the slow component was increased, and the time-course of this component was prolonged. The time-course of the fast component was either not affected, or shortened. In addition, the peak amplitude of the IPSC was reduced, especially at higher concentrations of VA. VAs produced all these effects at concentrations within the clinically effective range (≤0.69 mM ENF; ≤0.2 mM HAL; ≤0.7 mM ISO). We have shown previously that clinically relevant concentrations of these VAs cause increases in both the duration and amplitude of currents activated by exogenously applied GABA, and we therefore suspect that their depressant effects on the peak amplitude of IPSCs are presynaptic in origin, while the effects on IPSC decay kinetics reflect modulation of postsynaptic $GABA_A$ receptors.

102.3

ZINC BLOCKS POST- BUT NOT PRESYNAPTIC ACTIONS OF BACLOFEN IN AREA CA1 OF RAT HIPPOCAMPUS.

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Zinc is present in high concentration in the hippocampus, especially in the mossy fiber terminals. Zinc application to hippocampal slices elicits giant depolarizing potentials in CA3 pyramidal neurons (GDPs; Xie and Smart, Nature, 349: 521). It was suggested that this action of zinc results from the blockade of pre- and/or postsynaptic $GABA_B$ receptors; we have tested this hypothesis in the CA1 subfield of rat hippocampal slices (where there is little endogenous zinc) using intracellular and extracellular recording. Population excitatory postsynaptic potentials (pEPSPs) were evoked by stimulation of the Schaffer collateral/commissural fibers and monosynaptic inhibitory postsynaptic potentials (mIPSPs) were evoked in the presence of the excitatory amino acid antagonists, 25μM 6,7-dinitroquinoxaline 2, 3-dione and 50μM D,L-2-amino 5-phosphonovalerate.

Baclofen hyperpolarizes CA1 neurons (postsynaptic action) and depresses pEPSPs and mIPSPs (presynaptic action). All actions of baclofen are antagonized by CGP 35348 or 2-hydroxy-saclofen. In the presence of zinc (0.2mM), $GABA_A$ receptor-mediated GDPs occurred spontaneously or in response to stimulation; pEPSP slope and fast IPSP amplitude were unaltered. Zinc prevented baclofen-induced hyperpolarization but not the depression of pEPSP slope or mIPSP amplitude. These effects of zinc show significant overlap with those of barium, which blocks post- but not presynaptic actions of baclofen (Lambert et al., Soc. Neurosci. Abstr., 16: 1041) and 4-aminopyridine, which induces GDPs (Perreault and Avoli, J. Neurophysiol., 61: 953). We suspect that the actions of zinc are mediated by blockade of potassium channels on pyramidal neurons and interneurons.

102.4

DOES ETHANOL ENHANCE $GABA_A$-MEDIATED SYNAPTIC TRANSMISSION IN HIPPOCAMPAL CA1 CELLS?

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The effects of low concentrations of ethanol on synaptic transmission in rat hippocampal CA1 cells were investigated using the whole cell patch clamp configuration in an *in vitro* brain slice preparation. Experiments were performed at 35° C, using a pH and calcium buffered internal recording solution containing ATP and GTP. Under these conditions, ethanol (20-100 mM) significantly increased the amplitude of orthodromically evoked IPSP's but had minimal effects on EPSP's evoked in the same cells. In subsequent experiments, CNQX + APV (glutamate receptor blockers) were used to suppress EPSP's, leaving pharmacologically-isolated evoked IPSP's. These inhibitory responses were fully blocked by bicuculline and were presumably $GABA_a$-mediated potentials. In the presence of these blockers, no potentiation of the IPSP's was observed over the same concentration range that enhanced inhibitory potentials under normal conditions.

These initial results suggest that the potentiation of the evoked IPSP's observed under standard conditions may not be due to a direct action of ethanol on GABAergic synaptic transmission. (Supported by MRC and a Connaught Scholarship)

102.5

EFFECTS OF ADENOSINE ON SYNAPTIC ACTIVITY RECORDED IN THE PERFORANT PATHWAY TERMINAL ZONE OF THE DENTATE GYRUS OF THE RAT AFTER ENTORHINAL LESIONS. J. S. Kahle and C.W. Cotman, Department of Psychobiology, University of California, Irvine, CA 92717.

Adenosine acts at presynaptic receptors to inhibit the release of transmitter in many pathways in the brain. Binding to adenosine receptors has been shown to decrease dramatically in the molecular layer of the dentate gyrus after entorhinal lesions as perforant path fibers degenerate.

We studied the effects of adenosine on evoked synaptic activity recorded in the perforant path terminal zone in the dentate gyrus after partial lesions of the entorhinal cortex. Male rats were given electrolytic lesions in the full extent of the left entorhinal cortex and a vertical strip in the middle third of the right entorhinal cortex. After two weeks recovery period, slices were prepared from the right hippocampus, the remaining perforant path fibers were stimulated, and responses were recorded extracellularly from the middle third of the molecular layer of the dentate gyrus maintained *in vitro*. Additions of adenosine (50μM) reduced low frequency (0.05Hz) field potentials in a manner similar in control slices, but by ~55% as compared to ~25% in control slices. Also similar in control slices, additions of adenosine (50μM) blocked paired-pulse depression recorded with pairs of stimuli given 40 or 100msec apart. In contrast to control slices, paired-pulse depression was not blocked by adenosine when pairs of stimuli were given 400msec apart.

These results suggest that the physiological effect of adenosine receptor activation is not generally reduced, but may be enhanced two weeks following entorhinal lesions, despite an apparent decrease in adenosine binding. Paired-pulse depression recorded at short interpulse intervals remained sensitive to adenosine modulation, only paired-pulse depression recorded at long interpulse intervals was no longer sensitive to adenosine two weeks after lesions. Reactive synaptogenesis of another fiber system and/or adenosine receptor supersensitivity during the two week recovery period may allow for some recovery of function in the adenosine system.

102.6

MU OPIOID RECEPTOR-MEDIATED DISINHIBITION IN RAT DENTATE GYRUS. C.W. Xie, R.A.Morrisett and D.V.Lewis. Pediatric Neurology, Duke Unuv. Med.Ctr., Durham, NC 27710.

The effect of opioids on synaptic inhibition was examined in rat dentate gyrus. Whole-cell voltage-clamp recordings were undertaken in granule cells of hippocampal slices. In the presence of D-APV (NMDA antagonist, 40 μM) and DNQX (AMPA antagonist, 20 μM), monosynaptic inhibitory postsynaptic currents (IPSCs) were evoked in granule cells by stimulating in the outer molecular layer near to recorded granule cells. The IPSC was found to consist of 2 distinct components, an early component sensitive to the $GABA_A$ antagonist bicuculline or picrotoxin, and a late component blocked by $GABA_B$ antagonists. Bath application of the mu agonist PL017 (0.3-3 μM) significantly reduced the amplitude of the early IPSC by 21-56%, and the late IPSC by 43-81%. This effect could be antagonized by naloxone (1 μM) or by the selective mu antagonist β-FNA (10 μM).

These results suggest that the activation of mu receptors suppresses both $GABA_A$ and $GABA_B$-mediated IPSCs in the dentate, and the resulting disinhibition may be responsible for previously reported opioid enhancement of LTP at the lateral perforant path- dentate granule cell synapses. Supported by NS27488.

102.7

REDOX MODULATION OF SYNAPTIC FIELD POTENTIALS IN THE HIPPOCAMPUS. D. L. Tauck and J. E. Tullis*, Laboratory of Neurophysiology, Department of Biology, Santa Clara University, Santa Clara, CA, 95053.

Sulfhydryl redox reagents reversibly modulate currents evoked by N-methyl-D-aspartate (NMDA) [Aizenman, et al., 1989, *Neuron* 2:1257]. Similarly, chemical reduction increases the magnitude of long-term potentiation but has no effect on field potentials evoked by low frequency stimulation [Tauck, et al., 1990, *Brain Res.* 519:129]. The present study shows that the sulfhydryl reducing agent dithiothreitol (DTT) and the oxidizing agent 5-5-dithio-bis-2-nitrobenzoic acid (DTNB) reversibly antagonize each other's effects on the NMDA channel mediated component of synaptic field potentials in area CA1 of the hippocampal slice. To reveal the component of synaptic responses mediated by NMDA channels, field potentials evoked by single stimuli were compared to the responses generated after 5 stimuli at 10 Hz [Poolos et al., 1990, *Brain Res.* 508:7]. The NMDA antagonist D,L-2-amino-5-phosphonovaleric acid (APV), 100μM, blocked the potentiation induced by conditioning stimuli. DTT (1 mM) and DTNB (1 mM) were added to the perfusate repeatedly. DTT reversibly potentiated the NMDA component of the synaptic potentials while DTNB had the opposite effect.

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102.8

PUTRESCINE, α-METHYLORNITHINE AND α-DIFLUOROMETHYL ORNITHINE INCREASE NEURONAL EXCITABILITY IN HIPPOCAMPAL SLICES. P.A. Ferchmin♦, P. Di Scenna♣, M. Morales*♦, N.A. Lambert♣, E.M. Rivera*♦, V.A. Eterovic♦ and T. Teyler♣. ♦Dept. Biochem. Univ. Central del Caribe, Bayamon, Puerto Rico 00621 and ♣Dept. Neurobiol. Northeastern Ohio Univ. Col. Med. Rootstown, OH 44272.

Polyamines regulate neuronal activity. To study this we tested the effect of two inhibitors of ornithine decarboxylase (ODC), α-difluoromethyl ornithine (DFMO) and α-methylornithine (MO), and putrescine (PUT), on extracellular recordings in hippocampal slices. Cumulative dose-response curves were obtained by perfusing the slices with increasing drug concentrations. PUT (100 nM to 2 mM) increased neuronal excitability in a dose dependent manner. DFMO (2.5 to 5 mM) increased neuronal excitability, blocked paired-pulse (PP) inhibition and caused PP facilitation. The effects of DFMO were not mediated by ODC inhibition since they were also observed in the presence of PUT. MO increased neuronal excitability but did not affect PP inhibition. The effects were reversible but became irreversible after longer incubations. Ornithine seemed to be inactive. These results suggest that PUT and inhibitors of ODC have a complex effect on the nervous system.

Supported in part by NIH-RCMI RR03035 and NSF-EPSCoR RII-8610677

102.9

THE MONOSYNAPTIC PERFORANT PATH TO CA1 RESPONSE IS MEDIATED BY EXCITATORY GLUTAMINERGIC SYNAPSES. C.M. Colbert and W.B Levy, Dept. of Neurosurgery, Univ of Virginia, Charlottesville VA, 22908.

When stimulated under experimental conditions, the perforant path (PP) monosynaptically fires CA1 pyramids rather poorly compared to the Schaffer collaterals, although it maps as expected for a distally located excitatory response and is occasionally seen to fire individual cells when recording from s. pyramidal. In light of this fact, and because binding studies imply the presence of modulatory neurotransmitters in s. lacunosum-moleculare, it has been proposed that the PP input to CA1 is predominantly modulatory. Here, to the contrary, we report that 1) it is a glutaminergic system and 2) that it mediates the monosynaptic response in s. moleculare evoked by stimulation of the perforant path.

Transverse slices from 8 rats were maintained in an interface chamber. We stimulated the perforant path (PP) in the subiculum along its ventricular edge, and recorded postsynaptic potentials extracellulary in s. moleculare of CA1. To exclude any confounding DG responses, we gently dissected the DG from CA1 along the obliterated fissure in some slices. DNQX (20um) added to the bath greatly reduced the magnitude of the initial negativity (>1mV) evoked by PP stimulation in s. moleculare leaving a small positivity (<150uV). Subsequent application of picrotoxin (45uM) resulted in a small negativity (<100uV) to PP stimulation. Finally, addition of APV (100uM) resulted in no observable extracellular response to PP stimulation. At this same concentration, addition of APV alone did not alter the response.

These findings indicate that glutaminergic receptors of the NMDA and AMPA subtypes mediate the excitatory evoked synaptic potential recorded in s. moleculare, and suggest that this pathway may serve as a primary information-carrying input to the CA1 pyramids. Supported by NIH R01 NS15488, NIMH RSDA MH00622 to WBL and MH10019-01 to CMC.

102.10

PHARMACOLOGICAL CHARACTERIZATION OF EXCITATORY SYNAPTIC POTENTIALS IN RAT BASOLATERAL AMYGDALOID NEURONS. P.W. Gean and F.C.Chang*. Dept. of Pharmacology, College of Medicine, National Cheng-Kung University, Taiwan, R.O.C.

The pharmacological properties of synaptic responses in rat basolateral amygdaloid (BLA) neurons were studied using intracellular recording techniques. Three distinct types of synaptic potential were evoked by stimulation of adjacent ventral endopyriform nucleus: (1) a fast excitatory postsynaptic potential (f-EPSP); (2) a late EPSP (l-EPSP) following the f-EPSP; and (3) a multiphasic hyperpolarization following the initial depolarizing potential. Superfusion of 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX), a selective non-N-methyl-D-aspartate (non-NMDA) receptor antagonist, produced a concentration-dependent reduction of the f-EPSP with a shift of the input-output relationship downward and to the right. The ED_{50} for this effect was around 4 uM. In the presence of CNQX, however, a small depolarizing potential remained. This residual depolarizing component was markedly enhanced on removing Mg^{++} from the perfusing medium and could subsequently be abolished by DL-2-amino-5-phosphonovaleate (DL-APV, 50 uM) indicating its mediation via NMDA receptor-coupled ionophore. The l-EPSP was reversibly blocked by DL-APV. These results suggest that the pyriform cortex-amygdala pathway is mediated through excitatory amino acids acting on non-NMDA as well as NMDA receptors located on the BLA neurons.

102.11

RESPONSES TO EXTRACELLULAR ATP IN CULTURED RAT HIPPOCAMPAL NEURONS. K.Inoue, K.Nakazawa, T.Watano, K.Fujimori and A.Takanaka. Div.Pharmacology, Biological Safety Research Center, NIHS, Tokyo158

We have already reported that extracellular ATP stimulates P2-purinoceptors and activates Ca-permeable cation channels causing Ca^{2+} influx which evokes catecholamine release from PC12 cells (Neurosci.Let.106,294,1989; J.Physiol. 428,257, 1990 ; J.Physiol. 434,647,1991; Br.J.Pharmacol.102,581,1991 &102, 851,1991). We show here the effects of ATP on cultured rat hippocampal neurons.

ATP (100μM) evoked a rapid depolarization and transient inward currents (100 to 300 pA at -60 mV) in most cells (24 of 36 cells tested). The currents were reversed near 0 mV and abolished in the presence of 3μM tetrodotoxin (TTX). The transient currents were inhibited not by 2-amino-5-phosphono-valerate (AP5, 100 to 200 μM), an NMDA receptor antagonist, but by CNQX (30μM), a non-NMDA receptor antagonist. In a part of cells (6 of 36 cells tested), ATP evoked small (20 to 40 pA at -60mV) and sustained inward currents which resisted TTX. In almost all neurons, ATP induced an increase of intracellular Ca^{2+} which was dependent on extracellular Ca^{2+} and blocked by P2-purinoceptor blocker Suramin. These data suggest that ATP stimulates the hippocampal neurons directly via P2-receptor and trans-synaptically through a mechanism coupling to non-NMDA glutamate receptors.

102.12

PRE-ANESTHETIC CONCENTRATIONS OF HALOTHANE ALTER THE CARBACHOL-INDUCED FIELD ACTIVITY OF IN VITRO HIPPOCAMPAL NEURONS. S.H.Roth and J.Kohli*. Dept. of Pharmacology, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1.

We have demonstrated that carbachol can induce rhythmical field activity in the hippocampal slice preparation that is similar to in vivo hippocampal 'theta' rhythm. This activity has been shown to be associated with memory and sensory processing, features that are relevant to the state of anesthesia. The effects of halothane were examined on the field activity recorded from rat dentate granule neurons in vitro. It was observed that low concentrations, 0.2 to 0.4 vol%, of halothane significantly altered this activity; the MAC (minimum alveolar concentration) for halothane anesthesia in the rat is 1.0 vol%. Using power spectral analysis, it was shown that halothane shifted the relative power of the field activity to lower frequency ranges and also decreased total power. These data demonstrate that this activity is very sensitive to the effects of halothane at pre-anaesthetic levels, and may be a useful model to study the state of anesthesia and the mode of action of anaesthetic agents on the central nervous system. Supported by the Canadian Medical Research Council of Canada.

102.13

AMINO ACID COMPOSITION, IMMUNOREACTIVITY, AND SEQUENCE ANALYSIS OF BOVINE HIPPOCAMPAL METALLOTHIONEIN ISOFORMS. M.Ebadi*†, F. Perini**, R.F. Pfeiffer*†, V.K. Paliwal**, J.S. Garvey††, and K. Mountjoy^, Depts. of Pharmacol.* & Neurol.†, Univ. Nebr. Coll. Med., Eppley Inst., Univ. Nebr. Med. Ctr.^; Dept. of Int. Med., Yale Univ. Sch. of Med.**, & Dept. Biol., Syracuse Univ.††.

The mammalian hippocampi not only contain high concentration of zinc, but also exhibit subregional variation in this essential element, with concentration being highest in the hilar region and lowest in the fimbria. Studies from our laboratory first described the biochemical properties of metallothionein-(MT) in the hippocampus (Paliwal & Ebadi, Exp. Brain Res. 75, 477, 1989) and explored its involvement in the synthesis of GABA (Ebadi, et al. Annals New York Acad. Sci. 189, 1990). The amino acid composition of bovine hippocampal MT isoforms I and II consists of high concentration of cysteine, lysine, serine, alanine, being devoid of aromatic amino acids. Antibodies formed against both the sheep- and rat-hepatic MTs cross reacted completely with the hippocampal MT. These data along with sequence analysis of 60 residues of MT isoform I reveal that the immunologically dominant region of the NH2-terminal domain (residues 1-29) of hippocampal MT is conserved. The continuous synthesis of MT in the hippocampus of zinc untreated animals, its involvement in the synthesis of GABA and in the NMDA receptor-mediated channel currents are interpreted to indicate that MT isoforms appear to be involved in hippocampal neurotransmission. (Supported by a grant from USPHS ES 03949).

PHARMACOLOGY OF SYNAPTIC TRANSMISSION: NEUROTRANSMITTERS

103.1

EXCITATORY AMINO ACIDS (EAAs) MEDIATE FAST SYNAPTIC TRANSMISSION IN RAT LATERAL PARABRACHIAL NUCLEUS: DIRECT EVIDENCE FROM WHOLE-CELL SLICE PATCH RECORDING. J.A. Zidichouski and J.H. Jhamandas. Dept. of Medicine (Neurology & Div. of Neuroscience), University of Alberta, Edmonton, Alberta, Canada, T6G 2B7.

The brainstem lateral parabrachial nucleus (LPBN) is increasingly viewed as an important site for the integration of autonomic signals. Although glutamate-like immunoreactivity is present, the role of EAAs in neurotransmission in the LPBN is unknown. We have recently reported excitatory effects of exogenously-applied EAAs on LPBN neurons in vitro. We therefore directly assessed the role of EAAs in synaptic transmission in the LPBN by testing the effects EAA antagonists on fast excitatory post-synaptic potentials (fEPSPs) and currents (fEPSCs) recorded from pontine slices in vitro by means of whole-cell patch recordings. Stimulus-evoked fEPSPs or fEPSCs (fEPs) were recorded from 39 neufons. The fEPSC reversed at ≈0 mV and was reduced in low Ca^{2+} and high Mg^{2+} solution. All fEPs had a rapid onset followed by a slow decline to baseline. The non-selective EAA antagonist kynurenic acid (10μM) attenuated fEPs. Non-N-methyl-D-aspartate (NMDA) antagonists 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX; 1-60μM, dose-dependent) and 6,7-Dinitroquinoxaline-2,3-dione (DNXQ, 10μM) markedly reduced fEPs. In addition, a few cells displayed a slow Mg^{2+}-dependent component of the fEP that was sensitive to the NMDA-selective antagonist DL-2-amino-5-phosphonovalerate (APV, 10μM). These results suggest that endogenously-released EAAs act predominantly at non-NMDA receptors within the LPBN to mediate rapid excitatory synaptic transmission. The role of the NMDA-mediated responses is less clear but may facilitate neurotransmission at this site. These data reflect an increasingly important role for EAAs in central autonomic regulation. Supported by MRC Canada.

103.2

CHARACTERISTICS OF EXCITATORY AMINO ACID RECEPTORS IN SENSORY SYNAPSE. G. N. Akoev*, S. A. Dambinova*, A. I. Gorodinski*, G. N. Andrianov*, V. Ryzova*, & R. Stiles. I. P. Pavlov Institute of Physiology, Institute of the Human Brain, Academy of Sciences of the USSR, Leningrad, USSR.

The synaptic neurotransmission in electroreceptors ampullae of Lorenzini of scates *Raja clavata* was investigated by means of electrophysiological and ligand-binding techniques. Exogenous glutamate and aspartate and their agonists, N-methyl-D-aspartate, kainate and quisqulate, dose-dependently activated the receptors. This effect as well as the responses to electrical stimulation were suppressed by the selective excitatory amino acids antagonists 2-amino-5-phosphono-valeric acid, glutamic acid diethylester, kinurenate, glutamyl-glycine, piperidine carboxylate, and L-serine-O-phosphate. The synthetic analogue of enkephalin, dalargin, diminished the glutamate-evoked responses. Ligand binding analysis revealed one type of ^{3}H-L-glutamate binding sites with K_D 286 nM and B_{max} 2.1 pmol/mg of protein. These experiments confirm the electrophysiological data about the existence of excitatory amino acid receptors in the snyaptic membranes of ampullae of Lorenzini.

103.3

DO SOME M-CELL GLYCINE RECEPTORS INTERACT WITH GABA? D. S. Faber and H. Korn. Dept. of Physiology, SUNY, Buffalo, NY, 14214, and INSERM, Institut Pasteur, Paris.

Activation of the Mauthner (M-) cell's recurrent collateral network produces a somatic IPSC considered to be glycinergic and a later, longer lasting dendritic one, for which the transmitter(s) is (are) unknown. Both produce Cl-dependent conductance changes which can be easily quantified. A contribution of GABA to the latter is suggested by reports of GABAergic terminals and receptors, particularly on the main dendrites. In confirmation, we found in current- and voltage- clamp experiments that GABA-A antagonists (Bicuculline, BIC, 40 µM and Picrotoxin, PTX, 100 µM) can completely abolish the dendritic IPSC. This response is often followed in current clamp by a late hyperpolarization due to an outward current, which can be produced by iontophoretic applications of GABA but not of glycine. Yet BIC and PTX could also suppress, or completely block, the glycinergic M-cell inhibition, and strychnine (≤ 1µM) reduced both somatic and dendritic IPSCs and their associated conductance changes by 80% or more. When one class of antagonist produced only a partial block, the other eliminated the remaining IPSC. These overlapping effects suggested that the blockers and/or a fraction of the receptors linked to Cl-channels are non-specific. This prospect was tested with iontophoresis of glycine; in 5 of 6 preparations superfused with BIC, the somatic glycine response was reduced by 63 ± 12% (mean ± SD) but never was blocked completely. In 4 cases this effect was associated with an increase in the apparent Hill coefficient. It can be concluded that, in addition to the glycinergic component of collateral inhibition, there is a dendritic GABAergic IPSC. In addition, at least some "glycinergic" receptors may be sensitive to GABA, particularly in cases where the two transmitters are colocalized in presynaptic terminals.

103.4

DIFFERENTIAL ACTIONS OF ANABASEINE AND ITS 3-DMAB ADDUCT UPON BRAIN AND NEUROMUSCULAR NICOTINIC RECEPTORS. W. R. Kem, L. Abraham and B. Lin. Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, FL 32610.

Anabaseine, (2-(3-pyridyl)-3,4,5,6-tetrahydropyridine), an alkaloid occurring in certain marine worm venoms, possesses pharmacological actions similar to (-)-nicotine. We have investigated the abilities of anabaseine, its dimethylaminobenzaldehyde (DMAB) adduct, and nicotine to displace specific [^{3}H]methylcarbachol binding to rat cerebral cortex synaptosomes, to cause a characteristic nicotinoid prostration behavior when injected i.c.v., to relax the contractile response of the innervated rat colon longitudinal muscle to oxotremorine, and to stimulate frog neuromuscular nicotinic receptors. Relative to nicotine, anabaseine is 5 times more potent upon frog neuromuscular nicotinic receptors, but displays about 15-fold lower affinity (K_I 60 nM) for brain receptors, and is 5 times less potent in causing rat prostration. At physiological pH, anabaseine exists in three different forms: unionized cyclic imine, monocationic cyclic iminium and open-chain ammonium-ketone (Zoltewicz, Bloom and Kem 1989). We found that nicotinic activity of anabaseine resides in its cyclic iminium form. Comparing the cyclic iminium form of anabaseine with the nicotine monocationic species, anabaseine is 10 times more active on skeletal muscle, possesses 7.5 times lower affinity for rat brain nicotinic receptors, and is about 2.5 times less potent than nicotine in causing rat prostration. Relative to nicotine, anabaseine displays greater selectivity for muscle nicotinic receptors. DMAB-anabaseine acts as an agonist upon neuronal nicotinic receptors, but lacks nicotinic agonist activity at the neuromuscular junction. Addition of a benzylidene group at the 3 position of anabaseine reduces the coplanarity of the pyridyl and tetrahydropyridyl rings of anabaseine, and this apparently results in a preferential decrease in neuromuscular potency. (Supported by Taiho Pharmaceutical Co., Ltd.)

103.5

EFFECT OF PMSF IN THE PRESENCE OF TACRINE ON MEMBRANE-BOUND AChE. C.G. Triplett* and K.A. Skau. Div. of Pharmacology/Medicinal Chemistry, College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267-0004.

Tacrine has been shown to be a noncompetitive inhibitor of AChE. Its site of action on AChE has not been fully determined but has been suggested to be at a hydrophobic site. The inhibition of AChE by Tacrine has shown a possible use in treatment of memory loss associated with Alzheimer's Disease (AD) in its early stages and determination of how Tacrine inhibits AChE may provide information in developing better drugs for treatment of AD. We have studied the effect of Tacrine interacting with PMSF in solubilized AChE, membrane-bound AChE, and in vivo. Rat brain was homogenized and soluble forms of AChE removed. The membrane-bound AChE was pretreated with Tacrine followed by PMSF with samples taken at timed intervals, homogenized with Triton and assayed by the method of Ellman. Our results show an acceleration of inhibition by PMSF with pretreatment of Tacrine on solubilized AChE. Fifteen min. pretreatment with Tacrine (10 mg/Kg) in vivo, followed by PMSF (85 mg/Kg) showed no increase or acceleration of inhibition by PMSF. Studies with pretreatment of Tacrine followed by PMSF on membrane-bound AChE showed no acceleration of inhibition by PMSF. These studies show a difference in effect of Tacrine on soluble and membrane-bound AChE. Membrane-bound AChE may have a change in conformation altering the ability of Tacrine to bind to AChE.

103.6

MUSCARINIC RECEPTOR-MEDIATED EVENTS IN OPOSSUM LUMBAR PARAVERTEBRAL GANGLIA. W.H. Percy, Division of Gastroenterology, Winthrop-University Hospital, Mineola, New York 11501 & SUNY at Stony Brook.

In rabbit lumbar paravertebral ganglia a percentage of cells exhibit a slow excitatory postsynaptic potential (s-EPSP) mediated via muscarinic m1 receptors. This phenomenon is related to the number of preganglionic fibers impinging on the cell (Percy & Krier, JANS **18:** 195-205, 1987). The aim of the present study was to investigate whether a similar phenomenon is found in the paravertebral ganglia of the North American opossum. The lumbar sympathetic chains from above the L3 ganglion to below the L5 ganglion with attached inferior lumbar splanchnic nerves and rami communicantes were removed *en bloc* from opossums euthanized with pentobarbital (75mg/kg i.p.). Intracellular recordings were made from the largest ganglion in the L4 region. All fast excitatory postsynaptic potentials and action potentials were hexamethonium-sensitive. No cells of 9 tested exhibited either a s-EPSP or s-IPSP in response to stimulation of preganglionic fibers at frequencies ranging from 0.5-20Hz. In 7 of 7 cells McNeil-A-343 (10^{-4}– 10^{-3}M) caused no change in transmembrane potential or membrane input conductance but fast excitatory synaptic events were reversibly abolished. This was blocked by an equimolar concentration of atropine but not pirenzepine. In a further 4 of 4 cells oxotremorine (10^{-4}M) caused a membrane depolarization and continuous discharge of action potentials. These data suggest that preganglionic fibers in the paravertebral chains posses inhibitory presynaptic muscarinic receptors whereas excitatory muscarinic receptors are located postsynaptically. The lack of a muscarinic s-EPSP may reflect a sparse preganglionic cholinergic input to these cells.

103.7

2,4-DITHIOBIURET DECREASES OPEN-TIME OF AChR-CHANNELS IN CLONAL CELLS AND INTACT NEUROMUSCULAR PREPARATIONS. J.M. Spitsbergen and W.D. Atchison, Department of Pharmacology and Toxicology and Neuroscience Program, Michigan State University, E. Lansing, MI 48824

2,4-dithiobiuret (DTB) a thiourea derivative with moderate reducing properties, causes a delayed onset neuromuscular weakness when given chronically to rats. Previous two microelectrode voltage-clamp experiments demonstrated that the decay time constant (τ) for miniature end-plate currents (MEPCs), recorded in muscles taken from rats exhibiting muscle weakness following treatment with DTB, was decreased compared to τ_{MEPC} for control muscles. Agents known to decrease channel open-time for the nicotinic AChR-channel also cause a decrease in MEPC decay. Thus, the purpose of the present study was to determine whether DTB exposure alters the open-time for nicotinic AChR-channels present in muscle. Effects of bath application of DTB on single-channel currents were examined using patch voltage-clamp techniques in a clonal cell line and fluctuation analysis in intact neuromuscular preparations. Exposure of cultured G8 myotubes to 1 μM DTB caused a decrease in mean channel open-time, measured at a holding potential of -100 mV, from 1.82 ± 0.28 ms in control cells to 0.98 ± 0.28 ms in treated cells. Mean channel open-time, measured at -50 mV, was also decreased from 12.4 ± 5.2 ms in hemidiaphragms taken from control rats to 3.93 ± 1.06 ms following exposure to 1 μM DTB in the bathing medium. Thus, exposure, of both cultured myotubes and intact hemidiaphragm preparations, to DTB appears to decrease mean channel open-time of nicotinic AChR-channels. This DTB induced decrease in channel open-time may underlie the decrease in τ_{MEPC} observed in muscles taken from rats treated with DTB.

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103.8

SIGMA LIGANDS MODIFY CHOLINERGIC AGONIST-INDUCED Ca^{2+}-ACTIVATED K^+-EFFLUX IN DIFFERENTIATE PC-12 CELLS. H.Yamamoto[1]*, T.Yamamoto[1], N.Sagi[1]*, T.Hori[1]*, T.Moroji[1]*, T.Yamaguchi[2], [1]Dept. of Psychopharmacology, Psychiatric Res. Inst. of Tokyo, Tokyo156, [2]Medical Res.Lab. I, Central Res. Lab., Yamanouchi Pharmaceutical Co., Ibaraki 305, Japan.

PC-12 cells has been shown to express sigma-2 site as well as many properties of neurons, including neurite formation by NGF. Using differentiated PC-12 cells, the interaction between cholinergic receptors and sigma compounds was investigated. The cells treated with NGF(50ng/ml) were washed twice, preincubated for 20min at 37°C in the modified Locke medium(K^+ replaced by Na^+), finally incubated with drugs for 20min. The amount of K^+-efflux was measured using K^+-selective microelectrode. The response to carbachol application shows a dose-dependent increase of K^+-efflux. (Maximal response at 10^{-4}M-10^{-3}M conc. of carbachol; 2.6-fold of control). The stimulated K^+-efflux was markedly inhibited by phencyclidine(PCP), and moderately antagonized with 1,3-di-o-tolylguanidine(DTG) or haloperidol (35.0, 48.8 or 50.1 % of control, respectively). Antidepressants (desipramine or iprindole) also inhibited the carbachol-induced K^+-efflux (43.2 or 71.8% at the 10^{-4} M conc.) These results indicate that sigma-2 site modulates cholinergic receptor-coupled K^+-efflux in the differentiated PC-12 cells.

103.9

DOPAMINE-INDUCED GANGLIONIC BLOCKADE: ANTAGONISM BY DRUGS THAT INCREASE cAMP. C. A. Davis* and K. A. Alkadhi. Department of Pharmacology, University of Houston, Houston, TX 77204-5515.

Dopamine acting through DA_1 receptors is known to increase cAMP levels in a variety of tissues. However, measurement in sympathetic ganglia showed either a decrease or no change in cAMP levels on treatment with dopamine or fenoldopam both of which inhibited postganglionic spike (Alkadhi et al. *JPET* **238**:547, 1986; Sabouni et al. *JPET* **240**:93, 1987). The involvement of cAMP in the inhibition of the ganglionic spike was investigated in the isolated superior cervical ganglion (SCG) of rat. Suction electrodes were used to stimulate preganglionic nerve and record evoked compound postganglionic spike. Dopamine (1-566 µM) produced concentration-dependent inhibition of the spike. This effect was readily reversed after 30 min of wash. When the ganglia were pretreated with isoproterenol (1 µM) the dopamine-induced inhibition was markedly attenuated and there was a dextral shift of the dopamine concentration-inhibition curve. Similar results were obtained on pretreatment of ganglia with the adenylate cyclase activator arginine-vasopressin (10 mU). Isoproterenol or vasopressin alone had no significant effect on the spike (1 hr incubation). Preliminary results with adenylate cyclase inhibitors (e.g. SQ 22,536) and phosphodiesterase activators (e.g. imidazole) suggest that these compounds could enhance the inhibitory action of dopamine and antagonize the effects of isoproterenol or vasopressin on the dopamine-induced inhibition of the spike. The results indirectly suggest that the inhibitory action of dopamine may be due to a decrease in cAMP level.

103.10

DOPAMINE (DA) RELEASE IN INTERMEDIATE PITUITARY: REAL TIME MEASUREMENTS & PHARMACOLOGY. P.J.WILLIAMS, T.V.DUNWIDDIE, G.A.GERHARDT, Depts Pharmacology & Psychiatry, Univ Colorado Health Sciences, 4200 E 9th Ave, Denver, Colorado, U.S.A. 80220

We investigated the synaptic release of DA in intact intermediate pituitary (IL) maintained in vitro. This system is ideal for the study of DA neurotransmission since it contains only a single type of DA receptor (D2), & consists of only the axon terminals of the DA neurons & postsynaptic cells (melanotrophs). Electrochemical measurements were made using single 30µ carbon fiber electrodes coated with Nafion to enhance their sensitivity & selectivity to DA. When rats were pretreated with pargyline (100mg/kg) DA release was routinely detected in response to electrical stimulation of the pituitary stalk (0.5ms, 10Hz, 10V, 30 sec). In control conditions peak DA concentration following stalk stimulation was between 100 & 300nM. Release was abolished by the Ca^{++}-channel blocker Cd^{++} (1µM) & by 100nM tetrodotoxin. Release was modulated by presynaptic autoreceptors since the D2 antagonist sulpiride (1µM) increased DA release by 97± 30% (mean+S.D.). Quinpirole (10µM), a DA D2 agonist, however, had no effect on DA release, so released DA may be already maximally inhibiting these receptors. DA reuptake inhibitors markedly increased peak DA concentrations (20µM Nomifensine - 100±12%, 20µM Mazindol - 108±20%, 20µM Cocaine - 70±22%) thus confirming the importance of reuptake in removing released DA. The IL provides an excellent system for real time study of synaptic DA release. Supported by USPHS Grants NS09199 & DA02702 & Veterans Administration

103.11

RAT VENTRAL PALLIDAL NEURONS RECORDED *IN VITRO*: MEMBRANE PROPERTIES AND RESPONSES TO DOPAMINE. R.J. Maslowski, T.C. Napier and S.G. Beck. Department of Pharmacology, Loyola University Chicago, Stritch School Medicine, Maywood, Illinois 60153.

The ventral pallidum (VP) is comprised of a heterogenous cell population which includes cholinergic neurons. Extracellularly recorded VP neurons demonstrate both rate increases and decreases to microiontophoretic applications of dopamine[1] The present study was conducted to examine membrane properties of rat VP slices recorded *in vitro* and to determine the effects of bath-applied dopamine (100uM). In contrast to the adjacent striatal region, viable VP neurons were obtained only after utilizing tissue preparation methods of Aghajanian and Rasmussen[2]. VP neurons (N=18) were segregated into three categories based on the post-spike after-potentials: fast (39%) or slow (50%) hyperpolarizing (AHPs), or depolarizing after-potentials (11%). Neurons with slow AHPs were similar to those described as AChE-positive by Griffith and Matthews[3]. All three groups had a comparable resting membrane potential (68 ± 1.4mV) and input resistance (146 ± 9.5MΩ). Eleven neurons tested with dopamine demonstrated an average depolarization of +6 ± 1.5mV. In addition, dopamine often augmented spontaneous synaptic activity (9 of 11 cells). The response to the agonist did not distinguish among cell types. Thus, dopamine-induced increases in firing rate that are observed for these neurons with *in vivo* extracellular recording techniques may be attributed to the above *in vitro* effects. Supported by MH45180 to TCN; MH41917 and KO2-MH00880 to SGB.

1. Napier and Potter, Neuropharmacology 7:757, 1989.
2. Aghajanian and Rasmussen, Synapse 3:331, 1989.
3. Griffith and Matthews, Neurosci. Lett. 71:169, 1986.

103.12

NOREPINEPHRINE INHIBITS MITRAL CELL EVOKED EPSPS IN MAMMALIAN OLFACTORY BULB GRANULE CELLS IN CULTURE. Paul Q. Trombley & Gordon M. Shepherd. Section of Neurobiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

We are interested in plastic changes in the olfactory bulb that may underlie olfactory learning. Recent evidence suggests that alterations in the efficacy of excitatory and inhibitory synapses between mitral (M) and granule (GR) cells may be the bases of these changes (Brennan et al. 1990). Norepinephrine (NE) has been implicated in olfactory learning, and although the mechanism is not well understood it has been proposed that NE may act by reducing GR cell inhibition of M cells (Jahr & Nicoll, 1982). We examined the action of NE on synaptic potentials between pairs of monosynaptically coupled M and GR cells to reveal modulatory effects of NE that might be related to synaptic plasticity.

Dissociated olfactory bulb neurons from neonatal rat pups were grown in primary culture. Using simultaneous whole-cell recording from monosynaptically coupled M-GR cell pairs, an EPSP mediated by glutamate was evoked in the GR cell by intracellular stimulation of the M cell. These EPSPs invariably consisted of both CNQX and AP5 sensitive components. Flow pipe application of 30 μM NE reduced these EPSPs to 52 ± 14 % of control in 6 out of 6 pairs. Under voltage clamp, however, 30 μM NE had no effect on glutamate evoked currents in 5 out of 5 GR cells. In both M and GR cells NE did not affect currents evoked by voltage ramps from -60 to +40 mV nor currents evoked by discrete 10 mV steps between -60 and +40 mV.

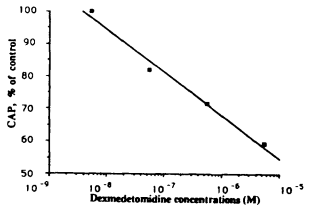
Concurrent results (Trombley & Westbrook, in preparation) demonstrate that NE acts presynaptically to inhibit high threshold calcium currents. These results suggest that NE may disinhibit M cells not by a direct action on GR cells but rather by reducing M cell excitation of GR cells. This effect would reduce GR cell inhibition of M cells at reciprocal dendrodendritic synapses. This suggests a mechanism in which arousal, mediated through NE, could affect synaptic plasticity associated with leaning. Supported by NIH 5-T32-NS07224-07 to PQT and NIH DC-00086 to GMS.

103.13

α_2 -ADRENOCEPTOR AGONISTS (AAA) MODULATE GANGLIONIC TRANSMISSION IN THE PERIPHERAL SYMPATHETIC NERVOUS SYSTEM (CNS). H.K. Schedewie*, N. Boban* and Z.J. Bosnjak. Dept. of Anesthesiology, The Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

The inhibitory effects of AAA on central sympathetic outflow at the spinal and cranial CNS level have been well established. However, AAA effects on the peripheral CNS remain to be elucidated. Therefore, we have studied the effect of dexmedetomidine (D), a selective AAA, and its antagonist yohimbine (Y) on synaptic transmission in the canine stellate ganglion (SG) *in vitro*.

Ten SG were desheathed and superfused with modified Krebs' solution (Ca^{++} 0.85 mM), equilibrated with 97% O_2 - 3% CO_2 (37°C, pH 7.4). The preganglionic T_3-ramus and the postganglionic ventral ansa subclaviae were used for stimulating and recording purposes, respectively. The percent compound action potential (CAP) change from control was measured following 15-min periods of SG superfusion with log step increments of D 5.5 x 10^{-9} to 10^{-6} M or equimolar concentrations of Y. Hexamethonium (10^{-4} M) was used at the conclusion of the experiment to verify the synaptic nature of CAP. D caused a dose-dependent CAP depression (fig) which was specifically and consistently reversed by Y. These findings suggest that AAA have potent inhibitory effects on the peripheral SNS in addition to their well known central effects. Peripheral AAA action may have important physiological and potential therapeutic implications for the control mechanism that regulates cardiopulmonary and nociceptive function.



SYNAPTIC TRANSMISSION

104.1

L-TYPE VOLTAGE SENSITIVE CALCIUM CHANNELS MEDIATE SYNAPTIC ACTIVATION OF IMMEDIATE EARLY GENES. T.H. Murphy, P.F. Worley, and J.M. Baraban. Dept of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Although L-type voltage sensitive Ca^{++} channels (VSCC) have been well characterized, their role in neuronal function remains unclear. Recent studies demonstrating localization of these channels to the soma suggest they may be involved in coupling synaptic activity to transcriptional regulation. Previously, we found that spontaneous synaptic activity present in cultured cortical neurons leads to the expression of immediate early genes (IEG), as TTX and Glu receptor antagonists reduce basal expression. We now report that treatment of these cultures with L-type VSCC blockers PN 200-110 or nitrendipine decrease basal c-Fos immunostaining and *zif268*, *jun*-B, and *fos*-B, mRNA. Conversely, (-)BayK-8644, a VSCC agonist, augments basal expression. These agents do not appear to be acting in a non-specific fashion as they do not reduce gene induction by phorbol esters. Furthermore, these agents do not suppress synaptic Ca^{++} transients, although they reduce kainate induced rises in intracellular Ca^{++} and c-Fos expression. Measurement of Ca^{++} during synaptic stimulation indicates contributions of both NMDA and kainate receptors. Our findings indicate that even though L-type VSCCs appear to contribute only a small fraction of synaptically-induced Ca^{++} transients, they play a key role in coupling synaptic activity to regulation of gene expression.

104.2

EXCITATORY POSTSYNAPTIC POTENTIALS EVOKED BY VENTRAL ROOT STIMULATION IN NEONATE RAT SYMPATHETIC PREGANGLIONIC NEURONS IN VITRO. E. Shen, S. Y. Wu*, C. Ren*, S. L. Dun* and N. J. Dun. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Intracellular recordings were made from sympathetic preganglionic neurons (SPNs) in neonate rat transverse spinal cord slices. Stimulation of ventral rootlets evoked in the majority of SPNs a depolarizing response and in a few SPNs a hyperpolarizing response. We have shown that the hyperpolarization was a di-synaptically mediated inhibitory postsynaptic potential (IPSP). The depolarizing response was eliminated in a low Ca (0.25 mM) solution and was reduced by the excitatory amino acid receptor antagonist CNQX, indicating it was an excitatory postsynaptic potential (EPSP). The amplitude of EPSPs increased with membrane hyperpolarization; the extrapolated reversal potential was between -10 to 0 mV; the responses were not significantly increased in Mg^{2+}-free solution. The estimated conduction velocity of the fibers transmitting the EPSPs was about 1 m/s. Dense fiber tracts and labelled cells were observed in the lateral horn and ventral horn following in vivo injection of DiI into the dorsal root ganglia. Our results indicate that SPNs receive glutaminergic inputs from ventral root afferents which may have their origin in the dorsal root ganglia. (Supported by NS18710).

104.3

Spatial and Temporal Studies of Electrochemical Oscillations of The Brain Extracellular Space. C. C. Turbes. Creighton University School of Medicine, Omaha, Nebraska 68178

In these studies, recordings from the brain extracellular space of cats are considered. Macro and microelectrodes are utilized. Special studies using visual, auditory stimuli and certain drugs are done. Drug studies involved amphetamine, ketamine and lidocaine with recordings from cerebral cortex, hippocampus, amygdala and thalamus. Coherence power and partial coherence spectra of wave field potentials and unit potentials are investigated.

There are several reasonable mechanisms whereby natural ac electrical oscillations arise in cells from cooperative dipolar oscillations fed by metabolic energy. This mechanism accounts for oscillations at very high frequencies in the multigigahertz range. We are interested in electrical fields present in the extracellular space of the brain. Our interest is in the relationship of the intrinsic electrochemical oscillations in the pericellular space of the brain as they change during induced chemical and electrical changes. We are interested in the frequency and coherence of these electrical fields. In these studies microelectrode recordings are compared with macroelectrode recordings in the extracellular space.

104.4

G_o AND GAP-43 ARE CONCENTRATED WITHIN AXON TERMINALS OF ADULT RAT VISUAL CORTEX THAT FORM ASYMMETRIC SYNAPSES. C. Aoki, L.I. Benowitz, K. Wu & P. Siekevitz, Center for Neural Science, New York Univ., NYC, 10003.

Visual cortex (area 17) of aldehyde-fixed adult rat brains were immunolabeled either for GAP-43 or for G_o, a GTP-binding protein, by the pre-embed avidin-biotin peroxidase complex method using previously characterized antisera (Benowitz et al., J. Neurosci. 8:339; Worley et al., PNAS 83: 4561). Light microscopic labeling for GAP-43 and G_o revealed most neuronal perikarya, including pyramidal cells, as unlabeled profiles within a rather homogeneously immunoreactive grey matter. In addition, intense immunoreactivity for G_o was evident within perikarya and dendrites of a few bipolar neurons. Electron microscopy showed that the antigenic sites for GAP-43 were almost exclusively within axons and terminals forming asymmetric synapses, many of which were perforated. G_o-immunoreactivity also occurred within axons and terminals forming asymmetric perforated synapses. These synapses resembled those associated with glutamate-immunoreactive terminals in previous studies. The results support the following ideas: (1) both G_o and GAP-43 are localized to axon terminals of adult cortex forming excitatory synapses; (2) these are sites where GAP-43 may stimulate GTP binding to G_o (Strittmatter et al., Nature 344: 836), perhaps in relation to the modulation of glutamatergic transmission. Future work are needed to determine whether single axons contain both G_o and GAP-43. (Supported by EY08055 to CA; NS25830 to LB)

ACETYLCHOLINE RECEPTORS: NEURONAL NICOTINIC I

105.1

PHARMACOLOGICAL PROPERTIES OF THE HOMO-OLIGOMERIC NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR A7. M Ballivet, S. Bertrand* and D. Bertrand Dpt of Physiology Medical School, 1, Rue Michel Servet, 1211 Geneva 4, Switzerland.

Two laboratories have recently isolated from chicken brain a cDNA encoding an α-bungarotoxin (α-Bgt) sensitive protein (α7). Functional neuronal nicotinic acetylcholine receptors (nAChR) can be expressed by nuclear injection of this cDNA alone, indicating that the protein forms homo-oligomeric nAChR. Physiological and pharmacological properties of α7 differ from those of other known neuronal AChRs. While ACh sensitivity of α7 is rather low (EC_{50} = 115 μM), its sensitivity to nicotine and cytisine is about ten fold higher. Curare (TC) is a potent antagonist of α7, but the block induced by TC is not competitive whereas TC is a competitive blocker of the major brain nAChR α4/nα1. Receptors formed by α7 resemble those of the neuromuscular junction in their sensitivity to α-Bgt and insensitivity to hexamethonium. However, the larger methonium salt decamethonium decreases the amplitude of ACh-evoked currents, suggesting that channels formed by α7 present a slightly larger apparent pore diameter than that of other neuronal nAChRs.

105.2

SINGLE CHANNEL CURRENTS THROUGH α-BUNGAROTOXIN-SENSITIVE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS. N. Hussy and D. Bertrand. Dpt de Physiologie, CMU, Univ. de Genève, 9 Av. de Champel, 1211 Geneva 4, Switzerland.

A gene encoding a neuronal nicotinic acetylcholine receptor α subunit (α7) sensitive to the snake toxin α-bungarotoxin has been recently cloned from chicken brain. Expression of this single gene into *Xenopus* oocytes yields functional nicotinic receptors indicating that α7 can form homo-oligomeric channels. We investigated the single channel properties of α7 receptors reconstituted into oocytes. *Xenopus* oocytes were injected intranuclearly with a vector containing the α7 cDNA under the control of a SV40 promoter. Oocytes presenting large responses to acetylcholine (>1μA) two days after injection were selected for patch-clamp recordings, in the cell-attached configuration. Receptors appeared highly clustered in the oocyte membrane, and presented a single slope conductance of 45pS in physiological solution in the presence of 10μM ACh. These channels were never observed in the absence of ACh in the pipet. No activity was observed at depolarized potentials, suggesting that the strong rectification exhibited by whole-cell recordings of α7 receptors may be due to a decreased channel open probability. The kinetics of the channel openings are currently investigated.

105.3

DISTRIBUTION OF ALPHA 7 NEURONAL NICOTINIC RECEPTOR mRNA IN THE RAT CNS. D.S. Johnson, H.M. Stroessner-Johnson, J. Boulter, D.G. Amaral, and S. Heinemann. The Salk Institute For Biological Sciences, La Jolla, CA 92037; Department of Neuroscience, UCSD, San Diego, CA 92122.

Alpha-bungarotoxin has proven a useful tool in the elucidation of the structure, distribution, and function of the nicotinic acetylcholine receptor at the neuromuscular junction. Specific binding sites for α-bungarotoxin also exist within the CNS. The recently cloned chicken nicotinic acetylcholine receptor α7 subunit has been shown to code for a functional receptor which is blocked by α-bungarotoxin. We have used a full length cRNA probe to the rat α7 subunit gene to carry out *in situ* hybridization studies in rat brain sections. The distribution of α7 RNA in rat CNS correlates strongly with total α-bungarotoxin binding, suggesting that this subunit accounts for most of the toxin binding sites. Many limbic areas are labeled, including: hippocampus (CA3 > CA2 > CA1); dentate gyrus; medial, cortical, and basal amygdaloid nuclei; and entorhinal, pyriform, and cingulate cortices. All layers in cerebral cortex are labeled, with the heaviest label occurring in the deepest layers. Many hypothamic nuclei are intensely labeled, including: supra-, medial, and lateral mammillary nuclei; lateral and anterior hypothalamic areas; supraoptic, arcuate, and ventromedial hypothalamic nuclei. The superior colliculus is labeled, as are many reticular and cranial nerve nuclei. One notable exception to the correlation between toxin binding and the distribution of α7 message is the absence of signal in the suprachiasmatic nuclei which exhibit high toxin binding.

Supported by: Tobacco-Related Disease Research Program, University of California; MDA Research Grant; and NIH Jacob Javits Award (S.H.)

105.4

IMMUNOHISTOCHEMICAL STUDIES OF THE DISTRIBUTION OF ALPHA-BUNGAROTOXIN BINDING PROTEINS AND NICOTINIC RECEPTORS IN THE CHICK VISUAL SYSTEM. L.R.G. Britto, K.T. Keyser, H.J. Karten, R. Schoepfer*, P.J. Whiting*, W.G. Conroy*, & J.M. Lindstrom*. Dept. Neurosci., Univ. California, San Diego, La Jolla CA 92093 and *Inst. Neurol. Sci., Univ. Pennsylvania, Philadelphia PA 19104.

Two subtypes of brain alpha-bungarotoxin binding proteins (BGTBPs) have been recently identified in the chick brain (Schoepfer *et al.*, ***Neuron*** 5:35-48, 1990). One BGTBP subtype contains only α1 subunits, whereas the other includes both α1 and α2 subunits. We have used monoclonal antibodies to study the localization of both α1 and α2 BGTBP subunits in the chick visual system. Their distribution was compared to that of nicotinic receptor-like immunoreactivity (nAChR-LI). All retinorecipient areas of the chick brain were observed to contain nAChR-LI, α1BGTBP-LI and α2BGTBP-LI. In some structures, both the somata and the neuropil staining for all three kinds of immunoreactivity was similar (e.g., ventral lateral geniculate nucleus, pretectal nuclei and the intergeniculate leaflet). In some other structures, however, we observed marked differences in the distribution of labeled somata and the intensity of neuropil staining with the different antibodies. For example, neuropil staining within the suprachiasmatic nucleus was more intense for nAChR-LI than for α1BGTBP-LI and α2BGTBP-LI, whereas staining for α2BGTBP-LI was by far most evident in the visual nuclei of the dorsal thalamus. In the isthmo-optic nucleus, the origin of the centrifugal system, no staining was observed for nAChR-LI or α2BGTBP-LI, but there was intense labeling for α1BGTBP-LI. Retinal lesions produced a marked reduction of nAChR-LI neuropil staining in retinorecipient structures. Neuropil staining for α1BGTBP-LI and α2BGTBP-LI was also reduced after retinal lesions, but to a lesser degree. These results reveal clear differences in the localization of nAChR-LI, α1BGTBP-LI and α2BGTBP-LI in the chick brain and suggest that both nAChRs and BGTBPs are transported from the retina to the brain.

Supported by EY07845 (KTK), EY06890 and NS24560 (HJK), Contract USP/BID and FAPESP (LRGB) and grants from NIH, MDA & CTR (JML).

105.5

ASSEMBLY AND FUNCTION OF α-BUNGAROTOXIN BINDING PROTEIN SUBUNITS EXPRESSED IN *XENOPUS* OOCYTES.
R. Anand, M. White and J. Lindstrom. Institute of Neurological Sciences and Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia PA 19104.

cDNA clones for two subunits of the chick brain α-bungarotoxin binding proteins (αBgtBP α1 and α2) have been recently identified (Schoepfer et al., *Neuron* 5:35-48, 1990). There are two subtypes: >75% of all αBgtBPs contain the α1 subunit, while ~15% contain both the α1 and α2 subunits, plus and undefined complement of structural subunits. Binding studies with synthetic peptides reveal that both α1 and α2 bind ^{125}IαBgt (McLane et al., 1991, *JBC*, in press). We find major differences in the ability of these subunits to assemble and function in the *Xenopus* oocytes. We confirm that the α1 subunit when expressed alone in oocytes forms acetylcholine-gated cation channels (Couturier et al., *Neuron* 5:847-856, 1990), but we find that the α2 subunit fails to do so. The expressed α1 subunits produce currents in response to bath application of acetylcholine (EC_{50}=90μM) which can be blocked by 10nM αBgt. The α2 subunit expressed alone gives no response to acetylcholine. The α1 subunit expressed by itself and in conjunction with the α2 is efficiently transported to the oocyte outer membrane where it binds ^{125}IαBgt with high affinity, whereas the α2 subunit by itself fails to do so. In addition, Triton X-100 solubilized αBgtBP formed from α1 subunits alone or from α1 co-expressed with α2, but not by α2 alone, cosediment on sucrose gradients with solubilized chicken brain αBgtBPs, and thus appear to form homo- or hetero-oligomers of the same size as native αBgtBP.

105.6

THYMOPOIETIN INTERACTS WITH AND REGULATES α-BUNGAROTOXIN BINDING SITES IN HIPPOCAMPAL CELLS IN CULTURE.
R. Afar, G. Goldstein and M. Quik. Dept. Pharmacol., McGill U., Montreal, Can. and Immunobiol. Res. Inst., NJ, USA.

Previous studies in our lab have shown that the thymic polypeptide thymopoietin (TPO) bound in a specific manner to the α-BGT binding site in brain, but did not interact with the high affinity nicotinic receptors. The present studies were done to investigate the regulation of the former sites by TPO in hippocampal neurons in culture, which provide a rich source of α-BGT binding sites. Hippocampal neurons in culture were obtained from day 18-19 old rat embryonic hippocampi. Inhibition curves with increasing concentrations of TPO showed that TPO inhibited [^{125}I]-BGT binding with an IC_{50} of 19 nM, compared to an IC_{50} of 550 nM for nicotine. The effect of TPO did not appear to be readily reversible, even though binding of TPO to whole brain homogenates is reversible within 30 minutes. In addition, long term exposure to TPO affected the α-BGT binding sites; a three day exposure to TPO resulted in a 73% decrease in B_{max} with no change in K_d. TPO can therefore interact with and regulate α-BGT binding sites in rat brain implying that this polypeptide may act as a ligand for this receptor.

105.7

SYNTHESIS, PURIFICATION, AND CHARACTERIZATION OF A PUTATIVE ENDOGENOUS LIGAND FOR THE α-BUNGAROTOXIN BINDING PROTEIN: THYMOPOIETIN.
D.D. Smith*, J. Petzel*, and B.J. Morley. Dept. of Biomedical Sciences, Creighton University Medical School Omaha, NE 68178, and Research Division, Boys Town National Research Hospital, Omaha, NE 68131.

Several laboratories have reported that thymopoietin (TPO), a polypeptide isolated from thymus, will alter muscle function, block α-bungarotoxin binding to neural and muscle-like cells in culture, and induce neurite formation in PC12 cells. TPO-like immunoreactivity is found in mammalian brain, and it has been hypothesized that TPO may be the endogenous ligand for the brain α-bungarotoxin binding protein.

Thymopoietin II was made using Merrifield's solid phase polypeptide, synthesis methodology. Butyloxycarbonyl-amino acid derivatives were double coupled as their hydroxybenzotriazole-active esters to the phenylacetamidomethyl polystyrene resin in the solvent, N-methylpyrrolidone. Unreacted amine groups were capped with acetic anhydride. The peptide was cleaved from the resin employing the low-high trifluoromethanesulphonic acid method and purified by gel filtration, ion-exchange and RP-HPLC techniques. Correct amino acid compositions and mass determinations were obtained. Further confirmation of the peptide's structure was gained after digestion of the peptide with S. aureas, V8 and identification of the resulting fragments by amino acid analysis and mass spectrometry.

The biological activity of synthetic thymopoietin and peptide fragments was tested using bioassays and *in vitro* receptor binding assays.

This research was supported in part by DA06482 to BJM.

105.8

IDENTIFICATION OF *CIS*-ACTING ELEMENTS REGULATING THE α3 AND α7 NEURONAL NICOTINIC AChR GENES.
L.Matter-Sadzinski*,M.C.Hernandez*,S.Couturier*, M.Ballivet and J.-M.Matter. Département de Biochimie, Université de Genève, 30, quai Ernest-Ansermet, 1211 Genève 4, Switzerland.

Ten neuronal chicken nAChR genes have been cloned and each of them exhibits a distinct pattern of expression in the developing central or peripheral nervous system. We have begun to define the *cis*-acting regulatory elements which govern the spatio-temporal expression of α3 and α7 nAChR genes during development of the chick nervous system. 5' flanking regions of both genes have been cloned and sequenced, and restriction and deletion fragments were fused to the reporter genes encoding CAT or SV40 T-antigen. Activity of these fragments was determined in transient transfection assays on cells freshly dissociated from different regions of the CNS (e.g., retina and optic tectum) at various stages of embryonic development. For both α3 and α7 we have delimited control elements which are active in neurons but inactive in glial cells. Furthermore, the control element of α7 was found to be active in identified retinal ganglion cells, i.e., in the cell population where α7 transcripts have been localized.

105.9

REGULATION OF NEURONAL NICOTINIC ACH RECEPTOR GENE EXPRESSION.
M. Hu*[1], N.L. Whiting*[1], J. Boulter*[2], E. Deneris*[2], R. Duvoisin*[2], S. Heinemann[2] and P.D. Gardner[1]. [1]Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756 and [2]Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138.

In situ hybridization studies utilizing recombinant DNA probes for neuronal nACh receptor subunit genes indicate differences in the cell-specific patterns of expression of the various members of this gene family. As part of a long term goal to understand the molecular events controlling nACh receptor expression, we have begun to identify the regulatory mechanisms involved in the cell-specific expression of these genes. Our initial studies have focussed on the gene encoding the β4 subunit of neuronal nACh receptors. The β4 subunit gene exhibits a very restricted pattern of expression in the nervous system. It is likely that this limited expression is due, at least in part, to transcriptional regulation of the β4 subunit gene. Consequently, we have begun to identify transcriptional regulatory elements in the β4 subunit gene. Initial studies indicated that 2.3 kb of 5' flanking DNA of the β4 subunit gene are sufficient to confer cell-type-specific expression of a reporter gene (luciferase) in transfected mammalian cells. Deletional analysis of this DNA indicated that a 391-bp region of the β4 subunit gene contains the sequence information necessary for the cell-type-specific expression. In addition, the deletional analysis revealed the existence of both positive and negative transcriptional regulatory elements in the 5' flanking DNA.

105.10

α3, β4, AND α5 HUMAN NICOTINIC RECEPTOR SUBUNITS GENES: GENETIC LINKAGE, CHROMOSOMAL LOCALIZATION AND DIFFERENTIAL EXPRESSION IN NEURONAL AND NON-NEURONAL CELLS.
B. Chini*, F. Rubboli*, P. Tarroni*, D. Fornasari*, E. Raimondi*°, F. Clementi. CNR Center of Cytopharmacology, Univ. of Milano, Milano, Italy; °Dept. of Genetics and Microbiology, Univ. of Pavia, Pavia, Italy

The cDNAs coding for the human α3, β4 and α5 human nicotinic receptor subunits were cloned from a neuroblastoma cell line. Using these clones as probes, several genomic clones were isolated for studying the organization of the α3, β4 and α5 genes in the human genome. By Southern blot and restriction mapping analysis of two overlapping genomic clones, we could demonstrate that the α3, β4 and α5 human genes are organized in a cluster, as already described in rat and chicken. Furthermore, by in situ hybridization on metaphase chromosomes using both cDNA and genomic probes, we localized the α3, β4 and α5 human genes in the same locus on chromosome 15.

We have also studied the expression of the α3 and α5 subunit genes in several human cell lines in culture. The expression of the α3 subunit was restricted to neuroblastoma and small-cell lung carcinoma cell lines; the latter have several neuro-endocrine properties. Transcripts corresponding to the α5 subunit were also found in several non neuronal cells of different embryological origin, a finding which opens a new perspective on the role of this subunit in non-excitable cells.

105.11

CHARACTERIZATION OF VARIANT THYMIC cDNA CLONES ENCODING FOR THE ALPHA-3 NEURONAL ACETYLCHOLINE RECEPTOR SUBUNIT: NOVEL EXON AND MULTIPLE 3' END NON-CODING ALTERNATES. M. Mihovilovic, A. Vaughan* C. Austin*and A.D. Roses. Depts. of Medicine & Neurobiology, Div. of Neurology, Duke University Medical Center, Durham, N.C.

We have isolated 13 cDNA clones encoding for the α-3 subunit of a thymic neuronal AcChR (M. Mihovilovic and A.D. Roses; Exp. Neurol. 111:175-180, 1991). Sequencing and PCR analysis have identified 2 clones that carry a 122bp insert downstream from exon 5. The insert not only shifts the reading frame of the normal α-3 transcript but also carries an in-frame stop codon. Translation of this variant transcript will result in a truncated peptide lacking the complete 4th membrane spanning domain. The domain is needed for the production of a functional AcCh gated ion channel. PCR analysis of genomic DNA suggests that the 122bp fragment represents a novel exon. RNase protection assays utilizing thymic poly (A)+RNA indicates that both the variant and normal transcripts of α-3 are expressed in thymus.

In addition to the 122bp variant, 3 alternative 3'end sequences are represented in the isolated thymic α-3 cDNA clones. The existence of multiple α-3 variant cDNA clones suggests that transcriptional regulatory mechanisms play an important role on the expression of thymic neuronal AcChR(s).

The roles played by the normal and the variant α-3 transcripts and their translation products in thymus are unknown. It is likely that the α-3 subunit associates with 1 or more subunits of the AcChR family to produce a thymic AcChR. A thymic AcChR may play a role in the transduction of signals originating in the CNS and transmitted to the thymus through autonomic innervation; and/or could represent a poorly understood cholinergic autoimmunogen in human acquired Myasthenia gravis(MG).

105.12

NICOTINIC RECEPTOR SUBUNIT mRNAS IN RAT PERIPHERAL GANGLIA. A. Mandelzys, E. Deneris*, and E. Cooper. Dept. of Physiol., McGill Univ., Montreal, Quebec, H3G 1Y6 and *Dept. of Neurosciences, Case Western Reserve Univ., Cleveland, Ohio,44106-4975

Recently, we have characterized functional nicotinic receptor (nAChR) current densities on neurons from both the sympathetic superior cervical ganglion (SCG) and the sensory nodose ganglion using whole-cell patch clamp techniques. All SCG neurons were sensitive to ACh and over 75% of the neurons had ACh current densities greater than 30 pA/pF. In contrast, most nodose neurons do not express functional nAChRs, and of those that were sensitive to ACh, the majority had ACh current densities below 8 pA/pF. However, we have shown that when nodose neurons develop in culture without other cell types, most nodose neurons express nAChRs and their ACh current densities can be up-regulated by NGF. We have now determined which nAChR subunit mRNAs are present in the two ganglia. Using the RNase protection assay, we examined total cellular RNA isolated from newborn rat SCG and nodose ganglia with antisense RNA probes to the mRNA for the α_2-α_5 and β_2-β_4 subunits. The results indicate that the major nAChR subunit mRNAs present in both ganglia are α_3 and β_4, with a lesser, although significant amount of β_2. This finding presents the possibility that the majority of nAChRs expressed by these neurons may be α_3/β_2, α_3/β_4, or a hybrid receptor incorporating α_3 and both β_2 and β_4. We are currently investigating whether the satellite cell influence and/or NGF are acting at the pre- or post-translational level on nodose neurons to specifically increase nAChR subunit mRNAs or to cause the appearance of novel nAChR subunit mRNAs. (Supported by the MRC of Canada, FRSQ, and FCAR).

105.13

β-NERVE GROWTH FACTOR ENHANCES ^{3}H-NICOTINE BINDING TO NICOTINIC CHOLINERGIC RECEPTORS ON PC 12 CELLS. T.C. Madhok and B.M. Sharp.* Endocrine-Neuroscience Laboratory, Minneapolis Medical Research Foundation, and Dept. of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55455.

Although nicotinic cholinergic agonists affect PC 12 cells, radioligand binding sites have not been described. We, therefore, studied PC 12 cells incubated in the presence of β-nerve growth factor (β-NGF). Specific ^{3}H-nicotine binding sites were induced approximately 3-fold above baseline in the presence of β-NGF (50 or 100 ng/ml); specific binding was maximal by the second to fourth day and these levels were detected for two weeks. By equilibrium analysis, K_d of 356.5 ± 6.9 nM, mean ± S.E., N=3 and B_{max} of 65 x 10^{-21} mole/cell were obtained using intact cells. Association and dissociation rate constants were 0.0023 min^{-1} nM^{-1} and 0.0709 min^{-1} at 22° C or 0.0012 min^{-1} nM^{-1} and 0.0101 min^{-1}, respectively, at 4°C, yielding an apparent K_d of 38.7 ± 12.8 nM, N=4). Intact cells and cell membrane yielded similar K_d values. ^{3}H-nicotine binding was inhibited on an equimolar basis by L(-)nicotine and N-methylcarbamylcholine. D(+) nicotine was 10-fold less potent while, α-bungarotoxin, mecamylamine and atropine show little or no inhibition. ^{3}H-nicotine binding was also inhibited quantitatively by monospecific polyclonal antibodies raised against the predicted α-3 subunit sequence (amino acids 130-139) of the rat neuronal nicotinic receptor. Sedimentation rate analysis showed that the NGF-sensitive ^{3}H-nicotine binding sites were macromolecules with MW ranging from >240K to approximately 60K. This study represents the first biochemical characterization of β-NGF stimulated-nicotine binding sites on PC 12 cells and confirms previous observations of the presence of functional nicotinic cholinergic receptors on these cells. (Supported by P.H.S. grants DA-04446 [T.C.M.] and DA-03977 [B.M.S.].)

105.14

SYNAPSE FORMATION IN CHICK LUMBAR SYMPATHETIC GANGLIA IN VIVO: TIMING OF PRESYNAPTIC INPUT AND PROJECTIONS TO TARGET. P. Devay, M. D. Listerud and L. W. Role. Dept. of Anatomy and Cell Biology in the Center for Neurobiology and Behavior, Columbia Univ., P & S, New York, NY 10032

To study potential developmental influences on the expression of acetylcholine receptors in sympathetic neurons, we need to know the time when these neurons establish connections with their pre- and postsynaptic partners.

Fibers growing out from the sympathetic neurons toward their targets as well as the arrival of preganglionic axons within the sympathetic ganglia were visualized by injecting DiI into the lumbar sympathetic chain of chicken embryos. By ED8 a number of fibers could be detected growing out of the chain towards peripheral targets. At this stage, examination of AChR subunit gene expression by Northern blot analysis and PCR indicates that the neurons express α_3, α_4, α_5, α_7, β_2 and β_4. By ED10 some lumbar sympathetic axons have reached the skin and blood vessels. At about the same time, the preganglionic fibers have entered the lumbar sympathetic ganglia, as indicated by retrograde labeling of the preganglionic neurons. Expression of synapse specific proteins is being examined to extend this analysis and determine the time-interval between axon ingrowth and formation of synapses.
This work was founded by grants from the NIH (PS22061, NS29071) and the Council for Tobacco Research.

105.15

CNS NICOTINIC ACHR CLUSTERING: HOT SPOTS AT NASCENT SYNAPSES. **M. Martinic and W.L. Klein. Neuroscience Institute, Northwestern University, Evanston, IL. 60208.** The developmental distribution of nAChR in the avian retina in vivo was examined by confocal microscopy. Labelling with monoclonal antibody 210 (generously provided by Dr. J. Lindstrom) showed that immunoreactivity appeared in the inner plexiform layer and on ganglion cell bodies after E7. The signal was highest at E18, but decreased by P0. The in situ receptor distribution was punctate, as determined by confocal analysis. For comparison, the distribution of nAChR was studied in cultures from dissociated E9 chicken retina. Immunoreactivity was present on cells with retinal ganglion cell morphology. Examination of permeabilized cells showed that retinal neurons contained an intracellular nAChR pool. The distribution of receptor within cell bodies was punctate, as if associated with intracellular vesicles. Confocal analysis of immunoreactive cells showed that cell surface receptor distribution also was punctate. Extracellular receptors were distributed on cell bodies, as well as on processes. Preliminary EM analysis of receptor distribution showed association of label with growth cones and filopodia, as well as with membrane which had been shed from the cells. Receptor clustering into "hot-spots" also was observed at points of contact between processes in culture. Confocal microscopy revealed that the distribution of immunoreactivity at points of contact also was punctate. The punctate nature of the label both in culture and in vivo suggests a one punctum, one synapse correlation, and that neuronal nAChR may cluster at focal points similar to those observed at the neuromuscular junction during synaptogenesis.

105.16

DENERVATION DOES NOT ALTER THE NUMBER OF NICOTINIC ACETYLCHOLINE RECEPTORS ON AUTONOMIC NEURONS IN THE FROG CARDIAC GANGLION. P.B. Sargent, G.K. Bryan, L.C. Streichert, and E.N. Garrett*. Depts. of Stomatology and Physiology, University of California, San Francisco, CA 94143

The binding of neuronal bungarotoxin (n-BuTX) was analyzed in normal and denervated parasympathetic cardiac ganglia of the frog, *Rana pipiens*. n-BuTX at 5-20 nM blocked both EPSPs and acetylcholine (ACh) potentials. Scatchard analysis of homogenates indicated that cardiac ganglia have two classes of binding sites for ^{125}I-n-BuTX -- a high-affinity site with an apparent K_d of 1.7 nM and a B_{max} of 3.8 fmol/ganglion and a low-affinity site with an apparent K_d of 12 μM and a B_{max} of 14 pmol/ganglion. α-Bungarotoxin does not appear to interfere with the binding of ^{125}I-n-BuTX to either site. The high-affinity binding site is likely to be the nicotinic acetylcholine receptor (AChR), given the similarity between its affinity for ^{125}I-n-BuTX and the concentration of n-BuTX required to block AChR function.

Light microscopic autoradiographic analysis of ^{125}I-n-BuTX binding to intact ganglia demonstrated that ^{125}I-n-BuTX bound selectively to synaptic sites. Scatchard analysis of the autoradiographic data revealed that ^{125}I-n-BuTX binding to synaptic sites has a K_d of about 1.5 nM.

Denervation of the heart increased the acetylcholine sensitivity of cardiac ganglion cells but did not change the number of high-affinity binding sites for ^{125}I-n-BuTX in tissue homogenates or the number of ^{125}I-n-BuTX binding sites on the ganglion cell surface. These results suggest that denervation alters neither the total number of nicotinic AChRs in the cardiac ganglion nor the number found on the surface of ganglion cells. The increase in acetylcholine sensitivity displayed by cardiac ganglion cells upon denervation cannot be explained by an increase in AChR number. (Supported by NIH NS24207)

105.17

DECREASED EFFECTIVENESS OF ACETYLCHOLINESTERASE UNDERLIES DENERVATION SUPERSENSITIVITY TO ACETYLCHOLINE IN THE FROG CARDIAC GANGLION. L.C. Streichert[1,2] and P.B. Sargent[2]. [1]Neurosciences Program, Stanford University, Stanford, CA 94305, and Depts. of Stomatology and Physiology, University of California, San Francisco, CA 94143-0512.

Sensitivity of the parasympathetic neurons in the cardiac ganglion of the frog, *Rana pipiens*, was assessed by measuring the peak depolarization elicited in response to the application of ligand emitted from a pipette positioned 10 μm from the neuronal surface. Denervation produced an increase in acetylcholine (ACh) sensitivity, but had no effect upon sensitivity to carbachol, a nonhydrolyzable cholinergic agonist. The inhibition of extracellular acetylcholinesterase (AChE) by echothiophate, a poorly-lipid soluble AChE inhibitor, resulted in similar responses to ACh in normal and denervated ganglion cells. These results suggest that denervation supersensitivity in the cardiac ganglion is caused by a reduction in ACh hydrolysis by AChE.

In normal ganglia, both AChE and ACh receptors (AChRs) are concentrated at the base of the cell, and the effectiveness of AChE is thereby maximized. Following denervation, AChR clusters disperse and are found over the entire cell surface, but the distribution and amount of extracellular AChE is apparently unchanged. Thus, denervation supersensitivity in the cardiac ganglion may be due to changes in the distribution of AChE relative to AChRs and not to an actual decrease in AChE. In skeletal muscle, increased ACh sensitivity is the result of a denervation-induced increase in AChR number. Therefore, the mechanisms underlying denervation supersensitivity may be fundamentally different at neuromuscular and at interneuronal cholinergic synapses. (Supported by NIH NS24207).

105.18

EFFECTS OF AGING AND NUCLEUS BASALIS LESIONS ON THE DENSITY OF NICOTINE BINDING SITES IN CEREBRAL CORTEX. N. Zawia, G. Arendash, and L. Wecker, Departments of Pharmacology and Biology, University of South Florida, Tampa, Florida 33612.

The main objective of these studies was to determine whether the density of nicotine binding sites, and activities of cholineacetyltransferase (ChAT) and acetylcholinesterase (AChE) in frontal-parietal cortex (fpc) are altered during normal aging, as well as in response to lesions of the nucleus basalis magnocellularis (nbm). To accomplish this, male Sprague-Dawley rats aged 2-3 and 23-24 months old were chosen for study. Unilateral lesions of the nbm were produced by injections of ibotenic acid. Two weeks later, the activities of ChAT and AChE were measured radioisotopically and spectrophotometrically, respectively, and the density of nicotine binding sites was determined by measures of the binding of L-(-)[N-methyl-^{3}H-nicotine) at concentrations of 5 and 25 nM. Neither enzyme activities nor nicotine binding site density were altered as a function of age. The loss of ChAT and AChE activities 2 weeks following unilateral lesions of the nbm was significantly greater in fpc from aged rats (71-74% decrease) as compared to young adult rats (55-57% decrease). Nicotine binding site density in fpc from young adult rats decreased by 23-25% following the lesion, whereas the density in fpc from aged animals decreased by 31-34%. Results indicate that although the activities of ChAT and AChE and the density of nicotine binding sites are not altered as a function of normal aging, basal forebrain cholinergic neurons in the aged brain are more severely affected by nbm lesions than neurons in the young adult brain, perhaps due to an age-related decrease in neuronal plasticity. Furthermore, a sizeable number of neocortical nicotine binding sites appear to be located presynaptically on the terminals of nbm cholinergic neurons (Supported by NIMH 33443 and STRC 0245).

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY I

106.1

EVIDENCE FOR DISRUPTION OF NEURON-ASTROCYTE METABOLIC INTERACTIONS IN HEPATIC ENCEPHALOPATHY. J. Lavoie and R.F. Butterworth, Lab. of Neurochemistry, André-Viallet Clin. Res. Centre, Hôpital Saint-Luc (University of Montreal), Montreal, Quebec, Canada H2X 3J4.

Chronic liver disease and portal-systemic shunting result in astrocytic changes characterized by mitochondrial swelling and, ultimately, Alzheimer type II astrocytosis. Studies of autopsied brain tissue from 9 cirrhotic patients who died in hepatic coma revealed severe astrocytosis in cerebral cortex and caudate nuclei, decreased activities of glutamine synthetase (GS) in caudate nuclei and increased densities of astrocytic ("peripheral-type") benzodiazepine receptors (PTBR's) in cerebral cortex and caudate nuclei. Similarly, brain tissue from rats following portacaval anastomosis (PCA) contains decreased activities of GS in cerebral cortex and hippocampus and a generalized 2 to 4-fold increase of PTBR's throughout brain. Ca^{2+}-dependent release of glutamate from hippocampal slices of rats following PCA is increased 2-fold and high affinity glutamate receptor densities are concomitantly decreased. Such changes probably result from decreased reuptake of glutamate into damaged perineuronal astrocytes. Thus, selective alterations of glutamatergic synaptic regulation resulting from disruption of neuron-astrocytic metabolic interactions appear to play a major role in the pathogenesis of hepatic encephalopathy in chronic liver disease. [supported by MRC Canada].

106.2

INFLUENCE OF N-METHYL-D,L-ASPARTATE (NMA) ON PROLACTIN (PRL) AND GROWTH HORMONE (GH) SECRETION IN THE PIG. C.R. Barb[1],[2], G.M. Derochers*[2], B. Johnson*[1], R.V. Utley*[2], W.J. Chang*[2], G.B. Rampacek*[2], R.R. Kraeling*[1], USDA-ARS[1], Athens, GA, and Animal and Dairy Science Dept., University of Georgia[2], Athens

Mature female pigs were ovariectomized and assigned to one of four treatments administered i.m.: corn oil vehicle (V; n=6); 10 μg estradiol-17ß/kg BW given 26 h before NMA (ES; n=6); 10μg estradiol-17ß/kg BW given 33 h before NMA (EL; n=6); 0.85 mg progesterone/kg BW given for 6 days prior to NMA (P_4; n=6). Blood was collected every 15 min for 6 h. Pigs received 10 mg NMA/kg BW i.v. 2 h after blood collection began and a GRF analog challenge (1 μg/kg BW) given i.v. 3 h after NMA. Treatment x period ($P<0.01$) interaction was detected for PRL and GH. NMA did not alter PRL secretion in P_4 pigs, while PRL increased ($P<0.0001$) from 2.9 ± 0.2, 4.7 ± 0.2 and 5.0 ± 0.2 ng/ml to 5.1 ± 0.3, 8.0 ± 0.3 and 7.5 ± 0.3 ng/ml for V, ES and EL pigs, respectively. NMA increased ($P<0.0001$) GH from 0.4 ± 0.3, 0.5 ± 0.3, 0.8 ± 0.3 and 0.5 ± 0.3 ng/ml to 8.9 ± 0.5, 10.4 ± 0.5, 7.1 ± 0.5 and 9.5 ± 0.5 ng/ml for V, ES, EL and P_4 pigs, respectively. GH response to NMA was greater ($P<0.001$) in ES and lower in EL pigs compared to V pigs. NMA is a potent secretagogue of PRL and GH secretion and ovarian steroids modulate this response.

106.3

CAPSAICIN SELECTIVELY RELEASES AMINO ACIDS FROM CULTURED SENSORY NEURONS IN A TTX-RESISTANT MANNER. B. Manwiller, K. Jeftinija*, F. Liu, and S. Jeftinija, Department of Vet. Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011, USA.

Capsaicin has been shown to selectively stimulate C-type sensory neurons which are involved in nociception. The specific objective of this study was to study the mechanism by which capsaicin releases amino acids (AA) from cultured dorsal root ganglion (DRG) neurons. DRG from 1 to 8-day-old rats were dissected and cultured on chicken plasma-coated slides for 6 to 8 days. Cultures were mounted in a perfusion chamber and perfused at a rate of 100µl/min, with aerated Ringer solution at 36±1°C. Quantification of amino acids was performed by high performance liquid chromatography utilizing fluorescence detection and pre-column OPA-derivatization. Following a period of culture equilibration 2 min, 200µl samples were collected. Perfusion application of capsaicin in concentration of 0.1 to 10µM for 1 to 2 min resulted in an increase of aspartate, glutamate, asparagin, serin, glutamin, and glycin release from cultured sensory neurons. This release of AA evoked by capsaicin was not abolished by pretreating the cultures with tetrodotoxin (TTX) or in zero Ca -EGTA solution. Pretreatment of the cultures with 1µM ruthenium red (RR) failed to antagonize capsaicin-evoked increase in the release of AA from cultured sensory neurons. However, cultures that were allowed to grow in media containing capsaicin in concentration of 1µM failed to respond with an increase of release of AA in response to perfusion of up to 10µM capsaicin. The same cultures, however, responded by increase in the release of AA in response to 50 mM K applied by perfusion. Our results show that capsaicin evokes the release of AA including aspartate and glutamate in TTX-resistant Ca-independent manner. This effect was selectively abolished in cultures grown in presence of capsaicin and was not blocked by RR. Work was supported by NIH Grant NS27751 and USDA Grant PL95-113.

106.4

ETHANOL ALTERS EXCITATORY AMINO ACID-INDUCED LUTEINIZING HORMONE RELEASING HORMONE SECRETION IN VITRO. W. Les Dees and J. K. Hiney*, Department of Veterinary Anatomy & Public Health, Texas A&M University, College Station, TX 77843.

In the present study, we evaluated the in vitro effects of ethanol (ETOH) on basal and excitatory amino acid (EAA) stimulated luteinizing hormone releasing hormone (LHRH) secretion from arcuate nucleus-median eminence (AN-ME) fragments removed from prepubertal female rats. Fragments were preincubated for 15 min in Krebs-Ringer bicarbonate glucose buffer in an atmosphere of 95% O_2-5% CO_2. These media were discarded and all AN-MEs were incubated for 30 min in media only. This media was collected to establish basal LHRH release, and replaced with media only (controls) or with media containing ETOH (30, 50, 70 mM). After a 60 min incubation, these media were collected to establish ETOH's effects on basal LHRH release by RIA. New media containing 20 mM N-methyl-DL-Aspartic Acid (NMDA) or NMDA plus each dose of ETOH was added, vials were incubated 30 min, and media collected. Another experiment was conducted as above, except that 1 mM kainic acid (KA) was used in place of NMDA and the amount of LHRH released was measured. Results indicate that both NMDA and KA stimulate LHRH 3-fold ($p < 0.01$) over basal secretion. ETOH did not alter basal LHRH release at any of the doses used, but compared with controls, blunted both the NMDA ($p < 0.01$) and KA ($p < 0.01$)-induced release of LHRH in a dose-related manner. Our results indicate that ETOH is capable of altering EAA-induced LHRH release from the AN-ME region of the prepubertal female rat in vitro, and suggest that this mechanism, at least in part, contributes to ETOHs inhibitory effect on LH secretion.

106.5

MUSCARINIC RECEPTOR ACTIVATION ENHANCES NMDA-STIMULATED DOPAMINE RELEASE FROM MESENCEPHALIC CELL CULTURES.

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Recent in situ hybridization histochemistry studies localizing muscarinic receptor mRNA indicate the presence of high levels of m5 mRNA in the ventral tegmentum and substantia nigra within dopaminergic neurons, while mRNA for m1, m2 and m4 receptors has been detected in the striatum (Vilaro et al., 1990; Weiner et al., 1990). In light of this, the present study examined some of the mechanisms involved in the functional modulation of dopamine (DA) release by cholinergic agents, using cell cultures of fetal rat mesencephalon as a model system. Both oxotremorine (OXO), a muscarinic agonist, and carbachol, a mixed muscarinic-nicotinic agonist, potentiated NMDA-stimulated DA release. The EC50 for OXO enhancement of the NMDA response was 2 μM. This effect of OXO was antagonized by 1 μM QNB, a muscarinic antagonist, while DMPP, a nicotinic agonist had no effect on NMDA-stimulated DA release, indicating that muscarinic receptor activation is responsible for enhancement of the NMDA response. OXO alone had no effect on DA release and OXO also had no effect on DA release evoked by kainate or quisqualate. Reversal of the OXO enhancement of NMDA-stimulated DA release by additional muscarinic antagonists was tested to examine muscarinic receptor sub-type involvement; pirenzepine (selectivity: M1> or =M3>M2) was the most potent antagonist tested, followed by 4-DAMP (M1=M3>M2) and AFDX 384 (M2>M4>M3>M1). This profile indicates that M2 and M4 receptor sub-types are not likely to be involved in muscarinic enhancement of NMDA-evoked DA release. Preliminary studies indicate that OXO enhancement of the NMDA response is reversed by a protein kinase inhibitor, staurosporine, suggesting that muscarinic enhancement of NMDA-stimulated DA release may by mediated by protein kinase activation. Supported by Fonds de la Recherche en Sante du Quebec.

106.6

RELEASE OF SEGMENTAL AMINO ACID NEUROTRANSMITTERS IN RESPONSE TO PRIMARY AFFERENT AND MOTOR CORTEX STIMULATION.

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The role of amino acid (AA) neurotransmitters in the spinal cord has been primarily studied using *in vitro* preparations. The technology required for *in vivo* study has only recently become available. Such an investigation could yield valuable information regarding the segmental neurochemistry. We measured the release of AAs into the rabbit lumbar spinal cord in response to sciatic nerve and transcranial stimulation with stereotactically placed micro-dialysis catheters. Samples were obtained periodically during 90 minutes of continuous stimulation of either the left or right sciatic nerve, or motor cortex. Quantification of γ-amino butyric acid (GABA), aspartate, glutamate, glycine, and taurine was performed using high pressure liquid chromatography. Adequate neural excitation was verified by recording somatosensory evoked potentials (SSEPs) or corticomotor evoked potentials (CMEPs). Sensory activation, at intensities sufficient to activate small and large diameter peripheral fibers, of the ipsilateral sciatic nerve produced a significant increase only in segmental glycine levels. Contralateral sciatic nerve stimulation failed to evoke significant changes in AA levels. A significant increase in the release of glycine and taurine, but not aspartate or glutamate, was measured after 90 minutes of transcranial stimulation. Although GABA was detected during sensory or motor stimulation, the levels remained consistently very low. SSEP and CMEP components repeatedly showed adequate activation of primary afferent, descending motor fiber pathways, and segmental interneuron pools during dialysis sampling. Glycine, and taurine, have been shown to inhibit motor neuron activity and both are closely associated with segmental interneuron pools. Furthermore, these AAs have been shown to modulate segmental afferent activity. Our data are consistent with the hypothesis that supra-segmental influence over peripheral afferent and motor activity may be, in part, through these inhibitory AA neurotransmitters in the rabbit lumbar spinal cord.

106.7

INDUCTION OF PRECOCIOUS PUBERTY IN FEMALE RATS BY N-METHYL-D-ASPARTIC ACID (NMDA).

C. Smyth*, M.C. MacDonald* and M. Wilkinson. Depts. of Obst. Gynecol. and Physiol. Biophys., Dalhousie University, Halifax, N.S. Canada B3H 4H7.

We have previously reported (Neuroendocr. 52: 143, 1990) that a single daily injection of NMDA (15 mg/kg; from d27 of life) synchronizes and slightly accelerates first ovulation (VO) in female Sprague-Dawley rats. Further investigation of this phenomenon reveals that a higher dose of NMDA (20 mg/kg), given from postnatal day 24, significantly advances the day of VO (CON: 34.1 +/- 0.7 days vs. NMDA: 31.5 +/- 0.3; n=10; p<0.01). Most rats ovulated coincident with VO, or were in proestrus, and cycled normally. NMDA was effective regardless of the time of injection (11.00 h, 14.00 h or 18.00 h). In an additional model of precocious puberty (i.e. MSG (2 mg/g) given on postnatal day 2) the combination of NMDA (20 mg/kg; from day 24) and neonatal MSG causes a greater precocity than MSG alone (CON: 36.1 +/- 0.6 (7) vs MSG: 34.0 +/- 0.5 (11) vs. NMDA/MSG: 31.3 +/- 0.6 (12)). This suggests that neonatal MSG treatment may induce some degree of NMDA receptor supersensitivity. As a prelude to a study of LH secretion in these models we have fully characterized the LH response to NMDA in prepubertal female rats. For example, maximal responses are seen at 8 mins. post-injection (s.c.) and are dependent on dose (ED50: 3.8 mg/kg) and age (zero response at 10 days, max. at 20 days). The non-competitive antagonist MK-801 (0.1 mg/kg) prevented the stimulatory effect of NMDA (15 mg/kg). The present data, together with a previous study on c-fos induction (Dev. Brain Res. 56: 294, 1990), implicate MBH NMDA receptors in the regulation of sexual maturation. Supported by Canadian MRC and Atlee Endowment.

106.8

GLIAL LOCALIZATION OF THE EXCITATORY AMINO ACID HOMOCYSTEATE.

P. Grandes*[1], Ph. Tschopp*[2], F. Ortega*[1], C. Decavel*[2], K.Q. Do[2], P. Morino*[2], M. Cuénod[2] and P. Streit[2]. [1]Dept. of Neurosci., Basque Country Univ., Bilbao, Spain; [2]Brain Res. Inst., Univ. of Zürich, CH-8029 Zürich, Switzerland.

L-Homocysteate (HCA) is an NMDA receptor preferring excitant, is released by K^+-induced stimulation in a Ca^{2+}-dependent manner from rat brain slices and, thus, has been proposed as transmitter candidate. In cerebellar slices, the HCA release depends on the presence of climbing fibers. To localize HCA, postembedding immunohistochemistry was performed using poly- and monoclonal antibodies. Surprisingly, HCA-like immunoreactivity (HCA-LI) was found predominantly in glial elements of several rat brain areas. In cerebellum, HCA-LI was most impressive in Bergmann glia. Furthermore, it was dramatically increased in the rat dorsolateral geniculate undergoing gliosis following cortical or combined retinal and cortical ablations. Astrocytes prepared from rat cerebral cortex showed strong HCA-LI; moreover, they contained high levels of HCA as determined by HPLC analysis in extracts. Since HCA fulfills certain transmitter criteria, but is localized predominantly in glia, it might be considered as 'gliotransmitter'. Supported by Proyecto del Gobierno Vasco and Swiss National Foundation.

106.9

EFFECT OF DEVELOPMENT AND CHRONIC NEUROLEPTIC TREATMENT ON KYNURENIC ACID SYNTHESIS IN THE RAT BRAIN.

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Kynurenic acid (KYNA) production has been shown to be reduced in the striatum of patients dying from Huntington's disease (HD), a movement disorder with early to mid-adult onset of clinical symptoms (J. Neurochem., 55:1327, 1990). We have therefore studied the ontogenetic profile of KYNA's biosynthetic enzyme, kynurenine aminotransferase (KAT), in rat brain. In all six regions examined, KAT activity increased consistently from postnatal days 3,7, 14,21 and 28 to young adulthood (3 months). However, cortical KAT, which was lowest at 3 days (0.17 pmoles/mg prot/h), rose 34-fold during the age range studied, whereas KAT in the substantia nigra and cerebellum (0.40 and 0.54 pmoles/mg prot/h at 3 days) increased much more slowly so that these regions contained only approx. one third of cortical KAT at 3 months. Hippocampus, olfactory bulb and striatum presented intermediate developmental curves. In order to evaluate drugs which are in current or possible future use as therapeutic agents in HD, rats were treated for 30 days with haloperidol (1.5 mg/kg/day), raclopride (10 mg/kg/day), clozapine (25 mg/kg/day) and SCH 23390 (0.5 mg/kg/day). None of the drugs influenced KAT in the cerebral cortex, striatum and substantia nigra. They also did not affect the extracellular levels of endogenous KYNA, assessed by striatal microdialysis in vivo (N = 7 per group). Supported by grant MH 44211.

106.10

IMMUNOHISTOCHEMICAL LOCALIZATION OF KYNURENINE AMINOTRANSFERASE IN THE RAT STRIATUM.

R.C. Roberts, F. Du, E. Okuno and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kynurenine aminotransferase (KAT) is the biosynthetic enzyme for kynurenic acid, an antagonist of excitatory amino acid receptors. The distribution of KAT -immunoreactivity (-i) was examined in the adult rat striatum at the light and electron microscopic levels. KAT-i was abundant in glia where the label was robust and homogeneous in both the nucleus and cytoplasm. The majority of neurons, both medium and large, exhibited granular KAT-i located in the cytoplasm of somata and proximal dendrites. The preadsorption control vastly reduced or eliminated specific staining at both the light and electron microscopic levels. At the ultra-structural level, KAT-i astrocytic processes dominated the neuropil. In neurons, label occurred in round membrane-bound organelles distributed throughout the cytoplasm. Cisternae and vesicles were identifiable in some of the labeled spheres. These data provide an anatomical basis for biochemical studies which have suggested the presence of striatal KAT in both astrocytes and neurons (Turski et al., J. Neurochem., 52:1629, 1989). Supported by USPHS grants NS28236 & MH44211 and a HDSA fellowship (to F.D.).

106.11

EXTRACELLULAR CONTENT OF POLYAMINES IN THE STRIATUM: A MICRODYALISIS STUDY IN AWAKE RATS. C. Speciale, M. Marconi*, L. Raimondi* and R.G. Fariello. Farmitalia Carlo Erba-Erbamont Group, R&D CNS Dept., 20014 Nerviano, Milano, Italy.

On the basis of biochemical and electrophysiological data, it has been suggested that endogenous polyamines (PA), putrescine (PUT), spermidine (SPD) and spermine (SPM), may play a modulatory role on the N-methyl-D-aspartate (NMDA) receptor. Several studies are available on PA in brain tissue, yet little is known on the extracellular content. Using the microdialysis tecnique in awake rats, we have studied the extracellular PA content in the rat striatum. Wistar rats (200-220 g) were implanted and perfused as described in Speciale et al., Neurosci. Lett. 104:345, 1989. PA were measured according to Yun and Zhong (Biomed. Chrom. 2:173,1987) with slight modifications. Under these experimental conditions, extracellular striatal PA were estimated 147±3, 301±32 and 73±12 nM for PUT, SPD and SPM, respectively (n=6). Upon perfusion with isotonic Krebs buffer containing 100 mM K^+, we observed 74±25%, 179±62% and 1432±236% of increase above basal levels for PUT, SPD and SPM, respectively (n=6). Data represent, to our knowledge, the first attempt to study the physiology of extracellular PA in the central nervous system and are relevant for investigating the modulatory role that endogenous PA may play on the NMDA receptor function.

106.12

HEAT SHOCK PROTEIN (HSP 72) RESPONSE TO NMDA ANTAGONISTS IS BLOCKED BY ANTICHOLINERGICS OR GABAMIMETICS. M.A. Sesma, M.T. Price and J.W. Olney, University of Missouri-St. Louis, MO 63121 & Washington University, St. Louis, MO 63110.

NMDA antagonists, including PCP, ketamine and MK-801, induce transient pathomorphological changes in cingulate and retropsplenial (C/RS) cerebrocortical neurons (Olney et al., Science, 244:1360, '89) in rats, mice and tree shrews. Associated with these changes is an abnormal expression of 72KD heat shock protein (HSP) (Sharp et al., NS Abst. 15:761, '90). The HSP response may be a sensitive indicator of this type of injury as Sharp et al. detected it not only in C/RS but in at least some other neocortical neurons and found it to be a long lasting reaction (up to 2 wks). The question arises whether these histological changes and the psychotomimetic effects of NMDA antagonists could be manifestations of the same toxic process, especially since the psychotic effects sometimes recur over a period of weeks (similar to the time course of the HSP response). We have found (Olney et al. NS Abst 1991) that the pathomorphological effects of NMDA antagonists can be prevented by co-administration of drugs having either anti-cholinergic (e.g. scopolamine) or GABAmimetic (e.g. diazepam or pentobarbital) actions, and here we report that these same drugs also prevent the HSP response. This strengthens the assumption that the pathomorphological and HSP responses have a common triggering mechanism and also supports the postulated link between the psychotomimetic and neurotoxic changes, since it is known from human anesthesia that the psychotomimetic effects of ketamine can be prevented by benzodiazepines, including diazepam. We propose that the pathomorphological changes induced by NMDA antagonists constitute a form of neuronal injury which induces synthesis of HSP, perhaps as a reparative response, and psychotic symptoms as a reflection of the dysfunctional status of neural networks integrally associated with the injured C/RS neurons. Supported by grants to M.A.S. (NSF 861 8448 & Univ. MO Weldon Spring Fund and Research Leave Award) and to J.W.O (RSA MH38894, DA05072, DA53568, AG05681).

106.13

FUNCTIONAL MAPPING IN THE BASAL FOREBRAIN USING EXCITATORY AMINO ACID-INDUCED PROTO-ONCOGENE EXPRESSION. K.J. Page,* A. Saha,* B.J. Everitt. (Spon: Brain Research Assn) Dept. of Anatomy, University of Cambridge, Cambridge CB2 3DY, U.K.

The effects of infusing excitatory amino acids (EAAs) into the ventral striatum (VS) or substantia innominata (SI) on direct and transynaptic immediate early gene (IEG) induction in ventral pallidal (VP) and cholinergic nucleus basalis (nbm) neurons was studied in rats.

Bilateral infusions into VS of AMPA (15mM) induced, 3h later, the *c-fos* protein, FOS, both within the VS and the sub-commissural VP/SI. The products of *c-jun* and *egr-1* were unchanged. Approximately 10% of the FOS-immunoreactive (IR) VP/SI neurons also contained choline acetyltransferase (ChAT)-IR. Thus, excitation of the VS leads to a change in state of both VP neurons and cholinergic neurons of the nbm.

Unilateral infusion into the nbm region of AMPA (1.5-15mM), Quisqualic acid (QUIS. 12-120mM), or NMDA (6-60mM) resulted, 1hr later in differential basal forebrain patterns of FOS-IR. AMPA induced a highly restricted, discontinuous pattern, while NMDA and QUIS resulted in a more widespread appearance of FOS-IR in pallidal and other structures. Double-staining revealed that the majority of ChAT-IR nbm neurons also contained FOS-IR following AMPA, but not NMDA or QUIS, infusions. Thus nbm ChAT-IR neurons appear preferentially to possess the AMPA sub-type of EAA receptor.

The results indicate that *c-fos* induction, in response to direct or indirect neuronal excitation, can be used to map aspects of the connectional and chemical characteristics of basal forebrain systems.

106.14

GLUTAMATE METABOTROPIC RECEPTOR ACTIVATION MODULATES THALAMIC NEURONAL ACTIVITY. M. von Krosigk and D. A. McCormick, Sec. Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510.

The dorsal lateral geniculate nucleus (LGNd) and reticular nucleus of the thalamus (nRt) receive presumed glutaminergic input from both cortical and subcortical afferents and contain mRNA for the glutamate (glu) metabotropic receptor (Masu et al., Nature 349, 760-765, 1991). Here we examined the consequences of activation of glu metabotropic receptors on neurons in the LGNd and nRt using standard *in vitro* slice techniques. Application of the glutamate metabotropic receptor agonist 1S,3R-ACPD resulted in prolonged membrane depolarization and a decrease in apparent input conductance. These effects were direct (not blocked by TTX) and due to a decrease of a "leak" current which reversed at E_K (-105 mV in 2.5 mM $[K^+]_o$). This K^+ current varied in a relatively linear manner with membrane potential and was not blocked by extracellular application of Cs^+, indicating that this current is distinct from I_M and I_h. The slow depolarizing effect of 1S,3R-ACPD persisted in solutions containing NMDA and non-NMDA antagonists (kynurenic acid, APV, CNQX). The response to 1S,3R-ACPD was occluded by a maximal activation of α_1-adrenoceptors by NA, and vice-versa, suggesting that these transmitters converge onto the same effector mechanism. Functionally, the 1S,3R-ACPD induced depolarization was able to shift neuronal firing from rhythmic bursting to single spike-tonic activity, similar to that seen *in vivo* in association with increases in arousal and alertness. Local electrical stimulation resulted in a slow excitatory postsynaptic potential which was similar to that seen with the application of 1S,3R-ACPD. We are currently investigating the possibility that endogenous release of glutamate may control the firing mode of thalamic neurons.

106.15

ACTIVATION OF GLUTAMATE METABOTROPIC, MUSCARINIC, AND α_1-RECEPTORS CONTROLS FIRING MODE IN NEOCORTICAL BURSTING NEURONS. Zhong Wang and David A. McCormick. Section of Neurobiology Yale Univ. Sch. Med. New Haven, CT 06510.

A subpopulation of layer V neocortical pyramidal cells generate action potentials in two distinct patterns characterized by burst firing and single spike activity. Here we investigated, using standard *in vitro* electrophysiological slice techniques, the possibility that activation of glu metabotropic, muscarinic, or α_1-noradrenergic receptors may determine which of these two firing modes is exhibited. Picodrop application of either the metabotropic receptor agonist 1S,3R-ACPD (400 μM in micropipette), the muscarinic agonist MCh (1-5 mM), or noradrenaline (400 μM) to layer V burst generating neurons in the guinea pig or rat sensorimotor or primary visual cortex resulted in membrane depolarization and a switch in firing mode from burst firing to single spike activity. The noradrenergic (NA) depolarization was blocked by the α_1-selective antagonist prazosin (1 μM) and mimicked by the α-agonist phenylephrine (400 μM), indicating that the NA effect is mediated by α_1-adrenoceptors. The shift in firing mode by these agonists appeared to result from the suppression of various potassium currents including I_M, I_{AHP} and a leak potassium current. All these effects were derived from the direct excitation of the neurons since they persisted after block of synaptic transmission with TTX. Furthermore, we found that brief trains of electrical stimuli to the white matter beneath the neuron were effective in switching the cell from the burst to single spike firing mode, suggesting that endogenous release of ACh, glu and/or NA may be capable of having this effect. We suggest that the firing mode of neocortical bursting neurons *in vivo* is controlled by glu, ACh and/or NA releasing neurons.

106.16

EFFECTS OF t-ACPD IN SIMULTANEOUS PATCH/SLICE, FURA-2 RECORDINGS OF THE NUCLEUS TRACTUS SOLITARIUS (NTS). S.R. Glaum, S.N. Murphy and R.J. Miller. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago IL 60637.

The NTS is the primary central nucleus coordinating respiratory, cardiovascular and gastrointestinal reflexes (Champagnat et al., 1986, J. Phys. 381, 551). The effects of glutamate (GLU) microinjected into the NTS on deglutative and baroreceptor reflexes are only partially blocked by the AMPA/KA antagonist kynurenate (KYN) or the NMDA antagonist AP5 (Leone & Gordon, 1989, JPET 250, 953; Hashim & Bieger, 1989, Neuropharm. 28, 913). We therefore examined the effects of the metabotropic EAA receptor agonist trans-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) on synaptic transmission and $[Ca^{2+}]_i$ in these cells.

Neurons in coronal slices of 3-6wk rat NTS were recorded at 25°C with K-gluconate filled patch electrodes containing (in mM): K-gluconate, 145; $MgCl_2$, 2; HEPES, 5; K_2ATP, 5 and the Ca^{2+} indicator fura-2 pentapotassium salt, 0.1.

ACPD (10-250μM) produced a depolarizing response (11 ± 3mV, $\bar{x}$ ± SD) and an increase in somatic $[Ca^{2+}]_i$ that paralleled Vm. Under voltage clamp (-60mV), ACPD produced an inward current with a reversal potential =-96mV and a decrease in slope conductance. ACPD reduced the peak amplitude of CNQX (10uM)-sensitive EPSPs and EPSCs (V_{hold}=-80mV) evoked by stimulation of the tractus solitarius by 38%. Bicuculline (10uM)-sensitive evoked IPSPs or IPSCs (V_{hold}=-50mV) were completely blocked by ACPD. ACPD effects were mimicked by GLU (50uM), preserved in the presence of the AMPA/KA antagonist CNQX (10uM) and D-AP5 (50uM), but occluded by 1-5mM $BaCl_2$. ACPD effects were not blocked by L-AP3 (50-100uM). These data suggest that the excitatory, KYN/AP5-insensitive effects of GLU in the NTS may be the result of direct depolarizing and indirect disinhibitory actions of metabotropic receptor activation.

106.17

SECOND MESSENGER CASCADE TRIGGERED BY GLUTAMATE RECEPTORS IN MESENCEPHALIC NEURONS. A. Ambrosini*, N. Brunello and G. Racagni. Center of Neuropharmacology, Inst. Pharmacol. Sci., Univ. of Milan, Italy.

Glutamatergic agonists act on dopamine neurons in midbrain by activating synaptic currents (Mereu et al, J. Neurosci. 4, 1991) and releasing dopamine (Mount et al., Soc. Neurosci.Abstr.,1988, 198.14). These effects seem to be mediated by glutamate receptors of both NMDA and non-NMDA ionotropic subtypes. However, the intracellular events triggered by glutamate stimulation have not been characterized yet.

We present here evidence that quisqualate metabotropic receptors are also present in midbrain neurons obtained from rostral mesencephalic tegmentum of 13 day-old mouse embryos. Indeed, 10 μM quisqualate (QUIS) induced inositol phosphate accumulation, reaching values about 170 % above basal after 10 min stimulation. This effect could be prevented (more than 90 % inhibition) by preatreating cells acutely with 80 nM phorbol dibutyrate, a protein kinase C activator. Further, we analysed cGMP production after neuronal stimulation with several glutamate receptor agonists, namely glutamate, NMDA, kainate and QUIS (100 μM). Kainate was the most potent stimulant (40 % above basal after 1 minute incubation), while no significant increase was observed with QUIS. The cross-talk between glutamate receptor subtypes and their different metabolic pathways is at present under investigation.

106.18

EXCITATORY SYNAPTIC TRANSMISSION IN NEOSTRIATUM AND ACTIVATION OF A GLUTAMATE AUTORECEPTOR BY T-ACPD. **D.M. Lovinger, Dept. of Molecular Physiol. and Biophys., Vanderbilt Univ. School of Medicine, Nashville, TN 37232.**

Glutamatergic afferents provide excitatory input to the neostriatum. The patch/slice technique was used to examine these glutamatergic synapses. Electrical stimulation in 400 μm thick striatal slices produced excitatory postsynaptic potentials (EPSPs) and currents (EPSCs) in current- and voltage-clamp recordings respectively. Transmission was mediated primarily by AMPA-type glutamate receptors, but NMDA receptor-mediated responses could be observed in CNQX and 0.5 mM [Mg^{2+}]o. The amplitude of EPSPs and EPSCs was reduced by t-ACPD (a metabotropic glutamate receptor agonist). The reduction in transmission increased in a concentration-dependent manner at 5-100 μM t-ACPD (IC_{50}=22.6 μM). t-ACPD appeared to act presynaptically as indicated by the findings that: 1) t-ACPD decreased AMPA and NMDA receptor-mediated transmission, but did not block current activated by agonist application; indicating that t-ACPD is not a glutamate receptor antagonist; 2) t-ACPD decreased transmission without altering postsynaptic cell properties. This t-ACPD-activated receptor differs from previously described amino acid autoreceptors as APB was without effect, and the action of t-ACPD was not reduced by the $GABA_B$ receptor antagonist 2-OH-saclofen. In sum, glutamatergic transmission in neostriatum is modulated by a t-ACPD-activated glutamate autoreceptor.

106.19

METABOTROPIC GLUTAMATE RECEPTOR ACTIVATES THE POTASSIUM CONDUCTANCE IN FRESHLY DISSOCIATED HIPPOCAMPAL CA1 NEURONS. N. Akaike, T. Shirasaki and N. Harata. Dep.of Neurophysiol., Tohoku Univ. Sch.of Med., Sendai, 980 JAPAN.

The presence of new type glutamate receptor coupled to inositol phosphate/Ca^{2+} signal transduction has been demonstrated in *Xenopus* oocytes injected the rat brain mRNA. Recently, the sequence of metabotropic glutamate receptor (mGluR) has also characterized. However, the functional role of mGluR has not been investigated in native mammalian CNS neurons. Hence, we studied the metabotropic Glu response with perforated patch clamp technique. Hippocampal pyramidal neurones were isolated from 1 to 4 week-old Wistar rats by mechanical dissociation following enzyme treatment. Drugs were applied by the "Y-tube" technique, which enables to rapid exchange of external solutions. Application of Glu and quisqualate (QA) induced inward current which was superimposed by an outward current. The outward current was 1) resistant to APV, CNQX and DNQX, and 2) disappeared once switched to the whole cell configuration. These results suggest that the outward current is mediated by the mGluR. Since this outward current was 1) abolished by pretreatment of caffeine, ryanodine and acetylcholine, 2) activated in Ca^{2+} free external solution, and 3) its reversal potential was close to E_K, it was concluded that mGluR in native rat hippocampal CA1 neurons activates Ca-dependent K channel *via* second messenger (probably IP_3) system. The concentration-response relationship of QA-induced I_K was characterized by followings. The threshold, Ka (apparent association constant) and Hill coefficient were 10^{-8} M, $1.1x10^{-7}$ M and 2.28, respectively. This Ka was 100 fold lower than that of inward current induced by QA. This suggest that mGluR may play important roles in physiological condition such as synaptic plasticity.

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY II

107.1

SEROTONIN DEPRESSES EXCITATORY AMINO ACID-MEDIATED EXCITATION OF RAT CEREBELLAR PURKINJE CELLS IN VIVO. J.G. Netzeband, J.C. Strahlendorf and H.K. Strahlendorf. Departments of Physiology and Medical and Surgical Neurology, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX 79430.

Serotonin (5-HT) can facilitate or depress neurotransmission mediated by excitatory amino acids (EAA), depending on the area of the nervous system under study. In the cerebellar slice, 5-HT was shown to depress glutamate- and quisqualate-induced excitation of Purkinje cells to a greater extent than that of N-methyl-D-aspartate (NMDA; Hicks et al., 1989, *Brain Research, 492*:371). More recently, however, it has been shown that quisqualate can activate both ionotropic and metabotropic receptors, which are activated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and trans-1-aminocyclopentyl-1,3-dicarboxylic acid (ACPD), respectively. Thus, we have extended these findings by including kainate, AMPA and ACPD in our studies. Extracellular activity of Purkinje cells was recorded from urethane-anesthetized adult male rats. EAA and 5-HT were applied by iontophoresis. We found that 5-HT (40 nA) was equally effective in depressing the magnitude of excitation produced by glutamate (70% decrease), kainate (62%), quisqualate (57%) and NMDA (56%), but that it had much less effect on AMPA (20%). 5-HT facilitated and depressed ACPD-mediated excitation, but these actions could be attributed to changes in cellular firing rates and were not significant when compared to the other agents studied. These results indicate that 5-HT preferentially modulates ionotropic EAA receptors, but that this modulation may require co-activation of intracellular second messengers, e.g. via metabotropic EAA receptors. NIH grant NS19296.

107.2

INDUCTION OF COBALT ACCUMULATION BY EXCITATORY AMINO ACIDS (EAA) IN THE HIPPOCAMPAL SLICE. J.F. Pregenzer*, E. Dunn, J.A. Oostveen, and L.R. Williams. CNS Diseases Res., The Upjohn Co., Kalamazoo, MI

Computer-assisted image analysis was used to establish the dose response of EAA analogs on the induction of cobalt accumulation within pyramidal and granule cell neurons in 400 μm slices of gerbil hippocampus. Slices were incubated 20 min at 22°C according to Pruss et al., (1990), in a solution containing 5 mM $CoCl_2$ and 0 to 1000 μM EAA analog. The cobalt was visualized by development in $(NH_4)_2S$, and the slices were digitized for quantitative densitometry. Kainic acid (KA) had the largest effect (ED_{50} = 30 μM), inducing cobalt accumulation in the dentate gyrus and CA1, 180% and 150% above control, respectively. Quisqualate (QA) induced accumulations in CA1 and hilar neurons, but had no effect on dentate granule cells. The accumulations induced by KA and QA were blocked by CNQX, but not by AP5. NMDA induced accumulation in the dentate and CA1 150% above control. The accumulation was blocked by both AP5 and CNQX. These data indicate that cobalt-permeable, ligand-activated ion channels are differentially distributed within the gerbil hippocampus, and have differential sensitivities to non-NMDA agonists. The NMDA effect is probably mediated through a secondary synapse acting on KA receptors.

107.3

NMDA INDUCES RHYTHMIC BURSTING ACTIVITY IN RAT MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs) IN VITRO. Charles W. Bourque and Bin Hu. *Centre for Research in Neuroscience, McGill University, Montreal, Québec, Canada H3G 1A4*

Phasic bursting activity is known to facilitate peptide release from the neurohypophysial terminals of hypothalamic MNCs. In this study we report a novel mode of burst firing which MNCs evolve when exposed to NMDA receptor agonists. Intracellular recordings were obtained from 48 MNCs impaled in the supraoptic nucleus of superfused hypothalamic explants. Bath application of NMDA (10-100 μM) from subthreshold membrane potentials induced intense (≈30 Hz) bursts of action potentials lasting 300-800 ms which recurred periodically every 1-2 seconds and which were associated with 10-20 mV oscillations in membrane potential. These effects could be mimicked by glutamate (n=3) or quinolinate (n=4), but not by quisqualate, kainate or AMPA (n=12). NMDA-evoked bursts were abolished in the presence of 10 μM APV (n=2), but persisted in the presence of CNQX (10 μM; n=2). Application of NMDA in the presence of TTX (300 nM; n=4) resulted in voltage-sensitive subthreshold oscillations (5-15 mV) recurring every 1-2 seconds. These results indicate that sustained activation of postsynaptic NMDA receptors can induce rhythmic bursting activity in rat supraoptic MNCs. *Supported by MRC and FCAR*

107.4

IONIC BASIS OF NMDA-INDUCED RHYTHMIC BURSTING ACTIVITY IN RAT MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs) IN VITRO. Bin Hu and Charles W. Bourque. *Centre for Research in Neuroscience, MGH, McGill University, Montreal, Québec, Canada H3G 1A4*

NMDA receptor activation in hypothalamic MNCs results in the appearance of high frequency (≈30 Hz) bursts of action potentials lasting 300-800 ms, recurring every 1-2 seconds. We have obtained intracellular recordings from 20 supraoptic MNCs in superfused explants of rat hypothalamus to examine the ionic basis of this response. In the presence of TTX, NMDA (10-100 μM) induced rhythmic subthreshold oscillations. Application of a voltage clamp within the same voltage range revealed no oscillations of membrane current, suggesting that voltage-dependent interactions are required for the genesis of the response. NMDA induced bursting was abolished by (i) removal of external Mg^{2+} (n=7), (ii) removal of external Ca^{2+} (n=4), (iii) intracellular Ca^{2+} chelation with BAPTA (n=2), or (iv) bath-application of apamin (100 nM; n=4). In each case, NMDA-induced oscillations and rhythmic bursts were replaced by steady depolarizing plateaus. We conclude that NMDA-induced bursting activity requires the intrinsic voltage-dependency of NMDA channels, Ca^{2+} influx, and the activation of apamin-sensitive Ca^{2+}-activated K^+ channels. *Supported by MRC and FCAR*

107.5

CALCIUM ENTRY EVOKED IN DORSAL HORN NEURONS BY GLUTAMATE: DOSE-DEPENDENT RELIEF OF MAGNESIUM ION BLOCK. D.B. Reichling and A.B. MacDermott. Department of Physiology and Cellular Biophysics and Center for Neurobiology and Behavior, Columbia University, New York, NY 10032.

Calcium ion (Ca^{2+}) entry into neurons through NMDA-gated channels has been implicated in mechanisms of synaptic plasticity. Blockade of this route of calcium entry by physiological concentrations of magnesium ions (Mg^{2+}) is relieved by depolarization of the membrane, for example when the endogenous transmitter L-glutamate co-activates NMDA and non-NMDA receptors. To quantify the effect of this unblocking action on the dose-response curve for Ca^{2+} entry evoked by glutamate, the intracellular concentration of calcium ions ($[Ca^{2+}]_i$) was measured using indo-1 in single cultured neurons isolated from the dorsal horn of embryonic rat spinal cord while glutamate was rapidly applied for a period of 3 sec. In all experiments the bath contained 5 uM glycine. At concentrations of glutamate that activate NMDA receptors alone (1 uM or less), 1 mM Mg^{2+} completely blocked Ca^{2+} entry in most neurons, while in the remaining neurons Ca^{2+} elevation was only partly blocked. As the concentration of glutamate was increased above 1 uM, and non-NMDA receptors were activated, the Mg^{2+} block was progressively relieved until, at 30 uM glutamate little block was evident. On the other hand, antagonism of the $[Ca^{2+}]_i$ response by 0.1 mM Mg^{2+} was much less potent, and was dramatically decreased by increasing concentrations of glutamate even below the level at which non-NMDA receptors are activated. Thus, 0.1 mM Mg^{2+} reduced the mean response to 0.3 uM glutamate by 75%, and to 1 uM glutamate by only 24%. These results demonstrate that, in physiological concentrations of Mg^{2+}, non-NMDA receptor activation is sufficient but not always necessary to relieve the Mg^{2+} block of NMDA receptors sufficiently to produce elevations of $[Ca^{2+}]_i$. We are now using co-cultures of dorsal root ganglion and dorsal horn neurons to determine if these conclusions also apply to the action of synaptically released excitatory amino acid transmitters upon postsynaptic receptors.

107.6

GLUTAMATE AND NMDA ACTIONS ON MESOPONTINE CHOLINERGIC NEURONS *IN VITRO*. R. Sanchez, A. Kahteb, M. Mühlethaler and C.S. Leonard, Center for Neural Science, NYU 6 Wash. Pl. NY, NY 10003 and Geneva Sch Med, CMU 1 rue Michel Servet, 1206 Geneva, Switzerland.

The actions of glutamate (Glu) and NMDA on pedunculopontine (PPT) and laterodorsal tegmental (LDT) neurons were studied with intracellular recording methods in a guinea pig brain slice preparation. Cholinergic neurons were identified by combined intracellular injection of biocytin and histochemical staining for NADPH-diaphorase. Focal application of Glu or NMDA produced a dose-dependent increase in firing rate and membrane depolarization in type II cells. Glu and NMDA depolarizations were of similar magnitude but had different time courses. Glu produced short latency depolarizations (50-200ms) which peaked rapidly and lasted for less than 15 seconds while comparable NMDA depolarizations had long latencies (800-1600 ms) and long durations (40-80S). The NMDA mediated depolarization was completely blocked by AP-5 (50μM) while the Glu responses were only marginally reduced by AP-5 (50μM) but were strongly antagonized by both DNQX (10-50μM) and CNQX (10μM) suggesting that a major part of the Glu response was mediated by non-NMDA receptors. It appears that Glu acts directly since neither TTX (1μM) nor low Ca/2mMCo Ringer, which blocks synaptic transmission, altered the response. NMDA may also act directly since its depolarization was unaltered by TTX (1μM) although possible indirect actions must be further considered. These findings imply that excitatory amino acid inputs may have both short and long-lasting effects on the integrative properties of mesopontine cholinergic neurons.

107.7

THE TIME COURSE OF GLUTAMATE IN THE SYNAPTIC CLEFT. J.D. Clements*, R.A.J. Lester*, C.E. Jahr & G.L. Westbrook. Vollum Institute, Oregon Health Sciences University, Portland, OR. 97201.

The concentration time course of free transmitter in the synaptic cleft for synapses in the vertebrate CNS is not known. We have estimated this time course for released glutamate by measuring the block of excitatory synaptic transmission produced by a rapidly dissociating competitive antagonist, D-aminoadipate (DAA). If free transmitter is present for only a brief period, agonist and antagonist binding will not reach equilibrium. Thus the synaptic response will be proportional to the number of receptors which are not initially occupied by a competitive antagonist such as DAA. Longer durations of free transmitter will increase the response as glutamate replaces DAA on the receptors.

To obtain the antagonist dissociation rate, pulses (1-40 ms) of glutamate (2 mM) were applied to outside-out patches of cultured hippocampal neurons in the continuous presence of saturating concentrations of DAA (200 μM). Control experiments revealed that solution exchange at the membrane patch was < 1 ms. As expected, increases in glutamate pulse length reduced the effectiveness of DAA in blocking NMDA channels. The response amplitude plotted as a function of pulse length followed a single exponential time course (τ = 8ms), implying a dissociation rate for DAA of 125 sec^{-1}. These results predict that in 200 μM DAA, a 2 ms pulse of 2 mM glutamate will reach only 10% of the response amplitude seen in the absence of DAA. In whole cell recordings from pairs of hippocampal neurons, synaptic activation of NMDA receptors was inhibited to 10% of control in 200 μM DAA. Therefore, assuming similar association rates for DAA and glutamate, free transmitter is present in the cleft at >200 μM for less than 2 ms. Using a kinetic model of NMDA receptor activation, the results predict that the transmitter concentration peaks at a value near 2 mM, then decays with a 1 ms time constant. Supported by grants from USPHS and the McKnight Foundation (CEJ & GLW).

107.8

NBQX REVEALS AN NMDA MEDIATED SYNAPTIC CURRENT. Paul A. Coleman, Weifeng Yu, Tage Honoré, and Robert F. Miller. Dept of Physiology, Univ of Minnesota, MN 55455; Novo Nordisk A/S, Soeborg, Denmark.

The effects of NBQX (2-3 Dihydroxy-6-nitro-7-sulfamoylbenzo (F) quinoxaline) were tested on amphibian inner retinal neurons. Whole cell currents were measured from these cells in the intact retinal eyecup or slice preparations. NBQX acted as a potent non-NMDA antagonist but like the other quinoxalines, it's selectivity is concentration dependent. Nevertheless, NBQX (0.7-25 μM) preferentially attenuated the depolarizing effects of non-NMDA over NMDA receptor agonists, in a manner superior to DNQX or CNQX.

In current clamp recordings, NBQX didn't reduce the amplitude of light-evoked EPSPs nor made their time course systematically slower. Likewise, blocking NMDA receptors (with D-AP7) resulted only in minor alterations of the EPSP. The changes seen with either of the selective antagonists were restricted to subtle alterations of the EPSP/IPSP complex. A substantial reduction in synaptic response amplitude was only observed when NMDA and non-NMDA antagonists were combined. Additionally, NBQX had no apparent effect on the response of the ON bipolar cell, indicating no antagonism of the L-AP4 receptor.

Under voltage-clamp conditions, NBQX revealed a voltage-dependent EPSC similar to classical NMDA-mediated responses, in that negative holding potentials reduced the inward current when compared to more depolarized holding potentials. In contrast, EPSCs recorded in the presence of D-AP7 varied linearly with holding potential.

NBQX permits the study of NMDA mediated EPSCs in relative isolation from non-NMDA contributions due to it's improved selectivity of non-NMDA over NMDA receptors. This has provided new insight on the role of these receptors in retinal ganglion cell function. Our results suggest that normal synaptic activation depends on the interactions between NMDA and non-NMDA receptor mediated currents. This research was supported by EY03014 and EY07376 to RFM.

107.9

QUISQUALATE INDUCES LONG-TERM CHANGES IN EPILEPTIFORM ACTIVITY IN HIPPOCAMPAL SLICES. W.W. Anderson and R.J. DeLorenzo, Dept. of Neurology, Medical College of Virginia, Richmond, VA 23298.

One goal of epilepsy research is to reverse the process of seizure development or epileptogenesis. Recent studies have shown that activation of a metabotropic glutamate receptor is necessary to produce long-term depression (LTD) of EPSPs (Chattarji et al., Neurosci. Abst. 16:660, 1990). Because seizure development is thought to be partially due to potentiation of EPSPs, we have begun to investigate the ability of metabotropic glutamate receptor agonists to depress epileptiform activity induced by electrical stimulation in rat hippocampal slices.

Interictal bursts (IIBs) and electrographic seizures (EGSs) were induced in physiological medium by repetitive tetanic stimulation of CA3 s. radiatum. To prevent further induction of epileptiform activity by NMDA receptor activation, 100 uM of the NMDA antagonist 2-amino-5-phosophonovalerate (APV) was applied. Then 10 uM (±) or 5 uM (+)quisqualate (QUIS), a metabotropic and AMPA receptor agonist, was added to the APV solution for 2 min. Following the washout of QUIS, a complex alteration of epileptiform activity occurred that continued for the remainder of APV application (30-60 min). Spontaneous IIBs increased in frequency and spikes/burst, but EGS activity was substantially reduced or blocked. The antidromic population spike (APS) amplitude was also reduced by one-third. When APV was washed out, IIBs decreased and EGSs increased to pre APV levels.

The results show that QUIS produces long-term changes in epileptiform activity during NMDA receptor blockade. These changes are not simply decreases in all types of activity, but include an increase in IIBs and a decrease in EGSs. The mechanisms of EGS decrease are not known, but could include inhibition by stronger IIBs, EPSP depression, and APS decrease. The reversal of QUIS activation after washout of APV is reminiscent of biochemical studies showing that activating NMDA receptors decreases the effects of metabotropic receptor agonists.

107.10

GLUTAMATE SYNAPSES ON RETROGRADELY-LABELLED SYMPATHETIC PREGANGLIONIC NEURONS IN THE RAT. P.M. Pilowsky1, L.J. Llewellyn-Smith1,2, K.D. Phend2*, J.B. Minson1* and J.P. Chalmers1*. 1Department of Medicine, Flinders University, Bedford Park, Australia and 2Department of Anatomy and Cell Biology, University of North Carolina, Chapel Hill NC 27599.

Physiological and anatomical evidence has suggested that L-glutamate (GLU) is involved in regulating the activity of sympathetic preganglionic neurons (SPN). In this study, we investigated whether or not GLU-immunoreactive synapses occurred on retrogradely-labelled SPN. Cholera toxin B subunit (CTB) was injected into the superior cervical ganglion or adrenal medulla of the rat. After fixation with 2.5% glutaraldehyde, CTB-labelled SPN were revealed by immunocytochemistry with avidin-biotin-peroxidase. GLU-immunoreactive axon terminals that directly contacted or synapsed on retrogradely-labelled neurons were visualized by post-embedding immunogold staining. GLU synapses were found on the cell bodies and dendrites of SPN projecting to the adrenal medulla or to the superior cervical ganglion. Close to two-thirds of the terminals that contacted or synapsed on sympathoadrenal SPN were positive for GLU. Double immunogold labelling for GABA and GLU indicated that GLU-immunoreactive profiles on SPN did not contain GABA and vice versa. These results suggest that GLU may be the major excitatory neurotransmitter regulating sympathetic outflow. This work was supported by the Australian National Health and Medical Research Council and National Heart Foundation.

107.11

A SLOW NMDA RECEPTOR-MEDIATED EPSP EVOKED BY REPETITIVE MOSSY FIBER STIMULATION IN TURTLE CEREBELLAR PURKINJE CELLS.
L.J. Larson-Prior, P.D. Morrison, and N.T. Slater. Department of Physiology, Northwestern University Medical School, Chicago, IL 60611 U.S.A.

A contemporary view of the neurotransmitter receptors involved in the mossy fiber-granule cell-Purkinje cell pathway in adult cerebellum indicates a predominate role for NMDA receptors at the mossy fiber-granule cell synapse, and for Quisqualate receptors at the granule cell-Purkinje cell synapse. We have examined this hypothesis by studying the actions of excitatory amino acid (EAA) receptor antagonists on synaptic potentials in cerebellar Purkinje cells activated by stimulation of the trigeminal nerve (Vn) or the ascending spinal lemniscus (SL).

Intracellular recordings were obtained in turtle Purkinje cells in an *in vitro* brainstem-cerebellum preparation with sharp microelectrodes. Single or repetitive (10-50 Hz) stimuli were applied to the Vn or SL with suction or tungsten electrodes, respectively. Single stimuli produced fast EPSPs (latency: 10-20 ms) only in response to SL stimulation; repetitive stimulation at either stimulus site evoked slow EPSPs (time-to-peak: 100-150 ms) which could drive 'pacemaker' discharges. The EPSPs differed in their sensitivity to EAA antagonists: the fast SL-activated EPSP was blocked by CNQX (10 μM), reduced by L-AP4 (100 μM), and was unaffected by D,L-AP5 (100 μM). In contrast, the slow Vn- and SL-evoked EPSP was not affected by CNQX, potentiated by L-AP4, and blocked by D,L-AP5. Both EPSPs were potentiated by bicuculline (10 μM). The pharmacologic profile for the slow mossy fiber-evoked EPSP also differs from a slow EPSP previously demonstrated in response to direct, repetitive parallel fiber stimulation (*J. Neurophysiol.* 63:637,1990). The parallel fiber-evoked slow EPSP was blocked by L-AP4 and CNQX, but not by D,L-AP5.

These data further indicate that while both NMDA and non-NMDA EAA receptors are required for the activation of Purkinje cells by mossy afferents, the selective effects of the EAA antagonists rule out the *exclusive* involvement of either receptor type at each synapse. Supported by NS 17489 and NS 25682.

107.12

EXTRACELLULAR AMINO ACID CONCENTRATIONS IN THE VENTROCAUDAL PAG OF FREELY MOVING RATS FOLLOWING VERATRIDINE AND/OR Ω-CONOTOXIN STIMULATION. W. M. Renno*, M. A. Mullett*, and A. J. Beitz. Dept. of Veterinary Biology, Univ. of Minnesota, St. Paul, MN 55108.

Glutamate, aspartate and GABA have been proposed as important neurotransmitters in the periaqueductal gray (PAG). The baseline concentrations and release of these amino acids in response to PAG neuronal depolarization were examined in the present study using *in vivo* microdialysis coupled to high-performance liquid chromatography (HLPC). A chronic guide cannula was implanted into the PAG of male Sprague-Dawley rats using stereotaxic coordinates. Seven to ten days later a microdialysis probe was inserted through the chronic guide cannula into the caudal ventrolateral PAG. The dialysis system was attached to a peristaltic pump and perfused with Ringer's solution (147 mM NaCl, 4.0 mM KCl, and 2.2 mM $CaCl_2$) at a flow rate of 2-5 ul/min. Samples were collected at 12 min intervals in polypropylene tubes and analyzed for amino acids, within 12 hours, by HPLC. PAG depolarization with 75 μM veratridine (ver, a Na channel activator) resulted in a 2,204% increase in aspartate, 2,006% increase in glutamate, 327% increase in glycine, 1,358% increase in taurine and 14,012% increase in GABA. Surprisingly, when 800 nM Ω-conotoxin (con, a calcium channel blocker) was co-administered with 75 μM ver through the dialysis probe, it potentiated the release of these amino acids (3,670%, 3,108%, 238%, 352%, 1,252%, and 21,417%, respectively). On the other hand 800 nM con by itself produced no significant increase in any of the amino acids under investigation. This study indicates that glutamate and aspartate are released by ver depolarization and these may serve as important excitatory amino acids in the PAG, while GABA appears to play an important inhibitory role in this region. The potentiation of ver induced release of these 3 amino acids by con may reflect a stimulatory effect of con on PAG neurons. Supported by DA06687, DE06682, and NS1920

107.13

ROLE OF EXCITATORY AND INHIBITORY AMINO ACIDS IN A PRIMARY SENSORY PROCESSING AREA, THE ELECTROSENSORY LATERAL LINE LOBE OF WEAKLY ELECTRIC FISH. J. Bastian, Dept. of Zoology, Univ. of Oklahoma, Norman, OK 73019.

The electrosensory lateral line lobe (ELL) receives the electroreceptor afferent projection as well as descending inputs from higher centers. The descending inputs terminate in a cerebellar-like molecular layer, the fibers of which make synaptic contacts with apical dendrites of the ELL interneurons and output (pyramidal) cells.

Pressure injection of Kainic acid, AMPA, or NMDA near a pyramidal cell's apical dendrite causes short latency excitatory responses as is expected given the prior autoradiographic demonstration of receptors for these substances (Maler and Monaghan, J. Chem. Neuroanat., 4:, 1991). These responses are antagonized by application of DNQX in the case of KA or AMPA, or APV in the case of NMDA. Injection of GABA or Baclofen causes short latency inhibition of pyramidal cells which are antagonized by Bicuculline and 2-Hydroxysaclofen respectively.

Responses to a given substance were sensitive to the position of the injection pipette. Changes in position of from 20 to 40 μm reversed glutamate mediated excitation of pyramidal cells to inhibition. Inhibitory responses to GABA also reversed to excitation with small changes in pressure injection pipette position. The reversal of a cell's response to a given transmitter agonist with movement of the injection pipette away from the neuron's apical dendrite tree is thought to reflect excitation or inhibition of nearby ELL inhibitory interneurons.

The normally rapidly adapting pyramidal cell responses to electrosensory stimulation were rendered more tonic after application of Bicuculline or DNQX but not APV. The phasic nature of pyramidal cell responses may be explained in part as due to activation of GABAergic interneurons via descending glutaminergic inputs to the ELL molecular layers. Supported by NIH NS12337.

107.14

GABAERGIC- AND GLUTAMATERGIC-MEDIATED SYNAPTIC INHIBITION AND EXCITATION IN RAT SUPRAOPTIC NEURONES FROM ACTIVATION OF ORGANUM VASCULOSUM LAMINA TERMINALIS (OVLT). C.R. Yang and L.P. Renaud. Neuroscience Unit, Loeb Research Institute, Ottawa Civic Hosp., Ottawa, Canada, K1Y 4E9.

Neuroanatomical studies have demonstrated that the rat OVLT, a circumventricular structure involved in body fluid homeostasis, projects to the supraoptic nucleus (SON). The present study evaluated the synaptic inputs from the OVLT to SON neurones recorded intracellularly (3M KAc-filled electrode) in superfused rat hypothalamic explants.

Both IPSPs and EPSPs were evoked by electrical stimulation of the OVLT. IPSPs evoked by single-pulse stimulation (50-200μA, 0.02ms duration pulses) displayed a mean latency of 9.3 ± 0.4 ms, a mean reversal potential (V_R) of -65 ± 2 mV, and were reversibly blocked by bath-applied bicuculline (10-25 μM), implying mediation by $GABA_A$ receptors. EPSPs and spikes elicited by single-pulse or repetitive stimulations displayed a mean latency of 8 ± 0.4 ms, a mean V_R of -26 ± 9 mV. The EPSP amplitudes were enhanced in Mg^{++}-free media, were partially blocked by APV (60 μM), an NMDA receptor antagonist, and were completely and reversibly blocked upon addition of CNQX (10 μM), an AMPA receptor antagonist, suggesting that both glutamate receptor subtypes mediate these EPSPs. Microinjection of glutamate (50μM) onto OVLT, to circumvent activation of fibres-of-passage, induced 3 - 7 mV depolarizations in SON neurones, thus supporting an excitatory OVLT input to the SON.

These results suggest the presence of a glutamatergic pathway linking OVLT neurones with neurosecretory neurones in the SON. This pathway may permit circulating substances acting through this circumventricular structure to influence the secretion of neurohypophysial hormones. (*Supported by FRSQ & MRC*).

107.15

SHORT-LATENCY SENSORY TRANSMISSION THROUGH NMDA RECEPTORS IN RAT SI BARRELFIELD CORTEX. *M.Armstrong-James, **E.Welker, and *C.A.Callahan *Physiology Dept, Queen Mary College, London E1 4NS, and **Institut d'Anatomie, Rue du Bugnon 9, Lausanne, Switzerland.

In adult rat neocortex *in vitro* NMDA-EPSPs play a minor and solely "late" role in transmission, neither of which appears to so for sensory transmission *in vivo*. Using rats lightly anaesthetized with urethane, post-stimulus time histograms (PSTHs) for SI cortical cells were generated in reply to 50 small abrupt deflections of principal whiskers both with or without iontophoresis of the NMDA antagonist APV. Spikes within successive epochs of PSTHs were counted at 0-9, 10-20, 20-50 and 50-100 ms post-stimulus. Relative to controls, APV reduced counts in the 0-9 ms epoch by a mean of 39% for layer IV cells and 83% for layer III cells, and by 80-100% (layer I-IV) for subsequent epochs (10-100 ms). Since the earliest evoked spikes occured at 6-8 ms post-stimulus, (4-6 ms in the thalamic relay nucleus), these results suggest that in lightly anaethetized rats monosynaptic thalamocortical fast EPSPs with a rise-time of <3ms to natural stimuli are about equally expressed through a mixture of NMDA and non-NMDA channels, with later (10-100 ms latency) intracortical relay largely being expressed through NMDA channels. *Support: Swiss NSF 3100.009468.*

107.16

SYNAPTICALLY MEDIATED COMPONENT OF THE NEOSTRIATAL FIELD POTENTIAL IS HIGHLY SENSITIVE TO DNQX AND CNQX. J.A. Wilson. Division of Physiology, Creighton Univ. Sch. of Medicine, Omaha, NE 68178

The neostriatum receives major inputs from the cortex, thalamus, and substantia nigra. There has been much interest in the nigral dopaminergic inputs due to their association with Parkinson's disease; however, the transmitters used in the other pathways have been more difficult to establish. Cordingley & Weight (Br. J. Pharmacol. 88 (1986) 847-856) tested many agents thought to act at a variety of synapses and suggested that a glutamate like neurotransmitter mediated cortico-striate synaptic transmission. Though they were unable to differentiate between classes of glutamate receptors, their findings are consistent with the hypothesis that the cortico-striate field potential is mediated by a kainate/quisqualate type receptor. To test this hypothesis, the newer antagonists CNQX and DNQX were applied to mouse nigrostriatal brain slices. ED_{50}s were estimated to be 1.25 μM for CNQX and 1.6 μM for DNQX. This finding is interpreted as strong support for Cordingley and Weight's suggestion that a glutamate like transmitter mediates cortico-striate synaptic transmission.

Supported by a grant from the Health Future Foundation.

107.17

EXCITATORY AMINO ACID (EAA) ACCUMULATING NEURONS WITHIN BRAINSTEM RESPIRATORY CIRCUITRY OF THE RAT. H.H. Ellenberger & J.L. Feldman. Systems Neurobiology Lab., Dept. of Kinesiology, UCLA, Los Angeles, CA 90024-1527.

Respiratory neurons that utilize EAAs for neurotransmission were selectively labeled in rats by retrograde transport of D-[^{3}H]aspartate (D-[^{3}H]Asp). D-[^{3}H]Asp is selectively internalized and accumulated within the parent cell body of neurons that possess a high-affinity uptake system for glutamate or aspartate along the synaptic region of their terminal bouton membranes. Injections of D-[^{3}H]Asp (~30 nl; 10-20 μCi) were made into the inspiratory portion of the rostral ventral respiratory group (rVRG) to identify the source(s) of presumptive EAA inputs to rVRG. Retrogradely labeled neuron somata were visualized by autoradiographic methods in Thionin counterstained tissue.

D-[^{3}H]Asp labeled neurons were distributed within several subnuclei of the ipsilateral nucleus of the solitary tract and bilaterally within the VRG. D-[^{3}H]Asp injections into rVRG labeled fewer neurons than we have previously observed utilizing non-selective retrograde tracers (e.g., rhodamine beads [Ellenberger & Feldman, Brain Res., 513:35-42, 1990]). We propose that these presumptive EAA neurons represent medullary sources of excitatory inputs to bulbospinal and propriobulbar inspiratory neurons of rVRG. The remaining neurons labeled by non-selective tracers but not by D-[^{3}H]Asp within medullary respiratory groups may represent inhibitory or non-EAA modulatory neurons that project to rVRG. Supported by NIH grant NS 24742 and American Lung Association grant J900701.

107.18

IMMUNOLABELING OF TERMINALS OF CORTICAL DESCENDING FIBERS J.Valtschanoff, R.J.Weinberg, A. Rustioni, Depts. of Cell Biology & Anatomy and of Physiology, University of North Carolina, Chapel Hill, NC 27599.

Previous work from our lab has found that a large fraction of cortical neurons with descending projections immunostain with an anti-glutamate antibody. While this suggests that these neurons may use glutamate as neurotransmitter, immunostaining of terminals, as reported here, provides more direct evidence. To identify these terminals, pressure injections of WGA-HRP were made in the sensorimotor cortex of anesthetized Sprague-Dawley rats. After 48 h survival, the animal was anesthetized and perfused with glutaraldehyde/formaldehyde/picric acid fixative. Fifty µm Vibratome sections of medulla and cervical spinal cord were cut, reacted for TMB with tungstate stabilizer (Weinberg & Van Eyck, *J. Histochem. Cytochem.* in press), and processed for light and electron microscopy.

Terminal labeling in the caudal medulla was concentrated in the dorsal column nuclei; in the spinal cord, labeling was concentrated in laminae 3, 4, and 5. Ultrastructurally, labeling in the form of electron-dense crystals and amorphous deposits was inside small dome-shaped terminals . In some cases, labeling could also be seen in postsynaptic elements, including dendrites, somata, and glia and in the extracellular space outside cortical terminals. Postembedding ICC (using methods described in Phend et al., *Soc. Neurosci Abst.* 18) revealed that the large majority of the labeled terminals were positive for glutamate, and did not stain above background for GABA.

This research was supported by NINDS award #NS-16264.

107.19

LOCALIZATION OF NMDA AND AMPA RECEPTORS IN RAT BARREL FIELD. D. Jaarsma, J.B. Sebens, G.J. ter Horst* and J. Korf., Depts. Biol. Psychiatry and Neurobiology*, University Groningen, P.O.Box 30.001, 9700 RB Groningen, The Netherlands.

In layer IV of the face portion of the rat primary sensory cortex (barrel field) the pattern of termination of excitatory afferents is highly organized, the thalamic and the cortical afferents ending in the barrel hollow and in the septa between the barrels, respectively. In an attempt to correlate the origin of excitatory input with a specific subtype of excitatory amino acid receptor, we studied the distribution of N-Methyl-D-aspartate (NMDA) and α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors in rat somatosensory cortex using in vitro receptor autoradiography. Freshly frozen brains from 8 male Wistar rats were used. Sections were cut in the transversal, horizontal or tangential plane, thaw-mounted, and incubated with the competetive NMDA antagonist [^{3}H]CGP39653 (20 nM), or [^{3}H]glycine (150 nM) in the presence of 1 mM strychnine to label NMDA receptors, or with [^{3}H]AMPA (100 nM) to label AMPA receptors. Autoradiograms were generated by apposing the sections against emulsion coated coverslips. NMDA, but not AMPA receptors were heterogeneously distributed throughout the barrel field with high density levels in the hollows and low levels in the barrel sides and septa. The table shows the densities of silver grains as a percentage of grain density in layer II/III of the SI cortex.

barrel subfield:	anterolateral		posteromedial	
	hollow	side	hollow	side
[^{3}H]CGP39653	87	44	82	55
[^{3}H]glycine	75	49	84	57
[^{3}H]AMPA	82	76	78	79

These results suggest that in rat barrel field the NMDA receptors are mainly present in the thalamo-cortical synapses, whereas AMPA receptors are present in both the corticocortical and the thalamocortical synapses.(Supported by NWO/MW, grant 552.076)

108.1

CNQX INCREASES SPONTANEOUS INHIBITORY INPUT TO CA3 PYRAMIDAL NEURONS OF THE NEONATE RAT HIPPOCAMPAL SLICE. J.V. EATON*, C.J. McBAIN, T.A. BROWN, & R. DINGLEDINE. Dept. Pharmacology & Curr. Neurobiology, Univ. of North Carolina, Chapel Hill, NC 27599-7365.

6-Nitro-7-cyano-quinoxaline-2,3-dione (CNQX) acts at non-NMDA receptors to block polysynaptic inhibitory input in the hippocampal slice (Davies and Collingridge, Proc. R. Soc. 236:373,1989). We investigated the effects of CNQX on spontaneous inhibitory input to CA3 pyramidal neurons in neonate rat hippocampal slices (4-10 days; 300 μm) using the whole-cell patch clamp technique. Instead of blocking spontaneous inhibitory input to CA3 pyramids, CNQX (3μM) increased the frequency of inhibitory post-synaptic currents (IPSCs). IPSC frequency was increased from a control of 2.24±0.6Hz to 4.37±1.1Hz (n=16 neurons) without affecting the holding current. CNQX did not significantly affect the amplitude (control= 12.7±0.8pA and 13.9±0.8pA after CNQX) or the decay time constant (τ) (control= 23.9±2.2ms and 23.0±1.3ms in CNQX) of the IPSCs. Other structurally related quinoxalines varied in effect as IPSC frequency was increased by DNQX but decreased by NBQX. The majority of IPSCs observed, both in control and following addition of CNQX, were eliminated by 1μM tetrodotoxin (TTX), suggesting that the majority of IPSCs in CA3 are driven by action potential firing in inhibitory interneurons. Recordings made from interneurons of *st. radiatum* revealed that CNQX blocked spontaneous EPSCs in the interneurons while leaving the IPSCs intact. CNQX also induced an inward current and concomitant burst firing in the interneurons, presumably the mechanism responsible for the increased frequency of IPSCs in CA3 pyramids. These results suggest that CNQX, while acting as a non-NMDA receptor antagonist, has an additional direct excitatory action on some hippocampal interneurons.

108.2

PATCH CLAMP RECORDINGS FROM INTERNEURONES OF HIPPOCAMPAL SUBFIELD CA3; RESPONSES TO EXCITATORY AMINO ACIDS. C. J. McBAIN and R. DINGLEDINE. Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, NC 27599-7365.

Using the whole cell patch clamp technique we have made recordings from interneurones of CA3 stratum radiatum located in the neonate (3-15 days) rat hippocampal slice (300um thick). Interneurones possessed a mean input resistance of 380 $\pm$ 52.2 megohm at a mean resting potential of -59 $\pm$ 2.6mV (n=10 neurones). In the presence of 5uM bicuculline, spontaneous excitatory events were small in amplitude (mean = 8.5 $\pm$ 1.4 pA) and had a frequency of 1.43 $\pm$ 0.47Hz. At a holding potential of -70mV the decay time constant of spontaneous EPSCs was described by a single exponential of 15.9 $\pm$ 3.2msec (n=8). This was significantly faster than the decay time constant observed in CA3 pyramidal neurones (mean= 21.9 $\pm$ 2.4msec; n=11 neurones).

The I-V relationship of the glutamate receptor agonists, kainate (100uM) and AMPA (30uM) are linear in CA3 pyramidal neurones and reverse polarity at ~0mV. The I-V relationships of these agonist responses in interneurones however were highly variable. The response to kainate was either linear or possessed marked inward rectification. The I-V responses to AMPA were more complex. Interneurones displayed either linear responses, inward rectification or outward rectification. These currents reversed polarity at ~10mV. These data suggest that the glutamate receptors present on inhibitory interneurones differ from those in CA3 pyramidal neurones in their responses to both exogenous and endogenous excitatory amino acids.

108.3

NBQX BLOCKS SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES. J.M. GOLDSTEIN, L.C. LITWIN* and A.J. SALAMA. ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897 USA

NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline) is a potent and selective inhibitor of binding to the quisqualate subtype of the glutamate receptor, with essentially no binding affinity at the N-methyl-D-aspartate (NMDA) or glycine sites. We examined the effects of NBQX on both NMDA and non-NMDA-mediated excitatory postsynaptic potentials (EPSPs) in area CA1 of the rat hippocampus, and compared its activity to the nonselective NMDA antagonist DNQX (6,7-dinitroquinoxaline-2,3-dione). In the presence of Mg++ (non-NMDA mediated synaptic transmission), bath application of NBQX caused potent and reversible antagonism (IC50=0.66 uM) of EPSPs evoked by stimulation of the CA3 Schaffer collateral-commissural fiber system. When the studies were conducted in Mg++-free medium (NMDA and non-NMDA transmission) concentrations of NBQX up to 30 uM reduced by 50% but did not abolish the EPSPs. These residual EPSPs could, however, be completely abolished by the addition of the competitive NMDA antagonist CPP (10 uM). When similar studies in zero Mg++ were conducted with DNQX (10 uM), this agent was also found to reduce but not abolish the synaptic response to CA3 stimulation. In addition, bath application of CPP (10uM) with DNQX again resulted in a further reduction of the EPSPs. However, in marked contrast to the inhibitory actions of NBQX, the inhibitory actions of DNQX could be reversed by bath application of D-serine (200 uM). The results of these studies suggest that NBQX is a selective antagonist of non-NMDA mediated synaptic transmission in the rat hippocampus. These data further support the hypothesis that DNQX is a non-NMDA antagonist with additional actions at the glycine modulatory site of the NMDA receptor complex.

108.4

QUINOXALINE DERIVATIVES (QD): INHIBITION OF EXCITATORY AMINO ACID (EAA) RECEPTOR CURRENTS AND SYNAPTIC POTENTIALS J.C.R. Randle♣, T. Guet*♣, C. Bobichon*♣, C. Moreau*♣, P. Curutchet*♣, B. Lambolez*♣, L. Prado da Carvalho*♣, A. Cordi*♦ and J. Lepagnol*♦, ♦FONDAX - Groupe Servier, 92800 Puteaux, ♦Inst. de Recherche Servier 92210 Suresnes, ♣Labo. Physiol. Nerveuse, CNRS, 91190 Gif/Yvette, FRANCE.

The relative EAA receptor inhibitory potencies of 11 QDs were evaluated. Kainate, AMPA and NMDA/glycine currents in voltage-clamped rat cortex mRNA-injected *Xenopus* oocytes were inhibited by all the QDs. Apparent K_i values for inhibition of kainate and AMPA currents were closely correlated over a >1000-fold range of potencies (CNQX = 0.16 uM --> QX = 300 uM) indicating that the two EAA agonists act at a single site. The QDs all potently inhibited NMDA/glycine current; K_i values varied only 20-fold (MNQX = 0.28 uM --> 5NQX = 6 uM). Excitatory post-synaptic field potentials were recorded in the CA1 region of hippocampal slices following electrical stimulation in the stratum radiatum in 1 mM Mg^{++} or Mg^{++}-free medium. QD IC_{50} values in 1 mM Mg^{++} correlated with K_i values against kainate and AMPA in oocyte experiments, but were 10-fold higher. QD IC_{50} values in Mg^{++}-free medium correlated with K_i values *vs.* NMDA/glycine in oocytes, but were 50-fold higher. Comparison of these two sets of results suggests that hippocampal synaptic transmission is mediated by near-saturating local concentrations of the endogenous ligands acting on kainate/AMPA- and NMDA-type receptors.

108.5

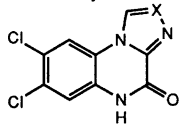
Regulation of Kainate/AMPA Currents in Cultured Hippocampal Neurons. E. J. Fletcher, Lu Wang and J.F. MacDonald. Depts. of Physiology and Pharmacology, University of Toronto, Toronto, Ont. M5S 2A8.

We have examined the possible regulation of kainate/AMPA receptors by cAMP-dependent kinase (PKA). Whole cell or perforated patch recordings were made from cultured hippocampal neurons. Kainate was applied by pressure from pipettes or individual neurons were constantly perfused and agonist applied using a motorized system. Kainate currents declined or "ran down" during whole cell patch recordings when conventional pipette solutions were employed. This rundown could be retarded by including an ATP regenerating system (ATP, creatine, creatine phosphokinase) within the patch electrode. Even more effective was the inclusion of cAMP and/or the catalytic subunit of PKA. Kainate currents recorded using the perforated patch technique were potentiated by the phosphatase inhibitor okadaic acid as well as by cAMP activators. In order to determine which pharmacological parameters were altered during rundown dose-response relations were determined relatively soon after break through and then again after 20 to 30 mins of whole cell recording. The rundown of kainate currents was associated with an average increase in the K_d value of approximately 60% (from 33 to 55 μM) as well as a 20% decrease in R_{max} values. In a separate group of neurons the patch pipette also included the ATP regenerating solution and no significant shift in the dose-response relationship was observed. In contrast to whole cell recordings kainate and AMPA currents did not demonstrate any time-dependent decline in amplitude. This evidence suggests that phosphorylation by endogenous PKA may be an important step in maintaining kainate/AMPA currents in these neurons. Supported by a grant to the MRC "Nerve Cell & Synapses" Group.

108.6

SYNTHESIS AND EXCITATORY AMINO ACID PHARMACOLOGY OF A SERIES OF HETEROCYCLIC FUSED QUINOXALINONES AND QUINAZOLINONES. E.C.R. Smith, L.A. McQuaid, A.J. Williams*, C.H. Mitch, P.L. Ornstein, K.K. South*, P.J. O'Malley*, D.O. Calligaro, D. Lodge, R.A. True*, and D.D. Schoepp. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 and Royal Veterinary College, London, NW10TU, UK.

As a part of our program aimed at identifying novel, potent antagonists of the AMPA excitatory amino acid receptor we have prepared a series of triazolo-, tetrazolo-, imidazo-, and pyrazolo-fused quinoxalinones and quinazolinones related to dichloroquinoxalinedione and dinitroquinoxalinedione. Among the derivatives evaluated, appropriately substituted triazolo[4,3-*a*]quinoxalin-4(5*H*)-ones exhibited the greatest affinity for the both the AMPA receptor and for the glycine site on the NMDA receptor. In fact, LY133578, represents a more potent antagonist of the glycine site (K_i = 0.63 μM) than of the AMPA receptor (IC_{50} = 7.0 μM). Additionally, 7,8-dichloroimidazo[1,2-*a*]quinoxalin-4(5*H*)-one, LY282261, was found to possess good affinity for the glycine site (K_i = 1.3 μM.) Antagonist activity at the glycine site was substantiated by a corresponding inhibition of [^{3}H]-MK801 binding. Compounds such as these which act by functionally inhibiting both NMDA and AMPA receptors may offer a distinct advantage in the treatment of ischemic episodes.



LY133578 X = N
LY282261 X = CH

108.7

DISCRIMINATIVE EFFECTS OF NBQX, (2,3-DIHYDROXY-6-NITRO-7-SULFAMOYL-BENZO(f)QUINOXALINE).

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NBQX, a potent and selective inhibitor of AMPA receptor binding, has anticonvulsant (1) as well as antiischemic properties (2). The present studies examined NBQX for substitution in rats discriminating phencyclidne (PCP; 3.0 mg/kg, i.p., 15 min) from no drug, morphine (3.0 mg/kg, i.p., 30 min) from no drug, or for antagonism in rats discriminating pentylenetetrazole (PTZ; 17.5 mg/kg, i.p., 15') from no drug. NBQX (3.0 - 30.0 mg/kg) produced no PCP-like effects (< 1%); there was a dose dependent depression of rate of responding with a max effect of 0.5 % of vehicle rates at 30.0 mg/kg. NBQX (1.0 - 30.0 mg/kg) also did not produce morphine-like effects up to doses that totally eliminated responding (30.0 mg/kg). NBQX (1.0 - 30.0 mg/kg) only slightly and non dose dependently attenuated PTZ with a max of 25% at 1.0 mg/kg of NBQX. These data indicate that NBQX is only marginally effective as an anxiolytic, and does not possess abuse liability of PCP or opiate type.

(1) Swedberg et al., Soc. Neurosci. Abstr., 1990.
(2) Sheardown et al., Science 247:571-574, 1990.

108.8

IN VIVO NEUROPROTECTIVE, AND ANTICONVULSANT ACTIONS OF 2,3-DIHYDROXY-6-NITRO-7-SULFAMOYL-BENZO(F)QUINOXALINE (NBQX).

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NBQX is a selective AMPA-type glutamate receptor antagonist, which has been shown to reduce global ischemic injury. The neuroprotective effects of NBQX were assessed in post natal day (PND) 7 rats that received a unilateral intrastriatal injection of 10 nmol/0.5 μl AMPA ((RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) by comparison of cerebral hemisphere weights on PND 12. Post-treatment (15 min) with single doses of NBQX (3, 10, 30 mg/kg, i.p.) did not reduce brain injury and higher doses increased mortality. Repeated post-treatment dosing reduced the severity of injury (20 mg/kg, i.p., 15, 60, 105 min; 35.5 ± 9.8% protection, $p<0.05$). Co-intrastriatal injection of NBQX with 10 nmol AMPA was most effective (40 nmol, 70.1 ± 7.9% protection, $p<0.001$).

NBQX produced rapid and short lasting (15-180 sec) suppression of tonic extensor seizures induced by low-intensity (ED_{60} = 6.0 mg/kg IV) and maximal (ED_{60} = 13.1 mg/kg IV) electroshock in mice. Pronounced behavioral side-effects were not noticed until doses of at least 30 mg/kg IV had been attained. These effects included ataxia, reduction of skeletal muscle tone, and decreases of spontaneous locomotor activity. The data show that NBQX is a short acting effective blocker of AMPA induced neurodegeneration in perinatal rat pups, and prevents electroshock induced tonic extensor seizures in mice.

108.9

IN VIVO ELECTROPHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF THE SELECTIVE AMPA ANTAGONIST, 2,3-DIHYDROXY-6-NITRO-7-SULFAMOYL-BENZO(F)QUINOXALINE (NBQX).

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NBQX is a selective antagonist of AMPA-type glutamate receptors in vitro. In the present studies, the in vivo CNS consequences of systemic administration of NBQX were evaluated. In electrophysiological experiments, the effects of NBQX were determined on the dentate gyrus e.p.s.p. and population spike evoked by electrical stimulation of the perforant path in urethane anesthetized rats. A current-response curve was generated every 15 min for 30 min preinjection and 1 h postinjection. NBQX (30 mg/kg i.v.) increased the latency to and reduced the amplitude of the evoked dentate gyrus population spike. The NBQX induced effects were present for the duration of the postinjection period. No effects were observed on the slope of the e.p.s.p. In behavioral experiments in rats, NBQX (30 mg/kg i.v.) inhibited spontaneous locomotion, produced catalepsy and produced analgesia (tail pressure assay). All these effects were determined 15 - 30 min postinjection. In the electrophysiological and behavioral studies lower doses of NBQX (3 - 10 mg/kg i.v.) had very little or no effect. These data indicate that systemically administered NBQX can block a CNS glutamate- mediated response and begin to characterize some of the behavioral consequences of this action.

108.10

DIAZEPAM WITHDRAWAL PREVENTED BY AMPA ANTAGONIST NBQX.

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The benzodiazepines (BDZs) are most commonly prescribed psychoactive drugs for the therapy of modern society disorders such as anxiety and sleep disturbances. Long term treatment leads to tolerance to and dependence on BDZs. Abrupt termination of BDZs administration gives rise to unpleasant withdrawal syndromes. Adult NMRI mice withdrawn from chronic administration of diazepam (15 mg/kg/day) showed a time related evolution of anxiety, muscle rigidity and seizures between days 4 and 21 after discontinuation of the treatment. The peak intensity of withdrawal symptoms was detected at days 5 and 7. A period between withdrawal days 1 and 3 was symptom-free. This "silent phase" was characterized by lowering of the threshold for ATPA (α-amino-3-hydroxy-5-tert-butyl-4-isoxazolepropionate; an AMPA agonist) and kainate seizures, and an increased magnitude of monosynaptic reflexes [mediated by non-N-methyl-D-aspartate (NMDA) mechanisms]. The "active phase" of withdrawal, between days 4 and 21, was characterized by a lowering of the threshold for NMDA seizures and enhanced magnitude of polysynaptic reflexes (which are NMDA dependent). Treatment of mice with the AMPA antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline; 10 mg/kg/h), but not with the NMDA antagonist CPP (3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate; 10 mg/kg/h) during the "silent phase" of withdrawal prevented or reduced the evolution of withdrawal symptoms. No muscle rigidity and seizures, and little anxiety were observed up to withdrawal day 21 in mice subjected to NBQX treatment during first three withdrawal days. These data indicate that excitatory neurotransmission mediated by L-glutamate may be differentially involved in withdrawal observed after abrupt termination of BDZ treatment. The non-NMDA phase of withdrawal is short and symptom-free, but essential for triggering clinical symptoms of withdrawal. Interference with non-NMDA mediated mechanisms during the "silent phase" of withdrawal by using AMPA antagonists such as NBQX may offer a therapeutic approach for preventing the evolution of withdrawal symptoms after BDZs, and perhaps other sedative drugs such as barbiturates and alcohol.

108.11

ANTICONVULSANT PROPERTIES OF THE NONNMDA RECEPTOR ANTAGONIST, 2,3-DIHYDROXY 6-NITRO-7-SULFAMOYL-BENZO(F)QUINOXALINE (NBQX).

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Antagonists of nonNMDA subtypes of excitatory amino acid receptors exhibit anticonvulsant properties *in vitro*. In certain of these models nonNMDA antagonists have greater efficacy than NMDA receptor antagonists. However, little is known about the properties of nonNMDA receptor antagonists *in vivo*. In this study, we examined the effects of NBQX, a potent antagonist of nonNMDA excitatory amino acid receptors, in two seizure models, electroshock (ES) and electrically-induced hippocampal afterdischarge (AD). Injection of NBQX (3 to 30 μg)into the lateral ventricle (ICV) failed to reduce the duration of ES-induced tonic hindlimb extension or the duration of the primary AD evoked by hippocampal stimulation. NBQX (10 μg) did reduce the number of wet-dog-shakes (from 22.0 ± 1.9 to 0.3 ± 0.2: $p < 0.003$) and the recurrent discharge (from 21.3 ± 3.1 to 3.0 ± 3.0 seconds; $p < 0.02$) evoked by hippocampal stimulation. NBQX (10 and 30 μg) also produced a behavioral toxicity (loss of muscle tone, ataxia and sedation) which resembled that produced by NMDA receptor antagonists. The inhibitory effects of NBQX on behavior, wet-dog-shakes and recurrent discharge were partially reversed by co-administration of D-serine. These findings demonstrate that NBQX has some anticonvulsant activity *in vivo*. However, since the behavioral and anticonvulsant profile of NBQX was found to be identical to that of 7-chloro-kynurenic acid (an antagonist of the glycine recognition site on the NMDA receptor) and since the actions of NBQX were partially reversed by D-serine (a selective agonist at the glycine site) these findings raise the possibility that at least some of the *in vivo* actions of NBQX are mediated by inhibiting glycing binding to the NMDA receptor.

108.12

ARCAINE NEGATIVELY MODULATES RESPONSES OF NMDA RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES.

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In addition to containing recognition sites for the coagonists NMDA and glycine (Gly), the NMDA receptor complex also harbors a polyamine modulatory site through which compounds such as spermine augment NMDA/Gly-induced cation flux. In the present study we tested the effects of several polyamines on NMDA receptor function using rat brain mRNA-injected *Xenopus* oocytes with particular emphasis on arcaine (1,4-diguanidinobutane), a putative polyamine antagonist. Spermine enhanced the response to the combined application of NMDA (100 μM) and Gly (3 μM), and increased the functional potency of Gly, but not NMDA. In contrast, both arcaine and 1,10-diaminodecane inhibited NMDA/Gly-induced currents. The inhibitory potency of arcaine was comparable in the absence (60.1 μM) and presence (52.8 μM) of spermine and, similarly, the potency of spermine to enhance NMDA/Gly-induced currents was unaffected by arcaine (49.8 μM and 43.5 μM in the absence and presence of arcaine, respectively). However, the maximal response to spermine was substantially reduced in the presence of arcaine. Putrescine and diethylenetriamine, reported polyamine antagonists, also inhibited NMDA/Gly-induced currents in the absence of spermine, and did not attenuate the inhibitory response to arcaine. These findings suggest that spermine and arcaine act at distinct, non-interacting sites on the NMDA receptor complex and that arcaine functions as a negative allosteric modulator or inverse agonist.

108.13

DUAL EFFECT OF SPERMINE ON THE N-METHYL-D-ASPARTATE-EVOKED [^{3}H]-NOREPINEPHRINE RELEASE. L. Facheris*, C. Speciale and R.G. Fariello. Farmitalia Carlo Erba-Erbamont Group, R&D CNS Dept., 20014 Nerviano, Milano, Italy.

A modulatory role for endogenous polyamines on the N-methyl-D-aspartate (NMDA) receptor has been suggested. Recently, the effect of spermidine on the NMDA-evoked release of [^{3}H]-Norepinephrine (^{3}HNE) in rat hippocampal slices has been studied. We now present experiments using 1 mM spermine (SPM) and extending data analysis to the post-NMDA fractions (tail). Hippocampi obtained from Wistar rats (200-220 g) were used and experiments perfomed according to Vezzani et al., J. Neurochem. 49:1438, 1987. Five minutes fractions were collected and fractional release (FR) expressed as per cent of ^{3}HNE present in the tissues at corrispondent times. Under these conditions, SPM decreased ^{3}HNE release to 60±7% of control (100% FR= 3.37±0.49, 100 μM NMDA). In the tail FR, measured up to 25 min, a prolongation of the NMDA effect was found (20'-25' FR= 0.33±0.02% and 1.00±0.08% without and with SPM, respectively). SPM was totally inactive on the ^{3}HNE release evoked by 200 μM kainate in the absence of Mg^{++}. In the presence of 50 mM K^+ and 1.2 mM Mg^{++} (100% FR= 12.38±1.51), SPM decreased ^{3}HNE release to 62±7%, but no effect was observed in the tail FR. Data suggest a dual component in the pharmacological action of SPM on the NMDA receptor function.

108.14

ONTOGENETIC PROFILE OF REGIONAL POLYAMINE EFFECTS ON THE NMDA RECEPTOR S. Subramaniam and P. McGonigle, Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Polyamines are thought to modulate the activation of NMDA receptors through a unique allosteric regulatory site. We have characterized the regional effects of polyamines on the NMDA receptor during development using a quantitative autoradiographic assay for [^{3}H]MK-801. The binding of [^{3}H]MK-801 in discrete brain regions was measured in the presence and absence of polyamines in 3, 7, 15, 25 and 60 day old Sprague-Dawley rats. In the absence of added polyamines, [^{3}H]MK-801 binding in different brain regions increased during development, peaking at 15 days and then gradually declining to adult levels. The effect of the polyamine agonist spermidine (75 μM) was greatest during early development, enhancing binding by 300% at 3 days. This effect declined between 5 and 25 days and then increased to 40% at 60 days. The pattern of regional variability observed in the effects of spermidine was similar to that in the adult except at 3 days when [^{3}H]MK-801 binding was extremely low. The weak partial agonist DET (1 mM) stimulated binding at 3 and 7 days and had a progressively greater inhibitory effect at later developmental stages. Spermidine and DET produced a complementary pattern of regional effects at 7, 15, 25 and 60 days, spermidine tending to enhance binding to a greater extent in the ventromedial striatum and DET producing a greater inhibitory effect in the dorsolateral striatum. In contrast, no regional heterogeneity was observed for the inhibitory effects of the inverse agonist DA-10. The varied profile of polyamine effects observed in this study suggests that the role of these endogenous substances may change during development. (Supported by USPHS GM 34781 and the Pew Charitable Trusts)

108.15

POLYAMINES MODULATE NMDA-INDUCED NEUROTOXICITY M. Munir, S. Subramaniam and P. McGonigle, Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104.

The presence of high concentrations of polyamines in the brain and their ability to modulate activation of the NMDA receptor-channel has led to the suggestion that they may have a role as endogenous modulators of NMDA receptor function. We have studied the ability of polyamines to modulate NMDA-induced neurotoxicity in the rat. Unilateral injections of NMDA in the presence or absence of polyamines were made into the striatum of 7 day old rat pups using the following coordinates: 2 mm lateral and 0.2 mm anterior to the bregma and 4 mm deep from the dura. After 5 days, the brains were removed and 20 μm thick sections were cut at the plane of injection and stained with cresyl violet. A computer-based image analysis system was used to densitometrically measure regions of necrosis and neuronal loss in the stained sections. These measurements were normalized with respect to the unlesioned contralateral side. Different doses of NMDA (5-35 nmol) were injected to determine the dose response relationship in this paradigm. A dose of 15 nmol produced a lesion of intermediate size and was chosen to determine the effects of co-injected polyamines. The polyamine agonist, spermine (10-200 nmol), offered marginal protection against neurotoxicity at lower doses whereas higher doses exacerbated the toxicity of NMDA in a dose-dependent fashion. The inverse agonist DA-10 produced only a small increase in the toxicity of NMDA at the highest doses tested. The divergence in the dose-response relationship between spermine and DA-10 suggests that the effect of spermine is a specific action mediated through the NMDA receptor. (Supported by USPHS GM 34781 and the Pew Charitable Trusts)

108.16

POLYAMINE EFFECTS ON THE NMDA RECEPTOR IN HUMAN BRAIN P. McGonigle, A. Dai, M.J. O'Connor and S. Subramaniam, Dept. of Pharmacology, University of Pennsylvania and Dept. of Neurosurgery, Graduate Hospital, Philadelphia, PA 19104.

Polyamines are thought to modulate the activation of NMDA receptors through a unique allosteric regulatory site. The effects of polyamines on the binding of [^{3}H]MK-801 were measured in cortical and hippocampal ttissue that was surgically removed to relieve intractable seizures. The polyamine agonist spermidine increased the binding of [^{3}H]MK-801 in the cortex in a dose-dependent manner and this effect could be blocked by the weak partial agonist diethylenetriamine (DET). Spermidine (75 μM) decreased the K_d of [^{3}H]MK-801 for the NMDA receptor from 4.3 ± 0.5 nM to 1.6 ± 0.3 nM but did not alter the density of receptors. Spermidine had essentially the same effect on the K_d and B_{max} for [^{3}H]MK-801 measured in the dentate gyrus. The binding of [^{3}H]MK-801 in human cortex was decreased by 30% by incubation with DET (1 mM). Pre-washing the tissue sections at room temperature for 1 hr also decreased binding by 30%, suggesting that the tissue contains an endogenous agonist that is removed by washing. In contrast, DET alone does not alter the binding of [^{3}H]MK-801 in rat cortex. Moreover, pre-washing sections of rat brain produces an increase rather than a decrease in binding suggesting that there are different endogenous modulators for the polyamine site in rat than in human tissue. The inverse agonist 1,10-diaminodecane decreased the binding of [^{3}H]MK-801 in a dose-dependent manner and this effect could also be blocked by DET. These results suggest that the fundamental modulatory properties of polyamines in rat and human tissues are essentially the same and that endogenous polyamines may regulate human NMDA receptors (Supported by GM 34781 and the Pew Charitable Trusts)

108.17

EVIDENCE OF DISSOCIATION BETWEEN THE MECHANISMS OF DOMOIC ACID AND KAINIC ACID TOXICITY IN VIVO. S.M. Strain* and R.A.R. Tasker. Dept. of Anatomy & Physiology, Atl. Vet. College, U.P.E.I., Charlottetown, P.E.I., Canada, C1A 4P3

Based largely on in vitro evidence the neurotoxins domoic acid (DOM) and kainic acid (KA) have been presumed to produce toxicity by an identical mechanism. In vivo, however, these compounds produce some recognizably different behaviours. We have investigated the actions of drugs known to interact with excitatory amino acid (EAA) systems on DOM and KA toxicity. Drugs were administered 15 minutes prior to TD50s of KA and DOM in groups of female CD-1 mice. Measures of overall toxicity and latency to onset of toxic behaviours were recorded. With most EAA antagonists tested the response to DOM and KA was similar, however CPP (15mg/kg), a competitive NMDA antagonist produced a significantly greater reduction in the toxicity of KA than DOM. Injection of the EAA agonists NMDA and Quisqualic acid significantly altered DOM toxicity while having no affect on KA toxicity. Low doses of morphine (2 & 4 mg/kg) produced the greatest reduction in DOM toxicity of all drugs tested but had minimal effects on the toxicity produced by KA. Finally, toxicity following co-administration of DOM and KA was not additive. We conclude that the mechanisms responsible for DOM and KA toxicity in vivo are pharmacologically similar but not identical.

108.18

ARCAINE BLOCKS OPEN *N*-METHYL-D-ASPARTATE (NMDA) CHANNELS. S.D. Donevan, S.M. Jones and M.A. Rogawski. Medical Neurology Branch, Neuronal Excitability Section, NINDS, NIH, Bethesda, MD 20892.

Recent studies have suggested that arcaine is an antagonist at the NMDA receptor complex (Reynolds, *Eur. J. Pharmacol.* **177**:215, 1990; Sacaan & Johnson, *Mol. Pharmacol.* **38**:705, 1990). Arcaine blocks polyamine-stimulated increases in NMDA-evoked [^{3}H]MK-801 binding in a competitive manner. In addition, arcaine inhibits NMDA-induced [^{3}H]MK-801 binding in the absence of added polyamines. In the present study, we examined the interaction of arcaine with the NMDA receptor using whole cell and single channel recordings from cultured rat hippocampal neurons. In whole cell recordings, arcaine caused a concentration-dependent block (IC_{50}=57 μM) of currents evoked by NMDA applied by flow pipe (V_H= −60 mV). Arcaine did not act directly at the NMDA or glycine binding site as the block could not be overcome by increasing the concentration of NMDA (3−50 μM) or glycine (0.1−10 μM). In addition, the block was not reduced by spermine (1−50 μM). The block by arcaine was, however, use dependent and could be prevented by prior application of Mg^{2+}. Like the open channel block of NMDA currents by Mg^{2+}, the block by arcaine was voltage dependent, and was almost completely relieved at positive holding potentials. In outside-out patches, arcaine caused a concentration-dependent decrease in apparent channel amplitude at negative holding potentials, which may, like the block seen with Zn^{2+} and Mn^{2+}, reflect unresolvable flickering. The reduction in channel amplitude was not observed at positive holding potentials as is consistent with the failure of arcaine to block outward currents in the whole cell recording experiments. These observations indicate that arcaine antagonizes NMDA responses by blocking the open NMDA channel and provide a likely mechanism by which arcaine reduces NMDA-evoked [^{3}H]MK-801 binding in the absence of exogenous polyamines.

108.19

ARCAINE AND MG^{2+} INHIBIT NMDA RESPONSES BY DISTINCT VOLTAGE-DEPENDENT MECHANISMS. J. Wang* and K.M. Johnson. Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX. 77550.

In the nominal absence of glutamate and glycine, both polyamines (e.g. spermidine, SPD) and divalent cations (e.g. Mg^{2+}) affect [^{3}H] TCP binding to the rat brain NMDA receptor ionophore complex in a biphasic manner. The ascending, but not descending, limb of the concentration-response curve for SPD and Mg^{2+} is competitively inhibited by arcaine. This study further characterizes arcaine's mechanism of inhibition. NMDA-induced release of [^{3}H] NE from hippocampal slices in either 3 mM or 7.5 mM KCl was used to assess the potential voltage dependence of arcaine's inhibition. The higher KCl concentration significantly diminished the inhibition produced by Mg^{2+} and arcaine, but had no effect on the inhibition by a competitive antagonist, CGS 19755. A multiple NMDA stimulus paradigm was used to examine possible use-dependency of the arcaine block. MK-801, used as a positive control, produced a stimulus dependent onset of blockade that showed only a slight recovery after washout over the subsequent NMDA pulses. In contrast, the block produced by both arcaine and CGS 19755 was very fast in onset and washout, with neither parameter being effected by the number of NMDA pulses. Thus, like Mg^{2+}, arcaine inhibition showed voltage-dependence, but not use-dependence. However, in washed rat cortical membranes the inhibition of [^{3}H] TCP binding by Mg^{2+} and arcaine was differentially affected by SPD, glutamate and glycine. Under non-equilibrium conditions, 100 μM SPD shifted the Mg^{2+} IC_{50} from 6.3 mM to 2.0 mM, but shifted the arcaine IC_{50} from 5.2 μM to 25 μM. Further, the addition of 10 μM L-glutamate and 10 μM glycine had no significant effect on the arcaine IC_{50} value, but shifted the Mg^{2+} IC_{50} leftward, from 6.3 mM to 1.0 mM. Thus, these data suggest that the inhibitory mechanisms for arcaine and Mg^{2+} involve binding to different voltage-sensitive sites, perhaps close to the mouth of the channel, rather than deep within the channel. Supported by DA-02073.

108.20

DIFFERENTIAL STIMULATION OF NMDA RECEPTOR ACTIVATION BY GLYCINE AND POLYAMINES: A KINETIC ANALYSIS. S.R. Zukin, D.C. Javitt, R. Sircar, M. Frusciante, J. Manheim* and T. Porzeline*, Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine and Bronx Psychiatric Center, Bronx, NY 10461.

Activation of *N*-methyl-D-aspartate (NMDA) receptors is regulated at separate sites by the allosteric potentiators glycine and polyamines. In order to determine the differential effects of glycine and polyamines on NMDA receptor activation, specific binding of [^{3}H]MK-801, serving as a probe of NMDA receptor activation, was measured at 12 time points between 5 min and 24 hr in the absence and presence of L-glutamate, glycine and the polyamine spermidine. We have previously demonstrated that association of [^{3}H]MK-801 to the PCP receptor is biexponential, the fast component representing binding to the activated conformation of the NMDA channel. In the present study, two components of association were again found. Under control conditions (absence of L-glutamate, glycine and spermidine), virtually all binding manifested slow kinetics. Addition of L-glutamate (0.3 - 100 μM) alone led to a dose-dependent 3-fold increase in total [^{3}H]MK-801 binding but did not significantly increase binding manifesting fast kinetics. Neither glycine nor spermidine alone led to significant increases in either fast or total [^{3}H]MK-801 binding. Both agents potentiated the degree to which L-glutamate increased binding manifesting fast kinetics of association. However, spermidine but not glycine increased total [^{3}H]MK-801 binding in the presence of L-glutamate. Both glycine and spermidine act primarily by increasing the interconversion of agonist-associated NMDA channels from the closed to the open state. Spermidine increases the total number of NMDA receptors that are accessible to binding of PCP-site ligands more effectively than does glycine, possibly implying a greater inhibition of NMDA receptor desensitization.

GABA RECEPTORS: FUNCTION I

109.1

$GABA_A$-RECEPTOR ANTIBODIES RAISED AGAINST A β_2 SUBUNIT-SPECIFIC SYNTHETIC PEPTIDE OF THE RECEPTOR. T.K. Machu[1], R.W. Olsen[2] and M.D. Browning[1]. [1]Dept. of Pharmacology, University of Colorado Health Sciences Center, Denver, CO. [2]Dept. of Pharmacology, University of California at Los Angeles Medical School, Los Angeles, CA.

The $GABA_A$ receptor is a multimeric subunit complex consisting of α,β,γ, and δ subunits. To date 3 β subunits have been identified as a result of cDNA library screening. The β_2 subunit has wide distribution in rat brain based on *in situ* hibridization studies measuring β_2 mRNA. We have raised polyclonal antibodies to the β_2 subunit peptide KKAAEKAANANNEKMRLDVN conjugated to thyroglobulin. This sequence is specific for the β_2 subunit and is contained in the large intracellular loop between the third and fourth membrane spanning regions of the receptor. The antibody which had been affinity purified recognized the β_2-subunit specific peptide in an immunoblot, whereas preimmune serum did not. Immunoblots with this purified antibody labeled two polypeptides of the purified $GABA_A$ receptor. One component had an M_r of ~56 kD whereas a second component, presumably a breakdown product, had an M_r of ~35 kD. In cortex homogenate immunoblots this antibody labelled three polypeptides. Two of these migrated similarly to those seen in the purified receptor. The third polypeptide had an M_r of ~68 kD. The 68 kD band may represent a glycosylated form of the β_2 subunit or an aggregate of the multiple subunits. The peptide which we had injected blocked, in a concentration dependent manner, the binding of the antibody to all three bands in cortex immunoblots. Moreover, a peptide representing another part of the large intracellular loop and not contiguous with the sequence we injected, failed to block the binding of antibody to cortex immunoblots. Supported by PHS grant NS26377 to MDB and NS28772 to RWO and an NRSA from NIAAA to TKM.

109.2

CYCLIC AMP-DEPENDENT PROTEIN KINASE DECREASES $GABA_A$ RECEPTOR-MEDIATED $^{36}Cl^-$ UPTAKE BY BRAIN MICROSACS N.J. Leidenheimer[1], T.K. Machu[1], S. Endo[2], R.W. Olsen[2], R.A. Harris[1,3] and M.D. Browning[1]. [1]Dept. of Pharmacology, Univ. of Colorado HSC., Denver, CO 80262; [2]Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024; [3]Denver VA Medical Center, Denver, CO 80220;

The effect of CAMP-dependent protein kinase (PKA) on $GABA_A$ receptor-mediated $^{36}Cl^-$ uptake was examined using mouse brain membrane vesicles (microsacs). Microsacs were transiently permeabilized by hypoosmotic shock to introduce the catalytic subunit of PKA. At a low concentration of muscimol (2μM), muscimol-stimulated $^{36}Cl^-$ uptake (nmoles $^{36}Cl^-$/mg protein/ 3 sec $\pm$SEM) in control (8.1 ± 1) and PKA (6.3 ± 1) treated microsacs was significantly different, $p < 0.05$. Muscimol-stimulated $^{36}Cl^-$ uptake in control (12.2 ± 0.7) and PKA (9.4 ± 0.9) treated microsacs was also significantly different, $p < 0.01$ at a maximally effective concentration of muscimol (50μM). No effects were observed with heat-inactivated PKA. To determine whether PKA treatment resulted in $GABA_A$ receptor phosphorylation, microsacs were loaded with PKA and [gamma-^{32}]-ATP and immunoprecipitation experiments were performed with a $GABA_A$ receptor anti-α subunit antibody (under nondenaturing conditions to precipitate the entire complex). PKA-loaded microsacs showed an increase in the phosphorylation of a variety of microsac proteins, including a 66 Kda polypeptide specifically precipitated by the anti-peptide sera. Phosphopeptide mapping of this 66 Kda polypeptide yielded a 14 kDa fragment similar to that obtained with the purified, PKA-phosphorylated $GABA_A$ receptor. These results show that the catalytic subunit of PKA inhibits brain $GABA_A$ receptor function concomitant with an increased phosphorylation of several proteins including a 66 kDa polypeptide believed to be a $GABA_A$ receptor subunit. Work supported by VA and ADAMHA grants AA06399 and AA03527 (RAH); NIH grants NS 28772 (RWO) and NS 26377 (MDB).

109.3

cAMP-DEPENDENT PROTEIN KINASE REDUCES SYNAPTICALLY ACTIVATED $GABA_A$-RECEPTOR CONDUCTANCES IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS *IN VITRO* MEASURED USING WHOLE-CELL RECORDING. B.L. Soldo, M.D. Browning, W.R. Proctor and T.V. Dunwiddie. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Recent studies indicate that purified $GABA_A$-receptors from rat brain can be phosphorylated by the catalytic subunit of cAMP-dependent protein kinase (PKA) (*Browning et al, 1990, PNAS 87: 1315*). Furthermore, it has been shown that PKA can decrease GABA-induced currents in mouse spinal cord neurons (*Porter et al, 1990, Neuron 5:789*). We were interested in whether PKA could also regulate GABAergic function in the hippocampal slice. The bicuculline-sensitive $GABA_A$-mediated IPSP evoked by local stimulation was pharmacologically isolated by using the specific glutamate receptor antagonists, DNQX (10 μM) and APV(40 μM). Whole-cell voltage-clamp recordings were made from CA1 pyramidal neurons to measure $GABA_A$-receptor responses to synaptically released GABA. When the catalytic subunit of PKA was included in the recording pipette, $GABA_A$-receptor conductances were significantly reduced within 30-50 minutes as compared to initial conductances measured following patch rupture. On average, PKA-treated cells had an approximately ten-fold lower $GABA_A$ conductance than control cells after 30 minutes. These data suggest that $GABA_A$-receptor function in hippocampal CA1 pyramidal neurons may be regulated by PKA phosphorylation.

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109.4

EFFECTS OF CPT-cAMP ON $GABA_A$ RECEPTOR BINDING AND FUNCTION **Patricia P. Edgar and Rochelle D. Schwartz Dept. of Pharmacology, Duke University Medical Center, Durham, NC** Our recent studies indicate that membrane-permeant cAMP analogs inhibit $GABA_A$ mediated $^{36}Cl^-$ flux and activate protein kinase A in synaptoneurosomes (Heuschneider and Schwartz, 1989; Schwartz et al.,1991). We have investigated the effect of 8-chlorophenylthio (CPT) cAMP, the most potent analog, on the binding of several $GABA_A$ receptor-associated ligands (^{3}H-muscimol, ^{3}H-diazepam, and ^{35}S-TBPS) and on the modulation of $GABA_A$ receptor function. Binding assays were performed in synaptoneurosomes under conditions identical to those used for ion flux. For ^{3}H-muscimol, this resulted in the identification of a low affinity site with a K_d (2 μM) comparable to the EC_{50} for muscimol-induced $^{36}Cl^-$ uptake (11 μM). CPT-cAMP did not alter the number or affinity of these binding sites, indicating that inhibition of $^{36}Cl^-$ uptake by CPT-cAMP is not the result of a decrease in $GABA_A$ agonist binding. Under identical conditions CPT-cAMP decreased the affinity of benzodiazepine (BZ) sites labeled with ^{3}H-diazepam by 30%. This was apparently due to the inhibition of endogenous GABA's ability to enhance BZ receptor affinity, since CPT-cAMP had no effect on BZ receptor affinity in the presence of bicuculline (BIC). In contrast, diazepam produced a greater enhancement of muscimol-induced (10 or 50 μM) $^{36}Cl^-$ uptake in the presence of CPT-cAMP. Finally, CPT-cAMP decreased the affinity of the chloride channel for ^{35}S-TBPS by 20% in both the presence and absence of BIC. We suggest that cAMP can cause allosteric changes in sites other than the functional GABA agonist recognition site. These results do not distinguish between a direct effect of cAMP on the chloride channel or an indirect effect via PKA-mediated phosphorylation. Supported by NSF Predoctoral Fellowship (P.P.E.) and by NS 24577 (R.D.S.).

109.5

SUSTAINED COMPONENT OF GABA-ACTIVATED Cl- FLUX: SELECTIVE MODULATION BY PROTEIN KINASE C. R.A. Harris, S.J. McQuilkin* and L. Hahner*. V.A. Medical Center and Dept. of Pharmacology, Univ. of Colo. Sch. Med., Denver, CO 80262.

Phosphorylation of $GABA_A$ receptor subunits by protein kinase C (PKC) occurs *in vitro*, but the functional significance is unknown (*TIPS 12:84, 1991*). We used the initial (3 sec) and sustained (30 sec) phases of GABA-stimulated uptake of $^{36}Cl^-$ (G-Cl) by mouse brain cortical microsacs (*FASEB J.*, in press) to study effects of activation and inhibition of PKC. PMA (10-100 nM), a phorbol ester that activates PKC, inhibited G-Cl at 30 sec, but not at 3 sec. The inactive analog, PMM, did not alter G-Cl. The kinase inhibitors staurosporine (10 nM) and sphingosine (3 μM) blocked the action of PMA. G-Cl of microsacs lysed and resealed in the absence of Ca^{++} and Mg^{++} (to abolish phosphorylation, *Mol. Pharmacol. 38:823, 1990*) was not affected by PMA. The protein phosphatase inhibitor okadaic acid (100 nM) mimicked the action of PMA and the actions of PMA and okadaic acid were not additive. Okadaic acid did not alter the rapid phase of G-Cl. We conclude that activation of PKC inhibits the sustained $GABA_A$ current without affecting the rapidly desensitizing current. PKC may selectively inhibit a non-desensitizing subtype of $GABA_A$ receptor or may slow receptor resensitization.

Supported by VA and NIAAA grants.

109.6

THE ROLE OF *N*-LINKED GLYCOSYLATION IN EXPRESSION OF THE RAT $GABA_A$ RECEPTOR IN *XENOPUS* OOCYTES

A.L. Buller*, G.A. Hastings*, E.F. Kirkness and C. M. Fraser, Section on Molecular Neurobiology, LPPS, NIAAA, Rockville, MD 20852.

The $GABA_A$ receptor, a ligand-gated chloride channel, represents the major inhibitory neurotransmitter receptor in vertebrates. The receptor is an oligomeric transmembrane glycoprotein complex. We have expressed functional $GABA_A$ receptors in *Xenopus* oocytes microinjected with RNA coding for the α_1, β_1 and γ_2 subunits. Bath application of GABA elicits inward chloride currents in voltage clamped microinjected oocytes with an EC50 of approximately 10 μM and a Hill coefficient of 1.25. GABA currents are potentiated by phenobarbital (200% of control) and by flunitrazepam (35% of control). Benzodiazapine potentiation is blocked by the benzodiazepine antagonist, RO15-1788.

Sequence analysis has revealed that the α_1 subunit contains two consensus sequences for *N*-linked glycosylation (Asn-X-Ser/Thr). Tunicamycin (TM) was used to investigate the role of *N*-linked glycosylation in expression of the GABA receptor in *Xenopus* oocytes. TM inhibits the transfer of the oligosaccharide precursor to the growing polypeptide chain and thus prevents the formation of all forms of *N*-linked glycosylation. Oocytes were injected with GABA receptor subunit-specific RNAs (α1, β1,γ2 mixed in equimolar stoichiometry) alone or in combination with TM (25 mg/ml) and incubated for 48 hr. TM completely abolished functional expression of GABA receptor. To further investigate the role of glycosylation in expression of the GABA receptor, glycosylation-deficient mutants have been generated. We have constructed mutant α_1 subunits containing only one of the two sites for glycosylation (changing residues Asn^{10} and Asn^{110} to Gln) or missing both sites. We are currently investigating the effects of these mutations on expression of the GABA receptor in *Xenopus* oocytes.

109.7

ONTOGENY OF $GABA_A$ RECEPTOR ION CHANNELS: REVEALED BY ZINC INHIBITION. T.G. Smart. School of Pharmacy, Department of Pharmacology, University of London, UK.

The sensitivity of $GABA_A$ receptors to inhibition by zinc declines with neuronal age. This aspect and the mode of zinc antagonism on single GABA ion channel currents were studied in cultured rat neurones using patch clamp techniques. Whole-cell GABA currents recorded from acutely cultured sympathetic neurones (<5 days *in vitro*) using embryonic (E21 days), postnatal (P3) and adult (P>90) rats to monitor development in vivo, were antagonised by zinc with mean inhibitions of 78, 43 and 16% respectively. A single exponential component described the reduction in zinc inhibition, with a $\tau_{1/2}$ of 48.2 days. Chronic culture (>4 months) of embryonic neurones (E21) also revealed a decline in zinc inhibition *in vitro*, proceeding at a slow rate of 0.45% per day. Single GABA channel currents recorded from large cerebellar neurones, exhibited multiple current levels with conductances of 8, 18, 23, 29 and 34pS. Zinc (10-50μM) did not affect the main conductance state (29pS), but reduced the opening frequency. The mean open time was unaffected by zinc, but the mean closed time increased. A small reduction in the mean burst duration and proportion of long duration bursts was also observed and correlated with noise analysis. The underlying developmental process rendering $GABA_A$ receptors insensitive to zinc apparently proceeds *in vivo* and *in vitro*. The major inhibitory effect of zinc is to reduce the GABA channel opening frequency.

This work was supported by the MRC and Wellcome Trust.

109.8

CENTRAL BENZODIAZEPINE RECEPTORS ARE SEQUESTERED IN BRAIN MEMBRANE VESICLES. E.M. Barnes, Jr. and M.H. Jalilian Tehrani. Dept. of Biochemistry, Baylor Col. of Med., Houston, TX 77030.

In order to study the intracellular trafficking of $GABA_A$/benzodiazepine receptors, we have synthesized an impermeant benzodiazepine, N-(4-sulfophenyl)thiocarbamoyl-1012S [SPTC-1012S]. The receptors which are sequestered within brain membrane vesicles were detected by displacement of specific [^{3}H]flunitrazepam binding by SPTC-1012S and the permeant homolog, 1012-S. The fraction of sequestered receptors was estimated by difference, using the ^{3}H which remained bound in the presence of SPTC-1012S and 1012-S. Sequestered benzodiazepine receptors represented 20-30% of the total in microsomal (P_3) membranes from rat brain, while a 1-2% level of internal receptors was observed for crude nuclear (P_1) and mitochondrial-synaptic (P_2) membranes. Similar findings were obtained with goat brain membranes. Exposure of P_3 vesicles to Triton X-100 reduced the level of sequestered receptors to <1%. Microsomal fractions enriched in clathrin-coated vesicles were isolated on discontinuous sucrose or ficoll-^2H_2O gradients. Of the receptors on crude coated vesicles, 17-26% were internalized. This approach may prove useful for the study of receptor down-regulation following exposure of animals to GABA and benzodiazepine agonists. Supported by DK 17436 and NS 11535 from NIH.

109.9

CHRONIC ETHANOL ADMINISTRATION INDUCES CHANGES IN $GABA_A$ RECEPTOR GENE EXPRESSION. M.K. Ticku and M. Mhatre. Univ. TX Hlth. Sci. Ctr., Dept. of Pharmacology, San Antonio, TX 78284-7764

In order to evaluate the mechanism underlying the structural and functional changes in $GABA_A$ receptor following chronic ethanol (alcohol) administration, we measured the steady state levels of the mRNAs for the α_1, α_2, α_3, α_4 and α_6 subunits of $GABA_A$ receptor following chronic ethanol administration to rats and the ethanol withdrawal for 24 h.. A marked decrease (40-60%) in the level of the $GABA_A$ receptor α_1 subunit mRNAs (3.8 and 4.3 kb), α_2 subunit mRNA (6 kb) and α_4 subunit mRNA (2.8 kb) was observed in the cerebral cortex in rats, while there was no change in the level of α_3 subunit mRNA (3 kb). On the other hand α_1 mRNA levels in cerebellum remained unchanged. The level of α_6 mRNA which selectively encodes Ro15-4513 binding sites was found to be increased by 263% in the cerebellum. Also, the photoaffinity labeling studies using [^{3}H]Ro15-4513 indicate a significant increase in the levels of various protein components of $GABA_A$ receptor in the cerebellum and the cerebral cortex (e.g. 50 and 56 KDa in the cerebellum and 41, 50 and 59 KDa in the cortex), following chronic ethanol treatment. These data indicate chronic ethanol induced alteration in the regulation of the expression of $GABA_A$ receptor subunit-encoding mRNAs. Supported by NIAAA grant AA04090.

109.10

AGE-RELATED ALTERATION IN THE BINDING AND SUBUNIT mRNA LEVELS IN $GABA_A$ RECEPTOR. M. Mhatre, G.Fernandes*† and M.K. Ticku. Univ. TX Hlth. Sci. Ctr., Depts. of Pharmacology and Medicinet, San Antonio, TX 78284-7764

The effect of aging on the binding of ligands to the GABA, benzodiazepine and picrotoxin binding sites as well as steady state mRNA levels of α subunit genes of γ-aminobutyric acid (GABA) receptor complex, was investigated in cerebral cortex and cerebellum of male Fischer F-344 rats. In aged (730-780 day-old) rats, the binding of [^{35}S]t-butyl-bicyclophosphorothionate (TBPS) was significantly reduced, whereas [^{3}H]muscimol and [^{3}H]flunitrazepam binding did not change compared with adult (6-mo-old) values. This decrease of TBPS binding derived from a reduced density of binding sites, rather than from affinity changes. In contrast, there was no alteration in the binding pattern of various ligands in the cerebellum in old animals. The allosteric interaction of GABA with the benzodiazepine and picrotoxin (TBPS) sites of the oligomeric $GABA_A$ receptor was not altered in aged animals. Also, α_1 mRNA level was markedly decreased in the cerebral cortex of old animals (86% suppression). In contrast, α_1 mRNA remained unchanged in cerebellum. These findings indicate a selective age-related structural change in $GABA_A$ receptor chloride ionophore complex in rat cerebral cortex.

109.11

IDENTIFICATION OF $GABA_A$ RECEPTOR ABNORMALITIES IN THE CEREBRAL CORTEX OF THE TOTTERING MOUSE. M.H. Jalilian Tehrani and E.M. Barnes, Jr., Biochemistry Dept., Baylor Col. of Med., Houston, TX 77030.

Previous electrophysiological studies of tottering mice (tg/tg) have suggested that reduced inhibition may contribute to their characteristic seizure activity. We have compared the properties of $GABA_A$ receptors in cortical membranes from tg/tg mice and co-isogenic controls (+/+). In +/+ microsacs, the rate of GABA-gated ^{36}Cl flux was nearly constant during the initial 5 sec of uptake and declined thereafter, while Cl entry into tg/tg microsacs faded more rapidly. Kinetic analysis of the muscimol dose response shows a higher affinity for tg/tg receptors (tg/tg: $K_{0.5}$ = 0.2 μM; +/+: $K_{0.5}$ = 1.3 μM) and a lower maximum rate of ^{36}Cl flux (tg/tg: Vmax = 6.1 nmol/mg·3s; +/+: Vmax = 12.1 nmol/mg·3s). Scatchard analysis of specific [^{3}H]muscimol binding suggests that affinities of both classes of tg/tg sites were higher than those for +/+ (tg/tg: Kd_1 = 3.8 nM, Kd_2 = 225 nM; +/+: Kd_1 = 5.4 nM, Kd_2 = 534 nM), while the densities of tg/tg sites were lower (tg/tg: $Bmax_1$ = 0.85 pmol/mg, $Bmax_2$ = 5.9 pmol/mg; +/+: $Bmax_1$ = 1.70 pmol/mg, $Bmax_2$ = 10.1 pmol/mg). The data suggest that the tg mutation leads to functional alterations in $GABA_A$ receptors which could produce dysinhibition. Supported by NS11535 from NIH and by the American Epilepsy Society.

109.12

EFFECTS OF CHRONIC MILD STRESS ON IN VIVO BINDING OF BENZODIAZEPINE RECEPTOR ANTAGONIST, [^{3}H] RO 15-1788, TO MOUSE BRAIN. M. MOSADDEHGI*, P.D. FELDMAN, AND J.M. MOERSCHBAECHER. 1901 Perdido St., LSUMC, Dept. of Pharmacology and Experimental Theraputics, New Orleans, LA. 70112

This study examined the effect of a brief mild stress on in vivo benzodiazepine (BZD) receptor binding in mouse brain. Mice in one group received daily single injection (i.p.) of saline (5 ml/kg) for 7 days (N=7). Mice in the second group were restrained for 5-6 sec by grabbing the back skin and holding the subject upside-down at 45 as if to be injected (sham control, N=7) for 7 days. Mice in the third group were neither restrained nor injected (acute control, N=7). On the 8th day BZD receptors were labelled in vivo by administering (i.p.) 3 μCi of [^{3}H] Ro 15-1788. The levels of in vivo binding of the radioligand to cortex (CX), cerebellum (CB), striatum (ST), hippocampus (HP), hypothalamus (Hy) and brain stem (BS) were determined. Results indicated that the level of binding was significantly ($p<0.01$) lower by (30 - 50% depending on the brain region) in saline injected or sham control groups as compared to acute control animals. The levels of binding in CX, CB, BS, ST, HP, and HY for acute control were 34.6 ± 2.6, 18.8 ± 0.5, 8.5 ± 0.5, 21.3 ± 2.4, 52.2 ± 4.0 and 41.4 ± 5.0 cmp/mg tissue, respectively. Whereas, the values in CX, CB, BS, ST, HP, and HY for saline injected mice were 15.2 ± 1.4, 10.0 ± 1.1, 5.9 ± 0.5, 11.6 ± 1.2, 31.9 ± 2.5, and 23.1 ± 1.9 cmp/mg tissue, respectively. The values for sham control were similar to the saline treated group. Our data suggest that exposure to chronic mild stress produces a decrease in in vivo binding of [^{3}H] Ro 15-1788 in mouse brain. Thus, these results support the hypothesis that chronic stress produces a decrease in BZD receptor binding sites. Supported by DA 03573 and DA 04775.

109.13

CHRONIC GABA EXPOSURE INDUCES DOWN-REGULATION OF THE GABA/BENZODIAZEPINE (BZ) RECEPTOR COMPLEX IN CULTURED CORTICAL NEURONS. A.K. Mehta and M.K. Ticku. Univ. TX Hlth. Sci. Ctr., Dept. of Pharmacology, San Antonio, TX 78284-7764.

The effect of chronic exposure (1-5 days) of GABA (500 μM) to cultured cortical neurons was investigated using [^{3}H]flunitrazepam, [^{3}H]Ro15-1788 and [^{3}H]Ro15-4513 binding, and GABA-induced ^{36}Cl-influx as paradigms for GABA-BZ binding sites and function, respectively. Treatment of cultures with GABA (500 μM) caused a decrease in [^{3}H]flunitrazepam, [^{3}H]Ro15-1788 and [^{3}H]Ro15-4513 binding. The decline in the specific binding was maximal (~40%) on the fifth day of GABA exposure, and was reversed by concomitant exposure of the neurons to RU5135 (1 μM), a $GABA_A$-receptor antagonist. GABA-induced downregulation was due to a decrease in the B_{max} of [^{3}H]flunitrazepam binding, without any effect on the K_D value. GABA induced ^{36}Cl-influx in control cultured cortical neurons, and the effect declined on prior exposure of the neurons to GABA. This effect was also susceptible to reversal by $GABA_A$-receptor antagonist, RU5135. These findings indicate that chronic GABA exposure induces down regulation of GABA/BZ receptor complex through $GABA_A$ receptors in cultured cortical neurons. Supported by NINDS grant #15339.

109.14

CHRONIC BENZODIAZEPINE (BZ) TREATMENT REDUCES GABA AND BZ AGONIST ACTIONS *IN VITRO* HIPPOCAMPUS. X-H. Xie and E.I. Tietz. Dept. of Pharmacology, Med. Coll. of Ohio, Toledo, OH 43699

Previous studies showed a significant reduction in GABA-mediated paired-pulse inhibition and subsensitivity to the $GABA_A$ agonist, isoguavacine (ISO) in hippocampal slices from chronic BZ treated rats. Behavioral studies had indicated differential subsensitivity to GABA agonists after chronic BZ treatment. Therefore, the potency and efficacy of other $GABA_A$ agonists (muscimol (MUS) and GABA) were examined 48 hr after 1 week flurazepam (FZP) treatment in *in vitro* superfused slices. The effect of diazepam (DZP) to potentiate ISO actions was also examined. Male rats (200-280 gm) were offered FZP in their drinking water (100 mg/kg X 3 dy; 150 mg/kg X 4 dy). Population spikes elicited by Schaffer collateral stimulation (.1 ms monophasic pulse) were recorded (2 M NaCl filled glass pipette, 2-5 mΩ) from CA1 pyramidal cells. MUS and GABA (in presence of 200 μM nipecotic acid) were delivered for 5 min via syringe pump (10-50 μl/min) to the superfusate (2 ml/min) in increasing concentrations. Responses, averaged over the last 2 min, were compared to the pre-drug baseline. MUS and GABA showed a significant decrease in potency to reduce CA1 evoked responses in treated (EC_{50}:MUS, 6.7 μM/n=5; EC_{50}:GABA, 195 μM/n=6) vs. control slices (EC_{50}:MUS, 2.4 μM/n=5; EC_{50}:GABA, 115 μM/n=6). DZP was tested in slices 48 hr after 1 week FZP treatment by its ability to shift the ISO dose-response curve. After superfusion of ISO (2.5-20 μM), DZP (300 nM, 20 μl/min) was superfused 20 min before a 2nd ISO curve was determined in the presence of DZP. DZP produced a significant parallel shift of the ISO dose-response curve in control ($p < .05$, n=6) but not treated (n=6) slices, indicating BZ tolerance. Supported by grants R01-DA04075 and S07-RR05700.

109.15

HORMONAL INFLUENCES ON SN RETICULATA RESPONSES TO GABA AND BENZODIAZEPINES IN RATS. Marlene A. Wilson, Dept. Pharmacology, Univ South Carolina Sch of Medicine, Columbia, SC 29208.

Prior studies have shown that administration of gonadal steroids can modify GABA and benzodiazepine (BZ) receptor sites in several brain areas. Progesterone metabolites can also directly modulate the GABA/BZ/chloride ionophore complex and physiological responses to GABA.

Extracellular single unit recording of substantia nigra pars reticulata (SNr) neurons was used to assess the influences of gender and gonadectomy on GABA effects and BZ responses. Groups of male, cycling female, ovariectomized (OVX) and orchidectomized (ORCH) rats were compared. Sensitivity to iontophoretically applied GABA (GABA IT_{50}) and the ability of iontophoretically (locally) applied BZ to enhance GABA responses (% BZ INCREASE) were comparable in all hormone groups. GABA effects on ^{36}Cl-influx into cortical synaptosomes were also similar in male, female, and OVX groups. Basal SNr firing rates were nonsignificantly elevated in MALES compared with other groups. The ability of systemic diazepam (DZ; 0.03-1.5 mg/kg, iv) to decrease SNr firing differed significantly between hormone groups. DZ-induced inhibition was greater in MALE rats than in other groups (see 1.0mg/kg effect below). Thus, neither gender nor castration modified local SNr responses to GABA or BZs. Since systemic BZ effects involve enhancement of GABA inputs, group differences in DZ responses suggest hormonal factors may be modulating endogenous GABAergic control of SNr neurons.

	N cells/rats	GABA IT_{50} (nA.sec)	% BZ INCREASE	FIRING RATE	%DECREASE SYSTEMIC DZ
MALE	19/9	198 ±23	40 ±6%	243 ±18	45 ±7%
FEMALE	16/8	164 ±31	38 ±4%	181 ±21	38 ±6%
OVX	22/10	161 ±17	37 ±3%	204 ±18	34 ±7%
ORCH	14/7	165 ±44	28 ±7%	183 ±21	23 ±6%

(support: 5 R29 DA05932-02 and Stefan Mironescu Grant from USC SOM)

109.16

EFFECT OF OVARIECTOMY OR STAGE OF ESTROUS CYCLE ON BENZODIAZEPINE BINDING IN FEMALE MOUSE BRAIN. J. V. Martin, N. Agrawal* and H. Lee*. Biology Dept., Rutgers Univ., Camden, NJ 08102.

To assess the effect of physiological fluctuations in ovarian hormones on benzodiazepine receptor availability, we measured the equilibrium binding of ^{3}H-diazepam to membranes from brains of 15 mice killed after ovariectomy or at different stages of the estrous cycle. Vaginal smears prepared daily from adult female CD-1 mice were classified by stage of the estrous cycle, until at least two complete cycles were recorded. The ovariectomized mice were used at two weeks after surgery. Separate binding analyses were performed in homogenates from the cerebral cortex and hypothalamus isolated from each mouse. As expected, uterine weight showed significant fluctations by stage of estrous cycle and after ovariectomy. Both brain areas showed significant differences in both apparent K_d and B_{max} according to phase of the estrous cycle and ovariectomy ($p < 0.02$ for each ANOVA). The patterns of differences were very similar for cortex and hypothalamus. (All data given are for cortex, presented as means ± S.E.M.) The apparent K_d (in nM) was significantly higher in the estrus phase (2.8 ± 0.4) as separately compared to other phases of the cycle (metestrus: 1.4 ± 0.4; diestrus: 1.3 ± 0.3; proestrus: 1.0 ± 0.2) using a post-hoc least-significant difference test ($p < 0.05$). After ovariectomy, the K_d was significantly lower (0.6 ± 0.3 nM) than in intact mice. The B_{max} (in fmoles/mg protein) was significantly higher in cortex from mice in estrus (564 ± 71) than in the other phases (metestrus: 310 ± 56; diestrus: 316 ± 129; proestrus: 282 ± 56). The B_{max} after ovariectomy was significantly lower (26 ± 3) than in any of the intact groups. These data suggest the influence of an endogenous ovarian factor on the binding characteristics of the benzodiazepine receptor. Supported by the Busch Fund and NIH (NS23200).

109.17

CHARACTERIZATION OF ANTAGONISTIC ACTIVITY AND BINDING PROPERTY OF SR 95531 IN RAT BRAIN. Y.Ito, T.Koshiba*, M.Doi* and H.Fukuda*. Dept. of Pharmacology, Coll. of Pharmacy, Nihon Univ., Funabashi-shi, Chiba 274, Japan.

Experiments were performed to characterize antagonistic activity and binding properties of ^{3}H-SR 95531, a synthetic $GABA_A$ antagonist, in rat brain. SR 95531 and bicuculline methiodide (BMI) inhibited the muscimol-stimulated $^{36}Cl^-$ uptake into cortical synaptoneurosomes. Inhibitory potency of SR 95531 for the muscimol-stimulated $^{36}Cl^-$ uptake was 15 times higher than that of BMI. The IC_{50} value of SR 95531 for the muscimol-stimulated $^{36}Cl^-$ uptake was in close agreement with the K_D value for low-affinity [^{3}H]SR 95531 binding sites. Pretreatment of the frontal cortical and cerebellar membranes with phospholipase A_2 (PLase A_2) invariably decreased [^{3}H]SR 95531 binding in a concentration-dependent manner. On the other hand, the treatment significantly increased [^{3}H]GABA binding in these regions. The products generated by the catalytic activity of Plase A_2 such as arachidonic acid and lysophosphatidylcholine mimicked the effects of PLase A_2. These results suggest that SR 95531 exerts GABA antagonistic action through the low-affinity binding sites of SR 95531. It is also suggested that $GABA_A$ agonist and antagonist bind differentially to $GABA_A$ receptors and that exogenously added PLase A_2 causes opposite modulation of antagonist binding to agonist binding.

109.18

UNCOUPLING OF GABA-BENZODIAZEPINE RECEPTORS IN CHICK CEREBRAL CORTICAL NEURONS REQUIRES CO-ACTIVATION OF BOTH RECEPTOR SITES. A. Prasad and J.N. Reynolds, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Nfld., Canada A1B 3V6.

Primary cultures of cerebral cortical neurons were prepared from seven day old chick embryos. After 5 days *in vitro*, culture dishes were exposed to one of four conditions: 1) control; 2) 1 μM Flurazepam; 3) 1 μM GABA; 4) 1 μM Flurazepam + 1 μM GABA. GABA-activated chloride currents and the interaction between flurazepam and GABA were examined using whole-cell voltage-clamp recordings. Cells were continuously perfused (1-2 mL/min) with a physiological saline containing (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, pH 7.3. Flurazepam HCl was dissolved in extracellular solution (0.1-10 μM) and applied by bath perfusion. Recording pipettes (3-5 MΩ) contained (in mM) 140 KCl, 2 $MgCl_2$, 10 HEPES, 4 ATP, pH 7.3. GABA (10-50 μM) was applied by brief (20-100 msec) pressure pulses directly to the soma. Cells grown under all four experimental conditions responded equally well to GABA, with no apparent differences in sensitivity. In control cultures perfusion with 0.5 μM flurazepam resulted in a strong potentiation of GABA-activated membrane currents. Cells exposed to 1 μM flurazepam for 5 days also had GABA-activated currents which were strongly potentiated by 0.5 μM flurazepam. Similarly, exposure of chick cortical neurons to 1 μM GABA did not alter the ability of flurazepam to potentiate GABA-activated membrane currents. In contrast, in cells exposed to flurazepam + GABA for 18 hours, no effect of flurazepam on GABA-activated membrane currents could be detected. These results suggest that co-activation of both GABA and benzodiazepine receptors is required in order to elicit changes in the functional coupling between these two sites.

Supported by the Medical Research Council of Canada.

OPIOIDS: ANATOMY AND PHYSIOLOGY I

110.1

ALTERATIONS IN N. ACCUMBENS EXTRACELLULAR DA AND BEHAVIOR FOLLOWING INTRA-VTA OPIOIDS. M.E. Hamilton and A. Pert. BPB / NIMH, Bethesda, MD. 20892.

Microinjection of opioids into the ventral tegmental area (VTA) has been shown to produce a variety of responses consistent with goal-directed behavior and intrinsic reward. The most dramatic goal-oriented responses have been observed following low pmole doses of the endogenous κ agonist, Dynorphin A (DYN). Indeed, other opioids produce similar effects only at those doses consistent with their relative binding affinities at the κ receptor. It was of interest to examine the relative effects of three naturally occurring opioids on both behavior and DA overflow in the n. accumbens. Male, Long-Evans rats were implanted with guide cannulae directed toward the medial n. accumbens and the VTA. At least one week following surgery, a microdialysis probe was inserted into the n. accumbens and each rat was placed in a sound-attenuated locomotor activity chamber. At 20 min intervals, dialysate was collected and photocell counts were recorded. When stable DA levels were achieved, rats received one of the Met-Enkephalin analog, DALA, the Leu-Enkephalin analog, DADLE, or DYN, at doses previously shown to elicit feeding in food-satiated rats (3 nmoles for the enkephalins; 3 pmoles DYN). Behaviors were recorded during the 20 min immediately following injection, and dialysate samples were further collected for two hours. Feeding durations following opioid injection were similar in all three groups; however locomotor activity was both more pronounced and more sustained following DALA and DADLE than after DYN. DADLE treatment was also characterized by a tight circling not observed in either of the other groups. DA overflow in the n. accumbens was also more pronounced and sustained following DALA or DADLE. A small, transient increase in DA following DYN accompanied the behavioral activation. It would appear that μ and/or δ receptor activation in the VTA probably contributes to a net disinhibition of DA cells, and that κ activation either is independent of mesolimbic DA or affects only a small subpopulation of DA cells.

110.2

EFFECT OF μ- AND δ-OPIOID AGONISTS ON SCHAFFER COLLATERAL AND MOSSY FIBER EVOKED RESPONSES IN CA1 AND CA3 OF HIPPOCAMPAL SLICE. L.S.Jones¶, S.Grooms¶, S.Salvadori*† and L.H.Lazarus*¶. ¶Dept. of Anat., U. So. Carolina, Columbia, SC 29208; †Dipt. Sci. Farm., Univ. di Ferrara, Ferrara, I-44100, Italy; *NIEHS, RTP, NC 27709.

Hippocampal slices from young rats were used to test physiological CNS responses to a μ-(dermorphin=DM) and a δ- (deltorphin A=DEL) agonist, which exhibit high affinity and selectivity. DM potentiated responses at 5 and 50 nM, while 100 nM caused evoked responses to become epileptiform, and 500 nM lead to low amplitude spontaneous epileptiform events more apparent in CA1 than CA3. The epileptogenic effects of DM were partially reversible with washing in ACSF. 5 μM Naloxone, a nonspecific opioid antagonist, markedly reduced the excitatory effects of 100 nM DM. The inactive enantiomer (L-ala^2)-DM had no effect. DEL had little effect on responses; potentiation was observed only at the highest concentrations (0.5-1 uM) tested. These data provide evidence that epileptogenic effects in rat hippocampal slices are triggered in part through μ receptors. Work supported by NS27903 and Minis. Publica Instruzione and C.N.R.+

110.3

ALFENTANIL-INDUCED HYPERMETABOLISM, SEIZURE, AND NEUROPATHOLOGY IN RATS; W.A.Kofke, R.H.Garman*,W.C.Tom*, M.E.Rose*, and R.A.Hawkins. Dept. of Anes/CCM and Dept. of Pathology, Univ. of Pittsburgh; Dept. of Physiology & Biophysics, Chicago Med. Sch.

Three experiments were performed on paralyzed ventilated rats to determine whether alfentanil (ALF) caused local seizures and neuronal damage. First, rats (n=15, 5/grp) were assigned to: 1) control (1h N_2O/O_2 70/30); 2) low dose ALF (150ug/kg/min followed by 15ug/kg/m); 3)high-dose ALF (1000ug/kg iv followed by 100ug/kg/min). Glucose consumption was measured by quantitative autoradiography with 6-^{14}C glucose as a tracer. Second, hippocampal electrodes were placed in 12 rats. They were assigned to control, low-dose and high-dose ALF grps. Depth and scalp EEG were recorded. Third, rats (n=15) were assigned to either a control grp (n=5) or an extra high dose grp (ALF 2000ug/kg iv followed by 33.3ug/kg/min). They were allowed to recover for 24hr before cerebral perfusion-fixation the next day. With high-dose ALF there was increased glucose consumption in the ventral hippocampus and lateral septal nuclei. Both the scalp and hippocampal electrodes showed epileptiform EEG patterns. Light microscopy showed neuronal damage in 6 of 10 high-dose ALF rats with amygala most affected. No control rats had lesions. We conclude that high-dose ALF rats produces limbic seizures with hypermetabolism and brain damage.

110.4

EFFECTS OF OPIOIDS ON EVOKED AND SPONTANEOUS CELLULAR EVENTS IN THE DENTATE GYRUS. JH Mayer, SC Steffensen*, SJ Henriksen. Dept. Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92117

In order to better understand the effects of opioids on hippocampal function, we studied the changes in dentate neuronal activity occurring spontaneously (unit activity) and after stimulation of the perforant path (population spikes) following systemic or local (iontophoretic) administration of opioids. Cells were differentiated as probable dentate granule cells (DGCs) or as probable dentate interneurons (INTs). Both DGCs and INTs were located from 50 to 200 um below reversal of the population EPSP. Cells were considered to be probable INTs when perforant path stimulation produced multiple cell firings extending beyond the envelope of the population spike and EPSP. The responsiveness of the dentate to stimulation of the perforant path was assessed via input/output curves (I/O) and paired-pulse (PP) paradigms. In each test we used either the iontophoretic administration of the mu-selective opioid agonist [D-Ala^2,NMe-Phe^4,Gly-ol]-Enkephalin (DAGO) or the systemic adminstration (IV or IP) of morphine sulfate (MS), 2.5 mg/kg.

DAGO and MS had similar effects on unit cell activity. There was a tendency for the spontaneous activity of DGCs to decrease or remain unchanged and for the spontaneous activity of INTs to increase or remain unchanged. However, DAGO and MS had differing effects on population spikes (PS) produced by stimulation of the perforant path. DAGO enhanced the response to stimulation with a shift to the left of the I/O curve and disinhibition in the PP paradigm. MS produced a very small decrease in primary PS amplitude as compared to baseline levels and no change in the PP paradigm. These results are consistent with previous work from our lab showing application of enkephalin produced decreased spontaneous activity of DGCs with increased responsiveness to perforant path stimulation. The current results suggest that opiates produce their effects on the dentate through multiple actions, and that INTs may be important in this expression. Furthermore, the difference in responses to stimulation between DAGO and MS is most likely due to effects of systemic MS on other inputs to the dentate. (Supported by DA 00143 to JHM)

110.5

MU-OPIOID RECEPTORS MODULATE CARDIOVASCULAR AND SYMPATHOADRENAL FUNCTION AT REST AND DURING STRESS.

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The role of brain mμ-opioid receptors in modulating cardiovascular and sympathoadrenal function was examined by studying the effect of mμ-receptor antagonist, ß-funaltrexamine (ßFNA), administered icv, on plasma catecholamines, blood pressure and heart rate under basal conditions and during restraint stress. Rats were prepared with chronic carotid and icv cannulae for blood pressure recording, blood sampling and drug injection. Two days later, rats were injected with either saline or ßFNA , 2.5 μg icv. After a further 48 hrs, rats were treated with mμ-opioid receptor agonist, D-Ala^2,Me-Phe^4,Gly-ol^5-enkephalin (DAMPGO), 1 nmole icv. Blood pressure and heart rate were recorded throughout the experimental period, and blood samples were taken 5 min prior and 20 min after DAMPGO treatment. Restraint stress was imposed 20 min after DAMPGO. DAMPGO produced analgesia that was completely blocked by pretreatment with ßFNA. DAMPGO increased blood pressure and plasma catecholamines and evoked a small bradycardia. Pretreatment with ßFNA reversed the DAMPGO-induced bradycardia and exposed a tachycardia and significantly blunted the increase in plasma epinephrine with only a small attenuation of pressor response. However, ßFNA did not alter the pressor response to restraint in DAMPGO-treated rats. Thus, stimulation of mμ-opioid receptors activates both sympathetic and parasympathetic outflow. Mμ-Opioid receptors tonically activate parasympathetic outflow to the heart at rest and mediate partially the parasympathetic cardiac response to restraint stress. These data further support roles for mμ-opioid receptors in modulating heart rate and sympathoadrenal responses under basal conditions and in response to stress.

This work was supported by the University of Kentucky Tobacco and Health Research Institute and the Veterans Administeration.

110.6

VENTRAL PALLIDAL AND SUBSTANTIA INNOMINATA NEURONS RESPOND TO SYSTEMIC AND MICRO-IONTOPHORETIC MORPHINE TREATMENT.

J.J. Chrobak & T.C. Napier. Department of Pharmacology, Loyola University Chicago, Maywood, IL 60153.

The ventral pallidum (VP) and substantia innominata (SI) receive an extensive enkephalinergic projection from ventral striatal regions. The present study examined the response of spontaneous or glutamate-activated VP and SI neurons to systemic or microiontophoretic morphine treatment. Neurons were monitored *in vivo* using extracellular recording techniques in chloral hydrate anesthetized Sprague-Dawley rats. A dose-dependent (1-30mg/kg) suppression of firing rate was observed in 89% of VP/SI neurons tested with intravenous administration of morphine (ED_{50} = 7.3±1.2mg/kg) which was antagonized by naloxone (1mg/kg). Iontophoretic application of morphine (75mM;20-80nA) resulted in current-related suppressions (40% of cells tested) and excitations (30% of cells tested) which were attenuated by naloxone (75mM;10-40nA). The prominence of responding cells corroborates the density of enkephalinergic input to the VP and SI. The present findings demonstrate the sensitivity of VP and SI neurons to morphine treatment and provide evidence of the physiological function of opiates in the region. (Supported by DA05255 to TCN).

110.7

ENHANCED SEROTONERGIC TRANSMISSION MAY ATTENUATE ACTIVATION OF LOCUS COERULEUS (LC) BY OPIATE WITHDRAWAL

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Noradrenergic neurons of the nucleus LC in morphine-dependent rats are strongly activated by opiate withdrawal (OW). We have recently shown that such activation of LC neurons may be mediated by direct excitatory amino acid (EAA) input to LC (Akaoka et al., 1990). On the other hand, we have also shown that the excitation of LC neurons induced by iontophoretic application of glutamate is strongly attenuated by co-iontophoresis of 5HT (Aston-Jones et al., 1991). Here, LC neurons were recorded extracellularly to examine the effects of direct and indirect agonists of 5HT on the OW-induced activation of LC neurons in halothane anesthetized, morphine-dependent rats. These 5HT drugs were injected intravenously (IV) about 2 min following the induction of OW precipitated by IV injection of naloxone (NLX, 0.1 mg/kg).

Intravenous administration of the 5HT releaser, d-fenfluramine (2mg/kg), substantially reversed the LC hyperactivity induced by OW (n=2, 50 and 70% blockade). The same dose of d-fenfluramine only slightly decreased the spontaneous activity of LC neurons in naive rats (mean decrease -0.3 ± 0.3 Hz, n=3). Sertraline, a 5HT reuptake-blocker (3 mg/kg IV), also attenuated the LC hyperactivity, but with a slower time course. The mean firing rate of LC neurons sampled during the 10-30 min period following the induction of OW was significantly lower in sertraline treated than in non-treated rats (1.7 ± 0.2 Hz, n=4, and 3.4 ± 0.5 Hz, n=9, respectively; p<0.04). Fluoxetine, another 5HT uptake blocker, (4 mg/kg IV) yielded qualitatively similar results (2 ± 0.3 Hz during the 10-30 min post-OW period, n=3). The effects of the 3 indirect 5HT agonists tested may not be mediated by 5HT1A receptors as buspirone (1 and 4 mg/kg IV) and 8OHDPAT (3 mg/kg IV) did not attenuate the LC hyperactivity (n=3). Supported by US PHS grant DA 06214.

110.8

EFFECTS OF MU AND DELTA OPIOID RECEPTOR AGONISTS ON SYNAPTIC RESPONSES IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS USING CONVENTIONAL AND WHOLE-CELL INTRACELLULAR RECORDINGS.

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Opioid agonists are thought to produce excitation in the hippocampus by reducing inhibitory GABAergic influences on pyramidal cells. However, in previous reports we demonstrated that while selective μ (DAGO) and δ (DPDPE) opioid agonists increased population spikes recorded in CA1 in a similar manner, the δ agonist DPDPE seemed to do so without reductions in recurrent or feed-forward GABAergic inhibition (*Lupica et al., 1990, Soc. Neurosci. Abst. # 448.1; Lupica & Dunwiddie, Synapse, in press*). In a further effort to elucidate the mechanism(s) of opioid-induced excitation in the hippocampus the effects of DPDPE and DAGO on intracellularly recorded EPSPs and IPSPs were characterized. DPDPE, DAGO and the $GABA_A$ receptor antagonist bicuculline all increased EPSPs, while only DAGO and bicuculline reduced evoked IPSPs. It was, thus, hypothesized that μ and δ opioids reduce a "fast" GABA-mediated inhibition that occurs concurrently with the EPSP. Further evidence that the enhanced excitation produced by these opioids was related to a reduction in GABAergic input to pyramidal cells came from the observation that DPDPE, DAGO and bicuculline all reduced the frequency of spontaneously occurring IPSPs, recorded using high intracellular Cl^- concentrations and whole-cell electrodes. These findings indicate that opioids may enhance pyramidal cell excitability by reducing a fast $GABA_A$ receptor-mediated conductance that is not observed using standard evoked intracellular potentials. Supported by NIDA grant DA 02702 and the V.A. Medical Research Service.

110.9

ACTIVATION OF EAA PATHWAYS IN THE RAT LOCUS COERULEUS DURING ACUTE MORPHINE WITHDRAWAL: AN *IN VIVO* VOLTAMMETRIC STUDY.

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The increase in neuronal firing in the locus coeruleus (LC) which occurs during morphine withdrawal may involve activation of excitatory amino acid (EAA) inputs to this area. To examine this during acute morphine withdrawal, LC neuronal activity was measured using differential normal pulse voltammetry (DNPV). In all groups, morphine (10 μg icv) significantly reduced LC activity ranging from 50.6 ± 1.3 % to 54.3 ± 3.1% of baseline 45 min following morphine. A subsequent injection of naloxone (1 mg/kg iv) resulted in a significant increase in LC activity: peak increase of 145.4 ± 10.1% of baseline. This naloxone-induced increase above baseline was completely attenuated by pretreatment with the EAA antagonists, gamma-D-glutamylglycine (DGG) (2.0-200 μg icv) or (-)-2-amino-7-phosphonoheptanoic acid (D-APH) (25 μg icv). In the presence of these agents, the naloxone-induced change in LC activity ranged from 88.3 ± 6.0% to 104.9 ± 2.5% of baseline. These data suggest that activation of LC activity during naloxone-precipitated acute morphine withdrawal is mediated at least in part by activation of EAA pathways to the LC.

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110.10

ENHANCED cAMP RESPONSIVENESS OF LOCUS COERULEUS (LC) NEURONS IN OPIATE DEPENDENCE: SINGLE-UNIT RECORDINGS IN RAT BRAIN SLICES

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Both extrinsic and intrinsic mechanisms have been proposed to play a role in opiate tolerance and dependence in the LC. To investigate the involvement of intrinsic factors, extracellular, single-unit activity was recorded in noradrenergic neurons of the LC in brain slices prepared from rats chronically treated with morphine. In contrast to previous reports, the basal rates of firing were two-fold higher in LC neurons from dependent animals compared to controls and they remained elevated for at least 7 hours (n=350, p<0.01). The maximal excitatory response of LC neurons from dependent animals to 8-Br-cAMP was found to be substantially greater than the response of controls with no change in the EC_{50} (n=354, p<0.01). This result parallels biochemical evidence of an up-regulation of the cAMP pathway in the LC of dependent animals. Since an excitatory amino acid (EAA) input appears involved in withdrawal activation of LC neurons *in vivo*, the response to glutamate was tested. However, no change in the responsiveness to EAAs was observed (n=150). These results show that an intrinsic increase in basal firing rate may contribute to the activation of the LC during opiate withdrawal *in vivo* and that this increase in firing rate may be mediated by an up-regulation of the cAMP pathway.

110.11

THE DIRECT EFFECTS OF DYNORPHIN A ON ISOLATED RAT CEREBRAL ARTERIES J. Chen*, S.H. Graham, and A.I. Faden, Department of Neurology, University of California, San Francisco, CA94121

Opioid peptides have been shown to constrict pial arteries *in vivo,* but whether this represents a direct effect upon cerebral arteries is not known. The purpose of this study was to determine the direct effects of dynorphin A (DynA) related peptides on rat cerebral arteries. In 16 rats, the first branch of middle cerebral artery was isolated and mounted in a wire myograph perfused with oxygenated artificial CSF. ED50 of the peptides was defined as the dose required to produce half of the tension produced by 10^{-4}M phenylephedrine. DynA(1-17), a proposed endogenous κ-receptor agonist, directly constricted the isolated vessels (ED50=0.45±.07μM). The DynA(1-8) fragment had less vasoconstrictive activity (80±40μM). The selective κ agonist U50,488H (3.2±0.7μM) and the selective μ agonist (D-Ala^2, N-Me-Phe^4,Gly^5-OL)-enkephalin (4.2±1.7μM), were less potent vasoconstrictors than DynA(1-17). DynA(2-17), which does not bind to opioid receptors, produced vasoconstrision but was less potent than DynA(1-17) (2.8±1.2μM). Furthermore, nalmefene (10^{-5}M), an opioid antagonist, inhibited the effect of U50,488H, but not that of DynA(1-17). These results demonstrate that DynA directly constricts cerebral vessels and suggest that DynA's actions on cerebral arteries are not predominantly mediated by κ-opioid receptors.

110.12

FREQUENCY-DEPENDENT EFFECTS OF MU OPIOID RECEPTOR ANTAGONISTS ON HIPPOCAMPAL MOSSY FIBER-CA3 RESPONSES. B.E. Derrick and J.L. Martinez, Jr. Dept. of Psychology, University of California, Berkeley, CA 94720.

Mossy fiber long-term potentiation (MF LTP) requires both high frequency stimulation (J. Neurophys., 64:948, 1990) and mu opioid receptor activation (Soc. Neurosci. Abstr., 16:980, 1990). In the present study we found that the mu opioid receptor selective antagonist CTOP (Cys^2-Tyr^3-Orn^5-Pen^7 amide; 3 nmol) has no effect on mossy fiber-CA3 responses evoked in the rat in vivo at 0.033 Hz. However, CTOP produces a dose-dependent attenuation of both MF LTP and short-term potentiation (STP) measured 5 sec following high frequency stimulation (100 Hz). The delta receptor selective antagonist naltrindole (3 or 10 nmol) has no effect on either STP or MF LTP. These findings indicate that the effects of mu opioid receptor activation by endogenous opioid peptides are expressed only following high frequency stimulation. That this stimulation is essential for MF LTP induction may reflect a requirement for frequency-dependent activation of mu opioid receptors. Because STP involves primarily presynaptic mechanisms (J. Neurophys., 63:491, 1990), and because treatments that augment presynaptic calcium influx enhance STP (Brain Res., 459:192, 1988), mu opioid receptors may participate in MF LTP induction via actions that enhance calcium influx presynaptically.

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110.13

OPIOIDS ACT AT PRE- AND POSTSYNAPTIC SITES IN THE SUBSTANTIA GELATINOSA OF THE SPINAL TRIGEMINAL NUCLEUS IN RAT. T.J. Grudt and J.T. Williams, Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Intracellular recordings were made from neurons in slices of brainstem cut in the horizontal plane containing the substantia gelatinosa of the spinal trigeminal nucleus pars caudalis (Sp5c). [Met^5]enkephalin (ME) (3 μM) hyperpolarized 32 of 36 cells. Under voltage clamp ME caused an outward current at -60 mV that reversed polarity at the potassium equilibrium potential. Increasing the external potassium concentration shifted the reversal potential as predicted by the Nernst equation for an increase in conductance to potassium. The μ-opioid receptor agonist [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO) (30 nM to 3 μM) hyperpolarized 12 of 12 cells with an EC_{50} of 69 nM. Focal electrical stimulation of the spinal trigeminal tract rostral to the Sp5c evoked a fast excitatory post-synaptic potential (epsp) that was sensitive to CNQX (10 μM). ME (3 μM) (5 of 8 cells) and DAMGO (1 μM) (2 of 3 cells) reduced the amplitude of the epsp by 40-90%. These results suggest that opioids acting at μ-opioid receptors are inhibitory at both pre- and postsynaptic sites in the substantia gelatinosa. Supported by NIH grants DA04523 and MH45003.

110.14

KAPPA OPIOIDS DECREASE EXCITATORY TRANSMISSION IN THE DENTATE GYRUS OF THE GUINEA PIG. J.J. Wagner*, R.M. Caudle, and C. Chavkin, Dept. of Pharmacology, University of Washington, Seattle, WA 98195.

Kappa opioid binding sites were localized in the molecular layer of the dentate gyrus in the guinea pig hippocampus using the kappa$_1$ selective radioligand [^{3}H]-U69,593. The effects of kappa$_1$ receptor activation in this region of the hippocampus were identified using extracellular and intracellular recordings of dentate granule cell responses in the hippocampal slice preparation. U69,593 reduced the amplitude of the stimulation-evoked population spike with an EC_{50} of 23nM. Schild analysis was used to determine the affinity of the kappa$_1$ selective antagonist, nor-binaltorphimine. The K_i obtained (0.2nM), agreed with the apparent dissociation constant of nor-binaltorphimine (0.2nM), as measured by binding displacement curves. As with U69,593, dynorphin B, an endogenous opioid peptide that is present in the dentate gyrus, also inhibited the population spike response. Mu and delta selective opioid agonists had no effect on the evoked response. Intracellular recordings of dentate granule cells showed no direct effects of U69,593 on the granule cells themselves. However, analysis of synaptic potentials revealed that U69,593 significantly reduced the amplitude of EPSP's evoked by afferent stimulation without affecting IPSP amplitudes. The specific effect of U69,593 application on granule cell EPSP's indicates that presynaptic kappa$_1$ receptor activation inhibits glutamate release from perforant path terminals in the molecular layer of the dentate gyrus. These results suggest that endogenous dynorphins present in the granule cells may act as feedback inhibitors of the major excitatory input to the dentate gyrus. Supported by DA 04123, GM-07750, and BNS-9012656

110.15

ENDOGENOUS OPIOID REGULATION OF NOREPINEPHRINE RELEASE IN GUINEA PIG HIPPOCAMPUS. M.L. Simmons, J.J. Wagner*, R.M. Caudle, and C. Chavkin, Dept. of Pharmacology, University of Washington, Seattle, WA 98195.

Previous studies have shown that exogenously applied opioids regulate norepinephrine release in the hippocampus. In this report, a radioligand displacement assay method was used to investigate the regulation of norepinephrine release by endogenous opioids. Guinea pig hippocampal slices were incubated with [^{3}H]-propranolol and depolarized by focal electrical stimulation of the dentate gyrus molecular layer to release both endogenous norepinephrine and endogenous opioids. Endogenous norepinephrine release was detected by a decrease in specific [^{3}H]-propranolol binding, due to the displacement of radioligand from ß-adrenergic receptors. Stimulation of adrenergic fibers caused a decrease in specific [^{3}H]-propranolol binding to 38% of the unstimulated control, and this effect was calcium dependent. In the presence of 1 μM PLO17, a mu opioid agonist, the stimulus-induced reduction in specific [^{3}H]-propranolol binding was attenuated, suggesting that PLO17 inhibited the release of endogenous norepinephrine. Additionally, stimulation-induced norepinephrine release was attenuated by endogenously released opioid peptides in the presence of peptidase inhibitors. The effects of both PLO17 and endogenously released opioids were blocked by naloxone. These results indicate that endogenous opioids can act to inhibit the release of endogenous norepinephrine in guinea pig hippocampus. Supported by DA 04123 and GM07266

110.16

SEXUAL DIFFERENCES IN KAPPA OPIOID RECEPTOR-MEDIATED REGULATION OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS. E.J. Wagner, J. Manzanares, S.D. LaVigne*, K.J. Lookingland and K.E. Moore. Dept. of Pharm. & Tox., Michigan State Univ., East Lansing, MI 48824.

The effects of kappa receptor blockade and activation on the activity of tuberoinfundibular dopaminergic (TIDA) neurons were examined. The basal activity of TIDA neurons (accumulation of 3,4-dihydroxyphenylalanine [DOPA] in the median eminence) was 2-3 times higher in female rats than in males. Following administration of the kappa antagonist nor-binaltorphimine (NOR-BNI), male rats exhibited a dose-related increase in TIDA neuronal activity. NOR-BNI had no effect on TIDA neuronal activity in intact diestrous female rats, but increased TIDA neuronal activity in ovariectomized females. In diestrous female rats, administration of the kappa agonist U-50,488 caused a dose-related decrease in TIDA neuronal activity. U-50,488 had no effect on TIDA neuronal activity in intact male rats, but decreased TIDA neuronal activity in orchidectomized males. These results suggest that gonadal steroids influence TIDA neuronal activity by altering tonic inhibition of these neurons by endogenous kappa opioids (supported by ADAMHA grant MH 42802).

110.17

SUCROSE DRINKING REDUCES DORSAL HYPOTHALAMIC β-ENDORPHIN LEVELS IN SHR BUT NOT IN WKY. T. Zhang and R. W. Rockhold. Dept. of Pharmacol. and Toxicol., Univ. of Miss. Med. Ctr., Jackson, MS.

The present study was performed to test whether drinking of a sucrose solution (S; 10%) would preferentially alter cardiovascular function in the spontaneously hypertensive rat (SHR) and if this effect correlated with changes in hypothalamic β-endorphin, a putative mediator of diet-induced changes in cardiovascular sympathetic nervous tone. Male SHR and Wistar-Kyoto (WKY) rats (12 wks of age) were trained to drink total 24 hr water consumption between 9-11 a.m. Catheters were implanted to record arterial blood pressure (BP) and heart rate (HR). Rats consumed 8 ml of either S or deionized (DI) water during a 10 min test period. Blood was removed for plasma glucose analysis 10 min before and at the end of test period. Rats were sacrificed and hypothalami removed for analysis of β-endorphin content (BE). Comparable increases in BP were noted in SHR and WKY during either S or DI drinking. Drinking-induced tachycardia was blunted in SHR-S. Plasma glucose levels were significantly elevated ($p < 0.05$) following S, but not DI. Dorsal, but not ventral, hypothalamic βE was reduced ($p < 0.05$) following S in SHR only. The S-induced changes in βE do not appear to correlate with altered BP in SHR or WKY. The results are consistent with exaggerated stimulation of βE release in the dorsal hypothalamus following S in SHR. (Supported by HL 39387 and the AHA-Mississippi Affiliate.)

110.18

THE ACTIVATION OF FUNCTIONAL AND STRUCTURAL HOMEOSTATIC PROCESSES BY SELECTIVE UNINVASIVE TRANSCRANIAL ELECTRO-STIMULATION (TES) OF BRAIN STEM OPIOID STRUCTURES (BSOS). V.P.Lebedev,Ya.S.Katznelson*,A.V.Krasjukov*,O.B.Illyinski*, A.V.Lebedeva* Pavlov Inst.of Physiol.,Leningrad,199034, USSR, Neuro Systems,Inc.,Dallas,TX,75238,USA

In screening experiments on different species of mammals the optimal regime of TES of BSOS was determined.TES with these parameters as demonstrated by [3-H]-2-Deoxy-glucose uptake selectively activated the BSOS. TES produced several central effects, namely deep analgesia and stabilization of bulbar vasomotor control. Emotional pain reactions, motor and autonomic nociceptive reflexes were inhibited or abolished by TES.The peripheral effects included the acceleration of repair processes in epithelium, connective tissue and in damaged peripheral nerve fibers. The obvious increase of immunity and some antitumor effect of TES were demonstrated in connection with activation of NK-cells and other phagocytic lymphocytes.The following data on the opioid nature of TES effects were obtained: the reversibility by naloxone, considerable increase of opioid neuropeptides level in plasma and cerebrospinal fluid, the potentiation by enkephalinase inhibitor, cross-tolerance with morphine analgesic effect. Clinical efficacy of our method of TES in anesthesiology, neurology and internal medicine coincided with experimental results obtained.

110.19

OPPOSITE EFFECTS OF OPIATE-RELATED AND NON-OPIATE-RELATED SIGMA LIGANDS ON BURST FIRING IN RUBRAL NEURONS. R.R. Matsumoto, L.N. Eisenman, W.D. Bowen* and J.C. Houk. Northwestern Univ. Med. Sch., Chicago, IL 60611 and *Brown Univ., Providence, RI 02912.

Bursts of red nucleus (RN) activity are associated with limb movements in behaving monkeys, cats, and turtles. Stimulation of the spinal cord or cerebellar cortex of the *in vitro* turtle brain also elicits bursts of RN activity, which can be used as a neurophysiological correlate of movement. The effects of sigma ligands on these burst responses were tested by bath-applying various concentrations (0.1 to 100 μM) of opiate-related [dextrallorphan (DEX), (+)-pentazocine (PENT)] and non-opiate-related [DTG, haloperidol (HAL)] sigma ligands while recording extracellularly (N=73) from the RN of the *in vitro* turtle brain. DTG and HAL increased the duration of RN bursts whereas PENT and DEX decreased the duration of the bursts. All of the effects were dose-dependent and a Student's t-test showed that the effects of the opiate-related sigma ligands differed significantly from those of the non-opiate-related compounds ($P<0.001$). Although the compounds affected the burst response in RN neurons, they had no consistent effect on spontaneous firing rate, suggesting a neuromodulatory role for the ligands. Scatchard and competition studies confirmed that sigma receptors labelled with 3H-DTG were present in the turtle brain and that all of the compounds used in this study displaced 3H-DTG binding. These data suggest that opiate-related and non-opiate-related sigma ligands affect brainstem motor circuits through different populations of sites and that activation of these sites may have different functional ramifications.

CATECHOLAMINES: HORMONAL REGULATION

111.1

SEXUAL DIFFERENCES IN THE RESPONSIVENESS OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS TO CENTRAL ADMINISTRATION OF BOMBESIN. J. Manzanares, T.W. Toney, K.J. Lookingland and K.E. Moore. Dept. Pharmacology & Toxicology, Michigan State University, East Lansing, MI 48824.

The purpose of the present study was to examine the effects of bombesin on the activity of tuberoinfundibular (TIDA) and tuberohypophysial dopaminergic (THDA) neurons, and the secretion of prolactin (PRL) and α-melanocyte stimulating hormone (αMSH) in gonadally-intact and castrated male and female rats. TIDA and THDA neuronal activity was estimated by measuring the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the median eminence and intermediate lobe of the pituitary, respectively. Bombesin (10 ng/rat; icv; 30 min) decreased pituitary secretion of PRL in both male and female rats, and this was accompanied by an increase in the activity of TIDA neurons in male, but not female rats. Orchidectomy increased TIDA neuronal activity in male rats, but failed to alter the ability of bombesin to increase in the activity of these neurons. On the other hand, ovariectomy decreased the activity of TIDA neurons in female rats and rendered these neurons responsive to the stimulatory actions of bombesin. Taken together, these results suggest that sexual differences in the responsiveness of TIDA neurons to central administration of bombesin are due to the presence of ovarian hormones. In contrast, bombesin increased the activity of THDA neurons and decreased αMSH secretion in both gonadally-intact and castrated male and female rats, indicating that gonadal steroids have no effect on the responsiveness of THDA neurons to bombesin. (Supported by NIH grant NS 15911 and ADAMHA grant MH 42802).

111.2

SEX DIFFERENCES IN STRIATAL DOPAMINE UPTAKE AND BASAL EXTRACELLULAR DOPAMINE CONENTRATIONS? S. A. Castner and J. B. Becker. Reproductive Sciences Program & Department of Psychology, The University of Michigan, Ann Arbor, Michigan 48104-1687.

The nigrostriatal dopamine (DA) system is sexually dimorphic. There are sex differences in both DA-mediated behaviors and the neurochemistry of the striatum. For example, there are sex-related and estrous cycle-dependent differences in amphetamine (AMPH)-induced rotational behavior and AMPH-stimulated striatal DA release. In addition, last year we reported a sex difference, independent of concurrent gonadal hormones, in basal extracellular striatal DA concentrations as assessed by *in vivo* microdialysis. Castrated (CAST) male rats had significantly higher basal extracellular striatal DA concentrations than ovariectomized (OVX) female rats. The present experiments were designed to determine if the sex difference in basal extracellular DA concentrations is due to a difference in striatal DA uptake.

Adult male and female rats were CAST or OVX for at least 1 month prior to experimental use. In the first experiment, the time course of (3H) DA uptake in striatal synaptosomes from CAST male and OVX female rats was determined. In the second experiment, dialysis probes were inserted through chronic guide cannulae aimed at the striatum. On the day of dialysis,12 to 18 hours after dialysis probe insertion, striatal dialysate samples were collected at 15 min. intervals following the sequential administration of increasing (.1-100) nM concentrations of DA through the dialysis probe. Percent DA recovered in dialysate was determined by high performance liquid chromatography with electrochemical detection. Results from these studies will be presented at the meeting. [Supported by USPHSNS25662 & BNS9021966 to JBB and 5T32HD07048-15)

111.3

SEX DIFFERENCES IN STRIATAL DOPAMINE RECEPTOR BINDING. J.B. Becker[1], T. Bazzett[1], & R. L. Albin[2].
[1]Psychology Dept, Neuroscience Prgm, and Reproductive Sciences Prgm, [2]Neurology Dept and Neuroscience Prgm, Univ. Michigan, Ann Arbor, MI 48104.

In female rats the ovarian hormones estrogen and progesterone modulate striatal dopamine (DA) release, striatal DA receptor binding, and DA-mediated behaviors. By contrast, in males estrogen does not affect striatal DA release and effects of estrogen on striatal DA receptor binding have been reported only after very high doses. This experiment was conducted to determine if there are sex differences in the effects of physiological doses of ovarian hormones on striatal DA receptor binding.

Adult male and female rats were castrated (CAST) or ovariectomized (OVX). Two weeks later animals received injections (s.c.) of: 1) peanut oil X 4 days; 2) 2 µg estradiol benzoate (EB)/100 g X 3 days, then 0.48 mg progesterone (P)/100 g 4 hr prior to use; or 3) peanut oil X 3 days, then 2 µg EB/100 g 30 min prior to use. Specific binding to [3H]SCH23390 and [3H]spiperone were determined in fresh frozen horizontal sections using quantitative autoradiography for D1 and D2 DA receptors.

Striatal binding (pmole/mg protein) to the D1 DA receptor antagonist [3H]SCH23390 was decreased by approximately 12% 30 min after EB in striatum of OVX ($p<0.011$). There was no effect of EB in CAST, nor was there an effect of EB + P in either sex. OVX and CAST animals receiving oil were not different, but D1 binding in the lateral striatum was lower in OVX than in CAST 30 min after EB ($p<0.04$). Experiments are in progress to determine whether these effects are due to changes in D1 DA receptor densisty or affinity. Results of binding to the D2 DA receptor antagonist will also be reported. These results demonstrate that the rapid down-regulation of striatal D1 DA receptor binding by EB is sexually dimorphic.

Supported by NS25662, BNS9021966, the Hereditary Disease Foundation, and Reproductive Sciences Training Grant HD07048.

111.4

THE EFFECT OF CASTRATION UPON NOREPINEPHRINE RELEASE FROM SUPERFUSED OLFACTORY BULB OF MALE RAT. X.-B. Guan, and D.E. Dluzen. Department of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

In order to investigate the possible relationship among the olfactory bulb (OB), norepinephrine (NE) and gonadal steroids, we measured basal and potassium stimulated (K^+-30mM) NE release from superfused anterior and posterior OB in intact and castrated male rats (Experiment I) as well as in castrated male rats implanted with either empty or testosterone filled silastic capsules (Experiment II). Both basal (pg/mg/min) and K^+-stimulated (area under curves-pg/50 min) release of NE was greater in posterior OB compared to anterior OB in Exp. I. (mean±SEM, basal release: anterior OB = 0.21±0.03, posterior OB = 0.29±0.04; K^+-stimulated release: anterior OB =1.45±0.16, posterior OB = 2.34±0.27, $n=18$, $p<0.05$). All groups were responsive to the 30 mM KCl stimuli showing increases in NE release. The degree of K^+-stimulated release was significantly greater in intact (mean±SEM = 2.31±0.22, $n=20$) compared to that of castrated rats (mean±SEM = 1.38±0.21, $n=16$, $P<0.01$). No differences in K^+-stimulated release were observed between castrated and castrated plus testosterone treated groups. These results demonstrate that castration of male rats significantly reduces OB noradrenergic responsiveness of K^+ stimulation, an effect which was not restored following administration of silastic capsules containing testosterone.

111.5

PUBERTAL-RELATED NOREPINEPHRINE UTILIZATION IN THE RAT HYPOTHALAMUS SuJean Choi and Carol K. Kellogg. Dept. of Psychology, University of Rochester, Rochester, N.Y. 14627.

Previous studies have indicated that at 28 days of age hypothalamic (HYP) norepinephrine (NE) turnover in rats is one third the adult rate, while in the cortex the NE turnover rate is at adult levels. To investigate this age related influence on NE utilization, catecholamine turnover was measured in male Long Evans rats at 28, 42, and 70 days of age. NE levels were measured at 15, 30, 45, 60, 120, and 240 min after inhibition of synthesis using alpha-methyl-p-tyrosine (MT; 250 mg/kg). NE levels decreased linearly over time in 28 and 70 day-old animals, with a greater rate of NE utilization in 70 day-olds. The pattern of NE utilization was markedly different at 42 days of age (midpuberty). NE levels decreased slightly over the first 45 min after inhibition (14% decrease). At 60 min, however, NE levels were 50% of basal levels, after which there was no further decrease. To estimate the degree of inhibition of tyrosine hydroxylase (TH) achieved by MT, DOPA accumulation was measured 30 min following inhibition of the decarboxylase enzyme with NSD-1015 (100 mg/kg). At both 28 and 42 days of age, DOPA accumulation decreased 70% 45 min after treatment with MT. Hence, the limited decrease in NE levels at 45 min in 42 day old rats treated with MT does not appear to be due to reduced inhibition of TH. Studies measuring the *in vitro* conversion of ^{3}H-tyrosine to ^{3}H-NE are underway to investigate the differential contributions of newly synthesized vs. stored NE to NE utilization at 28, 42, and 70 days of age. In summary, NE utilization is marked by a unique pattern in 42 day olds as compared to 28 and 70 day old animals. Grant No DA07080

111.6

NEUROENDOCRINE EFFECTS ON ADAPTATION OF NA-K ATPASE TO NOREPINEPHRINE DEPLETION. A.C. Swann, Dept. of Psychiatry, Univ. of Texas Med. Sch., Houston TX 77225.

Depletion of norepinephrine (NE) reduces brain Na,K-ATPase, but the enzyme recovers over time. We have investigated neuroendocrine effects on this adaptation. NE depletion was produced by injection of DSP4 (50 mg/kg ip) and verified by assay of NE in cerebral cortex. Most of the experiments described were also duplicated in rats receving lesions of the dorsal noradrenergic bundle. The number of Na,K-ATPase sites in cerebral cortex was reduced 25% by 2 wk after DSP4 but had returned to normal, or was even slightly increased, by six weeks. Thyroid hormone increases Na,K-ATPase in non-CNS tissues and is involved in the development of brain Na,K-ATPAse, but neither thyroid hormone nor experimental hypothyroidism altered the adaptation of Na,K-ATPase to depletion of NE. Dexamethasone prevented the reduction in Na,K-ATPase 2 wk after DSP4 but had no effect on the late recovery of enzyme activity. Adrenalectomy had no effect on Na,K-ATPase two weeks after DSP4, but adrenalectomized rats had a marked increase (40%) in Na,K-ATPase 8 weeks after DSP4. Intraventricular infusion of corticotropin releasing factor (CRF) also increased Na,K-ATPase after DSP4, but the effect was less than that of adrenalectomy. These results suggest that the hypothalamic-pituitary axis, particularly CRF, may be involved in the adaptation of Na,K-ATPase to depletion of NE.

111.7

CATECHOLAMINERGIC INNERVATION OF AROMATASE AND ESTROGEN RECEPTOR-IMMUNOREACTIVE CELLS IN THE QUAIL BRAIN. T. Bailhache*, A. Foidart, C. Surlemont*, N. Harada*, J. Balthazart.
Lab. Biochemistry, Univ. Liège, B-4020 Liège, Belgium and Molec. Genetics, Fujita Health Univ., Toyoake, Aichi 470-11, Japan.

Steroids, in particular estrogens modulate the activity of catecholaminergic systems in the brain. It has also been shown that catecholamines have direct effects on steroid-sensitive systems and namely modify the concentration of estrogen receptors (ER) and the activity of aromatase (ARO), the enzyme catalyzing estrogen synthesis in the brain. In quail, we recently showed that a noradrenergic denervation produced by the specific neurotoxin, DSP4 increases preoptic aromatase activity, suggesting that the noradrenergic system exerts a tonic inhibition on this enzyme activity (Brain Res. 1989, 492:163-175). We have now analyzed the morphological relationships between catecholaminergic innervation and estrogen-sensitive and aromatase containing cells using double label immunocytochemical procedures (see J. Neurobiol. 1991, 22:143-157 for the general procedure). The dopamine β-hydroxylase (DBH) and tyrosine hydroxylase (TH) fibers were labelled using commercial antibodies (Incstar), ER were identified by the monoclonal antibody H222 (Abbott) and finally ARO-immunoreactive (ARO-ir) cells were stained with a polyclonal antibody raised against human placental ARO and purified by affinity chromatography (J. Biochem.1988, 103:106-113; J. Comp. Neurol. 1990, 301:276-288). TH and DBH punctate structures were found in close association with ARO-ir cell bodies in all brain regions where these are present namely the medial preoptic nucleus, the ventral septum, the bed nucleus of the striae terminalis and the ventro-medial and tuberal hypothalamus. DBH fibers and punctate structures were also found in association with ER-containing cells throughout the hypothalamus, in the nucleus intercollicularis, the nucleus taeniae and the septum. Many cell bodies in the hypothalamus and septum were in addition completely surrounded by TH and/or DBH punctate structures but were not immunoreactive for ER or ARO. The punctate structures suggesting direct innervation of ARO-ir and ER-ir cells might represent the anatomical substrate for the regulation by catecholamines of aromatase activity and/or ER concentration.
Supported by NIH HD22064, FRFC (2.9003.91, 9.4601.90) and EEC (SC1-0230-C(TT).

111.8

STRESS AND β-CARBOLINES STIMULATE CATECHOLAMINE RELEASE IN RAT MEDIAL PREFRONTAL CORTEX, ASSESSED BY MICRODIALYSIS.

Deidra S. Atkins*, Jan Lavicky*, Xiao-Min Yang, R. Don Brown, and Adrian J. Dunn. Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Various stressful treatments increase the metabolism of catecholamines and serotonin (5-HT) in many brain regions. Norepinephrine (NE), and perhaps also dopamine (DA) and 5-HT appears to play an important role in the etiology of anxiety. Benzodiazepines (BZD) are the prototypical anxiolytic drugs. By contrast, inverse BZD agonists are anxiogenic. We have studied the effects of stress and benzodiazepines on extracellular concentrations of catecholamines, serotonin and catabolites in the prefrontal medial cortex (PFM) of freely moving rats using microdialysis. The inverse BZD agonist, FG 7142 (15 mg/kg i.p.) increased concentrations of NE and DA in the dialysate, as well as those of the catabolites 3-methoxy,4-hydroxyphenylglycol (MHPG) and dihydroxyphenylacetic acid (DOPAC). These effects were prevented by pretreatment with the BZD antagonist, Ro 15-1788 (20 mg/kg i.p.). Rats preconditioned to electric footshock, also showed increased dialysate concentrations of catecholamines and catabolites. The responses to stress were also studied in BZD-pretreated rats. The results suggest some interactions of benzodiazepines with cerebral catecholaminergic systems during stress.

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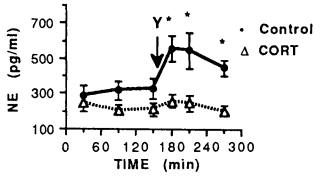
111.9

GLUCOCORTICOIDS INHIBIT YOHIMBINE-INDUCED RELEASE OF CATECHOLS IN POSTEROLATERAL HYPOTHALAMUS. K. Pacak*, I. Armando*, S. Komoly*, I. J. Kopin, D. S. Goldstein*. Clinical Neuroscience Branch and Laboratory of Experimental Neuropathology, NINDS, NIH, Bethesda, MD 20892.

Systemically administered yohimbine (YOH) increases sympathetic outflow and plasma levels of norepinephrine (NE); chronic hypercortisolemia markedly attenuates plasma NE responses to YOH. The present study assessed whether hypercortisolemia affects YOH-induced release of catechols in the brain--in particular, in the posterolateral hypothalamus (PLH), a region that participates in the regulation of the sympathetic outflow. Cortisol (CORT, 25 mg/kg/day) was infused s.c. for 7 days via osmotic minipumps, and release of endogenous catechols in the PLH was assessed in conscious, freely moving rats by in vivo microdialysis. Extracellular fluid concentrations of NE, its metabolites dihydroxyphenylglycol (DHPG) and methoxyhydroxyphenylglycol (MHPG), and the dopamine metabolite dihydroxyphenylacetic acid (DOPAC) were measured before and during YOH administration (1 mg/kg i.v.). Microdialysate collection (30-minute periods, 1 µl/min) began 24 hours after probe implantation. YOH markedly increased NE (Figure), DHPG, MHPG, and DOPAC levels in controls, whereas in CORT-treated animals the responses were significantly reduced. These results are consistent with the view that CORT treatment suppresses central noradrenergic mechanisms that contribute to sympathetic outflow.



111.10

NERVE GROWTH FACTOR ALTERS DOPAMINE UPTAKE KINETICS IN PC12 CELLS: RELATIONSHIP TO 2'ET-MPTP-INDUCED TOXICITY. H.M. Geller[1], A.N. Basma*, M.S. Saporito, R.E. Heikkila and W.J. Nicklas, Dept. of Neurology and [1]Pharmacology, UMDNJ-RWJ Med. School, Piscataway, NJ 08854.

The oxidative metabolite of MPTP, MPP^+, is a potent dopaminergic neurotoxin. MPP^+ and analogs such as 2'Et-MPP^+, are actively accumulated in mitochondria and inhibit NADH dehydrogenase of complex I, ultimately resulting in cell death. PC12 cells are a catecholamine-containing cell line which are neoplastic in nature and have a high rate of glycolysis accompanied by a large production of lactate and a low utilization of glucose carbon through the Krebs cycle. It has been reported that NGF-treated PC12 cells exhibited a marked increase in glucose utilization/energy consumption via the Krebs cycle to support the changes in morphology caused by the growth factor. If inhibition of mitochondrial respiration is indeed the mechanism of toxicity of 2'Et-MPP^+, then NGF treatment would be expected to enhance the toxicity of 2'Et-MPTP in PC12 cells. However, toxicity, measured by counting live cells following exposure to the neurotoxin was similar in both untreated and NGF-treated cells. Other parameters, such as uptake of the pyridinium were assessed. NGF treatment decreased the uptake capability of the cells significantly. For example, the V_{max} measured for uptake of dopamine in either the untreated or NGF-treated cells were 1409 ± 399 or 552 ± 94 fmol/mg protein/min, respectively. These results suggest that the change in morphology induced by NGF is accompanied by alterations in the dynamics of the DA uptake system which may make NGF-treated PC12 cells less susceptible to the neurotoxic actions of 2'Et-MPP^+. Supported by NIH NS 21469.

CATECHOLAMINES: ASCORBIC ACID

112.1

ASCORBIC ACID AS AN ADJUNCT TO NEUROLEPTIC THERAPY IN TREATMENT RESISTANT SCHIZOPHRENICS. L. K. White, J. Chiles* and G. V. Rebec, Dept. of Psychology, Indiana University, Bloomington, IN 47405

Several distinct lines of evidence indicate that abscorbate (AA) serves as a modulator of dopamine transmission in the mammalian brain (White et al., Pharm. Biochem. & Behav. 36:485, 1990). In animals, AA, when administered peripherally or infused directly into the neostriatum attenuates the behavioral response to amphetamine and potentiates the anti-amphetamine effects of haloperidol, a widely used neuroleptic drug. To the extent that these data are relevant for the treatment of schizophrenia, AA may potentiate the therapeutic effects of neuroleptics in a clinical population. To test this hypothesis, 30 schizophrenic inpatients maintained on standard neuroleptic therapy received 4-gram supplements of AA for 4 weeks followed by 4 weeks of placebo, with an intervening 3-week washout period. Psychiatric symptoms and extrapyramidal side effects were monitored weekly, along with daily patient behaviors. Although AA produced no consistent effects on positive psychotic symptoms, AA was associated with a decrease in irritability and agitation in a subgroup of patients.

112.2

IN VIVO VOLTAMMETRY IN FREELY MOVING RATS: DOPAMINE AGONISTS DECREASE EXTRACELLULAR ASCORBATE IN MEDIAL PREFRONTAL CORTEX. M.E. Taylor, R.C. Pierce, G.V. Rebec. Prog. Neural Science, Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Ascorbate (AA), which appears to modulate dopamine (DA)-mediated behaviors, increases markedly in neostriatum but not in nucleus accumbens in response to amphetamine or other DA agonists (Pierce & Rebec, Eur. J. Pharmacol. 191:295, 1990; Rebec et al., Soc. Neurosci. Abstr. 16:131, 1990). These results suggest that AA release mechanisms are not uniform in DA-rich forebrain areas. We extended this research to medial prefrontal cortex (mPFC), which like nucleus accumbens receives DA input from the ventral tegmental area. Electrochemically modified carbon-fiber electrodes were positioned in mPFC of awake, behaving rats that subsequently received either d-amphetamine (1.0-2.5 mg/kg) or the combination of SKF-38393 (10.0 mg/kg) and quinpirole (1.0 mg/kg), selective D1 and D2 agonists, respectively. Relative to saline controls, both amphetamine and the D1-D2 combination significantly lowered extracellular AA in mPFC. The drug-induced decline in AA typically ranged between 20-40% of the pre-injection baseline level. These results contrast with data obtained from neostriatum and nucleus accumbens and suggest that dopaminergic mechanisms do not exert uniform effects on AA release in different forebrain regions.

Supported by NSF grant BNS 87-11240.

112.3

INTRANEOSTRIATAL ACETYLCHOLINE AND GLUTAMATE, BUT NOT AMPHETAMINE OR DOPAMINE, INCREASE NEOSTRIATAL ASCORBATE LEVELS. R. C. Pierce, P.E. Langley* and G.V. Rebec, Prog. Neural Science, Dept. Psychology, Indiana Univ., Bloomington, IN 47405.

Ascorbate (AA), an important modulator of neostriatal function, increases markedly in the extracellular fluid of the neostriatum following systemic injection of compounds known to enhance acetylcholine, dopamine, or glutamate transmission. To assess the central mechanisms underlying this effect, we infused (0.33 µl/min) acetylcholine (50 µg/µl), dopamine (2 µg/µl) or glutamate (30 µg/µl) directly into the neostriatum of freely moving rats and simultaneously monitored AA release in this site via voltammetry. Relative to control infusions, both acetylcholine and glutamate significantly elevated neostriatal AA, but dopamine was without effect. In fact, intraneostriatal amphetamine (30 µg/µl) also failed to alter AA, though systemic injections of this drug nearly double AA levels in the neostriatum (Pierce & Rebec, Eur. J. Pharmacol. 191:295, 1990). Thus, neostriatal AA is not regulated by intrinsic dopaminergic mechanisms but may be controlled instead by cholinergic and glutaminergic neuronal systems.

Supported by NSF grant BNS 87-11240.

112.4

UNILATERAL NEOSTRIATAL KAINATE, BUT NOT 6-OHDA LESIONS BLOCK DOPAMINE AGONIST-INDUCED ASCORBATE RELEASE IN THE NEOSTRIATUM OF FREELY-MOVING RATS. D. W. Miller, R. C. Pierce, D. B. Reising, and G. V. Rebec. Prog. in Neural Science, Dept. Psychology, Indiana Univ., Bloomington, IN 47405.

Neostriatal ascorbate (AA), which is known to modulate dopamine-mediated behavior, increases following dopamine (DA) agonist treatment (Pierce & Rebec, Eur. J. Pharmacol., 1990, 191:295). In the present study, unilateral neostriatal kainate (KA) and 6-hydroxydopamine (6-OHDA) lesions were used to assess the role of the striatonigral and nigrostriatal pathways, respectively, on DA agonist-induced neostriatal AA release. Following a post-surgery recovery period (7-10 days), the subjects were challenged with either direct (a combination of 10.0 mg/kg SKF-38393 and 1.0 mg/kg quinpirole) or one of two indirect (2.5 mg/kg d-amphetamine or 20.0 mg/kg GBR-12909) DA agonists. Voltammetric recordings with electrochemically modified, carbon-fiber electrodes revealed that, relative to controls, unilateral neostriatal KA lesions abolished the ability of direct and indirect DA agonists to induce neostriatal AA release. In KA lesioned rats, AA levels decreased 20-60% following systemic drug administration. Histological analysis of the KA lesions indicated substantial gliosis in approximately 60-80% of the striatum. Conversely, 6-OHDA lesions (>95% depletion) of the nigrostriatal pathway had no effect on DA agonist-induced neostriatal AA release. These results suggest that the striatonigral, but not the nigrostriatal pathway is necessary for DA agonist- induced AA release.

Supported by NSF BNS 87-11240.

112.5

NEUROTOXIC EFFECTS OF ASCORBIC ACID ON HIPPOCAMPAL NEURONS IN CULTURE. M. B. Ghasemzadeh and M. A. Dichter. Depts. of Pharmacology and Neurology, U. of Pennsylvania School of Medicine, Philadelphia PA 19104.

Ascorbic acid (AA) is present at high concentrations (1-3 mM) in mammalian brain (ECF = 200-500 uM in rat brain); however, its significance in CNS functioning is still poorly understood. In rat fetal brain AA content almost doubles during the last week of gestation and thereafter is gradually reduced to adult levels. AA is an important biological antioxidant and free redical scavenger; although, it also possesses a paradoxical ability to act as a pro-oxidant. It has been proposed that lipid peroxidation and free radicals play a role in ischemic brain damage. Recently, it was shown that brain trauma and focal ischemia lead to a dramatic increase of extracellular AA. Given the results of the above studies, we decided to examine the effect of ascorbic acid on rat hippocampal neurons grown in primary dissociated cell culture.

A concentration dependent toxicity of AA was observed. Ascorbate at $\leq$200 uM did not exhibit any toxicity. Higher concentrations of AA produced cell death within 24 hours, with complete cell loss at $\geq$1 mM. The neurotoxic effect could be prevented by addition of catalase, a hydrogen peroxide scavenger. Inhibition of lipid peroxidation was also able to counteract the AA effect. Surprisingly, AA at the highest concentration tested (6 mM) was not cytotoxic to glial cells. These results indicate that AA produces profound neurotoxic effects on hippocampal neurons in culture which are mediated through production of hydrogen peroxide and membrane lipid peroxidation. Therefore, release of AA during trauma and ischemic episodes may contribute to neuronal degeneration and brain damage.

112.6

ASCORBIC ACID REGULATES TYROSINE HYDROXYLASE AND DOPAMINE LEVELS IN MESENCEPHALIC CULTURES. H.H. Kalir and C. Mytilineou, Dept. of Neurology, Mt. Sinai Sch. of Med., New York, N.Y. 10029.

Embryonic brain cells rapidly lose their ascorbic acid (AA) content when placed in culture, unless AA is supplied with the feeding medium (Kalir and Mytilineou, 1991). We have investigated the acute and long term effects of AA on the levels of dopamine (DA) and tyrosine hydroxylase (TH) immunocytochemistry in mesencephalic cultures prepared from E14 rat embryos and maintained in serum containing medium in the presence or absence of 200uM AA. After 12 days in vitro AA-treated cultures had DA levels 5-fold higher than controls and 10 times the number of TH+ neurons. The TH+ neurons in the AA-treated cultures were also more darkly stained and had more prominent processes than in the controls. When control cultures were treated with AA for 24 hrs, the levels of DA increased by 34% and the number of neurons that could be visualized by TH immunocytochemistry increased more than 3-fold. The intensity of the staining was also increased. Conversely, when AA was removed from AA-treated cultures, both DA levels and the number of TH+ neurons were reduced by about 50%. The staining intensity of individual neurons was also reduced after AA removal. The changes in the number of TH+ neurons and staining intensity after 24 hr exposure or removal of AA suggest a change in the amount of enzyme molecules rather than a change in the number of DA neurons. Taken together our results indicate that the levels of TH in the DA neurons are regulated by AA and that the increased levels of DA in the AA-treated cultures could result from increased enzyme levels. Supported by NIH grant NS-23017 and by the United Parkinson Foundation.

112.7

CHOLINERGIC AND GLUTAMATERGIC MODULATION OF AMPHETAMINE-INDUCED INCREASES IN ASCORBIC ACID IN RAT CAUDATE NUCLEUS. P. Kunko, K. Mueller and R. Saponjic. Dept. of Psych., Texas Christian Univ., Ft. Worth, TX 76129.

Ascorbic acid (AA) levels in the rat caudate nucleus are increased by low-dose amphetamine. However, AA levels may not be directly related to DAergic neurotransmission. Amphetamine also increases cholinergic and glutamatergic activity in the caudate. Therefore we tested the ability of scopolamine and MK-801 to block amphetamine-induced increases in AA in the rat caudate.

AA levels were measured in adult male albino rats by semidifferential linear sweep voltammetry. Locomotion was defined as line crossovers in the testing cage. One group of rats received scopolamine (0.6mg/kg,ip) and amphetamine (1.5mg/kg,sc). Three other groups received MK-801 (0.05,0.1, or 0.5mg/kg, ip) and amphetamine (1.5mg/kg, sc). Scopolamine reduced the amphetamine-induced AA increase by 50% while increasing crossovers. MK-801 reduced amphetamine-induced AA increases but did not alter crossovers.

AA levels and locomotor behavior appear to be mediated by different amphetamine-sensitive substrates.

112.8

RESTRAINT INCREASES RELEASE OF ASCORBIC AND URIC ACID IN CINGULATE CORTEX OF RAT. K. Mueller, Dept. Psych. TX Chrst. Univ., Ft. Worth TX 76129

Ascorbic acid (vitamin C) is plentiful in brain and is released into the extracellular space in response to a variety of stimuli. Ascorbic acid (AA) also modulates neuronal functions; thus AA may play an important role in brain. Uric acid (UA) is also plentiful in brain. Although UA is less likely to have any function in brain it may provide important information. Whether release of AA and/or UA are associated with particular neuronal events or behaviors is still unclear. Previous research suggested that increased release of AA in anterior cingulate cortex is an indirect result of stress. Therefore, the effects of restraint on release of AA and UA from anterior cingulate cortex (limbic) was compared to the effects in caudate (sensorimotor). Voltammetry in vivo was used to monitor release of AA and UA in conscious behaving rats. Restraint (40 min) increased both AA and UA in both brain areas, although the increase was greatest in cingulate cortex. Repeated restraint produced a gradually increasing AA response. The anxiolytic diazepam reduced resting AA and UA levels but failed to block the restraint-induced increase in AA.

112.9

ANOXIA-RESISTANT TURTLE BRAIN MAINTAINS ASCORBIC ACID CONTENT *IN VITRO*. M.E. Rice & J. Cammack, Dept. Physiology & Biophysics, New York University Medical Center, New York, NY 10016; Dept. Pharmacology, University of Kansas, Lawrence, KS 66045.

Tissue content of ascorbate in mammalian brain is 2-3 mM with an extracellular concentration of 200-400 μM. Total tissue ascorbate is rapidly lost, however, when incubated in ascorbate-free media *in vitro* (*Brain. Res.* 253: 353, 1982). We have previously reported that tissue and extracellular ascorbate concentrations in turtle brain (*J. Neurochem.* 49: 1096, 1987) are similar to those in mammals. Here we report that, in contrast to mammalian brain, isolated turtle brain tissue maintains intra- and extracellular ascorbate levels when incubated in ascorbate-free media for as long as 24 hours. After incubation for one hour, total tissue content of ascorbate in the turtle (*Pseudemys scripta* or *Chrysemys picta*) cerebellum was the same as in unincubated controls. After 20-24 hours, tissue ascorbate content remained at 65% of control levels, while extracellular ascorbate concentration, measured with carbon fiber voltammetric microelectrodes, was 56% of the initial value. For an intermediate incubation period of 6 hours, reduced ascorbate content was maintained at about 80% of control levels, regardless of whether incubation was under normal conditions or in the absence of glucose or oxygen. By contrast, only 4% of the ascorbate content of guinea pig brain slices remained after a 6 hour incubation. Maintenance of ascorbate concentration by the anoxia-resistant turtle brain suggests that this antioxidant is important in ameliorating anoxic injury. Inclusion of ascorbate in media used for *in vitro* studies of mammalian brain tissue is recommended. Supported by USPHS Grants NS-13742 and NS-28480 and NS-08740, BRSG S07 RR05399-29 and PHS Training Grant GM-07775.

112.10

INHIBITION OF CATECHOLAMINE AUTOXIDATION BY ASCORBIC ACID AND URIC ACID. W.H. Church, V. Ward*. Dept. of Chemistry, East Carolina Univ., Greenville, NC 27858-4353.

The inhibition of catecholamine autoxidation by ascorbic acid and uric acid is evaluated with regard to the production of cytotoxic agents. Previous work from this lab has shown that uric acid and ascorbic acid have a synergistic effect on the inhibition of dopamine autoxidation. The present work extends these findings to other catecholamine neurotransmitters, as well as other oxidizable biomolecules. UV photo-diode array and electrochemical detection are used to establish the kinetics and reaction mechanisms associated with these reactions in vitro. Relevance of in vitro results to in vivo processes is discussed. The ability of these anti-oxidants to inhibit aerobic oxidation of the cate-cholamines and, as a result, the generation of cytotoxic quinones and free radicals is interpreted in relation to the neuronal cell death associated with aging and Parkinson's disease.

112.11

PHYSIOLOGICAL AND CLINICAL EFFECTS OF ASCORBIC ACID ADMINISTRATION. L.C. Tolbert, J. Spollen*, and L.D. Middaugh[1]. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama at Birmingham, Birmingham, Al 35294 and [1]Dept. of Psychiatry Medical University of South Carolina, Charleston, SC.

A variety of experiments conducted over the last decade or so have produced results consistent with the hypothesis that one of the roles of this vitamin in the CNS might be that of a dopamine neuromodulator. These experiments have demonstrated, for example, effects on ligand binding and second messenger system in vitro as well as effects on neuronal firing rates, neurotransmitter turnover, and modulation of the effects of concomitantly administered dopaminergic pharmacologic agents, in vivo. Additionally, a series of these experiments have demonstrated that extracellular ascorbate levels in the CNS are dynamic and change in response to a variety of manipulators. Although alternative explanations for the results of individual experiments can be offered, but the recurrent conclusion, and most parsimonious explanation is that ascorbic acid can have pharmacologic effects consistent with the hypothesized role as a dopamine neuromodulator.

We have conducted two experiments to further evaluate this possibility. In one experiment we evaluated the ability of pharmacologic doses of ascorbate to impact the neuroendocrine system in rats; specifically the ability to modulate the L-Dopa-induced changes in prolactin and LH. Ascorbate significantly attenuated those L-Dopa-induced changes. In the second experiment we conducted a double-blind, placebo-controlled, cross-over design clinical trial of the ability of ascorbic acid to attenuate symptoms in a group of autistic subjects. Ascorbate significantly reduced total symptom scores and motor symptom scores. It had no effect on affectual symptom scores.

SEROTONIN RECEPTORS: $5HT_{1A}$ II

113.1

MONOCLONAL ANTIBODIES FOR THE VISUALIZATION OF BMY 7378-SENSITIVE 5HT1A RECEPTOR SITES. G.P. Martinelli[1], G.R. Holstein[2,3] D.W. Smith[5] F. D. Yocca[5], and S. Maayani[4]. Depts. of Surgery[1], Neurology[2], Anatomy[3] & Anesthesia[4] Mount Sinai Sch Med., NY, NY 10029; Preclinical CNS Res., Pharm. R&D Div., Bristol-Meyers Comp[5], Wallingford, Conn. 06492.

The goal of this work was the localization of BMY 7378 {(8-[2-[4-(2 methoxyphenyl)-1-piperazinyl] ethyl]-8-azaspiro[4,5]-decane-7,9-dione} as a marker for $5HT_{1A}$ receptor sites in the rat CNS. To obtain antibodies which might not interfere with the binding of BMY 7378 to an acceptor site, the compound 8-(4-aminobutyl)-8-azaspirol {4,5] decane-7,9-dione·HCl (BMY·Hpt) was synthesized and conjugated to keyhole limpet hemocyanin using 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (ECD). Such conjugate was used to immunize mice to raise monoclonal antibodies. Six stable clones producing antibodies with specificity against BMY·Hpt were thrice cloned. None of these showed cross-reactivity with ECD conjugates of various neuroactive substances to bovine serum albumin. The specificity of such antibodies for BMY 7378 was confirmed by immunoprecipitation with [^{3}H]BMY 7378. Subsequently, immunocytochemical studies of BMY 7378 localization were conducted in rats. Experimental animals received subcutaneous injections of BMY 7378 (10 mg/kg); controls received saline. Rats were sacrificed 40 min post-injection. Sections were exposed to the primary antibody for 16 hr, followed by the PAP procedure. Ultrastructural observations revealed immunoreactive elements in discrete brain regions of the BMY-injected rats. In the hippocampal CA1 region, most of the immunostain is present in unmyelinated axons, usually in apposition to long thin dendritic shafts, but without synaptic specializations. Many axon cylinders of myelinated profiles are also immunolabeled. Identically processed sections from saline-injected animals showed no specific immunostaining. Aided by Grants NS-24656, GM-34852.

113.2

CHARACTERIZATION OF ANTIBODY-BMY-7378 IMMUNE COMPLEX BINDING TO THE $5\text{-}HT_{1A}$ RECEPTOR IN RAT BRAIN. W.P. Clarke[1], G.P. Martinelli[2], G.R. Holstein[3,4], and S. Maayani[1,5]. Departments of Pharmacol[1], Surgery[2], Neurology[3], Cell Biology/Anatomy[4] and Anesthesiol[5], Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Ultrastructural localization of $5\text{-}HT_{1A}$ receptor sites with electron microscopy requires new methods to label the receptor. Martinelli *et al.*, (this volume) have developed a monoclonal antibody that binds to BMY-7378, a potent, selective and low efficacious $5\text{-}HT_{1A}$ agonist. The characteristics of binding to the $5\text{-}HT_{1A}$ receptor of an immune complex between this antibody and BMY-7378 were examined in rat hippocampal membranes. Immune complexes were formed by incubating the antibody (100 µl; 2-15 mg/ml) with BMY-7378 (20 µl; 1 mM) for 30 minutes at 37 °C. The immune complex was separated from free BMY-7378 by filtration with a Sephadex G-25 column and the void volume collected. Immune complexes labelled with ^{125}I were made by prelabelling the antibody with ^{125}I prior to incubation with BMY-7378. In homogenates of rat hippocampus, unlabelled immune complex (0.1-100 ug/ml) competed for [^{3}H]-8-hydroxy-2-(di-*n*-propylamine)tetralin (8-OH-DPAT; 1-2 nM) binding. Antibody alone (not complexed with BMY-7378) did not reduce [^{3}H]-8-OH-DPAT binding. The void volume collected when BMY-7378 alone (not complexed with antibody) was eluted from the G-25 column did not compete for [^{3}H]-8-OH-DPAT binding. Although non-specific binding (dctermined in the presence of 1 µM 5-carboxamido-tryptamine) was high, incubation of hippocampal membranes with 100 µg/ml of the ^{125}I-immune complex appeared to label $5\text{-}HT_{1A}$ receptor sites. These data suggest that the immune complex between antibody and BMY-7378 binds to the $5\text{-}HT_{1A}$ receptor. The high specific activity and selectivity of iodinated immune complexes and Fab fragment complexes should be useful for binding and autoradiographic studies of the $5\text{-}HT_{1A}$ receptor in brain. In addition, the complex will allow for ultrastructural localization of $5\text{-}HT_{1A}$ in brain. (Supported by grants USPHS GM-34852, MH-48125, NS-24656)

113.3

Phosphorylation may be involved in agonist-mediated desensitization of $5\text{-}HT_{1A}$ receptors. M.A. Harrington and R.D. Ciaranello. Nancy Pritzker Laboratory, Stanford University School of Medicine, Stanford, CA 94305.

Preincubation of rat cortical membranes in 1 nM 8-OH-DPAT, but not 1 nM (-)pindolol results in a significant decrease in the density (B_{max}) of ^{3}H-8-OH-DPAT binding to $5\text{-}HT_{1A}$ receptors without affecting the affinity (K_D). Preincubation in 100 uM cAMP or 10 uM forskolin has a similar effect on ^{3}H-8-OH-DPAT binding, decreasing the B_{max} while having no effect on the K_D. In addition, 8-OH-DPAT inhibition of forskolin-stimulated adenylate cyclase in rat hippocampal membranes is desensitized by preincubation in DPAT, cAMP or forskolin. Preincubation of HA7 cells (HeLa cells expressing $5\text{-}HT_{1A}$ receptors) with 5 pM 8-OH-DPAT results in a significant decrease in the B_{max} of ^{3}H-8-OH-DPAT binding without affecting the K_D, while preincubation with 5 pM (-)pindolol has no effect on the binding. Preincubation in either forskolin or cAMP also significantly decreases the B_{max} of ^{3}H-8-OH-DPAT binding. In HA7 cells, 8-OH-DPAT inhibition of forskolin-stimulated adenylate cyclase is desensitized by preincubation with either DPAT or forskolin. In addition preincubation with the catalytic subunit of protein kinase A results in a desensitization of DPAT-mediated inhibition of adenylate cyclase similar to preincubation with agonist. These results suggest that phosphorylation may play a role in regulation of the $5\text{-}HT_{1A}$ receptor..

113.4

ELEVATED BRAIN 5-HT LEVELS OR $5\text{-}HT_{1A}$ RECEPTOR AGONISM INDUCE C-FOS PROTEIN WITH SIMILAR PATTERNS IN NUCLEI OF RAT BRAIN. R.A. Leslie, T.P. Flanigan*, J.M. Harvey* and D. G. Grahame-Smith*. Oxford University - SmithKline Beecham Centre, Dept. of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE, U.K.

Many functions are mediated by CNS 5-HT, and most 5-HT cell bodies are in raphé nuclei of the brainstem. Little is known about the localization of 5-HT terminals which mediate specific functions. Immunocytochemistry has been used to localize c-fos protein to map brain activity associated with 5-HT functions. Increased 5-HT function produced by MAO inhibition and L-tryptophan results in the 5-HT hyperactivity syndrome and specific Fos-like immunoreactivity (FLI) in many limbic nuclei and in certain subdivisions of striatum, parasympathetic nuclei, paraventricular hypothalamus, dorsomedial thalamus and inferior olive. A similar pattern of FLI was seen in animals experiencing the 5-HT syndrome after being given 8-OH-DPAT, a specific $5\text{-}HT_{1A}$ receptor agonist. Anaesthesia with sodium pentobarbital to prevent locomotor effects of the syndrome resulted in a loss of FLI in striatal but not other brain regions. We conclude that Fos immunocytochemistry is a valuable technique for localizing 5-HT activity in rat brain.

113.5

EXPRESSION OF RAT 5-HYDROXYTRYPTAMINE$_{1A}$ RECEPTOR GENE IN CULTURED CELLS: CHARACTERIZATION BY [^{3}H]DPAT BINDING. Y.Fujiwara, I.Sora*, H.Tomita*, S.Otsuki*, H.I.Yamamura#, T.Ohnuki*##, Y.Hamagishi### and T.Oki*##, Dept. of Neuropsychiat. Okayama Univ. Medical Sch., Shikata-cho 2-5-1 Okayama 700, Japan, #Dept. of Pharmacol.,Sch. of Med., Univ. of Arizona, Tucson, AZ85724, USA, ##Bristol-Myers Squibb Research Institute, Tokyo 153, Japan.

We report the expression of the rat 5-HT$_{1A}$ receptor gene cloned by us (Fujiwara et al. 1990) and the characterization of this receptor by [^{3}H]DPAT binding. BamHI-XbaI fragment of this gene was cloned into Rc/RSV and transfected into HeLa cells by calcium phosphate method. The cells were scraped, centrifuged and resuspended in TEM buffer. For [^{3}H]DPAT binding, ligand (1nM for inhibition study) and 7.5ug protein of cell membrane in 500ul of buffer (50mM Tris-HCl pH=7.4, 2.5mM $MgCl_2$) were incubated at 30°C for 30 min. 5-HT(10uM) was used to define nonspecific binding, which was less than 10% of total binding at 1nM of [^{3}H]DPAT. Cells expressed specific and saturable binding of [^{3}H]DPAT with a K_D value of 0.6nM and a Bmax value of 2 p mol/mg protein. Specific binding was inhibited by partial agonists, which were expected to have anxiolytic effect. Ipsapirone, buspirone, SM3997 and gepirone displaced [^{3}H]DPAT binding at 10-100nM concentration, whereas spiperone and mianserin were effective only at 1 uM concentration. The displacement potencies of these compounds by [^{3}H]DPAT binding are similar to affinities obtained in rat hippocampus.

113.6

EXPRESSION AND FUNCTIONAL COUPLING OF THE SEROTONIN-1A (5-HT$_{1A}$) RECEPTOR IN HUMAN EMBRYONIC KIDNEY (293) CELLS. J.G. Hensler, D.B. Pritchett, A. Frazer and P.B. Molinoff. Depts. of Psychiatry, Pharmacology, and Pediatrics, Univ. of Penna. School of Medicine, and Dept. of Veterans Affairs Medical Center, Phila., PA 19104.

Human embryonic kidney (293) cells were transiently transfected by the method of Chen and Okayama (Mol Cell Biol 7:2745, 1987) with the BamHI-XbaI fragment of the human 5-HT$_{1A}$ receptor gene using a eukaryotic expression vector containing a cytomegalovirus promoter. Expression of receptor was detected 48 hr after transfection by the binding of the agonist ligand [^{3}H]8-OH-DPAT. Saturable binding of [^{3}H]8-OH-DPAT was measured with a K_D value of 2.5 nM, and Bmax values of 200 to 1000 fmol/mg protein. No specific binding was measured in untransfected cells. Specific binding of [^{3}H]8-OH-DPAT in transfected cells was inhibited by guanine nucleotides. Inhibition of forskolin-stimulated adenylyl cyclase by 5-HT, as determined by prelabeling cells with [^{3}H]adenosine and measuring the conversion of [^{3}H]ATP to [^{3}H]cAMP, was present in transfected but not in untransfected cells. 5-HT inhibited forskolin (1 μM)-stimulated adenylyl cyclase in a concentration-dependent manner (IC_{50}=20 nM) with a maximal inhibition of approximately 50%. The expression and coupling of the 5-HT$_{1A}$ receptor to G proteins, and the functional coupling of this receptor to the inhibition of the adenylyl cyclase system, suggest that this expression system may be useful for studying the structural requirements of the receptor protein and the regulation of this receptor. (Supported by research funds from the Dept. of Veterans Affairs, USPHS grants MH 48125, and MH 29094).

113.7

SEROTONIN INHIBITION OF CYCLIC AMP FORMATION IN XENOPUS OOCYTES INJECTED WITH RAT STOMACH FUNDUS RNA. J. Harts, J. Liu*, J.D. Kursar[1], M. Baez[1], D.L. Nelson[1], M.L. Cohen[1] and L. Yu Dept. of Med. and Mol. Genetics, Indiana Univ. School of Medicine, Indpls, IN 46202; [1]Lilly Research Laboratories, Lilly Corp. Center, Indpls, IN 46285.

Serotonin-elicited contractions in rat stomach fundus are mediated by a serotonin (5HT) receptor pharmacologically distinct from the known subtypes. Oocytes injected with fundus RNA, 5HT$_{1A}$ receptor RNA or tRNA were treated with either 100 μM forskolin or 100 μM forskolin and 1 μM 5HT. cAMP content was analyzed using a radioimmunoassay. Basal cAMP levels are 1.05, 1.41 and 1.78 pmoles for tRNA, fundus RNA and 5HT$_{1A}$ RNA-injected oocytes. Forskolin treatment gave significant increases in cAMP content in all groups. 5HT treatment decreased cAMP content by 85% and 28% in fundus RNA or 5HT$_{1A}$ receptor RNA-injected oocytes, but did not change cAMP levels in tRNA-injected oocytes. To determine the feasibility of studying receptors linked to inhibition of adenylyl cyclase by voltage-clamp methods, 5HT$_{1A}$ receptor RNA was injected into follicle oocytes. Membrane potential was held at -20 mV and currents were measured during application of either 1 μM forskolin or 1 μM forskolin and 1 μM 5HT. Forskolin elicited a weak, outward current which was reversed by 5HT. These methods will be useful in further studies of the fundus serotonin receptor and other receptors linked to inhibition of adenylyl cyclase.

113.8

MOLECULAR STRUCTURE AND FUNCTIONAL EXPRESSION OF α2-ADRENERGIC AND SEROTONERGIC RECEPTORS. N.J. Stam, R. Dijkema*, F. Van Huizen, J.A.D.M. Tonnaer. Microbiology and CNS Pharmacology R & D Labs, Organon Int. bv, PO Box 20, 5340 BH Oss, The Netherlands

In order to isolate individual α2-adrenergic and 5-HT receptor subtypes, a human genomic DNA library was screened under low stringency conditions with a set of degenerated oligonucleotide probes derived from consensus sequences (TMR segments) of known α2-AR and 5HT receptor genes. Three α2-AR subtypes were cloned and expressed in CHO cells. All three genes encoded a single high affinity receptor site with clearly different pharmacological characteristics. Exposure of α2-AR transfectants to the agonist clonidine attenuated forskolin induced cAMP levels by more than 40%, suggesting a functional coupling of the human receptors in the rodent cells to the intracellular second messenger system. Northern blot analysis with subtype specific probes indicated a differential expression of the receptor subtypes in various tissues. The complete coding sequence of the human 5-HT1a receptor was isolated. Rodent fibroblasts transfected with this 5-HT1a gene expressed a single receptor site for 8-OH DPAT with a Kd of 2-3 nM. Functional studies suggested a negative coupling of the 5-HT1a receptor to the enzyme adenylate cylase. A panel of independent transformants was generated that expressed the receptor at different densities. These clones are used to investigate the relationship between receptor densities and affinity state and coupling efficacy of the receptor. Preliminary results suggest that high affinity binding of 8-OH DPAT is independent of receptor density per cell.

113.9

CONSERVED ASPARTATE AND SERINE RESIDUES INVOLVED IN LIGAND BINDING OF THE SEROTONIN 1A (5-HT$_{1A}$) RECEPTOR. B. Y. Ho*, A. Karschin, N. Davidson and H. A. Lester Division of Biology, California Institiute of Technology, Pasadena CA 91125.

Aspartate[116] in the 2nd transmembrane region and serine[198] in the 5th transmembrane region of the human 5-HT$_{1A}$ receptor were mutated to asparagine[116] (D116N) and alanine[198] (S198A), respectively, to study their effects on ligand binding. The wild type and mutant receptors were expressed in COS-7 cells using vaccinia virus vectors and membranes were prepared for radioligand binding assays. Saturation binding experiments showed that the wild-type and mutant receptors were expressed in comparable levels (2-4 pmol/mg protein). Both mutants showed major decreases in affinity for serotonin but smaller changes in affinity for the antagonist pindolol. The K_i values for serotonin increased from 1 nM in the wild type to 148 nM in D116N and 246 nM in S198A. Those for pindolol changed from 18 nM to 28 nM in D116N and 32 nM in S198A. These findings suggest that aspartate[116], which is conserved among monoamine receptors, may form electrostatic interaction with the protonated amino group of serotonin, while serine[198], which is conserved among various serotonin receptors, may form hydrogen bond with the 5-OH group on the indole ring of serotonin. (supported by American Heart Association, Humboldt Foundation, NIH GM-29636, GM-10991).

113.10

EXPRESSION OF THE HUMAN 5-HT$_{1A}$ RECEPTOR GENE (G21) IN A NEURONAL CELL LINE.

F. Yocca[1,2], E. Baetge[1], M. Bachinsky*[1], G. Gaughan*[1], J. Torrente[1], and S. Maayani[2], [1]CNS Drug Discovery, Bristol-Myers Squibb Co., Wallingford, CT, and [2]Dept. Anesthesiology, Mt. Sinai School of Medicine, N.Y., NY.

The 5-HT$_{1A}$ receptor subtype has been described to date only in the CNS, but the human gene for this receptor (G21) has been expressed solely in non-neuronal cell lines. We have transfected the gene for the human 5-HT$_{1A}$ receptor into HT4 cells, a line derived from a mouse neuronal cell line and immortalized with temperature sensitive SV40 T-antigen (Whittemore, et al., *J.Neurosci.Res.* 28, 156-170, 1991). Using the published gene sequence, we employed the polymerase chain reaction of human genomic DNA to isolate an incomplete 5-HT$_{1A}$ coding sequence (Kobilka, et al., *Nature*, 329, 75-79. Complete restriction and partial DNA sequence analysis confirmed the identity of the 5-HT$_{1A}$ gene, which was subsequently subcloned into a plasmid expression/selection vector. The HT4 cells were transfected with the 5-HT$_{1A}$ receptor gene by use of calcium phosphate with osmotic shock by 25% glycerol (Schweitzer and Kelly, 1985).

Preliminary saturation studies indicate [^{3}H]-8-OH-DPAT, a selective 5-HT$_{1A}$ agonist, labelled with high affinity an apparently homogenous population of binding sites (K_D=1.6 nM). This value is comparable to that obtained utilizing rat hippocampal tissue and to the G21 expressed in non-neuronal cell lines. In competition experiments, 5-HT$_{1A}$ agents displayed monophasic displacement curves with nanomolar affinities. Further, when [^{3}H]-8-OH-DPAT binding to the 5-HT$_{1A}$ receptor binding site was performed in the presence of 5'-guanylylimidodi-phosphate (GppNHp), a concentration-dependent decrease in [^{3}H]-8-OH-DPAT binding was observed suggesting the site is coupled to a G-protein. This system offers the first cell line derived from neuronal tissue for studying the signal transduction mechanism initiated by activation of the 5-HT$_{1A}$ receptor (supported in part by USPHS6M-24852).

113.11

IDENTIFICATION AND LOCALIZATION OF 5-HT_{1A} RECEPTORS IN THE RAT BOWEL AND PANCREAS. A.L. KIRCHGESSNER, M. LIU* and M.D. GERSHON, Dept. of Anat.& Cell Biol., Columbia University, New York, NY 10032.

We tested the hypothesis that the rat enteric nervous system (ENS) contains 5-HT_{1A} receptors. [^{3}H]8-OH-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) was used as a radioligand. Binding of [^{3}H]8-OH-DPAT to membranes derived from the myenteric plexus and the pancreas was investigated by rapid filtration. Alternatively, radioautography was employed to locate [^{3}H]8-OH-DPAT binding sites in frozen sections of unfixed bowel or pancreas. An excess of 5-HT (10 µM) was used to define nonspecific binding. Saturable, high affinity binding of [^{3}H]8-OH-DPAT to enteric (K_d = 2.8 ± 1.1 nM; B_{max} = 39.9 ± 4.3 fmol/mg protein) and pancreatic (K_d = 6.6 ± 1.3 nM; B_{max} = 44 ± 2.2 fmol/mg protein) membranes was found. As is true of [^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors of the CNS, the binding of [^{3}H]8-OH-DPAT to enteric and pancreatic membranes was inhibited by 8-OH-DPAT, NAN-190, spiperone, and metergoline. In contrast, the binding of [^{3}H]8-OH-DPAT to enteric and pancreatic membranes was not inhibited by 5-carboxyamidotryptamine, or by a variety of compounds known to bind to other subtypes of 5-HT receptor. Radioautography revealed that the highest density of [^{3}H]8-OH-DPAT binding sites was found in the stomach and the colon. These sites were concentrated in the muscularis mucosa and myenteric ganglia. Fewer binding sites were observed in the muscularis mucosa and myenteric plexus of the esophagus, small intestine, and cecum. Pancreatic 5-HT_{1A} receptors were located on nerves, lymphoid tissue (especially the capsule of nodes), and on the tissue adjacent to ducts and some islets. It is concluded that the rat ENS and its extension in the pancreas contains 5-HT_{1A} receptors. The function of these receptors in the physiology of the entero-pancreatic innervation remains to be determined. Supported by American Diabetes Association & NS27645.

113.12

CHARACTERISATION OF THE GTP-SENSITIVE AND GTP-INSENSITIVE STATES OF THE $5HT_{1A}$ RECEPTOR. J. M. Elliott* & S. Phipps*. (SPON: Brain Research Association). Oxford University-SmithKline Beecham Centre for Applied Neuropsychobiology, Radcliffe Infirmary, Oxford OX2 6HE, U.K.

Binding of the agonist radioligand ^{3}H-DPAT to the $5HT_{1A}$ receptor is partially but not completely inhibited in the presence of GTP (Harrington & Peroutka, 1990; J. Neurochem. 54, 294-299). We have further investigated this phenomenon using ^{3}H-DPAT to label $5HT_{1A}$ receptors in rat cerebral cortex.

Specific binding to the GTP-sensitive state of the receptor was defined by the presence of 100µM GppNHp whereas specific binding to the GTP-insensitive state was defined by 10µM 5HT in the presence of 100µM GppNHp. Binding capacity of the two sites was similar but affinity was consistently higher at the GTP-insensitive site (Kd=0.90 ± 0.04nM; n=6) compared to the GTP-sensitive site (Kd=1.23 ± 0.13nM). Competitive binding affinity of other agonists and antagonists showed no significant difference at the two sites. However addition of monovalent cations reduced binding affinity of ^{3}H-DPAT at the GTP-insensitive site (Kd=2.13 ± 0.28nM) whilst not affecting affinity at the GTP-sensitive site.

We conclude that the GTP-sensitive and GTP-insensitive states of the $5HT_{1A}$ receptor are similar in ligand binding profile but differ in their sensitivity to guanine nucleotides and monovalent cations.

113.13

ALLOSTERIC INTERACTION BETWEEN BINDING OF HORMONES AND BINDING OF GUANYLNUCLEOTIDES TO Gi-LINKED RECEPTORS IS AFFECTED BY ISOFLURANE; A QUANTITATIVE AUTORADIOGRAPHIC STUDY.

J.H. Silverstein, C.D. Mahle and S. Maayani, Dept. of Anesthesiology and Pharmacology, Mount Sinai Medical Center, CUNY, New York NY 10029.

The initial steps of signal transduction mediated in neurons by hormone receptors may be perturbed by inhaled anesthetics. The binding of hormone to the receptor stabilizes a high affinity ternary complex between hormone, receptor and guanyl nucleotide regulatory proteins(G-proteins)(H-R-G). An early event is the destabilization of H-R-G by guanyl nucleotides. This perturbation may be receptor and brain region dependent. We have measured the destabilization of H-R-G by quantitative receptor autoradiography in 15 µ coronal sections from cow hippocampus. Guanosine 5'-(β,γ,imido)triphosphate(Gpp(NH)p), a non-hydropyzable analogue of GTP, destabilized H-R-G in a concentration dependent and saturable manner Two Gi-linked receptors were labeled with their respective ^{3}H agonists: 8-hydroxy-2-(di-n-propylamino)tetralin([^{3}H]-8-OH-DPAT) and R-phenylisopropyl-denosine([^{3}H]-R-PIA). Concentration-response curves to Gpp(NH)p were evaluated by a four parameter logistic equation to yeild basal level of agoniost binding, EC_{50}, E_{max}, and slope index. The first three parameters were not affected by 1.5% isoflurane while the value of the slope index was significantly decreased for ^{3}H-DPAT (0.98 to 0.72). Increased sensitivity of the H-R-G complex to lower concentrations of GppNHp was observed in the presence of isoflurane. Changes in attenuation of binding of ^{3}H-PIA by GppNHp were biphasic in the presence of isoflurane. The regional nature of these changes are evaluated. This study should enhance the delineation of the region and the receptor dependency of inhaled anesthetic action.

Supported by the Foundation for Anesthesia Education and Research with a grant from BOC Health Care/Anaquest and USPH GM-34852

113.14

BIOCHEMICAL PARAMETERS ASSOCIATED WITH DRUG EFFICACY: A QUANTITATIVE AUTORADIOGRAPHIC STUDY OF THE 5-HYDROXYTRYPTAMINE$_{1A}$ RECEPTOR IN RAT AND HUMAN HIPPOCAMPUS. S.Maayani[1,2], K.A. Hogan*[1], D.P. Perl[3], F.D. Yocca[4], and C.D. Mahle[2]. Depts [1]Anesthesiology, [2]Pharmacology and [3]Pathology, Mt. Sinai Sch. Med. CUNY, NY, NY 10029, and [4]CNS Neuropharmacology, Bristol-Myers Squibb Co., Wallingford, CT 06492.

The density of the ternary complex (hormone/receptor/G-protein; HRG) appears to be associated with the relative efficacy of ß-adrenoceptor agonists (Kent et al., Mol. Pharmacol. 17: 14, 1980). We have previously reported a similar finding in rat hippocampal (HIP) homogenates using full ([^{3}H]-8-hydroxy-2-(dipropyl-amino)tetralin; PAT) and partial ([^{3}H]-BMY-7378) 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) agonists (Mahle et al., Soc. Neurosci. Abstr. 16: 1036, 1990). Binding of the two 5-HT_{1A} agonists to 15 µ sections of rat and human HIP (8-13 subregions), was analyzed using quantitative receptor autoradiography (QRA). The current study was done in an attempt to discretely localize HRG to subregions of HIP, as well as test in human HIP the previously formulated hypothesis. Binding of the [^{3}H]-agonists in both species was saturable and high affinity. [^{3}H]-PAT and [^{3}H]-BMY-7378 displayed similar affinity (0.3-0.5 nM) for the 5-HT_{1A} receptor in both preparations and species. In subregions analyzed in both rat and human HIP, [^{3}H]-PAT displayed a 20-40% greater B_{max} than [^{3}H]-BMY-7378. Thus using QRA, we have demonstrated that, similar to findings in rat HIP membrane homogenates, [^{3}H]-PAT stabilizes a greater density of HRG in both rat and human HIP than does [^{3}H]-BMY-7378. Similar to the ß-adrenoceptor, the density of the high affinity ternary complex measured with a higher efficacy agonist is greater than that measured with a lower efficacy agonist. We propose that the greater density of HRG results in a greater amount of complex available for destabilization by guanine nucleotides, thus generating a greater functional response. (USPHS GM 34852, DA 06620, and MH 48125)

113.15

ALLOSTERIC INTERACTIONS BETWEEN BINDING SITES OF RECEPTOR AGONISTS AND GUANINE NUCLEOTIDES: A COMPARATIVE STUDY OF THE 5-HYDROXYTRYPTAMINE$_{1A}$ AND ADENOSINE A_1 RECEPTORS. C.D.Mahle[1], D.P.Healy[1], D.P.Perl[2], & S.Maayani[1,3]. Depts. [1]Pharmacol., [2]Pathol. & [3]Anesthesiol., Mt. Sinai Sch. Med., CUNY, NY, NY. 10029.

The high affinity ternary complex HRG (hormone/receptor/G-protein) is rapidly destabilized by guanine nucleotides (GN), e.g., guanosine 5'-(β,γ-imido)triphosphate (Gpp(NH)p), that convert R into a low affinity state. These allosteric interactions were characterized in membranes from rat hippocampus (HIP) and 15 µ sections of rat and human HIP (8-13 subregions) prepared for quantitative receptor autoradiography (QRA). Gpp(NH)p attenuated [^{3}H]-8-hydroxy-2-(dipropylamino)tetralin and [^{3}H]-R-phenyl-isopropyladenosine binding to the 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) and adenosine (AD) A_1 and receptor sites, respectively, in a concentration-dependent and saturable manner, described by three parameters: E_{max}, EC_{50}, and slope index. In rat HIP, for both receptors, the E_{max} was consistently greater in the tested subregions (80-90%) than in membrane preparations (50%). EC_{50} values were independent of subregion in both species, but were receptor system dependent in human HIP (CA1), with 5-HT_{1A} values consistently less (0.1-0.2 µM) than AD (0.3-0.4 µM). Virtually complete attenuation of binding (90-95%) was observed when sections were incubated for 60 min with either [^{3}H]-agonist in the presence of 100 µM Gpp(NH)p. In contrast, when 100 µM Gpp(NH)p was added to sections pre-incubated for 60 min with either [^{3}H]-agonist, the binding after an additional 60 min was attenuated only 70-80%. In addition, 100 µM Gpp(NH)p at the earliest time point (1 min) produced a substantially greater decrease in binding of either [^{3}H]-agonist, compared to displacement with agonist or antagonist. Steady-state and kinetic analyses of destabilization of HRG by GN can be discretely localized by QRA and used to measure the allosterism existing between binding of hormone and GN to RG. This technique may be useful in identification of the effect of perturbations such as aging, disease, and chronic drug treatment on specific events in receptor signalling. (USPHS GM 34852, HL 42585, DA 06620, and MH 48125)

113.16

DIFFERENCES IN THE CHARACTERISTICS OF ALLOSTERIC INHIBITION OF [^{3}H]AGONIST BINDING BY GppNHp AT PRE- AND POSTSYNAPTIC 5-HT_{1A} RECEPTORS IN COW BRAIN.

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Observed region-dependent variations of drug efficacy on the 5-HT_{1A} receptor subtype (Sprouse and Aghajanian, 1987) may reflect differences in the receptor system and not necessarily different receptor subtypes. Previously, we demonstrated region-dependent heterogeneity of the 5-HT_{1A} receptor in rat and cow brain (Yocca, et al, 1989), suggesting that a difference in regional receptor coupling to G proteins may explain variations in drug efficacy. To test this hypothesis, the allosteric inhibition of [^{3}H]8-OH-DPAT (agonist) and [^{3}H]BMY 7378 (partial agonist) by GppNHp was examined utilizing saturation binding studies at the presynaptic, dorsal raphe (DR), and postsynaptic, hippocampus (HIP), 5-HT_{1A} receptor binding sites. Pharmacological profiles generated for the displacement of both radioligands by selected 5-HT_{1A} agonists and antagonists reveal no differences in the recognition component of the pre- and postsynaptic 5-HT_{1A} receptor binding sites. Displacement curves for GppNHp in DR and HIP of [^{3}H]8-OH-DPAT and [^{3}H]BMY 7378 showed a distinct regional difference in potency. In HIP, they appear to be monophasic, while in DR the curves appear to be biphasic and are best fit by a two site model. Saturation studies indicate that GppNHp caused a greater reduction in B_{MAX} in DR, as compared to HIP, with the percent decrease being equal for both radioligands in DR. However, in HIP, GppNHp appeared to more potently inhibit the binding of [^{3}H]8-OH-DPAT than [^{3}H]BMY 7378. These results demonstrate that allosteric inhibition of agonist binding at 5-HT_{1A} receptors in DR vs. HIP occurs with lower concentrations of GppNHp, suggesting differences in regional receptor-G protein interactions. This phenomenon, as it relates to the concept of receptor reserve, will be discussed.

113.17

AFFINITY OF DRUGS FOR THE LOW AFFINITY STATE OF THE 5-HT-1A RECEPTOR OF RAT HIPPOCAMPUS. J. Chamberlain,* B.B. Wolfe and A. Frazer, Dept. of Psychiat., Univ. of Pa. Sch. of Med. and Dept. of Vet. Affairs Med. Ctr., Phila. PA 19104 and Dept. of Pharmacol., Georgetown Univ. Sch. of Med., Wash. D.C., 20007.

Previously (Soc. Neurosci. Abstr. 13:343, 1987), we reported that the affinity of serotonergic agonists for the high affinity state of ^{3}H-DPAT binding was 15-100 fold greater than the EC_{50} values of the same agonists for inhibiting forskolin-stimulated adenylyl cyclase. Potency in functional systems may reflect the affinity of these drugs for the low affinity state of the 5-HT-1A receptor. To test this, we developed a binding assay for the low affinity state. Saturation isotherms of ^{3}H-DPAT binding done in the absence of Gpp(NH)p and EDTA indicated two components of binding, one with a K_D of 0.82±0.05nM and the other with a K_D of 20±7nM. In the presence of Gpp(NH)p (100μM) and EDTA (1mM), the binding was fit by a single-site model (K_D = 12±1nM). Low affinity state binding was measured using 15nM ^{3}H-DPAT in the presence of Gpp(NH)p and EDTA. The affinity of drugs for the low affinity state were (K_i, nM): DPAT, 16±3; RU24969, 205±49; mCPP, 607±87. Their K_i values for the high affinity state were 1.4±0.2, 5±1 and 114±6, respect-ively. Previously calculated EC_{50} values (nM) for these drugs in the adenylyl cyclase assay were: DPAT, 16; RU24969, 71; mCPP, 9000. Thus, the potency of agonists to elicit 5-HT-1A-mediated responses may be quantitatively better related to their affinity for the low affinity state of ^{3}H-DPAT binding than for the high affinity state. (Supported by MH 14654 and Res. Funds from the Dept. of Vet. Aff.).

113.18

REGULATION OF BRAINSTEM 5-HT_{1A} RECEPTORS IN THE RAT. J.N. Murthy and M.R. Pranzatelli, Neurology, Pediatrics, and Pharmacology Depts., The George Washington University, Washington, D.C.

To study the regulation of 5-HT_{1A} receptors located on raphe nuclei in the brainstem, the region most relevant to serotonin-responsive human myoclonic disorders, we chronically treated rats with various 5-HT agonists and antagonists and labeled 5-HT_{1A} sites with [^{3}H]8-OH-DPAT. Ipsapirone and buspirone (presynaptic 5-HT_{1A} agonists), 8-OH-DPAT (pre- and post-synaptic 5-HT_{1A} agonist), L-propranolol,(±) pindolol and spiperone (5-HT_{1A} and 5-HT_2 antagonists), methysergide (5-HT_1 and 5-HT_2 antagonist), RU 24969 (5-HT_{1B} agonist), DOI and m-CPP (5-$HT_{1C/2}$ agonists) and MDL 7222 (5-HT_3 antagonist) or vehicle were injected once daily for 30 consecutive days at 10 mg/kg ip and the adult male Sprague-Dawley rats were sacrificed 24 hours after the last injection. There were no significant differences between vehicles (n=22): B_{max} = 4.7 ± 0.2 pmol/g, K_D = 2.5 ± 0.1, n_H = 1.0 ± 0.01, r_S = 0.98 ± 0.01. None of the drugs significantly altered B_{max} of [^{3}H]8-OH-DPAT specific binding and there were only a few small differences in K_D. These data demonstrate absence of down-regulation of presynaptic 5-HT_{1A} sites at doses which we have previously shown induce behavioral tolerance of 5-HT_{1A}-mediated behaviors of the serotonin syndrome and suggest changes in the post-synaptic cell rather than the receptor recognition site as the mechanism of tolerance. This phenomenon contrasts the marked down-regulation of 5-HT_2 receptors after chronic treatment with appropriate 5-HT agonists and antagonists.

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113.19

DELAYED EFFECT OF SPIPERONE ON 5-HT_{1A} RECEPTORS IN THE CA_3 REGION OF THE RAT DORSAL HIPPOCAMPUS. T. Dennis, G. Rizkalla*, P. Blier and C. de Montigny, Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University, Montreal, Quebec, Canada H3A 1A1.

Acute administration of BMY 7378 readily blocks the effect of microiontophoretically-applied 5-HT and 8-OH-DPAT on CA_3 pyramidal neurons (JPET, 246:359, 1988) in contrast to spiperone (SPIP) which blocks their effects on dorsal raphe 5-HT neurons, but not on CA_3 pyramidal neurons. In the latter region, antagonism of 5-HT becomes apparent only 24 h after a single dose of SPIP (Neurosci. Abst. 16:462.4, 1990). To elucidate the mechanism of this delayed effect of SPIP on postsynaptic hippocampal 5-HT_{1A} receptors, we investigated *in vitro* and *ex vivo* its effects on [^{3}H]8-OH-DPAT binding characteristics in rat hippocampal CA_3 membrane preparations and tissue sections.

In *in vitro* membrane binding studies, saturation analysis of [^{3}H]8-OH-DPAT binding yielded an apparent K_d of 1.48 ± 0.28 nM and a B_{max} of 223 ± 17 fmol/mg protein. Saturation isotherms in the presence of 200 and 500 nM SPIP showed 2- and 3-fold increases in K_d, accompanied in both cases by 55-60% reductions of B_{max}. In contrast, a 2-fold increase in K_d, with no change in B_{max}, was observed in the presence of 5 nM BMY 7378. Haloperidol, ritanserin and prazosin (500 nM) were without effect. Autoradiographic analysis showed significant increases in K_d in pyramidal and radiatum layers of the CA_3 region, but not in the dorsal raphe, 24 h after a single injection of SPIP (5 mg/kg i.p.).

The divergent *in vitro* effects of SPIP and BMY 7378 indicate a competitive antagonism of 5-HT_{1A} receptors by the latter, but not by the former. The delayed and regionally selective modification of the properties of 5-HT_{1A} receptors in the dorsal hippocampus by SPIP, detected by autoradiography, is consistent with our previous electrophysiological findings, although its nature remains to be elucidated.

TRANSMITTERS IN INVERTEBRATES: ARTHROPODS

114.1

PHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF GABA RECEPTORS ON MOTOR NEURONS IN THE MOTH *MANDUCA SEXTA*. Brian Waldrop. Dept. Zoology, Univ. Oklahoma, Norman, OK, 73019.

Motor neurons which innervate intersegmental muscles of the abdomen in the moth are retained through the larval and pupal stages during metamorphosis to promote two analogous reflex movements. The larva responds to tactile stimulation of its abdomen by bending, bringing its head towards the site of stimulation. In the pupa, the same muscles contract to close the gin traps, cuticular pits where the mechanosensory hairs are located. The pupal gin trap closure reflex consists of coordinated bouts of excitation and inhibition of the motor neurons, not seen in the larva. I wish to determine if and how inhibitory, presumably GABAergic, synaptic inputs might change during the metamorphic transition.

The motor neurons have large dorsal somata which make them easily identifiable, and large dorsally-located dendritic arbors which are accessible to application of pharmacological agents. Responses of larval and pupal motor neurons to pressure-ejection of GABA, and to GABA plus bicuculline, are recorded in the isolated abdominal nerve cord. GABA hyperpolarizes larval and pupal motor neurons, and this response is partially blocked by co-ejection of bicuculline (as methiodide). Muscimol also potently hyperpolarizes the motor neurons, but baclofen is without effect. By varying the duration of the GABA pressure pulse, a form of dose-response curve can be obtained. Application of bicuculline, either by co-ejection or superfusion, will allow a quantification of its effect on the GABA dose-response function. The actions of bicuculline on synaptically-mediated inhibition during the reflex responses initiated by stimulation of axons of sensory neurons will also be examined.

114.2

OCTOPAMINE BIOSYNTHESIS IN THE CNS OF *MANDUCA SEXTA*. H.K. Lehman, C. Murgiuc*, and J.G. Hildebrand. ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Octopamine is a widespread neurotransmitter/hormone in *M. sexta* and fluctuates in the brain and hemolymph on a daily basis (Lehman, H.K. *Soc. Neurosci. Abstr.* 16:1334, 1990). We have now characterized tyramine β-hydroxylase (the putative rate-limiting enzyme controlling octopamine synthesis) in CNS homogenates of *M. sexta* in order to examine the mechanisms responsible for altering octopamine titers during the diurnal cycle.

The assay for octopamine synthesis depends upon the hydroxylation of [^{3}H]tyramine to form [^{3}H]octopamine. Incubation of [ring-^{3}H]tyramine with crude brain homogenates results in the formation of three radiolabeled products. [^{3}H]octopamine was identified by comparison to octopamine standards in two HPLC systems. Octopamine synthesis is linear over time for at least 180 min and depends upon protein concentration and the presence of optimal levels of copper, ascorbate, and catalase. The optimum pH for the conversion of tyramine to octopamine is 7.0, and dopamine inhibits the formation of octopamine. The rate of tyramine hydroxylation as a function of the concentration of tyramine appears to follow Michaelis-Menten kinetics (Km=0.5 μM, Vmax=1.2 x 10^{-6} moles/min/mg protein). Levels of [^{3}H]octopamine produced by scotophase homogenates are 4-fold greater than levels produced by photophase homogenates. No detectable differences in dopamine β-hydroxylase (DβH)-like immunoreactivity have been observed in scotophase and photophase homogenates, but phosphorylation of photophase homogenates with the catalytic subunit of A-kinase slightly enhanced enzyme activity. Experiments are under way to examine ^{32}P incorporation into purified tyramine β-hydroxylase. Our findings suggest that an enzyme related to the mammalian enzyme DβH exists in *M. sexta*, and its activity is controlled diurnally.

114.3

MECHANISM OF ECLOSION HORMONE-STIMULATED CYCLIC GMP INCREASES IN MANDUCA SEXTA CNS. D.B. Morton and M. Giunta*, ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Eclosion hormone (EH) is a neuropeptide which triggers ecdysis behavior in insects. Previous work has shown that EH acts via the second messenger, cyclic GMP (cGMP). We have been investigating the mechanisms underlying EH-stimulated cGMP production.

EH will not stimulate guanylate cyclase activity in homogenates, suggesting that EH receptors are not directly coupled to guanylate cyclase. Some neurotransmitters elevate cGMP via the production of nitric oxide (NO). NO is generated by the conversion of arginine to citrulline which is blocked by arginine analogues. Incubation of Manduca nervous tissue with 0.5mM N-methyl arginine or nitro-arginine failed to prevent the EH-stimulated cGMP increase. Furthermore, no EH-stimulated production of 3-H-citrulline was observed when tissue was incubated in 3-H-arginine.

Another possible pathway is via the production of arachidonic acid from diacylglycerol which is generated from the hydrolysis of phosphatidylinositol bisphosphate (PIP2). LiCl depletes cells of inositol and hence reduces the hydrolysis of PIP2. Incubation of Manduca nervous tissue with 10mM LiCl for 30 minutes significantly reduced the EH-stimulated production of cGMP. This suggests that EH acts via the hydrolysis of PIP2 to stimulate guanylate cyclase.

114.4

MODULATING A MODULATOR: AMINERGIC POTENTIATION OF A PEPTIDERGIC RESPONSE IN A MOTH. K.R. Prier and N.J. Tublitz. Inst. of Neuroscience, U. Oregon, Eugene, OR 97403.

The CNS of the moth, *Manduca sexta*, contains a myoregulatory neuropeptide, CAP_2 (Cardioacceleratory Peptide$_2$), that is released during wing inflation and flight in adult moths. This peptide induces a physiologically important, dose-dependent increase in heart rate. The heart responds in a similar fashion to both octopamine and serotonin (5-HT), known insect neuromodulators. We report here that the CAP_2 response of the adult heart is greatly facilitated in the presence of subthreshold (nanomolar) concentrations of either amine. The CAP_2 response of the adult heart is increased to 170% and 180% of normal in the chronic presence of subthreshold concentrations of octopamine and 5-HT, respectively. The presence of subthreshold CAP_2 does not alter the heart's response to either amine.

We have begun to investigate the molecular mechanisms underlying this facilitation. Previous work on the adult heart has shown that CAP_2 works via an inositol 1,4,5 trisphosphate (IP_3) second messenger system. IP_3 added to individual, FURA2-filled heart cells causes an immediate rise in Ca^{2+}_{in}. This rise is partially blocked in the presence of 10mM Co^{++}_{out}, suggesting that the rise in Ca^{2+}_{in} is due both to the opening of membrane channels and to release by internal stores. In contrast to CAP_2, the cardioexitatory effects of the two amines are mediated by cAMP; application of either octopamine or 5-HT elicits a 3-fold increase in intracellular cAMP levels after pre-treatment with a phosphodiesterase inhibitor. CAP_2 has no effect on cAMP levels in the heart. The relationship between cAMP and Ca^{2+}_{in} is not yet known.

These results illustrate a mechanism by which the efficiency of a neurohormone can be increased with minimal cost. In *Manduca*, subthreshold levels of octopamine are found in the haemolymph during wing inflation and flight, and thus it is likely that octopamine up-regulates the CAP_2 effects during these important activities via a cAMP-dependent mechanism.

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114.5

STEROID-DEPENDENT MODIFICATION OF PRE-SYNAPTIC PATHWAYS IS REQUIRED FOR TRANSMITTER SWITCH IN INSECT PEPTIDERGIC NEURONS. P.K. Loi and N.J. Tublitz. Inst. of Neurosci., U. Oregon, Eugene, OR 97403.

What are the mechanisms that enable a fully mature neuron to alter its transmitter phenotype post-embryonically? The CNS of the moth, *Manduca sexta*, contains four lateral neurosecretory cells (LNCs) that switch their neurotransmitter phenotype from Cardioacceleratory Peptide 2 (CAP_2) to bursicon. The LNC transmitter conversion occurs at metamorphosis and is regulated by the ecdysteroids, the insect steroid hormones, which appear in two discrete pulses during the last larval instar. These two pulses, the commitment (CP) and prepupal peaks (PP), are apparently required for the normal CAP_2-to-bursicon transition. We have previously demonstrated that the second pulse, the PP, acts directly on the LNCs to increase bursicon levels. Here we describe experiments testing the role of and identifying the target of the CP, which is temporally associated with the major reduction of CAP_2 in the LNCs.

To test the role of the CP, the entire CNS was removed from a Vth instar larva prior to the CP (Day 2) and implanted into a second, intact Day 2 animal. Two days later, after the endogenous CP, the LNCs were dissected from implants and hosts, and assayed for both CAP_2 and bursicon. Under these conditions, both host and implanted LNCs showed a normal decrease in CAP_2 levels without any change in bursicon. This CAP_2 drop was blocked by application of juvenile hormone. That the CP is involved in this transmitter switch was confirmed by directly applying 20-HE to a complete CNS implanted into an isolated Day 2 abdomen. In these experiments, CAP_2 declined only in the implanted LNCs; no CAP_2 change was measured in the host LNCs. These results, combined with those from experiments with implanted brainless CNS, suggest that the brain is the target for the CP, and that direct neuronal connections between the brain and the rest of the CNS is required for this switch to proceed normally. Thus, the LNC transmitter shift is controlled by two separate steroid pulses: the CP which acts via the brain to promote a reduction in CAP_2, and the PP which directly induces a rise in bursicon.

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114.6

CHARACTERIZATION OF A PIGMENT-DISPERSING FACTOR FROM THE AMERICAN COCKROACH. C. J. Mohrherr*, K. R. Rao and J. P. Riehm*. Dept. of Biology, Univ. of West Florida, Pensacola, FL 32514.

The occurrence of a pigment-dispersing factor (PDF) in the cephalic nervous tissues of the American cockroach, *Periplaneta americana*, was indicated by previous studies involving bioassays (R. M. Dores and W. S. Herman, *Gen. Comp. Endocrinol.*, 43:76, 1981) and immunocytochemistry (U. Homberg *et al.*, in press). In the present study we have purified the PDF from lyophilized heads of the cockroach, *Periplaneta americana*, utilizing the method of Rao *et al.* (*J. Biol. Chem.*, 262:2672, 1987). The immunoreactivity of this peptide towards *Romalea* PDF antibody facilitated its detection by ELISA during purification. Automated sequencing established the primary structure of *Periplaneta* PDF as NSELINSLLGLPKVLNDA. A synthetic peptide, prepared as the C-terminal amide, gave a HPLC profile identical to the native form. Based on net charge and sequence similarity, *Periplaneta* PDF is more closely related to crustacean ß-PDH (NSELINSILGLPKVMNDA-NH_2) than to α-PDH (NSGMINSILGIPRVMTEA-NH_2). *Periplaneta* PDF differs from *Acheta* PDF by a single residue (Leu for Ile at position 4). *Periplaneta* PDF is several-fold more potent than *Acheta* PDF in assays for melanophore pigment dispersion in the fiddler crab, *Uca pugilator*. (This work was supported by NSF Grant DCB-8711403.)

114.7

BIOGENIC AMINES MODULATE SYNAPTIC TRANSMISSION BETWEEN VENTRAL GIANT INTERNEURONS AND THORACIC INTERNEURONS IN THE ESCAPE SYSTEM OF THE COCKROACH. J.L. Casagrand and R.E. Ritzmann. Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

In the escape system of the cockroach, *Periplaneta americana*, a population of uniquely identifiable thoracic interneurons (type A or TI_As) receive information about wind via short latency, functionally direct, and apparently cholinergic inputs from a population of ventral giant interneurons (vGIs). The TI_As are involved in the integration of sensory information necessary for orienting the animal during escape. It is likely that there are times in an animal's life (*i.e.* in the presence of alarm pheromone) when it is advantageous to modify the responses of some or all TI_As to vGI input, which would result in changes in the processing of the circuit, and thereby, its outputs. A great deal of evidence exists for the use of biogenic amines as neuromodulators in a variety of neural circuits, and the amines octopamine (OCT), serotonin (5-HT), and dopamine (DA) are present in cockroach nervous tissue. We, therefore, tested the ability of OCT, 5-HT, or DA to modulate synaptic transmission between vGIs and TI_As. We found that both OCT and DA significantly increased the amplitude of vGI-evoked excitatory postsynaptic potentials (EPSPs) in TI_As when bath-applied to a semi-intact animal at 10^{-4}-10^{-2}M and 10^{-3}M, respectively. On the other hand, 5-HT significantly decreased the vGI-evoked EPSPs in TI_As at 10^{-4}-10^{-3}M. These results indicate that OCT, 5-HT, and DA are able to modulate the responses of at least some of the neural elements in this circuit.

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114.8

MODULATION OF A CALCIUM CURRENT BY MUSCARINIC RECEPTORS IN AN INSECT MOTONEURONE. J.A. David and R.M. Pitman. Dept. of Biology and Preclinical Medicine, Gatty Marine Laboratory, St. Andrews, Fife, Scotland, KY16 8LB.

At membrane potentials more negative than -40 mV, ACh externally applied to the cell body membrane of the cockroach fast coxal depressor motoneurone (D_F) induces an α-bungarotoxin-sensitive inward current which is carried by Na^+, K^+ and Ca^{2+}. At membrane potentials more positive than -40 mV, externally-applied muscarinic agonists also induce an inward current which is insensitive to α-bungarotoxin but blocked by muscarinic antagonists. In the presence of TEA^+ the response to muscarinic agonists is reversed in direction; this residual current is blocked by externally applied Cd^{2+} indicating that muscarinic agonists induce a reduction in Ca^{2+} conductance and a consequent reduction in Ca^{2+}-activated K^+ conductance. The response appears to involve an increase in intracellular concentrations of cAMP since the effects of muscarinic agonists are mimicked by externally-applied dibutyryl cAMP and modified by the phosphodiesterase inhibitor theophylline.

114.9

THE PRODUCTION AND USE OF PCR PROBES TO STUDY CONSERVED SEQUENCES IN THE *dsk* GENE.

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In order to identify functionally important regions of the *dsk* gene in *Drosophila melanogaster* and the corresponding dsk neuropeptides (DSK-0, DSK-I, and DSK-II), the presence of the *dsk* gene in related species has been studied. The method of polymerase chain reaction (PCR) was used to amplify the dsk gene from genomic DNA of related species. Two PCR primers were designed to sequences present in the *D. melanogaster dsk* gene . Sequences corresponding to *D. virilis*, *D. pseudoobscura*, and C. *erythrocephala* have been amplified and sequenced. The PCR-generated dsk probes were then used to screen genomic libraries and the corresponding *dsk* genes have been sequenced. Sequences corresponding to all three dsk peptides are present. Significant sequence conservation is found among the DSK precursors. These data will be presented and discussed relative to neuropeptide biosynthesis and gene expression.

114.10

CHARACTERISATION OF A LOCUST NEURONAL OCTOPAMINE RESPONSE.

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Octopamine evokes changes in membrane potential when applied to the somata of insect neurones *in vitro* (Usherwood, P.N.R. *et al.*, In: *Insect Neurobiology and Pesticide Action*, p. 115, 1980; Suter, C., *Comp. Biochem. Physiol.* 84C:181, 1986). To investigate the voltage-dependence, ionic basis and pharmacology of the octopamine response, we voltage-clamped mechanically isolated neurones from the thoracic ganglia of *Locusta migratoria*. In unclamped somata at their normal resting potential of -50 to -60 mV, pressure micro-application of octopamine (10 mM, 100 - 200 ms) evoked a slow depolarisation accompanied by a small decrease in membrane resistance. In clamped somata, octopamine evoked a current that was inward over the membrane potential range -40 to -90 mV, with a peak amplitude at -70 mV. This current was blocked by 5 mM Cs and by Na-free saline. As an agonist, tyramine was ca. 100-fold less potent than octopamine, and the octopaminergic agonists clonidine and tolazoline were weak or inactive at 10 µM. The insecticidal compound desmethylchlordimeform was a highly potent and reversible agonist. Naphazoline reduced the octopamine response (EC50 value ca. 5 µM) but, in contrast to octopamine, it hyperpolarised somata at normal resting potential. Mianserin, an antagonist of octopamine, dopamine and serotonin receptors, blocked the response with an EC50 value in the nanomolar range. Maroxepine, another insecticidal compound, was antagonistic, with potency similar to that of mianserin. Phentolamine and promethazine were antagonists with micromolar EC50 values. Chlorpromazine, yohimbine and metoclopramide, which are antagonists of the locust muscle octopamine receptors (Evans, P., *J. Physiol.* 318:99, 1981), were without effect at concentrations of up to 0.1 mM. This neuronal pharmacological profile agrees well with findings from binding studies on membranes from the *Locusta* central nervous system (Roeder, T., *Europ. J. Pharmacol.* 191:221, 1990), but differs from the pharmacology of the three muscular octopamine receptor subtypes located in the locust (*Schistocerca*) hind leg extensor tibiae (Evans, P., *J. Physiol.* 318:99, 1981).

114.11

SEROTONIN CAUSES THE RELEASE OF [^{3}H]-SEROTONIN IN THE ABSENCE OF EXTRACELLULAR CALCIUM FROM THE ABDOMINAL NERVES OF *Rhodnius prolixus*

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In the blood-sucking bug, *Rhodnius prolixus*, there are extensive serotonergic neurohaemal areas, located on the abdominal nerves, whose stores of serotonin are released during feeding. The major contributors to these neurohaemal areas are 5 serotonergic, dorsal, unpaired, median neurons located in the terminal segment of the CNS, the mesothoracic ganglionic mass (MTGM).

We incubated an *in vitro* preparation, consisting of the MTGM and associated abdominal nerves, in 10^{-7}M [^{3}H]-serotonin for 3 h prior to experimentation. We have demonstrated that 10^{-6}M serotonin elicits a significant release of previously accumulated [^{3}H]-serotonin in the absence of Ca^{2+} in the bathing medium, during 5 min incubations. A significant release of [^{3}H]-serotonin,by serotonin, is also seen in Ca^{2+}-free saline which contains 10 mM $CoCl_2$. No significant release of [^{3}H]-serotonin is elicited by the application of 10^{-6}M octopamine, dopamine, acetylcholine or glutamate.

The second messenger cAMP does not appear to be involved in the mechanism of release. While 10^{-6}M serotonin elicits a significant increase in cAMP levels, in the abdominal nerves during 5 min incubations, in normal saline, it does not do so in Ca^{2+}-free saline. We are presently investigating the potential role of other second messengers in the mechanism of release and using serotonergic agonists and antagonists to characterize this putative serotonergic autoreceptor.

114.12

TACHYKININ-LIKE PEPTIDES IN THE CNS OF THE BLOWFLY: DISTRIBUTION AND PARTIAL CHARACTERIZATION.

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Neuropeptides of the tachykinin family have been isolated from a wide range of organisms including mammals, lower vertebrates and invertebrates. In insects such peptides have been isolated from brain and corpora cardiaca extracts on basis of their myotropic activity in a cockroach hindgut bioassay (Holman et al., *Ann. Rev. Entomol.* 35: 201-217, 1990). The mapping of tachykinin-like peptide(s) in the insect nervous system, however, relies on immunocytochemistry with antisera against substance P and bombesin. To extend these studies we have tested a large number of antisera against tachykinins of various vertebrates and of one insect, the cockroach *Leucophaea maderae*. We found three distinct populations of tachykinin immunoreactive neurons in the blowfly *Phormia terraenovae*: (1) one recognized by antisera against substance P, (2) another by antisera against the frog peptide kassinin and (3) a third with antisera raised against the cockroach peptide Leucokinin I (LK1). Two of these populations constitute unique sets of interneurons that were not detected previously with other antisera. The third, recognized by antisera against substance P, is a subpopulation of the FMRFamide immunoreactive neurons. The kassinin immunoreactive material has been partly characterized from fly head extracts by chromatography (rpHPLC) and radioimmunoassay and it is clear that it represents material distinct from that recognized by the substance P and LK1 antisera respectively. The results presented here indicate the presence of multiple forms of tachykinin-like peptides in the blowfly, present in at least three distinct populations of brain neurons. From the immunocytochemistry it appears that the insect tachykinins may be involved in a variety of modulatory functions in the CNS and in some cases they possibly act as neurohormones.

114.13

GALANIN-LIKE PEPTIDE IN THE BLOWFLY BRAIN: DISTRIBUTION, CHROMATOGRAPHIC CHARACTERIZATION AND ^{125}I-GALANIN BINDING.

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Galanin (GAL) is a 29 amino acid bioactive peptide originally isolated from upper small intestine of pigs (Tatemoto et al., *FEBS Lett.* 261: 397-401, 1983). GAL is present in several vertebrate species and GAL-immunoreactive (GAL-IR) neurons have been demonstrated in both mammals and submammalian species such as amphibians and fish. This is the first report on the presence of GAL-immunoreactivity (GAL-LI) in an invertebrate, the blowfly *Phormia terraenovae*. Immunocytochemistry indicated that there are about 160 GAL-IR neurons in the brain and subesophageal ganglia. In the brain GAL-IR fibers innervate the central body, superior protocerebrum, medulla of the optic lobe and tritocerebral neuropil. Neurosecretory cells of the median neurosecretory group (MNC) also display GAL-LI. GAL-LI is present in acetic acid extracts of fly heads as measured in RIA. Gel filtration indicated that the GAL-LI material is heterogeneous with components of about the same molecular weight as porcine GAL. The hydrophobic properties GAL-LI differ from that of porcine GAL as demonstrated by rpHPLC. Several immunoreactive components from fly head extracts can be seen in the chromatogram indicating the presence of several forms of GAL-like peptides. Autoradiography experiments with ^{125}I-labeled porcine GAL on fresh frozen brain sections revealed GAL binding sites in the central body and in deutocerebrum. The binding was displaced by porcine GAL at a concentration of 10^{-7}M. Our findings indicate the presence of GAL-like peptide(s) and putative binding sites in the fly central nervous system and suggest a role in neuromodulation in circuits of the brain as well as a neurosecretory role of the peptide(s).

114.14

OCTOPAMINE-LIKE IMMUNOREACTIVITY IN THE FUSED SPIDER CNS.

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We used antiserum against octopamine *(OA)* to map the serially organized system of OA-like immunoreactive (*OA-ir*) neurons in the fused ganglion complex of the wandering spider *Cupiennius salei*.

In the suboesophageal ganglion complex *(SOG)*, the total number of *OA-ir* somata varies and ranges from 56 to 73; all lie ventromedially. Surprisingly, there is no strict bilateral symmetry in soma number and location. Neurites from somata in the SOG ascend dorsally. Some processes end near longitudinal and anterior septa. Other *OA-ir* arborizations merge into plurisegmental tracts connecting the various neuromeres; collaterals project into lateral neuropil.

In the supraoesophageal ganglia *("brain")*, we find *OA-ir* somata merely in the 2 cheliceral neuromeres. Neurites from 2 bilateral clusters of 4 to 5 large somata (ca. 25µm in diameter) descend to the SOG where they continue into the opisthosomal neuromeres as 2 longitudinal tracts. Processes from smaller somata (<10µm) project into the anterio-dorsal brain. Profuse *OA-ir* projections end in the "central body" of the spider brain.

Except for fine varicosities at the roots of nerves, we find no *OA-ir* fibers leaving the CNS. Within the CNS, however, *OA-ir* terminals are concentrated near hemolymph spaces. Hence we suspect that OA acts directly at central synapses, while it reaches peripheral sites via the hemolymph system. [Supported by the DFG, SFB 45/A3]

114.15

PROCTOLIN AND OCTOPAMINE SYNERGISTICALLY ENHANCE cAMP LEVELS IN CRAYFISH TONIC FLEXOR MUSCLE. K. Obrietan*, C.A. Bishop and J.J. Wine. Dept. of Psychology, Stanford Univ., Stanford, CA 94305-2130.

Complex modulatory input can dramatically alter muscle function. For example, in the tonic flexor muscle of the crayfish, the cotransmitter proctolin and the hormone octopamine synergistically enhance both depolarization-induced tension and the activity of a large conductance plasma membrane Ca^{2+} channel (38 pS in 137 Ba^{2+}). CPT-cAMP also enhances activity of the Ca^{2+} channel, suggesting that modulation might occur via a cAMP pathway. Therefore, we examined the effects of proctolin and octopamine alone and together on cAMP levels in the tonic flexor muscles.

cAMP levels were measured in the presence of 0.5 mM IBMX using radioimmunoassay. Proctolin's enhancement of tension and Ca^{2+} channel activity is seasonal, with the lowest effect seen in winter, during which its effect can be restored by addition of octopamine. All of our experiments were performed between December and March, when proctolin's effect is predicted to be small. Addition of a maximal concentration of proctolin ($5x10^{-9}$ M, this concentration produces the greatest tension and channel responses) produced no significant increase in cAMP levels, whereas addition of a moderate concentration of octopamine (10^{-5} M) produced a 4-fold increase in cAMP levels. cAMP levels were measured as pmol/mg protein and are given as the mean±S.E.M). Results were:

control: 20.4±2.2 (n=16); **proctolin:** 26.7±5.0 (n=9), n.s.;
octopamine: 88.4±7.8 (n=6), $p < 0.05$.

For **proctolin + octopamine:** cAMP levels rose to 193.2±16.3 (n=4), ~10x the control level and 2x the level for octopamine alone ($p < 0.01$ and 0.05 respectively). These results demonstrate a synergistic enhancement of cAMP levels by the two neuromodulators, which parallels and may explain the synergistic enchancement of Ca^{2+} channel activity and muscle tension.

114.16

DEVELOPMENTAL AND GENDER DIFFERENCES IN PROCTOLIN AND DOPAMINE-LIKE IMMUNOREACTIVITY IN THE BLUE CRAB. Debbie Wood, Charles Derby, Ted Simon. Georgia State Univ., Dept. of Biology, Atlanta

We are interested in the modulation of simple motor systems by peptides and biogenic amines. Exogenously applied proctolin and dopamine produce rhythmic and postural components, respectively, of a courtship behavior in male blue crabs, and produce limited effects on juveniles and female adults. We have examined proctolin- and dopamine-like immunoreactivity across developmental and gender groups to understand these behavioral differences. Juveniles and adults show differences in the locations of cell bodies and numbers of fibers showing dopamine-like IR in the protocerebrum, optic nerve and circumoesophageal connectives. We are investigating the potential that proctolin may be expressed seasonally in the adult male.

Supported by NIMH Pre-doc Fellowship & Whitehall Foundation.

114.17

PROCTOLIN DEGRADATION BY THE CRAB NERVOUS SYSTEM. M.J. Coleman[1], M.P. Nusbaum[1] and B.S. Rothman[2]. [1]Neurobiology Research Center/Dept. of Physiol. & Biophys.; Univ. of Alabama, Birmingham; Birmingham, AL 35294; [2]Dept. of Biology; San Francisco State Univ.; San Francisco, CA 94132.

The pentapeptide proctolin (RYLPT: 10nM) excites the pyloric network in the stomatogastric ganglion of the crab, *Cancer borealis*. This proctolin effect is enhanced by co-application of amastatin (50uM), an aminopeptidase inhibitor (Nusbaum et al., Soc. Neurosci. Abstr. 15:366), suggesting that proctolin is enzymatically inactivated in the extracellular space. To test this hypothesis, we incubated this peptide with the larger, thoracic ganglion (TG) and used HPLC analysis to determine the fate of proctolin.

Over time (2-120 minutes at 13°C), the HPLC-detected proctolin peak diminished and three novel peaks appeared. Two of these peaks co-migrated with synthetic proctolin fragments YLPT and LPT. The third peak has yet not been identified. The YLPT fragment was produced in greatest abundance. Proctolin (10uM) degradation was reduced by co-applying amastatin (100uM). With amastatin present, the YLPT peak size was reduced to a greater extent than were those of the other two fragments. Conditioned saline (CS), which was incubated with the TG and then removed, was also effective in degrading proctolin. Incubating either the TG or CS with 10mM Co^{2+} reduced both the amount of proctolin degradation and the YLPT peak size. However, Co^{2+} increased the peak size of the other two proctolin fragments. These results suggest that proctolin is cleaved by at least two different peptidases in the crab CNS. These include an aminopeptidase that is suppressed by Co^{2+} and amastatin, and a second, Co^{2+}-insensitive peptidase.

Supported by BNS-8909613 (MPN) and NS24046 (BSR).

114.18

IMMUNOCYTOCHEMICAL IDENTIFICATION OF MULTIPLE CCK-RELATED PEPTIDES IN THE STOMATOGASTRIC NERVOUS SYSTEM OF SEVERAL SPECIES OF DECAPOD CRUSTACEA. Andrew E. Christie*, G.G. Turrigiano, S. Retter*, Wendi S. Neckameyer, and Eve Marder.
Dept. of Biology, Brandeis Univ., Waltham, MA 02254

Turrigiano and Selverston showed that a CCK-related molecule plays a role in modulating the feeding behaviour of crustacea (1990, Nature 344:866-868). Using a polyclonal antibody generated against mammalian CCK8 designated 243, these authors determined the distribution of CCK-related immunoreactivity in the stomatogastric nervous system of several species of decapod crustacea (1991, J. Comp. Neurol. 305:164-176).

Here, we compare the results of staining with two monoclonal antibodies, C36-9H and C37-4E, generated against CCK8 (Stretton laboratory, University of Wisconsin-Madison) with the results of Turrigiano and Selverston. In *Panulirus interruptus*, *Cancer borealis*, and *Homarus americanus*, each monoclonal antibody recognizes a different set of immunoreactive structures of which only subpopulations also stain with 243. For example, in the oesophageal ganglion of *P. interruptus* 243 detects no immunoreactive structures while C36-9H recognizes a diffuse neuropil, and C37-4E a set of brightly staining somata. All of these antibodies recognize an intense neuropil within the stomatogastric ganglion. The staining seen with these antibodies suggests a differential distribution of several CCK-related peptides within the stomatogastric nervous system. Supported by NS 17813.

114.19

BOTH GABA AND HISTAMINE ACTIVATE A CHLORIDE CONDUCTANCE IN MOTONEURONS OF THE LOBSTER CARDIAC GANGLION. J. Freschi, H. Hashemzadeh-Gargari, and J. Kerrison. Neurology Dept., Emory University, Atlanta, GA 30322.

The cardiac ganglion of decapond crustaceans is innervated by a single pair of inhibitory axons from the central nervous system. From indirect evidence, it is thought that the neurotransmitter released by the inhibitory fibers is GABA. GABA mimicks the IPSP, which has been shown to be a chloride-dependent potential, and picrotoxin blocks both the IPSP and the response to exogenously applied GABA. We found that GABA and histamine (neurotransmitters) have similar actions on motoneurons of the cardiac ganglion. Both neurotransmitters evoke a current that reverses at E_{Cl} under various conditions of altered internal and external [Cl^-]. The underlying Cl^- conductance in both cases is voltage-dependent. Both neurotransmitter-activated Cl^- conductances were inhibited by drugs known to interact at Cl^- channels, such as curare and picrotoxin. GABA responses were not inhibited by bicuculline. Histamine responses were best blocked by the H_2-blocker cimetidine. One difference between the effects of the two neurotransmitters was that GABA strongly inhibited the burst potentials whereas histamine did not. This suggests that GABA but not histamine may inhibit small pacemaker cells. Since it is not known whether histamine is released by inhibitory synapses or released from near-by pericardial organs into the hemolymph as a neurohormone, the hypothesis that GABA is the inhibitory neurotransmitter released by the inhibitory fibers must be re-examined by cross-desensitization experiments and by using pharmacological agents specific to the receptor rather than the Cl^- channel (supported by NIH NS-22628).

115.1

IDENTIFICATION AND LOCALIZATION OF DOPAMINE IN FRESHWATER *HYDRA* BY HPLC AND IMMUNOHISTOCHEMISTRY. M. Carlberg, University of Lund, Department of Zoology, Helgonavägen 3, S-223 62 Lund, Sweden.

Coelenterates are the most primitive animals known to have a nervous system. Different FMRFamide-like neuropeptides probably play an important role in chemical neurotransmission in these animals. The role of monoamines and other "classical" transmitters in coelenterate nervous systems is more uncertain. There is good chemical evidence for dopamine (DA) in coelenterate tissue, but its localization and function has not been established. In the present communication, quantitative determination of DA in single specimens of *Hydra* with HPLC is combined with immunohistochemical localization.

Single specimens of *Hydra attenuata* were analysed with HPLC for DOPA, catecholamines and 5-S-cysteinylDOPA (5-SC.D). DOPA generally occurred in minor amounts usually not exceeding 1 ng/specimen. The major metabolites of DOPA seemed to be DA (3.3 ± 4.0 ng/specimen, n=48) and 5-SC.D (3.2 ± 2.5 ng/specimen, n=24). A marked correlation between the amounts of DA and 5-SC.D indicated that the dopamine biosynthesis in *Hydra* is not protected from oxidative side-reactions.

To relate the DA content in single *Hydra* with immunohistochemical observations, living *Hydra* were cut in half - one half was analysed with HPLC and the other half was processed for immunohistochemistry. Occasional DA-immunoreactive neurites were found in specimens containing moderate to high amounts (>3 ng) of DA.

This investigation is the first evidence of a catecholamine in a coelenterate nervous system. Its sporadic occurrence suggests that it may have a function during certain physiological conditions rather than being a generally occurring neurotransmitter.

Supported by the Swedish Natural Science Research Council.

115.2

POLYCHLORINATED BIPHENYL (PCB) EFFECTS ON BIOGENIC AMINES IN THE PLANARIAN, DUGESIA DOROTOCEPHALA. R.F. Seegal and L.G. Hansen*. Wadsworth Center, NYSDOH, Albany, NY 12201 and College of Veterinary Medicine, University of Illinois, Urbana, IL 61801.

Planarians have a well-developed nervous system with many biochemical similarities to the vertebrate nervous system. Because planarians are fresh water organisms they may provide a valuable bioassay for determining toxic effects from environmental chemicals and mixtures. We used sensitive HPLC methods to determine if Aroclor 1254 induces neurochemical deficits in D. dorotocephala similar to those we have seen in mammalian preparations.

Solutions of Aroclor 1254 in acetone (10, 25, 50 or 100 mg/beaker) were deposited on 600 ml beakers, the acetone evaporated and a standard culture medium (10 ml/planarian) added. After 1,2, or 4 weeks planarians were weighed, frozen and homogenized in 0.2N perchloric acid containing 100 mg/l of EGTA. Only dopamine (DA) and serotonin (5-HT) were detected with their concentrations varying over the 4-week exposure period, due perhaps to changes in nutritional and developmental status. Aroclor 1254 reduced DA in a dose-dependent manner (resulting in a maximum 30% decrease) when control DA levels were less than 0.35 ng/mg wet weight. 5-HT levels, elevated at intermediate doses, were also reduced by 30% at the high-dose exposure.

Changes in biogenic amine concentrations after toxicant challenge indicate that D. dorotocephala can be used to monitor exposure to complex toxicants and elucidate their effects on basic neurochemical function.

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115.3

ASCARIS FMRFAMIDE-LIKE PEPTIDES. C.Cowden and A.O.W. Stretton. Dept. of Zoology, University of Wisconsin-Madison 53706

We are interested in the role of neuropeptides in the control of locomotion in Ascaris. We have been isolating FMRFamide-like peptides (FLPs) from Ascaris heads using an RIA that detects -RFamide peptides. AF1 and AF2 have similar chemical structures and similar effects on muscle tension including relaxation, contraction, and rhythmic activity. Two more Ascaris FLPs have been isolated by reverse phase HPLC and confirmed by microsequencing and FAB-mass spectroscopy. The effects on muscle tension of one of these, AF4, are strong contraction and rhythmic activity; there is no relaxation effect.

In addition, there is sequence data for 9 other FLPs which are not related by proteolytic cleavage or post-translational modification. Even though peptides are extracted from 10,000 heads, the amount of purified peptide is often less than 100 pmoles making it necessary to repeat the purification in order to confirm the chemical structure. HPLC analysis has shown that Ascaris FLPs are differentially distributed between heads and tails, between females and males, and between acetone and acid methanol extracts. These findings will be useful in confirming the structures of the other Ascaris FLPs. NS07954(CC),AI20355(AOWS)

115.4

DIFFERENTIAL DISTRIBUTION OF AF1, A FMRFamide-LIKE NEUROPEPTIDE IN ASCARIS NERVOUS SYSTEM REVEALED BY SPECIFIC MONOCLONAL ANTIBODIES. Paisarn Sithigorngul* and Antony O.W. Stretton, Department of Zoology, University of Wisconsin-Madison, WI 53706.

Monoclonal antibodies were generated from mice immunized with peptide AF1 (KNEFIRFamide, Cowden et al., 1989) conjugated to BSA or ovalbumen with paraformaldehyde. Four monoclonal antibodies specific to AF1 peptide were obtained. ELISA and dot blot tests showed that none of these antibodies bound to BSA conjugates of either FMRFamide or AF2 (KHEYLRFamide; Cowden and Stretton, 1990). These antibodies recognize a small subset of the neurons that are recognized by other monoclonal antibodies that bind to FMRFamide as well as to AF1 and AF2. This evidence suggests that FMRFamide-like peptides are differentially localized in different subpopulations of neurons in the Ascaris nervous system. (Supported by AI 20355).

115.5

THE EFFECTS OF BIOGENIC AMINES ON ASCARIS LOCOMOTION. C.A.Buchanan and A.O.W.Stretton. Neuroscience Training Prog and Dept. Zoology, Univ. Wisconsin, Madison, WI 53706.

We have developed a behavioral assay to investigate the influence of biogenic amines on Ascaris locomotion. Ligaturing the head induces Ascaris to generate anteriorly propagating body waveforms. This is called the head restricted behavior (HRB). Initiation of waveform propagation occurs near the egg-laying pore (ELP). Generally there are 3 complete waveforms anterior to the ELP.

Previously, Stretton and Johnson (Soc. Neurosci. Abstr. 11: 184.8) showed that there are two serotonin (5-HT)-like immunoreactive neurons in the pharynx with a putative neurosecretory function. Ascaris with heads ligatured posterior to the pharynx, were injected posterior to the ligature with 0.1 ml test aliquots and the effects on HRB scored. Injections of dye showed that there was a ca. 10X dilution in pseudocoelomic fluid. 5-HT (10^{-2} M) caused a paralysis of forward movement, the disappearance of waveforms anterior to the ELP, and a 3% increase in body length. Dopamine (DA) and octopamine (OA) (both at 10^{-2} M) disrupt HRB, but produced decreases in body length. Tryptophan, 5-hydroxytryptophan, tryptamine, histamine (all at 10^{-2} M), and Ascaris saline had no detectable effects on HRB. These results suggest the presence of 5-HT, DA, and OA receptors which affect Ascaris locomotion.

115.6

SEROTONIN AND MALE MATING IN THE NEMATODE *C. ELEGANS* C. M. Loer and C. J. Kenyon*. Dept. of Biochemistry and Biophysics, Box 0554, University of California, San Francisco, CA 94143.

We are seeking to identify genes required for specification of neurotransmitter phenotype in the nematode *C. elegans*. Mutations in genes that alter transmitter expression may be identifiable by specific behavioral defects in worms that carry such mutations. For example, mutants lacking immunocytochemically detectable serotonin entirely (*cat-4*) or from neuronal processes (*cat-1*) exhibit reduced male mating efficiency (Hodgkin, Genetics 103: 43, 1983; Desai et al., Nature 336: 638, 1988). Staining with serotonin antisera suggests that some CP and CA cells, male-specific motoneurons located along the ventral nerve cord, use serotonin (G. Garriga, pers. comm.). Preliminary observations suggest that these putative serotonergic neurons assist in one or more components of male mating behavior. Application of serotonin to adult males results in tight ventral curling of the tail, which contains the male-specific copulatory structures. This posture is reminiscent of a behavior exhibited by males during mating behavior: when an adult male moving its tail along the surface of a hermaphrodite reaches the end of its intended mate, it makes a tight ventral curl to place the tail in contact with the opposite surface. The male then continues moving its tail along that surface in search of the vulval opening. This behavior may be repeated many times before the vulva is located and copulation is initiated. Adult males in which precursors of CP and CA neurons were ablated appear to exhibit difficulty in executing the tight ventral curl; the defect is similar to that we have observed in *cat-1* mutant males. We are presently seeking to quantify the effects of mutations that alter serotonin expression and of ablation of CP and CA neurons on this aspect of male mating behavior. We also plan to identify additional mutations that alter serotonin expression or expression of genes required for serotonin synthesis.

115.7

IDENTIFICATION OF GLUTAMATE AND AVERMECTIN-SENSITIVE CHLORIDE CURRENTS IN *XENOPUS* OOCYTES INJECTED WITH mRNA FROM THE NEMATODE *CAENORHABDITIS ELEGANS*. D. F. Cully*, P. S. Paress*, K. K. S. Liu*, C. J. Cohen, and J. P. Arena*. Merck & Co. Biochemical Parasitology, Rahway, N.J. 07065.

The avermectins (AVM) are a family of naturally occurring macrocyclic lactones with anthelmintic and insecticidal activity. To investigate the mode of action of these compounds we have used as a model the free living nematode *Caenorhabditis elegans*. *C. elegans* is highly sensitive to the AVMs and contains a membrane associated high affinity AVM binding site (K_D= 0.2 nM). In this study we have used the *Xenopus* oocyte for expression of *C. elegans* mRNA and have identified an AVM-sensitive membrane current. Application of AVM to injected oocytes increased inward membrane current by 241±50 nA, when measured at -80 mV using a two microelectrode voltage clamp. The AVM-sensitive current was unchanged in oocytes injected with EGTA and exhibited a reversal potential of -19 mV that shifted in low chloride solutions as predicted for a chloride current. Injected oocytes were insensitive to GABA, picrotoxin or bicuculine. Oocytes injected with *C. elegans* mRNA also responded to glutamate with an increase in inward membrane current exhibiting properties consistent with a chloride current. When maximal concentrations of AVM and glutamate were used the response was less than additive, suggesting they both activate the same current. Size fractionation of mRNA showed that the AVM and glutamate-sensitive current was enriched in the 1.1-2.5 Kb mRNA class. A cDNA library has been synthesized and is being used to screen for expression in oocytes.

115.8

EFFECTS OF NEUROACTIVE ANTHELMINTICS AND METABOLIC INHIBITORS ON MUSCLE FUNCTION AND ATP LEVELS IN HAEMONCHUS CONTORTUS. J.P.Davis*, E.M.Thomas* and D.P.Thompson. Parasitology Research, The Upjohn Co., Kalamazoo, MI 49001.

To determine the relationship between muscle function and energy charge in the gastrointestinal nematode *Haemonchus contortus*, we measured the effects of several neuroactive anthelmintics and metabolic inhibitors on motility, axial muscle tension and ATP during *in vitro* incubations. Concentration-dependent effects of drugs on motility were measured using an automated recording system consisting of a 4-channel Micromotility Recorder (B&P Inst., East Lansing, MI) and a Zymark® robotics system. ATP levels were measured using a bioluminescence assay. Axial muscle tension in 2-3 mm neuromuscular strips were recorded using a modified suction electrode-balance beam system. Results of these tests showed that ATP levels were not affected by the neuroactive anthelmintics ivermectin, milbemycin D, levamisole or pyrantel; all of which caused rapid paralysis of the axial muscle at therapeutic concentrations. ATP and motility levels were reduced in a concentration- and time-dependent manner by closantel and carbonyl cyanide phenylhydrazone, agents which inhibit oxidative phosphorylation in nematodes, and by potassium antimony tartrate, which inhibits the enzyme phosphofructokinase in nematodes. Isotonic tension recordings showed that the metabolic inhibitors cause a flaccid paralysis of *H.contortus* segments, while the neuroactive agents cause a spastic paralysis (levamisole, pyrantel) or do not affect tension (ivermectin, milbemycin D). These results suggest that the anthelmintic actions of the neuroactive agents tested do not depend on ATP depletion.

115.9

BIOSYNTHESIS OF RFAMIDE PEPTIDES IN LEECH CNS NEURONS. B.D. Evans, J.L. Sperring and R.L. Calabrese. Dept. of Biology, Emory Univ., Atlanta, GA 30322.

Recently, five RFamide peptides (containing the carboxyl terminal sequence -Arg-Phe-amide) were isolated and identified from central nervous system extracts of the leech Hirudo medicinalis (Evans et al., 1991). Three of these peptides contain methionine (FMRFamide, YMRFamide, & GGKYMRFamide). Upon depolarization, some or all of these peptides are released from neural processes on heart tubes in a Ca^{++}-dependent manner. Cropper et al. (1987) and Church & Lloyd (1990) have incorporated ^{35}S-methionine into newly synthesized peptides to demonstrate the biosynthesis of methionine-containing peptides by individually identified CNS neurons of the mollusc Aplysia.

Several CNS neurons in leech, including heart motor neurons, contain RFamide peptides as indicated by immunocytochemistry (Kuhlman et al., 1985). Whole nerve cords *in vitro* incorporate ^{35}S-methionine into a large number of peptides as indicated by rpHPLC elution profiles. Heart motor neurons also incorporate ^{35}S-methionine into peptides. A radioactive peak obtained from rpHPLC analysis of heart motor neuron extracts coelutes with synthetic FMRFamide, indicating that these neurons synthesize FMRFamide. We are presently analyzing extracts from large numbers of heart motor neurons (10 or more) to verify our results. Other identified neurons which contain RFamide peptides, such as heart modulatory neurons or anterior pagoda cells, will be analyzed in a similar manner. We suggest that heart motor neurons synthesize FMRFamide and possibly YMRFamide and GGKYMRFamide.

115.10

IDENTIFICATION OF CATECHOLAMINES, INDOLEAMINES AND THEIR METABOLITES IN THE SEA PANSY, RENILLA KOELLIKERI. A.K. Pani*, D. Umbriaco and M. Anctil. Dépt. de sciences biologiques and CRSN, Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

Several monoamines were reported to activate or modulate light emission and/or muscular activities in this colonial cnidarian species. These studies prompted the present biochemical investigation of such amines in the sea pansy using HPLC with electrochemical detection and radioimmunoassays. Among the catecholamines, dopamine, norepinephrine and epinephrine were present in variable amounts, and there was a correlation between the detected levels of the latter two amines and the intensity of their immunohistochemical reactivity in the same colonies. Among the indoleamines, both serotonin and a melatonin-like substance were detected. In addition, metabolites such as DOPAC, normetanephrine, metanephrine, N-acetylserotonin and 5-HIAA were occasionally detected. In view of the key phylogenetic position of Cnidaria regarding the evolutionary emergence of nervous systems, these results suggest that all major vertebrate monoamines and their oxidation, methylation and acetylation pathways are evolutionarily ancient.

BEHAVIORAL PHARMACOLOGY II

116.1

REDUCED IMMOBILITY FOLLOWING ANTIDEPRESSANTS IN A GENETIC ANIMAL MODEL OF DEPRESSION. D.H. Overstreet, G D. Schiller*, O. Pucilowski*. Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27514.

The Flinders Sensitive Line (FSL) rats exhibit exaggerated immobility in the forced swim test, increased sensitivity to cholinergic agonists, & increased numbers of muscarinic receptors (Overstreet et al., Experientia 44:465-472, 1988). The present project assessed whether this immobility could be overcome by chronic treatment with classical antidepressants (imipramine, desmethylimipramine), a novel antidepressant (sertraline), or an anticholinesterase (DFP). Imipramine, a classical antidepressant with strong inhibiting effects on the uptake of both serotonin and norepinephrine, almost completely counteracted the exaggerated immobility in the FSL rats. Desmethylimipramine, with more selective inhibition of norepinephrine uptake, & sertraline, with more selective inhibition of serotonin uptake, had only partial counteracting effects. DFP was without any counteracting effect. None of these agents, at the dose regimens used, significantly altered the immobility of control, Flinders Resistant Line rats. The findings confirm the utility of the FSL rats as an animal model of depression, since the mixed spectrum antidepressant produced the greatest "therapeutic" effect, as is the case in human depressives. In addition, these findings call into question the postulated association between elevated muscarinic receptors and immobility. Rather, changes in serotonin and/or norepinephrine are more likely to underlie the exaggerated immobility of the FSL rats.

116.2

5-HT_1 MODULATION OF SENSORIMOTOR REACTIVITY AND SPONTANEOUS ACTIVITY IN MICE. M. Schmidt, D. Helton, D. Modlin*, and J. Tizzano. Lilly Research Laboratories, Eli Lilly and Company, Greenfield, Indiana 46140.

In rats, $5HT_{1A}$ agonists increase startle while 5-HT_{1B} agonists decrease startle amplitude, and both types of 5-HT_1 agonists decrease activity levels. In the present study, auditory startle and spontaneous activity in male CD-1 mice were evaluated after treatment with: 5-HT_{1A} receptor subtype agonists, LY228729 (0, 0.05, 0.5, 5.0 mg/kg, PO) or LY165163 (PAPP, 0, 0.05, 0.5, 5.0 mg/kg, PO); the partial 5-HT_{1A} agonist 8-OH-DPAT (0, 1.0, 3.0, 10.0 mg/kg, IP); or the preferential 5-HT_{1B} agonist, 1-(m-chlorophenyl) piperazine (MCPP, 0, 1.25, 2.5, 5.0 mg/kg, IP). Spontaneous activity was measured for 2 hours in a novel home-cage environment (Multi-Varimex). Auditory startle was measured in automated SDI chambers. Each startle test session involved a 5-minute acclimation period at a background noise level of 70 dBA prior to 50 presentations of 120 dBA noise bursts at 8-second intervals. LY228729 and LY165163 produced dose-related decreases in auditory startle amplitudes and decreased initial activity levels, at 5.0 mg/kg. 8-OH-DPAT decreased startle and produced a biphasic effect on activity levels. In contrast, at 5.0 mg/kg MCPP increased startle amplitude but produced a dose-related decrease in activity levels. These data indicate that the effects of 5-HT_1 agonists are dependent not only on the specific receptor subtype involved and the behavior measured, but also on the species tested.

116.3

POTENTIAL ANXIOLYTIC ACTIVITY OF 5-HT1A PARTIAL AGONISTS MDL 102,181 AND MDL 73,005EF IN ANIMAL MODELS OF ANXIETY SENSITIVE TO BUSPIRONE. J.M. Hitchcock, T.C. McCloskey*, R.A. Padich*, D.R. McCarty*, M.W. Dudley, J.S. Sprouse, J. Freedman, and J.H. Kehne, Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215.

Compounds acting at the 5-HT1A receptor represent a new class of anxiolytics devoid of the sedative and muscle relaxant effects seen with the benzodiazepines. The 5-HT1A partial agonist buspirone is representative of this anxiolytic class. Although buspirone is a clinically effective anxiolytic, it does not show anxiolytic activity in all animal models of anxiety. Two models in which buspirone does show anxiolytic activity are the fear-potentiated startle paradigm, in which rats exhibit an enhanced acoustic startle reflex in the presence of a conditioned stimulus that has previously been paired with shock, and the separation-induced ultrasonic vocalization paradigm, in which rat pups emit ultrasonic vocalizations when separated from the litter. These paradigms were used to assess potential anxiolytic effects of novel compounds with activity at 5-HT1A receptors.

MDL 102,181 (2-[1-(2-isopropoxyphenoxy)-2-phenyl]ethylimidazoline), an aryloxyimidazoline chemically unrelated to any known anxiolytic drug, and MDL 73,005EF (8-[2-(2,3-dihydro-1,4-benzodioxin-2-yl-methylamino)ethyl]-8-azaspiro [4, 5]decane-7,9-dione methyl sulphonate), a 1,4-benzodioxan, displayed agonistic properties at dorsal raphe autoreceptors and higher potency than buspirone at the 5-HT1A binding site. MDL 73,005EF has shown potential anxiolytic activity in other animal models (Moser et al., *Br. J. Pharm., 99,* 1990). MDL 102,181, like MDL 73,005EF (Kehne et al., *Eur. J. Pharm., 193,* 1991), suppressed separation-induced vocalizations and did not affect performance on an inclined plane test. Furthermore, both compounds decreased fear-potentiated startle without depressing baseline startle. Thus, these animal models appear to be sensitive to drugs acting at the 5-HT1A site, even those in different chemical classes than buspirone. The data indicate that the 5-HT1A partial agonists MDL 102,181 and MDL 73,005EF have profiles of selective potential anxiolytics lacking sedative or muscle relaxant activity.

116.4

BEHAVIORAL PHARMACOLOGY OF THE 5-HT_{1A} RECEPTOR LIGANDS IPSAPIRONE AND 8-OH-DPAT IN RATS AFTER REPEATED ADMINISTRATION. J. De Vry*, R. Schreiber* and T. Glaser. Institute for Neurobiology, Troponwerke, Berliner Straße 156, 5000 Köln 80, FRG

Acute administration of the 5-HT_{1A} receptor ligands ipsapirone (I, partial agonist) and 8-OH-DPAT (D, full agonist) [8-hydroxy-2-(di-n-propylamino)tetralin] results in anxiolytic, anti-aggressive and antidepressive effects in several animal models [De Vry et al.(1991).In Briley M and File SE (Eds) New Concepts in Anxiety. MacMillan Press, London, chapter 7]. Rats treated for two weeks with low to moderate doses of I (0.5 - 10 mg/kg i.p.) and D (0.03 - 1 mg/kg i.p.) do not develop tolerance to the anxiolytic effects in a shock induced ultrasonic vocalisation test. With regard to the antidepressive effects in a rat forced swimming test, repeated treatment with low doses either do not affect (D), or even produce sensitization (I), whereas repeated treatment with higher doses results in weak (I, $\geq$ 10 mg/kg i.p.) and strong tolerance (D, $\geq$ 0.3 mg/kg i.p.). Tolerance to the antidepressive effect of D (higher doses), as well as to its capacity to induce a 5-HT behavioral syndrome, develops already after 1 or 2 applications; a finding not observed in the case of its hypothermia and hyperphagia effects. The data suggest that the behavioral effects of 5-HT_{1A} ligands after repeated treatment are the result of a complex interaction of tolerance and sensitization processes. It is hypothesized that the development of tolerance/sensitization depends on (a) the particular behavioral effect (reflecting the activation of a particular population of pre- and/or postsynaptic 5-HT_{1A} receptors), (b) the dose and (c) the intrinsic activity of the compound at these receptors.

116.5

LY53857 BLOCKS RESPONSE SUPPRESSION (RS) INDUCED WITH 5-HTP AND DOI BUT NOT WITH 8-OH-DPAT IN AN ANIMAL MODEL OF DEPRESSION. E.A. Engleman, J.N. Hingtgen, J.M. Murphy, F.C. Zhou, and M.H. Aprison. Depts. Psychiat; Biochem; Psychol; & Anat; Prog. Med. Neurobiol.; Inst. Psychiat. Res.; Indiana U. Sch. Med. & Purdue Sch. Sci. IUPUI, Indianapolis, IN 46202.

Studies from this laboratory have demonstrated that administration of the selective 5-$HT_{2/1C}$ antagonist LY53857 can block 5-HTP induced RS (Hingtgen et al., Biol. Psychiat., 20:592 1985). To further investigate the serotonergic mechanisms involved in this effect we tested the ability of LY53857 to block RS induced with DOI and 8-OH-DPAT (agonists selective for 5-$HT_{2/1C}$ and 5-HT_{1A} receptors, respectively). Rats trained to press a lever for milk reinforcement on a VI 1' schedule were given 1.0 mg/kg IP injections of DOI or 8-OH-DPAT to induce RS after a 15 minute baseline period. IP injections of 1.0 mg/kg LY53857 1 hour before DOI or 8-OH-DPAT resulted in a 100% blockade of DOI induced RS but did not block RS induced with 8-OH-DPAT. These results indicate that RS can be induced with agonists selective for 5-$HT_{2/1C}$ or 5-HT_{1A} receptors, but 5-HTP induced RS shows more pharmacological similarity to DOI induced RS and may be mediated primarily through 5-HT_2 and/or 5-HT_{1C} receptors. (Indiana Dept of Mental Health, IUPUI Research Investment Fund, and AA08553).

116.6

SELECTIVE ATTENUATION OF THE DOI-INDUCED HEAD SHAKE RESPONSE IN RATS BY DEFEAT IN TERRITORIAL AGGRESSION. **D.J. Knapp, D. Benjamin, & L.A. Pohorecky,** Rutgers University Center of Alcohol Studies, Piscataway, NJ 08855-0969.

Head shakes induced by DOI (1-(2,5,-dimethoxy-4-iodophenyl aminopropane)-2) reflect 5-HT_2 receptor function. Neurochemical changes induced by antidepressant drugs attenuate 5-HT_2 receptor function; an effect correlated with antidepressant efficacy. To characterize the effects of non-pharmacological manipulations, such as stress, on 5-HT_2 receptor function, the effects of four qualitatively different stressors on DOI-induced head shakes were investigated. Male Long Evans rats were exposed to one of the following conditions at least 24 hours prior to a 30 minute observation period which began with DOI injection (0.64 mg/kg, ip): 1) no manipulation, 2) repeated exposure to startle-inducing air puff, 3) 2 hour exposure of resident rats to intruder rats, 4) 2 hour exposure to a novel environment similar to the resident's cage, or 5) 2 hour exposure of intruder rats to resident rats. Head shake responses following these manipulations were 12.6±1.9, 10.6±1.6, 9.9±2.1, 8.7±2.4, 2.8±0.5, respectively. Analysis of variance of these data indicate a significant overall effect ($p < .05$), the variance of which was accounted for entirely by the intruder rat group ($p < .0026$). Pre-exposure to the novel environment alone, air puff stimuli, or status as a resident were not sufficient to reduce head shakes. It is concluded that exposure to a stressful encounter with an aggressive resident produces a decrement in the function of 5-HT_2 receptors which may reflect a specific adaptive response to stress induced by conspecific attack. (Supported by USPHS AA05306, AA08499, and a Smithers Foundation Grant)

116.7

DIFFERENTIAL ANTAGONISM BY KETANSERIN OF BEHAVIORAL EFFECTS OF 5HT$_2$ VERSUS 5HT$_{1B}$ AGONISTS ON A COUNTING PROCEDURE. **M.A. Kautz, R. Preston*, G. Agritellis*, Z. Ritch*, and G. Galbicka*. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.**

The behavioral effects of the 5HT antagonist ketanserin were examined alone and in combination with the 5HT$_{1B}$ and 5HT$_2$ agonists TFMPP and DOI, respectively, in a complex operant task designed to mimic control by response number, or "counting." Rats pressed the left of two response levers one or more times and then made one press on the right lever to end a trial. The probability of reinforcement increased systematically as the number of left lever responses (a "run") more closely approximated the target value of 12. Under one procedure the current run was reinforced if it was closer to the target than two-thirds of the most recent 24 runs. This maintained a constant reinforcement probability of 0.33. Under a second procedure, the reinforcement probability was fixed such that runs around the target would result in the same overall reinforcement probability, but any drug-induced disruption in run length decreased reinforcement probability.

The 5HT$_{1B}$ and 5HT$_2$ agonists TFMPP and DOI both produced decreases in run length at a dose of 3.0 mg/kg. Ketanserin (0.3-30.0 mg/kg) administered in isolation only decreased run length when reinforcement probability was held constant. Administered in combination with the agonists, ketanserin substantially antagonized the effects of DOI, but not TFMPP. These behavioral results are consistent with differential sensitivity of ketanserin at the different 5HT receptor subtypes obtained in receptor binding studies. Antagonism of DOI's behavioral effects was obtained at doses that were ineffective administered alone. This suggests that ketanserin pretreatment may be useful therapeutically in blocking the behavioral side-effects of 5HT$_2$ compounds.

116.8

EVALUATION OF 5-HT3 ANTAGONISTS ON SCHEDULE-CONTROLLED PERFORMANCE IN RATS. P.C. Mele and J.H. McDonough. Behavioral Sciences Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

5-HT3 antagonists are effective antiemetic agents; they may have antianxiety, antipsychotic and nociceptive properties as well. The present study extended the behavioral evaluation of three 5-HT3 antagonists to schedule-controlled performance in male Sprague-Dawley rats. Zacopride, GR38032F and ICS 205-930 did not alter responding under a multiple fixed-interval 2 min, fixed-ratio 20 (mult FI FR) schedule of milk presentation at 0.01-10 mg/kg IP. These doses inhibit emesis induced by ionizing radiation and chemotherapeutics, and are active in paradigms used to evaluate drugs for antianxiety and antipsychotic efficacy. A higher dose (32 mg/kg) of GR38032F and ICS 205-930 reduced both FI and FR response rates. Higher doses of zacopride (32 and 56 mg/kg) did not alter either FI or FR responding consistently. The effects of each of the 5-HT3 antagonists differed from those of several reference compounds (metoclopramide, haloperidol, buspirone and chlordiazepoxide) that were examined because of their known antiemetic, antianxiety, and/or antipsychotic effects. ICS 205-930 was administered in combination with each of the reference compounds, caffeine, scopolamine, and morphine.

116.9

ALPHA-2 RECEPTOR ANTAGONIST POTENTIATES BARBITURATE ANESTHESIA IN RATS. D.Stenberg, T.Kauppila* and Z.Lelkes*. Department of Physiology, University of Helsinki, Siltavuorenpenger 20 J, 00170 Helsinki, Finland.

Alpha-2 receptor agonists are increasingly being used as adjuvants to anesthesia, and specific antagonists are developed to end the effect. Thus the highly specific alpha-2 receptor antagonist atipamezole (A) at 1 mg/kg reverses completely the sedative-anesthetic effect in rats of 0.3 mg/kg medetomidine (M), a highly specific alpha-2 receptor agonist. We found, however, that A greatly prolonged anesthesia induced by 40 mg/kg pentobarbital ip. in rats. When A was given 20 minutes after B, the duration of EEG spiking was prolonged from a mean of 35 min to 90 min, and the loss of righting reflex from 90 to 200 min. The combination of B+M induced loss of righting for a mean of 440 min, and was only partly antagonized by A given 20 minutes after the combination. We conclude that the pharmacological occupancy of alpha-2 receptors is highly potentiating to the depressing effect of barbiturates, as evidenced by the paradoxical action of the antagonist after barbiturate.

116.10

ATIPAMEZOLE, AN ALPHA-2-ADRENOCEPTOR ANTAGONIST, INCREASES SEXUAL BEHAVIOR IN MALE MONKEYS. I.Linnankoski*, M.Grönroos*, S.Carlson and A. Pertovaara. Dept.Physiol.,Univ.Helsinki, 00170 Helsinki, Finland. (SPON: ENA)

Atipamezole is a novel imidazole-type alpha-2-adrenoceptor antagonist. The alpha-2/alpha-1 selectivity ratio of atipamezole is 200-300 X higher than that of either idazoxan or yohimbine. In the present study the effect of atipamezole on sexual behavior was studied in three male stumptail macaques (Macaca Arctoides). Following i.m. administration of atipamezole (0.01-0.30 mg/kg) or saline control the male monkey was put together with a female monkey and the behavior observed for 30 min. Atipamezole produced a significant dose-dependent increase in the number of ejaculations in all three monkeys, including an old one with decreased sexual activity in control conditions. No side-effects, except increased alertness, were observed. It is concluded that atipamezole is highly effective in increasing sexual behavior in male monkeys.

116.11

ANTAGONISM OF THE BEHAVIORAL EFFECTS OF MEDETOMIDINE BY ATIPAMEZOLE IN THE DOG. S.G.Kamerling, L.Berryhill*, C.Bagwell*, and W.R.Kilgore. Dept. of Vet. Physiol. Pharmacol. Toxicol., Sch. of Vet. Med., Louisiana St. Univ., Baton Rouge, LA 70803.

Atipamezole (ATI), a novel alpha-2 adrenoceptor antagonist, is currently being evaluated as a reversal agent for the sedative/analgesic, medetomidine (MED), in the dog. Vaha-Vahe (1990) reported that ATI (80-240 ug/kg i.m.) rapidly and effectively reversed medetomidine-induced sedation, analgesia, bradycardia, and recumbency in dogs. The purpose of the present study was to extend these observations using (1) a more extensive assessment of sedation amd ambulation, (2) a broader range of doses of ATI, and (3) mixed breed hounds of similar weight and size.

Eight male and 8 female mixed breed hounds received a 1mg/m^2 dose of MED followed 30 minutes later by ATI (1, 5, and 10 mg/m^2 and saline) according to a latin square crossover design. All three doses of ATI significantly shortened time to arousal, standing, and ambulation. Significant differences between the 1 and 10 mg/m^2 doses of ATI were observed for standing and walking times. No differences were observed between the 5 and 10 mg/m^2 doses. All three doses of ATI significantly antagonized MED-induced sedation and reduction in walking ability. The 5 and 10 mg/m^2 doses produced greater antagonism than the 1 mg/m^2 dose. However, there were no statistical differences between the 5 and 10 mg/m^2 doses. ATI produced dose related antagonism of MED-induced bradycardia. Significant reversal was observed at the 5 and 10 mg/m^2 doses.

These data support the use of atipamezole as an effective antagonist for the sedative and bradycardic effects of medetomidine in the dog. (Supported by a grant from Smithkline Beecham Animal Health)

116.12

Intracerebroventricular minocycline inhibits amphetamine-induced increases in rearing in rats. O. Kofman* [1], R.H. Belmaker[1]* and Y. Shavit[2]. Psychiatry Research Unit, Ben-Gurion University[1], Beer-Sheva & Dept. Psychology, Hebrew University[2], Jerusalem, ISRAEL.

The tetracycline derivative minocycline, like lithium, inhibits agonist-induced stimulation of cAMP in vitro and ex vivo and inhibits motor activity and amphetamine hyperactivity in rats. To verify that these behavioural effects are centrally mediated, baseline and amphetamine-induced activity were measured following injections of minocycline in the lateral ventricle. Rats were injected with minocycline HCl (50 µg) or vehicle (2µl) icv, and with either d-amphetamine (1 mg/kg) or saline ip. Amphetamine increased locomotor, total and rearing activity ($p<.001$). Minocycline significantly reduced rearing ($p<.05$), and there was a significant interaction between amphetamine and rearing ($p<.05$). Minocycline (100 µg) did not reverse hypokinesia induced by the cAMP phosphodiesterase inhibitor, rolipram. Thus, while minocycline has central effects on motor behaviour, it remains unclear whether these effects are due to its actions on cAMP.

116.13

A DOSE-RESPONSE FOR D-AMPHETAMINE AND BEHAVIORAL THERMOREGULATION IN RATS. Cheryl Chancellor-Freeland and Harry J. Carlisle . University of California, Santa Barbara, CA 93106.

Past studies have shown that hyperthermic animals under the influence of amphetamine approach heat sources while hypothermic animals avoid heat. These paradoxical effects of amphetamine on behavioral thermoregulation have been attributed to changes in thermal set-point (Yehuda & Frommer, 1978). The present experiment reexamines this notion by allowing rats to select preferred ambient temperature in a thermal gradient. Eight female rats were administered 0, 1, 5, and 10 mg/kg d-amphetamine sulfate and tested for 60 minutes. Thermal preferences as well as core temperatures were used to assess the thermic effects of amphetamine. With doses of 1 and 5 mg/kg animals responded to the thermogenic properties of the drug by lowering temperature preference. However, in contrast to previous reports (Bushnell & Gordon, 1987; Yehuda & Wurtman, 1974), these dosages also produced dose-dependent decreases in core temperature. The high dose of amphetamine (10mg/kg) produced an inconsistent pattern of thermoregulation. Some animals preferred the cold and became hypothermic, while others preferred warmth and became hyperthermic. The present findings tend to support the view that amphetamine reduces the set-point, although this effect was observed at much lower doses than previously reported (Yehuda & Wurtman, 1974).

116.14

TOLERANCE, WITHDRAWAL AND SUPERSENSITIVITY TO DOPAMINE MEDIATED CUES IN A DRUG-DRUG DISCRIMINATION PARADIGM. R.J. Barrett* & W.F. Caul. Veterans Administration Medical Center and Dept. of Psychology, Vanderbilt University, Nashville, TN 37204

Rats were trained to discriminate between 0.25 mg/kg amphetamine (AM) and 0.03 mg/kg haloperidol (HD) in a two-lever drug discrimination task. In order to test for a drug-induced withdrawal state, Ss were assigned to one of three groups and given 10 consecutive, daily injections of 10 mg/kg AM, 1.0 mg/kg HD or distilled water (DW). Ss from each treatment condition were then tested either 24, 48 and 72 hrs after the final injection. Results showed that at the 24 hr retest interval Ss injected with AM responded as though administered an acute dose of HD (0.02 mg/kg) and Ss injected with chronic HD responded as though administered an acute dose of AM (0.15 mg/kg). By 72 hrs choice behavior had returned to pre-treatment values.

To determine whether the rebound observed after 10 daily injections was present after a single injection, independent groups of subjects were injected with single doses of either 10 mg/kg AM or 1.0 mg/kg HD and then retested either 4, 6, 12, 20, 24, 30, 36, or 42 hrs later. The data showed that single doses of both AM and HD produced significant rebounds that peaked between 20 hrs (AM) and 24 hrs (HD) following administration.

In a third experiment, Ss tested with acute doses of HD 24 hr after a single 1.0 mg/kg dose of HD demonstrated enhanced AM lever choice (supersensitivity), while Ss tested with acute doses of AM 20 hr after a single 10.0 mg/kg dose of AM showed diminished AM lever choice (tolerance). Taken together the data suggest a common mechanism of action for the observed phenomena.

116.15

AGE-RELATED CHANGES IN ATTENTIONAL ABILITIES IN FISCHER-344 RATS: EFFECTS OF BENZODIAZEPINE RECEPTOR LIGANDS AND AMPHETAMINE.
K. Quigley, H.Moore*, P. Dudchenko, J.P. Bruno, and M. Sarter. Dept. Psychology, The Ohio State University, Columbus, OH 43210.

While studies on the age-related changes in cognitive functions in animals have focused on impairments in learning and memory, the human data suggest that attentional processes are predominantly affected by age. Fischer-344 rats (4, 12, 18 months) were trained in a sustained attention task that partly resembles the continuous performance task frequently used in human studies. Animals were trained to report detection of an unpredictably and rarely occurring brief visual signal (0.1 sec) by pressing a lever which remained active for 3 sec after stimulus onset. The probability for a hit and a false alarm (an operation during the prestimulus 3 sec bin) were used for the calculation of indices of signal sensitivity (SI) and response bias (RI). In comparison to 4 and 12 month old rats, aged rats showed a decrease in vigilance (SI) but not in response bias (RI). Middle-aged and aged rats were slower in responding than 4 month old animals. While these data suggested that this task model age-related changes in sustained attention, drug testings did not support this hypothesis. In contrast to human data (Koelega, 1989), chlordiazepoxide (1,3,5 mg/kg) did not affect SI and RI. Likewise, neither the nootropic ZK 93426 (1,3,5 mg/kg) nor amphetamine (0.25 mg/kg) were effective. Thus, the animals' performance appeared insensitive to these pharmacological manipulations. The nature of the effects of age remains unclear as cognitive, locomotor, and motivational demands were not varied. Future attempts to model age-related changes in attention will have to be based on a more precise analysis of the factors that provoke age-related effects in humans, particularly on the integration of recently acquired information about changes in spatial and temporal stimulus characteristics. Furthermore, conclusions on the cognitive nature of age-related effects should be based on variations of task demands.

116.16

CHARACTERIZATION OF THE SEXUAL DIMORPHISM IN STRESS-INDUCED CHANGES IN RENAL PERIPHERAL BENZODIAZEPINE RECEPTORS IN RAT
R.C. Drugan, J. Chang*, R. Park* and P.V. Holmes. Schrier Research Laboratory, Department of Psychology, Brown University, Providence, R.I. 02912

Last year we reported a gender difference in inescapable shock-induced changes in [3H]Ro 5-4864 binding to renal peripheral benzodiazepine receptors(PBR) in rat. Subsequent scatchard analysis has indicated that both genders exhibit a reduction in PBR density in kidney following inescapable shock stress, with females showing an attenuated response in comparison to males.

We have begun to characterize the etiology of these gender differences by surgical gonadectomies in both groups. Castration of male rats does not appear to influence the magnitude of the stress-induced reduction in renal PBR density. However, preliminary evidence suggests that ovariectomy in females may potentiate the stress-induced change in this group in comparison to sham surgery controls. We have also recently observed a gender difference in stress-induced alterations in both behavior and renal PBR binding following the forced swim behavioral despair test. We are currently confirming and expanding our findings in an effort to characterize the etiology of this phenomenon as well. These differences in renal PBR changes in response to stress may shed light on the cause of certain behavioral and physiological gender differences in stress responsivity. Uncovering the mediators of these gender differences in PBR responsivity to stress may aid in the discovery of the functional significance of this receptor. This research was supported by an NIMH Grant # MH 44034 and an Alfred P. Sloan Research Fellowship to RCD.

116.17

LOCALIZATION OF A BENZODIAZEPINE RECEPTOR MEDIATED, CONTROL-DEPENDENT INCREASE IN ANXIETY FOLLOWING STRESS IN THE RAT.
K. R. Short and S. F. Maier, Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

This laboratory has reported that an inescapable stressor but not an escapable stressor produces a decrease in social interaction, an animal model of anxiety, 24 hrs later. This control-dependent anxiety was blocked by the benzodiazepine receptor antagonist Ro15-1788 (flumazenil) when systemically administered prior to stress treatment but not when given prior to social interaction measurement. We now report that Ro15-1788 (200 ng in 1.0 µl) or an equal volume of vehicle microinjected into the third ventricle immediately prior to inescapable shock, via a chronically implanted cannula, reproduces the blockade of stress-induced anxiety observed following intraperitoneal injections. This result confirms that a central mechanism mediates the production of anxiety associated with inescapable stress. The search for the brain areas responsible was further narrowed to noradrenergic and serotonergic nuclei that would be capable of activating numerous cortical and diencephalic regions to produce anxiety. Cannulae were chronically implanted either on the midline into the dorsal raphé or bilaterally into the locus cœruleus. Ro15-1788 or vehicle was injected into each site (50 ng in 0.5 µl in each cannula) prior to inescapable or escapable shock exposure. Social interaction measured 24 hrs later revealed a blockade of anxiety only in inescapably-stressed rats that had received the benzodiazepine receptor antagonist in the dorsal raphé. This result suggests that neurons synapsing onto the dorsal raphé release an endogenous benzodiazepine receptor inverse agonist (anxiogenic) in response to inescapable stress but not in response to escapable stress. BNS 8808840

116.18

A COMPARISON OF THE SCHEDULE-INDUCED EFFECTS OF BEHAVIORAL TOLERANCE IN TRIAZOLAM AND COCAINE.
S.E. Bowen*, M.J. Kallman and M.R. Durnam*. Depts. of Psychol. & Pharmacol., Univ. of Mississippi, University, MS 38677.

This investigation explored the effects of repetitive oral exposure to triazolam (TZ) and cocaine (CO) on behavioral tolerance and withdrawal. Forty male rats were trained to respond on a multiple fixed ratio 30: differential reinforcement of low rate 20 sec. schedule during daily 20 min. sessions. An initial dose effect curve was obtained with 16 rats for TZ (0.05-1.0 mg/kg) or CO (2.0-24.0 mg/kg) and redetermined following 30 days of chronic exposure for each group. During the chronic exposure period half of the rats from both drug groups (N=8/drug) were gavaged presession (PRE) and the other half were gavaged postsession (POST). The remaining 8 rats were tested as saline treated controls. Disruptions in operant performance were assessed for 15 days following the termination of drug exposure. FR and DRL performance was assessed with measures of response rate (RR), response duration(RD), interresponse times (IRT), and reinforcements earned. Both TZ and CO produced greater tolerance when the rats were given the opportunity to practice under the drug state (PRE group). Repetitive exposure to CO was more susceptible to conditioned tolerance effects than repetitive TZ exposure. The severity of disruption following drug withdrawal was also enhanced for the PRE animals for both TZ and CO exposure. Supported by NIDA funds DA05253.

116.19

Incremental Shock in the Conflict Procedure Provides a Behavioral Baseline Sensitive to Bidirectional Changes in Anxiety.
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Pollard and Howard's (1979) modification of the Geller-Seifter conflict procedure was used to create a baseline of operant responding sensitive to both increases and decreases in anxiety. Rats were trained under a multiple schedule using food reward. Sessions consisted of three, six-m periods under a RI 80-s schedule alternated with three, tone-signalled, two-m periods under a CRF schedule. In addition to reinforcement, each response during the tone period was punished using an incremental shock procedure whereby every other lever press incremented the shock intensity. Animals made an average of 12 responses/conflict period for a total of 36 punished responses/session; the twelfth response corresponded with the midpoint in the range of shock intensity. The benzodiazepine agonists diazepam, chlordiazepoxide (CDP) and lorazepam produced dose-related increases in punished responding. The antipunishment effect of CDP (10 mg/kg) was antagonized by flumazenil. The putative anxiogenic compound pentylenetetrazol produced a selective suppression of punished responding while the inverse agonist FG 7142 suppressed responding under both schedules. Additional anxiogenic compounds are being tested, these include: ßCCE, DMCM and picrotoxin. Experiments investigating the usefulness of this model for studying BZ withdrawal are underway. Supported by DA 05413 and DA 05186.

117.1

MORPHOLOGY OF BULLFROG SYMPATHETIC PREGANGLIONIC NEURONS. D. Peruzzi and C.J. Forehand. Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Bullfrog sympathetic preganglionic neurons are segmentally segregated according to function (Horn and Stofer, J. Comp. Neurol. 268:71, '88). Preganglionic neurons that project to lumbar ganglion cells that innervate blood vessels are known as C cells; they are located in spinal segments seven and eight. Preganglionic neurons that project to lumbar ganglion cells that innervate non-vascular targets are known as B cells; they are located in spinal segments five and six. Whether these two classes of preganglionic neurons have similar dendritic arborizations within the spinal cord is unknown. To address this issue, we examined the morphology of these preganglionic neurons by intracellular injection with neurobiotin.

Of the five cells labeled thus far, two were C cells and three were B cells. In general, B and C cells were similar. They averaged 18 µm in cell body diameter and had an average total dendritic length of 3253 µm arising from three to four primary dendrites. However, differences were noted between B and C cells in both axonal and dendritic parameters. Axons of B cells had a larger diameter at their origin (three to four µm) than axons of C cells (one µm). Both C cell axons arose from a dendrite, while two of the B cell axons arose directly from the cell soma. One of the C cell axons branched extensively within the spinal grey. Both cell types had extensive dendritic projections into the lateral funiculus. In contrast to B cells, C cell dendrites also extended into the dorsal horn. This dorsal extension of C cell dendrites may be involved in specific central connections for vascular control.

117.2

GENE EXPRESSION OF TYROSINE HYDROXYLASE AND NPY IN PREVERTEBRAL GANGLIA OF RENAL HYPERTENSIVE RATS. T.L. Krukoff and Y. Zheng*. Dept. of Anatomy & Cell Biol., Fac. of Medicine, Univ. of Alberta, Edmonton, Canada T6G 2H7.

Gene expression of tyrosine hydroxylase (TH) and neuropeptide Y (NPY) was studied in prevertebral ganglia and adrenal glands of adult male rats during development of one kidney/one clip (1k/1c) Goldblatt hypertension. Tissues from 1k/1c rats with arterial pressures significantly elevated by day 3 were compared to those from sham controls. Four or 5 days after renal surgery, superior cervical ganglia, celiac-mesenteric plexus, adrenal glands, and stellate ganglia were surgically removed from non-fixed rats for Northern blot analysis or from perfusion-fixed rats for 'in situ' hybridization. In all tissues, levels of TH mRNA were decreased in hypertensive rats; cells with decreased levels were scattered throughout each tissue. In contrast, levels of NPY mRNA were unchanged in hypertensive rats compared to controls. Changes in TH mRNA levels suggest that the developing phase of renal hypertension is associated with a decrease in sympathetic outflow to the periphery. The failure of NPY mRNA levels to change suggests different regulatory mechanism for NPY expression or a different role for NPY in sympathetic neurotransmission.

Supported by the Medical Research Council of Canada.

117.3

CLASSIFICATION OF STELLATE GANGLIA (SG) NEURONS IN NEONATAL SWINE. **A.L. Sica, J.A. Armour, N.A. Kaplan*, F.M. Pisana* and P.M. Gootman. Albert Einstein Coll. Medicine, Long Island Jewish Med. Ctr., Schneider Children's Hosp., Dept. of Peds., New Hyde Park, NY 11042.**

In anesthetized (pentobarbital or saffan), paralyzed, thoracotomized and artifically ventilated (100% O_2) neonatal swine, extracellular potentials of SG neurons were recorded along with phrenic nerve (monitor of central inspiratory activity), EKG, intratracheal and arterial blood pressure signals. While neurons with spontaneous discharges related to phrenic nerve activity were not observed, some neurons had discharges during the inflation or the deflation phases of the ventilator cycle. Surprisingly, few neurons had discharges related to the cardiac cycle. In contrast to the relatively smaller number of neurons with spontaneous discharges, many neurons were evoked by ipsilateral mechanical stimulation (i.e. probing, or stroking) of their receptive fields located on a) the ventricular surface; b) the lateral surface of the thorax; c) the hairy regions of the upper and lower arms. The finding of few cardiac-related SG neurons was unexpected and suggested that such activities may emerge in an age-dependent fashion.

117.4

ANATOMY OF CANINE INTRINSIC CARDIAC NEURONS. Yuan, Bing-Xiang*, D.A.Hopkins, J.A.Ardell*, J.A.Armour. Depts. of Physiology / Biophysics and Anatomy, Dalhousie University, Halifax, N.S., B3H 4H7, and Physiology, University of South Alabama, Mobile, Alabama, 36688.

Ganglionated plexi were identified on the ventral and lateral surfaces of the right and left atria, the dorsal surface of the two atria, as well as the dorsal surface of the atria adjacent to the origin of the inferior vena cava by means of microscopic analysis of methylene blue-stained fresh and fixed tissues. Ganglia were identified on the cranial surface of the ventricles surrounding the aortic root and extending ventrally and dorsally adjacent to the origins of the major coronary arteries. Ganglia were also identified near the origin of the right and left marginal coronary arteries. The somata of single neurons were identified along the course of nerves. Some of these, arising from nerves like a row of grapes, appeared to be similar to pseudounipolar neurons. The majority of neurons were multipolar. About 40% of histologically identified neurons contained multiple nucleoli. The canine intrinsic cardiac nervous system contains many more neurons than has been considered previously. These neurons are widely distributed and appear to include a number of different cell types. Supported by the MRC of Canada (MT-10122), as well as Nova Scotia and Alabama Heart Foundation grants.

117.5

ELECTRON MICROSCOPIC STUDY OF DOG CARDIAC GANGLIA AFTER CHRONIC DENERVATION. M.H. Mostafa, J.X. Thomas Jr., R.D. Wurster and E.J.Neafsey. Neuroscience Graduate Program and Department of Physiology. Loyola University Chicago, Maywood IL 60153.

Cardiac ganglia were dissected from the pulmonary vein fat pads of three groups of dogs: Intact, selective vagal denervation (VagX), and total sympathetic and vagal denervation (TotalX). After processing the ganglia for EM, the number of boutons and synaptic contacts per primary ganglionic neuron were counted. As seen from the table below, both types of denervation produced statistically significant (*, $p < .05$)

	boutons/cell (sem)	synapses/cell (sem)
Intact	2.53 (0.36)	1.26 (0.13)
VagX	0.10 (0.002)*	0.03 (0.01)*
TotalX	0.09 (0.03) *	0.02 (0.01)*

decreases in both bouton number and synapse number compared to Intact dogs. There was no significant difference between the two denervation groups, implying that there is little or no sympathetic innervation to these primary ganglionic neurons. In both denervation groups the ganglionic neurons also displayed an obviously high accumulation of intracellular glycogen, as well as a slight increase in lipofucsin granules. Their cell nuclei had normal ultrastructure, but the nuclear membrane did appear convoluted compared to cells in Intact dogs. However, only 3% of the cells showed signs of degeneration (large vacuoles and severely dilated cisternae). (NIH HL 27595)

117.6

DECREASED VAGAL SENSORY INNERVATION OF THE HEART IS ASSOCIATED WITH PROLONGED QT INTERVALS IN THE ELECTROCARDIOGRAM. M.J. Mulroy and T.H. Harrison, Dept. of Anatomy, School of Medicine, Medical College of Georgia, Augusta, GA 30912.

Longer than normal QT intervals in electrocardiograms are associated with cardiac arrhythmias which can be fatal. Chicks with long QT intervals can be experimentally produced by ablation of the nodose placode early in development (Christiansen et al., 1989; Mulroy et al., 1990). We will present morphometric evidence from experimental chicks with long QT intervals and control chicks with normal QT intervals indicating a reduction in the sensory innervation to the heart provided by the nodose ganglion in chicks with long QTs.

We speculate that in this animal model a decrease in vagal sensory innervation to the heart during development can induce the long QT syndrome. This could occur directly by interfering with the development of normal cardiac sensory pathways or indirectly by interfering with the development of normal cardiac motor innervation, that is, an appropriate balance between sympathetic and/or parasympathetic innervation. We are currently investigation these alternatives. (Supported by NIH PO1HL36059).

117.7

CARDIAC MYOCYTE MET-ENKEPHALIN-IMMUNOREACTIVITY (ir). B.A. Barron, J.F. Gaugl*, J.L. Caffrey*. Dept of Physiology, Texas College of Osteopathic Medicine, Ft Worth, TX 76107.

We find met-enkephalin-ir uniformly distributed in the canine heart. When combined with reports of concentrated enkephalin mRNA in rodent hearts, these observations suggest cardiac opioids are not confined to nerves and may originate in the myocytes. The myocytes and intact left ventricle (LV) were acid extracted for estimation of total opioids and met-enkephalin-ir by RRA and RIA, respectively.

Tissue Assayed (n=5)	Met-enkephalin-ir: fmol/mg protein	Total Opioids: fmol etorphine equivalents/mg protein
Left ventricle	3.11 ± 0.42	143.0 ± 33.60
LV myocytes	11.53 ± 4.07	34.3 ± 3.99

The enkephalin content of the isolated myocytes is 3-4 X that found in intact ventricle, despite a 75% decrease in total opioids. This suggests that enkephalin and the larger pool of total opioids may be functionally independent. The large enkephalin increase in isolated myocytes suggests that other influences in intact tissue supress synthesis and/or processing of enkephalin. HPLC of extracts indicates approximately 12% of myocyte and 45% of LV met-enkephalin-ir chromatographed with authentic met-enkephalin. These results suggest that the myocytes will be useful in determining the characteristics of heart enkephalin synthesis, processing and release.

117.8

EFFECT OF OPIOID RECEPTOR ANTAGONISTS ON VASODILATOR NERVE ACTIONS IN THE PERFUSED RAT MESENTERY. YJ. Li* and S. P. Duckles. Dept. of Pharmacology, College of Medicine, University of California, Irvine., CA 92717.

We have previously shown that opioid peptides inhibit sensory nerve effects in the rat mesentery. To explore the possibility that endogenous opioids modulate sensory nerve function, we investigated the effects of opioid receptor antagonists. In the presence of 5×10^{-6} M guanethidine and 10^{-5} M methoxamine, transmural nerve stimulation (TNS) causes a vasodilator response. This response was significantly potentiated by 3×10^{-7} M naloxone. Naloxone did not alter vasodilator responses to exogenous calcitonin gene related peptide, suggesting that potentiation by naloxone is mediated by a prejunctional action. To further characterize the opioid receptors involved, selective antagonists were used. 3×10^{-7} M ICI 174.864, a selective δ antagonist, had no effect on vasodilator responses to TNS. In contrast 3×10^{-7} M CTOP, a selective μ receptor ligand, significantly inhibited vasodilator responses to TNS. However, this effect was reversed by naloxone, suggesting that CTOP is a partial agonist at μ receptors. In preparations pretreated with the irreversible μ antagonist β-funaltrexamine (2×10^{-7} M, 30 min) naloxone did not further potentiate vasodilator responses to TNS. These results confirm that opioid receptors are involved in regulation of sensory nerves and suggest that potentiation of vasodilator responses to TNS by naloxone may be due to blockade of μ receptors, resulting in reduced inhibitory modulation by endogenous opioids.

117.9

NEUROPEPTIDE Y PRODUCTION IN RAT MYOCYTE CULTURE IS REGULATED BY SYMPATHETIC NEURON CO-CULTURE. K.L.Marek AND T.Niven-Fairchild, Dept. of Neurology, Yale Univ. School of Medicine, New Haven CT 06510

The regulation of Neuropeptide Y (NPY) production and secretion was examined in primary cell cultures from rat atrium, ventricle, superior cervical ganglion (SCG) and in myocte-SCG co-cultures. NPY mRNA levels were quantitated by Northern analysis, NPY synthesis was measured after incubation in medium containing [^{3}H]tyrosine followed by immunoprecipitation and SDS-PAGE, and NPY content was measured by radioimmunoassay. NPY was stable in spent medium for at least 48 hours. Cultures were maintained in complete serum free medium for up to 21 days.

NPY production and secretion was reduced in SCG-atrial cultures by 3-fold and SCG-ventricular cultures by 7-fold as compared to atrial and ventricular cultures alone. The reduction in NPY secretion shows a dose-dependent response to the number of neurons added to the co-cultures. In previous studies we have shown that NPY is rapidly secreted from myocte cultures, but minimally secreted from SCG cultures alone.

Thus innervation of myocyte culture dramatically alters myocyte peptide expression. Experiments are underway to further elucidate the mechanism of this neuronal-target interaction and to identify the developmental significance of cardiac NPY expression.

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117.10

MODULATION OF VASCULAR SMOOTH MUSCLE GROWTH BY A SYMPATHETIC NEUROTRANSMITTER, NEUROPEPTIDE Y. Z. Zukowska-Grojec*, C. Colton, J. Yao*, S. Abi-Younes*, A. K. Myers*, J. I. Koenig and C. Wahlestedt. Department of Physiology & Biophysics, Georgetown University Medical Center, Washington, D.C. 20007

Neuropeptide Y (NPY) is a 36 amino acid peptide present with norepinephrine (NE) in all sympathetic postganglionic nerves innervating the cardiovascular system, in the adrenal medulla and in platelets. NPY evokes direct vasoconstriction via Y1 receptors in many vessels (e.g. vena cava) and potentiates NE-induced vasoconstriction (e.g. aorta). Since sympathetic nerves are presumed to play a role in vascular smooth muscle (VSM) growth, we studied the effects of NPY (and NE) on proliferation of cultured VSM cells (C) from aorta and vena cava of female rats. Passaged cells (passage 5-8) were plated into 96-well dishes at a density of 2×10^4 cells/well in DMEM containing 0.5% fetal calf serum (FCS). Following 48 hrs of treatment of aortic VSMC with NPY in 0.5% FCS, [^{3}H] thymidine uptake tended to decrease at 10^{-9}M of NPY, whereas at 10^{-7} and 10^{-6}M, it significantly increased (+19±5 and 35±1%); NE, at 10^{-5}M, stimulated proliferation by 42+9%. Similar results were found with venous VSMC. At 30 min of treatment with NPY, 80% of NPY-like immunoreactivity (NPY-LI) was taken up by the cells, but at 48 hrs, cell media still contained 25% of NPY-LI (RIA). NPY-specific, high affinity binding sites were present on cultured VSMC and corresponded to Y1 receptor type on aortic and Y1- and Y2-type on venous cells. NPY had no effects on basal but inhibited forskolin-stimulated cAMP accumulation in VSMC. We propose that NPY, a sympathetic and platelet-derived vasoconstrictor, has a novel action on VSM: stimulation of cell proliferation, probably mediated by specific NPY (Y1) receptors.

117.11

LOCATIONS AND PEPTIDE CONTENT OF POSTGANGLIONIC NEURONS INNERVATING HINDLIMB VEINS, ARTERIES AND FOOTPADS OF RATS. N.S. Dehal*, A. Kartseva* and L.C. Weaver. J.P. Robarts Res. Inst., London, Ont., Can. & Bogomoletz Inst., Kiev, U.S.S.R.

The ganglionic location and peptide content of sympathetic neurons innervating hindlimb arteries, veins or footpads have not been compared. The different responses of these targets to sympathetic activation may relate to differences in the peptides contained in specific hindlimb sympathetic neurons. Retrograde transport of fluorescent dyes was used to identify, separately, postganglionic neurons innervating femoral arteries or veins in 28 rats. Footpad neurons were studied in 6 additional rats without distinguishing sweat gland neurons from footpad vasomotor neurons. Proportions of dye-labelled neurons containing NPY -and VIP-like immunoreactivity (LI) were compared in 24 of these rats. Venous vasomotor neurons were found in the T13-L6 ganglia with 62% in L1 and L2. Few were in L4-L6. Arterial vasomotor neurons were also found in the T13-L6 ganglia with most (81%) located in L1-L3. Veins and arteries were not innervated by the same cells. Footpad neurons were mostly in L4-6. NPY-LI was identified in 8% of 143 venomotor neurons, 94% of 637 arterial neurons and 24% of 267 footpad neurons. VIP-LI was found in 4% of 130 venomotor neurons, 8% of 385 arterial neurons and 44% of 305 footpad neurons. In summary, hindlimb venous, arterial and footpad neurons are anatomically distinct and differ markedly in their content of NPY-LI. The high proportion of footpad neurons containing VIP-LI likely reflects innervation of sweat glands; those containing NPY-LI may innervate footpad arteries.(Support: MRC Canada)

117.12

ENDOTHELIAL-DERIVED NITRIC OXIDE (NO) INFLUENCES BASAL CORONARY VASCULAR TONE IN ANESTHETIZED RATS. Leslie F. Jones and Michael J. Brody. Dept. Pharmacology & Cardiovascular Ctr., Univ. Iowa, Iowa City, IA 52242.

The present study evaluated the role of NO in determining basal coronary vascular tone. Sprague Dawley rats were anesthetized and instrumented for recording arterial pressure (AP), heart rate (HR) and coronary blood flow velocity (CBF; Doppler). In rats without ventricular pacing L-N-nitro arginine (LNA) and L-N-nitro arginine methyl ester (LNAME), inhibitors of NO synthase, produced a L-arginine reversible increase in AP and decrease in HR and CBF. Stellate ganglionectomy and adrenal medullectomy did not alter the decrease in CBF but attenuated the decrease in HR produced by LNAME. Sinoaortic denervation inhibited the LNAME-induced bradycardia. Dose-response curves were performed for LNA and LNAME (0.3-300 μM/kg, iv) in rats with ventricular pacing. LNAME produced a more potent increase in AP and coronary vascular resistance (EC_{50}=4.7±0.2; 6.3±1.1 μM/kg, iv, respectively) compared to LNA (EC_{50}=8.0±1.1; 9.8±1.1 μM/kg, iv, respectively). However, LNAME and LNA produced similar maximal responses. These results indicate that NO has a tonic action on coronary vessels *in vivo* and that bradycardia produced by LNAME is due to baroreceptor mediated withdrawal of sympathetic tone. (Supported by HL-32295)

117.13

CARDIOVASCULAR REFLEXES IN PATIENTS WITH TENSION-TYPE HEADACHE. T. Pogačnik*, M. Perovič*, S. Šega* and T. Kiauta. Dept. of Neurology, University Medical Centre, YU-61105 Ljubljana, Slovenia.

The role of the autonomic nervous system in the etiopathogenesis of functional headaches has been investigated for a number of years, with equivocal results. The aim of this study was to apply a standardized battery of cardiovascular tests to a sizable group of functional headache patients and healthy controls.

The Valsalva manoeuvre, deep breathing test, sustained handgrip test and orthostatic test were performed in a group of 51 patients of both sexes aged 21 to 50 years (average age 35.6 years) and in an age-matched control group of healthy volunteers. Data were acquired and processed by an IBM PC/AT-compatible computer.

Diastolic blood pressure increase and particularly heart rate increase during sustained handgrip were significantly reduced in the headache group when compared to the control group, while the results of the remaining tests were not significantly different. No significant differences were found between the episodic (19 patients) and chronic (32 patients) tension-type headache subgroups.

It is concluded that sympathetic function is impaired in tension-type headache patients.

117.14

THE RELATIONSHIP OF CEREBRAL LATERALIZATION TO HEMIBODY AUTONOMIC FUNCTION AS EVIDENCED BY THE HISTAMINE WHEAL RESPONSE. S.S. Avery,* K.J. Meador, S.L. Wise,* D.L. Loring,* G.P. Lee,* N. Thrash,* R.E. Figueroa* and B.B. Wray*. Department of Neurology, Medical College of Georgia, Augusta, GA 30912.

In a prior study, we found differential left/right cerebral effects on heart rate following unilateral cerebral inactivation via intracarotid amobarbital (Neurology 1990;40:1408-1411). In order to further investigate possible differential left/right cerebral effects on autonomic function, two studies were performed. In the first study, the effects of cerebral lateralization on histamine skin test response were examined in 177 patients from the allergy clinic. Wheals were larger on the left than right forearm for dextrals with dextral family history ($p<.02$), equal in dextrals with primary sinistral relatives, and larger on the right in sinistral/ambidextrous patients ($p<.005$). In the second study, histamine skin testing was performed before and during Wada testing in 14 epilepsy surgery patients. Preliminary results reveal no differences in wheal size for left vs. right Wada test and for ipsilateral vs. contralateral arm to injection. However, intracarotid amobarbital appears to suppress right arm and enhance left arm responses ($p<.03$). Additional studies are needed to further delineate the relation of cerebral lateralization to autonomic function.

117.15

ALTERATIONS WITH AGE IN ADRENAL MEDULLARY TYROSINE HYDROXYLASE EXPRESSION IN RESPONSE TO COLD STRESS. N. Tumer*, P.J. Scarpace*, and G. Rajakumar. GRECC, VA Med. Center; Dept. of Pharmacology, University of Florida, Gainesville, FL 32608.

One of the most serious consequences of the aging process is an inability to respond to environmental stimuli. Cold exposure is known to increase tyrosine hydroxylase (TH) expression in the sympathetic nervous system. We have recently shown that exercise, another modulator of TH activity, reduces TH expression in young but not in old rats. We assessed TH activity with age in adrenal medullae of 3- and 24-month old F-344/BN rats (n = 5-6) following 48 h cold exposure at 5°C. TH activity was measured by a coupled decarboxylase assay. Concentration of L-tyrosine was 40 µM, 6-MHPH$_4$ was 3mM and pH was 6.5.

TH Activity with Age and Cold Exposure (pmol/h/adrenal)

	Room Temperature (26°C)	Cold (5°C)
Young	5171 ± 490	5548 ± 280
Old	8350 ± 745	8939 ± 626

There was an age-related increase in TH activity in control rats ($p < 0.01$). Surprisingly, TH activity did not increase following 48 h of cold exposure in either age group. However, there remained an age-related increase in TH activity in the cold exposed rats ($p < 0.001$). Cold exposure for a 48 h period normally increases the expression of TH activity in several rat strains. The present results indicate that the induction of TH activity in F-344/BN rats is not as sensitive to cold exposure as other rat strains. However, the increase with age in TH activity is much larger (72% vs 21%) compared with our previous study in F-344 rats. The F-344/BN strain has recently been made available by the NIA for aging research. Our results indicate there are differences between the F-344 and the F-344/BN cross in both the age-related increases in TH activity and the sensitivity to cold exposure. Supported by Med. Res. Service of Dept. of Veterans Affairs and Center for Neurobiology of Aging, UF.

117.16

DIURETIC AND NATRIURETIC RESPONSES AFTER A WATER LOAD IN HUMANS UNDER DIFFERENT SODIUM BODY CONTENT CONDITIONS. EVIDENCES OF A SODIUM HOMEOSTATIC MECHANISM. M.P. Rosas-Arellano*, R. Guevara-Guzmán and L.P. Solano-Flores. Dept. Fisiología, Medicina-U.N.A.M. A.P. 70-250, 04510 México.

The present study was focused to observe the diuretic and natriuretic responses after a water load (2% body weight) in four groups of young volunteers submitted previously during three days to an hyper- (500 mEq Na/day), hypo- (35 mEq Na/day), and normosodic diet (200 mEq Na/day) or treated with furosemide (Lasix 40 mg/day). During the three days of treatment the urine of each day was collected. The fourth day in the morning the bladder was emptied, the water load was drank and the urine was collected during ten periods of 20 minutes each. The urinary, sodium and chloride flows were determined. The four groups displayed diuretic curves following a similar pattern. In contrast, the natriuretic curves of the four groups were completely different one from the other: totally flat in low values for the furosemide group and a large initial natriuretic curve for the hypersodic group with a gradual decrease to maintain high values. The results indicate that the way the organism compensates the excess of water by means of urinary water loss is independent of the body sodium content, meanwhile, the way in which the sodium loss is accomplished is determined by the body sodium content and is independent of the way in which the water is lost. *Supported by CONACyT 47969.

117.17

SYMPATHETIC NERVOUS SYSTEM (SNS) INNERVATION OF WHITE ADIPOSE TISSUE (WAT): POTENTIAL ANATOMICAL BASIS FOR REGIONAL DIFFERENCES IN LIPOLYSIS. T. G. Youngstrom and T. J. Bartness. Depts. of Psychology and of Biology, Georgia State Univ., Atlanta, GA 30303.

We previously demonstrated regional differences in lipid content and metabolism of WAT in Siberian hamsters during the short day-induced decreases in body weight (fat). We tested whether the innervation of two WAT pads that had differential lipid mass loss in response to short day exposure had unique SNS innervation. Male Siberian hamsters were injected with the retrograde tracers Fluoro-Gold (FG; 1% solution) or rhodamine-labeled microspheres (RLM; 1:4 dilution). The inguinal subcutaneous WAT (IWAT) and epididymal (EWAT) depots were injected (five, 1ul injections) at the apparent site of entry of SNS fibers and killed 10-20 days later. RLM labeled few cells and no fibers; therefore the results pertain to FG injections only. Labeled cells were more readily apparent in hamsters killed 10 vs 20d postinjection. Fibers were intensely labeled bilaterally from both injections sites throughout the extent of the sympathetic chain (SC); however their origin and destination were not apparent. Although some overlap in labeled cells occurred for these pads, a distinct pattern of labeling was seen with tracer injected into IWAT labeling cells in the SC predominantly in the celiac ganglion and extending caudally to L3. In contrast, tracer injected into EWAT labeled cells in the SC predominately in L1 and extending caudally to L3. Labeled cells from both injection sites were frequently as large as 50µm. These results form one possible neuroanatomical basis for differential control of lipid metabolism in these fat pads. Supported by grants NIMH RSDA MH-00841 and NIH DK-35254.

118.1

MORPHOLOGY OF VAGAL AFFERENT NEURONS IN THE NODOSE GANGLION OF THE CAT. L. Kubin, R. Rogers* and R.O. Davies. Department of Animal Biology and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA. 19104.

Although vagal afferent pathways have been well studied, a comprehensive description of the morphology of nodose ganglion cells in relation to their electrophysiological and functional properties is not available. To fill this gap, horseradish peroxidase was injected intracellularly into nodose ganglion cells after determining the conduction velocities of their peripheral axons and (where possible) their peripheral receptor modalities in decerebrate, paralysed and artificially ventilated cats. Cell bodies and up to 4 mm of their peripheral and 0.7 mm of their central axons were visualized. Of the 11 cells analyzed to date, 4 had conduction velocities over 19 m/s (3 pulmonary stretch receptors, one baroreceptor) while the remaining had conduction velocities below 1.4 m/s (receptor modality undetermined). The peripheral axonal diameters of the fast conducting, myelinated axons were 5.4 μm ± 0.9(SD), only about twice that of the slowly conducting axons. The central axons of the fast conducting cells were 0.7 ± 0.15(SD) that of their peripheral axons; the corresponding ratio was 0.3 ± 0.2(SD) for the slowly conducting cells. The latter cells had longer common portions of the axon (between the cell body and the bifurcation into the central and peripheral axons) than the former. We did not find anatomical evidence for bifurcations of the peripheral axons. However, the central axon of one slowly conducting cell branched within the ganglion. This study should provide useful morphological correlates for electrophysiological properties and developmental patterns of vagal afferent cells of different modalities. (Supported by HL-36621 and the U. of PA. Res. Found.)

118.2

DEVELOPMENT AND ELECTRICAL MEMBRANE PROPERTIES OF NODOSE GANGLION CELLS GROWN IN CULTURE. I. Schmiedel-Jakob* and S.Tyc-Dumont. Unité de Neurocybernétique Cellulaire, CNRS UPR 418, 280, Bd Ste Marguerite, Marseille, France.

Dissociated nodose ganglion cells from adult rat were cultured for up to 5 weeks in DMEM/F12 medium supplemented with foetal calf serum and hormones. Neurite outgrowth started within 3 days in culture (DIC). Although the cells are always pseudounipolar in vivo, in culture they exhibit more anatomical diversity including bipolar or multipolar cells. Using whole-cell patch-clamp technique active and passive membrane properties of the ganglion cells were studied from 1 to 28 DIC. Prolonged depolarizing current steps evoked one or several action potentials (AP). At their maximal response some neurons gave only one AP while other increased frequency with increasing current intensities. APs had a mean threshold of -31 mV and a mean amplitude of 48 mV. They overshot 0 mV with a mean of 32 mV. On their basis of spike duration there appeared to be 2 types a short <3 ms and a longer duration AP. Voltage-current relations revealed mild anomalous rectification for some neurons and input resistances in the range of 50-520 Mohm. In voltage-clamp step depolarizations elicited transient inward and sustained outward currents. The results suggest that following dissociation and culturing the neurons retain most of their electrical properties.

118.3

PROJECTION OF NODOSE GANGLION CELLS TO THE UPPER CERVICAL SPINAL CORD IN THE RAT. M.J. Chandler, D.L. McNeill, Q.-G. Fu and R.D. Foreman. Depts. of Physiology and Biophysics and Anatomical Sciences, University of Oklahoma, Oklahoma City, OK 73190.

Noxious input from myocardial ischemia classically is believed to enter the central nervous system via sympathetic afferent fibers. After sympathectomies, however, angina pectoris still may be referred to the neck and inferior jaw. These observations suggest that vagal afferent fibers may participate in transmitting information to upper-cervical spinal cord segments. The purpose of this study was to determine the density and pathway of vagal afferent nerve fibers to the upper cervical spinal cord. Male Sprague-Dawley rats were divided into 3 groups. Group 1 rats received a unilateral injection of 2 µl of 2% WGA-HRP into the cervical (C) 2, 3 or 4 dorsal spinal cord. Group 2 rats received a similar injection followed by an ipsilateral cervical vagotomy. For Group 3 rats, a spinal cord hemisection was performed cranial to the WGA-HRP injection site. Following a 2 day survival, the nodose ganglion was removed and processed for WGA-HRP reactivity. Approximately 5.8% of the nodose ganglion cells from Group 1 rats contained WGA-HRP reaction product. In the Group 2 rats, cervical vagotomy did not produce a diminution in the density of WGA-HRP-labeled cells in the nodose ganglion. However, no labeling was observed in nodose ganglia from Group 3 rats. These data suggest that a portion of vagal afferent neurons project from the nodose ganglion to the upper cervical spinal cord. In addition, vagal afferent fibers reach the spinal cord via a supraspinal pathway, rather than through peripheral nerves. (National Heart, Lung and Blood Institute, HL-22732.)

118.4

EXCITATORY VAGAL INPUT TO THE UPPER CERVICAL NEURONS IN THE RAT. Q.-G. Fu*, M. J. Chandler, D. L. McNeill, R. D. Foreman. Depts. of Physiology and Biophysics and Anatomical Sciences, University of Oklahoma HSC, P.O. Box 26901, Oklahoma City, Ok 73190

Our recent anatomical study showed a direct projection of nodose ganglion cells to the upper cervical cord. This result suggested that vagal afferents may activate upper cervical neurons. In this study, we tested this possibility. Effects of electrical stimulation of cervical vagus nerve on 80 cervical dorsal horn cells were examined in 27 rats anesthetized with pentobarbital. Sixty-three of 80 cervical cells were excited by ipsilateral cervical vagal stimulation (ICVS) and most of them (47/63) were located in C_1 segment. Twelve of 47 C_1 cells also were excited by contralateral cervical vagal stimulation (CCVS). The spontaneous activity of 8 cervical cells excited by ICVS was inhibited by CCVS. CCVS also inhibited the increased cell activity by ICVS in 6 cervical cells. Some properties of C_1 cells were summarized according to responses to ICVS and CCVS: the cells not affected by ICVS and CCVS were most often LT cells with lower spontaneous activity; cells activated by both ICVS and CCVS were most often located in deeper laminae in dorsal horn and had higher spontaneous activity, compared with the cells activated only by ICVS. Excitatory vagal input to the C_1 cells was not significantly affected by cutting C_2 and C_3 dorsal roots. These results demonstrate that a large group of cells located in the upper cervical cord were excited by vagal afferent input. This excitatory vagal input reaches the C_1 segment probably via a supraspinal route. (National Heart, Lung, and Blood Institute, HL-22732)

118.5

X-IRRADIATION-INDUCED EMESIS IN SUNCUS MURINUS. N.Matsuki[1], Y.Torii*[1], M.Shikita*[2] and H.Saito[2]. 1)Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo 113, 2)Natl. Inst. Radiol. Sci., Chiba 260, Japan.

We have shown previously that Suncus murinus (house musk shrew), a species of insectivore, vomits in response to emetic drugs and motion stimulation. Cisplatin-induced emesis was inhibited by surgical vagotomy or pre-treatment with 5-HT_3 antagonists. In the present study we investigated whether or not X-irradiation caused emesis in the suncus. Whole body X-irradiation (800 cGy) caused emesis in all animals studied with mean frequency 10.0 times/90 min, and mean latency was 20 min. The strength of X-ray which causes emesis in 50 % of animals was calculated to be 429 cGy. Abdominal X-irradiation caused vomiting in all animals whereas the same irradiation to the head had no emetogenic effect. The surgical vagotomy completely abolished the emesis. Subcutaneous injection of ICS205-930, a selective 5-HT_3 antagonist, prevented the emesis with ID_{50} value of 29 μg/kg. These results indicate that 1) Suncus murinus can be a new animal model for X-irradiation-induced emesis, 2) peripheral 5-HT is involved in X-irradiatin-induced emesis, which is very similar to that caused by cancer chemotherapeutic agents.

118.6

PUTATIVE NEUROTRANSMITTER AGENTS IN SENSORY NEURONS OF THE CAROTID SINUS NERVE (CSN) OF THE RAT. C.J. Helke, A. Rabchevsky and H. Ichikawa. Dept. of Pharmacol., Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

The origin of calcitonin gene-related peptide (CGRP), substance P (SP), tyrosine hydroxylase (TH) and vasoactive intestinal polypeptide (VIP) in the rat carotid body and carotid sinus was examined by retrograde labelling of the CSN with Fluoro-gold (FG) and by immunohistochemistry. At 3 days after application of FG to the central cut end of the CSN, many petrosal and some jugular neurons were labelled with FG. The nodose ganglion contained few, if any, FG-labelled neurons. CGRP- and SP-immunoreactivities (-ir) were observed in many petrosal and in a few jugular neurons labelled with FG. In the petrosal ganglion, 25.0±6.5 of FG-labelled neurons showed CGRP-ir and 15.8±4.8% of FG-labelled neurons showed SP-ir. Only a few FG-labelled cells in the petrosal ganglion showed TH- or VIP-ir. No FG-labelled neurons in the jugular ganglion were immunoreactive for these substances. The present study indicates that subpopulations of petrosal neurons innervating the carotid sinus and carotid body contain CGRP or SP. The failure to see significant TH-ir in FG-labelled neurons at 3 days may result from an axotomy-induced reduction of TH-ir (Helke and Rabchevsky, 1991). [Supported by NIH grant NS20991]

118.7

CYTOCHROME OXIDASE ACTIVITY IN THE NODOSE AND PETROSAL SENSORY NEURONS OF THE RAT AFTER PERIPHERAL NERVE SECTION. H. Ichikawa and C.J. Helke. Dept. of Pharmacol., Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Axotomy of the peripheral axons of bipolar sensory neurons alters the synthesis and content of putative transmitter agents. Changes in cellular metabolism in response to injury or to loss of afferent inputs may be involved in these axotomy-induced transmitter effects. Using enzyme histochemistry for cytochrome oxidase, an endogenous metabolic marker, axotomy-induced changes in metabolic activity were investigated in visceral sensory neurons of the rat nodose and petrosal ganglia. Most neurons in control nodose and petrosal ganglia were stained darkly. By 3 days after section of all peripheral nerves from the nodose and petrosal ganglia, darkly-stained neurons decreased in number and lightly-stained neurons which were not observed in control ganglia began to appear in the nodose ganglion. At 7 days after peripheral nerve section, the average population of these lightly-stained neurons increased to 29% in the nodose ganglion. Subsequently, the population decreased so that at 14 days and at 21 days, 19% and 7% respectively of neurons were lightly stained. In the petrosal ganglion, no remarkable change was observed at any stages after peripheral nerve section. These results suggest that the metabolic activity decreases in some nodose neurons after peripheral nerve section.

[Supported by NIH grant NS20991]

118.8

ALTERATION OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) EXPRESSION IN VISCERAL SENSORY NEURONS OF THE NODOSE AND PETROSAL GANGLIA OF THE ADULT RAT FOLLOWING PERIPHERAL AXOTOMY. E.K. Tayo, J.T. McCabe and C.J. Helke. Depts. of Pharmacol. & Anatomy, Uniformed Services Univ. of the Health Sci., Bethesda, MD 20814.

Visceral sensory neurons of the glossopharyngeal and vagus nerves are located in the petrosal and nodose ganglia respectively. Peripheral axotomy of visceral sensory neurons causes differential changes in the content of putative transmitters (Helke and Rachevsky, 1991).

In situ hybridization histochemistry with ^{32}P- or ^{35}S-labeled oligonucleotide probes was used to study CGRP mRNA in control and axotomized (7 d survival) ganglia. Transections of the cervical vagus and superior laryngeal nerves, or of the glossopharyngeal and carotid sinus nerves comprised peripheral axotomies of the nodose and petrosal ganglia, respectively. In control nodose ganglia, a few CGRP mRNA-containing cells were seen in the rostral and caudal poles. Axotomy caused little or no change in the numbers of these CGRP mRNA-containing cells. CGRP mRNA was observed in a large number of neurons distributed throughout the control petrosal ganglia. Axotomy caused a pronounced decrease in the number of these CGRP mRNA-containing cells. Reductions in CGRP mRNA were also seen in somatic sensory neurons of the jugular ganglion. These findings suggest that CGRP expression decreases in visceral sensory ganglia following peripheral nerve section and are consistent with previously reported axotomy-induced decreases of CGRP immunoreactivity in visceral sensory neurons.

118.9

SPINAL CAPSAICIN EXACERBATES 1K RENAL WRAP HYPERTENSION IN THE RAT. M. Burg*, D.S. Zahm, Q. Gan*, and M.M. Knuepfer, Dept. of Pharmacol. and Physiol. Sciences and Dept. of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104

Afferent renal nerves (ARN) in the rat contain substance P (SP) and calcitonin gene-related peptide (CGRP). We examined whether spinal depletion of SP and CGRP affects the development of renal hypertension. One week after intrathecal administration of capsaicin (70 µg) or vehicle at spinal level T12, right nephrectomy and left renal figure-8 wrap or sham wrap were performed. After 8-10 weeks, renal wrap induced an increase in arterial pressure that was greater in capsaicin-treated rats. Diamidino yellow was placed on the central cut renal nerve 3 days before processing spinal cord and dorsal root ganglia (DRG) at T9-L1 for immunocytochemistry. Two thirds of ARN cells in DRG contained CGRP-immunoreactivity (ir) and approximately half of these also contained SP-ir. The percentage of CGRP- and/or SP-ir ARN soma in DRG were not significantly affected by capsaicin treatment or renal wrap. In contrast, spinal CGRP- and SP-ir were substantially reduced in capsaicin-treated rats. These results suggest that spinal SP and/or CGRP may play a role in reducing renal hypertension. Furthermore, intrathecal capsaicin depletes SP- and CGRP-ir in spinal cord but not in DRG. (Supported by USPHS Grants HL38299 and NS23805).

118.10

REFLEX ACTIVITY OF THE RAT VAGINAL ORIFICE. M. Martínez-Gómez; R. Chirino*, P. Carrillo* and P. Pacheco. CIRA, Univ. Auton. Tlaxcala-CINVESTAV, Panotla, Tlax.; CIB, Univ. Veracruzana; IIB-UNAM, México, D.F.

As a part of a study on the reflex components involved in the vaginal orifice dilation (VOD), we analyzed on ovariectomized rats the effects of genital area denervation, adrenergic influence and temperature on VOD. In addition the effect of direct electrical stimulation of ischiocavernosus muscle (icm) was analyzed. RESULTS: Denervation: Bilateral transection of genitofemoral, pudendal or pelvic nerve does not block evoked perineal tapping VOD; however, pudendal nerve transection maintains longer VOD (10-20sec) as compared to controls (3-5sec). Adrenergic effects: Noradrenaline (NA: 25,50 or 75ug) intracerebroventricularly injected provokes a dose-related intermittent, spontaneous VOD during a 90 min period. NA (25ug) administered intrathecally provoked a continuous VOD for 2 h. Temperature: VO stimulation with a cold (15^{o}C) wet cotton device induces sudden closing when the VO is open or an increment in closure if the VO is partially closed. Muscular stimulation: Unilateral or bilateral stimulation of icm induces the closure of VO with a velocity and intensity-pulse/frequency related fashion. The caudal third of vaginal internal wall presented contractions. CONCLUSIONS: a) Different innervation to that here studied and perhaps vascular related, determines VOD. b) Central adrenergic mechanisms regulate VOD. c) VO closure appears to be regulated via reflex muscular mechanisms. CONACyT D111-903851; SEP C90-01-0439 (M.M.G.).

118.11

EFFECTS OF SPINAL CORD TRANSECTION AND MK-801 ON CGRP-IMMUNOREACTIVE FIBERS IN THE URINARY BLADDER OF THE RAT. D.L. McNeill, R.L. Shew and R.E. Papka. Dept. of Anatomical Sciences, Univ. of Oklahoma, Oklahoma City, OK 73190.

If supraspinal control of bladder function is removed, micturition is often restored, at least partially, via spinal reflex mechanisms. In theory, increasing the density of sensory nerve fibers in the bladder wall may elicit a more effective reflex contraction. MK-801, an NMDA receptor antagonist, increases the density of CGRP-immunoreactive (I) primary afferent nerve fibers in the spinal cord, stomach and uterine cervix. In this study, the effect of MK-801 on CGRP-I bladder fibers following spinal cord transection was examined. Rats received either a spinal cord transection at the L2 level (n = 8) or a transection plus a daily i.p. injection of MK-801 (1.0 mg/kg, n = 6). Additional rats served as untreated controls (n = 5). Twelve days post-surgery, bladders from all rats were processed for CGRP-I. In control rats, CGRP-I nerve fibers were present as perivascular plexi and as a dense, non-vascular network of fibers throughout the detrusor muscle. In bladders from transected rats, the density, number of varicosities and the fluorescence intensity of CGRP-I fibers associated with vessels and the musculature were greatly reduced. Bladders from cord-transected rats receiving MK-801, showed a marked increase in the density of varicose, intensely fluorescent CGRP-I fibers throughout the detrusor muscle. However, the density of non-vascular CGRP-I nerve fibers remained less than that in bladders from control rats. These data suggest that MK-801 treatment following spinal cord transection increases the intensity of immunostaining, the density of CGRP-I fibers and the number of varicosities in the urinary bladder. (Supported by the Paralyzed Veterans of America Spinal Cord Res. Found. and OCAST)

118.12

IN VITRO CHARACTERIZATION OF MESENTERIC MECHANORECEPTORS OF THE SMALL INTESTINE. J.Y. Wei. CURE/VA Wadsworth MC, Dept. of Medicine and BRI, UCLA, CA.

The purpose of the current study was to develop a splanchnic nerve - mesentery in vitro preparation to characterize the mechanosensitivity of the nerve endings under a controlled experimental environment. Urethane anesthetized rats were used. A segment of abdominal aorta, with superior mesenteric artery and the celiac-superior mesenteric ganglionic complex attached to it, the splanchnic nerves connecting suprarenal and celiac ganglia, the greater splanchnic nerve, and the attached mesentery were isolated and removed. The preparation was transferred into a perfusion bath flowing with oxygenated normal rat saline (2-3 ml/min). Unitary activity was recorded from a thin splanchnic nerve filament. The receptive field was searched and defined with a camel's hair brush and/or von Frey hairs. Of the 22 units studied 11 had irregular and one regular low frequency on-going discharge; 2 had rhythmic while 8 had no resting activities. Location of the receptive fields includes 11 units near the main vascular nerve bundle, 8 on the mesentery, 1 near the aorta and 2 unknown. Fourteen units were slowly adapting and 6 rapidly adapting; after-discharge occurred in 10 units. A short after-inhibition following termination of the stimulus was observed in 6 units. Conclusion: this preparation can be used to study afferent terminals in the mesenteric membrane and their interaction with the surrounding environment. (Supported by NIH grant NS28433)

118.13

IMPROVED TRACER LABELING: FAST BLUE IN DORSAL ROOT GANGLIA AFTER INJECTIONS INTO REPRESENTATIVE GUT SEGMENTS. M.-Q. Zhang, J.Y. Jew, Y.-F. Wang and T.H. Williams. Dept. of Anatomy, Univ. of Iowa, Coll. of Medicine, Iowa City, IA 52242.

Previous investigations have provided information about the sources of sensory nerve fibers servicing selected portions of the gastrointestinal system but have not systematically addressed comparative aspects of the sensory innervation between the different gut segments. This report represents preliminary findings for stomach, jejunum, ileum, cecum, ascending colon, and descending colon of the rat. The retrograde fluorescent tracer Fast Blue (FB) was injected into the wall of portions of the gut as listed above of male Sprague-Dawley rats (anesthetized with Nembutal, 40 mg/kg b.w.). Eight days later the animals were reanesthetized and sacrificed by perfusion fixation. Dorsal root ganglia (DRG) from levels T1-S4 were dissected out and serial sections were cut and processed for fluorescence microscopic examination. Labeled perikarya were counted and measured to establish differences between DRG neurons that innervate different parts of the gut. We also compared the effectiveness of retrograde labeling when a given amount of FB tracer was administered via numerous versus few multiple injection sites. The greatest number of FB labelled perikarya was found in T9 after injections into the wall of stomach; in T11 for jejunum; in T11 for ileum; in T12 for cecum; in T13 for ascending colon; and in L1 and S2 for descending colon where there was a double distribution peak. Preliminary results indicated that DRG neurons innervating stomach, cecum, and colon were larger than those innervating jejunum and ileum. When FB tracer was injected via numerous (200) sites into a specific gut segment, the number of labeled perikarya per DRG was greater than that following the use of few (20) injection sites. This information is intended to lead to transmitter correlation experiments utilizing combinations of retrograde tracers and immunohistochemical techniques. Supported by grant DK38123.

118.14

DEVELOPMENT OF A RETROGRADE DYE LABELING TECHNIQUE FOR THE IDENTIFICATION AND FUNCTIONAL STUDY OF LUNG-SPECIFIC VISCERAL C-FIBER NEURONS. K.E. Naper, E.P. Christian, G. Koschorke, G.E. Taylor*, B.W Forbush* and D. Weinreich. Dept. of Pharmacol., ICI Americas, Inc., Wilmington, DE 19878, and Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD, 21201.

The nodose ganglion contains cell bodies which give rise to visceral afferent C-fibers innervating the respiratory, cardiovascular and gastrointestinal systems. To study airways-specific C-fiber neurons, a 100 ul volume of (w/v ratio) 10% rhodamine conjugated dextran, 1-2.5% fast blue, or 1% fluorogold is instilled into the trachea of anesthetized (mg/kg: 30 ketamine, 5 xylazine, 2 acepromazine) male guinea pigs (450-550g) by either intubation or catheterization below the cricoid cartilage. After allowing 5-10 days for retrograde transport, label can be visualized in viable 100 um ganglion sections, and is retained following acute dissociation of neurons. Equivalent dye administrations directly into the esophagus or carotid artery fail to label neurons; thus providing evidence that neurons extrinsic to the airways are not inadvertently labeled by tracheal instillations. Passive and active electrophysiological properties do not differ between unlabeled and rhodamine-labeled dissociated neurons, and are not altered in labeled neurons by 5-15 s illumination by the 530-560 nm excitation wavelength for rhodamine. This methodology should provide new information on the physiological, biochemical and pharmacological properties of *airways-specific* C-fiber neurons. (NIH NS22069 to D.W)

SUBCORTICAL SOMATOSENSORY PATHWAYS: BRAINSTEM

119.1

DIFFERENTIAL LABELLING OF CLASSES OF PRIMARY AFFERENT SYNAPTIC TERMINALS IN THE RAT NUCLEUS GRACILIS AFTER INJECTION OF EITHER WGA-HRP OR B-HRP INTO THE SAPHENOUS NERVE. S.E. Kapadia, C.M. Shapiro, and C.C. LaMotte. Section of Neurol. Surgery, Yale Univ. Sch. of Med., New Haven, CT. 06510.

The saphenous nerves of anesthetized rats were labelled by injection of either 0.5 mg of WGA-HRP or 0.05 mg of B-HRP. After 72 hours the animals were sacrificed by perfusion; the lumbar roots, lumbar cord and the medulospinal junction were removed, sectioned, and reacted with a combined TMB-DAB procedure for EM visualization of the label. Counts of labelled axons in the roots showed that 98% of the axons labelled by B-HRP and 16% of the axons labelled by WGA-HRP were myelinated. The two labels were differentially localized within some, but not all, afferent terminal types in the nucleus gracilis: 1. simple terminals with round vesicles and few mitochondria (B-HRP and WGA-HRP); 2. larger simple terminals with many mitochondria (B-HRP); 3. small terminals with few mitochondria, having 1-2 postsynaptic targets and often postsynaptic to other terminals (B-HRP); 4. large glomerular terminals with several central mitochondria and no neurofilaments (WGA-HRP); 5. large glomerular terminals with neurofilaments and many mitochondria (B-HRP and WGA-HRP); and 6. large glomerular terminals with no neurofilaments and many mitochondria (B-HRP and WGA-HRP). Some of these terminal types resemble the terminal classes associated with myelinated and nonmyelinated afferents in the spinal dorsal horn. (NIH NS 13335)

119.2

CALCITONIN GENE-RELATED PEPTIDE (CGRP) IMMUNOREACTIVE FIBERS ARE PRIMARILY RESTRICTED TO A "MIDDLE" REGION OF THE CUNEATE NUCLEUS IN THE RAT. D.P. Crockett, S.L. Harris*, S. Maslany, and M.D. Egger. Dept of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

Contrary to earlier reports, the cuneate nucleus (CN) in the rat appears to have a functional "middle" region, extending from approximately 0.2 mm to 0.8 mm caudal to the obex. Within this middle region, there is maximal differentiation of cutaneous primary sensory afferent termination zones (particularly from the glabrous skin of the forepaw digits), corresponding topographically to dense patches of cytochrome oxidase staining. CGRP-like immunoreactivity (n = 5 rats) was largely confined to this "middle" region of the CN, with a few fibers being found more caudally. In contrast, substance P-like immunoreactive fibers (n = 6 rats) were located chiefly outside of this region, most abundantly in the caudal CN near the spinomedullary junction, extending caudally into the internal basilar nucleus. Our evidence that the CN in the rat has a distinct "middle" region indicates that it is organized more like that of other mammalian species than previously recognized.

119.3

POST-SYNAPTIC DORSAL COLUMN TERMINALS IN THE CUNEATE NUCLEUS OF THE RAT: MORPHOLOGY AND PUTATIVE TRANSMITTER. S. De Biasi, L. Vitellaro-Zuccarello*, P. Bernardi* and A. Rustioni. Dip. Fisiol Biochim gen, Univ.Milano, Italy; Dept Cell Biol & Anat, Univ North Carolina, Chapel Hill,NC.

The dorsal column nuclei (DCN) receive two ascending inputs that may subserve different sensory modalities: 1) primary afferent terminals from dorsal root ganglion cells and 2) post-synaptic dorsal column (PSDC) terminals from neurons in the ipsilateral dorsal horn.Multiple injections of WGA-HRP were made in the cervical spinal cord of rats 6 days after unilateral cervical rhizotomy and lesion of the dorsal quadrant at T1. After 24-36h, rats were reanaesthetized and perfused. Vibratome sections of brainstem, processed for HRP visualization, were embedded in plastic and blocks of cuneate nucleus (CN) from 3 rats were thin sectioned. At the EM, HRP reaction product is in numerous medium-size terminals (mean area 1.5um^2) containing many small clear vesicles. HRP+ terminals synapse on small- to medium-size dendrites and, at variance with the large-size primary afferent terminals, are not contacted by GABAergic endings of interneurons. In thin sections of CN processed with a post-embedding immunogold method using anti-glutamate (Glu) serum, about 2/3 of the HRP+ terminals are Glu+. Since most of the primary afferents to CN are Glu+ (DeBiasi and Rustioni,J Histochem Cytochem 38:1745,1990) it is likely that Glu is the major transmitter for both ascending pathways to DCN. *Supported by NIH grants NS27827 and 12440.*

119.4

CONNECTION RELATIONSHIP OF SPINAL NEURONS WITH BOTH THE POINT ZUSANLI AND THE SOLITARY TRACT NUCLEUS. G.W.Lu,and Z.Meng*,Dept.of Neurobiol. Capital Institute of Medicine, Beijing 100054, China.

We have demonstrated that the point zusanli (tsu-san-li, St-36,ZSL) is innervated by the peroneal nerve (Amer J Physiol 245:606-612,1983) and that spinal dorsal horn neurons project to the solitary tract nucleus (STN)-spinosolitary tract neurons (SST) (Chin Sci Bull 35: 1474-1479, 1990). The present study aims at studying the connection among the ZSL, SST and STN.

Experiments were performed on 27 Wistar rats anesthetized with sodium pentobarbital. Microelectrode recording was made from the lumbosacral dorsal horn of the spinal cord. Electro- stimulation was delivered to the STN and ZSL to antidromically and orthodromically activate the nucleus and the point, respectively. Natural stimulation of the receptive fields (RF) was used to identify the physiological category of the recorded neurons.

A total of 57 neurons was intracellularly recorded and identified as SST neurons in laminae III-V of the lumbosacral dorsal horn. Synaptic responses were also shown in 34/57 neurons. All these neurons orthodromically responded to electrical stimulation of the ZSL. An equal number of LTM and WDR neurons were categorized based on RF stimulation. Most LTM neurons responded to ZSL electrical stimulation to the similar degree at 10T and 50T and almost all the WDR neurons discharged greater at 50T than at 10T.

These results indicate that single spinal dorsal horn neurons receive somatic information and transmit it to the viscerosensory nucleus, STN, and that the somatic and visceral input may converge on common SST neurons as well as within the STN.

119.5

THE TOPOGRAPHIC ORGANIZATION OF THE RAT ZONA INCERTA. **R.C.S. Lin, M. A. L. Nicolelis, J. McLean, and J. K. Chapin. Department of Physiology and Biophysics. Hahnemann University, Philadelphia, PA 19102-1192, USA.**

Using the cytochrome oxidase (CO) staining method the rat zona incerta (ZI) can be subdivided, throughout its rostro-caudal extension, into two laminae: a distinctly dark ventral layer and a much lighter dorsal zone. This clear subdivision can be observed since the day of birth and is correlated with the topography of the main afferents to the ZI. Here, the connectivity and physiology of ZI neurons were investigated by combining the use of anterograde/retrograde fluorescent tracers with mapping and multi-single unit recording techniques. Injections of rhodamine dextran (RD) into the somatosensory (SI) and motor cortices, deep layers of the superior colliculus, trigeminal complex and dorsal column nuclei revealed the presence of anterogradely labeled terminals within the ZI. These terminals were mainly distributed around the ventral portion of the ZI, within the darkest CO-stained lamina. Less dense terminals were also found into the dorsal subdivision of the nucleus. Conversely, injections of RD in the deep cerebelar nuclei labeled terminals in the most dorsal, lighter CO zone whose cells project to the SI. Injections of (RD) into the ZI demonstrated that its cortical afferents derive mainly from layer V pyramidal neurons and that incertocortical neurons may project to layer I. Mapping experiments revealed the existence of a crude somatotopic map of the rat body within the ZI. This map seems to parallel the somatotopic map of the ventral posterior thalamus, being mainly characterized by large contralateral facial RFs. Large contralateral, circular, and non-direction selective visual RFs were also observed in the ZI. These results suggest that the rat ZI may be involved in sensorimotor integration related to the control of gaze. ***Sponsored by grants NS29161, NS26722, and FAPESP 88/4044-9.***

119.6

CODING OF NOVELTY BY MULTISENSORY NEURONS IN THE SUPERIOR COLLICULUS OF THE HAMSTER. M. M. Nikoletseas. Dept. of Anatomy, Univ. of Puerto Rico, Sch. of Med., San Juan, PR 00936.

Although multisensory neurons in the tectum have been known for several decades, details of their fine morphology and physiology are still lacking. The problem of coding stimulus novelty presents more facets for these cells, since afferent input comes from more than one sensory systems. Hamsters were anesthetized and paralyzed. Stimulation parameters were programed in strict temporal sequence: Interstimulus interval 30 ms and intertrial interval 1 s. Spikes were preamplified and fed into a computer for on-line acquisition and analysis. Spike activity was monitored for periods of 20 s or more. The main findings are: 1. A novel stimulus induces a decrease in interspike intervals in the baseline of spontaneous activity. 2. An increase in number of spikes is not a good predictor of novelty. 3. As novelty fades, the system lapses into longer interspike intervals. 4. This coding mechanism operates when a cell is processing either a stimulus of one sensory modality, or a compound stimulus consisting of stimuli from two sensory modalities.

119.7

PARCELLATED ORGANIZATION IN THE TRIGEMINAL AND DORSAL COLUMN NUCLEI OF PRIMATES. A.L. Noriega* and J.T. Wall. Department of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Previous studies have used cytochrome oxidase (CO) staining to describe patterns of parcellated organization in the trigeminal nuclei of rodents and cats. The present studies used CO histochemistry to test if parcellated organization patterns also occur in the brainstem of adult monkeys. The results demonstrate that a parcellated pattern of neural organization exists in the primate trigeminal nucleus principalis. In the more caudal spinal trigeminal nuclei, interruptions in CO staining are also seen, but the overall patterns are more uniform and do not reflect the parcellated pattern seen in the nucleus principalis. In contrast, the organization in nucleus principalis resembles the parcellated organization in nuclei of the dorsal column complex. Taken together with findings of previous studies in rodents and cats, these results suggest that aggregated or parcellated neural organization is a common characteristic of specific ascending somatosensory projections from the face and other body parts in many mammals including primates. Supported by NIH Grant NS21105.

119.8

DISTRIBUTION AND MORPHOLOGY OF EFFERENT PROJECTIONS FROM NEURONS IN DORSOMEDIAL SUBDIVISIONS OF RAT TRIGEMINAL NUCLEUS INTERPOLARIS. M. S. Cook* and W. M. Falls, Dept. Anat., Michigan State Univ., East Lansing, MI 48824.

Efferent projections from dorsomedial (DM) subdivisions of rat trigeminal nucleus interpolaris (Vi), were studied using the PHA-L method. Contralateral projections were to ventral posteromedial thalamic nucleus (VPM), zona incerta (ZI), parvocellular red nucleus (RPC) and anterior pretectal nucleus (APT). Projections to superior colliculus (SC), medial accessory oculomotor nucleus (MA3) and portions of inferior olivary nucleus (IO) were bilateral with contralateral predominance while those to facial motor nucleus (VII), sensory trigeminal complex (SVC), cervical spinal cord (CSC), as well as cerebellum (Cb) and deep cerebellar nuclei (DCN) were bilateral with ipsilateral predominance. No more than one type of efferent ended in APT, RPC, MA3, VII, SVC, IO or CSC, while more than one type was located in VPM, ZI, SC and DCN. Efferents to Cb were mossy fibers with some extending into Purkinje cell layer. These results suggest DM subdivisions play a major role in conveying orofacial tactile input to functionally diverse areas along the neuraxis and support Vi subdivisional organization by showing that major differences exist in DM efferent projections when compared to those originating from neurons in ventrolateral subdivision. (Supported by B.R.S.G. Grant, College of Osteopathic Medicine.)

119.9

ULTRASTRUCTURE OF PRIMARY DEAFFERENTATION IN THE NEONATAL FELINE TRIGEMINAL SYSTEM. [1,2,3]L.E. Westrum, [1,2,3]M.A. Henry, and [1]X-M. Dong*. Depts. [1]Neurol. Surg., [2]Biol. Struct., [3]Restor. Dent. University of Washington, Seattle, WA 98195.

We are investigating the age-related, morphological effects of total retrogasserian rhizotomy on the synaptic organization and remodeling in the brainstem spinal trigeminal nuclei (STN) in felines. Other previous publications by ourselves and by F.W.L. Kerr have suggested that plasticity in the STN is greater in neonates than in older felines following primary deafferentation. Our several past publications have described time-dependent changes in the adult system and here we present preliminary findings on STN alterations in the immature system.

Felines of postnatal age (PN) 3 days, under deep anesthesia, received total unilateral retrogasserian rhizotomy. Following survival times varying from 16 hours to one week the subjects were reanesthetized, perfused and tissue from STN prepared for study by conventional electron microscopy. The preparations show striking alterations of the axons and synapses in STN. Even in the shortest (16 hours) survival, extensive degeneration occurs in the tract and disintegration of axon terminals and synapses can be seen in large numbers. Within 1-2 days postsurgery, deafferented postsynaptic specializations are frequently apposed by one or more profiles. The latter may be glia, dendrites or axons. Some of these profiles contain vesicles, vacuoles, and smooth endoplasmic reticulum suggestive of "growth processes". The observations demonstrate an exceedingly rapid post deafferentation degenerative process as compared to the adult system with even earlier evidence of competitive reoccupation of denervated postsynaptic sites, possibly by growth processes, than occurs in adults. These findings could explain the age-dependent plasticity occurring in the system. (Supported by NIH Grants DE04942 and DE00219. L.E.W. is a research affiliate of the CDMRC at the University of Washington).

119.10

EFFECTS OF "LATE" INFRAORBITAL NERVE TRANSECTION UPON CYTOCHROME OXIDASE PATTERNS IN TRIGEMINAL NUCLEUS PRINCIPALIS AND SUBNUCLEUS INTERPOLARIS OF THE RAT. R.W. Rhoades, C.A. Bennett-Clarke and N.L. Chiaia. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

The vibrissa-related cytochrome oxidase patterns in nucleus principalis (PrV) and subnucleus interpolaris (Spl) are both completely disrupted by neonatal transection of the infraorbital nerve (ION). This effect has been interpreted as being the result of a loss and/or disruption of the normal primary afferent innervation of these nuclei. Since the same primary afferent axons innervate both PrV and Spl, it follows that a peripheral lesion that disrupts the CO pattern in one nucleus should have the same effect in the other nucleus. ION lesions made between postnatal days (P) 3 and 8 have different effects upon the CO pattern in PrV and Spl. In rats that sustain ION lesions on these days and are killed 2-6 days later, the density and clarity of the CO pattern in Spl is significantly reduced while the pattern in PrV is essentially unaltered. Such differential effects are not observed in rats that sustain ION transection on P-0 to P-2, or on P-10 and later ages. The differential effects of ION damage in PrV and Spl are not likely to be the result of differential transganglionic degeneration in these two nuclei; transganglionic tracing with HRP in rats that sustained ION damage on P-4 and P-5 indicated essentially equivalent deafferentations of PrV and Spl. These results indicate that the CO patterns in PrV and Spl have different sensitive periods for ION damage. They also support the conclusion, at least for PrV, that normal primary afferent input, while necessary for the development of the vibrissa-related CO pattern, need not be maintained for that pattern to persist. NS 28888, DE 07734

119.11

NORMAL DEVELOPMENT AND EFFECTS OF INFRAORBITAL NERVE LESIONS UPON THE INNERVATION OF THE TRIGEMINAL BRAINSTEM COMPLEX BY CGRP-POSITIVE PRIMARY AFFERENTS. F.A. White., C.A. Bennett-Clarke and N.L. Chiaia. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Previous experiments have shown that neonatal infraorbital nerve (ION) transection results in the death of many trigeminal ganglion cells that contain substance P-like immunoreactivity (SPLI), but only a small and transient decrease in the density of SPLI in subnucleus caudalis (SpC) of the rat's brainstem trigeminal complex. In an effort to determine the extent to which the effects observed in the brainstem result from primary afferent reorganization or alterations in central SP projections to SpC, we evaluated the normal development and lesion induced alterations of CGRP-like immunoreactivity in SpC. This peptide co-localizes extensively with SP in primary afferents and has not been reported to exist in any central projections to SpC. In normal adults, CGRP-positive fibers form a dense plexus in lamina I and outer lamina II. A smaller number of fibers extend into layers III-V. Essentially the same pattern of CGRP immunoreactivity is present in newborn rats. Neonatal ION transection produces no substantial alteration in this pattern in rats killed between one and 60 days after the lesion. Densitometry indicated a slight (5%-11%), but non-significant reduction in the density of CGRP-like immunoreactivity ipsilateral to the ION lesion on postnatal days 4-6. Thus, neonatal ION transection has little effect upon the distribution and density of CGRP-positive fibers in SpC. Since this lesion is likely to kill numerous CGRP-positive trigeminal ganglion cells, our results further suggest that neurons surviving neonatal axotomy increase their innervation of SpC. DE 07734, NS 28888

119.12

INNERVATION OF SPARED FOLLICLES AFTER DAMAGE TO THE VIBRISSA PAD IN FETAL RATS. R.S. Crissman, N.L. Chiaia and F.A. White. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Partial damage to the vibrissa pad on embryonic (E-) days 15 through 18 increases the representations of undamaged follicles in the trigeminal brainstem complex (Bennett-Clarke et al., this meeting). This study used anatomical and electrophysiological methods to determine whether such lesions altered the trigeminal innervation of surviving vibrissa follicles in adult rats that sustained vibrissa pad lesions on E-17. We recorded single trigeminal ganglion cells from 12 rats with fetal vibrissa pad lesions verified by both direct examination of the face and by CO staining of flattened sections through the cerebral cortex contralateral to the lesion. As is the case in normal rats, all of the 49 vibrissa-sensitive ganglion cells recorded from the lesioned animals were responsive to deflection of one and only one vibrissa. We dissected 11 deep vibrissal nerves from intact follicles in rats that sustained fetal vibrissa pad damage and counted numbers of myelinated axons in 1 μm plastic sections using the light microscope. These counts were compared with counts from the corresponding follicle on the intact side of the face and with previous results from normal adult rats (R.S. Crissman et al., Somatosensory and Motor Res., in press). The average number of myelinated axons from deep vibrissal nerves innervating intact follicles in the damaged vibrissa pad was 197±28 and that for nerves on the intact side was 195±26. These results suggest that damage to vibrissa follicles on E-17 does not significantly affect the trigeminal innervation of undamaged follicles and that increases in the central representations of these follicles need not be correlated with changes in their peripheral innervation. NS 28888, DE 07734

119.13

DAMAGE TO VIBRISSAE FOLLICLES IN FETAL RATS INCREASES THE SIZE OF CYTOCHROME OXIDASE PATCHES CORRESPONDING TO INTACT FOLLICLES IN THE TRIGEMINAL BRAINSTEM COMPLEX. C.A. Bennett-Clarke, N.L. Chiaia, and R.W. Rhoades.
Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Damage to vibrissa follicles in newborn rats and mice does not alter the brainstem representations of the remaining vibrissa as demonstrated by cytochrome oxidase (CO) staining. This study asked whether this lack of effect might be due to the fact that trigeminal primary afferents in rodents are already quite well developed at birth. We made partial lesions of the vibrissa pad on embryonic (E-) days 15-20 and on postnatal (P-) day 0 (at least 5 and an average of 11 animals for each age), killed pups on P-6 or P-7, and measured the size of the CO patches in trigeminal subnucleus interpolaris (Spl) on both sides of the brainstem. All vibrissa pad lesions were verified by silver staining sections through the face and the correspondence between CO patches and clusters of primary afferent terminal arbors was verified in some animals by combining transganglionic HRP tracing and CO staining in alternate sections. Vibrissa pad damage on E-15 through E-18 resulted in significant (20%-37%) increases in the average area of the remaining CO patches in Spl ipsilateral to the lesion. Furthermore, there was a significant inverse relationship between the average increase in patch size and the number of patches that remained in Spl. Vibrissa pad damage on E-19, E-20, and P-0 produced small (6%-9%), but non-significant, increases in patch size in Spl ipsilateral to the lesion. These data suggest the possibility that competitive interactions among the central arbors of trigeminal primary afferents in fetal life may influence the development of central vibrissa representations. DE 07734, NS 28888

119.14

ORGANIZATION AND RESPONSE PROPERTIES OF NEURONS IN THE CAUDAL TRIGEMINAL COMPLEX OF THE MUSKRAT. W.M. Panneton and M.F. Jacquin. Dept. of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

The caudal subnucleus interpolaris and rostral medullary dorsal horn of the muskrat were explored electrophysiologically to establish data on the response properties of neurons to orofacial and upper respiratory tract (URT) stimulation. Muskrats were anesthetized with chloralose-urethane, tracheotomized, paralyzed and artificially ventilated. Single units were recorded with glass micropipettes (DC resistances 35-60 megohms) filled with 6% HRP in 0.05M Tris-buffered KCl to mark recording sites. All units responded to electrical stimulation of the ipsilateral trigeminal ganglion and were tested with innocuous and noxious stimuli to orofacial areas, ammonia vapors to the nasal cavity, and electrical stimulation of the superior laryngeal nerve.

None of the 58 cells studied were activated antidromically from the thalamus or the dorsolateral pons. Their topographical organization was similar to that previously described for the cat and rat. 91% of the neurons responded to innocuous stimulation with a mean latency of 1.49 ms to ganglion shock. 9% responded to noxious or URT stimulation with a latency of 2.3 ms. 17% were spontaneously active; 30% of these were inhibited by stimulation of their receptive field surrounds. 57% gave phasic responses to tactile stimulation, while 43% had tonic responses. 34% responded to stimulation of mystacial vibrissa(e), 29% to guard hairs, 16% to hairy skin, 16% to nasal vestibule or nasal mucosa with ammonia, 9% to nasal glabrous skin, 7% to oral mucosa, and 2% to superior laryngeal nerve. These data establish baseline data for more concentrated efforts to characterize trigeminal neurons responsive to URT stimulation. NIH HL38471, DE07762.

119.15

PLASTICITY OCCURS IN THE SPINAL TRIGEMINAL NUCLEUS OF THE ADULT MOUSE UPON PARTIAL PERIPHERAL DEPRIVATION.

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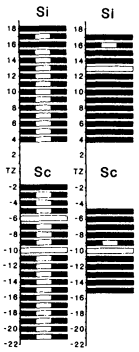
Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

After injection of ^{14}C-deoxyglucose (DG), vibrissae of rows B and D were stimulated. In 3 mice the nerve innervating the follicles of row C had been divided 60 days earlier; 4 mice served as controls. Autoradiograms of transverse brainstem sections showed stimulus-evoked DG uptake in nucleus principalis, and in subnuclei oralis, interpolaris (Si) and caudalis (Sc). Representations of rows B and D were consistently observed along a major extent of Sc and Si only, except for their rostral and caudal poles. They were either separated (sections displayed as ▬) by an area of low uptake (row C) or fused (▬) due to high uptake over row C. Unusable sections: ▭. The left "keyboard" represents a control mouse; the right, a deprived one. Transition zone between Si and Sc (TZ) serves as reference point. Numbers refer to serial sections. Per animal we determined an "index" for each subnucleus by dividing the number of fused sections by the total number of usable sections. For controls, the mean index for Sc = .1 (range: .0 - .3) and for Si =.3 (.2 - .7); for the deprived mice, the mean index for Sc = .6 (.3 - .9) and for Si = .9 (.9 - 1). We conclude that the increase in the functional representations of the B and D whiskers occurs without preference for a rostrocaudal level. The effects in Si are more marked, with smaller interindividual variability. For similar signs of plasticity in the barrel cortex see Melzer et al., 1988. Does the functional enlargement occur in brainstem and cortex independently? Support: Swiss NSF 3100.009468.



119.16

COMPUTER SIMULATIONS OF SIGNAL PROCESSING IN THE RAT TRIGEMINAL BRAINSTEM AND THALAMUS. D.W. Doherty, R. Granger and H.P. Killackey. Dept. of Psychobiology, University of California, Irvine, CA 92717.

A model of the trigeminal neuroaxis, from periphery to thalamus, has been developed. The model simulates a 3 by 3 array of 9 whiskers. Thirty primary afferents connect each whisker to a corresponding barrelett of 12 trigeminothalamic neurons in the trigeminal nucleus principalis (PrV). The bifurcating primary afferents also synapse with trigeminothalamic neurons of both the corresponding and the surrounding whiskers in the spinal trigeminal nucleus interpolaris (SpVi). There are 3 SpVi neurons for each whisker. Synapses from individual barreletts in PrV or SpVi are restricted to neurons in their corresponding barreloid in the thalamic ventral posterior medial nucleus (VPm). Each of the 30 VPm neurons in a barreloid receives input from both PrV and SpVi and sends collaterals to a restricted portion of the thalamic reticular nucleus (TRN). Six inhibitory neurons in TRN synapse with their VPm input neurons and neurons in adjacent barreloids.

An equivalent circuit model based on ion fluxes was implemented to mimic cellular voltage dynamics. The physiological characteristics of each neuron type were matched with available data. A mix of rapidly and slowly adapting primary afferent response types was simulated.

Stimulation of a single simulated whisker for 4 ms elicited responses in the thalamic barreloid of the principle whisker with latencies of between 3 to 6 ms and a mean of 1.0 spikes per stimulus. Neurons in the surrounding barreloids responded with latencies between 4 to 6 ms and a mean of 0.2 spikes per stimulus. When PrV was lesioned the high amplitude (center) response in the barreloid of the principle whisker disappeared and was replaced by the low amplitude (surround) response typically seen in the surrounding barreloids. When SpVi was lesioned the center response remained intact but the surround response disappeared. The model demonstrates that the parallel trigeminal pathways through PrV and SpVi that converge onto VPm are sufficient to account for the principle whisker (center) and non-principle whisker (surround) response characteristics recorded from thalamocortical neurons in vivo.

120.1

CHARACTERIZATION OF THE CARDIOVASCULAR AND VISCEROMOTOR RESPONSES TO ESOPHAGEAL DISTENTION IN THE RAT. S.T. Meller and G. F. Gebhart. Deptment of Pharmacology, University of Iowa, Iowa City, IA, 52242

The aim of this study was to characterize the cardiovascular (CV) and visceromotor (VM) responses to graded levels of esophageal distention (ED; 0.5-1.5 ml, 30 s) produced by a Swan-Ganz catheter inserted intra-orally into the middle third of the esophagus in the lightly-pentobarbital anesthetized male Sprague-Dawley rat (420-460 g).

The CV response during ED was consistently a robust, intensity-dependent pressor response (0-35 mmHg) and a tachycardia (0-50 bpm). Following distention, arterial blood pressure showed a small, but consistent decrease (5-15 mmHg). The VM response, which was measured from the EMG recorded in the masseter muscle, was a vigorous intensity-dependent contraction (2-5 s) followed by a smaller sustained contraction throughout ED (30 s). Peak magnitude of this response with graded levels of ED was 110 μV. The VM response was completely abolished by bilateral transection of the cervical vagus or neonatal capsaicin treatment. The CV response was unaffected following bilateral vagotomy. In neonatal capsaicin-treated rats (50 mg/kg, s.c.), the pressor response to ED was unaffected, but the depressor response following distention was abolished. The VM response to esophageal distention was abolished in a dose-dependent manner by morphine (0.5-4 mg/kg, i.v.) and reversed by naloxone (1 mg/kg, i.v.). The CV responses were unaffected by 4 mg/kg morphine.

These results suggest that the nociceptive responses to graded levels of ED are mediated by capsaicin-sensitive vagal afferents.

120.2

CHARACTERIZATION OF NOXIOUS VISCERAL INPUT TO MEDIAL THALAMIC NEURONS. R.M. Danzebrink, S.T. Meller and G.F. Gebhart. Dept. Pharmacology, Univ. Iowa, Iowa City, Iowa 52242.

Anatomical and physiological studies suggest that various nuclei of the medial thalamus are involved in nociceptive processing. This study characterized the location and responsiveness of neurons in the medial thalamus to a natural, noxious visceral stimulus, colorectal distension (CRD), in pentobarbital-anesthetized rats. The locations of 133 units responding to noxious CRD in the medial thalamus were histologically verified to be distributed throughout nuclei such as medialis dorsalis, medialis and lateralis centralis, lateralis medialis and ventralis, paracentralis, reunions and submedius. The spontaneous activity of 55 units was inhibited by noxious CRD; 78 units were excited by CRD. About half of the units gave reproducible responses to repeated CRD for at least 90 minutes. These units also responded to CRD in an intensity-dependent manner. 61% of the 133 units exhibited large, bilateral convergent cutaneous receptive fields. Noxious distension of the esophagus also activated some (n=34/64) of the neurons which responded to noxious CRD. These results reveal that visceral nociceptive input to medial thalamic nuclei is widespread and that medial thalamic neurons receive convergent input from other visceral as well as somatic structures.

120.3

CEREBRAL CORTICAL NEURONS RESPOND TO NOXIOUS VISCERAL STIMULATION. K.A. Follett, R. Hadley, and G.F. Gebhart. Depts. of Neurosurgery and Pharmacology, University of Iowa, Iowa City, IA 52242

The role of cerebral cortex in visceral nociception has not been well-studied. We have undertaken studies to determine whether cortical neurons respond to a physiologic noxious visceral stimulus (balloon distension of the colon) and have characterized the responses.

Sprague-Dawley rats were anesthetized with pentobarbital. Spontaneously active single neurons were isolated in somatosensory cortex using standard microelectrode recording techniques. Their responses to graded distension of the colon (20-100 mm Hg) and cutaneous stimuli were recorded.

Thirteen neurons responding to colon distension were identified. Ten units (77%) were excited upon distension (112-415% of control, mean 166%), 3 were inhibited (35-65% of control, mean 48%) with thresholds as low as 20 mm Hg. Contralateral cutaneous receptive fields were identified for 3 units.

We have demonstrated that cerebral cortical neurons respond to noxious visceral stimulation. Convergence of visceral and somatosensory input occurs in a subset of these neurons. The responses of these cells are similar to those observed in spinal cord and thalamus.

120.4

RAT LOW BACK DYSFUNCTION ASSOCIATED WITH AMBULATION IN AN ABNORMAL POSTURE. J Hulse Neufeld. VA Medical Center, San Diego, CA 92161.

An animal model of low back dysfunction was developed in order to study the mechanism of induction and maintenance of low back pain. Male Sprague-Dawley rats (2 mo) ambulated 1 hr/day for 4 - 6 weeks in motorized apparatus. Two groups of rats were compared: rats that ambulated on a flat surface (AF) and in a rotating cylinder (AC). While ambulating, the AF rats held their low back in the normal kyphosis and the AC rats in a relative extension. Using repeated measures design significant interactions were obtained in body weights, ambulation in an open field test, and surface temperatures at the base of the tail. The results of these significant findings and of trends in a tail flick assay of nociception were consistent with the hypothesis that the AC group had experienced increased levels of general stress (body weight results) and disability (ambulation) associated with hyperalgesia in the lower body (tail flick) and increased sympathetic output (tail temperature). Also, a significant inverse correlation between ambulation and concentration of calcitonin gene-related peptide in the dorsal part of the caudal spinal cord was observed. The correlation related sensory pathways to locomotion in rats.

120.5

ASSESSMENT OF OROFACIAL PAIN FOLLOWING NEONATAL INFRAORBITAL NERVE TRANSECTION USING THE FORMALIN TEST. **B.G. Klein and C.F. White*. Dept. of Biomedical Sciences, College of Veterinary Medicine, VA Tech, Blacksburg, VA 24061.**

Much literature is devoted to the anatomical and physiological effects of neonatal infraorbital nerve (ION) transection in rat. Effects of such damage on sensation in conscious animals has received little attention. We therefore employed the formalin test for orofacial pain (Clavelou et al., 1989, Neurosci. Let., 103:349-353) in normal adult rats and adults subjected to neonatal ION cut. Formalin (5% in saline, 0.05 ml) or saline vehicle was injected into the vicinity of the C4/D4 vibrissae. Rats were placed in an observation tank and videotaped for 45 min. Continuous records of whiskerpad rubbing were made from the tapes, which were examined blind. As previously described, normal rats (N=12) injected with formalin exhibited an early, short-lasting response (0-3 min) and a later, prolonged response (18-39 min), separated by a period of relative inactivity. No such pattern was observed for ION cut rats injected with formalin (N=11) or saline vehicle (N=11). Total duration of rubbing in formalin injected ION cut rats ($\bar{x}$=65,SEM=11 s) was significantly less than that in formalin injected normal rats ($\bar{x}$=205,SEM=29 s), and did not differ from that of ION cut rats injected with saline ($\bar{x}$=67,SEM=9 s). Thus, neonatal ION cut appears to render rats insensitive to the noxious stimulation of subcutaneous formalin. **Support: NIDR DE08966 to BGK.**

120.6

ACTIVATION OF LOCUS COERULEUS NEURONS BY DORSAL COLUMN (DC) STIMULATION (st). S.J. Jabbur, E. El-Chaer*, S.F. Atweh and N.E. Saadé. Faculty of Medicine, American University of Beirut, Beirut, Lebanon.

Pain inhibition by activation of a DC-brainstem-spinal loop can be partially antagonized by α-adrenergic antagonists (S.F. Atweh et al., Soc. Neurosci. Abstr. 12:617, 1986). This study investigates the possible involvement of locus coeruleus (LC) in a descending adrenergic path of the above loop. In decerebrate-decerebellate cats with cervical spinal cuts (at C_1 and C_3) that allow activation of (and/or interactions between) isolated DCs and antero-lateral columns (ALCs), the responses of 55 LC neurons were examined. Forty-four neurons were discharged by DCst (mean latency 10.36±0.72 ms) and 41 neurons were discharged by ALCst (mean latency 16.54±1.69 ms) and 35 neurons discharged to both inputs. In neurons discharging only to ALCst (n=5), conditioning DCst inhibited the evoked and spontaneous firing. Peripheral receptive fields could be determined mainly in spinal cuts that allowed the ALC to connect the periphery to the brain and typically involved large areas of the body surface, bilaterally or ipsilaterally.

Our results substantiate the lack of anatomically-demonstrable direct projections from the DC nuclei to LC and support an indirect connection through relays in the brainstem. (Supported by grants from LNRC and DTS Fund).

120.7

RECEPTOR SUB-TYPE AND SECOND MESSENGER MEDIATING SEROTONIN HYPERALGESIA IN THE RAT. Y. O. Taiwo and J. D. Levine*. Procter & Gamble, Cincinnati, Ohio 45239-8707 and UCSF San-Francisco, CA 94143-0724.

The intradermal injection of serotonin, a hyperalgesic inflammatory mediator, in male Sprague-Dawley rats, produces a dose-dependent lowering of mechanical paw-withdrawal thresholds that was not attenuated by procedures known to interrupt the known indirect mechanisms of hyperalgesia.

Serotonin hyperalgesia was selectively mimicked and antagonized by several 5-HT_{1a} agonists [8-OH-DPAT and DP-5-CT] and antagonists [spiroxatrine and spiperone]. 5-HT_{1b} [CGS-12066B; and TFMPP], 5-HT_{2+1c} [DOI, α-m-5-HT; mesulergine and ketanserin] and 5-HT_3 [2-m-5-HT, phenylbiguanide; quipazine and ICS 205-930,] agonists and antagonists, respectively, were without effect.

The hyperalgesia produced by the 5-HT_{1a} agonist [8-OH-DPAT] was markedly enhanced by GTP-γ-S and cholera toxin but not significantly altered by pertussis toxin. Furthermore, 8-OH-DPAT hyperalgesia was significantly prolonged by the phosphodiesterase inhibitor- rolipram.

These data suggest that serotonin hyperalgesia is mediated by the activation of 5-HT_{1a} receptors and is positively linked to intracellular c-AMP through stimulatory guanine regulatory proteins - a mechanism similar to that of other directly acting hyperalgesic agents

Supported by NIH grant NS21647.

120.8

CORRELATION OF CAPSAICIN-INDUCED CHANGES IN PAIN RATING WITH LASER EVOKED POTENTIAL AMPLITUDE. A. Beydoun, T.J. Morrow [1] and K.L. Casey [1]. Depts. of Neurology and Physiology[1], Univ. of Michigan and Neurol. Res. Labs., VA Med. Center, Ann Arbor, MI 48105.

Cutaneous stimulation with infrared laser pulses activates finely myelinated (A delta) nociceptive afferents and evokes a cerebral potential (LEP: 250-350 ms; 20-90 uV). If the LEP reflects the activation of cerebral mechanisms mediating pain, then changes in pain rating of the laser stimulus should correlate with LEP amplitude. Capsaicin depletes putative neurotransmitters of unmyelinated (C) nociceptive afferents. If topically applied capsaicin also affects A delta afferents, it should systematically attenuate LEP amplitude and laser pain rating. .Methods: In a single blinded experiment, five normal volunteers applied capsaicin (0.75%) to the dorsum of one hand and vehicle cream to the other 3 times daily for up to 6 weeks. At weekly intervals before, during and after discontinuation of capsaicin, we determined the thresholds for light touch (Von Frey), deep pain (pressure algometer), pinch pain (hemostat), heat pain (contact thermode) and cold pain (Peltier device). Equal intensity laser stimuli (60 ms pulses) were delivered to the dorsum of the right and left hands of each subject, who rated pricking pain intensity on a visual analog scale. The LEP were averaged (n=25, 0.2-40Hz, CZ-A1A2) and the amplitude of the potential was measured. An analysis of variance was performed to compare right-left differences for the psychophysical measurements and the LEP. A linear regression analysis was used to correlate the laser pulse sensory rating and the amplitude of the evoked potential. Results: Capsaicin produced highly significant right-left differences ($p<0.001$) for laser pulse sensory rating and the amplitude of the LEP. There was also a highly significant correlation between the laser pulse sensory rating and LEP amplitude ($r=0.72$, $p<0.001$). There was no statistically significant right-left difference for the light touch, pinch pain, heat pain, cold pain and deep pain thresholds (ANOVA). Conclusions: 1) With the stimulus parameters used here, LEP amplitude is highly correlated with the intensity of laser-induced pricking pain. 2) In this study, LEP amplitude changes were not correlated with the thresholds of other thermal or mechanical sensory modalities. 3) Capsaicin reversibly inactivates A delta nociceptive afferent transmission.

120.9

PRICKLE AND PAIN IN NORMAL AND HYPERALGESIC SKIN: EVIDENCE THAT LOW THRESHOLD MECHANORECEPTORS ARE RESPONSIBLE FOR THE PAIN OF SECONDARY HYPERALGESIA. F. Cervero*, R.A. Meyer and J.N. Campbell. Dept. of Neurosurgery, School of Medicine, The Johns Hopkins University, Baltimore, MD 21205.

We have studied in ten normal volunteers the sensation of prickle evoked by woollen fabrics applied to the skin of the forearm before and after an intradermal injection of 25μg of capsaicin, a procedure known to induce a large area of mechanical hyperalgesia around the site of injection. Five woollen fabrics (two non-prickly, two very prickly and one intermediate) were presented, in a blind manner, to the volar skin of the forearm. The subjects were asked to rate fabric-induced prickle and pain using visual-analog scales from 0 to 100. Each fabric was presented 5 times in pseudorandom order so that each fabric was preceded once by every other one. On day one the fabrics were applied to normal skin. On day two the test was presented immediately before the intradermal injection of 25μg of capsaicin. Twenty minutes after the injection the fabrics were presented again in the region of secondary hyperalgesia induced by capsaicin. On day 3 or 4 the test was carried out again once the capsaicin-induced hyperalgesia disappeared. The results showed that sensations of fabric evoked prickle remained unchanged in hyperalgesic skin. In contrast, all fabrics, including those judged as non-prickly in normal skin, evoked pain when applied to hyperalgesic skin. These results are consistent with the hypothesis that low threshold mechanoreceptors mediate hyperalgesia to mechanical stimuli, and moreover, that nociceptors that serve prickle sensation are not sensitized to mechanical stimuli in skin rendered hyperalgesic by injection of capsaicin.

120.10

SYMPATHECTOMY DOES NOT ABOLISH BRADYKININ-INDUCED HYPERALGESIA TO HEAT IN HUMAN. R.A. Meyer, K.D. Davis, S.N. Raja, and J.N. Campbell, Johns Hopkins Univ., Baltimore, MD 21205

Bradykinin is an endogenous peptide that is thought to be involved in the hyperalgesia associated with injury. Based on paw withdrawal data in chemically sympathectomized rats, Levine, et al. (Nature 323, 1986, 158-160) proposed that hyperalgesia following bradykinin injection is dependent on an intact peripheral sympathetic nervous system. We sought to test this hypothesis in human subjects. Since intradermal injections of bradykinin produces a dose-dependent hyperalgesia to heat (but not mechanical) stimuli in human subjects (Raja, et al., Pain, Suppl. 5, 1990, S130), we tested for heat hyperalgesia in a patient diagnosed to have sympathetically maintained pain (SMP) in the left leg. An ipsilateral surgical lumbar sympathectomy relieved her pain. Three years later her SMP returned in her left leg, although her right leg was completely normal. Following a contralateral (right) surgical sympathectomy her SMP was again eliminated. Immediately before and 6 months after this sympathectomy on her right side, we tested her verbal pain ratings to heat stimuli before and after intradermal injection of bradykinin (10 nmoles in 10 μl saline) or saline near her right ankle. Pronounced hyperalgesia to heat stimuli occurred after the bradykinin (but not after saline) injection. The magnitude of the hyperalgesia produced by bradykinin after the sympathectomy was similar to that produced before. We conclude that the heat hyperalgesia produced by bradykinin does not require an intact sympathetic nervous system in human. (Supported by NIH NS-14447 and NS-26363)

120.11

A-FIBER NOCICEPTORS WITH A FAST RESPONSE TO HEAT ARE ABSENT IN MONKEY GLABROUS SKIN. R.-D. Treede*, R.A. Meyer, and J.N. Campbell, Johns Hopkins U., Baltimore, MD 21205, Univ. Hospital Eppendorf, D-2000 Hamburg 20.

Human behavioral data indicate that first pain to heat can only be elicited in hairy skin, but not in glabrous skin (Brain Res 266, 1983, 203-208). We now demonstrate that this is parallelled by an absence of A-fibers in monkey glabrous skin with a fast response to heat. Standard teased-fiber techniques were used to record from mechano-heat sensitive A-fiber nociceptors (AMHs) in glabrous (n=64) and hairy skin (n=58) of anesthetized monkey. Fibers were classified based on their response to a 30 s, 53°C stimulus, delivered by a laser (0.1 s rise time). All 64 AMHs in glabrous skin responded to this stimulus with a latency of more than 2 s and a discharge rate that increased with time and reached a maximum near the end of the stimulus. In contrast, only 1/3 of the hairy skin AMHs had similar properties. The remainder started to discharge within 1 s. A subset of these hairy AMHs (n=14) had their peak discharge within 1 s and a mean activation latency of 170 ms and are thought to account for first pain sensation (Pfluger's Arch Suppl 415, 1990, R108). The slow heat response of AMHs in glabrous skin does not appear to be due to the thick epidermis, since a fast response to heat is observed for C-fiber nociceptors (CMHs) in glabrous skin. The occurrence of the maximum discharge at the end of the stimulus for the glabrous AMHs may reflect the time course of sensitization, which is chemically mediated. Fast heat responses, which would be consistent with a direct transducer mechanism, occur only for a subset of the hairy skin AMHs, and for CMHs in both skin types. (Support: NIH NS-14447 and DFG Tr236/1-1).

120.12

PAINFUL THERMAL STIMULATION PRODUCES DECREASED BLOOD FLOW IN CONTRALATERAL PARIETAL CORTEX IN AWAKE HUMANS. R.A. STEA*, N.M. SZEVERENYI*, S.H. MANGLOS*, R.T.STEVENS, F.D. THOMAS*, A.V. APKARIAN. Depts. Neurosurg. and Radiology, SUNY HSC, Syracuse, N.Y. 13210.

Single Photon Emission Computed Tomography (SPECT) with Tc-99m HMPAO is a measure of regional cerebral blood flow (rCBF) and neural activity. In this study, SPECT with MRI localization was used to measure rCBF changes during pain perception. Volunteers were fitted with a headholder to allow reproducible positioning in SPECT and MRI scanners. SPECT images were obtained while the subject's hand was immersed in moderately painful hot water (46-48°C). Control images were obtained with neutral temperature water. Software was developed to objectively normalize, translate and rotate data in identical orthogonal planes. SPECT stimulus minus control images were superimposed on corresponding MRI sections to provide a change distribution analysis.

In all three subjects rCBF decreased an average of 25% (>2SD) in the contralateral parietal cortex in proximity to primary somatosensory cortex. This decrease was 14 counts/pixel by ROI analysis (p-value 0.022, df=16). In one subject, the painful stimulus was given to the opposite hand as well. This produced a contralateral decrease in rCBF of 30% (33.3 counts/pixel, p-value 0.0017, df=10 ROI analysis). In another subject a vibratory stimulus to the left third digit produced an increase in rCBF in the contralateral parietal cortex (10.1 counts/pixel, p-value 0.006, df=6 ROI analysis). There was no significant change in frontal, temporal or occipital lobes or cingulate gyrus. These results suggest, in contrast to an earlier PET study (Talbot et al., Science 251:1355, 1991), that painful stimulation primarily produces inhibition in the contralateral parietal cortex while innocuous stimulation produces activation in this area.

120.13

HYPERALGESIA AND ALLODYNIA-TWO TYPES OF PAINFUL ALTERED SENSATION WITH DIFFERENT PATHOPHYSIOLOGY. U.Lindblom*,P.Hansson,* and H.Fields[1], Dept. of Neurology, Karolinska Hospital, 104 01 Stockholm, Sweden and [1]Dept. of Neurology, UCSF,San Francisco, CA, 94143.

Hyperalgesia may be described as a steepened stimulus-response function. In patients with acute pain and sensitized nociceptors the function will be shifted towards lower stimulus strengths. In patients with neurogenic pain, on the other hand, it is reasonable to expect an increased threshold to pain due to loss of afferent fibres or damage to central pain pathways.

Magnitude estimation of pain intensity as a function of graded thermal pain stimuli, in patients with neurogenic pain and increased threshold to thermal pain, resulted in a steeper slope in the painful region as compared to the control area. Based on our findings we propose that the IASP definition of hyperalgesia may be amended to "An increased response to a nociceptive stimulus". This definition would apply also to patients with increased pain due to sensitization of nociceptors.

For allodynia, clinical characteristics are different from hyperalgesia and a monotonous stimulus-response function may not apply.

120.14

SENSATIONS EVOKED BY MICROSTIMULATION IN THE AREA OF HUMAN VENTROCAUDAL NUCLEUS (Vc). FA Lenz, M Seike, YC Lin, RT Richardson, FH Baker, RH Gracely*. Dept Neurosurg, Johns Hopkins Hosp, Baltimore, MD 21205 and NAB-NIDR-NIH, Bethesda, MD 20892.

It has been suggested that an area near the postero-inferior border of Vc is involved in nociception. However, sensations evoked by stimulation of this area vary between investigators and between patients. We have now employed cross-modality training to measure sensations evoked by threshold microstimulation (TMS) in unanesthetized patients undergoing thalamotomy for tremor.

Vc was identified as the region where a majority of cells had 'lemniscal' response properties including small receptive fields and a constant response to repeated stimulation. Along trajectories through Vc from antero-superior to postero-inferior, recordings of spontaneous activity were used to identify three distinct regions: b - where numerous cells were recorded, c - where cells were sparser and firing rates were slower than b, d - where only fibers were recorded. Along these trajectories, cells with 'lemniscal' properties were almost exclusively located within b, and the transition from cells with 'lemniscal' properties to those without occurred within -0.1 to 0.5mm of the b/c border.

Four patients were trained to use a questionnaire to describe sensations evoked by different types of cutaneous stimulation preoperatively and by TMS intraoperatively. Evoked sensations were usually paresthetic (50/64 sites) but sometimes had a thermal component (13/64). A thermal component was observed more frequently in c (8/21) than in Vc (3/35, $p<0.05$) and was always warm in c. Thermal sensations were evoked in c and/or at the posterior margin of Vc in each patient studied. The sensation of pain was evoked once, in region d. These results demonstrate that, in psychophysically trained patients, TMS reproducibly evokes thermal but not painful sensations from an area near the postero-inferior border of Vc. (E Lilly Corp, NIH NS28598 K08-NS1384)

120.15

NEURONS IN HUMAN Vc RESPOND TO NOXIOUS HEAT STIMULI. M Seike, FA Lenz, YC Lin, FH Baker, RH Gracely*, RT Richardson. Dept Neurosurg, Johns Hopkins Hosp, Baltimore, MD 21205 and NAB-NIDR-NIH, Bethesda, MD 20892.

The previous abstract suggests that neural elements signalling thermal and pain sensations might be located in an area near the postero-inferior border of Vc. In order to test whether sensations evoked by TMS were due to excitation of neurons we examined cells for evidence of a response to noxious stimulation.

Sites within thalamus were characterized by: the neuronal receptive field to innocuous mechanical stimulation (RF) and the projected field evoked by TMS (PF). RFs were found for most cells in Vc but for no cells in c. During recordings from cells in Vc noxious heat and a control stimulus were applied within the RF and PF identified for that site. In c, stimuli were applied within the PF. At some sites in c stimuli were also applied within the V2, V3, ulnar and median nerve distributions. Both stimuli were applied (duration approx. 5s) several times in random order. The noxious heat stimulus was a probe heated to 51-53°C which was rated at 5-8/10 on a visual analog scale but which did not produce hyperalgesia. The control stimulus was a thermal neutral probe otherwise identical to the noxious heat probe. The change in firing rate (response) during applications of heat were compared to control responses.

Responses to noxious heat were significantly greater than control for 4 of 49 cells in Vc and 0 of 21 cells in c. Noxious heat responses were up to 6 times greater than control. No evidence of sensitization of the responses to heat or control stimuli was observed. Thermal sensations were not evoked by TMS at any of the sites where cells responding to noxious heat were recorded. These results suggest that the input of thermal and pain signalling pathways to neurons in Vc is at least as strong as that to neurons in c, postero-inferior to Vc. (E Lilly Corp, NIH NS28598 K08-NS1384)

PAIN MODULATION: OPIOIDS II

121.1

EFFECTS OF EPIDURAL MORPHINE AND LIDOCAINE ON SOMATIC AND VISCERAL PAIN. M. Kaneko*, Y. Saito, Y. Kirihara* and Y. Kosaka* Dept. of Anesthesiology, Shimane Med. Univ., Izumo, Shimane, 693 JAPAN.

The purpose of this study is to evaluate the analgesic effects of epidural anesthesia with morphine, lidocaine and a mixture of morphine and lidocaine.

This protocol was approved by Animal Care and Use Committee of Shimane Medical University. Female Sprague-Dawley rats weighing 250-300g were implanted epidural catheter at the level of Th13/L1. Tail flick(TF) test was employed for response to somatic stimuli. Colorectal distention(CD) test which were modified from Ness and Gebhart(Brain Res, 1988,153) was used for response to visceral stimuli. The thresholds in TF and CD test were measured before and 5 to 180 min after the epidural injection of saline, morphine of 1 to 10 μg, lidocaine of 200 to 800 μg or a mixture of morphine and lidocaine in a volume of 40 μl.

Morphine and lidocaine increased the threshold of CD test as well as TF test in dose-dependent fashion. The mixture of low dose morphine and lidocaine that produced little analgesic effect alone produced greater analgesic effects in both TF test and CD test. The results suggested the possibility of the synergistic interaction of epidural morphine and lidocaine.

121.2

EFFECT OF MORPHINE WITH COCAINE ON ANALGESIA, ALERTNESS, AND CARDIO-RESPIRATORY PARAMETERS A.Pertovaara, T.Kauppila* and E.Mecke*. Dept. of Physiol.,Univ.Helsinki, Helsinki, Finland.

Cocaine enhanced morphine-induced analgesia in the formalin test, hot plate test and heat-induced tail withdrawal test in intact rats. However, in spinal rats a similar combination of cocaine with morphine had no effect in the tail withdrawal test. The formalin test was the most sensitive one to the drug effects. Morphine at the dose 6 mg/kg produced maximal analgesia in the formalin test accompanied by significant hypolocomotion/sedation, bradycardia and bradypnea. At an equianalgesic dose of morphine (3 mg/kg)-cocaine (5 mg/kg)-combination no significant changes in heart rate, respiratory rate or locomotion/alertness were observed. Also, skin blood flow changes determined by the laser Doppler flow method were not significant. The results indicate that cocaine enhances morphine-induced analgesia, due to supraspinal/spinopetal mechanisms. However, the morphine-induced bradypnea, bradycardia and hypolocomotion/sedation are concomitantly attenuated by cocaine. It is possible to combine low,subanalgesic doses of cocaine and morphine to produce powerful analgesia with minimal undesirable side-effects.

121.3

EFFECTS OF SPINAL [DAla^2, NMePhe^4, GLY^5-ol]ENKEPHALIN (DAG) ON THERMALLY-EVOKED CARDIOVASCULAR AND CATECHOLAMINE RESPONSES IN HALOTHANE ANESTHETIZED RATS. J. G. Hamra, T.L. Yaksh and M.R. Brown. Dept. of Anesthesiology, Univ. of California, San Diego, La Jolla, CA 92093.

In many species, surgical stress evokes hypertension, tachycardia and catecholamine secretion. Using DAG, a mu opioid agonist, the role of opioid receptors in the sympathetic response to activation of small unmyelinated somatic afferents was examined. Male Sprague-Dawley rats were implanted under halothane anesthesia with chronic intrathecal (IT) catheters. Following a 5-7 day recovery period, the rats were anesthetized with 3.0% halothane and the tail artery cannulated for monitoring blood pressure. Rats were subjected to one of three experimental protocols: anesthesia (1.5% halothane), anesthesia followed by immersion of the distal 1/3 of the tail in 60°C water for 60 sec, and anesthesia plus IT injection of 10 µg of DAG, followed by tail immersion 30 minutes following IT injection. Heart rate (HR), blood pressure (BP) and catecholamine levels remained stable during the one hour of anesthesia. Tail immersion resulted in a marked increase in BP and HR (Δ=30.4±5.8 mm Hg and 49±1.5 bpm, respectively, $P<0.05$), while norepinephrine but not epinephrine levels increased (Δ=115.2±48.2 ng/ml). The IT injection of DAG resulted in a mild, transient decrease in baseline BP but not HR, and blocked the rise of BP and HR noted after tail immersion. IT injection of DAG significantly decreased norepinephrine levels following tail immersion. Thus, it appears that opioid receptors in the spinal cord play a role in modulating pain-evoked sympathetic responses to activation of small unmyelinated afferent fibers. (NS07329)

121.4

DO DIFFERENT KAPPA OPIOIDS MEDIATE ANALGESIA VIA DIFFERENT RECEPTORS AT SPINAL AND SUPRASPINAL SITES? P Max Headley* and Juan F Herrero* (SPON: Brain Research Association) Dept Physiology, Medical School, University Walk, Bristol BS8 1TD, UK.

Kappa opioids reduce the responses of spinal neurones to peripheral noxious stimuli (Parsons & Headley, 1989, Brit. J. Pharmac. 98: 523-551). We have now examined electrophysiologically (a) the relative degrees to which kappa actions are produced at spinal *vs* supraspinal sites and (b) whether the effects may be mediated at more than one receptor. Reflexes to noxious pinch stimuli were recorded as single motor unit responses in hindlimb flexor muscles of rats anaesthetized with alpha-chloralose. The spinal cord was either sectioned or left intact with sham spinalization surgery. ED_{50} values in spinalized animals for 5 selective kappa opioid ligands were 7mg/kg for U-50,488, 5mg/kg for U-69,593, 3mg/kg for PD-117,302, 0.08mg/kg for CI-977 (see Hunter et al, 1990, Brit. J. Pharmac. 101: 183-189), and 0.3mg/kg for GR-103,545A (see Hayes et al, 1990, Proc. I.N.R.C., Int. Cong. Ser. 914: 214-215). With the cord intact, potencies were greater - 1.5-2 fold for U-69,593 and PD-117,302, 4-6 fold for U-50,488, 12-16 fold for CI-977 and 80-120 fold for GR-103,545A. All drug effects were reversed by i.v. naloxone; U-50,488, U-69,593 and PD-117,302 required less than 0.5mg/kg; CI-977 sometimes needed 1mg/kg and GR-103,545A up to 10mg/kg. These different agonist and antagonist potencies indicate that the various agents were activating different populations of receptors and that these varied between spinal and supraspinal sites. If these agents are selective for kappa receptors then this implies actions at kappa receptor subtypes. [Supported by the Wellcome Trust and the Spanish Ministry for Education and Science. We thank Upjohn, Parke-Davis Research Unit and Glaxo Group Research for gifts of compounds].

121.5

CLOCINAMOX: CHARACTERIZATION OF A SYSTEMICALLY-ACTIVE IRREVERSIBLE OPIOID RECEPTOR ANTAGONIST IN THE MOUSE TAIL-WITHDRAWAL ASSAY. Timothy F. Burke*, Sandra D. Comer*, James H. Woods and John W. Lewis*. Depts. of Psychology and Pharmacology, University of Michigan, Ann Arbor, MI, and Reckitt and Coleman Pharmaceutical Division, Kingston-upon-Hull, England.

Morphine and fentanyl produced dose-dependent increases in tail-withdrawal latency and were fully effective at doses of 32 and 1 mg/kg, respectively in the mouse warm water (55°C) tail-withdrawal assay. Naltrexone (1-100 mg/kg) produced parallel rightward shifts in the dose-response curves for both agonists with pA_2 values of 5.85 ± 0.53 (95% C.L.) and 5.39 ± 0.83, respectively. Clocinamox (14β-(p-chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethyl normorphinone mesylate; 0.32 mg/kg) also shifted these dose-response curves to the right. The highest dose tested (32 mg/kg) produced an insurmountable antagonism up to lethal doses of the agonists. Interestingly, clocinamox (3.2 mg/kg) produced a shift down in the morphine dose-effect curve but only produced a parallel rightward shift in fentanyl's dose-effect curve. This result may indicate a difference in efficacy between morphine and fentanyl. The duration of antagonist action of clocinamox (32 mg/kg) was found to be approximately 8 days with its peak effect at 1 hour. Naloxone, when co-administered with 32 mg/kg clocinamox 2 days prior to determination of morphine's dose-effect curve, prevented the shift down produced by clocinamox in a dose-dependent manner. Thus, clocinamox may be producing its antagonist effect *in vivo* by irreversibly inactivating opioid receptors. *Research supported by USPHS Grants DA-00254 and DA-05405.*

121.6

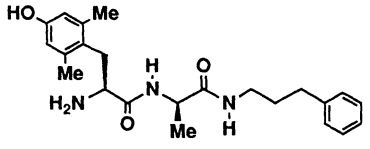
MORPHINE ANTAGONIZES U50,488-INDUCED ANTINOCICEPTION IN THE SQUIRREL MONKEY. R. M. Craft, L. A. Dykstra and J. J. Yarbrough. Psychology Department, University of North Carolina, Chapel Hill, NC 27599-3270.

In a previous study, we showed that several kappa opioid agonists antagonized the antinociceptive effects of mu opioid agonists in a squirrel monkey analgesia procedure (Craft and Dykstra, 1990). The purpose of the present study was to determine whether a mu agonist would antagonize the antinociceptive effects of a kappa agonist. The mu agonist morphine was administered in combination with a dose of the kappa agonist U50,488 that produced a 75% of maximal antinociceptive effect. Morphine (0.03-0.3 mg/kg) did not antagonize U50,488's effects during the first component of the session (15-25 min after injection of U50,488), but dose-dependently antagonized U50,488's effects during the second, third and fourth components of the session (40-100 min after injection of U50,488). The effects of morphine in combination with U50,488 were then redetermined in morphine-tolerant monkeys. As in non-morphine tolerant monkeys, morphine dose-dependently antagonized U50,488's effects during components 2, 3 and 4; however, the doses required to antagonize U50,488's effects in morphine-tolerant monkeys were 1 log unit higher than those in non-tolerant monkeys. When the pretreatment time for morphine was increased from 30 to 60 min, morphine still only antagonized U50,488's effects during components 2, 3 and 4. In contrast, when the pretreatment time for U50,488 was increased from 10 to 35 min, morphine antagonized U50,488's effects during all four components of the session. In summary, the mu agonist morphine partially antagonized the antinociceptive effects of the kappa agonist U50,488, suggesting that U50,488's antinociceptive effects may be mediated by multiple mechanisms in the squirrel monkey shock titration procedure. The fact that chronic morphine administration produced tolerance morphine's kappa antagonist effects suggests that the antagonism of U50,488's antinociceptive effects by morphine may be mu receptor-mediated. Supported by NIDA Grants DA02749, 05386.

121.7

ENKEPHALIN ANALOGS AS SYSTEMICALLY ACTIVE ANTINOCICEPTIVE AGENTS: 0- AND N-ALKYLATED DERIVATIVES OF THE DIPEPTIDE AMIDE SC-39566. Barnett S. Pitzele, Robert W. Hamilton*, Kathleen D. Kudla*, Awilda Stapelfeld, Michael A. Savage*, Michael Clare*, Donna L. Hammond, Don W. Hansen, Jr., Janice M. Thompson, Elaine Rohrbacher, Linda L. Tam, Patricia C. Contreras, Searle, 4901 Searle Pkwy, Skokie, IL 60077.

The Dipeptide Amide SC-39566, a simplified enkephalin analog, has been shown to be a parenterally and orally active opioid antinociceptive agent. A number of O- and N-alkylated analogs and derivatives of SC-39566 were synthesized. Mouse behavioral data show that substitutions at the tyrosine and propylamide nitrogens reduce potency, while substitution at the alanyl nitrogen is less critical. Biochemical and behavioral data will be discussed.



SC-39566

121.8

INTRAVENOUS (IV) MORPHINE-INDUCED INHIBITION OF THE TAIL-FLICK (TF) REFLEX AND SPINAL NOCICEPTIVE TRANSMISSION: PERIPHERAL AND CNS SUBSTRATES. A.Randich, C.L.Thurston, P.S. Ludwig*, J.D.Robertson*, C.Rasmussen* and G.F.Gebhart.Dept. of Psychology., UAB, Birmingham, AL, 35294 and Dept. of Pharmacol., UI, Iowa City, IA, 52242.

Inhibition of the TF reflex produced by 1.0 mg/kg of IV morphine in pentobarbital-anesthetized rats was significantly attenuated by bilateral cervical vagotomy, local anesthesia (0.5 µl of 4% lidocaine) of the nucleus tractus solitarius (NTS), somatotoxic lesions (0.5 µl of 10 µg of ibotenic acid) of the nucleus raphe magnus (NRM), or complete spinal cold block. The antinociception was unaffected by either local anesthesia of the periaqueductal grey (PAG) or bilateral transections of the dorsolateral funiculi (DLFs).

IV administration of 0.5 mg/kg of IV morphine resulted in significant inhibition of responses of lumbosacral spinal dorsal horn neurons to noxious heating of the hindpaw and this effect was significantly attenuated by bilateral cervical vagotomy. This research was supported by NIH grants 22966 and 24958.

121.9

A ROLE FOR PERIAQUEDUCTAL GREY (PAG) H_2 RECEPTORS IN THE SUPRASPINAL ACTIONS OF SYSTEMIC MORPHINE. J.W. Nalwalk* and L.B. Hough. Dept. Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208.

Previous studies support the hypothesis that brain histamine (HA) and H_2 receptors are mediators of morphine (MOR) antinociceptive responses, although the anatomical localization of the relevant H_2 receptors is unknown. To assess the importance of PAG H_2 receptors, the effect of the H_2 antagonist tiotidine (TIOT) was determined on nociceptive thresholds in the presence and absence of MOR in rats. Intracerebral TIOT (1 ng, 10 min, PAG at the level of the dorsal raphe) significantly attenuated antinociception (52°C hot plate) induced by systemic MOR (5.6 and 10 mg/kg, 30 min, sc). The inhibitory effect of PAG TIOT was surmounted by a higher dose of MOR (17.8 mg/kg, 30 min, sc). In contrast to hot plate results, PAG TIOT at all doses tested had no significant effect on MOR-induced changes in tail flick latencies. PAG TIOT (up to 30 ng) produced no effect on hot plate or tail flick responses in the absence of MOR. Microinjection mapping studies of the PAG showed TIOT to be most effective in the caudal ventrolateral PAG, an area previously shown by microinjection to exhibit MOR antinociception. Within the PAG, this overlap between TIOT-sensitive sites and antinociceptive MOR sites suggests that MOR may act, in part, in the PAG to release HA. Taken together with previous studies showing PAG HA injections cause analgesia, these data strongly suggest a role for PAG HA and H_2 receptors in supraspinally mediated MOR antinociception (Supported by DA-03816).

121.10

EFFECT OF MORPHINE ON HISTAMINE RELEASE IN THE RAT PERIAQUEDUCTAL GREY. K.Barke and L.B.Hough Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.

Previous studies have demonstrated that histamine (HA) microinjections into the periaqueductal grey (PAG) cause analgesia. Further, HA H_2 receptor antagonists block both HA and morphine (MOR)-induced analgesia when given into the PAG. Taken with recent anatomical evidence for the existence of histaminergic nerve terminals in the PAG, these studies suggest that MOR may act in part by releasing HA in the PAG, but no direct evidence for this exists. Thus, the hypothesis that analgesic doses of MOR increase extracellular HA levels in the PAG was tested in conscious rats by in vivo intracerebral microdialysis. Sequential doses of 5.6 and 12.8 mg/kg of MOR (s.c.) caused a highly significant dose-dependent increase ($p < 0.001$) in PAG HA levels (239% ± 64% and 294% ± 71% respectively, mean ± SEM, n=5). In a different group of animals in which the probe was placed more laterally in the midbrain reticular formation, no significant increase in MOR-induced HA release was observed (n=5). In addition, a separate experiment, with sequential saline injections demonstrated no significant effect on HA release in the PAG (n=3). These results demonstrate that MOR can increase HA release in the rat PAG, a site where MOR and HA are known to have analgesic action. The cellular origin and mechanism of this MOR effect remains to be explored (Supported by DA-03816).

121.11

RESPONSE OF NEURONS IN ROSTRAL VENTROMEDIAL MEDULLA TO LOCAL NANOLITER INFUSIONS OF MORPHINE. M.M. Heinricher, M.M. Morgan and H.L. Fields. Depts. of Neurology and Physiology, Univ. of Calif., San Francisco, CA 94143

Neurons in the rostral ventromedial medulla (RVM) are known to contribute to opioid antinociception. Two classes of physiologically characterized RVM neurons that show distinct responses to morphine (MOR) given systemically, intrathecally or by microinjection into the PAG have been identified: "off-cells," which are thought to have a net antinociceptive action, are activated by MOR, whereas "on-cells," postulated to have a pro-nociceptive role, are invariably depressed. Because microinjection of morphine into the RVM is itself antinociceptive, the present study used local infusion of MOR to characterize the role of connections among RVM neurons in this effect.

Activity of on- and off-cells was recorded before and after local infusion of MOR (0.5-2 µg in 50-200 nl). These injections, which generally had no effect on tail flick latency, produced variable decreases in on-cell firing (10% to complete suppression). Changes in off-cell firing, when present, were generally modest (10-20% increase, although larger effects were seen). These effects were partially antagonized by naloxone (1 mg/kg, i.p.). In contrast, when higher MOR concentrations were used (5 µg in 100 nl), both on- and off-cells showed a progressively broader and lower amplitude action potential that eventually disappeared. This effect was not reversed by naloxone, indicating a local anesthetic effect.

Thus, local infusion of small quantities of MOR variably depresses on-cell firing, and results in a modest increase in off-cell firing. This reinforces the importance of the on-cell as the focus of direct opioid action within the RVM. In addition, since iontophoretically applied MOR has no effect on off-cell firing, these results are consistent with the suggestion that on-cells in RVM are inhibitory to off-cells.

Supported by PHS grant DA01949 and a Pain Research Grant from the Bristol-Myers Squibb Foundation. MMM was supported by DA05399.

121.12

KAPPA OPIOID AGONISTS PRODUCE HYPOALGESIA ON THE TAILFLICK TEST AFTER APPLICATION TO THE AMYGDALA IN PENTOBARBITAL ANESTHETIZED RATS

F.J. Helmstetter , E.L. Brozoski & J.A. Frost*, Department of Psychology, University of Wisconsin, Milwaukee, WI 53211

Prior work has shown that the amygdala is a critical forebrain structure involved in some environmentally-mediated forms of antinociception (Helmstetter et al, Neurosci Abst, 1988). This study represents our first attempt to directly modulate nociceptive reflexes by manipulating the amygdala. Adult male rats were prepared with chronic cannulae aimed at the amygdala. Subjects were anesthetized with sodium pentobarbital (50mg/kg i.p.) and their latency to tailflick in response to a radiant heat source was recorded at 2 min intervals. After baseline trials animals received a bilateral infusion (1.0 ul/ 1 min) of 0, 5.0, 10.0 or 20.0 ug of the kappa opioid agonist MR2034. This resulted in a dose-related elevation in response latency lasting more than 25 min. In a similar experiment bremazocine HCl, a kappa agonist with reported mu antagonist properties, also produced a long-lasting hypoalgesia arguing against a mu-dependant effect. We propose that kappa agonists directly or indirectly activate amygdalar projections to brainstem antinociceptive systems and that this circuit is involved in some forms of stress-induced hypoalgesia.

RETINA AND PHOTORECEPTORS: PHOTORECEPTORS

122.1

BINDING OF TRANSDUCIN ($G_t\alpha$-[^{35}S]GTPγS) TO CYCLIC GMP PHOSPHODIESTERASE SUBUNITS IMMOBILIZED ON NITROCELLULOSE MEMBRANES. J.J. Erdos and J.K. Northup*. Dept. of Pharmacology and Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06510.

($G_t\alpha$-[^{35}S]GTPγS) at a specific activity of approximately 1000 Ci/mmol was able to bind its effector, cyclic GMP phosphodiesterase (cGMP PDE $\alpha\beta\gamma$) immobilized on nitrocellulose at dilutions of 1 - 10 nM. Binding was not decreased by extensive washing and crosslinking the $G_t\alpha$-[^{35}S]GTPγS to the cGMP PDE $\alpha\beta\gamma$ with 1 mM disuccinimdyl suberate prior to washing did not increase the signal. Likewise there was no increase in sensitivity visualizing the crosslinked $G_t\alpha$ using polyclonal antisera to $G_t\alpha$ and ^{125}I secondary antibody. $G_t\alpha$-[^{35}S]GTPγS at 9.5 nM was able to detect a minimum of 2.1 ng cGMP PDE $\alpha\beta\gamma$ with the binding being linear from 70 ng to 70 ug. Treatment of cGMP PDE $\alpha\beta\gamma$ with 10 ug/ml trypsin which proteolyzes the γ subunit essentially eliminated binding. These results support the theory that the site of interaction of cGMP PDE heteromer with $G_t\alpha$ is the γ subunit. Further the ability of $G_t\alpha$-[^{35}S]GTPγS to remain stably associated with cGMP PDE immobilized on nitrocellulose suggests that Gα subunits can be used to identify effectors expressed in E. Coli.

122.2

PHOSPHORYLATION OF THE MAJOR LIGHT-DEPENDENT SUBSTRATE IN THE LIMULUS PHOTORECEPTOR IS MEDIATED BY CA^{2+} BUT NOT BY PROTEIN KINASE C. BG Calman* and B-A Battelle, The Whitney Laboratory, St Augustine, FL 32086.

The major light-dependent phosphoprotein in the Limulus photoreceptor, a 46 kD protein, (Edwards et al., 1989) can be phosphorylated in vitro in the presence of Ca^{2+} and CAM (calmodulin) (Wiebe et al., 1989). We have examined the possibility that the 46 kD protein is a substrate for PKC using homogenates of Limulus lateral eyes and ventral photoreceptors. Stimulation of endogenous PKC-like activity with PTS, DAG, and 100 µM Ca^{2+} resulted in the consistent phosphorylation of proteins with approximate molecular weights of 210, 185, 160, and 130 kD. Phosphorylation of the 46 kD protein was marginally enhanced in 10 of 16 experiments on the lateral eye. A specific PKC inhibitor, pseudosubstrate peptide PKC(19-36), reduces the PTS/DAG/Ca^{2+} stimulated phosphorylation of all substrates but does not inhibit the phosphorylation of the 46 kD protein stimulated by 1 mM Ca^{2+}. Ca^{2+}-stimulated phosphorylation of the 46 kD protein is blocked by calmodulin inhibitors, including mastoparan and calmidizolium. We conclude that the major light-dependent phosphorylation in the Limulus photoreceptor is not mediated by PKC, but by another Ca^{2+}-regulated kinase, probably a Ca^{2+}/CAM-regulated kinase.

Supported by NIH (EY06232) and NSF (BNS-8607660).

122.3

ION CHANNELS UNDERLYING REGENERATIVE AND PROLONGED DEPOLARIZATIONS IN CONE PHOTORECEPTORS. Steven Barnes, Micheline C. Deschenes* and L.W. Haynes, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Cone photoreceptors may generate a Ca-dependent action potential followed by a prolonged depolarization in response to current injection or surround illumination. Lasansky (J.Physiol. 310:205, 1981) implicated a Cl conductance increase during the prolonged depolarization and Thoreson and Burkhardt (J.Neurophys. 65:96, 1991) supposed that Ca-activated Cl channels mediate this conductance change. In patch-clamped isolated cones we confirm that L-type Ca channels are responsible for the action potential and show that Ca-activated Cl channels together with elevated $[Cl]_i$ sustain the prolonged depolarization.

Ca-activated Cl current ($I_{Cl(Ca)}$) is seen in most voltage-clamped tiger salamander cones dialysed with CsCl or KCl intracellular solution following step depolarizations that activate L-type Ca channels in normal NaCl saline. Only cells exhibiting $I_{Cl(Ca)}$ under voltage-clamp can produce prolonged depolarization under current clamp. 100 μM niflumic acid blocks $I_{Cl(Ca)}$ and eliminates the prolonged depolarization, even though the cone still musters a shortened, Ca-dependent spike. Addition of Ba, which produces larger inward currents through Ca channels, blocks several types of K channels and does not activate $I_{Cl(Ca)}$, supports brief (~250 ms) action potentials with no prolonged depolarization.

If E_{Cl} is made negative to the cones rest potential (as shown by Maricq and Korenbrot, 1988, Neuron 1:503) prolonged depolarization does not occur, rather, $I_{Cl(Ca)}$ contributes to membrane hyperpolarization. Unless E_{Cl} is normally high (e.g. >-20 mV) prolonged depolarization is likely an artifact of $[Cl]_i$ enhancement due to dialysis, as here, or leakage from KCl-filled microelectrodes.

Supported by the Alberta Heritage Foundation and the MRC.

122.4

EFFECTS OF IODOACETATE ON $[H^+]_o$ OUTSIDE ROD PHOTORECEPTORS IN CAT. F. Yamamoto and Y. Honda*. Dept. of Ophthalmology, Kyoto University, Kyoto, 606 Japan

Measurements of $[H^+]_o$ by intraretinal microelectrodes showed that pH outside dark-adapted rods was relatively acidic in the dark, with maximum acidity (pH 7.04) in the outer nuclear layer. Hypoxia in the dark further acidified the extracellular space surrounding rods (Yamamoto, F. and Steinberg, R.H., Soc. Neurosci. Abstr. 15:206, 1989). Illumination produced intraretinal alkalinization that was largest (up to 0.2 pH units) in outer nuclear layer (Borgula, G.A. and Steinberg, R.H., IOVS Suppl. 25:289, 1984). It is commonly known that iodoacetate is an inhibitor of triose phosphate dehydrogenase and causes a lowering of the metabolic energy production (Noell, W.K., 1951). We studied the effect of iodoacetate on subretinal pH, hypoxic acidifications and light-evoked alkalinizations using double-barrel H^+-selective microelectrodes in the intact cat eye, *in vivo*. Intravenous infusion of iodoacetate (5 mg/kg) produced rapid and prominent intraretinal alkalinization in the subretinal space, with an increase of trans-epithelial potential in the dark. After the injection, the hypoxic acidifications were completely suppressed while there was a little change in the amplitude of light-evoked alkalinization. These results suggest that the hypoxic acidification originates from the glycolysis of rod photoreceptors and also the subretinal acidification in the dark is related to rod glycolysis.

122.5

SELECTIVE FLUORESCENT STAINING OF CONE OUTER SEGMENTS WITH CHLORIDE TRANSPORT BLOCKERS. J. Kleinschmidt. Dept. of Ophthalmology, New York University Medical Center, New York, NY 10016.

SITS, DIDS, and other stilbene disulfonates are widely used, effective blockers of many chloride membrane transport systems and chloride channels. These compounds at 0.5 mM concentration strongly hyperpolarize salamander horizontal cells and abolish their light responses (J. Kleinschmidt, Invest. Ophthal. Vis. Sci. (Suppl.) **30**, 124, 1989). Since SITS and DIDS fluoresce blue when excited with UV light, fluorescence microscopy might reveal the distribution of binding sites for these compounds. I now report that when isolated goldfish, salamander or Xenopus retinae are incubated with 25 μM DIDS or 250 μM SITS under the same conditions which block horizontal cell responses, UV-excited blue fluorescence is found to be localized selectively to cone outer segments. Different cone types appear to be stained to different extents. Under the same incubation conditions, 10 mM Procion Yellow M4RAN (see Laties & Liebman, Science **168**, 1475-1477, 1970) or 5-500 μM FITC do not stain cone outer segments. Goldfish and human red blood cells or rbc ghosts (10^6 anion exchangers/cell) show no visible fluorescence at these concentrations of DIDS or SITS under the same excitation and viewing conditions. These findings indicate a high density of SITS/DIDS binding sites on cone outer segments. Whether these sites are anion binding sites of an anion transporter, anion channel or some other anion binding protein, remains to be determined.

Supported by NIH grant EY05213.

122.6

IDENTIFICATION OF 11-*cis* RETINAL IN THE CHICKEN PINEAL J.H. Sun*[1], R.J. Reiter[1], N.L. Mata*[2], A.T.C. Tsin[2] [1] Dept. of Cell & Struct. Biol., Univ. Texas Health Science Center, San Antonio, Tx 78284; [2] Div. of Life Sciences, Univ. Texas at San Antonio, San Antonio, Tx 78249

Direct evidence is not available that 1) rhodopsin-like photopigment exists in the chicken pineal and that 2) this visual pigment is responsible for the light sensitive mechanism in the gland. Therefore, the objective of this study was to test for the existence of visual pigment in the chicken pineal through the identification of 11-*cis* retinal. 11-*cis* and all-*trans* retinals were extracted from light and dark adapted chicken pineals and analyzed by high performance liquid chromatography (HPLC) using the formaldehyde method (Suzuki et al., *Vision Res.* **26**:425,1986). 11-*cis* retinal was initially identified by coelution with an authentic standard. Further characterization was carried out by collecting the retinal from the HPLC eluant, subjecting it to reduction by sodium borohydride and then identifying the derived 11-*cis* retinol using HPLC. Proportions of 11-*cis* retinal to total pineal retinals were also studied from decapitated heads after light and dark adaptation. Analyses of dark adapted, pooled chicken pineals revealed equal proportions of 11-*cis* and all-*trans* retinals at two hours after dark and at night. Two hours of light adaptation resulted in the reduction of the 11-*cis* proportion (from 50%) to 26% of total retinals. These observations suggest that 11-*cis* retinal exists in the chicken pineal and that it undergoes light-induced *cis* to *trans* isomerization in a manner similar to the visual pigment chromophores in the vertebrate retina. (Supported by grants from the NSF, NIH and the San Antonio Area Foundation)

122.7

DEPOLARIZING PHOTORESPONSES IN PHOTORECEPTORS OF THE PARIETAL EYE OF LIZARDS. G. A. Engbretson and E. Solessio*. Institute for Sensory Research and Department of Bioengineering, Syracuse University, Syracuse, New York 13244.

The parietal eye of lizards is a highly organized photoreceptive organ. Its cone-like photoreceptors synapse directly onto ganglion cells. Despite the lack of interneurons this simple eye encodes information about the intensity and chromatic qualities of light stimuli.

Local ERG's recorded in the lumen (analogous to subretinal space) consist of a negative-going 4 to 6 mV response to monochromatic stimuli. Chromatic adaptation was used to isolate two antagonistic spectral components with maximal sensitivities at 440 and 495 nm. Responses to green stimuli were characterized by a rapid initial transient and exponential decay at "OFF". Blue stimuli limited the amplitude of the initial transient and yielded a strong lumen-negative "OFF" response. Superfusion with low Ca^{2+} Ringers solution slowed the ERG but did not eliminate the components. We believe the source of the signal lies in the mass activity of photoreceptor cells.

We have recorded from, stained, and identified parietal eye photoreceptor cells. Typical resting membrane potentials were -50 to -60 mV. Responses to light were depolarizing with an amplitude of up to 20 mV. The latency of the response was similar to that of the ERG. As observed in the ERG, responses to green stimuli exhibited a fast initial transient whereas responses to blue stimuli were characterized by a strong "OFF" transient. We have not yet determined if the presence of the two spectral mechanisms in the intracellularly-recorded photoresponse originates in synaptic interactions between two populations of photoreceptors, or in the presence of a bistable photopigment.

Supported by NIH grant EY03359 and the Department of Bioengineering, Syracuse University.

122.8

CAN BIREFRINGENCE EXPLAIN THE POLARIZATION ANALYZING CAPABILITY OF TELEOST DOUBLE CONES?

M. Rowe*, N. Engheta*, and E.N. Pugh, Jr. Inst. of Neurological Sciences and Depts. of Electrical Engineering & Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Behavioral experiments have demonstrated that double cones mediate a differential polarization sensitivity in the green sunfish, *Lepomis cyanellus* (Neurosci. Abs. **16**:406). In an attempt to understand the mechanism by which double cones confer this ability to obtain information about the polarization of light, mathematical models have been created to determine if birefringence in these structures can explain the animals' differential sensitivity to light linearly polarized at different angles. Solving Maxwell's equations for a highly simplified geometry, and then expanding plane waves with orthogonal polarizations into the solutions thus obtained indicates that geometrical birefringence is a viable mechanism which may underly the behavioral responses. Although promising, the results obtained with this model, which has a step index of refraction profile, indicate that such an effect cannot account for the behavioral data, since the difference in transverse electric field strength between waves excited by the two initial polarization states would not be large enough if the photoreceptors have a uniform refractive index as in this step index model. Electron micrographs from the retina of *L. cyanellus* indicate that the index of refraction in the double cones may vary continuously from a maximum in the center to some minimul value at the periphery (S.S. Easter, personal communication). We anticipate that a graded index model which more closely approximates the double cone structure will lead to a larger difference in the transverse electric field strength for the two types of incident wave. Currently models which incorporate this assumption of a smoothly graded profile are being developed, along with measurements of the guiding properties of double cones in whole mounted retinas.

Supported by NIH grant EY-08260

122.9

IDENTIFICATION OF RED-GREEN AND BLUE SENSITIVE CONES IN THE GOLDFISH RETINA BY PHOTORECEPTOR-CLASS SPECIFIC ANTIBODIES. S.C. Sharma. Department of Ophthalmology, New York Medical College, Valhalla, NY 10595.

Antisera raised against synthetic peptides generated from amino acid sequences for cDNAs of human pigment polypeptides (Lerea et al., Neuron 3:367, 1990) was used in the present study to discern the photoreceptor subtypes in the retina of common goldfish. These polyclonal antibodies selectively label red- and green-sensitive cone opsin (antisera 4942A) and blue-cone opsin (antisera 108B) in human retina and cross react well with goldfish cones.

Based upon labelling pattern in whole-mounted and sectioned goldfish retina, only 12-15% of the entire retina was comprised of blue-sensitive cones. Nearly 80% of the retina was comprised of red-green cones. Distribution of both red-green and blue cone was found to be higher in the ventral than the dorsal retina. Supported by N.I.H. EY 01426.

122.10

LATERAL SIGNAL PROPAGATION VIA CONE TELODENDRITES IN TIGER SALAMANDER RETINA. Vaishali Merchant* and Steven Barnes, Neuroscience Research Group, University of Calgary Faculty of Medicine, Calgary, Alberta, Canada T2N 4N1.

Fine neurites extending laterally up to 45 μm from cone pedicles could provide pathways by which signals from distant cells might modifiy the cones' receptive field. The extent to which synaptic input arising distally in a telodendrite is electrotonically decremented at the cell body could limit this form of lateral interaction and was studied using computer simulations of the cone membrane.

Patch-clamped, lucifer yellow-filled cones in slices were morphologically and electrically analysed to determine passive and active properties. Cones had on average five telodendrites extending from the pedicle for 14.5±11.2 μm (mean ± S.D., N=5 cones), an input resistance of 911±680 Mohm at -40 mV and capacitance of 26.3±8.9 pF (N=14). An earlier model (Kraft and Burkhardt, 1988, JCN 249:13) indicated that communication between walleye cone cell bodies and telodendrites was good bidirectionally. Our work suggests a much higher value of membrane resistivity (40 kohm-cm^2) than the previous microelectrode work, where low input resistance shunted the signal-shaping actions of voltage-gated ion channels. Even so, when implemented with homogeneous membrane properties and ionic currents I_h, I_{Kx}, and I_{Ca} (using the program Manuel with thanks to David Perkel of UCSF), our simulations give predictions similar to the earlier work (e.g. coupling ratios >88%). But inclusion of inhomogeneities yields different results. For example, increasing telodendrite Ca channel density leads to distinct synaptic responses in the different cone regions. Strategic placement of synaptic conductances or addition of leak conductance to the cell body increases electrical separation of telodendrites and the soma. The simulations suggest mechanisms that could modify telodendrite-cell body coupling. [Supported by the AHFMR and the MRC].

122.11

COMPUTED SPREAD OF VOLTAGE BETWEEN CONES IN MONKEY FOVEA. A. Hsu, R.G. Smith, and P. Sterling, Inst. for Neurological Sciences and Dept. of Anatomy, Univ. Pennsylvania, Phila., PA 19104.

Spatial resolution by the foveal cone array is apparently limited by optical blur (5.4μm diam. Gaussian at 1/e amplitude, 2mm pupil), summation across the cone aperture (3μm diam.), and cone spacing (3μm, center to center). Evidence of gap junctions between neighboring pedicles of foveal cones suggests that electrical coupling might cause additional signal spread. We computed the degree of spread using a compartmental model. A cone's outer segment and inner segment plus soma was simulated as 2 spheres; the axon was simulated as a cable (250μm long, 1.5μm diam.); and the pedicle was modeled as a sphere (7μm diam.). Membrane Resistance was 15$k\Omega$–cm^2. Cones were square-packed in a 15x15 array and coupled to 4 nearest neighbors with inter-cone conductance at $2.7 \times 10^3 pS$.

The voltage arising from current evoked at a cone outer segment spread to neighboring cone pedicles, decaying exponentially with a space constant of 3μm. Convolving this exponential with the blur due to optics and summation across the cone aperture gave a spread function with a Gaussian peak and an exponential skirt (diam. at 1/e 9.3μm). Changing membrane resistance by 2-fold altered the diameter of the spread function by less than 1μm; changing coupling conductance by 2-fold altered the diameter of the spread function by less then 2μm.

Psychophysical experiments suggest that signal blur is entirely accounted for by optical factors at high luminance. Conceivably, therefore, cone coupling occurs at low luminance. Since low luminance is served mainly by M ganglion cells, modest spatial averaging between cone pedicles might improve contrast sensitivity at no cost to spatial acuity. Supported by EY08124.

122.12

SIGNAL-DETECTION CHARACTERIZATION OF NEURAL CODE EFFICACIES IN SINGLE PHOTORECEPTORS: EFFECT OF STIMULUS INTENSITY AND LIGHT ADAPTATION. Zixi Cheng* and Gerald S. Wasserman. Sensory Coding Laboratory, Dept. of Psychological Sciences,Purdue University, West Lafayette IN 47907-1364.

The multiple meaning theory of neural coding and its extension, the task dependence theory, both suggest that different features of identical neural signals (i.e., codes) may represent information (see Nisly and Wasserman, *Psychological Bulletin*, 1989, 106, 483-496). Why should more than one neural code exist? One possibility, suggested by the work of Cohn (*IEEE Transactions on Systems*, 1983. 13, 873-881), is that different codes are differentially efficacious in different circumstances. We therefore measured the efficacy of several neural codes in different circumstances. We recorded intracellularly from photoreceptors of *Limulus* and characterized code efficacy by measuring the detectabilities (i.e., by measuring d') of various codes while varying stimulus intensity and the state of adaptation. We found reliable differences in code efficacy which were reliably altered by intensity and adaptation.

122.13

ULTRASTRUCTURE OF *LIMULUS* PHOTORECEPTORS IN EXPERIMENTAL PROTOCOLS: PHYSIOLOGISTS' FAVORITE RECIPES. Brad Hanna[1], Robert N. Jinks[2], Hui-juan Zhang[3], Leonard Kass[3], George H. Renninger[1], and Steven C. Chamberlain[2,4]. [1]Biophysics Group, U. of Guelph, Guelph, ONT, [2]Dept. of Bioengineering and [3]Dept. of Zoology, U. of Maine, Orono, ME; and [4]Institute for Sensory Research, Syracuse U., Syracuse, NY.

Most physiological experimentation using the *Limulus* ventral eye has used simple excision and incubation in seawater. One significant alternative approach has been excision and incubation in an organ culture medium (*J. Gen. Physiol.* 72:539). We have compared the ultrastructure of photoreceptors excised in light and maintained for 5 hrs in darkness in seawater or organ culture with that of cells fixed *in situ* (*J. Gen. Physiol.* 80:839). Organ culture incubation produces cells with ultrastructure similar to those fixed *in situ*. Two major differences are observed in cells incubated in seawater. The layers of glia covering the R-lobe are ultrastructurally disrupted, and the fused quintuple membrane between adjacent microvilli normally seen in dark adapted rhabdom is absent. The reduction of microvillar fusion is reminiscent of structural light adaptation in the lateral eye (*J. Neurosci.* 4:2792) and probably accounts for the lower total resistance of cells maintained in seawater compared to those maintained in organ culture. Recent enzymatic treatments to enable whole-cell recording from ventral photoreceptors have yielded cells with essentially normal light responses (*ARVO Abstr.* 31:389). Many of these cells have nearly *in situ* ultrastructure, except that the glial covering is vastly attenuated and the mitochondrial matrix is vacuolized suggesting that mitochondria may have relatively minor roles in the light responses measured. Similar enzymatic treatments to dissociate the retina of the lateral eye have produced promising whole-cell recordings (*ARVO Abstr.* 31:389). The ultrastructure of these cells varies over a range from essentially normal to trauma response characterized by extensive vacuolization, breakdown of the rhabdom, and a number of lysosomal pathway structures.

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122.14

CORRELATION OF PHOTORECEPTOR STRUCTURE AND LIGHTING ENVIRONMENT: IMPLICATIONS FOR PHOTOSTASIS. Eric P. Hornstein* and Steven C. Chamberlain. Institute for Sensory Research and Department of Bioengineering, Syracuse University, Syracuse, NY.

Why is photosensitive membrane shed and synthesized? A common assumption is that cumulative age and light damage require its periodic replacement. Alternately, the daily cycling of photosensitive membrane may serve to compensate for seasonal changes in light flux, i.e. to maintain photostasis. If the main purpose of photoreceptor membrane turnover is the maintenance of photostasis, then species living in environments with no seasonal changes in light intensity might lack this process. Indeed, recent studies of the photoreceptors of the deep sea species, *Bathynomus giganteus* and *Rimicaris exoculata*, suggest that they may have minimal or no turnover of light sensitive membranes. Specifically, the absence of conventional membrane turnover is suggested by the hypertrophy of the rhabdomeral segment (R-segment) and the atrophy of the arhabdomeral segment (A-segment) so that there is essentially no room for the shedding and synthesis processes to proceed.

We have surveyed the photoreceptor structure of fifteen invertebrate species from a variety of habitats. Our morphometric measure is the ratio of the volume of the R-segment to the volume of the A-segment. This ratio is typically significantly less than one for species living in cyclic lighting with seasonal changes in daily light flux. For deep sea species living in noncyclic lighting, the ratio is greater than one. Absent cyclic lighting, there seems to be no significant cycling of the phototransductive membrane. Animals living in noncyclic lighting include both those such as *R. exoculata* which live in extremely dim environments and those which prey on bioluminescent species such as *B. giganteus* that live in relatively bright environments. Aging or deleterious effects of cumulative light absorption do not themselves appear to require cycling of photosensitive membrane. Perhaps photostasis is a general principle in visual systems which mandates membrane turnover for animals living in seasonally varying cyclic lighting. Supported by NIH EY03446.

122.15

CONSEQUENCES OF RETINAL DETACHMENT ON GENE EXPRESSION IN XENOPUS LAEVIS EYECUPS. G. Glaesener-Cipollone[1], M.W. Kaplan[2], C.B. Sterritt[2*] and R.D. Fernald[3]. [1]Inst. Gen. and Microbiol., Univ. Wuerzburg, FRG, [2]Dow Neurol. Sci. Inst., Portland, OR, [3]Stanford, CA.

Retinal photoreceptors require an intimate connection with pigmented epithelial cells (RPE) for normal functioning. In many species, detachment of the retina from the RPE produces pronounced changes in photoreceptors, for example the inhibition of disk shedding and the disorganisation of disk renewal (Kaplan et al. 1990).

We have studied whether retinal detachment affects gene regulation in photoreceptors by analyzing the production of opsin, transducin and retinal regeneration slow protein (RDS) mRNA. Experiments were carried out in Xenopus laevis retinas that had been maintained in eyecup cultures for up to 4 days. The mRNA abundance in attached and isolated retinas was measured by Northern blot analysis. Additionally, in situ hybridization on retinal tissue sections were carried out to reveal the specificity of the RNA probes and to support the quantitative data found with the Northern blots.

In order to exclude changes in RNA transcription induced by a day-night rhythm we collected freshly excised Xenopus laevis retinas at different times of the day and investigated the opsin, transducin and RDS mRNA abundance by Northern blot analysis. No profound changes in the abundance of any of the investigated mRNAs could be detected indicating that the regulation of the mRNA transcription does not underlie a cyclic rhythm.

The Northern blot analysis indicated that neither the opsin nor the RDS transcription were affected by retinal detachment. In contrast, a clear decrease in transducin mRNA abundance appeared. This suggests that the apposition of the retina with the RPE is not a necessary precondition for the normal expression of opsin and RDS but plays an important role in the regulation of transducin gene expression.

1. Kaplan et al. (1990), IOVS 31:1-8

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122.16

REGULATION OF MELATONIN SYNTHESIS AND NAT ACTIVITY BY A PHOTORECEPTOR-LIKE PHOSPHODIESTERASE. M. Max, T. Silliman*, M. Menaker. Deptartment of Biology., University of Virginia, Charlottesville VA 22901

Zaprinast, (M&B 22,948) has been shown to have a dual effect on purified photoreceptor phospodiesterase (PDE). Zaprinast competitively and specifically inhibits the light- or trypsin-activated form of PDE; but partially stimulates the dark form of PDE[1]. We find that Zaprinast, at doses similar to those used to inhibit purified PDE, blocks the light-induced suppression of melatonin production from perifused photosensitive trout pineal glands, (*Salmo trutta*). It also partially suppresses melatonin production in darkness. These results suggest that a photoreceptor-like PDE mediates the light-induced suppression of melatonin.

We also find that Zaprinast blocks light-induced suppression of the activity of trout pineal serotonin N-acetyltransferase (NAT), the penultimate enzyme in the melatonin synthesis pathway, without increasing its activity in darkness. When 10μM Zaprinast is combined with a threshold light stimulus, NAT activity is elevated above dark levels. In darkness, with 10μM Zaprinast, NAT activity is partially suppressed. We hypothesize that: In darkness a photoreceptor-like PDE is active at a low rate and sets the upper limit of NAT activity; the dark PDE activity is stimulated by Zaprinast, which results in a partial suppression of NAT activity and melatonin production; in light, the PDE becomes sensitive to inhibition by Zaprinast, which therefor increases NAT activity and blocks the suppressive effect of light on melatonin synthesis.

Because photosensitve pinealocytes contain many of the same molecules known to mediate the effects of light in retinal photoreceptors, it has often been suggested that they also share similar phototransduction cascades. If our interpretation of the effects of Zaprinast reported above is correct, it supports this idea and suggests that a critical feature of retinal phototransduction, the activation of PDE by light, is involved in the photic regulation of pineal melatonin synthesis.

[1]Gillespie, P.G., & J.A. Beavo (1989), *Mol. Pharm.* 36:773-781.

122.17

A VITAMIN A_2-BASED VISUAL SYSTEM IN A FULLY TERRESTRIAL VERTEBRATE. I. Provencio, A.C. Bizzell* & R.G. Foster, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Current dogma states that freshwater vertebrates possess vitamin A_2-based photopigments (pigments containing 11-*cis*-3,4-didehydroretinal as the functional chromophore). Conversely, marine and terrestrial vertebrates have vitamin A_1-based pigments (11-*cis*-retinal chromophore). Those animals that only spend a portion of their life cycle in freshwater, such as migratory fish and amphibians, possess both chromophores, but regulate the relative concentrations with respect to the environment. Using high performance liquid chromatography (HPLC), we have demonstrated the first example of an exclusively vitamin A_2-based visual system in a completely terrestrial vertebrate, *Anolis carolinensis*.

HPLC analysis of retinoid oxime extracts from *Anolis* eyes reveals a vitamin A_2-derived chromophore. No vitamin A_1-derived chromophore was detectable. Spectral analysis of the *Anolis syn* 11-*cis* oxime isomer yields a λ_{max} of 368 nm with a "cis peak" absorbance of 304 nm. The elution time and spectral profile of this extract corresponds precisely with vitamin A_2-derived retinoid oxime standard. This study suggests that a long wavelength photopigment may reside within the retina of *Anolis*. (Supported by an NSF Grad. Fellowship to IP and Jeffress Memorial Trust J-213 to RGF.)

122.18

RESTORATION OF RHODOPSIN LEVELS IN RETINOID-DEPRIVED RATS AFTER VITAMIN A REPLETION. D.-M. Chen*[1], M.L. Katz*[2] and W.S. Stark[1]. [1]Div. of Biol. Sci. and [2]Dept. of Ophthalmol., U. of Missouri, Columbia 65212.

In rats deprived of the retinoid precursors of the visual pigment chromophore 11-*cis* retinal, rod cell pigment rhodopsin levels gradually decline. After 23 weeks of deprivation, rhodopsin levels are less than 15% of normal. The density of the protein component of the visual pigment (opsin) remains unchanged, although outer segment volume is reduced by about 60%. Thus, rod cells of retinoid-deprived rats contain a large amount of chromophore-free opsin. Experiments were conducted to determine whether the effects of deprivation could be reversed by rapidly repleting retinoid-deprived animals with vitamin A. Dark-adapted rats that had been fed a diet lacking retinoids other than retinoic acid for 23 weeks were given a single i.m. injection of all-*trans* retinol. At various times following injection, electroretinograms (ERGs) were recorded from a number of animals. Before injection, ERG thresholds of the deprived animals were an average of 3.5 log units higher than those of rats that had been fed a retinoid-adequate diet. Maximum ERG response amplitudes were decreased by an average of 37%. Partial recovery of visual sensitivity was observed as early as 4 hrs after retinol administration. By 7 days after injection, the ERG thresholds and maximum amplitudes had returned to almost control levels. Direct measurement of rhodopsin levels in the retinas of treated animals also indicated that visual pigment levels recovered quickly after retinol administration. An increase in rhodopsin levels was observed as early as 3 hrs after vitamin A injection. The early rapid recovery of rhodopsin levels once chromophore precursors were made available suggests that the chromophore-free opsin synthesized by retinoid-deprived animals can be converted to functional rhodopsin if 11-*cis* retinal becomes available. Continued recovery until normal sensitivity is achieved suggests that outer segment size eventually returns to normal.

122.19

ONTOGENETIC LOSS OF UV PHOTO-SENSITIVITY IN RAINBOW TROUT, DETERMINED USING OPTIC NERVE COMPOUND ACTION POTENTIAL RECORDING. L. Beaudet, H.I. Browman, and C.W. Hawryshyn. Dept. of Biology, University of Victoria, P.O. Box 1700, Victoria, B.C., Canada V8W 2Y2.

We characterized the photopic spectral sensitivity of ON and OFF responses from small (< 20 g) and large (> 55 g) rainbow trout (O. mykiss, RT) using various adapting backgrounds. The ON responses of three small RT exhibited sensitivity peaks corresponding to UV, S, M, and L wavelength photoreceptor mechanisms. The UV sensitivity peak was one log unit higher than those for the M and L mechanisms, and 0.5 log unit higher than the S mechanism peak. When a UV and S wavelength adapting background was added, the relative sensitivity of the UV peak was depressed by two log units, the S peak by one log unit, while the sensitivities of the M and L photoreceptor mechanisms were unchanged. The ON response from three large RT did not exhibit a selectively depressed UV peak, indicating that sensitivity to these wavelengths was not generated by a UV mechanism; these fish were not UV photo-sensitive. The relative sensitivity of the OFF response was always lower than that for the ON and was characterized by broader peaks in the UV and M parts of the spectrum. L.B. was supported by a NSERC Canada Postgraduate Scholarship, H.I.B. by a MRC Canada Postdoctoral Fellowship, and C.W.H. by a NSERC Canada URF and Operating Grant (# URF 0043984).

122.20

THYROXINE-INDUCED LOSS OF ULTRAVIOLET PHOTO-SENSITIVITY IN RAINBOW TROUT, Oncorhynchus mykiss. H.I. Browman and C.W. Hawryshyn. Dept. of Biology, Univ. of Victoria, Victoria, B.C., Canada V8W 2Y2.

We used thyroxine to induce a precocial loss of UV sensitivity in rainbow trout (RT). During their normal ontogeny, RT are UV sensitive (360 nm peak) to 40-50 g and, over this weight range, a transition to loss of UV sensitivity occurs. Three fish (12-17 g) were treated with 300 ug/l thyroxine (in their aquarium water; the solution was changed daily) for 35 days (treatment fish). The other three fish (12-17 g) were handled in an identical manner, but no thyroxine was added to their aquaria (control fish). The spectral sensitivity of **the same six RT** was measured, using a heart-rate conditioning protocol, before treatment, and after 15 and 35 days. Prior to treament, all fish exhibited sensitivity peaks corresponding to UV, S, M, and L photoreceptor mechanisms. At 15 days, the UV peak in treatment fish was shifted towards 400 nm, broadening the S peak, while the M and L peaks were unchanged. There was no change in control fish. At 35 days, treatment fish exhibited sensitivity peaks corresponding to S, M, and L photoreceptor mechanisms; there was no UV peak. Control fish were unchanged. We conclude that elevated levels of thyroxine induces a precocial transition in spectral sensitivity identical to that which occurs during normal ontogeny. H.I.B. was supported by a MRC Canada Postdoctoral Fellowship, and C.W.H. by a NSERC Canada URF and Operating Grant (# URF 0043984).

123.1

GABA IMMUNOREACTIVE NEURONS AND TERMINALS IN THE AUDITORY BRAINSTEM AND THALAMUS OF THE NORTHERN LEOPARD FROG, RANA PIPIENS PIPIENS. J.C. Hall. Zoology Dept., University of Tennessee, Knoxville, TN 37996.

Immunohistochemical localization of γ- aminobutyric acid (GABA) was used to identify GABAergic neurons and terminals in the auditory brainstem and thalamus of *Rana p. pipiens*. The antiserum against GABA that I used was kindly provided by Dr. R.M. Buijs and Dr. C.W. Pool of the Netherlands Institute for Brain Research, Amsterdam.

Preliminary observations revealed immunoreactive puncta surrounding unlabeled cell bodies in all primary auditory nuclei including the dorsolateral nucleus, superior olivary nucleus and, superficial reticular nucleus in the caudal brainstem; the laminar nucleus, principal nucleus and, magnocellular nucleus in the torus semicircularis, or auditory midbrain; and the posterior nucleus and central nucleus in the thalamus.

In the caudal brainstem, GABA immunoreactive cell bodies were found only in the superior olivary nucleus. In the torus semicircularis, immunolabeled cell bodies were observed in both the principal nucleus and the magnocellular nucleus; though, the vast majority of labeled cell bodies were located in the magnocellular nucleus. Both the posterior and central thalamic nuclei contained immunopositive cell bodies but, they were few in number.

GABA has been identified as an inhibitory neurotransmitter in the auditory system of birds and mammals. The results of this study indicate that GABA likely mediates inhibitory interactions in the auditory pathway of frogs as well. I am currently conducting studies to test this hypothesis. (Supported by BRSG RR-07088)

123.2

ACETYLCHOLINESTERASE DIFFERENTIALLY STAINS PARALLEL PATHWAYS IN THE BARN OWL'S AUDITORY BRAINSTEM. R. Adolphs, Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

Auditory nuclei in the brainstem of the barn owl, Tyto alba, stain differentially with acetylcholinesterase (AChE) histochemistry. At the level of the cochlear nuclei, nucleus angularis showed very intense staining of fibers and cell bodies, whereas n. magnocellularis showed only a weak staining of cell bodies and no fiber staining. Nucleus laminaris, which receives bilateral input from n. magnocellularis, showed very faint staining of terminals surrounding cell bodies. In the lemniscal nuclei, nucleus ventralis lemnisci lateralis pars posterior (VLVp) stained more intensely than the adjacent pars anterior (VLVa). In the inferior colliculus (IC), the shell of the central nucleus, which is a terminal field both of n. angularis and VLVp, stained more intensely than did the core of the central nucleus, which is a terminal field of n. laminaris. The external nucleus of IC stained intensely also.

These staining patterns suggest that nuclei or subdivisions of nuclei which process interaural level differences (n. angularis, VLVp, the shell of IC) stain more strongly for AChE than do those nuclei which process interaural time differences (n. magnocellularis, n. laminaris, VLVa, the core of IC). Moreover, this staining pattern is complementary to that seen with calbindin-immunohistochemistry (Takahashi et al, J. Neurosci. 7(6): 1843-1856 (1987)).

Results from baby owls indicate that the staining pattern seen with AChE has already formed at 15 days post hatching.

R.A. is a Howard Hughes Medical Institute Fellow.

123.3

GABA AND GLYCINE RELEASE FROM GUINEA PIG INFERIOR COLLICULUS AFTER ABLATION OF DORSAL NUCLEUS OF LATERAL LEMNISCUS. S.J. Potashner, A. Shneiderman, M.B. Chase*, C. Benson, and J.M. Rockwood*. Dept. of Anatomy & Center for Neurological Sci., Univ. Connecticut Health Center, Farmington, CT, 06030.

The dorsal nucleus of the lateral lemniscus (DNLL) receives many of the auditory projections that ascend to the inferior colliculus (IC) and projects bilaterally to the IC as well as to the contralateral DNLL. The projection to the contralateral IC may be inhibitory, as over 90% of its axonal endings have pleomorphic vesicles (J. Comp. Neurol. 286:28 '89) and many DNLL cells contain GABA-like immunoreactivity (Brain Res. Bull. 13:585 '84). The projection to the ipsilateral IC provides half the number of synaptic endings furnished by the contralateral projection, and only 70% have pleomorphic vesicles.

To determine if GABA and glycine could be transmitters of DNLL neurons projecting to the IC, we measured the electrically-evoked release of exogenous ^{3}H-GABA and ^{14}C-glycine from each IC *in vitro* 2-4 days after destroying neurons in the left DNLL with injections of kainic acid. The release of both compounds was Ca^{++}-dependent. Glycine release was unaffected by DNLL lesions. GABA release was depressed in direct proportion to the degree of neuronal loss in the lesioned DNLL. After complete ablation of the left DNLL, GABA release in the contra- and ipsilateral IC was reduced by 51% and 25% respectively. These findings are consistent with the hypothesis that DNLL projections to the IC which contain pleomorphic vesicles mediate the synaptic release of GABA. (Supported by DC00199 from NIH-NIDOCD)

123.4

THE ORGANIZATION OF GABAergic NEURONS IN THE CAT MEDIAL GENICULATE BODY: A QUANTITATIVE IMMUNOCYTOCHEMICAL STUDY OF POST-EMBEDDED MATERIAL. D.M. Huchton*, D.T. Larue, J. Y.-M. Sun*, and J.A. Winer. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-2097.

The cat medial geniculate body contains three main divisions and many smaller subdivisions that differ in their neuronal architecture, patterns of cortical and midbrain connectivity, and functional organization. Previous work in several species has shown that GABA is the likely transmitter for many thalamic local circuit neurons. The purpose of this study is to compare the intrinsic organization of these nuclei using 1 μm-thick sections of plastic-embedded material immunostained with an antiserum to GABA. If the number, type, and arrangement of GABAergic cells is similar between subdivisions, perhaps thalamic local circuitry is highly conserved in mammals despite otherwise important species differences in the absolute number of GABAergic neurons.

The major findings are (i) that divisions differ significantly in the proportion of GABAergic cells, the ventral division having 34%, the dorsal division 26%, and the medial division 21%. We find (ii) striking differences in the proportion of GABAergic neurons along a caudal-to-rostral axis in each division: the number in the ventral division increased (from 29 to 38%), the dorsal division decreased (32 to 16%), and the medial division increased four-fold (9 to 39%). There is (iii) a statistically significant difference in cell size between the GABAergic and immunonegative cells only in the dorsal and the medial divisions. Finally, (iv) GABAergic cells average about 12 μm in diameter, and have a spindle-shaped perikaryon from which slender dendrites arise without a preferred orientation.

These results suggest a basic similarity in the form, though not the number, of GABAergic neurons in different medial geniculate body divisions. These regional differences may be correlated with caudal-to-rostral differences in physiological processing reported in the auditory thalamus (C. Rodrigues-Dagaeff et al., *Hearing Res.* 39:103-126, 1989).

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We thank Dr. R.J. Wenthold for the antiserum.

123.5

GLYCINE AND GABA IMMUNOSTAINING DEFINES FUNCTIONAL SUBREGIONS OF THE LATERAL LEMNISCAL NUCLEI IN THE MUSTACHE BAT. D.T. Larue, T.J. Park, G.D. Pollak, and J.A. Winer. Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA 94720 and Dept of Zoology, Univ. of Texas, Austin, TX 78712.

We studied the distribution of GABA and glycine immunoreactive cells and axons in the mustache bat to find the sources of inhibitory neurons projecting to the central nucleus of the inferior colliculus (ICC). We injected WGA-Apo-HRP-Gold (WAHG) into the ICC and immunostained both free-floating 50 μm-, and post-embedded 1 μm-thick sections.

The intermediate nucleus (INLL) is very prominent and contains many cells and puncta immunoreactive for GABA, glycine, or both. The dorsolateral region of the INLL that projects to the 60 kHz sonar signal representation in the ICC has a remarkable concentration of both GABA+ and Gly+ puncta as well as Gly+ somata. The region projecting to the 90 kHz representation in the ventrolateral part of the nucleus (J. Wenstrup et al. unpublished observations) has a similar pattern. No such neurochemical differentiation of a tonotopic subregion has been previously reported.

The ventral nucleus of the lateral lemniscus is divided into dorsal and ventral parts. The dorsal part of the ventral nucleus (VNLLd) has both GABA+ and Gly+ cells and puncta without the striking columnar organization seen in the ventral part. Both GABA+ and Gly+ cells project to the IC. The columnar (ventral) part of the ventral nucleus (VNLLv) consists of rows of cells that are almost exclusively Gly+, and receive many GABA+ and fewer Gly+ puncta. Tonotopically projecting bands of Gly+ cells across the columns in VNLLv are labeled by colocalization with WAHG injected into the ipsilateral ICC. This projection is the major source of Gly+ axons and puncta in the mustache bat IC as it is in the guinea pig and chinchilla, (Saint Marie and Baker, *Brain Res.* 524:244-253 1990).

The Gly+ cells from the monaural nuclei, INLL and VNLL, project ipsilaterally to the IC. An ipsilateral projection from Gly+ cells also occurs in the superior olivary complex, (see Saint Marie et al.,*J. Comp. Neurol.* 279:382-396 1989; Park et al.,1991, this vol.). No crossed projection to the IC from Gly+ cells has been reported. How these ipsilateral glycinergic influences might shape acoustic response properties in the ICC awaits further study.

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We thank Dr. R.J. Wenthold for the antisera.

123.6

GLYCINE AND GABA IN THE SUPERIOR OLIVARY COMPLEX OF THE MUSTACHE BAT: PROJECTIONS TO THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS.

T.J. Park, D.T. Larue, J.A. Winer, and G.D. Pollak. Dept. of Zoology, Univ. of Texas, Austin, TX 78712 and Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA 94720

The superior olivary complex (SOC) is the first binaural region of the auditory system. Both the lateral (LSO) and medial (MSO) nuclei are highly developed in the mustache bat and both send major projections to the central nucleus of the inferior colliculus (ICC). We studied the distribution of GABA and glycine immunoreactive cells and axons in the SOC to assess the inhibitory inputs to these nuclei and the inhibitory projections they send to the ICC. To examine projection cells from SOC to the ICC, we combined immunostaining with retrograde labelling from ICC injections of WGA-Apo-HRP-Gold in both 50 um- and 1um thick post-embedded sections.

In the LSO, about 30% of the cells are Gly+ and project ipsilaterally to the ICC as in the cat (Hutson et al., *Soc. Neurosci. Abstr.* 13: **548**, 1987; Saint Marie et al., *J. Comp. Neurol.* **279**: 382-396, 1989). There is a dense array of Gly+ fibers in the neuropil and axosomatic puncta on Gly+ and immunonegative cell bodies. The distribution of GABA, however, is striking: in the lateral limb there are a few GABAergic cells in a sparse GABA+ neuropil, while medially there is an abrupt change to a dense plexus of GABA+ fibers and puncta devoid of GABA+ cells. Adjacent, post-embedded senithin sections show that, unlike the cat, many immunonegative somata receive both Gly+ and GABA+ puncta.

In the MSO, roughly half the cells are Gly+, or GABA+, and sometimes both, and all cells receive axosomatic Gly+ puncta. Gly+ cells as well as immunonegative cells project to the ipsilateral ICC, in contrast to the guinea pig and chinchilla, in which MSO projection cells did not transport ^{3}[H]glycine (Saint Marie and Baker, *Brain Res.* **524**: 244-253, 1990).

In light of the specialized nature of the mustache bat's auditory system,both the parallels and differences from other mammals in neurochemical circuitry may provide insights into the role of the two inhibitory transmitters for information processing including mechanisms for sound localization. Supported by U.S.P.H.S. grants RO1 DC00268 (GDP) and RO1 NS16832-11 (JAW).
We thank Dr. R. J. Wenthold for the antisera.

123.7

NEURONS OF LAYER VI IN CAT PRIMARY AUDITORY CORTEX (AI): STUDY WITH GOLGI METHOD AND GABA AND GAD IMMUNOCYTOCHEMISTRY. J.J. Prieto and J.A. Winer. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-2097.

In cat primary auditory cortex layer VI is unique since it is the origin of four different types of efferent neural pathways: ipsilateral corticocortical, commissural, corticothalamic, and corticotectal. The aim of this study is to describe its cell types in Golgi material, and to suggest which are GABAergic.

Layer VI contains two sublayers, VIa and VIb. The former has a predominantly vertical internal arrangement, dominated by the apical dendrites of pyramidal cells; the latter appears more horizontal due both to the disposition of the dendritic arbors of the cells in it and a dense fiber plexus running parallel to the border with the white matter.

Several cell types occur in Golgi material. Besides the classical small and medium-sized pyramidal cells, which are common in sublayer VIa, there are atypically-oriented pyramidal cells whose apical dendrite runs parallel to the pia (tangential pyramidal cells), or is perpendicularly directed towards the white matter (inverted pyramidal cells). Both cell types were more common in sublayer VIb. Fusiform pyramidal cells display two principal dendrites, arising from the somatic poles; these cells are oriented either perpendicular or parallel to the pia. Among the non-pyramidal cells, small, medium-sized and large multipolar cells were found, together with bipolar cells.

The distribution, form, and number of GABA- or GAD-immunoreactive neurons in layer VI were studied in adult animals in 25 µm-thick sections. All of the non-pyramidal cell types contain GABAergic members. Strikingly, many of the cell profiles in our GAD material resembled inverted pyramidal cells. In other cortical areas spinous inverted pyramidal cells may have corticocortical axons while smooth inverted pyramidal cells project locally. Perhaps these two populations have different connections, and use different neurotransmitters.

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123.8

EXPRESSION OF GLUTAMATE RECEPTOR SUBUNIT mRNAS IN DEFINED CELL TYPES IN THE RAT COCHLEAR NUCLEUS. C. Hunter, T. Yu*, N. Yokotani*, K. Wada, and R.J. Wenthold. Lab of Neurochemistry, NIDCD, NIH, Bethesda, MD. 20892.

A number of cDNAs for the ionotropic class of the glutamate receptor (GluR) have recently been identified (Keinanen et al, Science 249: 1990), and these subtypes form heteromeric complexes with different physiological responses to applied agonists in vitro. These complexes, present on primary neurons in the cochlear nucleus, likely mediate the excitatory responses to complex sounds. We have used *in-situ* hybridization histochemistry to localize GluR receptor subtypes to cells in the rat cochlear nucleus. Within the dorsal cochlear nucleus (DCN), GluR-A and GluR-C oligodeoxy- nucleotide probes hybridized primarily to mRNAs in medium sized cells in layer 1, likely cartwheel cells. GluR-B and GluR-D probes by contrast, hybridized to multipolar shaped cells and a limited number of cells in layer 1. Large cells in the deep layer of the DCN were also labeled with GluR-D. The posterior division of the ventral cochlear nucleus (VCN) showed hybridization to medium sized, round cells in the globular bushy cell area with GluR-C and GluR-D probes. GluR-C, in addition, bound to a small group of cells in the vicinity of the octopus cell area. At rostral levels in the VCN, moderate hybridization to large spherical cells was observed with GluR-C and GluR-D probes. Cells within the granule cell cap of the VCN were seen to hybridize primarily with GLUR-B and GluR-D probes. The correlation of GluR receptor subtypes with physiologically and neuroanatomically identified cell types within the cochlear nucleus complex may help to delineate the molecular basis for sensory processing within the auditory system.

123.9

GLUTAMATE STIMULATED PHOSPHATIDYLINOSITOL METABOLISM IN THE AVIAN COCHLEAR NUCLEUS. *L. Zirpel, N.M. Nathanson and R.L. Hyson.* Hearing Development Laboratories and Depts. of Pharmacology and Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

Neurons of the second-order auditory nucleus, nucleus magnocellularis (NM), of young chicks undergo dramatic changes in morphology and metabolism upon cessation of excitatory afferent input from the eighth nerve. The cellular mechanisms of this afferent regulation are not known. One possible mechanism is via neurotransmitter-mediated activation of a second messenger system. Synaptic transmission from eighth nerve terminals to NM neurons is mediated by an excitatory amino acid. The excitatory amino acid, glutamate, has been shown to stimulate phosphatidylinositol (PI) metabolism in rat hippocampal slices and cultured cerebellar neurons. PI metabolism generates the intracellular second messengers DAG and IP_3. It is therefore plausible that glutamate stimulation of PI metabolism is involved in afferent regulation of NM neurons by eighth nerve activity. The goal of this study was to establish the functional presence and pharmacology of glutamate stimulated PI metabolism in NM neurons.

Samples of NM isolated from transverse brain slices of 4- to 16-day-old chicks were maintained in oxygenated Ringer's and incubated with ^{3}H-inositol in the presence of LiCl. Antagonists, when used, were added 10 min. prior to agonist. The water soluble products of PI metabolism (including IP_3) were quantified and normalized to total lipid incorporation of ^{3}H-inositol. Our results show that glutamate (2.5 µM -1 mM) reliably increases PI metabolism in NM 20-50% above control levels. Addition of the kainate/AMPA receptor antagonist, CNQX and the NMDA receptor antagonist, APV potentiates this response to glutamate. In addition, aminocyclopentyl-1,3-*trans*- dicarboxylate (ACPD, 2.5-100 µM), increases PI metabolism in NM 50-80% above control levels. Thus, glutamate can stimulate PI metabolism in the chick brainstem auditory system and the pharmacology of this phenomenon is similar to that reported for other preparations. (Supported by DC00858, GM07108, and DC00395.)

123.10

LOCALIZATION OF CALBINDIN, PARVALBUMIN AND GABA IMMUNOREACTIVITY IN NEURONS IN THE GERBIL BRAINSTEM AUDITORY NUCLEI. I.R.SCHWARTZ and P.R.EAGER*Sect. of Otolaryngology, Yale Univ. Sch. of Med., New Haven, CT 06510

In a continuing study of chemical markers which may distinguish subsets of GABAergic neurons we have examined the distribution and colocalization of calbindin (CaBP), parvalbumin (PV) and GABA immunoreactivity (IR) in frozen and vibratome sections of adult gerbil auditory brainstem.

In the superior olivary complex (SOC) virtually all neurons in the medial nucleus of the trapezoid body (MNTB) showed colocalization of PV-, CaBP- and GABA-IR. PV also produced heavy labeling of neurons in the lateral and medial superior olives (LSO, MSO), while CaBP labeled fewer LSO neurons and a few MSO neurons, both more lightly than MNTB neurons. Only an occasional neuron was GABA-IR in the MSO or LSO, although many were in the ventral nucleus of the TB. No neurons were stained by PV or CaBP in the dorsomedial periolivary nucleus (DMPO) although DMPO neuropil was consistently stained by both. SOC labeling differed from that reported in the rat by Webster et al (Neurosci. Letts. 111: 252-257, 1990) where only terminals labeled with CaBP in the MSO and LSO.

PV heavily stained neurons in all lateral lemniscal (LL) nuclei, while CaBP stained a smaller number in the ventral and intermediate nuclei of the LL (VNLL, INLL) and only a few scattered cells in the dorsal nucleus (DNLL). With PV the labeled neurons appeared in columnar arrays, especially in INLL and VNLL.

In the inferior colliculus (IC) results were similar to those reported by Coleman et al (ARO Absts. 14:43, 1991) in the rat with PV labeling many cells in the central nucleus and CaBP labeling a different population of cells in the external nucleus and dorsal cortex of IC. GABA labeling was similar to the pattern seen with PV. PV also labeled neurons and processes in the deep layers of the superior colliculus (SC). GABA and CaBP had similar staining patterns in the SC, small neurons were stained in a heavily stained neuropil in the SC superficial layers.

There are consistently different patterns of colocalization observed in different auditory nuclei for GABA, CaBP and PV.

Supported by NIH grant DC00132.

123.11

C-FOS IMMUNOHISTOCHEMISTRY REVEALS NO SHIFT OF TONOTOPIC ORDER IN THE CENTRAL AUDITORY SYSTEM OF DEVELOPING RATS. E. Friauf. Dept. of Animal Physiology, Univ. of Tübingen, D-7400 Tübingen, Fed. Rep. Germany.

Developmental alterations in the tonotopic maps of the mammalian central auditory system have been described in only a few species (gerbil: Sanes et al, JCN 279:436, '89; bat: Rübsamen et al, J Comp Physiol 165:755, '89; mouse: Romand and Ehret, Dev Brain Res 54:221, '90). I have identified isofrequency bands in rat central auditory nuclei by labeling them immunohistochemically for Fos. Fos is the protein product of the proto-oncogene c-fos, which can be expressed in neurons following sensory stimulation. Awake, unrestrained rats between postnatal day 10 (P10) and P30 (onset of hearing is P12) were stimulated with pure tone pulses (1, 4, 16 or 50 kHz; 100 ms on/off at 80 dB SPL) for 1.5 hrs. Brains were processed for Fos-like immunoreactivity (Fos-ir) using the PAP technique. Fos-ir neurons were present at all ages, but isofrequency bands became apparent only after P14. Isofrequency bands were prominent in the inferior colliculus and in all three subdivisions of the cochlear nucleus (dorsal, posteroventral and anteroventral) and were further analyzed. Their locations at P30 coincided remarkably well with those described by electrophysiology and [^{14}C]-2-deoxyglucose in adult animals. Age-related changes of the location of isofrequency bands, i.e. alterations in the tonotopic maps, were not detected in any auditory nucleus. In addition, high-frequency areas were responsive as early as low frequency areas, indicating that cochlear transduction of high frequencies is functional as soon as hearing is. These findings contradict the earlier reports. Since systematic shifts in the spatial encoding of frequency have been found in the developing cochlea of gerbils (Echteler et al, Nature 341:147, '89) and are thought to be the underlying process for changes in central auditory nuclei, it is interesting to see if these shifts occur in the rat cochlea, too. This may clarify whether the hypothesis of shifting place code holds for all vertebrate species.

123.12

DISTRIBUTION AND ORIGIN OF SEROTONIN IN GUINEA PIG COCHLEAR NUCLEUS. K.R. Moore*[1], H.B. Britton*[1], A.M. Thompson[1,2], C.D. Ross[1], and G.C. Thompson[1,2]. Depts. of [1]Otorhinolaryngology and [2]Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

With immunohistochemical staining, we have localized the putative neurotransmitter serotonin in all three divisions of the guinea pig cochlear nucleus. Serotonin-like immunoreactivity was present in fibers and terminals whose distribution varied among cochlear nucleus divisions: the dorsal and anteroventral cochlear nuclei contained the highest density. No immunoreactive cell bodies were observed in the cochlear nucleus or in any other auditory regions. Therefore, we have initiated experiments to determine the non-auditory source of the positive staining in cochlear nucleus.

The retrograde tracer HRP was pressure-injected into cochlear nucleus, and we looked for labeled neurons in regions containing serotonergic cell bodies. After large HRP injections, neurons of the caudal interstitial nucleus situated within the medial longitudinal fasciculus were retrogradely labeled. A few other cell bodies scattered along the midline were also retrogradely labeled. Therefore, preliminary evidence indicates that a non-auditory nucleus may be a source of serotonin in the cochlear nucleus.

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123.13

PHARMACOLOGY OF BINAURAL INTERACTION IN THE MOUSE LATERAL SUPERIOR OLIVE. Shu Hui Wu* and Jack B. Kelly. Laboratory of Sensory Neuroscience, Psychology Department, Carleton University, Ottawa, Canada K1S 5B6.

The pharmacology of evoked responses in the lateral superior olive (LSO) was examined in a brain slice preparation of the mouse superior olivary complex. Brain slices (400 μm thick) were taken through the superior olive of C57 BL/6J mice and were maintained in a continuously circulating, oxygenated saline solution. Glass micropipettes (50-80 MΩ) were inserted into the LSO under visual control. Stimulating electrodes were placed on the trapezoid body ipsilateral and contralateral to the superior olivary complex. Pharmacological agents were applied to the slice by redirecting the flow of solution in which the tissue was emersed. Results indicate that ipsilateral excitatory responses are mediated largely by non-NMDA receptors. Application of non-NMDA antagonists, CNQX or DNQX, had the effect of blocking extracellular spikes elicited by ipsilateral trapezoid body stimulation. In contrast, the NMDA antagonist, APV, had no effect on the vast majority of cells tested in either normal or 0 Mg^{++} solution. Simultaneous ipsilateral and contralateral stimulation of the trapezoid body resulted in the inhibition of ipsilaterally produced spikes. This inhibitory effect was mimicked by application of glycine and was completely blocked by application of strychnine to the bath. This result supports the growing body of evidence that contralateral inhibition in LSO is glycinergic. Unexpectedly, application of NMDA in relatively low concentrations completely blocked the inhibition produced by contralateral stimulation. These data indicate that NMDA as well as non-NMDA receptors play a role in regulating binaural interactions in the superior olivary complex.

123.14

Comparison between AChE histochemical staining and ChAT immunocytochemistry in the rat cochlear nuclei.

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The cochlear nuclei (CN) contain a rich complement of acetylcholinesterase (AChE) positive and choline acetyltransferase (ChAT) positive structures. Osen et al. (1984) demonstrated four AChE positive elements: 1) scattered cell bodies in VCN and in granule cell domains; 2) a fiber plexus consisting of irregularly oriented axons and mossy fibers; 3) a diffuse neuropil staining; and 4) dense patches, usually in the granule cell domain of DCN. By light microscopic ChAT immunocytochemistry, we demonstrate the occurence of scattered immunoreactive cell bodies and fibers in the major subdivisions of the CN. Axon terminals surround non-immunoreactive cells and their proximal dendrites in AVCN and PVCN, while in DCN the terminals are more diffusely arranged in the deep layers. The DCN molecular layer contains scattered immunoreactive terminals and fibers while the deeper layers contain scattered immunoreactive cell bodies. The AChE positive diffuse neuropil staining may correspond to ChAT positive terminals. Dense patches in DCN are absent after ChAT immunocytochemistry and may not, therefore, represent enzyme related to neurotransmission. Pre-embedding immunoelectron microscopy indicates that ChAT positive terminals constitute a heterogeneous population. Some of these terminals synapse on granule cells as rosettes and have an extrinsic origin.

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123.15

DEVELOPMENTAL PHARMACOLOGY OF EXCITATORY AMINO ACID TRANSMISSION IN THE CHICK COCHLEAR NUCLEUS (NUC. MAGNOCELLULARIS). N. Zhou and T. N. Parks, Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

During development, excitatory amino acid (EAA) receptors can, in addition to their role in normal signal processing, influence the differentiation and synaptic connectivity of neurons. To examine developmental changes in EAA receptors in auditory neurons, we studied the pharmacology of transmission between the cochlear nerve and nuc. magnocellularis (NM) in chickens aged embryonic day (E) 14 to posthatching day (P) 40, using previously-described methods for bath application of drugs and recording of electrically-evoked field potentials in brain slices (*Neurosci. 16*: 171). Postsynaptic responses in NM (reflected by the N1 potential) are insensitive to the N-methyl-D-aspartate (NMDA) receptor antagonists 3-((-)-2-carboxypiperazin-4-yl)propyl-1-phosphonate (CPP) and dibenzocycloheptenimine (MK-801) but can be blocked reversibly at all ages by the competitive non-NMDA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX). The EAA agonists kainic acid (KA), a-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) and NMDA can also block postsynaptic currents in NM in a reversible and dose-dependent fashion via depolarization block. The suppressive effects of NMDA, but not of KA or AMPA, are blocked completely by CPP or MK-801. The potency of NMDA decreases greatly with increasing age; the IC_{50} is about 85 μM at E14 but at E21 2 mM NMDA produces only a 6% reduction in the N1 amplitude. The potencies of AMPA and NBQX decrease by about 3-fold and 10-fold, respectively, between E14 and hatching, whereas the potency of KA appears to increase slightly across the ages studied. The results demonstrate the existence of functional NMDA receptors on NM neurons and suggest that during the last week of embryonic life, when NM neurons undergo dramatic morphological and functional transformations (*Curr.Top.Dev.Biol. 21*: 309), there are concurrent changes in the number, affinity and/or efficacy of both NMDA and AMPA receptors.

Supported by USPHS grant DC00144. We thank Dr. T. Honore for the NBQX.

123.16

NMDA-RECEPTORS IN SOUND-AZIMUTH ANALYZING NUCLEI OF CAT HINDBRAIN. B. N. Baker, K. K. Glendenning and R. B. Masterton. Dept. Psychology, Florida State University, Tallahassee, FL 32306.

In cat, the lateral superior olive (LSO) provides a neural representation of the interaural spectrum difference for sound localization (Boudreau & Tsuchitani, '70). The medial superior olive (MSO) acts as a 'coincidence detector' providing an analysis of interaural time differences for the same purpose (Yin & Chan, '90). Both LSO and MSO are known to receive excitatory glutamatergic and inhibitory glycinergic input, with the glutamatergic input acting mostly on quisqualate-type (and not kainate-type) receptors.

In further pursuit of the nature of the excitatory input to LSO and MSO, we have now found receptor-binding evidence for the presence of NMDA receptors both in LSO and MSO. Two putative NMDA-receptor ligands, CGS19755 and CGP39653, show significant binding both in LSO and MSO and each is displaced by glutamate itself.

Although the density of the NMDA receptors is only about 10% of the density of the quisqualate receptors, it suggests the presence of voltage-sensitive synapses. Therefore, LSO and MSO may have the capacity for modifying their input-output relationships perhaps in a manner useful for maintaining accurate sound localization during head and pinna growth. Supported by NIH DC197.

123.17

PATTERNS OF GAD AND GLYCINE IMMUNOREACTIVITY IN THE INFERIOR COLLICULUS OF TWO SPECIES OF BAT. B.R. Johnson*, E. Covey and J.H. Casseday, Department of Neurobiology, Duke University, Durham, NC, 27710.

Inhibitory mechanisms are involved in auditory signal processing, and the two amino acids, glycine and GABA, appear to be major inhibitory transmitters in the auditory system. We examined the distribution of glycine-and GABA-like immunostaining in the inferior colliculus of two FM bats, *Eptesicus fuscus* and *Nycticeius humeralis*, by using immunoglobulins directed against glycine and the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD). In both species, the immunostaining patterns were almost identical for a given immunoglobulin. There were no glycine-reactive cells in the IC. Glycine-reactive fibers and puncta occupied a crescent-shaped band that extended from the dorsolateral to the ventromedial part of the central nucleus of the inferior colliculus (ICc). In the rostrocaudal dimension, this region occupies approximately the central one-half of the ICc. These fibers appear to be continuous with glycine-reactive fibers in the lateral lemniscus. GAD-positive cell bodies are distributed in patches mainly in the ventromedial and lateral parts of the ICc and in the pericentral areas of the IC. Staining of puncta with the GAD antibody is dense except in the area that contains glycine-reactivity. In fact there is little overlap of GAD and glycine immunoreactivity. Most of the overlap is in the ventromedial region. This unequal distribution raises the possibility that there may be different regions of the ICc in which one or the other of these putative inhibitory amino acid neurotransmitters predominates. Research supported by NIH grants DC 287 and DC 607.

123.18

INFERRED CONTRIBUTIONS OF LOW AND HIGH SPONTANEOUS-RATE AUDITORY NEURONS TO THE COMPOUND ACTION POTENTIAL E.M. Relkin and J.R. Doucet*. Institute for Sensory Research and Dept. of Bioengineering, Syracuse Univ., Syracuse, NY 13244.

The recovery of auditory neurons from prior stimulation depends on the spontaneous rate (SR) of the neuron. Low SR neurons recover more slowly compared to high SR neurons. Thus, it is possible to reduce the relative contribution of low SR neurons to a population response by preceding a test stimulus with a conditioning stimulus, and recording the response to the test stimulus at a time after the conditioner when the high SR neurons are mostly recovered and the low SR neurons are not.

Compound action potentials (CAP) were recorded in chinchillas for the response to a 12 kHz tone-burst preceded by an 80 dB SPL conditioning toneburst at the same frequency. The CAP was recorded for many time delays of the test stimulus relative to the conditioner and for many test-stimulus intensity levels. CAP waveforms were reduced relative to control waveforms recorded with no conditioner for delays up to 400 ms. Recovery of the waveform is consistent with the recovery of two populations of neurons with different latencies and recovery rates. The inferred properties of the two populations are also consistent with direct measurements of recovery for single auditory neurons.

123.19

REPRESENTATION OF THE COCHLEA IN THE AUDITORY BRAINSTEM OF THE OPOSSUM, *MONODELPHIS DOMESTICA*. F.H. Willard, Melissa C. Stenner,* and Frank. J. Daly*, Department of Anatomy, University of New England, Biddeford, ME 04005.

The development of cochleotopic order in the auditory system of ***Monodelphis domestica*** is being investigated. To provide an end point in this study, the topographic pattern of the projections from cochlea through cochlear nucleus (CN) to midbrain in the adult has been examined. Small pledgets of HRP were placed in the apex or base of the cochlea in a series of 15 adult animals. In a second series of 10 animals, small injections of HRP from a glass micropipette were made into the inferior colliculus of the midbrain. Survival of all animals was 24 hours. Tissue was sectioned frozen or with a vibratome, and reacted with tetramethylbenzidine. In the first series of animals, labeled sheets of fibers were found coursing through the ventral and into the dorsal CN consequent to the cochlea placement of HRP. Specifically, apical placements resulted in labeled caudolateral sheets of auditory nerve fibers in the CN, while basal placements labeled rostromedial sheets of fibers. In the second series of animals, ventromedial placements of HRP into the midbrain resulted in labeled rostromedial sheets of neurons in contralateral CN, while dorsolateral placements labeled caudolateral sheets of neurons. When data from the two studies were collapsed onto single plots, the labeled sheets of midbrain-projecting neurons were in register with the sheets of labeled auditory nerve fibers. The close spatial alignment of auditory nerve laminae with those of midbrain-projecting neurons, demonstrates a mechanism for maintaining the fidelity of the cochleotopic map as it passes through the cochlear nucleus and into the midbrain.

123.20

RESPONSE OF AUDITORY NEURONS TO APPARENT AUDITORY MOTION. W.W. Wilson* and W.E. O'Neill, Program in Neuroscience, Univ. of Rochester Med. Ctr., Rochester, NY 14642

The interaural intensity difference (IID) of a sound between the two ears is thought to be a cue to the location of that stimulus. Dynamically changing IID would be produced by a moving sound source and may serve as a cue to auditory motion. We tested this possibility by recording the response of single units in the inferior colliculus (IC) of an awake mustached bat to a dichotic stimulus simulating a sound moving between the ears.

Best frequency (BF) tone bursts were presented through earphones to the contralateral (CL) ear at 10 dB above minimum threshold (MT) and simulatneously to the ipsilateral (IL) ear at intensities changing sequentially in 10 dB increments to produce IIDs from -30 to +30dB and back to -30dB IID condition. The response of the neuron to a particular IID was compared for increasing IID trains, decreasing IID trains, and a static train of stimuli at the same IID presented in succession.

Increasing IID trains generally elicited a greater response at most IIDs than decreasing IID trains. Most units showed a greater response in the increasing IID case than at that same IID in the static case. In the decreasing IID case, these same cells had a response similar to or lower than static responses. These units were excited by low IIDs and inhibited by high IIDs, suggesting facilitation when changing from excitatory to inhibitory IIDs and inhibition in the opposite direction. (Sponsored by NRSA fellowship 1 F31 MH10107-01 from NIMH)

AUDITORY SYSTEM: CENTRAL PATHWAYS II

124.1

A COMPARISON OF ONSET LATENCIES OF AUDITORY RESPONSES TO CLICK IN DIFFERENT BRAINSTEM AREAS OF THE CONSCIOUS CAT. B. Hoang, C.D. Woody, X.F. Wang, E. Gruen*. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Recordings from the following areas showed increases in discharge to 1ms, 70db clicks: cochlear nucleus (CO), dentate (D), interpositus (IP), cerebellum (C), inferior colliculus (IC), medial geniculate (MG), suprageniculate (SG), lateral superior olive (OSL), superior colliculus (SC), and lateral geniculate (LG). Latencies of response compared within a single animal ranged from 4-8ms (CO, OSL, MG and D) to 20-28ms (C, IP, SG and LG). Other latencies were 12-16 ms (IC) and 16-20 ms (SC). Latencies of response from within-animal recordings were substantiated by combined data from more than one animal for the following areas: C, CO, OSL, D, LG, and IP. The across-animal latency for MG was 12-16 ms. The latency for C in one other animal with more lateral recordings was 12-16 ms. Other investigators have shown auditory responses of comparable latencies to those in our conscious-animal recordings in the following areas in anesthetized cats: IC (Rose et al., J. Neurophysiol., 1963 - with shorter latencies in some IC regions), MG (Nelson et al., J. Neurophysiol., 1963), CO (Kiang, Acta oto-laryng., 1965), and OSL (Galambos et al, Amer. J. Physiol., 1959). (Supported by NS25510)

124.2

EVIDENCE FOR A NEW, PRIMARY AUDITORY TRANSMISSION PATHWAY BETWEEN THE COCHLEAR NUCLEI, THE SUBCEREBELLAR DENTATE NUCLEUS, THE ROSTRAL THALAMUS, AND THE MOTOR CORTEX OF CATS. C.D. Woody, X.F. Wang, V. Chizhevsky*, J. Landeira-Fernandez and E. Gruen*. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Short latency (4-10 ms) responses to 70 db click stimuli were recorded at the above loci in conscious cats. The response latencies in motor cortex (Sakai and Woody, J. Neurophysiol., 1980) were as short as those reported in classical auditory receptive cortex. Injections of PHA-L were made in the dentate nucleus. Fibers were traced between dentate nucleus and rostral thalamus, a region shown recently to respond at latencies of 6-8 ms to click stimuli (Woody et al., J. Neurosci., 1991). Fibers were also traced between dentate and dorsal and ventral cochlear nuclei. Since cell bodies were also observed, these fillings were thought to be retrograde. (Previously described fiber connections with vestibular, caudate and VA nuclei were also found.) The evidence suggests that an ascending auditory transmission pathway exists between cochlear nuclei, dentate nucleus, rostral thalamus and motor cortex. The proposed auditory pathway appears to have functional significance since ablation of classical auditory receptive regions of cortex that receive classical collicular-geniculocortical projections does not prevent acquisition of short latency Pavlovian conditioned blink responses to click CS that depend on the motor cortex for development (Woody et al, J. Neurophysiol., 1974). (Supp. NS25510, HD05958.)

124.3

APPARENT EXPECTATION IN INTERSTIMULUS-INTERVAL-SPECIFIC EVENT RELATED POTENTIALS TO OMITTED STIMULI IN THE ELECTROSENSORY PATHWAY OF ELASMOBRANCHS. T.H. Bullock and M.H. Hofmann*. Dept. Neurosci., Univ. Calif., San Diego, La Jolla, CA 92093-0201 and Dept. Anatomy, Univ. Göttingen, Germany.

Comparing a lateral line modality with the previously studied (Bullock et al., J Neurophysiol 64:903-914) visual omitted stimulus potentials (OSP), we recorded multiunit activity and slow local field potentials (LFP) in the electrosensory system in rays (*Platyrhinoidis triseriata*) during and after trains of microvolt pulses in the bath, with special attention to end-of-train effects, at four levels from peripheral nerve to cerebrum. LFPs occur after the due-time of the first omitted stimulus, visible on each sweep down to 2 Hz, larger at higher frequencies. They resemble OFF response to single long current pulse. Independent of stimulus intensity, OSPs are on schedule, with a constant, long (60-100 ms) latency from the due-time - as though the system has an expectation. The OSP depends on a sufficient duration and regularity (vs jitter) of the conditioning train. OSP appears already in the nerve; dorsal nucleus of medulla, mesencephalic lateral nucleus and a deep nucleus in cerebrum show progressively later, broader first main OSP peaks, plus labile late waves, to >700ms. Our telencephalic loci cannot follow >0.1 Hz; their OSP arises after trains causing no EPs except to the first stimulus. Inhibitory processes dominate until excitatory rebound due to omitting a stimulus. One or two induced rhythms (IR) of the frequencies: 4-7, 16-20 or 55-75 Hz often follow the main OSP for >1s. Each is labile, most prominent in medulla; shorter and smaller in midbrain and inconspicuous at best in forebrain. IR tend to synchronize in medulla and midbrain. ERPs such as OSP and IR and properties such as apparent expectation on schedule over a range of ISIs do not require higher brain levels or the complexities of the retina. They can appear in primary medullary sensory nuclei of the octavolateralis system, to be modified in midbrain and forebrain.

124.4

ELEVATION SENSITIVITY OF AZIMUTH SENSITIVE NEURONS IN CAT PRIMARY AUDITORY CORTEX (AI). F.R. Samson*, P. Barone, J.C. Clarey, and T.J. Imig, Dept. of Physiol., Kansas University Med. Ctr., Kansas City, KS 66103.

Responses of single units (CFs 6 - 28 kHz) were studied in AI of barbiturate anesthetized cats using broadband noise burst stimuli that varied in azimuth, elevation and sound pressure level (SPL). Unilateral ear occlusion revealed that some cells derived azimuth sensitivity strictly from binaural cues, and others exclusively or predominantly from monaural cues. Responses to sound source elevations between ±67.5° using SPLs that ranged between 0 and 90 dB were obtained for 30 cells at best azimuth. Elevation functions, averaged over SPL, were obtained for each cell, and those with elevation functions modulated ≥75% were considered elevation sensitive. Spatial receptive fields (SRF) were obtained for 15 cells using a single SPL that in the case of nonmonotonic cells was the best level. Monaural and binaural cells differed in elevation sensitivity. The vast majority of monaural cells (12/14) were elevation sensitive, most were tuned to relatively narrow ranges of elevation, and the elevation tuning of 2 cells that were tested with ear occlusion was shown to derive from monaural cues. In contrast, binaural cells were less sensitive to elevation. Most (4/6) EI (excitatory input from one ear, inhibitory input from the other) cells were elevation insensitive, and the sensitive ones were broadly tuned to elevation. A hemifield SRF was obtained for one EI cell. Binaural cells receiving excitatory or facilitatory inputs from each ear varied in elevation sensitivity. Five of 15 were insensitive, and those that were sensitive could be either broadly or narrowly tuned. Supported by NIDCD grant # DC00173.

124.5

EFFECT OF BANDWIDTH ON NEURONS' AZIMUTH TUNING IN CAT MEDIAL GENICULATE BODY (MGB) AND PRIMARY AUDITORY CORTEX (AI). P. Barone, J.C. Clarey, W.A. Irons* F. Samson* and T.J. Imig. Dept. Physiol., Univ. Kansas Med. Ctr., Kansas City, KS 66103, USA.

The azimuth-level response areas to free-field broadband noise (BBN) and tone-burst stimulation at characteristic frequency (CF) were compared in 41 MGB and 130 AI high-CF neurons in barbiturate-anesthetized cats. A small number of forebrain cells (N=23) was also tested with bandpass noise (BPN) of various bandwidths. Azimuth *sensitivity* was assessed by the degree of modulation of the average azimuth function, and azimuth *selectivity* was assessed by the range of azimuths that produced greater than 75% responsiveness relative to maximum. The vast majority of forebrain cells showed the same azimuth preference to the two stimuli (i.e., contralateral, ipsilateral or midline), although many showed greater selectivity (92/171: 53.8%) or sensitivity (59/171: 34.5%) to BBN compared to CF-tones. These effects were particularly apparent in cells that responded to a narrow range of azimuths near the midline or the lateral poles ($\pm 90^0$). Some cells required only narrow BPN centered about CF to generate an azimuth response similar to BBN and more selective than tones; fewer cells required a wider BPN that encompassed excitatory and non-excitatory regions of the cell's frequency response area. The latter result suggests that inhibitory frequency domains may contribute to the directional selectivity of some cells. In a few thalamic cells the presence of such domains was confirmed using two-tone stimulation. The fact that cells responded at a significantly lower discharge rate to noise compared to tones (t=4.14, df=170, pr<0.001) also suggests an inhibitory influence, apparent only with broadband stimulation. Acute ear occlusion manipulations showed that cells that derived their directional selectivity principally from monaural spectral cues showed quite dramatic decreases in selectivity and sensitivity when stimulated with a CF-tone. In contrast, cells that derived their selectivity from binaural excitatory-inhibitory interactions tended to show similar azimuth responses to noise and tones.

124.6

VARIABLITY IN DISCHARGE RATE OF COCHLEAR NUCLEUS NEURONS DURING DEVELOPMENT. J.L. Fitzakerley, J. McGee* and E.J. Walsh. Boys Town National Research Hospital, Omaha, NE 68131.

Many theories regarding information coding in sensory systems are based upon discharge rate analyses, and variability in neuronal discharge rate is an inherent complication in these measures. As a result of the need to use rate-based analyses in ongoing investigations, this study was designed to assess the variability of both acoustically-evoked and spontaneous discharge rates in developing animals. Standard extracellular recording techniques were used to study neurons in the cochlear nucleus of kittens throughout postnatal development. Overall levels of spontaneous activity were low in the youngest animals, and many neurons exhibited marked variability in spontaneous rate (SR). At all ages, SR variability was dependent upon rate, with SR being less stable in low-SR units than in high-SR units. Acoustically-evoked discharge rates were also more variable early in development, with the largest differences between immature and mature animals occurring at near threshold intensities. This variablity was apparently related to a decreased ability to respond to succeeding stimulus presentations, suggesting that fatigue and/or a lack of synaptic security may be responsible for the observed variability in acoustically-evoked discharge rate.

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124.7

AN AUDITORY AFTEREFFECT FOR MOTION IN THE FREQUENCY DOMAIN. *Z. Shu*, N. V. Swindale, C. A. Laszlo*,† and M. S. Cynader, Departments of Ophthalmology and †Electrical Engineering, Univ. of British Columbia, Vancouver, B.C., Canada V5Z 3N9.*

Adaptation aftereffects in the visual system have been much studied and used as tools in investigating the functional mechanisms of vision. In audition, however, although there have been studies on adaptation to frequency- and amplitude-modulated signals, little work has been done on other adaptation aftereffects. This has been partly due to difficulties in generating analytic stimuli analogous to those used in studies of visual adaptation. By using a novel auditory stimulus, which has either a notch- or a peak-shaped spectrum with the position of the notch or peak varying with time over a certain frequency range, we have been able to demonstrate an auditory motion aftereffect analogous to the well known visual motion aftereffect.

A two-alternative forced choice experiment was designed to measure the velocity of the moving peak or notch in a brief test stimulus at which the subject perceived the stimulus as stationary, under different adaptation conditions with different adapting velocities. The task of the subject was to judge the direction of motion of the peak or notch in the test stimulus. The adapting stimulus, which typically lasted for 3 minutes, was either a peak or a notch which moved up or down at a constant velocity. The results show that after adaptation to motion in one direction, a stationary test stimulus will be more likely perceived as moving in the opposite direction. This aftereffect, analogous to the visual motion aftereffect, indicates that the auditory system may process time-varying stimuli with mechanisms functionally similar to those in the visual system.

124.8

SALICYLATE INDUCED CHANGES IN LOUDNESS THRESHOLDS AT VARYING FREQUENCIES IN RATS. J.F. Brennan[1] and P.J. Jastreboff[2]. [1]Dept Psychology, Univ of Massachusetts/Boston, MA 02125 and [2]Dept Surgery, Univ of Maryland Sch Med., Baltimore, MD 21201.

Shifts in auditory intensity thresholds for 31 different frequencies (1-16 kHz) were tested in 15 pigmented rats using bar press behavior. Rats were trained in 5 progressive stages of difficulty until they were able to detect a standard tonal probe (10 kHz, 62 dBC) by depressing the response bar for 2 s (S+) or to detect no probe (S-) by releasing the bar after the onset of a light signal. Groups of 5 rats received either saline injections or salicylate injections of either 200- and 300-mg/kg 2 hrs prior to the 45-min testing session. A total of 7 tonal frequencies (S+ probes) were interspersed with about a third as many S- probes on each session, and the order of frequencies was counterbalanced across testing days and subjects. For each frequency series initial probes began at 62 dB and decreased in 5 dB units until an error was recorded, at which time the series increased in 2.5 dB units until detection, followed by a decrease in units of 1 dB. The results indicated that salicylate injections induced threshold shifts at selected frequencies. (NIH DC00299).

124.9

LOCAL INTERNEURONS IN THE AUDITORY BRAINSTEM OF THE BARN OWL. T. Walford* and C. E. Carr. Dept. of Zoology, Univ. Maryland, College Park, MD 20742.

In the barn owl, sensitivity to interaural time differences (ITDs) arises in the brainstem nucleus laminaris (NL). NL receives auditory input from the cochlear nucleus magnocellularis (NM). GABAergic terminals surround both NM and NL neurons. The role of the GABAergic terminals in NM is unknown, while the GABAergic terminals in NL play a role in shaping the response to ITDs (Fujita and Konishi, 1991). Furthermore, models of binaural interactions in NL suggest that inhibition is required for ITD sensitivity (Gruen et al, 1990).

Since the source of these GABAergic terminals in the barn owl is unknown, we used immunohistochemical and tract tracing techniques to identify the cell bodies of origin and to suggest the nature of the inhibitory input.

GABAergic terminals in NM originate from 3 sources: the superior olive (Carr et al., 1989), neurons dorsal to NM in the floor of the 4th ventricle and a population of interneurons caudal and lateral to NL. The olivary neurons may receive binaural inputs, while the other 2 groups are located in or around the auditory nerve root.

GABAergic terminals in NL appear to originate from interneurons in the magnocellular efferent tract, lateral to the medial vestibular nucleus and medial to NL. Medium-sized fusiform spiny cells extend their dendrites parallel to the ipsilateral magnocellular axons, and the dendrites of small ovoid cells ramify below NL.

Supported by NIH DC00436.

124.10

AXONAL ARBORIZATIONS IN THE MEDIAL GENICULATE NUCLEUS OF FERRETS: COMPARISON OF ARBORS FROM THE INFERIOR COLLICULUS WITH INDUCED INPUTS FROM RETINAL GANGLION CELLS. S.L. Pallas, J. Hahm and M. Sur. Dept. of Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139.

In a previous study (Pallas et al., Soc. Neurosci Abstr. '89), we reported on the morphology of adult ferret retinal axon arbors which project into the auditory thalamus (MGN) following partial removal of retinal targets and deafferentation of the MGN in neonates. In the present study, we describe the morphology of arbors in the MGN from inferior colliculus (IC) axons in normal ferrets, and compare them to the retinal axon arbors in MGN of the "rewired" ferrets.

We used the in vitro method of bulk-filling axons with HRP in the brachium of the IC to label individual axon arbors in the MGN. These axons have well-formed terminal arbors in the MGN, with a wide variety of bouton shapes and sizes. Many boutons are quite large (10-30 μm) and crenulated. The arbor volume varies from 0.63 to 26.56 x 106 μm^3 (mean= 7.34 x 106 μm^3, SE= 2.39, n=11). Axon diameters vary from 0.4 to 1.4 μm (mean= 0.9 μm, SE= 0.1, n=11).

Although IC axons to the MGN in normal ferrets and retinal axons to the MGN in rewired ferrets have similar axon diameters, IC arbors are much larger in volume (retinal axon arbor volume range 0.02 to 1.68 x 106 μm^3, mean= 0.72 x 106 μm^3, SE= 0.10, n=26) and branch/bouton density is qualitatively much lower.

These results suggest that axon arbor morphology is somewhat independent of target identity. Also, since the induced retinal axon arbors are much more spatially restricted than arbors of normal IC axons, it is likely that the two-dimensional spatial information from the retina is preserved as a retinotopic map in the MGN of rewired ferrets.

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124.11

CYTOLOGY AND SYNAPTIC RELATIONSHIPS IN THE COCHLEAR NUCLEUS OF THE ALLIGATOR LIZARD. D.D. Wright*, M.R. Szpir, D.K. Ryugo. Center for Hearing Sciences, Departments of Otolaryngology-HNS and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

In order to understand the structural basis for acoustic signal processing in vertebrates, we have been investigating the organization of neurons and synaptic inputs in the cochlear nucleus of the alligator lizard (*Gerrhontus multicarinatus*). The cochlear nucleus of this species receives primary afferent information from two morphologically and functionally distinct types of cochlear nerve fibers (Szpir et al., JCN 295:530-547, 1990). In the present study we describe the anatomy of cell types and synapses in the cochlear nucleus of the alligator lizard.

The neurons of the cochlear nucleus were characterized on the basis of their light microscopic appearance in Nissl preparations and their corresponding ultrastructural features. We found seven morphologically distinct cell types: four large- and three small-cell types. Each cell type was confined to one of the four subdivisions of the cochlear nucleus. In addition, we investigated the ultrastructure of HRP-labeled cochlear nerve terminals as well as that of unlabeled terminals. Cochlear nerve terminals have round synaptic vesicles and form somatic and dendritic synapses with the neurons in all subdivisions of the cochlear nucleus. All subdivisions also contained three types of unlabeled synaptic terminals exhibiting either round, pleiomorphic or flattened vesicles.

The presence of different cell populations in the four subdivisions suggests that acoustic information may be further channeled into several functionally specialized pathways that originate in the cochlear nucleus.
(Supported by NIH grants DC00232, DC00979 and DC00119)

124.12

TOPOGRAPHIC DISTRIBUTION OF THE PROJECTION FROM THE PRIMARY AUDITORY CORTEX TO THE INFERIOR COLLICULUS IN ALBINO RATS.

Carrascal, E.*(1), Garcia-Méndez, J. A.*(2), Lanciego, J. L.*(1), and Collia, F. (1). 1- Cátedra de Histologia, Fac. de Medicina. Univ. de Salamanca.(España) 2- Cátedra de Histologia, Colegio Univ. de Avila. Univ. de Salamanca (España).

PHA-L was injected into the primary auditory cortex (Te1), in order to determine the distribution of cortical fibres in the inferior colliculus (IC). The location of the injections were stereotaxically determined according to Zilles et al.(1985) and the injections were placed in a rostro-caudal sequence through Te1. Small to medium size zones of injection in Te1 (ranged from 360 µm. to 900 µm.) afforded in the IC an arrangement of the fibres in restricted groups of axonal fascicles which gave it the appareance of bands. The number of bands varied, depending on the extent of the injection in Te1 although small-medium size injections gave 1 or 2 bands and in the greatest injections up to 3 bands. The rostral most zone of Te1 projects to the caudal-most part of the external cortex, the medial part of the dorsal cortex and the medial part of the central nucleus. The anterior zone of Te1 projects to the central zone of the dorsal cortex and forms 2 to 3 bands of labeled axons in the medial and central parts of the central nucleus. The fibres from the central zone of Te1 strongly innervates the external cortex, like the posterior middle of the medial part of the dorsal cortex with the dorsal superficial cortex being practically free of fibres, and scattered fibres in the central nucleus, and the lateral periacueductal gray matter. The caudal part of Te1 supplies fibres to the superficial layers of the external cortex at the rostral and middle levels, the dorsal part of the central nucleus, and the central part of the dorsal cortex. The results demonstrate a topographically and highly organized projection from Te1 to all subdivisions of the IC, including the central nucleus, each of which has a distribution of the fibres forming definite and specific bands.
(Supported by FISS grant Nº 88/2040, and by Castilla-León grant Nº 1115/90).

124.13

MORPHOLOGY OF THE DORSAL COCHLEAR NUCLEUS (DCN) IN YOUNG AND OLD C57BL/6J AND CBA/J MICE. J.F. Willott, L.S. Bross*, S.M. McFadden, and P. Burke*. Dept. Psychol., Northern Illinois Univ., DeKalb. IL 60115.

In C57 mice, which exhibit progressive age-related cochlear sensorineural degeneration, the size of neurons in each layer of the DCN decreased gradually between age 2 and 29 mos.(old age). The number of neurons in layer 3 (but not layers 1 and 2) decreased significantly between 1.5 and 7 mos. and between 12 and 24 mos. Volume of DCN layers 1 and 3 decreased with age, but volume of layer 2 remained stable. In CBA mice, which hear well during old age, neuron size and number showed only a trend toward a decrease in all three DCN layers and only in the oldest mice. Volume of layers 1 and 3 increased during the first year of life, then decreased during the second year. Layer 2 volume showed little age- related change.

Layer 3 receives more 8th nerve input than other layers, so the effects of age-related loss of 8th nerve fibers in C57 mice would be expected to impact layer 3 in particular. In fact, cell counts and volume decreased most markedly in layer 3 of aging C57 mice.

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124.14

TONOTOPIC ORGANIZATION, ARCHITECTURE AND CONNECTIONS OF AUDITORY CORTEX IN MACAQUE MONKEYS. A. Morel, P.E. Garraghty, M.K. Schwaber, P. Burch-Sims, and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Detailed microelectrode best-frequency maps of macaque monkey auditory cortex revealed two complete frequency representations in the lateral bank of the lateral sulcus, primary area A-I, and the rostral area, R. Confirming previous observations, we found a progression from high to low best frequencies in a caudal to rostral direction in A-I. Rostral to A-I, in R, best frequencies reversed and progressed from low to high in a caudal to rostral direction, so that A-I and R share a low-frequency border. A-I and R together are coextensive with a densely myelinated zone that reacts darkly for acetylcholinesterase and cytochrome oxidase, and has intense uptake of 2-deoxyglucose under normal ambient sound conditions. Several distinct architectonic subdivisions compose a belt of cortex surrounding A-I and R. Connection patterns with A-I and R, as demonstrated by injections of several different tracers (fluorescent dyes, WGA-HRP) in physiologically identified loci, and best frequencies of neurons recorded in a limited number of sites suggest that portions of the belt cortex medial and lateral to A-I and R are tonotopically organized. Belt auditory cortex also differs from A-I and R in having long cortical connections, such as with the prefrontal cortex, as well as projections from the medial pulvinar.
(Supported in part by the Deafness Research Foundation.)

124.15

DO DORSAL COCHLEAR NUCLEUS PARALLEL FIBERS EXCITE FUSIFORM CELLS VIA AN EXCITATORY AMINO ACID NEUROTRANSMITTER? P.S.Palombi, P.M. Backoff* and D.M. Caspary. Dept. of Pharmacology, SIU School of Medicine, Springfield, IL 62702

Fusiform cells of the dorsal cochlear nucleus (DCN) appear to receive excitatory inputs on their basal dendrites from VIIIth nerve fibers and on their apical dendrites from the parallel fiber axons of granule cells; they also receive inhibitory inputs onto their proximal dendrites and somata. These neurons often display buildup (BU) or pauser/buildup (PBU) temporal response patterns. In forward masking paradigms, while most cochlear nucleus neurons display a masked response, BU/PBU neurons often show no masking or even a potentiation of the probe response (Boettcher et al. Hearing Research, 48(1990) 125-144). In studies of anesthetized chinchilla DCN neurons, we presented two tones at characteristic frequency, 20 to 40 dB above threshold, separated by 0 to 75 ms. Extracellular single unit recordings from 14 of 17 DCN cells with characteristic BU/PBU temporal response patterns displayed an increased discharge rate during the second, or probe, tone; only one cell showed masking of the probe tone. Several cells also displayed altered temporal response patterns to the probe with induction of or change in chopping rate. The molecular layer of the DCN displays high concentrations of the excitatory amino acid (EAA) glutamate, selective uptake and release of D-aspartate, and high levels of zinc-containing neurons. These characteristics, which are similar to the cerebellar granule cell system/parallel fiber system, and In vitro electrophysiologic data suggest that an EAA is a good candidate for the neurotransmitter used by DCN parallel fibers. One possible mechanism for the potentiation of DCN cells seen in the masking paradigm described above is potentiation of excitatory input to the basal dendrites of DCN fusiform cells by EAA-mediated excitatory input to the apical dendrites from parallel fibers, just as depolarization of hippocampal neurons is potentiated by NMDA receptor activation. This mechanism could account for the increasing activation of fusiform cells observed in DCN, resulting in units displaying BU/PBU temporal discharge patterns, potentiation in a forward masking paradigm, and increased chopping. Iontophoretic studies are underway to further test this hypothesis. (Supported by NIH DC00151-10)

124.16

PHYSIOLOGICAL CORRELATES OF THE VOICE ONSET TIME BOUNDARY IN AUDITORY CORTEX. M. Steinschneider, C. Schroeder, J. Arezzo and H.G. Vaughan, Jr. Albert Einstein College of Medicine, Bronx, NY, 10461.

Voice onset time (VOT) is the interval between stimulus onset and onset of a speech sound's periodic segments. A short duration VOT produces a percept of a voiced consonant such as /d/, whereas longer durations elicit the percept of an unvoiced consonant such as /t/. The VOT duration which elicits an ambiguous percept is called the boundary, and for the /d/-/t/ distinction is about 30 msec. We investigated the cortical encoding of VOT by examining laminar profiles of auditory evoked potentials, current source density and multiple unit activity elicited by the syllables /da/ and /ta/ in auditory cortex of an awake macaque. VOT was varied from 0 to 80 msec in 20 msec increments. We hypothesized that response differences would distinguish between voiced (/d/) and unvoiced (/t/) consonants.

VOT is reflected by two different response patterns which are time-locked to the onset of the voiced and unvoiced speech segments. In the first, responses time-locked to the unvoiced and voiced segments occur at all VOT durations and do not distinguish between voiced and unvoiced consonants. In the second, response bursts time-locked to the later onset of voiced segments are accentuated to the unvoiced consonant /t/ with VOTs 40 msec or longer. Auditory evoked potentials recorded in the far-field also display the second response pattern.

We conclude that voiced and unvoiced consonants elicit responses in auditory cortex that are differentiated by time-locked activity to voicing onset. This activity may play an important role in speech discrimination.
(supported by DC00657, MH06723 and the J.S. McDonnell Foundation)

124.17

REPRESENTATION OF MULTI-FREQUENCY SOUNDS IN THE AUDITORY CORTEX OF CATS. I. Nelken*, Y. Prut* and E. Vaadia. Dept. of Physiology, Hadassah Medical School, Jerusalem, Israel 91010.

Properties of single unit and population activities in the primary auditory cortex (AI) of cats have been studied in response to multi-frequency sounds. The stimuli spaces consisted of two-frequency signals, four-frequency signals and quasi-harmonic complexes composed of nine frequencies. Since the number of possible combination of parameters was very large, new methods for studying the response to large stimuli spaces have been developed. One of them is based on an analysis of variance of the single responses to large number of combinations of parameters. The second is an automatic procedure for finding combinations eliciting large responses. The results show strong interactions between the sound components. Usually, the strongest response is for a frequency combination, of which none of the components is exactly at the best frequency of the unit. Also, the distance between components of the best combinations tend to be relatively large, much beyond the width of the single-frequency receptive field. It is proposed that the units in AI are not simple bandpass filters, but are "searching" for combined spectral features, approximating matched filters in the spectral domain. This research was supported by the Fund for Basic Research administered by the Israel Academy of Sciences and Humanities.

124.18

TEMPORAL ANALYSIS OF AMPLITUDE MODULATED SIGNALS: A COMPUTER SIMULATION OF PERIODICITY CODING NEURONS. G. Langner, Zoological Institute, Technical University of Darmstadt, 6100 Darmstadt, Germany

In the range of typical fundamental frequency of many biological sound sources, i.e. below 1kHz, periodic envelope fluctuations elicit the perception of periodicity pitch. Such fluctuations or modulations are temporally coded in the peripheral auditory system and analyzed by neuronal correlation mechanisms in the central auditory system, resulting in a computational periodicity map at the level of the auditory midbrain (Langner and Schreiner, Soc., Neurosci. Abstr. 15, Part 1, p. 1116, 1989). A computer model has been presented composed of a trigger neuron, a triggered integrator ('reducer'), a triggered oscillator, and a coincidence detector (Langner et al., Soc., Neurosci. Abstr. 13, Part 1, p. 546, 1987). It includes only components resembling major neuron types of the auditory brainstem, like chopper and pauser neurons. The present paper compares results obtained from the computer model with those obtained in various recordings of periodicity coding neurons. The simulations are successful in describing modulation transfer functions (MTFs) of neurons, details of temporal response patterns (onsets and time courses of responses), and variation of MTFs with variations of signal parameters (carrier frequency).

124.19

DENDRITIC MORPHOLOGY AND LOCAL COLLATERALIZATION OF INFRAGRANULAR PYRAMIDAL NEURONS IN CAT PRIMARY AUDITORY CORTEX. H. Ojima[1], C.N. Honda[1,2], E.G. Jones[1,3]. [1]Neural Systems Laboratory, Frontier Research Program, RIKEN, Wako, 351-01 Japan. [2]University of Minnesota, Minneapolis, MN 55455. [3]University of California, Irvine, CA 92717.

Nine pyramidal neurons in infragranular layers of cat primary auditory cortex (AI) were intracellularly penetrated under physiological control and injected with biocytin. Cells were located in relation to the tonotopic map in the AI.

Layer V pyramids could be subdivided into two types on the basis of dendritic morphology and collateral distribution. Layer VI neurons were more heterogenous.

Cells, located at the border between layers IV and V, had a large cell body and a thick dendrite extending to layer I where it branched considerably. These cells had few or no recurrent collaterals and, unlike neurons in layer II and III, no terminal bushes were formed in the vicinity of the cell body. Several long horizontal collaterals emerged from the main axon and extended in all directions in the tangential plane. Along these horizontal collaterals, many boutons were distributed at short intervals.

Cells located in the deeper portion of layer V emitted no long horizontal collaterals but had a dense bush of recurrent collaterals above the cell body, extending into layers II and III. A thin dendrite reached layer I where it branched little.

Cells in layer VI had a thin ascending dendrite that branched more frequently than that of layer V neurons and terminated in layer III. Collateral axon distributions varied from cell to cell; some cells had both terminal bushes in the vicinity of the cell and horizontal collaterals and others formed only recurrent bushes distributing in layer III.

124.20

A RESONANCE MODEL OF MICROSECOND TIME SENSITIVITY IN NUCLEUS LAMINARIS OF THE BARN OWL. J.C. Pearson, C.D. Spence*. David Sarnoff Research Center, CN5300, Princeton, NJ 08543.

In the barn owl, the azimuth of sound source direction is largely determined by the interaural phase difference (IPD) spectrum. This binaural information is first represented in nucleus laminaris, whose neurons are tuned in frequency and IPD up to ~10kHz, with corresponding temporal sensitivity of ~10 μsec. They have low spontaneous rates that equal the out-of-phase binaural rate, and their in-phase binaural rate is twice the monaural rate. They receive ~50 synapses from each nucleus magnocellularis, whose frequency tuned neurons spontaneously fire at ~100sec^{-1}, and noisily phase-lock with little change in the average rate.

How can μsec sensitivity be achieved in the face of msec synaptic integration time constants and the noise in the inputs? We present simulations that demonstrate that standard neuronal biophysics cannot account for this phenomena because the noisy oscillating synaptic input produces a very small ripple in membrane potential that is swamped by random fluctuations.

Perhaps laminaris neurons contain some kind of neuronal resonator that, in effect, amplifies the oscillating synaptic potentials relative to the noise. This is suggested by the fact that the hair cells of some animals resonate, albeit at much lower frequencies than 10kHz. We present simulations that show that this idea is theoretically feasible. We determine the relationships between the "Q" of the resonator and the filtering characteristics of the membrane needed to jointly satisfy the conflicting constraints of signal amplification and short response latency.

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124.21

OLIVOCOCHLEAR NEURONS IN THE ADULT HAMSTER. D.D.Simmons[1,2], J.Raji-Kubba*[1], and J.K.Moore[2]. [1]Dept. of Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024 and [2]House Ear Institute, Los Angeles, CA 90057

The mammalian olivocochlear system is morphologically, spatially, and chemically heterogeneous. In order to study the development of olivocochlear (OC) efferent neurons in the hamster, the morphological and chemical identities of these neurons have to be established in the adult. The OC cell bodies were labeled retrogradely via biocytin injections. Such injections labeled cells bilaterally in periolivary (PO) regions but only ipsilaterally within the lateral superior olive (LSO). The cells labeled in PO regions had areas that ranged from 250-700 μm^2 and shapes that varied from fusiform to spherical. The majority (roughly 60%) of PO cells were found ipsilaterally and were intensely staining. Within the LSO, cells labeled tended to be smaller (200-400 μm^2), had generally spherical shapes and were stained lightly. In all species studied to date, both PO and LSO efferent neurons are ChAT positive but only the lateral OC system shows immunoreactivity to CGRP. In hamster, immunoreactivity to CGRP antibody was found mostly in LSO and only rarely in PO regions. The CGRP-positive cells in LSO had similar morphology and distribution as the retrogradely biocytin-labeled cells. In the cochlea, CGRP-positive terminals were found underneath inner hair cells from base to apex. The densest staining occurred toward the middle regions. These results suggest that the hamster is similar to other rodent species in the locations, morphology and chemistry of OC neurons. These data will be compared with other immunocytochemical results.

(Supported by the NSF [8719610], the Deafness Research Foundation and the Sloan Foundation)

124.22

IDENTIFICATION OF CELLS IN THE COCHLEAR AND DENTATE NUCLEI AFTER ELECTROPHYSIOLOGICAL RECORDING IN CONSCIOUS CAT. J. Landeira-Fernandez, C.D. Woody, X.F. Wang, V. Chizhevsky*, and E Gruen*. UCLA, Depts. Anatomy, Psychiatry, and Psychology, MRRC, BRI, Los Angeles, CA 90024.

Cells in the cochlear and dentate nuclei were marked by intracellular injection of biocytin made immediately after eletrophysiological recording in a conscious cat. Elongate, fusiform cells of 20 u length were found in the fusiform layer of the dorsal cochlear nucleus, and a non-fusiform cell of 10 u diameter resembling a granule cell was found in the molecular layer of the dorsal cochlear nucleus. The morphologies were in agreement with those described by Brawer et al (J. Comp. Neurol., 155, 251-300, 1974) and Rhode et al (J. Comp. Neurol., 213, 426-447, 1983). Most cells showed pauser responses to click stimuli. Among the cells identified in the dentate nucleus, using Chan-Palay's classification (Cerebellar Dentate Nucleus, Springer, 1977), were a large asymetrical cell of somewhat smaller size than that illustrated (fig. 3-15,E2) by Chan-Palay and a small multipolar cell of approximatly 10 u in diameter. Both were located in the ventromedial portion of the dentate nucleus. A small bridging cell with a perikaryon 10 u in width was also identified in the dorsomedial portion of the dentate nucleus. Preliminary results suggest that some of the cell types identified in the dentate nucleus may have a short latency auditory response. (Supported by NS 25510 and by a Brazil CNPq fellowship.)

125.1

LEARNING OF SENSORI-MOTOR SEQUENCES BY PREFRONTAL CORTICAL CIRCUITS: A MODEL

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Inst. Neurosc. Paris and URA CNRS 1121, Paris XI, Orsay, France.

We propose a neural network model of prefrontal (PF) circuits which can learn temporal sequences with 5 constraints:
1. *The training protocol is the same as that used in monkeys to learn a spatial delayed response task.* **2.** *Computation and learning are done by units which model local circuits of neurons in the cortex,* with two processing steps: (i) inputs of common origin are tuned through learning within specific layer-divisions of the unit, (ii) the mutual interactions between layers are also adjustable by learning. **3.** *The maps which model the PF cortex are part of a coherent architecture which includes visual and visuo-motor processing.* Properties of these cortical microcircuits account for a common adaptive mechanism to learn the different aspects of behavior such as sensori-motor coordinate transformations and invariant recognition. **4.** *The specific properties of processing units modelling PF circuits are compared to other cortical regions.* We suggest that PF neurons have two specific properties to process conditional sequences with greater efficacy: (i) a bistable state as a the substratum of long-lasting activities (working memory) and (ii) a three-step learning mechanism controlling this bistable state. **5.** *The temporal properties of units are related to those of ionic channels and synapses of PF neurons.* We discuss the specific activation rules in relation with the recent findings in PF neurons of a I_A-like potassium current (I_D). Its inactivation results in a steady firing (C.Hamon & F.Crepel, this volume). Furthermore, learning rules take into account the role of NMDA receptors in synaptic plasticity in PF neurons (J.Hirsch & F.Crepel, 1991, Exp.Brain.Res., in press).

125.2

FUNCTIONAL RESPONSES OF DORSOLATERAL PREFRONTAL NEURONS: AREAL DISTRIBUTION AND RELATION TO DELAYED ALTERNATION PERFORMANCE.

S. Carlson, H. Tanila*, I.Linnankoski*, H.Kahila*, and M. Grönroos*. Dept. Physiol., Univ. Helsinki, 00170 Helsinki, Finland.

In the present work we studied the responses of single prefrontal neurons to various visual, auditory, and somatosensory stimuli and correlated the neuronal firing to movements of the eyes, face and limbs of the monkey (Macaca arctoides) with the method described by J. Hyvärinen (Brain Res 1981:206:287-303). Several of the neurons were also studied while the monkey performed a spatial delayed alternation task. About 45% of the neurons could not be activated by any of the stimuli used. Approximately 30% responded to visual stimulation only and about 7% to visual and some other type of stimulation. Pure auditory (3%) and somatosensory (3%) responses were also found and about 13% of the neurons fired in relation to the movements of the eyes or limbs. The areal distribution of the functional responses is largely in line with anatomical studies of the connections of the prefrontal cortex.

125.3

SIMULTANEOUS RECORDING OF SINGLE UNITS IN THE FRONTAL CORTEX OF MONKEYS PERFORMING A DELAYED RESPONSE TASK

E. Vaadia, M. Abeles, A. Aertsen, H. Bergman, H. Cohen, I. Halman, Y. Prut and E. Shtoyerman Dept. of physiology, The Hebrew University - Hadassah Medical school, Jerusalem 91010, Israel.

In a previous study we reported that coherent activity of pairs of neurons in the frontal cortex may be related to behavioral events. This study was designed to further test the hypothesis that the neural code for higher brain function resides in correlated activation of selected neurons.

Two monkeys were trained to perform arm reaching task with two paradigms; The monkey touched a central key to switch on a central light. After a delay of 3-6s ("*pre-cue*" period), one of two target keys was illuminated for 200ms. Then, after 1-32s ("*delay*" period) the central light dimmed ("*Go signal*"). In the first paradigm ("GO"), the monkey had to release the central key within 600 ms and touch the target key. In the "NO-GO" paradigm the monkey had to maintain touch for 1200ms after the GO signal. The activity of up to 16 neurons was recorded simultaneously by an array of 6-10 electrodes arranged in a circle of 1000μ diameter. Raster-displays, time interval histograms, auto-correlograms, cross-correlograms and joint-PST histograms were computed separately for the "*pre-cue*" and the "*delay*" periods in each of the behavioral paradigms. The raster displays indicated that in each recording site neurons firing rate is modulated in relation to several behavioral events while others are "indifferent". Preliminary crosscorrelation analysis demonstrated that spike trains of some of the neurons may be uncorrelated during performance of one behavioral paradigm and become correlated during performance of the other paradigm.

125.4

SIMULTANEOUSLY RECORDED ACTIVITY IN MOTOR AND PREMOTOR CORTICES OF MONKEY DURING ARM-MOVEMENT SEQUENCES.

JK Marcario, RE Kettner and MC Clark, Dept. Psych., Prog. Neural Science, Indiana University, Bloomington, IN 47405.

Pairs of simultaneously recorded single-units were recorded from the motor and premotor cortices of two rhesus monkeys during a movement-sequence delay task described in the companion abstract. Individual neurons were isolated using spike sorting algorithms on stored waveforms. Neural activity was categorized according to the average amount of activity during each of four task periods, yielding 15 possible classifications: initiation (I), sensory (S), delay (D) and motor (M) responses, plus all combinations of the four primary categories (i.e. IM, SD, ISD, etc.). Three main groups were observed: units that fired during arm movements (the I and M periods), units that fired during the periods between arm movements (the S and D periods), and units that responded both during and between arm movements. 142 neurons were recorded simultaneously from the same electrode tip in 50 pairs and 14 triples. Each triple was considered to consist of 3 pairs of units, thus yielding a total of 92 pairs of simultaneously recorded units analyzed. 27% of unit pairs were considered *equal* because both units of the pair belonged to the same category (e.g. M-M), 26% were considered *similar* because both units of the pair belonged to the same group (e.g. IM-M), 20% were considered *complementary* because one unit belonged to the arm-movement group and the other to the between-arm-movement group (e.g. IM-SD), and the remaining unit pairs (27%) were deemed *mixed*. These results suggest a variey of responses among pairs and triples of simultaneously recorded units that may reflect a diversity in local processing in the motor and premotor cortices, including motor preparation and sensory-motor integration. Examination of single-trial histograms further supports this view. (Supported by NSF grant BNS-8919867)

125.5

POPULATION CODING OF MOVEMENT DIRECTION IN PRECENTRAL CORTEX DURING A MOVEMENT-SEQUENCE DELAY TASK

RE Kettner, JK Marcario and MC Clark, Dept. Psych., Prog. Neural Science, Indiana University, Bloomington, IN 47405.

Past work indicates that many single-neuron responses in precentral cortex are correlated with the direction of movement. Even so, individual neurons are only broadly tuned to a particular direction of movement called the neuron's preferred direction, and cannot account for the precisely directed movements that are observed. To explain movement accuracy one must assume that directional information is stored in a distributed fashion over a population of broadly tuned neurons. This view has been demonstrated using a population code that sums vector contributions from large populations of neurons; each neuron's contribution is in its preferred direction with a magnitude related to its change in firing rate from baseline. This population code works well in describing both single-direction and tracking movements.

The current experiments show that the code also works for a rapid sequence of directed movements. Two rhesus monkeys were trained in a movement-sequence delay task that was divided into 2 sensory, 2 delay, and 3 movement periods. Analyses of 328 neurons indicate neurons with statistically significant tuning during each period (18, 14, 19, 26, 47, 46, 45%). Population analyses based upon these tuned neurons indicate that there is accurate directional information during all 7 periods. The average angular difference between observed and predicted movement direction was 6, 22, 9, 7, 7, 10, and 9 degrees. Preferred directions was generally similar when more than one movement period was tuned. Population analyses based upon the average preferred direction during the three preferred directions also predicted movement direction accurately. The average error was 18, 20, 26 degrees for the movement periods. (Supported by NSF grant BNS-8919867)

125.6

COMPARISON OF NEURONAL RESPONSES IN THE PRECENTRAL CORTEX WITH EMG ACTIVITY DURING AN ARM-MOVEMENT SEQUENCE DELAY TASK.

M.C.Clark, J.K.Marcario, and R.E.Kettner, Prog. in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

EMGs were collected from arm and trunk muscles of rhesus monkeys and human subjects performing a movement-sequence delay task. Each trial was initiated by pressing a center button when a cue light was illuminated. Two out of four target buttons were illuminated during a sensory period while the center button remained depressed. After a 2.5-3.5 second random delay period, the subject pressed the "remembered" target buttons in the appropriate sequence for a reward. The task allows the separation of initiation, sensory, delay, and movement responses. We have compared the EMG data with the neuronal activity recorded from the motor and premotor cortices of rhesus monkeys. Neuronal single-unit activity was classified into different categories based upon statistically significant levels of firing activity. We have found basically two groups of neuronal responses: those that fire during movements and those that fire during the sensory-delay period. EMG patterns were found to be similar to a subset of neuronal responses in the precentral cortex. Cortical responses were more varied. EMG activity was observed primarily during movement periods and did not show sensory-delay period activity without accompanying movement period activity. Thus, the precentral neurons active only during the sensory-delay period, had no counterpart in the EMG responses. This suggests that the sensory-delay period responses represent processing in the precentral areas that is not directly involved in muscle control, but may instead indicate other processes including sensory-motor integration and motor preparation. (Supported by NSF grant BNS-8919867)

125.7

PREPARATORY NEURONAL ACTIVITY IN PREMOTOR CORTEX DURING AN INSTRUCTED-DELAY PERIOD: RELATION TO CONTRA- AND IPSILATERAL ARM MOVEMENTS. D.J. Crammond and J.F. Kalaska. CRSN, Dépt. de physiologie, Université de Montréal, Montréal, Canada, H3C 3J7.

Experiments using Instructed-Delay (ID) tasks have implicated neuronal activity recorded in the premotor cortex (PM) in the preparation of contralateral arm movement. This DELAY period activity may be encoding either details of the impending movement such as joint motion or muscle activity, or spatial attributes of the movement such as its trajectory and target location. In an attempt to distinguish among these possibilities, we studied PM discharge while a monkey made movements with identical trajectories in an ID task, with contralateral (CL) and ipsilateral (IL) arms. A rhesus monkey made movements from a central starting position in 8 directions to targets when a red LED was illuminated (GO, CONTROL task). In the ID task a green LED (CUE) was illuminated at the target in the second half of the centre hold period after which the target GO signal was illuminated. Results were obtained from 22 PM neurones: 19/22 (86%) cells discharged after the CUE in the ID task when using the CL arm, which in 18/19 cells was tuned and centred on a preferred direction (PD). Of these 18 DELAY cells, 17 (94%) cells were also active in the DELAY period when the IL arm was used. Three measures of neuronal activity during the DELAY period did not vary significantly between the IL or CL arm. These were: PDs (mean difference 1.4^{o} per cell), level of cell discharge (26.1 & 24.7 imp/sec), and latencies after CUE onset (165.6 & 169.1 msec). Likewise, only small variations in cell PDs (1.4^{o} per cell), discharge levels (26.8 & 26.9 imp/sec), and onset latencies (146.7 & 148.3) were recorded between CL and IL arms after the GO signal in the CONTROL task. In comparison, motor cortex (MC) cells rarely showed preparatory activity in the ID task. They were strongly active after the GO signal in the CONTROL task using the CL arm and weakly active with IL arm movements, and the PD of this activity differed widely with the two arms. Thus PM activity seems related to more general aspects of the movement, such as its spatial trajectory or endpoint, while MC activity is more specifically related to the details of the response. Supported by MRC Group Grant (JFK) and FRSQ (DJC).

125.8

MOTOR CORTICAL CELL ACTIVITY IN A MEMORIZED DELAY TASK. N. Smyrnis*, J. Ashe, M. Taira*, J.T. Lurito, and A.P. Georgopoulos. Bard Labs., Department of Neuroscience, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

We trained a monkey to move a handle from the center towards lights on a planar working surface. The information concerning the direction of the movement was provided by the peripheral light, and the triggering of the movement by turning off the center light. We recorded the activity of 121 cells in the arm area of the motor cortex during 3 main tasks: In the 1st task, the center light was turned off at the same time as the peripheral light was turned on. In the 2nd task, the peripheral light was turned on for 300 ms, after which it was turned off. This marked the beginning of the memorized waiting period (450-950 ms) during which there was no peripheral light. Then the center light was turned off to trigger the movement in the memorized direction. In the 3rd task, the peripheral light came on for 300 ms, as before, but remained on throught the trial. We found that 52/121 (43%) cells changed activity during the memorized waiting period (2nd task). Moreover, 27/52 (52%) cells changed activity in the memorized (2nd) task, but not in the simply delayed (3rd) task. Finally, the neuronal population vector calculated every 20 ms pointed in the memorized direction (2nd task). These results indicate that the motor cortex is involved in processing information concerning memorized movement directions. Moreover, this processing can be visualized using the neuronal population vector analysis. (Supported by NIH, ONR, HSFP.)

125.9

MOTOR CORTICAL CELL ACTIVITY IN A VISUALLY GUIDED ISOMETRIC FORCE PULSE TASK.

M. Taira*, J. Ashe, N. Smyrnis*, and A.P. Georgopoulos. Dept. of Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD 21205.

We trained a monkey to produce force pulses (Fs) on a 2-D isometric handle (Massey et al., Exp. Brain Res. 83: 446, 1991) in a direction (Fv) indicated by a visual target, both in the absence of force bias and under 8 static force bias conditions (Fb) of different directions. A force feedback cursor indicated on the display the net force (Fn = Fs + Fb) exerted on the handle: The task was to exert such Fs so that Fn = Fv. In the absence of force bias (Fb = 0), Fs was in the direction of Fv; however, in the presence of force bias, as the force exerted increased, Fs changed continuously in direction and magnitude, so that at any moment Fn = Fs + Fb = Fv. Thus in these cases the net force, Fn, was dissociated from the active force, Fs, exerted by the animal. We wanted to find out whether the neuronal population vector calculated in time would reflect the direction of Fs or that of Fn. For that purpose we recorded the activity of 132 cells in the arm area of the motor cortex during performance of the task. The activity of 57% cells during the reaction time was directionally tuned. The population vector, calculated every 10 ms following the onset of the target, pointed in the direction of the net force, Fn (which coincided with Fv) and not in the direction of Fs. This result suggests that when a dynamic force pulse is initiated, the motor cortex might be involved with the specification of the visually-defined net force rather than with the actual total force output. (Supported by NIH, ONR, UCP.)

CORTEX II

126.1

BEHAVIORAL INVARIANTS ENCODED IN MOTOR CORTICAL ACTIVTIY. A.B. Schwartz, A. Kakavand* and J.L. Adams. Div. of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013

We examined two behavioral correlates associated with the production of drawn figures. The speed of drawing is inversely correlated to the 2/3 power of the curvature of the drawn figure. This exponent is consistently found in human adults but varies greatly in young children. These movements are produced in segments, demarcated by points in the trajectory where angular acceleration is zero.

Having developed an algorithm leading to a neural representation in the motor cortex of movement trajectory, we asked the question: Are the behavioral features characteristic of the movement present in the activity of motor cortical cells? Monkeys drew spirals and figure eights on a touchscreen monitor as unitary activity was recorded from motor cortex. The directionally-sensitive unitary responses from different cells were added vectorially to form a time series of population vectors throughout the movement. Population vectors were added tip-to-tail, producing neural images of the trajectory. These images resembled closely the figures drawn by the monkeys. The movement trajectory and the neural image both obeyed the 2/3 power law. Also very similar were the number of segments and the duration of each segment in both the movement and the neural representation of the movement. This strongly suggests that the behavioral features, to which these drawing movements conform, are present in the activity of motor cortical cells. (NIH 26375)

126.2

DIRECTION CONTROL IN HUMANS WITH MOTOR CORTEX LESION. J.W. Martin*, J.P Donoghue and J. N. Sanes Center for Neural Science and Laboratory of Motor Control, Brown University, Providence, RI 02912.

One current hypothesis about the function of the primary motor cortex (MI) is that it controls movement direction. In support of this notion is the finding that MI neurons have discharge patterns that are tuned to the direction of voluntary movement. A corollary of this hypothesis might be that humans with lesion of MI would show deficits in direction control of voluntary movement. Previously, we showed (Kerman et al. 1990) that an MI lesion did not dramatically affect directional errors for straight line or parabolic shaped movements performed at a subjects' chosen speed. However, the chosen speed of MI lesion subjects was significantly slower than that of normal subjects. It is possible that movement direction control of MI lesion subjects is disrupted when they perform movements at speeds equal to those obtained by normal subjects.

Subjects with CT or MR defined focal lesion involving MI and normal controls performed planar reaching via-point movements for which normal subjects commonly employ parabolic paths; these paths by their nature have continuously changing direction. The movements were instructed to be done as fast as possible or at one's own speed. Path curvature and movement time of the first half of each of 30 movements at each speed were analyzed, with curvature measuring the ability to reproduce the presumed ideal path. For the movements performed at their own speed, MI subjects had movements that were slightly straighter, but were significantly slower (1 sec vs 0.5 sec) than normal. When the MI subjects performed movements in about the same time as normal, the curvature of their movements approximately that of normal subjects. Therefore, from these data, we would reject the hypothesis that MI lesion does not disrupt direction control of parabolic reaching movements performed rapidly. This hypothesis rejection casts doubt on the role of MI in participating in overall movement direction control. Nevertheless, MI may participate in other important aspects of preparation and execution of movement direction. Supported by NIH NS 25074, 22517, March Of Dimes 5-562, Culpeper and Whitehall Foundations.

126.3

PARIETAL CORTEX ACTIVATION DURING VISUALLY GUIDED REACHING MOVEMENTS IN HUMANS MEASURED WITH PET. C. Kertzman, U. Schwarz, T. Zeffiro, and M. Hallett. Human Motor Control Section, MNB, NINDS and LSR, NEI, NIH, Bethesda, MD 20892.

Lesions of the posterior parietal cortex (PPC) in humans can result in difficulty in visually guided reaching within the proximal extrapersonal space. However, it is not clear to what extent this reflects a disruption in the process of computing information about current hand position and its intended movement in space, compared to spatial information concerning target location. To test the hypothesis that parietal cortex is involved in the motor component of reaching, we measured regional changes in cerebral blood flow (rCBF) in six control subjects using PET under 6 conditions: fixation with visual targets presented in either the right or left visual field and during reaching with the left or the right hand in either visual field. Subjects maintained central fixation while reaching, and visual targets were presented randomly on a screen within arm's reach. The subject's blood flow data was registered in 3D with corresponding brain MRI data. PET images obtained from corresponding fixation and reaching conditions were subtracted, and regions of interest were examined using a ART2 type neural network that clustered local maxima. A center of mass was determined for each cluster, and its location was projected back onto the corresponding MR image.

Bilateral activation was found in the superior and inferior parietal lobules in all subjects, particularly along the intraparietal sulcus and in precuneus. Some subjects showed asymmetric enhancement of the hemisphere contralateral to the arm used and not contralateral to the visual field in which the targets appeared; changing the arm changed the side of enhanced activity. Additional regions of focal rCBF were found in frontal cortex, cingulate gyrus, and occipital cortex.

These results confirm the role of PPC in the guidance of hand movements toward visual targets and suggest that its mapping of the target is more in limb coordinates than in field coordinates.

126.4

FIRING PATTERNS AND PROPERTIES OF NEURONES IN PRIMATE TONGUE MOTOR CORTEX (MI) IN RELATION TO SWALLOWING. R.E. Martin*, G.M. Murray and B.J. Sessle, Faculty of Dentistry, Univ. of Toronto, and *The Toronto Hospital, Canada M5G 1G6.

Our recent studies have shown that some neurones in tongue MI of awake monkeys alter their firing rates during swallowing. The aim of this study was to determine the swallow-related firing patterns and mechanoreceptive field properties of these neurones. Extracellular single neurone recordings were made from tongue MI (defined by intracortical microstimulation; $\leq 20\ \mu A$) while the awake monkey (M. fascicularis) swallowed a fruit juice reward associated with successful performance of a tongue protrusion task. EMG activity from the genioglossus muscle was simultaneously recorded and swallowing was defined by a characteristic EMG activity pattern. For each of 37 tongue MI neurones examined, firing rates during swallowing and the 50ms period immediately preceding the swallow, were compared statistically with those during a control period (ANOVA and post hoc comparisons). Swallow-related activity patterns occurred in 28 (76%) of the neurones; of these, 36% (10/28) showed a significant ($p<.05$) alteration of firing rate only during the 50ms period preceding the swallowing, 25% (7/28) only during the swallow, and 39% (11/28) exhibited altered firing during both the swallow and the preceding 50ms period. In addition, 54% (15/28) had a mechanoreceptive field on the superior tongue surface. These findings suggest that tongue MI may be involved in the initiation and/or regulation of oral movements associated with swallowing. Supported by Canadian MRC.

126.5

DISCHARGE PROPERTIES OF NEURONES WITHIN PRIMATE FACE SOMATOSENSORY CORTEX SI DURING TRAINED MOTOR TASKS. L.-D. Lin, G.M. Murray, and B.J. Sessle, Fac. of Dentistry, Univ. of Toronto, Toronto, Canada, M5G 1G6

Our recent studies in awake monkeys have shown that most tongue SI neurones alter their activity during a trained tongue-protrusion task but not during a trained biting task. The aim of this study was to determine if neurones in face SI also alter their activity in these two trained orofacial movements and if they do so in a selective manner. Extracellular single unit recordings were made in two awake monkeys (M. fascicularis) trained to perform the tongue-protrusion and biting tasks. A total of 71 face SI neurones with a mechanoreceptive field localized to the hairy upper or lower lip were recorded and 17 of them analyzed in both tasks. The activity of these neurones was related to both tasks (30%, 5/17), to the tongue task only (35%, 6/17), or unrelated to both tasks (35%, 6/17). The probability was significantly greater (Sign test, $p<0.05$) that a neurone was related to the tongue task than to the biting task. In addition, 8 of the 11 tongue task-related neurones showed an increase and 3 a decrease in activity during the tongue task whereas 4 of the 5 biting task-related neurones showed an increase and 1 a decrease in activity during the biting task. These data suggest that some neurones in face SI as well as in tongue SI may selectively alter their activity in relation to different trained orofacial tasks and that these activity patterns may be utilized in the sensorimotor integration of orofacial movements. Supported by Canadian MRC.

126.6

INACTIVATION OF SENSORY CORTICO-CORTICAL INPUT AFFECTS NEURONAL ACTIVITY IN CAT MOTOR CORTEX. R. Izraeli and L.L. Porter. Dept. of Anatomy, USUHS, Bethesda, MD 20814.

The effects of input from a functionally distinct region of somatosensory cortex (area 2), on the ipsilateral motor cortex (area 4γ) were studied. Extracellular single and multi-unit activity was simultaneously recorded from area 2 and area 4γ of anesthetized cats. Cells with matching or overlapping receptive fields (RFs) were isolated in both cortices. Subcutaneous or deep stimuli which evoked cell responses in both sites, were delivered to the RF area. Evoked potentials (EPs) and cell activity were monitored before and after injection of lidocaine in area 2. Lidocaine reversibly abolished cell activity and cell responses at the injection site, which in turn affected the EPs recorded in area 4γ. In several sites a reversal in polarity from positive-negative to negative-positive was noted. In other sites the EP amplitude was affected. The positive component usually decreased, while the negative phase increased at some sites, and decreased in others. Our results indicate that input from area 2 affects motor cortex activity, but further studies are needed to determine how it interacts with other sources of information.

126.7

GATING OF CUTANEOUS INPUT TO SELECTED MOTOR CORTEX NEURONS DURING PREHENSION. N. Picard, G. Cadoret* and A.M. Smith, C.R.S.N., Université de Montréal, Québec, Canada H3C 3J7.

Three *M. fascicularis* monkeys were trained to grasp, lift and hold within a position window an object of varying weight and texture. The monkeys scaled the grip and lifting forces appropriately for the object weight and texture. Single units with cutaneous receptive fields were recorded from the hand area of the motor cortex where low-threshold (< 30 μA) intracortical microstimulation (ICMS) elicited discrete digit movements. The neural activity was recorded in blocks of trials in which different combinations of object weight and texture were displaced. In an other condition, a force-pulse perturbation was delivered to the object during holding to produce a slip force on the fingers. Nearly two-thirds (67/128) of cutaneous cells were sensitive to object texture. The contribution of cutaneous afferents to the cell discharge was suggested by the a failure to find a correlation with grip force, by the responses to object slips induced by the perturbation and by the increased discharge frequency of certain neurons with rougher textures. A small number (11) of cells with cutaneous receptive fields on the glabrous skin of the digits were not active during the task despite the fact that their receptive fields were directly stimulated by the pinch. In all cases, ICMS delivered at these recording sites evoked contraction of muscles which, although active during grasping, had actions which would reduce grip force. Examples include extensor pollicis longus, abductor pollicis and extensor digitorum communis. Some of these cells were identified pyramidal tract neurons (PTN). Cutaneous afferents to the motor cortex provide information about texture and slip of surfaces in contact with the skin. In addition, these afferents appear to be selectively prevented from exciting neurons related to actions opposed to the goal of the task.

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126.8

EFFERENT NEURONS AND SUSPECTED INTERNEURONS IN MOTOR CORTEX OF THE AWAKE RABBIT. H. A. Swadlow. Dept. of Psychology, University of Connecticut, Storrs, CT 06268.

Efferent neurons and suspected interneurons (SINs) were studied in rabbit motor cortex. Microstimulation of this region yielded low-threshold movements of the rostral vibrissae, sinus hairs or philtrum. Axonal properties and sensory receptive fields were examined in four efferent populations: callosal (CC) neurons, neurons projecting to ipsilateral S-1 (C-IC), and descending corticofugal neurons of layer 5 (CF-5) and layer 6 (CF-6). Suspected interneurons (SINs) responded with a hi-frequency (> 600 hz) burst of spikes to stimulation of ventrolateral thalamus.

Receptive field position within this area corresponded roughly to the position of twitches produced by microstimulation, and receptive field sizes were much larger than seen in S-1. SINs had the largest receptive fields and the highest spontaneous firing rates of any population. CF-5 neurons had high axonal conduction velocities (median value ~ 12 m/s), intermediate spontaneous firing rates (median = 3.5 spikes/sec), and had the largest receptive fields of any efferent population. In contrast, CC, C-IC and CF-6 populations each had low axonal conduction velocities (medians all < 2 m/s), low spontaneous firing rates (medians all < 0.5/sec), and had smaller receptive fields . Whereas nearly all SINs and CF-5 neurons responded to peripheral sensory stimulation, many CC, C-IC and CF-6 neurons did not.

These differences among efferent neurons and SINs of motor cortex mirror similar differences among corresponding populations studied in S-1, S-2 and V-1 (Swadlow, H. A., J. Neurophysiol., 59: 1162-1187, 1988; 62: 288-308; 63: 1477-1498; in press, 1991). These data suggest that a common physiological plan underlies the operations of functionally and morphologically diverse neocortical regions and that efferent analysis may be pivotal to understanding this plan.

126.9

Functional consequences of branching in primate cortico-motoneuronal (CM) cells. K.M. Bennett* and R.N. Lemon, Anatomy Dept., Cambridge University, England, CB2 3DY.

Primate CM cells branch to produce monosynaptic facilitation of several hand muscles. As this system is important for fractionated finger movement we examined CM cell activity and its functional output during precision grip. Two M.nemestrina monkeys performed a low force (0.5-1.5N) precision grip. Levers moved by the index finger and thumb were then held within a target zone for 1-2s. PTNs were used for the spike-triggered averages of up to 8 forelimb muscles. Post-spike facilitation (PSF) revealed a CM connection. 16 CM cells, with PSF of 2 target muscles, were analysed. The muscles cocontracted during the hold periods. During movement they contracted independently of each other. With low discharge, each CM cell produced PSF of both muscles during the hold phase. Maximum discharge was seen with the independent activity of ONE muscle. PSF of this muscle was enhanced in this period. 8 cells produced no PSF of one muscle when it was independently active.

Thus each CM cell exerts PSF of its target muscles both when they are cocontracted and during periods of EMG fractionation. Modulation in the relative activity of target muscles is associated with clear changes in the firing pattern and facilitatory influence of the CM cell. This contributes to the fractionation of muscle activity during independent finger movement.

126.10

MOTOR CORTICAL ACTIVITY DURING VOLUNTARY GAIT MODIFICATIONS MAY SPECIFICALLY ENCODE MUSCLE ACTIVITY AT A SINGLE JOINT. T. Drew. Dept. Physiologie, Université de Montréal, Québec, Canada, H3C 3J7.

The discharge patterns of 91 identified pyramidal tract neurones (PTNs) in the forelimb region of area 4 were recorded in a task in which unrestrained, chronically implanted cats were required to make a co-ordinated modification of their gait in order to step over moving obstacles attached to a treadmill belt. The results showed that 52% of these cells (48/91) increased their discharge rate by greater than 20% during the swing phase of the contralateral limb when the cat stepped over the obstacle. An analysis of the discharge patterns of the task-related cells over different kinds of obstacles showed that some cells were temporally related to the onset and offset of activity in specific muscles rather than discharging throughout the movement. 22 cells discharged during early and mid-swing and appeared to be principally correlated with the periods of activity in either elbow flexors or shoulder protractors, while a further 12 cells increased their discharge frequency only in late swing and were better correlated to the changes in wrist dorsiflexor muscle activity required to control the placement of the foot. These results suggest that some PTNs specifically code parameters of muscle activity around single joints in these multi-articulate movements, despite the widespread spinal branching of PTNs, and despite the fact that microstimulation through the recording electrode during locomotion frequently evoked transient increases in the activity level of muscles acting around different joints. (Supported by the MRC and FRSQ).

126.11

SYNCHRONIZED 25-35 HZ OSCILLATIONS IN SENSORIMOTOR CORTEX OF AWAKE MONKEYS. V. N. Murthy and E. E. Fetz. Department of Physiology & Biophysics and Regional Primate Research Center, University of Washington, Seattle, WA 98195.

Local field potentials and activity of some units recorded in sensorimotor cortex of awake rhesus monkeys showed transient synchronous oscillations between 25 and 35 Hz. These oscillations occurred more frequently during performance of tasks involving fine finger control (eg., retrieving raisins from a Klüver board) than during repetitive alternating wrist movements. During the former tasks, oscillatory spindles typically consisted of 5-15 cycles and recurred once or twice per second. More sustained oscillations could also be evoked by cutaneous stimulation of the arm. In the motor cortex the spindles did not appear in any regular relation to the EMG activity of forelimb muscles, but cycle-triggered averages of EMG sometimes revealed synchronous effects in both flexor and extensor muscles. The polarity of the oscillations reversed at a cortical depth between 250 and 500 μm, suggesting an intracortical source of these fields. Simultaneous recording from cortical sites separated by up to 10 mm in the anterior-posterior direction [estimated 20 mm tangential intracortical distance] or up to 8 mm mediolaterally revealed that oscillations could occur coherently over these distances. The spindles occurred most robustly and extensively in the precentral gyrus; these appeared to be intermittently phase-locked with more localized spindles in the postcentral gyrus. During the oscillatory episodes, single units often discharged preferentially during a particular phase of the oscillations and could become synchronized with other units separated by estimated distances up to 15 mm. Thus, neurons in the pre- and post central cortex can be transiently recruited into coherent activity during the oscillatory events. [Supported by NIH: NS12542 & RR00166.]

126.12

INTRACELLULAR CORRELATES OF OSCILLATORY ACTIVITY OF CORTICAL NEURONS IN AWAKE BEHAVING MONKEYS D.-F. Chen & E.E. Fetz. Regnl. Primate Res. Ctr. & Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195

Synaptic interactions between pairs of neurons in sensorimotor cortex were documented by spike-triggered averages (STAs) of membrane potentials (MPs) in awake monkeys performing a step-tracking task. Intracellular (IC) recordings were obtained with K-methylsulfate electrodes, while extracellular spikes from neighboring "EC" cells were recorded with carbon-fiber electrodes. To analyze the interspike membrane potential trajectories, we also compiled averages of the MPs for various fixed intervals between pairs of IC action potentials (APs). The 14 IC cells analyzed to date all had stable resting MPs of at least -50 mV, overshooting APs and a wide range of firing rates during rest or task performance. These neurons can be divided into three groups according to their AP width and interspike MP trajectories. Cells of one group (n = 4) had narrow spikes (0.5 ms) with a fast initial repolarization, which suddenly changed to a slower repolarization lasting 4 - 9 ms, followed by a 20 - 25 ms depolarizing rebound to the resting level or to a subsequent AP. These four cells had a strong tendency to fire around 25 - 35 Hz. The other two groups showed no significant rebound trajectories and did not fire preferentially around 30 Hz. In three of the four "30 Hz" IC cells, STAs of MPs revealed subthreshold oscillatory potentials at 25 - 40 Hz, due to common synaptic input to the EC and IC cells. This evidence suggests that some cortical neurons have a distinctive membrane potential trajectory that may play a "pacemaking" role in making them fire preferentially at about 30 Hz. In addition, subthreshold oscillatory common input potentials suggest synaptic interconnections between these cells that would further contribute to this tendency. [Supported by NIH grants NS12542 & RR00166]

126.13

LATERALIZATION OF GAMMA BAND EEG ACTIVITY DURING ATTENTION-DEMANDING TASKS IN 23 MEDICAL STUDENTS. M.B.Levin*, and M.L.Reite. Colo. Psych. Hosp.,Univ. of Colo. Hlth. Sci. Cntr.,Denver, CO 80220.

High frequency (30-80 Hz) electroencephalographic (EEG) activity has been attributed to several aspects of brain intercommunication, from the mediator of attention to the means by which areas of cortex communicate with one another and with other areas of the brain. This study examined the power in the 36-44 Hz EEG band (referred to here as 40 Hz activity) over areas of cortex in normals during attention demanding tasks. Twenty-three right handed medical students, 11 females and 12 males, were recruited for this study. Six electrodes, referenced to linked ears, were placed on the scalp; over left and right frontal, central, and parieto-occipito-temporal cortex. EEG data was obtained during a visual-motor task such that it represented the subject's being in a "state of readiness" to push a button. The data was adjusted for muscle activity via a mathematical technique which treated activity in the 36-44 Hz and 62-78 Hz bands as amplitude modulated signals with 40 Hz and 70 Hz carrier waves respectively. The power in the 40 Hz band due to brain independent of muscle was calculated as the difference between the 40 Hz and 70 Hz "demodulated" EEG data averaged over time. Normalized laterality values of 40 Hz activity were determined over time for the frontal, central, and parietal areas. Statistical analysis demonstrated significantly increased left, over right, central 40 Hz power just prior to motor response. There were no significantly different normalized laterality values for the parietal or frontal regions. Furthermore, a figure rotation task for which no motor response was required demonstrated no significant changes in laterality value. This lateralization of 40 Hz power over the central cortex may represent a component of the event-related desynchronization described as a decrease in alpha activity over left central cortex during right hand voluntary movements.

126.14

LOCALIZED OSCILLATORY ACTIVITY IN THE SENSORIMOTOR CORTEX IN MOTIONLESS PERIODS OF A REPETITIVE MOTOR TASK

F. Lado, U. Ribary, L. Lopez, R. Jagow, A. Mogilner, R. Llinás. Center for Neuromagnetism, Dept. of Physiology and Biophysics, New York University Medical Center, New York, NY 10016, USA.

Using a 14 channel MEG system (BTi), the magnetic field pattern produced by neural activity was recorded while human subjects performed a simple movement once every few seconds. Several trials were combined to yield measurements from 30 - 35 scalp locations. Analysis of the data demonstrated that there is a consistent increase in average signal power at higher frequencies (>10 Hz) during pre- and post-motor periods, relative to the average power observed at the time of movement onset. The averaged movement evoked magnetic field revealed no fast activity. The topographic distribution of signal power over the scalp was consistent with that of a current source located in the sensorimotor region. If the assumption is made that a single source generated the signal, it is then possible to compute the average phase relationship between all scalp locations by using cross correlational techniques. Given these assumptions, it could be shown that the two maxima evident in the topographic maps of field power corresponded to the positive and negative field extrema of a localized source. Recent experiments using a 37 channel MEG system (BTi) have permitted simultaneous recording of a large area of scalp, over the motor region. Analysis of this data revealed well organized but complex relationships between signals recorded from different locations. These complex relationships force a reevaluation of the traditional single dipole source as a model for understanding cortical activity and suggest new interpretations for focal activity in the cortex based on the analysis of phase. From these findings it is clear that well localized and task related neuronal activity occurs that cannot be observed in averaged evoked recordings. Moreover, the richness of the phase relationship between signals recorded from different scalp locations calls for an equally rich model of cortical activity.

126.15

LAYER V CONTAINS A SUBSTRATE FOR REORGANIZATION OF MOTOR CORTEX MAPS. K.M. Jacobs, B.W. Connors and J.P. Donoghue Center for Neural Science, Brown University, Providence, RI 02912

Blocking inhibition via local cortical application of bicuculline (bic) leads to reorganization of motor cortex (MI) similar to that seen after nerve lesion or changes in sensory feedback (Jacobs and Donoghue, 1991). These results suggest that the cortex contains a substrate for MI reorganization. We hypothesize that MI map changes are due to alterations in horizontal connections between adjacent motor cortex representations. In order to test this hypothesis and identify the cortical architecture which could contribute to reorganization, we recorded field and intracellular potentials from in vitro coronal slices of rat MI. Field potentials were recorded at regularly spaced intervals through the depth of cortex, while stimulating electrically in layer V, 500 µm medial to the recording sites. The largest amplitude potential was a negativity recorded in layer V. Using current-source-density analysis this was identified as the site of the largest current sink, suggesting that a strong horizontal pathway exists within layer V. Next, the recording electrode was moved laterally in layer V away from the stimulation site in steps of 250 µm . Area, peak amplitude, and slope of the field negativity all decayed with distance. At distances greater than 1.0 mm, the field response was nearly absent. When bic was applied locally within 100 µm of the layer V recording site (which was 1.0 to 1.5 mm lateral to the stimulation site), the field negativity increased, revealing a connection between horizontally-distant layer V sites which is normally suppressed by inhibition. Intracellular recordings showed that epsps could be evoked by stimulation in layer V, 1.0 mm away from the recorded cell. The stimulus-evoked epsp amplitude was enhanced and firing elicited after local application of bic within 100 µm of the recorded cell. These results demonstrate that strong excitatory connections exist between horizontally-distant sites in layer V and that these connections are normally masked by inhibition. Therefore, layer V appears to contain a substrate sufficient to reorganize motor cortex representations. Supported by NIH NS 22517, 25074 and March of Dimes 5-562.

126.16

PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES OF EXCITATORY SYNAPTIC POTENTIALS FROM LAYER II-III TO LAYER V CELLS OF THE CAT MOTOR CORTEX IN VITRO. C. Capaday and E.E Fetz. Department of Physiology & Biophysics, University of Washington, Seattle, WA, 98195.

Intracellular data was obtained from 60 cells in Layer V of neocortical slices from area 4-gamma of the cat. To date the results from 14 cells have been analyzed. Layer II-III was electrically stimulated by 100 µs square pulses at 1.5 to 2 times threshold for eliciting an EPSP in layer V cells. These EPSPs were completely eliminated by perfusion of solution containing 5 mM Kynurenate, which blocks all 3 types of excitatory amino acid receptors. The EPSP amplitude of most (n= 11) layer V cells was independent of the membrane potential over the range from -65 mV to -95 mV. Perfusion of Mg^{++} free solution increased the amplitude and duration of the EPSPs 1.5 to 2.5 times. In the Mg^{++} free solution the amplitude of the EPSPs increased with hyperpolarization, in marked contrast to their behaviour in normal solution. This observation implies that NMDA-receptors normally contribute to the excitatory synaptic current of these cells. Furthermore, when APV (a specific NMDA-receptor blocker) was added to the Mg^{++} free solution the enhanced EPSPs were reversibly reduced. These results may be relevant to mechanisms of motor learning and provide a basis for the observation of LTP in pyramidal tract neurons.

126.17

CONTRIBUTION OF APICAL DENDRITES TO SOMATIC MEMBRANE PROPERTIES OF LAYER V PYRAMIDAL CELLS IN NEOCORTEX. A.E. Telfeian, L.J. Cauller and B.W. Connors. Section of Neurobiology, Brown University, Providence, RI 02912.

A unique but enigmatic feature of large pyramidal cells is the apical dendrite. To explore the role of apical dendrites we recorded and stained (with biocytin) pyramidal cells of layer Vb in slices of rat SI cortex. Cuts were made along the layers IV-V border to dendrectomize neurons. Populations of cut and control pyramidal neurons had similar proportions of intrinsic firing properties (about 40% regular-spiking, 33% intrinsically bursting and 48% oscillating), spike sizes, V_ms and time constants (τ_0 and τ_1). R_{in}s for nonbursting neurons in cut and uncut slices were very similar, however the mean R_{in} for dendrectomized bursting cells was about double that of control bursting cells. The effects of dendrectomy were also tested in a compartmental model of a large layer V pyramidal cell using the program NEURON (M. Hines); simulated removal of the apical dendrite only increased R_{in} by 5% to 20% (depending upon choice of specific internal resistivity, from 350 to 75 Ω·cm). We conclude that 1) amputation of most of a large apical dendrite does not impair the viability of a neuron, 2) the contribution of the apical dendrite to the active and passive behavior of the soma is usually small; in particular, intrinsic bursting does not depend upon the apical dendrite. Supported by NIH and ONR.

126.18

DENDRITIC ELECTROGENESIS IN NEOCORTICAL NEURONS **IN VITRO.** Y. Amitai[1], A. Friedman[1*], B.W.Connors[2] and M.J. Gutnick[1*]. [1]Dept. Physiology, Ben-Gurion University of the Negev, Beersheva, Israel, and [2]Div of Biology & Medicine, Brown University, Providence, RI, USA.

The complexity of the neocortical neuropil has hindered study of dendritic electrical properties. We recorded intracellularly from pyramidal cell dendrites in coronal slices of rat and guinea pig parietal neocortex, maintained in vitro. In some recordings, the dendritic impalement site was confirmed anatomically by dye injection; in others, it was inferred from the distinctive pattern of activity. In all recordings, depolarizing pulses evoked at least two types of spikes, fast and slow, which arose from different voltage thresholds and were clearly distinguishable from typical somatic spikes. TTX caused the disappearance of fast spikes, leaving repetitive generation of broad spikes.

These data indicate that in neocortex, the dendritic membrane contains voltage-dependent channels that are sufficient to initiate and sustain regenerative events.

Supported by the National R & D Council, Israel, and the EEC.

126.19

SIMULATIONS OF DENDRITIC INTEGRATION IN RECONSTRUCTED PYRAMIDAL AND NONPYRAMIDAL NEURONS OF NEOCORTEX. L.J. Cauller, M.S. Wehr*, I. Bülthoff* and B.W. Connors. Sect. Neurobiology, Brown Univ., Providence, RI 02912.

The diverse shapes and physiology of neurons in the neocortex suggest that each cell type may process synaptic inputs in a distinctive way. We recorded from spiny and smooth neurons from layers II to V in the rat SI barrel-field *in vitro*, measured R_{in}, τ's and intrinsic firing properties, and synaptically activated distal dendrites via axons of layer I. Each cell was filled with biocytin, its morphology was quantified, and a passive electrotonic model was generated with the program NEURON (kindly supplied by M. Hines). Likely ranges of membrane parameters were determined for each cell, and steady-state and transient analyses were performed. Nonpyramidal cells were the most electrotonically compact, followed closely by the proximal regions (basal and apical oblique dendrites) of pyramidal cells: e.g. unitary excitatory synaptic conductances (1 nS peak) applied to the dendrite tips generated EPSPs of about 0.1-1 mV in the somata. The distal apical tufts of large layer V pyramidal cells were strikingly different, however; somatic EPSPs generated by simulated tuft (layer I) synapses were extremely small and slow. Because layer I synapses can generate strong, fast excitation experimentally (Cauller & Connors, NS abst., 1990), we suggest that active conductances in apical dendrites facilitate distal synaptic inputs. Supported by NIH and ONR.

126.20

Morphological characteristics of double-labelled axotomized corticospinal neurons studied in vitro. G.-F. Tseng, D.A. Prince. Dept. of Anat., Col. of Med., Nat'l. Taiwan U. and Dept. Neurol.& Neurol. Sci., Stanford U., Sch. of Med., Stanford, CA 94305

Identified normal and axotomized corticospinal neurons were studied in a slice preparation using a double-labelling technique (Tseng et al., '91, J. Neurosci. Meth., in press). Axotomy and labelling were performed at C2-3 cervical levels on 4-5 week old albino rats. Axotomized cells were studied intracellularly at 3, 9, and 12 months after surgery. Physiologically, axotomized differed from normal cells by higher input resistances, lower rheobase currents, steeper f-I relationships, higher steady state spiking frequencies, and fewer cells had identifiable sAHP. Other passive membrane properties were comparable to those of normal neurons. Synaptically, there seems to be a decrease in the incidence of IPSPs been evoked in response to layer I stimuli.

Morphologically, axotomized corticospinal neurons had smaller somata and maintained a pyramidal shape. Sholl's analysis at 40 micron increments revealed a basal dendritic arborization comparable to that of normal cells. In addition, the basal dendritic domain, the apical dendritic width, measured at 80 microns from the center of the soma, and the maximal number of distal, apical dendritic branches were also comparable to those of normal cells. Like normal cells, superficial layer ascending axon collaterals were only rarely identified in axotomized neurons.

These results indicate that distantly axotomized corticospinal neurons maintained their dendritic and local axonal arbors while their somata became smaller. Their high input resistance and steep f-I slope will make them hyperexcitable (as compared to normal cells) upon stimuli. Thus both physiological and morphological data suggests that axotomized cortical neurons may participate actively in cortical functioning instead of regress into an inactive state. Supported by US NIH grants NS06477 and NS12151 from NINDS and a Pimley Postdoctoral Fellowship to G.-F.T.

127.1

PARABOLIC FLIGHT STUDIES IN ASTRONAUTS: OCULAR TORSION IN NOVEL GRAVITY STATES PREDICTS SPACE MOTION SICKNESS. Shirley G. Diamond and Charles H. Markham. Dept. Neurology UCLA School of Medicine, Los Angeles, CA 90024-1769.

Studies in hypo- and hypergravity of parabolic flight examined ocular torsion during 10 to 20 parabolas in 9 former astronauts seated upright. Those who had space motion sickness (SMS) on prior space missions had high scores of disconjugate eye torsion on NASA's KC-135 aircraft, while those free of SMS had low scores. This finding suggested a predictive test of SMS. A follow-up asked: 1) Would tilting the prior subjects null their torsional asymmetry in hypergravity? 2) Could a predictive test be obtained in fewer parabolas? 3) Would results in additional astronauts support the earlier findings? Four new and 4 prior high score astronauts were studied in 5 positions: 4 parabolas each in upright, 5° and 10° right and left ears down. Results showed 1) tilting did not alter torsional asymmetry and a tilted protocol was not as clear a discriminator of SMS propensity as was the upright; 2) four parabolas in the upright position were not sufficient to effectively differentiate those who had had SMS from those who had not; 3) the 4 new astronaut subjects showed 2 with high scores and 2 with low scores of torsional asymmetry on the KC-135. The high scorers had had SMS on their space missions and the low scorers did not, supporting results of the earlier study. We conclude eye torsion on the KC-135 may offer a simple predictive test of SMS.

127.2

DO LABYRINTHINE AFFERENTS EXCITE HAIR CELLS? S.L. Cochran. Dept. Life Sciences, Indiana State University, Terre Haute, IN 47809.

Glutamate (but not NMDA agonists), when bath-applied to the isolated labyrinth of the frog, results in an increase in EPSP and action potential frequency, as well as afferent depolarization. The increase in EPSP frequency suggests that hair cells have non-NMDA receptors, which depolarize the hair cells and increase transmitter release. This increase in EPSP frequency precedes afferent depolarization and persists in 10 nM TTX (which blocks afferent action potentials) suggesting that glutamate acts by directly depolarizing hair cells, rather than indirectly depolarizing hair cells due to K^+ release accompanying afferent spiking and depolarization. Since the afferents are 'glutamatergic' and since reciprocal synapses have been reported between hair cells and afferents (Dunn, 1980, J.Comp. Neurol. **193**: 255-264), it is possible that afferent activity can modify transmitter output from hair cells. However, an analysis of the intervals between EPSPs indicates no increase in the probability of occurrence of an EPSP following an action potential and no correlation between EPSP amplitude and either the interval between EPSPs or the amplitude of the subsequent EPSP. Preliminary ultrastructural investigations confirm the presence of vesiculated profiles in the afferents, but typical active zones (from afferent to hair cell) seem to be absent. These findings suggest that afferent activity does not synaptically modulate release of transmitter from hair cells, although hair cell activity may influence adjacent hair cell activity through the glutamate-like transmitter released at hair cell-afferent synapses.

Supported by NSF (BNS 8616738) and NASA (NAG-498) grants to SLC.

127.3

DISCHARGE PROPERTIES OF AFFERENTS INNERVATING THE POSTERIOR CRISTA IN THE ISOLATED HALF HEAD OF THE TURTLE. A.M. Brichta and J.M. Goldberg. Dept. of Pharmacological and Physiological Sciences, Committee on Neurobiology, University of Chicago, Chicago, IL 60637.

As a first step in developing an *in vitro* preparation of the posterior crista of the turtle, *Pseudemys scripta*, we have recorded afferent activity in the isolated half-head. Fibers innervating this end organ were identified by their responses to pitch rotations. Most units have a background discharge of 5-30 spikes/s. Some are regularly and others irregularly discharging. A coefficient of variation (cv*), normalized to a mean interval of 50 ms, varies between units from 0.1-0.8. Different fibers respond to sinusoidal rotations with maximum sensitivities of 0.1-30 spikes·s^{-1}/ deg·s^{-1}. Based on responses to 0.1-3 Hz rotations, the units can be divided into three groups. Group 1 consists of regular, intermediate and irregular units whose gain and phase leads re head velocity both increase with cv*. Discharge leads head velocity by <45°. The most regular units of the group have response dynamics resembling the torsion pendulum model. Group 2 are irregular units whose gain and phase leads are larger than those of irregular group 1 afferents. Group 3 are irregular units responding to a combination of head jerk and head acceleration.

There are four kinds of afferents terminating in the turtle posterior crista: calyx, dimorphic, and two varieties of bouton units (Brichta and Peterson, Soc. Neurosci. Abstr. 16: 735). Intra-axonal labelling studies are needed to match the morphological and physiological groups. (Supported by ONR Contract N00014-88-k-0381).

127.4

ORGANIZATION OF EIGHTH NERVE EFFERENTS IN THE TURTLE, *PSEUDEMYS SCRIPTA*. A. Fayyazuddin, A.M. Brichta, and J.J. Art. Dept. of Pharm. & Physiol. Sciences, Committee on Neurobiology, The University of Chicago, Chicago. IL 60637.

As part of a larger study aimed at understanding the efferent contribution to auditory and vestibular control we have begun to characterize the organization and location of the C.N.VIII efferent axons and cell bodies in the turtle, *Pseudemys scripta*.

Following unilateral extracellular injections of horseradish peroxidase (HRP) into the posterior branch of the eighth nerve we observed retrogradely labelled cells confined to both ipsi- and contralateral medial reticular nuclei. Efferent cell bodies ranged in size from 96 to 389 μm^2 (mean ± s.d.; 199 ± 96), but there was a bimodal distribution in their size spectra, suggesting the presence of two groups. The larger group (89%) had a mode of ≈160 μm^2 and the smaller group (11%) had a mode of ≈330 μm^2. Further evidence of two groups of efferents came from measurements of parent axon diameters, prior to bifurcation, as they entered the midline bundle within the medial longitudinal fasciculus (MLF). Efferent axon average diameters (1.37 ± 0.45 μm) size spectra revealed the presence of two groups with modes of ≈1.6 μm and ≈2.8 μm respectively. To ascertain if these differences may reflect variations in efferent terminal patterns we have begun a quantitative analysis of individual anterogradely filled efferent terminals following HRP injections into the MLF. Our preliminary results suggest that small diameter axons have few if any bifurcations as they course within the peripheral nerve and supply only one end organ. In contrast, large diameter axons were seen to bifurcate extensively and supplied more than one end organ.

Taken together these results suggest that eighth nerve efferents may be subdivided into two possible subgroups based on their structure and spatial organization. (Supported by NINCD DC 00454, and ONR N00014-88-k-0381).

127.5

COMPARISON OF HAIR-CELL POPULATIONS AND AFFERENT INNERVATION PATTERNS IN THE CRISTAE OF THE SQUIRREL MONKEY AND THE CHINCHILLA. C. Fernández*[1], A. Lysakowski[2] and J.M. Goldberg[2]. Depts. of [1]Surgery and of [2]Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637.

The structural organization and afferent innervation patterns in the chinchilla cristae have been described (J. Neurophysiol. 60:167-181, 1988). We now report that the organization of the cristae in the monkey differs substantially from that in the chinchilla. In the latter species, there is an ≈1:1 ratio of type I and type II hair cells in all regions of the the epithelium. Hair-cell counts indicate that in the monkey type I outnumber type II hair cells by a ratio of ≈3:1. The ratio is higher in the central zone (≈5:1) than in the peripheral zone (≈2:1). As might be expected from the differences in hair-cell ratios, innervation patterns are distinctive in the two species. Comparisons are based on data from 301 monkey and 368 chinchilla units labeled with horseradish peroxidase. In both species, calyx units are concentrated in the central zone and bouton units in the peripheral zone; dimorphic units are seen throughout the sensory epithelium. Three differences reflect the relative paucity of type II hair cells in the monkey: a) the percentage of calyx units units is 3x larger in this species; b) the percentage of bouton units is 1.5x smaller; and c) central dimorphic units have half as many bouton endings. Calculations indicate that in both species each type II hair-cell receives an average of ≈20 afferent boutons. This suggests that the afferent innervation in each species is matched to its hair-cell population. (Supported by NIDCD DC00070, NASA NAG 2–148, ONR N00014-88-k-0381 and by the American Otological Society. A.L. is a NASA Research Associate.)

127.6

TOPOGRAPHIC DISTRIBUTION OF FIBERS PROJECTING TO THE SEMICIRCULAR CANAL CRISTAE IN THE CHINCHILLA. V. Honrubia[1], E. Naito[1]*, Y. Naito[1]*, M. Ross[2]*, & L. Hoffman[1]. [1]Victor Goodhill Ear Center, UCLA School of Medicine, Los Angeles, CA 90024 and [2]NASA Ames Research Center, Moffett Field, CA 94035.

The number and distribution of nerve fibers in the semicircular canal cristae of chinchillas were investigated through thin (1 μm) plastic-embedded histologic sections, serial reconstructions and computer-based graphic methods.

There is a systematic organization of fibers reflecting the topography of the receptor area in the crista. Nerves projecting to individual cristae branch first from the single canal nerve into two bundles, one for each transverse half of the crista. Thereafter each "heminerve" subdivides again in two, one bundle for half of the utriculopetal and utriculofugal slopes of the crista. As the fibers enter the stroma of the receptor organ, the fibers beneath the center of the crista further subdivide into small bundles containing 30-40 fibers. These bundles are comprised of fibers with a broad distribution of diameters, comparable to that of the entire canalicular nerve (Carney et al., ARO Abstracts 13:364, '90). Fibers proceed in a straight trajectory and without bifurcaion in the direction of the crista apex. Fibers within each bundle were found to innervate an apex-to-perimeter slab of the neuroepithelial surface, 30-50 μm in width. Individual fibers followed a straight course until they reached the level along the crista slope of their eventual innervation destination, at which time they turned sharply toward the neuroepithelial surface. The bundles destined to the periphery of the crista run in a lateral position in the canalicular nerve and innervate hair cells in the lateralmost ends of the crista. The majority of fibers in these bundles are of smaller diameter (<3 μm).

The morphological organization of the nerve suggest that information from differents areas of the crista travels in the vestibular nerve in discrete channels according to crista location. In view of the well known size dependent differences in fibers response dynamics, the vestibular nerve not only sends information about different types of head motion, but this information might also represent a topographical map of the cristae within the CNS.

127.7

EYE MOVEMENTS AND BRAINSTEM NEURONAL RESPONSES EVOKED BY CEREBELLAR AND VESTIBULAR STIMULATION IN CHICKS. S. du Lac and S.G. Lisberger, Dept of Physiology, UCSF, San Francisco CA 94143.

Inputs from the cerebellar floccular onto brainstem vestibular neurons are essential for adaptive changes in the vestibulo-ocular reflex. As a step toward developing an *in vitro* preparation for studying the cellular and synaptic nature of those changes, we have characterized floccular and vestibular interactions in the intact chick. We placed stimulating electrodes in the horizontal vestibular ampulla and the flocculus of 3-6 week old ketamine anesthetized chicks. Stimulation-evoked eye movements were monitored with a scleral search coil and brainstem neuronal responses were recorded extracellularly.

Electrical shocks to the vestibular ampulla evoked brief, contralaterally directed movements in both eyes. Although single shocks to the flocculus elicited no response, conjunctive floccular and vestibular stimulation significantly reduced the vestibularly-evoked movement. Trains of current pulses applied to the flocculus and ampulla evoked eye movements directed toward and away from the side of stimulation, respectively. Recordings from the brainstem revealed a number of neurons that were activated by ipsilateral vestibular stimulation (at latencies of 0.7 to 3.1 msec) and inhibited by ipsilateral floccular stimulation. In units with no spontaneous activity, we found that floccular stimulation reduced the probability of a vestibularly-evoked spike. The duration of floccular inhibition in spontaneously active neurons ranged from 9 to 38 msec. Our sample included neurons in the lateral, medial, and superior vestibular nuclei. Similarities between these findings and those of comparable studies in mammals indicate that the chick will provide a good model sytem for cellular and synaptic studies of the vestibulo-ocular reflex. (supported by NIH training grants EY-07058 and EY-06318 and by the McKnight Foundation)

127.8

LATENCY OF ADAPTIVE VOR SHOWS RESPONSES WHICH ARE DEPENDENT ON THE LENGTH OF ADAPTATION. T.T. Khater, K.J. Quinn, J.F. Baker and B.W. Peterson, Northwestern University Medical School, Chicago, IL 60611

When an animal is first exposed to a new visuo-vestibular stimulus, its VOR adapts in a way that minimizes retinal slip. To measure VOR latencies during development of adaptation, we recorded daily the eye movements evoked by randomly occurring velocity transitions between ±19°/s earth-horizontal whole body rotation (HWBR) in total darkness with a CNC eye coil system in 5 cats. The cats were outfitted with x0.25 or x2.2 magnifying lenses for up to 5 days, and subjected to 2 h of HWBR daily. In another series of experiments, cats were trained using 2 h "cross-axis" coupling of HWBR with a synchronous vertical rotation of a projected random spot pattern that had 1.5X the amplitude.

Vertical eye movements elicited by HWBR after 2 h of cross-axis adaptation had latencies of 13-15 msec and in 7/15 cases included a second component beginning at 70-80 msec. These two components also appeared with latencies of 11-14 msec and 67-77 msec in the adaptive changes elicited by x0.25 or x2.2 lenses, which were revealed by subtracting control from adapted horizontal eye movements. The early component increased strongly during the initial and subsequent daily periods of HWBR but declined while the cat wore the lenses in its cage. The longer latency component increased slowly both during HWBR and unrestrained wearing of the lenses. These data suggest that adaptation occurs most rapidly in the shortest (presumably brainstem) pathway, which, however, requires continuous strong mismatched visual-vestibular signals to maintain its activity. Supported by EY05289, EY06485, EY07342.

127.9

EFFECTS OF MK-801 ON ADAPTIVE VESTIBULO-OCULAR REFLEX MODIFICATIONS IN THE GOLDFISH

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Goldfishes can produce large and rapid adaptive vestibulo-ocular reflex (VOR) gain modifications which can serve as a model for studying neuroplastic changes in the sensori-motor system. VOR modification experiments investigated the influence of the NMDA antagonist, MK-801, which is known to affect other neuroplastic changes. After initial calibration measurements of the VOR in light and dark, goldfishes received either an intramuscular control injection of fish Ringer's solution or MK-801 (1mg/kg). This dose of MK-801 did not alter the unmodified gain of the VOR in the light or dark. VOR gain modification was initiated approximately 30 minutes after the injection and continued for a 3 hour period. Training towards a gain of 2X was accomplished by presenting visual stimuli 180 degrees out of phase with the vestibular platform rotating about the vertical axis (1/8 Hz ± 20 deg). Control injected fishes immediately produced a significant gain change (0.82 gain change/hr). For the fishes that received the MK-801 injection, there was no gain change during the first 30 minutes. The subsequent VOR gain change was reduced considerably (0.34 gain change/hr). However, after 3 hours these fish produced a gain change comparable to that of the control animals. Another group of fishes received a similar injection of MK-801. However, VOR modification did not commence until at least 2 hours after the injection. For this 2 hour delayed modification group, the MK-801 had a more pronounced effect. No increase in VOR gain above baseline occurred during the first 60 minutes of modification. Subsequently, the rate of gain change was less (0.2 gain change/hr) than observed in the first set of experimental animals. Even after 5 hours of modification the final gain achieved by these fishes was less than that reached by the other animals that were modified for 3 hours. Thus, intramuscular injection of MK-801 is able to hinder modification of VOR without affecting the unmodified VOR. (Supported by a grant from NIH DC 01094)

127.10

ALTERATION IN THE VESTIBULO-OCULAR REFLEX (VOR) PRODUCED BY MUSCIMOL INJECTIONS INTO THE VESTIBULAR NUCLEI. J.Yokota, H.Reisine, T.Raphan , B.Cohen Depts. of Neurol. & Physiol., Mt Sinai Sch. of Med., NY10029 & Dept. of CIS, Brooklyn College, CUNY, NY11210

Slow phase eye velocity (SP Vel) related to velocity storage can be elicited by electrical stimulation of the vestibular nuclei (VN; Yokota et al,1990). In this study we recorded single units and electrically stimulated VN to determine locations from which velocity storage could be induced. We then injected 0.5µl of muscimol, a $GABA_A$ agonist. Animals developed spontaneous nystagmus with downward rotatory horizontal slow phases. The downward component was affected by head position re gravity; the minimum position was ipsilateral side down.The most common pattern of VOR dysfunction was a bilateral reduction in the VOR time constant (Tc) with preservation of VOR gain. In this condition steady state SP Vel during off-vertical axis rotation (OVAR) as well as OKAN were also lost bilaterally. Quick phases of vestibular nystagmus were not affected, but there was some inability to hold eye position. A second pattern of dysfunction occurred after injection into areas where neurons responded to otolith input. There was an isolated loss of SPVel of OVAR with preservation of VOR and OKAN gain and Tc. The first pattern with bilateral reduction in VOR and OKAN Tc with preservation of VOR gain suggests that areas of VN associated with the velocity storage integrator had been inactivated. The isolated loss of ipsilateral OVAR SP Vel with preservation of OKAN and the VOR Tc suggests that areas of VN associated with head velocity estimation from the otoliths had been disrupted. Supported by NS00294,EY04148,EY01867

127.11

DISTINCTIVE DYNAMICS OF FLOCCULUS RECEIVING NEURONS IN THE RABBIT VESTIBULAR NUCLEUS. J.S. Stahl and J.I. Simpson, Dept. Physiology & Biophysics, NYU Med. Ctr., New York, N.Y. 10016.

We have demonstrated (SFN Abst. 15: 206.6) that for 0.2 Hz rotation in the light, the average firing rate of vestibular neurons receiving direct floccular inhibition (FRNs) leads that of vestibular neurons lacking a floccular input (nonFRNs). We now report that this phase lead is present for a wide range of stimulus conditions. Neurons were recorded in awake rabbits. The sample was restricted to cells that received from the flocculus and/or projected to oculomotor midbrain structures. For sinusoidal rotation in the light, FRNs (n=26) led nonFRNs (n=14) at all frequencies in the tested band (0.05-0.8 Hz). The lead increased monotonically, from 8.6° at 0.05 Hz to 19.4° at 0.8 Hz. The phase differences were significant ($p<0.05$ or better). For rotation in the dark, FRNs (n=22) led nonFRNs (n=10) by 13.7° at 0.05 Hz and 22.1° at 0.8 Hz, and again the phase difference was significant at all frequencies. The fundamentals of the firing rates of the two cell groups were also compared for triangular optokinetic stimulation at 0.1 Hz. FRNs (n=52) led nonFRNs (n=20) by 18.7°, and the phase difference was significant at $p<0.0001$. For both FRNs and nonFRNs, the relationship of firing rate to eye movement could be modeled by a 2-pole, 1-zero transfer function, differing only in that the time constants were longer for the FRNs. If the phase lead of FRNs is produced by the signal contributed by the floccular Purkinje cells, then these observations suggest that one role of the flocculus is to control the phase of the net premotor signal. The flocculus could sample vestibular nucleus signals, enhance the velocity component, and return the altered signals. It would thereby counterbalance an excessive intergration (in the mathematical sense) postulated to occur in the brainstem circuitry. (Supported by NIH Grant NS-13742.)

127.12

LECTIN BINDING PATTERNS OF VESTIBULAR HAIR CELLS.

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Vestibular hair cells exhibit regional differences in hair bundle morphology and physiological response properties. To see if these cells express distinct surface glycoconjugates, we applied lectins with different carbohydrate specificities to vestibular endorgans of the bullfrog and guinea pig.

In the bullfrog sacculus, three lectins - CON A, RCA-I, and WGA - labeled only supporting cells while a fourth, VVA, indiscriminately labeled the hair bundles of saccular hair cells. CON A and RCA-I labeled the hair bundles of hair cells in the utriculus and semicircular canals, regardless of their epithelial location. The labeling of WGA and, to a lesser extent, VVA, was confined to specific regions. In the utriculus, these lectins labeled the hair bundles of extrastriolar, but not striolar, hair cells. In the semicircular canals, they labeled the hair bundles of peripheral, but not central, hair cells. Similar patterns of staining of both Type I and Type II hair cells were seen in the utriculus and semicircular canals of the guinea pig. These staining patterns were preserved in isolated hair cells after enzymatic digestion.

Our results indicate that receptors for a variety of lectins exist on vestibular hair cells and suggest that lectins can be useful probes for identifying vestibular hair cells from central and peripheral epithelial regions. Supported by NIDCD 00355, NASA 2-651, and OLSHF.

127.13

CYTOCHROME OXIDASE (CO) STAINING IN SCARPA'S GANGLION AND THE MEDIAL VESTIBULAR NUCLEUS (MVN) POST-HEMILABYRINTHECTOMY (HL). A.A. Perachio[123], G.A. Kevetter[13] and R. Kassir*[1]. Dept. Otolaryngol.[1], Physiol. & Biophy.[2], Anat. & Neurosci.[3]. Univ.TX Med.Br., Galveston, TX 77550.

We have previously reported that, following HL in the gerbil, the activity rates and dynamic responses of horizontal canal-related MVN neurons are not restored during vestibular compensation in the number of active neurons or in dynamic function, especially in the nucleus ipsilateral to the damaged labyrinth. We have now used CO staining to examine how this measure of oxidative metabolism may be used to assess vestibular afferents and the MVN at various stages recovery from HL. End organs of one labyrinth were removed by aspiration in adult gerbils. Animals were sacrificed at 1 or 72 hrs, 2 weeks, 1 month and 3 months post-HL. Microdensitometry, using a computer-assisted image analysis system, was performed on 500 primary afferents. Areal measures of staining density were made from multiple sites bilaterally in the rostral MVN that contain vestibulo-ocular neurons some which were retrogradely labelled. Primary afferents innervating the intact labyrinth at 1 hr post-HL stained darker than those on the injured side. However, by 72 hrs post-surgery, the two sides stained equivalently. In the MVN, CO staining was statistically equal bilaterally at 1 hr and 72 hrs. By 2 weeks post-HL both primary afferents and rostral MVN exhibited significantly greater CO activity on the intact side. This difference remains significant at 3 months post-HL. These findings support the results of electrophysiological evidence of a persistent asymmetry in central vestibular and peripheral afferent neurons following behavioral signs of vestibular compensation. (Supported by NASA Grant NAG 2-26).

127.14

EFFECTS OF ROLL TILT ON PITCH VERTICAL VESTIBULO-OCULAR REFLEX (VOR). S.A. Rude, K.D. Powell, J.F. Baker. Department of Physiology, Northwestern Univ., Chicago, IL 60611.

The horizontal vestibulo-ocular reflex (VOR) during low frequency oscillations is more accurately compensatory when tested in body orientations that introduce a dynamic gravity stimulus (Rude and Baker, '88). For pitch vertical VOR, however, a dynamic gravity stimulus *increases* accuracy of the VOR in the upright position and *decreases* accuracy of the VOR in the supine position (Rude, et al., NS abstr '88). Inaccurate non-upright position vertical VOR is related to a predominance of upward direction slow phase eye movements. We are studying slow phase eye movement asymmetry and eye position drift at various roll tilts.

Using search coils and electro-oculography, eye movements were recorded in the dark from 4 cats during static tilt, or during inter-aural axis pitch in their sagittal plane (0.01 Hz ±30°), after rolling the cats to angles from 0°(upright) to 180°(supine).

Peak slow phase velocities were greater at all angles for upward vs. downward eye movement in the orbit. Asymmetry of slow phase velocity was slight at the 0° tilt, substantial at ±90° tilt, and at 180° tilt vertical eye movements showed a continuous upward slow phase regardless of sinusoidal rotation stimulus phase. Furthermore, experiments employing a static tilt with no oscillation revealed a consistent upward drift of the eyes in all cats, varying in magnitude with angle of tilt from 0° (smallest) to 180° (largest). The drift was not present in the light.

Upward drift may be a linear VOR response to the altered saccular stimulus during tilt. Asymmetrical slow phase eye velocity responses to rotation were a close fit to a sinusoid with an offset term, suggesting that the observed steady drifts were involved in producing the asymmetries.

Supported by EY05289, EY06485, EY07342.

127.15

MULTIPLICATIVE LEARNING RULES SIMULATE PROPERTIES OF VOR PLASTICITY. K.J. Quinn, N. Schmajuk, S.A. Rude, J.F. Baker and B.W. Peterson. Northwestern Univ., Chicago, IL 60611

Errors requiring adaptation of the vestibulo-ocular reflex (VOR) can be characterized in terms of gain and/or phase deficits. We are exploring how the CNS can use the only known available error signal (retinal image slip velocity - RSV) to adjust these properties. This credit assignment problem facing an adaptive VOR network is solved by a multiplicative learning rule, utilizing three signals likely to be available to brain stem neurons participating in VOR function: head position (HP), head velocity (HV) and RSV (Soc. Neurosci. Abstr., 1990, 304.13). Here we describe the characteristics of the algorithm in greater detail and compare its function with known characteristics of VOR plasticity.

The algorithm has been implemented in a neural network based on a control systems representation of the VOR. It uses two rules: the product of RSV*HP is used to adjust the weight of a pathway carrying a position signal; the product of RSV*HV is used to adjust the weight of a pathway carrying a velocity signal. This learning rule is sufficient to account for adaptive responses required across the complete spectrum of possible gain and phase changes. Sensitivity of these products to gain or phase errors changes with frequency of head movement but the algorithm still provides proper compensation. Topography of the model response is similar to that described for phase reversal experiments and cross-axis adaptation acquisition. A modified implementation of this algorithm using products of pre- and post-synaptic activation levels, also predicts differential acquisition of phase advanced vs phase lagged cross-axis adaptive responses. (see Powell, et al. this session). Supported by EY05289, EY06485, EY07342, NS07223.

127.16

OTOLITH RESPONSES TO LINEAR OSCILLATIONS IN ALERT SQUIRREL MONKEYS. C.J. Somps, and D.L. Tomko Vest. Res. Fac., NASA Ames Res. Ctr., Moffett Field, CA 94035

The linear vestibulo-ocular reflex (LVOR) stabilizes visual images on the retina during linear displacements of the head (Paige and Tomko, 1991). How vestibular neural mechanisms produce the compensatory eye movements of the LVOR is unknown. In the present study, the activity of otolith afferents was recorded in alert squirrel monkeys subjected to earth-horizontal, sinusoidal linear oscillations, a stimulus known to produce a robust LVOR. All units were spontaneously active, and modulated by linear oscillations. Discharge waveforms were triphasic, and amplitudes averaged 100 to 200 uV. Discharge rates in upright, stationary animals ranged from 16 to 126 spikes/sec and averaged 60 spikes/sec. Interspike intervals were regular in most units (coefficients of variation (CV) between 0.03 and 0.1), and were less regular in others (CVs>0.1). For 0.5 Hz oscillations, regular units exhibited sensitivities to linear oscillations averaging 22 spikes/sec/g and small phase leads averaging 10°. Sensitivities (23 spikes/sec/g) remained similar and phases slightly lagged the stimulus (8°) with 1.5 Hz oscillations. Units with CVs greater than 0.1 had higher sensitivities (118 spikes/sec/g) and larger phase leads (27°) than regular units. For units whose polarization vectors were calculated, vectors were found to lie roughly in either the utricular or saccular plane. Units sensitive to angular accelerations (canal afferents) were not sensitive to linear oscillations. Given LVOR gains of 10-20°/sec/g and phases slightly leading head velocity (30°), a phase shift of ~90° and a gain of 0.1-1°/impulse must be provided central to the otolith afferents.

Support: NASA Space Med. Tsk 199-16-12-02 & -03.

127.17

PRIMATE LINEAR VESTIBULO-OCULAR REFLEX (LVOR) DURING HORIZONTAL MOTION ALONG AXES BETWEEN NASO-OCCIPITAL (NO) AND INTERAURAL (IA). D.L.Tomko and G.D.Paige. Vestibular Research Facility, NASA Ames Research Center, Moffett Field, CA 94035-1000

We previously described squirrel monkey LVORS during motion in the horizontal plane along NO and IA axes. NO LVOR amplitudes increased as gaze eccentricity relative to the motion axis increased and as binocular fixation distance decreased. Further, to fixate visual targets during forward head motion and rightward gaze, eyes must move to the right, but when looking left, the eyes must move to the left.

In this study, the LVOR was measured (binocular search coils) during horizontal motion (5.0 Hz) along several axes between and including NO and IA. This reorients the head, and therefore otolith input, relative to the axis of motion. We hypothesize that the LVOR would still follow the same gaze-dependent kinematics relative to the axis of motion, regardless of eye position in the head or the orientation of the head relative to the motion axis. This requires integration of eye position (e.g., efference copy) with otolith inputs in order to determine the angle of the eye relative to the axis of linear motion. Results confirmed that the LVOR is indeed governed by eye position relative to linear motion.

Supported by: NASA Space Med Tasks 199-16-12-02 & -03

127.18

SPATIAL ORIENTATION OF VELOCITY STORAGE DURING POST-ROTATORY NYSTAGMUS. M.J. Dai, T. Raphan, B. Cohen, C. Schnabolk, Dept. of CIS, Brooklyn College, CUNY, NY 11210, and Depts of Neurol. & Physiol. Mt. Sinai Sch of Med., NY 10029

Studies of cross-coupling during OKN and OKAN in tilted positions show that the yaw axis eigenvector lies close to the spatial vertical (Dai et al. 1991). In this study we determined whether the same principles apply when the vestibular system is activated by stopping in a tilted position after head rotation. Velocities were induced during off-vertical axis rotation (OVAR) that exceeded the saturation velocity of velocity storage, and yaw and pitch components were measured after monkeys were stopped in side down positions. Steady state slow phase eye velocity during OVAR was predominantly in yaw with oscillating pitch and roll components. When stopped side down, the yaw time constant of the post-rotatory nystagmus was shorter than when upright, and a pitch component developed whose vector direction was a transformation of the vector from the yaw axis in the body frame to the corresponding direction in the spatial frame. A model of velocity storage from OKAN predicted the behavior of the pitch component during vestibular stimulation. Thus, the vestibular system utilizes velocity storage according to the same organizational principles of orientation as during OKN and OKAN. Supported by EY04148, NS00294.

127.19

CONVERGENCE OF COMPLEMENTARY SEMICIRCULAR CANAL INPUTS ONTO VESTIBULAR NUCLEI NEURONS. J.D. Dickman. Depts. of Surgery (Otolaryngology) and Anatomy, Univ. of Mississippi Medical Center, Jackson, MS 39216.

How do vestibular nuclei neurons synthesize the information from the paired complementary semicircular canals into a unified output signal? Experiments were conducted to answer this question by recording extracellular single fiber responses from vestibular nuclei neurons elicited by individual stimulation of ipsilateral and contralateral horizontal semicircular membranous ducts using mechanical micropushers (Dickman & Correia, *J. Neurophysiol.*, 1989, 62, 1090-1101). Experiments were conducted in awake, decerebrate pigeons that were paralyzed (Pancuronium) and ventilated (250 ml/min O_2/CO_2). Sinusoidal (0.01 - 10 Hz) displacements (± 1 - $\pm 2.5\mu$) of the exposed ipsilateral horizontal membranous duct were delivered first, followed by identical stimulations of the contralateral horizontal duct. To date, complete protocols have been recorded in only a few vestibular nuclei neurons, with all cells responding to independent ipsilateral and contralateral horizontal canal mechanical stimulation. Mean ($\pm$SEM) gain and phase values (N = 7) from responses produced by ipsilateral canal sinusoidal stimulation (0.1 Hz, $\pm 2.5\mu$) were 8.3 (± 3.3) spikes$\cdot$sec$^{-1}/\mu$ and 34 ° (± 5.4) *lead* relative to peak inward pusher position, respectively. Contralateral horizontal duct stimulation (0.1 Hz, $\pm 2.5\mu$) produced much smaller responses with a mean gain of 1.6 (± 0.5) spikes$\cdot$sec$^{-1}/\mu$ and a mean phase of 185 ° (± 3.5) *lag* re peak inward pusher position. This corresponds to an approximately 5:1 gain ratio (ipsi:contra) and a 219 ° difference in response phase between ipsilateral and contralateral stimulation. Higher frequency stimulations of the ipsilateral canal produced responses similar to those observed at 0.1 Hz. However, contralateral canal stimulations above 1.0 Hz either silenced the neuron or produced no detectable modulated response. Supported in part by NIH BRSG grant 2S07RR05386.

127.20

VESTIBULO-OCULAR REFLEX (VOR) DIRECTION ADAPTATION TO PHASE ADVANCED VS PHASE DELAYED OPTOKINETIC STIMULI. K.D. Powell, K.J. Quinn, S.A. Rude, B.W. Peterson, J.F. Baker. Dept. Physiology, Northwestern Univ., Chicago, IL 60611.

The VOR can adjust its direction and gain and introduce phase lags (Powell et al., NS abstr '89) to reduce retinal slip. Are signals available to VOR neurons to allow adaptive VOR phase advances? To study this, 7 alert cats were VOR adapted to either a phase advanced or phase delayed vertical optokinetic stimulus.

VOR adaptation consisted of 2 hr of horizontal whole body rotation at 0.25Hz coupled to vertical rotation of a field of spots. The phase of the optokinetic sinusoid was 45° advanced or 45° delayed with respect to the vestibular stimulus. Vertical and horizontal VOR at .02 - 2.5Hz were measured with EOGs in the dark before and after adaptation. The adaptive response was calculated as the difference between the pre and post adaptation vertical VOR during horizontal rotations.

The average adaptive VOR gain from .02-1.0Hz was greater in phase lag experiments than in phase advance experiments At 0.25Hz, adaptation to phase delay had an average gain of .09, phase of -22°, and adaptation to phase advance had an average gain of .04, phase of +11°. Highest gains for phase delay experiments were recorded at low frequencies while highest gains for phase advance experiments were seen at high frequencies. The diminished ability of the VOR to adapt its direction in response to phase advanced stimuli is shared by certain VOR models (Quinn et al., this vol.) and suggests that phase delayed signals are more readily available to VOR neurons than phase advanced signals. Supported by EY05289, EY06485, EY07342.

VESTIBULAR SYSTEM II

128.1

SPATIAL AND TEMPORAL PROPERTIES OF OTOLITH-SENSITIVE HORIZONTAL CANAL (HC) NEURONS IN THE VESTIBULAR NUCLEUS AS A FUNCTION OF LINEAR ACCELERATION FREQUENCY. G.A. Bush[1], A.A. Perachio[1,2], D.E. Angelaki[3], Depts. of Otolaryngol.[1] and Physiology & Biophysics[2], Univ. Tex. Med. Br., Galveston, TX 77550, Dept. of Physiology[3], Univ. of Minn., Minneapolis, MN.

Otolith-sensitive HC vestibular nuclei neurons were characterized for their responses to sinusoidal linear translation in the horizontal head plane over the frequency range 0.2-1.4 Hz (peak acceleration, ± 0.10g) in the decerebrated rat. The spatial response properties of the neurons were determined by systematically varying the direction of the applied force vector by statically repositioning the head in the horizontal plane with respect to the direction of travel at each frequency. 89% of the neurons (operationally referred to as broadly tuned neurons) exhibited response gains that were not proportional to the cosine of the angle between the direction of the applied force vector and the cell's maximum sensitivity vector. Each neuron could be described by a maximum (S_1) and a minimum (S_2) sensitivity vector which were in spatial and temporal quadrature. There was a significant linear correlation between the S_1 and S_2 magnitudes. The slope of the linear regression was frequency dependent. Two classes of neurons were distinguished by their S_1 response vectors as frequency was increased: 1) *Dynamic* neurons exhibited a gain enhancement of S_1 with increased phase lags, a moderate attenuation of S_2 and a decreasing S_2/S_1 and 2) *Low Dynamic* neurons which exhibited a moderate gain attenuation of S_1 with small phase lags, a gain enhancement of S_2 and an increasing S_2/S_1. Therefore, given the temporal quadrature and the frequency dependent linear relationship between S_1 and S_2, broadly tuned neurons encode two dimensions where either S_1 or S_2 could be the time derivative of the other depending on the cell's dynamics. (Supported by NASA NAG 2-26, NIH DC00385)

128.2

VESTIBULAR SYSTEM DEVELOPMENT IN THE FLATFISH, Pseudopleuronectes americanus. W. Graf, B. Evans and S. Gallager. The Rockefeller University, N.Y., NY 10021; Inst. Neuroscience, Univ. Oregon, Eugene, OR 97403; Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

During metamorphosis, flatfish undergo a 90° side tilt to become bottom-adapted adults. Simultaneously, the eye on the down side is pushed over to the up side. Compensatory eye movements during swimming in the now asymmetric animals are subserved by a unique central nervous connectivity involving rearranged second-order vestibular neuron connections.

The ontogeny of this flatfish-specific connectivity was studied in larval winter flounders using [^{3}H]thymidine autoradiography. There is widespread cell proliferation throughout the entire brain at day 1 post hatching. Specific cell divisions involving vestibular neurons occur at about day 26-30 post hatching. Occasional labelling in vestibular neurons is present in subsequent stages before metamorphosis. An increase in cell division activity is noticeable around the period leading to the metamorphic climax (day 54-68 post hatching).

Although there is a clear indication of newly appearing vestibular neurons prior to and during metamorphosis, further experiments are necessary to ascertain whether these particular cells are the ones which provide the new neural circuitry to support the adult flatfish's compensatory eye movements.

Supported by NIH grant DC-00239.

128.3

VISUAL SUPPRESSION OF TORSIONAL VESTIBULAR NYSTAGMUS IN RHESUS MONKEYS. Straumann D, Suzuki M*, Henn V, Hess BJM, and Haslwanter Th*. Neurology Dept, University Hospital, Zürich, Switzerland, and Otolaryngology Dept, Dokkyo University, Koshigaya Hospital, Saitama, Japan.

Juvenile rhesus monkeys, placed on a motorized turntable, were rotated at constant velocity and then decelerated about an earth-vertical axis. The animals were implanted with dual search coils to measure eye movements in three dimensions. By changing the monkey's body position (upright, ear-down, supine), postrotatory nystagmus was elicited in the horizontal, vertical, or torsional direction. Peak slow phase eye velocity (Vp) and time constant of velocity decay (Tc) were compared between decelerations in the dark and in the light. In all nystagmus directions, illumination reduced the time constants (Tc) to values around 5 s. Peak velocities (Vp) were markedly attenuated in the horizontal and vertical directions (around 50%), but the effect of light on Vp in the torsional direction was small (less than 20%). These findings were independent of the velocity step size. We conclude that the two components of optokinetic nystagmus not only differ in their dynamics, but also in their dimensionality: The indirect pathway (optokinetic system) operates in all three dimensions, while the direct pathway (pursuit system) is only effective in the horizontal and vertical directions. Supported by SNF 3100-28008.89 and 31-25239.88(ESPRIT-MUCOM 3149).

128.4

NMDA Receptor Antagonist Microinjected Unilaterally into the Vestibular Complex Induces Postural Syndromes in Normal Rats. Jennifer D. Porter and Merle E. Meyer, Dept. Psychology, Univ. of Florida, Gainesville, FL 32611.

Neurotransmitters involved in the vestibular system are largely uncharacterized. However, Glutamate and Aspartate have been suggested as possible neurotransmitters between primary vestibular afferents and second-order vestibular neurons. The purpose of our study was to evaluate the roles of NMDA and non-NMDA receptor antagonists microinjected unilaterally into the vestibular nuclei. Microinjections of AP-5, a specific NMDA receptor antagonist, into the vestibular nuclei of the normal rats induced postural disorders which were similar to those observed following unilateral labyrinthectomy.

128.5

EFFECTS OF CHRONIC HYPER-GRAVITY ON THE RIGHTING REFLEX AND VESTIBULAR ENDORGAN MORPHOLOGY IN THE RAT. N.G. Daunton, M.D. Ross*, R.A. Fox, M.L. Corcoran*, L.K. Cutler*, and L.C. Wu*. Life Science Division, NASA Ames Research Center, Moffett Field, CA 94035

As part of an effort to identify the neural mechanisms underlying adaptation to altered gravity, behavioral and morphological studies have been carried out on rats exposed to hyper-gravity for a maximum of 16 days. To assess changes in vestibular function and neuromuscular coordination induced during adaptation to 2-G, the righting reflex was tested by dropping each rat 45 cm from a supine position into a tank of warm water. The landing attitude of each animal and time to resurface following the drop were determined from video images. Animals were tested within 20 - 30 minutes of removal from the centrifuge, and then again after 48 hours and 8 days. Immediately after removal, half of the 2-G and none of the Control animals failed to right. In addition, the 2-G animals took significantly longer to resurface following the drop than the Controls, with some 2-G animals swimming in a downward rather than upward direction. Righting and resurfacing returned to normal over the recovery period.

To assess the possibility that these behavioral changes may be due in part to changes in processing of vestibular (particularly otolithic) input at the periphery, the neuroepithelium of the utricular maculas of centrifuged and control animals was studied. Results indicate that there was a 30% reduction in the number of synapses of macular Type II hair cells in the 2-G animals, but no change in the Type I hair cells. Since this reduction was not seen in the Rotation Control group, it is likely that the increased G component, rather than the rotational component of centrifugation, was responsible for this change. Additional work is required to determine whether this change in morphology results in an increased threshold for linear acceleration and thus underlies the deficits seen in righting and resurfacing in 2-G-adapted animals.

128.6

VELOCITY CHARACTERISTICS OF OPTOKINETIC AND VESTIBULAR EYE MOVEMENTS IN NORMAL HUMANS. M.J. Morrow, R.W. Baloh, K.M. Jacobsen*, C. Mai*, J. Burrows*. Department of Neurology, UCLA, Los Angeles, CA 90024

We measured OKN, VOR and VOR fixation-suppression (VOR-Fix) in normal subjects. A full-field striped drum was used to test OKN and VOR-Fix. VOR was measured in darkness. Subjects were examined with low frequency (0.025-0.2 Hz) sinusoidal stimuli at multiple peak velocities up to 120°/sec. We analyzed plots of smooth eye velocity vs. stimulus velocity to assess linear (gain) and non-linear (saturation) elements of responses. Linear portions of eye vs. stimulus velocity plots had slopes averaging 0.95 for OKN and 0.02 for VOR-Fix. OKN responses saturated at velocities of 70-80°/sec. OKN velocity limits were independent of drum accelerations up to 150°/sec^2. VOR-Fix eye velocities did not exceed 10°/sec; VOR never saturated. VOR-Fix peak eye velocities were close to those predicted by subtraction of OKN peak velocities from VOR peak velocities.

At low stimulus frequencies, gain approaches ideal values of unity for OKN and zero for VOR-Fix; responses to high velocity stimuli are limited by velocity saturation. Velocity dynamics of VOR fixation-suppression can be explained by cancellation of vestibular signals with oppositely directed smooth eye tracking signals.

128.7

SPATIO-TEMPORAL CONVERGENCE (STC) IN OTOLITH NEURONS. D.E. Angelaki, Dept of Physiology, Univ of Minnesota, Minneapolis, MN 55455.

It has been recently demonstrated that some primary otolith afferents (Dickman et al., Brain Res, in press) and most vestibular nuclei neurons (Bush and Perachio, Soc Neurosci Abstr 14:330, 1988) encode two spatial dimensions and can be described by two vectors that are in temporal and spatial quadrature (Angelaki, IEEE TBE, in press). These neurons are called broadly-tuned (BT) and are characterized by a non-zero *tuning ratio* which is defined as the ratio of the minimum over the maximum sensitivity of the neuron. BT neurons exhibit response gains that do not vary as the cosine of the angle between the stimulus direction and the cell's maximum sensitivity vector and response phase values that show a dependence on stimulus orientation. These responses were observed with pure linear acceleration; thus spatio-temporal convergence (STC) between primary otolith afferents and/or otolith hair cells could be possible. Simulations of STC in the inputs to primary otolith afferents and vestibular nuclei neurons have revealed several interesting points. First, when BT neurons linearly summate inputs from two narrowly-tuned (NT) units, the greatest tuning ratio is achieved with equal input gains. The smaller the phase difference between the input vectors, the larger orientation differences are required to produce a tuning ratio of a certain value. Orientation and phase differences of 30-40° would create tuning ratios of approximately 0.10-0.15 in primary otolith afferents. Second, when multiple inputs are considered, the greater the number of converging inputs, the smaller the tuning ratio of the BT neuron. The tuning ratio is very sensitive at small numbers of input units, whereas the larger the number of converging inputs, the smaller the effect. For more than 10-20 input units, the tuning ratio can be considered independent of the number of inputs. Further, if the inputs comprise two populations (with different gain and phase values at a given stimulus frequency), the greatest tuning ratio is obtained when the largest population has the lowest gain. Supported by NASA NGT 50581.

128.8

INTRAVENTRICULAR INJECTION OF CALMIDAZOLIUM CHLORIDE DECREASES SPONTANEOUS NYSTAGMUS FOLLOWING UNILATERAL LABYRINTHECTOMY IN THE GUINEA PIG. A.J.Sansom*, C.L. Darlington*, C.J.Keenan*, P.F.Smith*, D.P.D.Gilchrist* & D.K.Bilkey. Department of Psychology and the Neuroscience Centre, University of Otago, Dunedin,New Zealand.

In order to determine whether Ca^{2+}-mediated intracellular pathways are involved in the behavioural recovery which occurs following a unilateral labyrinthectomy (UL),(vestibular compensation) we examined the effects of intraventricular injections of calmidazolium chloride (R24571),a calmodulin antagonist and inhibitor of several Ca^{2+}-dependent enzymes,on the time course of compensation of spontaneous nystagmus (SN).Each experimental animal (n=4) received 3 injections of calmidazolium chloride (1 µL,0.5mM,dissolved in DMSO and artificial cerebrospinal fluid (ACSF),pH 7.0), beginning 30 mins after a UL and spaced at 2 h intervals thereafter.Control animals received injections of the vehicle,DMSO plus ACSF (n=2), or ACSF alone (n=1),using identical procedures to the experimental group. Animals which received calmidazolium injections showed reduced SN compared to control animals ($p < 0.05$, t-test) during the first 15 hs post-UL, following which SN compensated at a normal rate. During the period of SN suppression, animals still showed vestibulo-ocular eye movements. These results suggest that intracellular pathways associated with Ca^{2+} may contribute to vestibular compensation.

128.9

RELATION BETWEEN C1 TERMINAL ARBORS AND SPATIAL PROPERTIES OF MEDIAL VESTIBULOSPINAL TRACT NEURONS (MVSNs). S.I. Perlmutter, L.D. Barke*, Y. Iwamoto*, K.D. Powell, J.F. Baker, B.W. Peterson, Northwestern Univ. Med. School, Chicago

Directional sensitivity of neck muscles for 3-D rotations depends on spatial properties of vestibulospinal cells and appropriate distribution of their signals in the spinal cord. In decerebrate cats, HRP or Neurobiotin was injected intraaxonally at C1 in 21 MVSNs monosynaptically activated from ipsi- or contralateral labyrinth. A vector representing the rotation producing maximal activation was derived from responses to 0.5 Hz rotations in 2-11 vertical planes and yaw. Neurons were tested for antidromic activation from IIIrd nucleus and C6 ventral funiculus.

MVSNs terminating above C6 had many C1 collaterals (1/1.5 mm, on average), which usually projected to similar regions of the gray matter. Axons preferentially innervated the ventral horn (especially motor nuclei, laminae VII and VIII), but some branches extended toward the central cervical nucleus or intermediate laminae. Cells with excitation vectors near the horizontal canal axis projected to wide areas of the ventral horn, including extensor and flexor motor nuclei. Neurons with vectors near one vertical canal axis had more restricted projections to motor nuclei, interpretable as excitatory and inhibitory substrates for compensatory movements. Collaterals of cells with convergent canal input arborized within narrower regions. Axons activated from C6 had fewer collaterals in C1 (1/3.8 mm); 4/6 (2 with significant type II yaw responses; 2 with spatio-temporal convergent behavior) did not branch within motor nuclei.

Significant divergence of single canal, and narrower distribution of more processed, signals occurs at the spinal level, suggesting functional specialization of MVSNs, and substrates for spatial transformations in the vestibulocollic reflex. NS17489, EY06485, EY05289, EY07342

128.10

VESTIBULAR DISORDERS IN CHORNOBYL CLEAN-UPPERS. Trinus K. RCA Foundation, Lukianivsky Str.27,Apt.47, Kiev-71, Ukraina, GSP252601.

Vertigo is one of the frequent early complaints of the nuclear lesion. This study was to elucidate if it is related to the vestibular lesion. 273 persons the Chornobyl clean-uppers were examined before going to the station.We used mostly the routine tests: Ujemura, Fucudas: writing and stepping; tracking and indicating tests. The abnormality degree was expressed in the score table with the range from 0 to 20, the greater the score – the more expressed disorder. The 5 score was decided to be the sign of the disorder. From all the group there was allowed to work at the station 187 or 68.5% persons. From the persons examined 197 (72.2%) were working at the station during the accident (the doses are unknown), and 76 (27.9%) never been in contact with radiation before. All the persons examined have passed the medical commission and were allowed to work in the zone. Among them 32.5% of those were in the zone beforehand and 32.9% of those who never contacted radiation were considered to be practically absolutely healthy. According to our data out of those who were in the zone 37.1% were not allowed to work, and out of those who had not been in the zone only 14.5% were not allowed to work. The difference is statistically significant. The total # of those who had well expressed vestibular disorders (10 and more) was 18 persons, that means 6.6% from all the examined, among them were in the zone 17 persons (94.4%). In most part of this group of people were diagnosed vascular disorders by therapeutists. The data presented allowed us to suspect the vestibular lesion to be one of the earliest symptoms of the nuclear damage.

128.11

PHYSIOLOGICAL RESPONSES TO VISUALLY INDUCED MOTION SICKNESS

Benton D. Lawson, Fred A. Sunahara, and James R. Lackner
Ashton Graybiel Spatial Orientation Laboratory, Brandeis Univ., Waltham, MA. 02254.

We studied the relationship between severity of motion sickness (MS) and heart rate (HR), blood pressure, forearm blood flow, respiration rate, and gastric rhythmn (electrogastrograms).

14 Subjects received 10 min. of optokinetic stimulation at 60 deg/sec. Symptoms of MS and physiological responses were monitored throughout stimulation, and during 10 min. baseline and recovery periods.

Subjects were divided into high, medium, and low MS groups. Analyses of variance with contrasts were performed on 4 time periods: baseline, early stimulation, late stimulation, and recovery. Only HR showed a significant change that was related to MS group (ANOVA $F=7.1$, $p<.05$ for high vs low MS from baseline to late stimulation). HR increased 9bpm +/- 5 for the high ms group and lbpm +/- 3 for the low ms grp. 4/5 cases of HR >100 bpm occured during onset of stimulation and prior to onset of MS. All 5 cases were in naive subjects (regardless of MS group). HR and the other physiological responses varied considerably within and across subjects.

The physiological responses did not reliably distinguish individuals, subject groups, or time periods based on severity of MS.

128.12

NMDA-MEDIATED LTP AND LTD IN THE MEDIAL VESTIBULAR NUCLEI. S. Grassi*, G. Capocchi, G. Della Torre*, M. Zampolini, V. Pettorossi* Ins. Human Physiology* and Cl. Neurology, Univ. Sch. of Med. Perugia. Italy

We studied the effect of High Frequency Stimulation (HFS) of ipsilateral primary vestibular afferents on the field potentials recorded in different portions of Medial Vestibular Nuclei (MVN). The involvement of NMDA receptors was assayed using DL-APV. The experiments were performed on rat brain-stem slices maintained in vitro with artificial liquor.

In agreement with previous reports the field potential test (0,05 Hz) was not modified during perfusion with DL-APV. Long Term Potentiation (LTP) of the monosynaptic component was observed in the ventral portion of the MVN following HFS (4 trains 200 Hz, 1 sec., 5 sec. interval) in 6 out of 9 slices; the mean increase of the field potential amplitude was 27.4.%. The induction of LTP was blocked under APV perfusion (100μM). In the dorsal portion of MVN HFS did not significantly affect the monosynaptic component but induced Long Term Depression (LTD) of the polisynaptic component in 6 out of 7 slices. LTD was not observed under DL-APV and completely disappeared under bicuculline perfusion (50μM).

Our results show that LTP and LTD can be induced in different portions of MVN and are dependent on the activation of NMDA receptors. LTD could be due to potentiation of the GABAergic interneurons which modulate the excitatory polisynaptic transmission. We suggest that LTP and LTD could play a role in the vestibular plasticity phenomena such as habituation to prolonged stimulation, rebalancing after hemilabyrinthectomy and visuo-vestibular recalibration.

128.13

RETICULOSPINAL NEURONS AND VERTICAL VESTIBULOSPINAL REFLEXES. P. Bolton, T. Goto*, R.H. Schor, V.J. Wilson, Y. Yamagata*, B.J. Yates. Rockefeller University, New York, NY 10021; Eye & Ear Insitute, Pittsburgh, PA 15213.

To examine the contribution of the reticulospinal (RS) system to vestibulospinal reflexes, we have studied, in the decerebrate cat, responses of medial ponto-medullary RS neurons to natural vestibular stimulation. Neurons projected to neck, lower cervical, thoracic, or lumbar levels, as determined by antidromic stimulation of medial and lateral reticulospinal tracts. Sinusoidal stimuli in vertical planes were used to determine response vector orientation and nature of the vestibular input (otolith, O; canal, C; otolith-canal convergence, O+C; spatial-temporal convergence, STC). Features of the responses of 67 RS neurons were prominent otolith input (32 neurons), less frequent otolith-canal convergence (12 O+C, 6 STC), and only 1 cell with pure canal input; 16 cells could not be fully characterized, although canal input was not apparent. This pattern of vestibular input contrasts sharply with that of vestibulospinal neurons, where the most frequent input is from vertical canals (Wilson et al, JNP 1990). Vector orientations were almost exclusively in the roll quadrants. Our results suggest the RS system plays an important role in the production of gravity-dependent postural reflexes.

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128.14

OPIOID RECEPTORS NEUROPHARMACOLOGY IN THE VESTIBULAR AFFERENTS. R. Vega*, E. Soto and M.E. Pérez*. *Ciencias Fisiológicas, Universidad Autónoma de Puebla, P.O. Box 406, Puebla 72000, México.*

This study was designed to determine the effects of opiate drugs on the electrical activity of the semicircular canal afferents of the vestibular system.

Experiments were done on larval axolotls (*Ambystoma tigrinum*). Multiunit spike activity was recorded from the anterior vestibular nerve by a suction electrode. Naloxone, D-Ala-Leu-Enkephalin (DALE), D-Ala-Met-Enkephalin (DAME) and D-Ala-Gly-Phe-Ol Enkephalin (DAGO) were applied by pressure, ejecting 20 μl each time. Naloxone (10^{-4} to 10^{-8} M), produces a dose-dependent increase of the vestibular afferents discharge. Both DAME and DALE (10^{-10} to 10^{-4} M) induced a biphasic effect. DALE inhibited at low doses (less than 10^{-5}) and excited at greater doses. In contrast, DAME excited at low doses (less than 10^{-4}) and inhibited at greater doses. DAGO (10^{-10} to 10^{-5} M) elicited a dose-dependent long lasting (> 5 min) excitatory effect at all the doses tested.

Our data indicate that the activity of vestibular afferent fibers may be regulated in a complex fashion by the activation of opioid receptors.

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128.15

THE INITIAL VESTIBULO-OCULAR REFLEX AND ITS CANCELLATION IN HUMANS WITH UNILATERAL VESTIBULAR DEFECTS J.L.Johnston and J.A.Sharpe, The Toronto Hospital Neurological Centre and Playfair Neuroscience Unit, University of Toronto, Toronto, Canada M5T 2S8

We investigated the horizontal vestibulo-ocular reflex (VOR) in 5 patients, 1 to 51 months after unilateral vestibular deafferentation, using transient, unpredictable chair movement (Mean head velocity: 34 deg/s, Mean head acceleration: 331 deg/s/s) and magnetic search coil recordings of head and eye motion. Trials were conducted in darkness (VOR) and with a head-fixed target (VOR cancellation). Initial VOR gains during the first 80 ms after head motion were asymmetric, with lower gains for head motion toward the side of the lesion. Initial vestibular smooth eye movement accelerations decreased at peak head acceleration, both toward and away from the side of the lesion. All patients had significantly worse cancellation of the VOR when head motion was toward the lesioned side; the severity of this deficit correlated inversely with the age of the lesion. Unilateral loss of vestibular information impairs cancellation of the reduced contralateral VOR during combined eye-head tracking toward the side of the lesion. These results suggest that the VOR cancellation system employs disfacilitation of the intact side to drive cancellation when head motion is toward the lesioned side.

Supported by the R.S.McLaughlin Foundation and MRC of Canada Grant MT5404 and ME5509.

128.16

EFFECTS OF GINKGO BILOBA EXTRACT ON THE GUINEA PIG VESTIBULAR SYSTEM. T. Yabe, M. Chat*, E. Malherbe* and P.P. Vidal. Lab. de Physiologie Neurosensorielle, CNRS, Paris, FRANCE.

Previous studies have demonstrated that the administration of Ginkgo biloba extract (EGb) improves the compensation of the vestibular syndrome induced by VIIIth nerve transection. In order to investigate the mechanisms at play, the lateral vestibular nucleus of alert normal guinea pigs were perfused unilaterally with EGb. This perfusion always induced a stereotyped reversible postural syndrome which was the mirror image of the syndrome produced by the unilateral lesion of the otolithical receptors. This result supports the hypothesis that EGb has a direct excitatory effect on lateral vestibular nucleus neurons. In a second step, we measured the horizontal vestibulo-ocular reflex (HVOR) of normal and hemilabyrinthectomised guinea pigs following intraperitoneal (IP) injection of EGb. In the normal guinea pig, IP administration of EGb led to a reversible dose-dependent decrease of the HVOR gain without affecting the phase of the reflex. The same diminution of the gain was not found in hemilabyrinthectomized animals. These data help to explain the therapeutic effects of EGb in vestibular syndromes and strongly suggest an impact at the neuronal level.

128.17

CONJUGATE AND NON-CONJUGATE 3D EYE MOVEMENTS DURING STATIC AND DYNAMIC OTOLITH STIMULATION IN THE MONKEY. BJM Hess, Th Haslwanter*, Neurology Dept, University Hospital, CH-8091 zürich, Switzerland

Rhesus monkeys were implanted on both eyes with dual scleral search coils for recording horizontal, vertical, and torsional eye position (3d ep), They were tested in the dark in 90° right/left side down, in supine/prone position, and during constant velocity rotations on a three-axes turntable. In side down position, vertical (only in dark) and torsional (in dark and light) left and right ep differed, resulting in a skew deviation. No such deviation was present in supine (prone) position. Pitch rotation evoked a sustained vertical nystagmus with cyclically modulated beating field. The horizontal but not the vertical or torsional component of the difference between the right and left ep was modulated, resulting in a change of the vergence angle (peak convergence in phase with gaze down). Yaw rotation in side down position (barbecue spit) or roll rotation elicited a sustained horizontal and torsional nystagmus, respectively. It was superimposed on a non-conjugate cyclic modulation of horizontal, vertical, and torsional ep. These findings will be discussed in the light of the presumed spatial arrangement of disynaptic excitatory utricular inputs to the oculomotor plant (Hess & Dieringer, J Neurophysiol, 1991).

128.18

ACTIVATION OF BRAINSTEM NUCLEI FOLLOWING CENTRIPETAL ACCELERATION IN THE RAT. G. D. Kaufman, J. H. Andersont, A. J. Beitz, Departments of Veterinary Biology and Otolaryngologyt,University of Minnesota, St. Paul, MN 55108.

A sheep polyclonal antisera to the Fos protein (Cambridge) was used to identify activated neurons in rat brainstem following 90 minutes of earth-horizontal centripetal acceleration at 2 G. In one set of experiments, 250 gram Long-Evans (pigmented) rats were partially restrained inside metal cones in darkness and positioned either on-axis or ~50 cm eccentric from the axis of rotation which was earth vertical. Within one hour after the centripetal stimulus, the rats were anesthetized, perfused, and the brains processed for immunohistochemistry. Compared to the on-axis animals, the off-axis rats had significantly greater Fos labeling in the area postrema (AP), solitary nucleus (sol),vagal nucleus, vestibular nuclei, interstitial nucleus of Cajal (inC), and the dorsomedial cell column (DMCC) of the inferior olive. On-axis animals had no Fos-immunolabeling in the DMCC, but did have some in areas associated with stress (Locus coeruleus, central grey, and adrenergic cell groups), similar to the off-axis rats. Room controls had little to no Fos immunolabeling in any of the nuclei. In another set of experiments, rats were restrained inside the cones with a skull cap so that the position of the vestibular labyrinth was fixed relative to the gravitoinertial force. When rats were lightly anesthetized (Chloral hydrate, ~300mg/kg, IP) for the duration of the spin, the off-axis animals showed no DMCC labeling, but did label trochlear neurons. Anesthetized on-axis rats revealed intense, AP and sol immunolabeling, and light immunostaining of the beta subunit in the medial accessory olivary nucleus. Our data defines some of the temporal and spatial CNS sequelae to sustained, hypergravity stimulation, and describes an otolith-olivary pathway which may be important in vestibular adaptation and compensation. Supported by NASA/NGT-50563, NIH DC01086, DC00110, DA06687.

128.19

AUGMENTATION OF CALORIC NYSTAGMUS BY VISUAL FIXATION IN PATIENTS WITH SPINOCEREBELLAR DEGENERATION AND PROGRESSIVE SUPRANUCLEAR PALSY. H. IMAI, N. ISHIKAWA*. Dept. of Neurology, Juntendo Univ. Sch. of Med., Tokyo 113, Japan.

Caloric nystagmus is suppressed by visual fixation in normal subjects. This visual suppression of horizontal caloric nystagmus is lost or significantly reduced by flocculus destruction. However, it is known that visual suppression of caloric nystagmus is abolished and, furthermore, caloric nystagmus is augmented by visual fixation frequently in patients with spinocerebellar degeneration. On the other hand, in patients with progressive supranuclear palsy (PSP), it was reported that the caloric test evoked tonic deviation of the eyes and head. We examined and observed both signs in 4 cases of multiple system degeneration in the wide sense: PSP, dentatorubropallidoluysian atrophy, Joseph disease and familial spastic paraplegia. For the caloric test, the ear was irrigated with 50 ml of water at 20°C for 20 sec. From our results, the responsible neuronal groups of lesions will be in the neuronal circuit generating the quick phase of vestibular nystagmus: excitatory burst neurons (EBNs) in the PPRF and/or burster-driving neurons (BDNs) in the medullary reticular formation, a direct afferent to the EBNs.

128.20

THE EFFECTS OF TARGET DISTANCE ON COMPENSATORY PITCH HEAD MOVEMENTS PRODUCED DURING LOCOMOTION. J. J. Bloomberg*[1], M. F. Reschke*[1], B. T. Peters*[2] and W. P. Huebner[2] (SPON: F. Kutyna) [1]Space Biomedical Research Institute, NASA Johnson Space Center, Houston, TX, and [2]KRUG Life Sciences, Houston, TX.

Berthoz and Pozzo (1988) have suggested that gait motor programs operate in a "top-down" fashion to preserve gaze stability during locomotion. To further investigate this hypothesis we had three subjects walk (6.4 km/h) on a motorized treadmill while visually fixating an earth-fixed target positioned in the center of view either near (30 cm) or far (2 m) from the head. Head movements were recorded using a video-based motion analysis system. During gaze fixation of both near and far targets, pitch head movements were compensatory for linear Z-axis head motion. The mean peak to peak amplitude of compensatory pitch head movements for all 3 subjects increased from 2.24 ± 0.07° (mean ± S.E.) in the far target condition to 3.31 ± 0.22° in the near target condition, despite no significant change in mean peak to peak linear Z-axis head motion (3.03 ± 0.77 cm, far and 2.99 ± 0.82 cm, near). This result confirms that compensatory pitch head movements are driven by the need to stabilize gaze during locomotion. This stabilization is achieved through a synergistic combination of goal-directed eye and head movements. Gait instabilities experienced by astronauts upon return to Earth may be caused by in-flight adaptive acquisition of new motor strategies designed to maintain head and gaze stable in microgravity, but may be inappropriate for a terrestrial environment.

128.21

NMDA AND APAMIN INDUCED OSCILLATIONS IN THE GUINEA PIG MEDIAL VESTIBULAR NUCLEI : AN IN VITRO AND IN VIVO STUDY. M. Serafin, C. de Waele*, A. Khateb, P.P. Vidal, and M. Mühlethaler, *Dept. de Physiologie, CMU, 1211 Genève 4, Switzerland and *Lab. de Physiologie Neurosensorielle, CNRS, Paris, France.*

We have recently identified in the guinea pig medial vestibular nuclei (MVN) two main neuronal cell types, A and B MVNn, differing by their intrinsic membrane properties. In addition, one subtype of B MVNn was distinguished by the characteristic presence of low threshold calcium spikes (LTS). Both A and B MVNn were depolarized by **NMDA**, which also induced a decrease in membrane resistance and an increase in the spontaneous firing rate. These effects could be blocked by D-AP5, a specific antagonist of NMDA receptors. Long-lasting NMDA application resulted in a persistent oscillatory bursting behavior when the cells were maintained under a sustained membrane hyperpolarization of 10-30 mV. These oscillations were however restricted to the subtype of B MVNn without LTS. Neither type A nor type B+LTS MVNn could ever be brought to oscillate in presence of NMDA. These NMDA-induced oscillations were TTX-resistant, but could be eliminated either by D-AP5, or by replacing sodium with choline. Another similar type of oscillation could be triggered with **apamin**, presumably by blocking calcium-activated potassium conductances. However these oscillations were resistant to D-AP5 and could be eliminated by TTX. In vivo, perfusion of the MVN with apamin resulted in long-lasting head oscillations. It is therefore speculated that these oscillations might be of functional importance and might in particular play a role in the rhythmic firing of vestibulospinal neurones during locomotion (Supported by a Swiss NSF grant nº 31-26495.89 and the French Ministère des Affaires Etrangères).

128.22

PHARMACOLOGY OF MEDIAL VESTIBULAR NEURONES IN GUINEA PIG BRAINSTEM SLICES: PRELIMINARY RESULTS. N. Vibert*, M. Serafin, A. Khateb, P.P. Vidal and M. Mühlethaler, *Department of Physiology, CMU, 1211 Geneva 4, Switzerland.*

We have recently identified in the guinea pig medial vestibular nuclei (MVN) two main neuronal cell types, A and B MVNn, differing by their intrinsic membrane properties (Serafin et al.; Exp. Brain Res. 1991). These cells, which had already been shown to respond to histamine and NMDA (Serafin et al.; Soc. Neurosci. Abst. 1990, 1991), were selected for further pharmacological studies. Both A and B MVNn were depolarized by serotonin, which also induced a decrease in membrane resistance and an increase in the spontaneous firing rate. At least on B MVNn, these effects seemed to be mediated through several receptor subtypes, including 5-HT 1 and 5-HT 2 receptors. Preliminary results also indicated that trans-ACPD, a new specific agonist for glutamate metabotropic receptors, also strongly depolarized and excited both types of MVNn. In contrast, they were apparently both hyperpolarized by application of GABA and glycine, which also provoked a decrease in their spontaneous firing rate. The effects of substance P were also studied; though it seemed to have no effect on A MVNn, it clearly depolarized and excited B MVNn. Together with preliminary studies concerning other neurotransmitters (including acetylcholine, norepinephrine and dopamine), these results point to a good pharmacological homogeneity of type B MVNn, whereas A MVNn appear to present much more heterogeneous responses. (Supported by the Swiss NSF, with N. VIBERT being a Swiss Confederation fellow)

129.1

INVESTIGATION OF THE NEURO-MUSCULAR SYSTEM OF CONDYLACTIS GIGANTEA (Cnidaria: Anthozoa). C. DellaCorte and W.O. McClure. Dept. Biol. Sci., Univ. of Southern Calif., Los Angeles, CA 90089.

The phylum Cnidaria has traditionally been considered structurally primitive. Recent studies, however, have shown specializations that are unexpectedly complex. We now report an ultrastructural study of the neuro-muscular system which is in agreement with more sophisticated organization. Transmission electron microscopy shows the presence of both discrete and bundled longitudinal muscle processes on the ectodermal surface of the mesoglea. Scanning electron micrographs show nerve tracts along the top of the muscle bundles. Many of the neurons appear unipolar, with cell bodies which lie above the neuronal fibers. Along their length, the fibers have varicosities which are presumed to provide synaptic contacts to surrounding structures. The cellular location of several neuron-related antigens was investigated using immunocytochemistry. Distribution of the immuno-stained product at the level of light microscopy shows highly selective localization at the ectodermal-mesogleal interface. Work is in progress to confirm the localization and function of this neural network. (Supported by the National Institutes of Health.)

129.2

EFFECTS OF LESIONING THE TERMINAL NERVE ON GNRH IMMUNOCYTOCHEMISTRY IN THE ROUND STINGRAY, D.E.Wright and L.S.Demski. Sch. of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506.

In order to distinguish the distribution of GnRH-immunoreactive (ir) fibers of the terminal nerve (TN) from those of mesencephalic GnRH cells, (Wright & Demski, J. Comp. Neurol., 307: 49-56, '91) the white body (WB) ganglion and fibers of passage of the TN were lesioned via cauterization (unilateral, n=3; bilateral, n=5). Animals were sacrificed after survival times of 14-62 days. GnRH systems were immunocytochemically labeled with anti-salmon GnRH antisera. After 28 days, WB ablation resulted in loss of most of the immunoreactivity in TN GnRH fibers entering the dorsal forebrain. Scattered bipolar GnRH-ir cells (10-15um) remained intact along the original TN pathway, contributing a few fibers to the TN system. GnRH-ir was virtually eliminated in the dorsolateral pallium, nucleus septi caudoventralis, rostromedial preoptic area, and diffuse areas of the forebrain. GnRH-ir fibers persisted in the habenula, fasciculus retroflexus, posterior hypothalamus, thalamus, lateral tegmentum, periaquaductal grey, tectum, ventral medulla and spinal cord. Thus, in lesioned animals, remaining labeled fibers probably originate from the mesencephalic GnRH cells.

129.3

LOCALIZATION OF MONOAMINERGIC AND ENKEPHALINERGIC CELLS IN THE BRAINSTEM AND DIENCEPHALON OF A CARTILAGINOUS FISH. S.L. Stuesse and W.L.R.Cruce. Neurobiol. Dept., N. E. Ohio Univ. Col. Med., Rootstown, OH 44272.

There are two major radiations of cartilaginous fish: elasmobranchs and holocephalians. We studied the organization of the brainstem and diencephalon of a holocephalian, *Hydrolagus colliei*, and compared it to our previous results on elasmobranchs. We used antibodies against three substances, tyrosine-hydroxylase (TH), leu-enkephalin (LENK), and serotonin (5-HT) and the peroxidase-antiperoxidase method to visualize immunopositive cells. TH+ cells were found in three locations, diencephalon (thalamus and hypothalamus), locus coeruleus, and myelencephalon (A1/A2 cell group). No substantia nigra (A9), ventral tegmental area (A10), or A5 cell group was seen. LENK+ and 5-HT+ cells were concentrated in raphe nuclei (raphes magnus, pallidus, obscurus, and centralis superior). The rostral ventral metencephalon contained a large 5-HT+ cell group (putative B7/B9), but the dorsal metencephalon did not (no dorsal raphe). The pre-tectal area contained numerous 5-HT+ and LENK+ cells. These cells have been described in teleosts and a lizard, but not in mammals. Thus the brains of these fish contain some, but not all, of the immunoreactive cell groups described in other vertebrates. An analysis of selected traits may give us insights into the evolution of these immunopositive cell groups. NIH grant NS25895.

129.4

THE OCTAVOLATERAL REGION OF AN ADVANCED RAY, *DASYTIS SABINA*. R. L. Puzdrowski and R. B. Leonard, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

In elasmobranchs, the medullary octavolateral region is characterized by a pair of cell plates termed C1 and C2 (Smeets, '83), which lie at the level of entrance of the posterior lateral line nerve (PLLN) and the trigeminal nerve, respectively. These cell plates lie within the terminal field of the primary afferents of the anterior lateral line nerves (ALLNs) (Bodznick and Schmidt, '84) and project to the midbrain (Barry, '87). In the Atlantic stingray, *Dasyatis sabina*, the presence of a third cell plate which we term C3, prompted us to examine the primary projections of the octavolateral nerves and the second order octavolateral projections to the midbrain using HRP and cobalt-lysine techniques.

The organization of the octavolateral nuclei in *D. sabina* is similar to that described in other elasombranchs. The eighth nerve projects to the octaval column, the reticular formation, and to the vestibulolateral lobe of the cerebellum. The PLLN projects to the lateral portion of the intermediate nucleus. The ALLNs project to the medial portion of the intermediate nucleus, including cell plates C1 and C2, and to the magnocellular nucleus of the octaval column. Cell plate C3 lies just beneath the medial border of the cerebellar crest and extends from the level of the entrance of the eighth nerve, caudally to the rostral pole of the solitary nucleus. Cell plate C3 lies outside the terminal fields of the octavolateral nerves and thus appears not to receive primary projections. Injections of wheatgerm-HRP into the medial and lateral mesencephalic nuclei labels cells in the anterior octaval nucleus and C1, C2, and C3 contralateral to the injection site. The full connectivity of C3 remains to be elucidated. Supported by NIH grants DC00036 and NS11255.

129.5

THE VALVULA CEREBELLI RECEIVES PRIMARY LATERAL-LINE AFFERENTS FROM THE ROSTRUM OF THE UPPER JAW IN A SPINY EEL. M.F.Wullimann, M.H.Hofmann* and D.L.Meyer*. Brain Res. Institute, Univ. of Bremen, Postfach 33 04 40, 2800 Bremen 33, FRG & Dept. of Neuroanatomy, Sch. of Med., Kreuzbergring 36, Univ. of Göttingen, 3400 Göttingen, FRG

In the spiny eel, Macrognathus aculeatus, lateral-line nerve labeling with horseradish peroxidase (Böhringer) reveals that anterodorsal and, to a lesser degree, anteroventral lateral-line nerves project massively to the granular layer of the valvula cerebelli, throughout its rostrocaudal extent. The posterior lateral-line nerve terminates heavily in the corpus cerebelli. Thus, valvula and corpus cerebelli are supplied with mechanosensory input of different peripheral origins. The massive lateral-line input to the valvula in Macrognathus originates mostly in mechanoreceptors located in the elongated rostrum of the upper jaw which is characteristic of mastacembeloid fishes. This projection to the valvula, therefore, is likely to represent a unique specialization which arose with the evolution of the peculiar rostrum.

Supported by the Swiss National Science Foundation, the Janggen-Pöhn-Stiftung (MFW) and the German Science Foundation (DLM).

129.6

CENTRAL TOPOGRAPHY OF ELECTRO- AND MECHANOSENSORY LATERAL LINE AFFERENTS IN THE CHANNEL CATFISH, *Ictalurus punctatus*. S. Singh* and J.G. New, Dept. of Biology, Loyola University of Chicago, Chicago, IL 60626

Afferent anterior lateral line nerve (ALLN) fibers innervating electro- and mechanoreceptor organs terminate within medullary and cerebellar nuclei. Electrosensory fibers terminate principally in the electrosensory lateral line lobe (ELLL) whereas mechanosensory fibers terminate primarily in the medial octavolateralis nucleus (MON). The purpose of this study is to determine the central projections of fibers in the principal ALLN branches to determine the topography within these nuclei.

Branches of the ALLN were exposed, transected, and horseradish peroxidase applied to the proximal root. After survival times of 10-14 days the fish were transcardially perfused, the brains removed, sectioned and the tissue developed according to standard HRP protocols.

Fibers of the superficial ophthalmic (SO) branch terminate laterally in the ELLL and medially in the MON. The hyomandibular (HM) nerve fibers terminate medially in the ELLL and laterally in the MON. In both the ELLL and MON fibers from the buccal (B) branch of the ALLN terminate between those of the HM and SO branches.

Our studies reveal that in both the MON and ELLL there exists a topographic organization of ALLN fibers. The patterns of the terminal fields within the two nuclei is reversed along the mediolateral axis of the two nuclei. The electrosensory system in the catfish may have evolved as a specialization of the mechanosensory system. The common origin of the ELLL and MON may explain the similar topography of ALLN projections, notwithstanding that the two systems detect different stimuli.

129.7

INNER EAR ENDORGAN PROJECTIONS IN THE CATFISH Ictalurus punctatus. C.A. McCormick and M.R. Braford, Jr. Dept. of Biology, Oberlin College, Oberlin, OH 44074

Previous studies revealed that eighth nerve projections in catfish species are specialized relative to those of non-teleost fishes (Finger and Tong '84; McCormick and Braford '88; Fritzsch et al. '90). This study extends these observations by examining individual projections of the 7 otic endorgans. Horseradish peroxidase applied to a given endorgan nerve branch was allowed to migrate for 18-27 hrs in exsanguinated specimens. HRP-labeled fibers were visualized in transverse sections using TMB (Mesulam, '78). The largest octaval nucleus, the descending, is composed of lateral and medial portions. The medial portion, which corresponds to the medial auditory nucleus of Finger and Tong ('84), receives input predominantly, and possibly exclusively, from the saccule. The lateral portion of the descending nucleus has 3 zones. The dorsal zone receives a heavy saccular input and a very light lagenar and utricular input. The intermediate zone receives input from all otic endorgans. The ventral zone receives input mainly from the semicircular canals. The anterior octaval nucleus also has a lateral portion supplied by all otic endorgans and a medial portion in which saccular input predominates. The magnocellular nuc. receives input from all otic receptors. The tangential nuc. is supplied mainly by the semicircular canals.

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129.8

THE DISTRIBUTION OF AUTONOMIC AND VISCERAL SENSORY NERVE FIBERS IN THE BRAINSTEM, SPINAL CORD, AND GUT OF THE CATFISH, ICTALURUS PUNCTATUS. L. E. Goehler. Dept. C&S Biology, UCHSC, Denver, CO 80262.

The extrinsic nerves (vagal, splanchnic, sensory) innervating the vertebrate abdominal viscera provide the means by which the CNS regulates visceral functions. The aims of the present study were to trace the distribution of visceral nerves in the CNS and gut of the catfish, using the carbocyanin dye, DiI. In the brainstem, crystals of DiI placed on the abdominal branch of the vagus labeled fibers in the general visceral nucleus, as well as numerous somata in the caudal dorsal motor nucleus of the vagus. In the rostral spinal cord, crystals of DiI placed on the splanchnic nerve labeled fibers travelling in a dorsal tract, terminating in the lateral funicular nucleus and in a ventromedial subnucleus of the medial funicular nucleus. In the gut, crystals of DiI placed on the combined vagus/splanchnic labeled numerous fibers of the myenteric plexus, innervating unlabeled ganglia, as well as the submucosal plexus, oxyntic cells, and subepithelium.

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129.9

LOCALIZATION OF THYROTROPIN-RELEASING HORMONE-LIKE IMMUNOREACTIVITY IN THE CHINOOK SALMON. S. Matz* and T.T. Takahashi. Institute of Neuroscience, University of Oregon, Eugene, OR 97403

We studied the distribution of thyrotropin-releasing hormone (TRH)-like immunoreactivity in the brain of juvenile chinook salmon (*Oncorhynchus tshawytscha*) with the use of a polyclonal antibody. We found TRH-positive cell bodies in the preoptic area of the forebrain. Based on the size, morphology and position of these cell bodies, we assigned them provisionally to the posterior parvocellular preoptic nucleus. We also found TRH-positive cell bodies in the internal cellular layer of the olfactory bulb. Fine, beaded, TRH-positive fibers were also present in various regions throughout the brain, but were especially dense in the ventral hypothalamus and in the medial and posterior regions of the dorsal forebrain. TRH-positive fibers were also found in the areas surrounding the anterior commissure rostrally into the ventral telencephalon and caudally into the preoptic area. The staining described above required perfusion with 0.5% glutaraldehyde and 4% paraformaldehyde (in 0.1 M PBS). Perfusions with paraformaldehyde alone resulted in no staining. Preabsorption of the antibody with TRH eliminated all of the staining described above.

The localization of TRH-like immunoreactivity in the preoptic and hypothalamic regions is of particular interest since this system may control the release of thyroxine, a hormone involved in the imprinting of salmon to their homestream odor as well as many of the aspects of smoltification in salmon. The localization of TRH-positive cell bodies in the olfactory bulb and the wide distribution of fibers lends credence to the notion that TRH also has a neurotransmitter role.

129.10

MELATONIN BINDING SITES IN FISHES. A.J. Vernadakis*, W.E. Bemis* and E.L. Bittman, Department of Zoology, Univ. of Massachusetts, Amherst.

Through its secretion of melatonin, the pineal complex of lower vertebrates exerts a range of physiological effects including regulation of diurnal rhythms, reproduction, metamorphosis, and body color change. Little is known about evolutionary trends in the distribution and characteristics of melatonin binding sites. In vitro autoradiography was used to examine binding in 20-micron frozen brain sections of amphioxus (Branchiostoma lanceolatum), hagfish (Myxine glutinosa), adult and larval lamprey (Petromyzon marinus), little skate (Raja erinacea), and rainbow trout (Salmo gairdneri). Tissue was incubated with 2-[^{125}I]-iodomelatonin (IMEL) under previously described conditions (J. Neuroscience 9:2581) in the presence or absence of unlabeled melatonin (1uM, in order to assess nonspecific binding). A concentration of 32pM IMEL was used for single point assays and competition studies.

No specific binding was found in hagfish or amphioxus, which lack a pineal complex. In skate and trout optic tectum, IMEL binding is highly specific (melatonin>>NAS>5-MT>>5-HT). Scatchard analysis revealed that tectal IMEL binding is of high affinity (K_d of 36, 38 and 50pM) and low capacity (B_{max} of 8.1, 19.8, and 21.8 fmol/mg protein in lamprey, skate and trout, respectively). In adult lampreys, intense specific IMEL binding is found in the optic tectum (layer I>II>III) and preoptic nucleus (pars parvocellularis>magnocellularis). Binding was less intense and consistent in the same areas of ammocoete brain. In skates and trout, intense specific binding is found in optic tectum and diencephalic preoptic and suprachiasmatic nuclei. These results indicate that specific melatonin binding sites represent a phylogenetically primitive vertebrate characteristic whose functions might include regulation of visual and endocrine responses to light. (Supported by NSF BNS86-16935 and NIMH RO1-44132.)

129.11

GNRH IN THE SEXUALLY POLYMORPHIC TELEOST FISH, *PORICHTHYS NOTATUS*: IMMUNOCYTOCHEMISTRY AND *IN SITU* HYBRIDIZATION. M.S. Grober, D. Myers*, T. Myers* and A. Bass. Section of Neurobiology and Behavior, Cornell University; and Department of Physiology, New York State College of Veterinary Medicine, Ithaca, New York, 14853.

The plainfin midshipman, *Porichthys notatus*, exhibits two alternative male reproductive morphs: Type I males establish nests from which they call to attract females to spawn; smaller, and apparently earlier maturing Type II males attempt to sneak fertilizations with females that are paired with nesting Type I males. The organization of the gonadotropin releasing hormone (GnRH) system is being studied because of its known role in controlling the ontogeny of hormone-dependent sexual traits.

Using a monoclonal antibody to GnRH, it was found that females and both male morphs have similar distributions of GnRH-like immunoreactivity in the nervus terminalis ganglion (NT) and the preoptic area (POA). However, females had significantly more GnRH POA cells than either male morph. When corrected for body size, the POA of Type I males had significantly fewer GnRH-positive cells compared to females or Type II males, which were similar. Preliminary data indicate that juveniles have fewer GnRH POA cells than adults. These differences in GnRH POA cell number may reflect differential rates of development, and result in the alternative reproductive behaviors exhibited by the two male morphs.

To identify the mechanisms underlying GnRH dimorphisms, *in situ* hybridization is being used to probe for cells containing GnRH-mRNA. Thus far, using a DNA oligonucleotide probe developed to the putative nucleotide sequence of salmon GnRH, only the NT has been labelled. These preliminary results suggest that sexual polymorphisms may result from the differential distribution of GnRH-positive cells and/or the cell-specific expression of different GnRH peptides.

129.12

ALLOMETRY OF THE TELEOST VISUAL SYSTEM IN RELATION TO ENVIRONMENT AND BEHAVIOR. Nico A.M. Schellart* (SPON: European Neurosc. Association). Lab. of Medical Physics, Acad. Med. Centre, Univ. of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam.

With a mathematical model, based upon the allometry of the eye, and the Stratum opticum (SO) and Stratum fibrosum et griseum superficiale (SFGS) of the optic tectum, the spatial visual performance of 28 species was estimated. To allow interspecific comparison, bodyweight was standardized. To establish allometric relations, the allometric function $y = kx^{d(1-a\log x)}$ is introduced (d depends on the dimensions of x versus y, k and a are constants to be fitted). This function was used in addition to the classical allometric function. The four variables of the model, i.e the focal length of the eye, the surface of SO + SFGS, the thickness of SO + SFGS and the density of the optic projection (latter datum gathered from literature), together constituting the visual index, show mutually a high positive correlation. The visual index was obtained by multiplication of four parameters, each indicating the factor by which the respective variable deviates from the interspecific, body weight dependent mean.

It was found that the volume of SO + SFGS is, irrespective of fish size, proportional to the retinal surface. On average, this also holds interspecifically. The visual index shows a relation with published data about the psychophysical determined minimum resolvable angle.

The visual index, with a range of 1.7 log unit, appears to be interrelated with behavioral and environmental characteristics. Species with low indices are often benthic. However, the period of foraging during the diurnal cycle is not related in a simple way to the visual index. High indices are found among predatory and especially piscivorous species and among pelagic species living in clear waters.

129.13

RETINAL PROJECTIONS IN A CLUPEOMORPH TELEOST. Ann B. Butler and R. Glenn Northcutt. I.T.N.I., 4433 N.33rd St., Arlington, Va. 22207 and Scripps Inst. Oceanog. and Dept. Neurosci. A-001, Univ. Ca. San Diego, La Jolla, Ca. 92093.

Retinofugal projections were studied in four Atlantic herrings (*Clupea harengus*) which received intraocular injections of HRP (Sigma) under MS222 anesthesia. After six days survival, the animals were perfused under anesthesia with phosphate buffer followed by 4% glutaraldehyde in buffer. Sections cut at 35μm were reacted with the Hanker-Yates [1977] protocol. A cytoarchitectonic analysis of nuclei was carried out on Bodian and cresyl violet serial sections before charting the projections.

Contralaterally, the retina projects sparsely to nuclei suprachiasmaticus (SC), anterior (A), and ventromedialis (VM) and to the periventricular nucleus of the posterior tuberculum (TPp). Dense terminal fields lie over nuclei intermedius (I), pretectalis superficialis pars parvocellularis (PSp), corticalis (NC), pretectalis centralis (PC), and pretectalis periventricularis pars dorsalis (PPd). The dorsal and ventral accessory optic nuclei also receive contralateral projections. Ipsilateral projections reach I and PPd. A sulcus divides the tectum into two lobes. Contralateral retinal projections reach the dorsomedial lobe via the dorsal optic tract (DOT) and the caudolateral lobe via the ventral optic tract (VOT).

While the distribution of retinal projections to diencephalic and pretectal nuclei is similar to that in other teleosts, the presence of two tectal lobes innervated separately by the DOT and VOT appears to be apomorphic.

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129.14

EXPERIMENTAL EMBRYOLOGICAL EVIDENCE OF THE PLACODAL ORIGIN OF GnRH AND FMRF-amide NEURONS OF THE TERMINAL NERVE AND PREOPTIC AREA IN SALAMANDERS. R.G. Northcutt and L.E. Muske. Dept. Neurosciences, UCSD, La Jolla, CA 92093; Dept. Biology, Franklin and Marshall College, Lancaster, PA 17604.

To test the hypothesis that neurons of the terminal nerve and preoptic area arise from the olfactory placode, this placode was bilaterally extirpated in 30 stage 26-29 axolotls (*Ambystoma mexicanum*) and the embryos reared for at least four months. Experimental and control juveniles were anesthetized and perfused with cold 4% buffered paraformaldehyde (pH 7.4). The brains were removed, embedded in 10% gelatin and postfixed for 12h. Experimental and control brains were paired and run in tandem. Transverse or horizontal frozen sections were cut and incubated with commercial antisera made in rabbit to GnRH or FMRF-amide (1:4000). Antibodies were localized using a metal intensified peroxidase-antiperoxidase reaction. About half the experimental cases exhibited no olfactory organs, nerves or bulbs and no forebrain GnRH reactive cells or fibers; in the same cases, no FMRF-amide reactive cells or fibers occurred in the position normally occupied by the terminal nerve but did occur in other positions. In control cases, GnRH and FMRF-amide reactive cells and fibers of the terminal nerve and preoptic area were observed in the same blocks. This is the first direct experimental evidence that ganglionic cells of the terminal nerve and preoptic area arise from the olfactory placode. (Supported in part by NIH grants NS24669 and NS24869.)

129.15

INTRASPECIFIC VARIATION IN IMMUNOREACTIVE GnRH IN THE POSTERIOR HYPOTHALAMUS OF AN AMPHIBIAN. L.E. Muske, A. Loeffler*, R.J. Dreher*, F.L. Moore. Biology Dept., Franklin & Marshall College, Lancaster PA 17604; Zoology Dept., Oregon State Univ., Corvallis OR 97331.

Recent reports of GnRH-immunoreactive (ir) neurons in the posterior hypothalamus or midbrain prompted a re-examination of the distribution of these cells in a urodele amphibian. Adult male and female T. granulosa were captured in March (breeding) or August (sexually inactive) and brains processed for immunocytochemistry. Sections were collected across sets of slides and treated with one of several GnRH antisera or with anti-FMRFamide. Antibodies were localized with a commercial avidin-biotin-HRP preparation. Anti-mammalian GnRH #635 (Dr. L. Jennes) revealed a large population (up to 50 somata/25 uM section) of CSF-contacting neuronal perikarya in the ventral diencephalon of a few individuals. Where staining was robust, GnRHir periventricular neurons extended from the preoptic recess to the posterior margin of the infundibular recess. There was no apparent anatomic relationship between these neurons and the terminal nerve/septal/POA GnRH population. Since FMRFamide immunolabeling was uniform across subjects, it appears that individual variability in GnRHir staining in the posterior hypothalamus is not an artifact of technique, nor is there any apparent association with sex or reproductive state. Supported by a Faculty Research Grant from Franklin and Marshall College.

129.16

ANATOMY OF THE OLFACTORY AND VOMERONASAL SYSTEMS IN *AMPHIUMA TRIDACTYLUM* AND *SIREN INTERMEDIA*. H.L. Eisthen, D.R. Sengelaub, and D.M. Schroeder. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Jurgens (1971) and Bertmar (1981) suggest that the vomeronasal (VN) system arose in tetrapods as an adaptation to terrestriality, a view which has led to the assumption that the VN system is absent in aquatic amphibians. Conversely, Broman (1920) and Parsons (1971) conclude that presence of the VN system is plesiomorphic in vertebrates and that the olfactory system is evolutionarily newer. We are conducting studies of the phylogenetic distribution of these chemosensory systems in aquatic salamanders. We have previously described the anatomy of the olfactory and VN systems in relatively derived aquatic salamander species. However, we have found that the VN system is absent in mudpuppies, leaving open the possibility that mudpuppies represent the ancestral condition and that the presence of the VN system in larval and neotenic salamanders is a derived character-state.

We have examined the forebrain and nasal cavities of two salamander species from families that are considered primitive relative to mudpuppies. In the three-toed amphiuma (*Amphiuma tridactylum*, family Amphiumidae) olfactory epithelium is located in grooves throughout the medial portion of the nasal cavity. As in other salamanders, the lateral portion of the nasal cavity contains VN epithelium. Preliminary evidence suggests that the nasal cavity of lesser sirens (*Siren intermedia*, family Sirenidae) lacks a lateral diverticulum, but *Siren*, like *Amphiuma*, possesses a clearly-defined accessory olfactory bulb dorsocaudal to the main olfactory bulb, indicating the presence of a VN system.

The presence of olfactory and VN systems in derived and primitive salamanders indicates that the presence of both systems is the ancestral condition in aquatic salamanders. We conclude that the VN system did not arise as a terrestrial adaptation.

129.17

BRAIN ATLAS OF TRH mRNA EXPRESSION IN DEVELOPING AND ADULT FROG: CONTIGUOUS ROWS SPANNING ANTERIOR-TO-POSTERIOR FOREBRAIN SHOW ORIGINAL RELATIONSHIPS BETWEEN DISCRETE NUCLEI. Y.P. Loh and W.P. Hayes. Laboratory of Developmental Neurobiology, NICHD, Bethesda, MD 20892.

Little is known about how discrete vertebrate brain nuclei have evolved and diversified, or about their phylogenetic relationships. Comparative findings in cytoarchitecture and connectivity remain the basis for arguing CNS homology, but as discussed elsewhere this approach can be equivocal (Northcutt, 1984 Amer. Zool. **24**). Recent molecular findings showing that gene structure and CNS distribution are highly conserved for neuropeptides have led us to examine how adult patterns of expression for several neuropeptides are established in *Xenopus*. To this end, embryonic to adult brains were examined by *in situ* hybridization histochemistry using oligonucleotide probes (Hayes and Loh, 1990 Development **110**). The focus here is on the gene encoding thyrotropin-releasing hormone (TRH), because its incipient pattern in forebrain at embryonic day 3.5 (stage 42) arises from bilateral rows of TRH cells (Loh and Hayes, 1989 Neurosci. Abstr. **15**).

Serial sections showed that the embryonic anterior-to-posterior TRH rows continue to undergo progressive anteriorization during larval development; non-contiguous expression was also seen in discrete areas of olfactory bulb, optic tectum, hindbrain brainstem and retina. Adopting the new forebrain nomenclature of Northcutt (1981, An. Rev. Neurosci. **4**) and Neary and Northcutt (1983, JCN **213**), TRH cells formed a path spanning over ten contiguous ventral nuclei from lateral septum to posterior tuberculum, crossing the telencephalic-diencephalic border at the anterior preoptic area to the lateral amygdala and thalamic eminence, and continuing in lateral pallium, nucleus accumbens and ventral striatum. Thus, TRH expression forms a continuum mapping embryonic relationships between adjacent brain nuclei. This in turn, suggests that in phylogeny the sculpting of discrete forebrain nuclei was preceded by the simpler TRH expression pattern.

130.1

NUCLEUS BASALIS SINGLE UNIT ACTIVITY AND CONDITIONED CORTICAL AROUSAL IN THE RABBIT. P.J. Whalen, B.S. Kapp and J.P. Pascoe. Dept. of Psychology, Univ. of Vermont, Burlington, VT 05405

Our current research is concerned with the neurobiological substrates of conditioned arousal. The cholinergic basal forebrain neurons within the sublenticular substantia innominata (SI; Grove, 1988), referred to also as nucleus basalis (NB) are believed to contribute to cortical arousal. This arousal response is indexed by a decrease in EEG high voltage slow activity (0-4 Hz; delta) and the presence of EEG low voltage fast activity (Buzsaki et al., 1988). The present study sought to determine if the activity of SI neurons is correlated with the conditioned cortical EEG arousal response.

New Zealand rabbits received differential Pavlovian conditioning trials in which a tone of one frequency (the CS+) immediately preceded an electrical stimulus (the US) to the pinna, while a tone of another frequency (the CS-) was never followed by the US. A Fourier analysis of the neocortical frontal EEG revealed that a significantly larger EEG arousal response emerged over trials to the CS+ than to the CS- ($p < .05$).

The majority of recorded neurons were located in the SI at the level of the anterior commissure. Many of these neurons increased their firing rate significantly more to the CS+ than to the CS- ($p < .05$), similar to the discrimination seen in the EEG arousal response. Furthermore, the spontaneous activity of these same neurons was found to correlate negatively with spontaneous fluctuations in the EEG. That is, neuronal activity increased with decreased EEG slow activity, and decreased with increased EEG slow activity ($p < .05$). Supported by NSF grant BNS 9010760.

130.2

CHOLINERGIC CONTRIBUTION TO CORTICAL ELECTRO-ENCEPHALOGRAPHIC (EEG) CONDITIONED RESPONSES (CRs) IN THE RABBIT. B.S. Kapp, P.J. Whalen and S.L. Thurm*, Dept. of Psychology, University of Vermont, Burlington, VT 05405.

We have reviewed evidence suggesting that the amygdaloid central nucleus (ACe) contributes to the expression of CRs indicative of arousal in the rabbit (Kapp et al. 1990). This hypothesis is supported by the observation that electrical stimulation of the ACe in rabbit elicits EEG arousal, manifested in an inhibition of high voltage slow activity (0-4 Hz) and the presense of low voltage fast activity. This arousal is blocked by atropine sulfate (Kapp et al. 1990, 1991). In the present study, one of a series designed to assess the role of the ACe in conditioned arousal, we examined the effects of cholinergic antagonists on the expression of conditioned EEG arousal in the rabbit.

New Zealand rabbits received Pavlovian differential conditioning trials in which a tone of one frequency (the CS+) immediately preceded a brief electrical stimulus (the US) to the pinna while a tone of another frequency (the CS-) was never followed by the US. A Fourier analysis of the frontal cortical EEG demonstrated that over conditioning trials a significantly larger EEG arousal response emerged in response to the CS+ than to the CS-. Atropine sulfate (8 mg/kg i.v.) administered following the establishment of this discrimination blocked the EEG response to both CSs, whereas the peripherally-acting atropine methyl nitrate (8 mg/kg) exerted little effect. The results suggest a significant cholinergic contribution to conditioned EEG arousal. An examination of the contribution of the ACe to conditioned EEG arousal is now in progress. Supported by NSF grant BNS 9010760.

130.3

SINGLE UNIT ACTIVITY IN MESOPONTINE PERIBRACHIAL AREA (PB) NEURONS THAT PROJECT TO THE THALAMUS DURING PAVLOVIAN CONDITIONING IN RABBITS. J.P.Pascoe & B.S.Kapp. Dept. Psychology, The University of Vermont, Burlington, VT 05405 USA.

We reported previously that activity in many PB neurons is altered significantly during presentations of both novel stimuli and an auditory stimulus (CS) that has acquired affective significance consequent to Pavlovian conditioning procedures (Pascoe & Kapp, 1990). We are now testing the hypothesis that such activity occurs in at least some PB neurons that project to the thalamus, and in this way may contribute to learning-related changes in the activity of thalamic neurons.

PB neurons were activated antidromically by stimulation of one or more thalamic sensory relay nuclei with an average latency of 1.8 ms. Activity in these neurons during CS presentations was either unchanged, increased at a short latency (<15ms), or decreased at a longer latency (>100ms). For a given neuron, responses to novel stimuli were of the same pattern as those to the CS but were more robust initially and habituated rapidly. Other neurons with similar patterns of sensory-evoked activity were activated orthodromically by stimulation of the thalamus at an average latency of 3.0 ms, including some that also were activated orthodromically by stimulation of the amygdaloid central nucleus. This research demonstrates learning-related phenomena in PB neurons that (a) project to the the thalamus and (b) may serve to modulate sensory processing and transmission within the thalamus during learning. Supported by the American Heart Association and the AHA Vermont Affiliate, Inc.

130.4

AIR PUFF EVOKED PURKINJE CELL COMPLEX SPIKE ACTIVITY IS DIMINISHED DURING CONDITIONED RESPONSES IN EYEBLINK CONDITIONED RABBITS. D. J. Krupa, C. Weiss, and R. F. Thompson, Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Previous Purkinje cell recording studies in eyeblink conditioned rabbits found very few cells which responded with a complex spike to an air puff unconditioned stimulus (US). Also, recordings from the dorsal accessory inferior olive (DAO) demonstrate decreased activity on paired CS-US trials in trained rabbits. These data suggest that conditioning related inhibition of the DAO, and/or its afferents, results in a reduced probability of US evoked complex spikes.

In the present study, Purkinje cells from HVI and surrounding areas were recorded in rabbits during paired tone-airpuff (isi=250 ms) conditioning trials. Interspersed throughout the paired trials were airpuff alone trials. In well trained rabbits, Purkinje cells which responded to the airpuff alone with a complex spike ($n=10$, $p=.8$) did not respond to the airpuff with a complex spike ($p=.2$) on paired CS-US trials. Five more airpuff responsive cells have been recorded from rabbits very early in training. On paired trials (0% CRs), these cells continued to respond to the US with a complex spike.

Five cells were found which responded with a complex spike to the tone CS as well as the sound of the airpuff US (airpuff was aimed away from the rabbit). However, on paired trials in well trained rabbits, these complex spikes were not diminished.

These data suggest that inhibition of the US input to the cerebellum is mediated by the CR pathway and is selective for somatosensory responsive Purkinje cells. Supported by the McKnight Foundation, NSF BNS-8718300 and ONR N00014-88-K0112 to RFT.

130.5

SINGLE UNIT RECORDINGS OF SOMATOSENSORY AND AUDITORY EVOKED RESPONSES IN THE ANTERIOR INTERPOSITUS NUCLEUS IN THE NAIVE RABBIT.
J. Tracy, C. Weiss and R.F. Thompson, Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

The cerebellar deep nuclei have been implicated as necessary for classical conditioning of the rabbit nictitating membrane response (NMR). Previous studies have shown that lesions as small as 1 cubic mm in the anterior-lateral portion of the anterior interpositus nucleus (IPA) are sufficient to abolish NMR conditioning (Lavond, et al. 1984). We are undertaking a single unit mapping of the IPA of naive rabbits to determine which areas respond to the auditory and somatosensory stimuli typically used during conditioning trials.

Preliminary results show that responses to either auditory stimulation, somatosensory stimulation, or both occur consistently in the IPA. Several response types have been recorded. Evoked excitatory responses tend to occur within a 5-50 msec latency and inhibitory responses within 20 to 100 msec latencies. The majority of cells show a variety of "on" responses to the effective stimulus. A few cells exhibit an on-off response pattern. These cells, typically found 1.0 mm anterior to lambda, increase in firing frequency and then shortly thereafter (200-400 msec) turn off. The most responsive region ranges from 0.5 mm posterior to 1.0 mm anterior to lambda. Dorsal/ventral positions within the interpositus appear to contribute little to response specificity.

Thus, while no specific topographic arrangement of afferent response is yet apparent, there are clearly areas of response with greater and lesser complexity. Cells that respond to more than one type of stimuli are seen in the anterior-lateral area while the those recorded in the more anterior, posterior, and medial regions respond more selectively, or not all.

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130.6

SUBICULAR CELLS THAT PROJECT TO THE MEDIAL PREFRONTAL CORTEX ARE NOT NECESSARY FOR CLASSICALLY CONDITIONED BRADYCARDIA IN RABBITS. Mark E. Chachich, Brian Maxwell*, Conrad Terry* & D.A. Powell, VA Medical Center, Department of Psychology, University of SC and Department of Neuropsychiatry and Behavioral Science, University of SC School of Medicine, Columbia, SC.

Rabbits and rats received horseradish peroxidase injections in the medial prefrontal cortex and retrograde label was examined in the ventral hippocampus and associated subiculum. As previously reported (e.g., Ferino et al, Exp Brain Res, 1987, 65, 421-426), labeled cells were observed in dorsal and ventral subiculum and in the CA1 hippocampal field in the rat. Although scattered cells were observed in the dorsal subiculum of the rabbit, no labeled cells were seen the hippocampus proper or the ventral subiculum. Instead the strongest projection to the medial prefrontal cortex in the rabbit appears to arise from the postsubiculum. Separate groups of rabbits with sham, postsubicular, or dorsal subicular lesions were subjected to classical heart rate conditioning in which a 4 sec, 75 db tone was the conditioned stimulus and a 3 mA paraorbital shock was the unconditioned stimulus. However, no significant differences were observed between the lesion and sham groups in either the acquisition or magnitude of conditioned bradycardia.

Supported by VA Institutional Research Funds

130.7

AN ANALYSIS OF NEURONAL ACTIVITY IN THE MEDIAL PREFRONTAL CORTEX AND THE AMYGDALA DURING CLASSICAL HEART RATE CONDITIONING IN RABBITS. D.A. Powell, Brian Maxwell* & Conrad Terry*. VA Medical Center, Columbia, SC 29201, Department of Psychology, University of SC and Department of Neuropsychiatry and Behavioral Science, University of SC School of Medicine, Columbia, SC.

Multiple unit recording electrodes were chronically implanted in either area 32 of the medial prefrontal cortex (PFC) or the lateral, central, or basolateral nucleus of the amygdala in rabbits. Multiple unit activity was recorded during adaptation, acquisition and extinction of classical heart rate conditioning in which a 4 sec tone served as the conditioned stimulus (CS) and a 250 msec paraorbital shock was the unconditioned stimulus. As expected, conditioned heart rate decelerations were obtained. These cardiac changes were accompanied by CS-evoked increases in neuronal output in both the medial PFC and the amygdala that were significantly greater than that obtained during either adaptation or extinction. However, in general the latency to peak response and the magnitude of the response was greater in all the nuclei of the amygdala than in the prefrontal cortex. These data provide further support for the participation of both the medial PFC and amygdala in classically conditioned bradycardia.

Supported by VA Institutional Research Funds

130.8

EFFECTS OF INTERPOSITUS NUCLEUS OR RED NUCLEUS LESIONS ON RABBIT CLASSICAL CONDITIONING-RELATED THALAMIC AND HIPPOCAMPAL NEURAL ACTIVITY. L. L. Sears and J. E. Steinmetz. Program in Neural Science, Indiana University, Bloomington, IN 47405.

The cerebellum and hippocampus appear to be involved in rabbit classical eyelid conditioning. Both structures evidence neural activity that precedes and models the topography of the learned behavioral response. Acquisition of the learned response, however, and the development of training-related neural activity in the hippocampus requires an intact cerebellum (Sears & Steinmetz, Beh. Neurosci.; 5, 1990). In an initial attempt to determine how important training-related activity from the cerebellum may be transmitted to higher brain areas, a series of lesion and recording studies were completed.

Multiple-unit recordings from the ventral lateral thalamus in rabbits chronically implanted with either interpositus (N=7) or red nucleus (N=7) lesion electrodes revealed neural activity that formed an amplitude-time course model of the learned response. The neural activity and conditioned eyelid responses were abolished following interpositus nucleus electrolytic lesions. Red nucleus lesions, however, blocked conditioned responses without altering ventral lateral thalamic neural activity suggesting that the observed thalamic activity was dependent on direct cerebellar efferents. In a second group of rabbits with chronically-implanted hippocampal recording electrodes, lesions were made in either the ventral lateral thalamus (N=6) or red nucleus (N=6) following acquistion of learned responses. Lesions in the thalamus or red nucleus did not effect training-related activity in the hippocampus. The results of these studies indicate that training-related activity in the hippocampus is not dependent on cerebellar pathways to the red nucleus or ventral lateral thalamus.

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130.9

DIFFERENCES IN ACQUISITION AND EXTINCTION OF CONDITIONING-RELATED NEURONAL ACTIVITY IN RABBIT CEREBELLAR CORTEX AND INTERPOSITUS NUCLEUS. T. J. Gould, D. P. Miller & J. E. Steinmetz. Dept. of Psych., Prog. in Neural Science, Indiana University, Bloomington, IN 47405.

Neural recordings from lobule HVI of cerebellar cortex and the interpositus nucleus (INP) during classical eyelid conditioning revealed increases in activity that formed an amplitude-time course model of the learned response (McCormick & Thompson, J. Neurosci; 4, 1984). These data and a number of cerebellar lesion studies have suggested that the cerebellum may be a critical site of plasticity associated with classical conditioning. The goal of the present study was to compare rates of acquisition and extinction of CR-related activity in the INP and HVI. After implanting recording electrodes into both the INP and HVI, 11 animals were classically conditioned with a tone CS and an air puff US until a conditioning criterion was reached on 3 consecutive sessions. Animals then received unpaired extinction presentations of the US and CS until a baseline eyelid response (i.e., less than 5 % CRs) was observed on CS alone trials for three consecutive sessions. During all phases of training, behavioral and neural responses were monitored. In 2 rabbits, neuronal models were observed in both the INP and HVI. CR-related activity developed in the INP about one-half a session prior to its development in HVI and simultaneously with the production of behavioral CRs. In 9 other animals, CR-related activity was not seen in both areas due to improper placement of one of the electrodes. These rabbits were trained to criterion, however, and given extinction training. Four animals showed CR-related activity in the INP and five animals showed CR-related activity in HVI. At 5 of 6 INP sites, the neuronal model disappeared at the same time the behavioral CR extinguished. At 6 of 7 HVI sites, however, the neural model decreased to some extent but some CR-related activity remained for several sessions after the behavioral CR disappeared. In sum, although both the INP and HVI showed CR-related activity, the excitatory neuronal model did not develop at the same time (i.e., CR-related activity in the INP developed about one-half session prior to that in HVI). Furthermore, CR-related activity at the two sites did not extinguish at the same time (i.e., CR-related activity in the INP extinguished with the behavioral CRs while CR-related activity in HVI remained for a period of time after extinction of behavioral CRs). [Supported by NIMH Grant # 44052 to J.E.S.]

130.10

RABBIT CLASSICALLY CONDITIONED EYELID RESPONSES FAIL TO REAPPEAR AFTER INTERPOSITUS LESIONS AND EXTENDED POSTLESION TRAINING. J.E. Steinmetz and S.S. Steinmetz*. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

A number of laboratories reported complete abolition of classically conditioned eyelid responses (CRs) after cerebellar interpositus nucleus lesions (e.g., McCormick & Thompson, Science, 223, 1984). Recently, the relative permanence of this lesion effect has been challenged (Welsh & Harvey, J. Neurosci., 9, 1989; Kelly et al., Behav. Brain Res. 38, 1990). One possibility is that the reappearance of CRs in lesioned rabbits is related to the amount of postlesion training given. To test this hypothesis, to date, we have trained 3 rabbits to asymptotic responding (15 days) using a 350 ms tone CS and a 100 ms coterminating air puff US. The rabbits were then given electrolytic lesions of the left interpositus and paired training reinstated for an extensive number of sessions. During all sessions, the CS-US interval on 108 paired trials and a 2.5 sec period after tone onset on 12 tone-alone test trials were examined for CRs. After 100 postlesion sessions, training was switched to the right side for 5 sessions then switched back to the left side. Our results to date have shown a permanent abolition of CRs after interpositus lesion. One rabbit has received more than 180 postlesion sessions (over 20,000 trials), a second rabbit, more than 100 postlesion sessions (over 12,000 trials) and a third rabbit, more than 25 postlesion sessions (3000 trials). For the 3 rabbits combined, percent CRs has exceeded 10% on paired trials only 18 times with a maximum of 19% reached on 1 session. On test trials, eyeblinks have been generally observed on 0-2 trials per sessions (M = 1.1). Postlesion responding on test trials exceeded 16.7% on only 19 sessions distributed somewhat randomly across training and rabbits. The number of responses observed on paired and test trials are similar to spontaneous blinking rates that would expected over the respective time intervals. Training of the right eye produced rapid and robust acquisition of CRs. These data provide evidence that extended postlesion training fails to produce classically conditioned responses after interpositus lesions, a finding that argues against the possibility that postlesion changes in the brain, capable of supporting classical eyelid conditioning, occur after destruction of the interpositus nucleus. [Supported by NIMH Grant #44052 to J.E.S.]

130.11

EFFECTS OF RED NUCLEUS STIMULATION ON A PAVLOVIAN UNCONDITIONED RESPONSE (RABBIT EYEBLINK) T. Canli, K. Whitney*, and N.H. Donegan. Dept. of Psychology, Yale Univ., New Haven, CT. 06520.

A core assumption of an influential class of Pavlovian conditioning models (Rescorla-Wagner, SOP; see Donegan, *et al., Psych. Learn. & Motiv.*, 23, 1989) is that signaling a Pavlovian US by an associated CS diminishes US processing. This is thought to contribute significantly to phenomena such as the negatively accelerated acquisition curve, the Kamin blocking effect, and the conditioned diminution of the UR. Within the context of rabbit eyeblink conditioning, we proposed that one way a CS can associatively diminish US processing is by activating the projection from the magnocellular division of the red nucleus to the contralateral trigeminal spinal nuclei, that, from the work of Dostrovsky, is assumed to be inhibitory (Donegan *et al.*1989).

To begin evaluating these proposals, we assessed the effect of stimulating the red nucleus on the ability of a peri-orbital shock US to elicit an eyeblink UR. Twenty three male New Zealand rabbits had pairs of stimulating electrodes chronically implanted in the magnocellular region of both red nuclei. Each electrode site was tested by giving subjects 22 US-alone and 22 stimulation -> US trials presented in an intermixed, counterbalanced sequence. Brain stimulation consisted of a 100 msec train of .2 msec pulses at 200 Hz. Current intensity ranged from 100 to 400 μA, depending on the amount and kind of behavior elicited. The US was a 50 msec train of 5 msec pulses at 100 Hz, 2.5 to 4 mA. On stimulation -> US trials, the onset of the stimulation train preceded the US by 150 msec (Weiss *et al, Neurosci. Abs.*, 11: 182, 1985). Stimulation of the posterior or central regions within the red nucleus, or the dorsolateral border of the posterior region, in most cases produced a diminution of the peak UR amplitude compared to US-alone trials. When both eyes were tested, diminution was more consistently seen on the contralateral eye. Stimulation of a small region at the ventral border of the anterior portion of the red nucleus tended to potentiate UR amplitude. In pilot studies, we are finding that similarly stimulating the region of the anterior, lateral interpositus nucleus of the cerebellum consistently diminishes the amplitude of the eyeblink UR in the ipsilateral eye.

130.12

CONTRIBUTION OF NMDA-MEDIATED NEURONAL ACTIVITY TO CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE AND CONDITIONING-RELATED POTENTIATION OF PERFORANT PATH-GRANULE CELL RESPONSES. G.B. Robinson, University of New Brunswick, Fredericton, New Brunswick, E3B 6E4 Canada.

We previously demonstrated that the NMDA antagonist MK801 impairs acquisition of the classically conditioned rabbit nictitating membrane (NM) response (Stillwell and Robinson, Neurosci. Abstr., 1990). MK801 also reduces the magnitude of long-term potentiation (LTP), a candidate neural learning and memory mechanism. Several LTP-like effects occur with NM conditioning that, if similar to electrically-induced LTP, also should be affected by MK801.

MK801 (0.05 and 0.10 mg/kg, sc) was administered to New Zealand rabbits 75 minutes prior to each daily conditioning session (108 trials). A 400 ms tone (1 KHz, 82 db) served as the CS and coterminated with a corneal airpuff US (5 psi, 100 ms). Hippocampal granule cell responses were evoked by perforant path stimulation midway between each trial and 220 ms after tone onset within each trial.

MK801 decreased the rate of acquisition of the NM conditioned response but did not disrupt conditioning-related potentiation of either the perforant path-granule cell population spike or EPSP. MK801 also did not alter the suppression of spike amplitude during tone presentations. These results raise the possibility that the conditioning-related potentiation is not dependent on NMDA-mediated neuronal activity. (Supported by MRC and NSERC)

130.13

ROLE OF SUBCEREBELLAR DENTATE NUCLEUS IN SHORT LATENCY AUDITORY TRANSMISSIONS USED AS CS FOR PAVLOVIAN EYEBLINK CONDITIONING. X.F. Wang, C.D. Woody, and E. Gruen*. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Patterns of activity were recorded from single units of the dentate and cochlear nuclei during conditioned blinking produced by forward pairing of a 70 db click as CS with glabella tap and hypothalamic electrical stimulation (570-10ms ISI; see Hirano et al., Br. Res. 1987)

The proportion of units of the dentate nuclei that responded to the CS with increased discharges of 4-8 ms latency was significantly greater (X^2 5.47, p = 0.02) after conditioning with forward pairing of the CS than after pseudoconditioning with backward pairing of the CS with UCS. This finding suggests that these adaptations are sensitive to associative features of stimulus presentation and may thus support the conditioned behavior.

Earlier studies by other investigators failed to show adaptive changes in activity of neurons of the inferior colliculus with conditioning to auditory CSs. In contrast, the dentate nucleus represents an auditory relay nucleus at which associatively induced changes in activity are found. The changes in activity also permit discrimination of a forward paired click CS from a backward paired hiss DS. (Supported by NS25510.)

130.14

MULTI-CHANNEL SINGLE UNIT RECORDING IN THE BRAINSTEM DURING ACQUISITION OF THE CONDITIONED NICTITATING MEMBRANE RESPONSE IN THE RABBIT. V. Bracha*, M. Webster* and J.R. Bloedel, Barrow Neurological Institute, Phoenix, AZ 85013.

A new method was developed to record simultaneously from up to 12 channels of single or multiple unit data in order to monitor neuronal activity during the acquisition of the classically conditioned nictitating membrane/eyelid response in the rabbit. The chronically implantable microdrive houses three vertical, independently-moveable electrode systems each with the capacity to carry a bundle of four 13-25 μm microwires. The microdrive together with the electrodes and the preamplifiers can remain mounted on the head when the animal is returned to its cage between recording sessions.

Albino New Zealand rabbits were trained using a standard delay paradigm for conditioning the nictitating membrane reflex. The experiments were designed to record from the same neurons before, during, and after the acquisition of the conditioned behavior and to compare the responses obtained during these three periods. In each naive animal, training was initiated after the isolation of up to 10 neurons. If the rabbit did not learn the task in one session, recordings from the same cells or from neurons in the same area were obtained the next day as training continued. Recordings performed in the red nucleus, spinal trigeminal nucleus, and lateral tegmental field demonstrated that this technique could be used to reliably isolate multiple single units for recording sessions lasting up to 240 trials. The data indicate that modifications of the cell discharge patterns accompany the acquisition of the conditioned behavior in all three recording sites. NIH Grant NS21958

130.15

Effects of CS presentation on US-elicited activity in the deep cerebellar nuclei during the early stages of NM response conditioning in rabbit. B. Y. Yang and D. J. Weisz. Departments of Neurological Surgery and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Lesion studies have revealed that the anterior interpositus nucleus (AIP) in the cerebellum is critical for the acquisition of conditioned nictitating membrane (NM) responses in rabbit. Because of its role in acquisition, we chose this nucleus for electrophysiological investigations of the potential interactions of the conditioned stimulus (CS) and unconditioned stimulus (US) during the early stages of NM response conditioning. Specifically, we hypothesized that CS presentation would alter US-elicited activity in this nucleus. This experiment is based on our model of conditioning which states that neuronal plasticity underlying behavioral conditioning grows out of CS alterations in US-elicited neuronal activity.

Thus far, we have recorded from 24 single cells in the anterior interpositus and 34 single cells in the cerebellar dentate nucleus (DN) in a total of 32 animals. All recordings were made on the first day of conditioning prior to the appearance of CRs. Ten cells in the AIP and 13 cells in the DN exhibited excitatory responses to both CS and US presentation. In 10/10 of these AIP cells the CS facilitated the US-elicited response at a 500 msec CS-US interval, which supports NM response conditioning. Either no effect or an inhibition by the CS of US-elicited activity was seen at a 30 msec CS-US interval, which does not support conditioning. In 13/13 DN cells the CS inhibited US-elicited activity at the 500 msec CS-US interval. These results indicate that CS-US interactions in the deep cerebellar nuclei begin very early in training, depend on the CS-US interval, and vary depending on the anatomical locus. The initial CS-US interactions in the AIP and DN may represent the early stages of CR acquisition, and the AIP and DN may have different roles during this process. (Supported by MH42800)

130.16

HIPPOCAMPAL RESPONSES TO A COMPLEX TONE DURING DISCRIMINATION/REVERSAL JAW MOVEMENT CONDITIONING. L. Dreshfield and S. Berry, Dept. of Psychology, Miami University, Oxford, Ohio 45056.

In order to assess the generality of conditioned hippocampal unit responses obtained during NM and CJM conditioning, bilateral recordings were made from CA1 during go/no go jaw movement discrimination/reversal training. Electrodes were implanted under general anesthesia (Ketamine 50 mg/kg; Rompun 10 mg/kg IM) in 10 New Zealand white rabbits. In the task, one tone (CS+) signalled delivery of a saccharin solution 500 msec later; another tone (CS-) signalled no saccharin. One kHz and 8 kHz tones were counterbalanced as CS+ and CS-. Neural responding was greater to the 1 kHz tone throughout training, and was significantly elevated in the group that received 1 kHz-saccharin pairing during reversal ($F(1,8)=5.26$, $p<.05$). Behaviorally, discrimination or reversal was significantly faster when the 1 kHz tone was the CS+ ($t(6)=4.0$, $p<.01$). Animals given unpaired stimuli (n=3) did not display any changes in neural activity. Post hoc evaluation of the tones showed that chamber acoustics added a 2.5 kHz component to the 1 kHz tone, perhaps making it more salient than the 8 kHz tone. Salience differences between stimuli affect learning rate (Scavio & Gormezano, 1974), and may alter tone-evoked hippocampal activity (Best & Best, 1976). These results provide clear evidence for hippocampal involvement during learning and suggest that stimulus salience is coded by hippocampal neurons.

130.17

NORMAL ACQUISITION AND EXTINCTION OF EYEBLINK CONDITIONING IN HIPPOCAMPECTOMIZED RATS. Beth A. Christiansen and Nestor A. Schmajuk. Department of Psychology. Northwestern University. Evanston, IL 60208.

The effect of hippocampal lesions on acquisition and extinction of eyeblink conditioning in rats was examined with the procedure described by Schmajuk and Christiansen (1990). Male albino rats received either sham, cortical control or hippocampal lesions. After a two week recovery period and two days of habituation, animals were trained in a delay conditioning paradigm. The conditioned stimulus was a 2,000 Hz, 98 dB, 500 ms tone; the unconditioned stimulus was a 4 psi, 150 ms air puff; the interstimulus interval was 350 ms; and the intertrial interval was 60 s.

Animals received 50 trials daily. After each animal reached a criterion of 8 out of 10 conditioned responses (CR) in a given block, extinction began and continued until the animal achieved a criterion of one CR in a block of 10 consecutive trials. No significant lesion effects were found during acquisition ($F[2,5] = .30$, $p > .05$). Although there was a trend toward slower extinction in hippocampal animals, no significant lesion effects were found during extinction ($F[2,5] = 1.88$, $p > .05$). Similar results were reported for acquisition (e.g., Solomon & Moore, 1975) and extinction (e.g., Berger and Orr, 1983) of the rabbit nictitating membrane response.

In both rabbits and rats, acquisition of the delay conditioned eyeblink/nictitating membrane response seems to be preserved after hippocampal lesions, but impaired after cerebellar lesions (Skelton, 1988; Thompson, 1989). Therefore, similar neural substrates might be involved in both species.

130.18

SORTING OF SINGLE-UNITS FROM MULTI-UNITS: LIMBIC CORTICAL AND THALAMIC CORRELATES OF DISCRIMINATIVE AVOIDANCE LEARNING. **Y. Kubota, E. Kang, A. Poremba and M. Gabriel. Dept. of Psych. and Beckman Institute, Univ. of Illinois, Urbana IL 61801.**

Past studies of the effects of lesions have implicated the cingulate cortex (CC) and limbic thalamus (LT) in mediation of discriminative avoidance conditioning in rabbits. Neurons in these areas encode the associative significance of the positive (footshock predictive) and negative (not shock predictive) tone conditional stimuli (CS+ and CS-, respectively) and processes leading to initiation of the conditioned avoidance response (CR, stepping in an activity wheel). Here, CC and LT multi-unit activity in rabbits performing at an asymptotic level was submitted to waveform classification using microcomputer based software (BrainWave Systems, Inc.). Sorted single-units were examined for differential responses to CS+ and CS- and for firing preceding and coinciding with the onset of the CR. Seventy-four percent of cells fired more to one CS than to the other. These differences could be pre-wired, but significantly more cells discriminated in favor of CS+ than in favor of CS- ($p<.02$), indicating that the discrimination was a product of conditioning. Consistent with a previous study of isolated single units (Kubota, Y., et al., Neurosci. Abstr., 12:518, 1986), two patterns of firing were observed in relation to CR production: increase in firing rate (FR) preceding CR and lower FR in trials with CR than in trials without CR, the latter found only in area 24 and the mediodorsal (MD) nucleus. These consistencies provided preliminary validation of the waveform sorting routine. Additional results not previously seen were: 1) cells in anterodorsal and laterodorsal nuclei showed most massive pre-CR firing; 2) virtually no pre-CR increase was seen in area 24 cells in well-trained rabbits; and 3) cells with higher FR to CS- than to CS+ were found in the MD nucleus and in the CC. (Supported by AFOSR and NIH).

130.19

PREDICTION OF CONDITIONED AVOIDANCE RESPONSES BY CS-RELATED NEURONAL DISCHARGES IN CINGULATE CORTEX AND LIMBIC THALAMUS OF RABBITS. E.Kang and M.Gabriel, Dept. of Psychol. and Beckman Inst., Univ. of Illinois, Urbana, IL 61801.

This study concerns the neural mediation of discriminative avoidance conditioning of rabbits wherein a conditioned response (CR, stepping in an activity wheel) to an auditory positive conditional stimulus (CS+) prevents a footshock 5 sec. after CS+ onset. This conditioning results in the development of discriminative activity, i.e., greater neuronal discharges 15 - 500 msec. after CS+ onset, than after a negative CS (CS-) which does not predict shock. The discriminative activity develops in limbic areas (below) essential for learning. Here are reported analyses of neuronal data of trained rabbits indicating that the discharges evoked by CS+ presentations followed by CRs did not differ in magnitude from the discharges evoked by CS+ presentations not followed by CRs. This outcome was obtained in parvocellular and magnocellular anteroventral thalamic nucleus (N=9, 16), medial dorsal nucleus (N=23), anterior cingulate cortex (area 24, N=21) and in four layers in each of two regions of the posterior cingulate cortex (areas 29c/d and 29b, N=26, 31). These results are incompatible with motoric, arousal, or attentional interpretations of the elicited activity, but they are consistent with a mnemonic retrieval interpretation. A consistent elevation of baseline (pre-CS) neuronal activity occurred prior to CS- presentations only, on trials in which CRs occurred, relative to no-CR trials, in certain layers of area 29 and in all thalamic areas. No such elevation of the baseline occurred in area 24. The elevated baseline activity may reflect activation preparatory to locomotion, accounting for the erroneous CRs on CS- trials. The appearance of this activational process in certain areas signifies distinctive functions of these areas (Supported by NIH and AFOSR).

130.20

AMYGDALA LESIONS BLOCK ACQUISITION OF DISCRIMINATIVE ACTIVE AVOIDANCE LEARNING IN RABBITS. A. Poremba and M. Gabriel, Dept. of Psych. and Beckman Inst., Univ. of Ill., Urbana, IL. 61801

Multi-unit recording and lesion studies have shown that the rabbit anterior cingulate cortex (ACC) and the mediodorsal thalamic nucleus (MDTN) comprise an early learning circuit involved in mediating the initial stages of active avoidance learning, wherein a conditioned response (CR) of stepping in an activity wheel to a CS+ tone prevents a footshock US 5 sec. after CS+ onset. (No footshock follows a CS- tone presentation on trials randomly sequenced with the CS+). In contrast, the anteroventral thalamic nucleus (AVTN) and the posterior cingulate cortex mediate asymptotic performance, but not original acquisition (Gabriel, M., Progress in Brain Research, 85:467-83, 1990). Amygdaloid basolateral nucleus (BLN) neurons are interconnected with the ACC and the MDTN and they exhibit early training-induced activity (TIA: increased CS evoked excitation and development of greater neuronal discharges to the CS+ than to the CS-), suggesting that BLN neurons participate in the early learning circuit. To test this hypothesis, bilateral electrolytic lesions were centered in the BLN (small lesion group), and larger lesions included BLN and other amygdaloid areas (large lesion group). As expected, BLN lesions impaired behavioral acquisition in the early training stages ($p<.01$), and reduced TIA in the ACC ($p<.01$) and MDTN ($.1>p>.05$). Unexpectedly, small and large lesions impaired TIA in the AVTN ($p<.01$) and the large lesions blocked acquisition of the CR ($p<.01$). These results are compatible with the hypothesis that BLN neurons are involved in mediating the early stages of CR acquisition. However, the severe learning impairment due to the large lesions and the attenuation of AVTN TIA suggest that amygdala neurons are involved in learning-relevant processes in addition to those mediated by the early learning circuit. (Supported by NIH and AFOSR).

130.21

A SYSTEM FOR MULTICHANNEL NEURONAL RECORDING DURING DIFFERENTIAL APPETITIVE CONDITIONING OF RABBITS. M. Gabriel, D. Tcheng*, Y. Kubota, E. Kang, A. Poremba and C. Cuppernell*. Dept. Psych. and Beckman Inst., Univ. of Ill., Urbana IL 61801.

A new microcomputer-based data collection system was implemented for recording brain neuronal correlates of appetitive conditioning, in order to directly compare the activity with extensively documented neuronal correlates of avoidance conditioning(Gabriel, M. Prog. in Brain Res., 85:467-483, 1990). In both tasks, an instrumental response (stepping in a large activity wheel to avoid shock and head extension performed in a restraining box to contact a drinking tube) yields the desired outcome when performed in response to a .5 sec. tone conditional stimulus (CS+). Non-response to a second tone (CS-) which does not signal reinforcement is also learned. Trials are presented daily (60 with each CS in a quasi-random sequence). Appetitive training of water-deprived rabbits involves drinking tube insertion 5 sec after CS presentation. Contact with the tube following the CS+ yields 3 ml. of water but responses following the CS- induce tube-withdrawal and no water. Multi-unit activity and EEG records from six interconnected brain areas are sampled during training. Five rabbits trained to date reached asymptotic discrimination in 4-6 training sessions, responding on an average of .50 more of the CS+ than of the CS- trials. Average post-CS multi-unit histograms and field potential profiles in posterior cingulate cortex, the anteroventral thalamic nucleus and hippocampal subfield CA1 were similar to those recorded in other rabbits during avoidance learning, for which those areas are essential. In both tasks, the discharges became discriminative (i.e., greater in response to the CS+ than to the CS-) during training. However, hippocampal discrimination during appetitive conditioning was much greater than that seen during avoidance conditioning. Future studies of the neuronal activities in concurrently trained rabbits are planned to elucidate the neural mediation of contextual control of the learned behavior. (Supported by AFOSR and NIH.)

MONOAMINES AND BEHAVIOR: DEVELOPMENT, INGESTION AND SEX

131.1

HYPERACTIVITY IN THE OFFSPRING OF NICOTINE TREATED RATS: ROLE OF THE MESOLIMBIC DOPAMINERGIC PATHWAY. S.A. Richardson, V.J. Massari, and Y. Tizabi. Dept. of Pharmacology, Howard University, College of Medicine, Washington, D.C. 20059

Recent reports have linked hyperactivity in children to cigarette smoking by the mother during pregnancy. Since it has been shown that nicotine-induced hyperactivity in adult rats is associated with increased dopamine (DA) concentation in the nucleus accumbens (NACC), this study was designed to determine whether similar biochemical changes may also occur in the hyperactive offspring of nicotine treated dams. Timed-pregnant Sprague Dawley rats were implanted subcutaneously on gestational day 4 with osmotic-mini pumps releasing saline or nicotine (3mg/kg/day) for 14 days. Male offspring were tested twice (at 19 and 21 days) for their locomotor activity using a Digiscan Monitor and sacrificed 24 hours after the last test. The NACC, frontal cortex (FC), and ventral tegmental area (VTA) were microdissected and DA concentration was measured in the following groups: hyperactive offspring of nicotine treated dams (Nic-H), non-hyperactive offspring of nicotine treated dams (Nic-NH) and non-hyperactive offspring of saline treated dams (Sal-NH). DA concentration in the NACC of the Nic-H (49.2±2 pg/ugPr) was not significantly different from the Sal-NH (55.5±6pg/ugPr) or the Nic-NH (38.6±2pg/ugPr). However, there was a significant decrease in the DA concentration in the NACC of the Nic-NH compared to the Sal-NH ($p<0.05$). DA concentration in the VTA of the Nic-H (26±4pg/ugPr) was significantly higher ($p<0.05$) than the Nic-NH (15.7±1pg/ugPr) but did not show a significant difference compared to the Sal-NH (20.8±2). DA concentrations in the FC were similar in all groups (Sal-NH, 1.9±0.3pg/ugPr; Nic-NH, 1.7±0.3; Nic-H, 1.7±0.1). These data suggest that the biochemical basis of hyperactivity in the offspring of nicotine treated dams is different from nicotine-induced hyperactivity in the adult rats.

131.2

HETEROGENOUS NEURONAL DAMAGE OF RAT BRAIN DOPAMINERGIC SYSTEMS FOLLOWING NEONATAL INTRACISTERNAL 6-HYDROXYDOPAMINE TREATMENT. H.OKAMURA, C.YOKOYAMA, Y.IBATA. Dept. of Anatomy and Psychiatry, Kyoto Pref. Univ. Med., Kyoto 602, Japan.

Neonatal intracranial treatment of 6-hydroxydopamine (6-OHDA) causes the destruction of the dopaminergic neurons and induces various behavioral changes at adult age after loading of dopamine agonists. Previous biochemical studies have revealed the severe depletion of dopamine and its metabolites in the basal ganglia, but the affected region has not been fully investigated. In the present study, we investigated the damaged area of dopaminergic neurons by ABC-immunocytochemistry using anti-tyrosine hydroxylase serum. Desipramine pretreated (20 mg/kg) neonatal Wistar rats were injected with 6-OHDA (100 ug/5 ul, 2 times) intracisternally, and their behavior after Ro-4-4602 (50 mg/kg) and L-DOPA loading (100 mg/kg, i.p.) was evaluated at 6 weeks of age. Only the rat showing self-mutilation behavior was used in the present study. Dopaminergic neurons and terminals were severely depleted in the caudate-putamen, the substantia nigra, the nucleus accumbens, the ventral tegmental area and the tuberculum olfactorium, but almost unaffected in the insula of Calleja, limbic cortex, hypothalamus, and raphe nuclei. The result has revealed that the neonatal 6-OHDA treatment induces region-specific neuronal damage on dopaminergic neurons, and suggests that this heterogenous destruction is a base of abnormal behavior induced by dopamine agonist loading.

131.3

THE EFFECT OF NEONATAL 6-HYDROXYDOPAMINE LESIONS IN RATS ON HABITUATION TO A NOVEL ENVIRONMENT. S. S. Moy*, D. A. Eckerman*, H. Criswell, and G. R. Breese. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599

Selective dopamine lesions were given to ten rat pups at three days of age, while sham lesions were performed on ten control animals. At four months of age, the activity levels and spatial behavior of these subjects were measured in an activity pattern monitor. Rats were given hour-long sessions, one per day, across five days. Midway through the fifth session, the animals were given an acute stressor (handling for 30 seconds). In contrast to previous reports, the lesioned group was significantly hypoactive in comparison to the control animals. In addition, examination of locomotor paths indicated that the lesioned animals failed to develop the increasingly organized and patterned spatial behavior observed in control animals, demonstrating a deficit in normal habituation to a novel environment. No significant effect of the brief handling was observed. The animals are not yet available for postmortem analyses, which will provide the correlations between extent of dopamine depletion, level of activity, and degree of habituation (measured by the patterning of locomotor paths) both within sessions and across sessions.

131.4

PRENATAL STRESS ALTERS BRAIN CATECHOLAMINERGIC ACTIVITY AND POTENTIATES STRESS-INDUCED BEHAVIOR IN ADULT RATS. L. K. Takahashi, J. G. Turner,* and N. H. Kalin. Dept. Psychiatry, Univ. Wisconsin Med. Sch., Madison, WI 53792 and Middleton Veterans Hospital, Madison, WI 53705.

We demonstrated previously that prenatally stressed (PS) rat pups have increased hormonal and behavioral responses to stress. This study examined whether these characteristics persist into adulthood. Due to the importance of brain catecholaminergic (CA) systems in mediating stress responses, we also measured the activity of NE and DA systems.

PS and control male rats (80-100 days) received 5 foot shocks (1.0 mA, 1 s). During testing, defensive freezing was measured. ACTH and corticosterone were measured by RIA. Brain CA levels were determined by HPLC.

PS rats showed a high level of freezing ($p < .05$). Cortical MHPG/NE and DOPAC/DA ratios were also elevated in PS rats ($p < .05$). Hormone concentrations, however, did not differ between groups. Prenatal stress produces persistent behavioral alterations suggestive of increased defensive responsiveness accompanied by increased CA activity. These changes may compromise an adult's ability to adapt to stressful events.

Supported by NIMH grant MH-43986.

131.5

RATS DOPAMINE LESIONED ON DAY 3 OF NEONATAL LIFE ARE SUBSENSITIVE TO 6-HYDROXYDOPAMINE TREATMENT AS ADULTS ON TESTS OF BRAIN STIMULATION REWARD. Sidhu, K.S., Levesque, C.P.*, and Stellar, J.R. Northeastern University Dept. of Psychology, Boston, MA 02115.

Previously, adult rats dopamine (DA) lesioned at day 3 of neonatal life were shown to be subsensitive to D1 and D2 DA receptor blockade in a test of lateral hypothalamic self-stimulation (LHSS) reward (Sidhu et al., Neuroscience Abstracts, 1990). In this study, adult rats previously DA lesioned at day 3 of life received adult ICV injections of 25ug/ul 6-OHDA after pretreatment with pargyline (40mg/kg) and desmethylimiprimine (25mg/kg). Following the adult lesion, these rats showed an initial LHSS reward deficit, but recovered in three days using the rate-frequency method. Three of four control animals with sham neonatal lesions given the 6-OHDA adult lesion, stopped responding for LHSS. Neonatal DA lesioned animals exhibited transient weight loss following the adult lesion, but stabilized after 10 days post lesion. Control animals lost weight following the adult lesion, did not maintain eating and drinking behaviors, and eventually died within one week.

(Supported by the Whitehall Foundation)

131.6

IS.DENSITY OF REINFORCEMENT RESPONSIBLE FOR THE DIFFERENTIAL EFFECT OF DA ANTAGONISTS ON RAT PUPS DURING TWO INGESTION TESTS? A. Tyrka* and G.P. Smith. Bourne Laboratory, NY Hospital-Cornell Medical Center, White Plains, NY 10605.

The D_1 antagonist, SCH 23390, and the D_2 antagonist, raclopride, significantly inhibit intake of 10% sucrose in independent ingestion tests (II, pups continuously lick sucrose from the bottom of a beaker) but not when sucrose is continuously infused in oral catheter (OC) tests on postnatal days (PN) 7 and 14 (Tyrka & Smith, 1991). Since pups normally ingest twice as much in OC tests as in II tests, it is possible that DA antagonists do not decrease intake in OC tests because reinforcement is more dense in OC tests.

To test this possiblity, PN 7 and 14 pups were deprived for 4 h and orally infused with 10% sucrose at a rate that produced intakes equivalent to those in II tests. Vehicle, SCH 23390, or raclopride (doses were the ID_{50} for II or 4X ID_{50}) was given ip at -15 min. Only SCH 23390 (ID_{50} on PN 7) decreased intake significantly (see Table).

	PN 7		PN 14	
Drug	Dose (ug/kg)	Intake	Dose (ug/kg)	Intake
Saline		1.9 ± 0.1		2.4 ± 0.1
SCH 23390	60	1.4 ± 0.1*	14	2.4 ± 0.2
SCH 23390	240	2.3 ± 0.1	57	2.5 ± 0.2
Raclopride	194	1.6 ± 0.2	208	2.8 ± 0.2
Raclopride	776	2.0 ± 0.1	832	2.3 ± 0.3

Note. Intake values are mean ± SEM percent body weight gained for 8-10 pups per condition. *less than saline, $p < 0.05$.

Since no dose of either antagonist produced decreased intakes comparable in magnitude to decreases in II tests, the differential effect of DA antagonists in the two tests is not explained by the density of reinforcement.

Supported by MH15455 and MH00149 (GPS).

131.7

CHRONIC DOPAMINE ANTAGONIST DURING DEVELOPMENT ALTERS THE D1 AND D2 MEDIATION OF SENSORIMOTOR BEHAVIOR. J.P. Bruno, B.J. Johnson, D.R. Abrams, T. Mitra, T.A. Reader, and K.M. Dewar Dept. of Psychology, Ohio State Univ. Columbus, OH 43210; Dept. de Physiologie, Univ. de Montreal, Que.

Age at the time of DA denervation differentially affects the contributions of D1 and D2 receptors to sensorimotor behavior. 6-OHDA-induced DA depletions on Day 3 enable D1 and D2 receptors to mediate sensorimotor function independently whereas activity of both D1 and D2 receptors is required in controls and rats depleted on Day 20. The present study determined whether temporary blockade of D1 and D2 receptors during various periods of development can replicate the effects of 6-OHDA-induced denervations.

Male rats were injected every 12 hrs with the mixed D1/D2 antagonist flupentixol (1.0 mg/kg/day) or saline from Days 3-20 or Days 20-37 and then tested, at varying ages, for sensorimotor deficits after D1, D2, or D1 + D2 antagonists. Rats treated from Days 3-20 and tested 1 week later resembled rats that had been denervated with 6-OHDA. They were insensitive to the behavioral effects of D1 or D2 antagonists, but just as sensitive as controls to the effects of combined D1 + D2 antagonists. This insensitivity declined with time. In contrast, flupentixol administration from Days 20-37 only slightly altered the sensitivity of animals to DA antagonists. These data suggest that receptor stimulation during certain early stages of postnatal development are necessary for the normal development of D1 and D2 mediation of sensorimotor function. We are presently studying the age-dependent effects of chronic flupentixol on DA receptor density and distribution.

131.8

DOPAMINE DEPLETIONS IN NEONATAL RATS ALTER THE D1 AND D2 CONTRIBUTIONS TO MOTORIC BEHAVIOR.

D.R. Abrams and J.P. Bruno. Dept. of Psychology, Ohio State University, Columbus, OH 43210

Residual DA neurons remain critical for the expression of sensorimotor function in rats depleted of DA as neonates. However, blockade of either D1 or D2 receptors is sufficient to induce sensorimotor deficits in normal rats whereas both receptor subtypes must be blocked to produce deficits in rats depleted of DA as neonates. These results suggest that D1 and D2 receptors are independently capable of supporting sensorimotor functions in rats depleted of DA as neonates but not in controls. The present experiments examined this hypothesis by studying the behavioral effects of D1, D2, and mixed D1 + D2 agonists.

Three-day-old male rats received intraventricular injections of 6-OHDA (100 µg + DMI) or its vehicle and were tested for agonist-induced behaviors as adults. The data reveal both quantitative and qualitative differences between the two groups of animals. Both vehicle- and 6-OHDA-treated rats exhibited locomotion and low-level stereotypy after the D2 agonist quinpirole and high-level stereotypy after the mixed D1 + D2 agonist apomorphine. DA-depleted animals were far more sensitive than controls. In contrast, the D1 agonist SKF 38393 produced locomotion and high-level stereotypy in depleted animals while producing intense grooming in controls. The ability of specific antagonists to block these effects will also be discussed.

131.9

NEURAL MECHANISMS IN THE CONTROL OF SENSORIMOTOR BEHAVIOR IN RATS DEPLETED OF DOPAMINE AS NEONATES. B.J. Johnson and J.P. Bruno.
Dept. of Psychology, Ohio State University, Columbus, OH 43210

Rats depleted of dopamine (DA) with 6-OHDA as neonates are spared from the sensorimotor deficits seen in comparably damaged adults. Residual striatal DA neurons are involved in the expression of these behaviors in rats depleted of DA as neonates but the functional interactions between D1 and D2 receptors differ qualitatively from those seen in controls. In normal animals intrastriatal injections of either D1 or D2 antagonists induce deficits whereas administration of both antagonists is required to impair rats depleted as neonates. The present data extended this observation in two ways. First, we observed similar roles for accumbens D1 and D2 receptors in mediating sensorimotor behavior in controls and rats depleted as neonates. Microinjections of the D1 antagonist SCH 23390 or the D2 antagonist clebopride into the accumbens produced sensorimotor deficits in adult controls but had no effect on rats depleted of DA as neonates. Injections of both antagonists synergistically produced deficits in each group of animals.

Second, we determined the role of striatal D1 and D2 receptors in mediating acetylcholine (ACh) release in order to determine whether the depletion-induced changes at the behavioral level paralleled at the synaptic level. Using in vivo microdialysis in awake animals, we determined that in both adult controls and rats depleted of DA as neonates, D2 agonists inhibit ACh release while D1 agonists stimulate ACh release. Thus, the depletion-induced changes in receptor mediation of sensorimotor behavior do not coincide with receptor mediation of striatal ACh release.

131.10

CONSUMPTION OF A PALATABLE MEAL PREFERENTIALLY INCREASES INTERSTITIAL CONCENTRATIONS OF DOPAMINE IN THE NUCLEUS ACCUMBENS. G.G.Nomikos, C.Wilson, G.Damsma, H.C.Fibiger.
Division of Neurological Sciences, University of British Columbia, Vancouver, Canada.

Interstitial concentrations of dopamine (DA) and its metabolites were examined in the nucleus accumbens and the striatum of rats during feeding behavior using on-line brain microdialysis. Rats were trained to consume under freely feeding conditions a palatable liquid meal (Sustacal). Both the training and the test sessions were performed in a chamber that was partitioned by a screen. The animals were first confined to one compartment (anticipatory phase) and then they were allowed to drink the Sustacal (consummatory phase). At the test session and during the anticipatory stage DA from the nucleus accumbens increased to 110% in groups that were trained and tested with Sustacal, trained but not tested with Sustacal, and trained and tested with water. Subsequently after removal of the screen and initiation of feeding, DA gradually increased to 130% only in the group trained and tested with Sustacal. Striatal DA was enhanced to 110% during the anticipatory phase, but did not increase significantly further after Sustacal consumption. These data provide neurochemical evidence that DA transmission is preferentially enhanced in the nucleus accumbens during consumption of a palatable meal.

131.11

DIFFERENCES IN THE BRAIN MONOAMINE LEVELS IN THE BLOW FLY UNDER THREE FEEDING CONDITIONS. S. Ye, W.J. Bell and N.A. Dahl. Department of Physiology and Cell Biology, and Department of Entomology. University of Kansas, Lawrence, KS 66045.

HPLC systems with electrochemical detection were used to determine the levels of 3,4-dihydroxyphenylethylamine (dopamine) and 5-hydroxytryptamine (5-HT) in the brains of satiated, starved, and recently food-stimulated starved flies, Phormia regina Meigen. Our preliminary data indicate that the brain dopamine level is lower in starved flies than in satiated flies. No significant difference in the 5-HT level was found among the three treatment groups. The short-term feeding regulation mechanism in the blow fly seems to be associated with an unidentified compound found in the fly's brain. The level of this compound increases significantly after the fly ingests a small amount of food (sucrose).

131.12

BASELINE-DEPENDENT FEEDING RESPONSES TO AMPHETAMINE: EFFECTS OF INTRA-ACCUMBENS ALPHA-FLUPENTHIXOL ADMINISTRATION. T. L. Sills, J.P. Baird & F. J. Vaccarino.
Department of Psychology, University of Toronto, Toronto, Ont., M5S 1A1.

Previously we have shown that a low dose of amphetamine (AMP) stimulates sugar intake in low baseline feeders and inhibits intake in high baseline feeders. Furthermore, there is evidence suggesting that nucleus accumbens (ACB) dopamine (DA) mediates AMP's effects on feeding. The present study examined the effects of intra-ACB DA blockade, using alpha-flupenthixol (FLU), on the feeding response to AMP, administered to high and low baseline feeders.

Baseline chow and sugar intake was measured for one hour each day for seven days in male Wistar rats with bilateral ACB cannulae implants. Subsequently, all rats received either systemic saline or AMP (0.125 mg/kg) in combination with intra-ACB saline or FLU (10μg) in a counterbalanced order. Following injections, animals were returned to their home cages and their intake of chow and sugar measured for 1.5 hours.

Animals were divided into high and low feeders based on a median split of their baseline sugar intake. Consistent with previous results, AMP stimulated feeding in low baseline feeders. This effect was blocked by intra-ACB FLU administration. AMP had no effect on feeding in high baseline feeders, though intra-ACB FLU significantly attenuated intake and this was reversed by AMP administration. These results support the notion that increased DA transmission mediates AMP-induced increases, as well as naturally elevated levels of sugar intake.

This research was supported by a NSERC grant to FJV.

131.13

N-0923, A SELECTIVE DOPAMINE D2 RECEPTOR AGONIST, DECREASES FOOD INTAKE AND BODY WEIGHT IN RATS. J.D. Belluzzi, D. McKenna* and L. Stein*, Whitby Research Inc., Irvine, CA 92715 and *Department of Pharmacology, University of California, Irvine, CA 92717.

The anti-obesity effects of the dopamine D2 receptor agonist (-) 2-(N-propyl-N-2-thienylethyl)amino-5-hydroxytetralin HCl (N-0923) were evaluated in rats. Intraperitoneal injections of N-0923 prior to the daily 2-hr feeding period produced anorexia at all doses tested (0.3-3 mg/kg) and induced body weight loss up to 12% within 3-4 days at the highest dose. N-0923 was more potent than either *d*-amphetamine or apomorphine in producing weight loss and its effects were stereoselective. The (+) isomer (N-0924) failed to reduce body weight at 10 times the lowest effective dose of N-0923.

Continuous 24-hr infusion of N-0923, using Alzet implantable osmotic pumps or transdermal patches in free-feeding animals, induced a similar pattern of anorexia and weight loss at both doses tested (50 and 200 μg/hr). Anorexia was produced at doses that did not induce stereotypy or hyperactivity. The anti-obesity effects were independent of starting body weight or state of food deprivation and the weight loss could be maintained by continuous infusion for nearly 1 month. As in the case of acute dosing, continuously administered N-0923 was more potent than *d*-amphetamine and its effects were stereoselective. N-0924 (500 μg/hr) did not significantly reduce body weight.

131.14

NIGROSTRIATAL DOPAMINERGIC ACTIVITY, AS DETERMINED BY *IN VIVO* ELECTROCHEMICAL DETECTION, DURING FOOD CONSUMPTION AND CONDITIONED CONSUMATORY BEHAVIOR. T.J. Brozoski, K. Stenvers, A. Athorp*, G. Skeen, E.L. Brozoski, K.M. Oeth, and J. Green*. Dept. of Psychology, Grinnell College, Grinnell, IA 50112.

Dopaminergic activity, as measured by *in vivo* electrochemical detection of dopamine metabolites, was quantified during feeding and conditioned consumatory behavior without food, in the nigrostriatal system of food-deprived rats. Following 24-hr food deprivation, dopamine activity increased during feeding periods in both the dorso-anterior caudate-putamen and the substantia nigra *pars compacta*. In contrast, no increase in dopamine activity occurred during conditioned consumatory behavior when food was not available. Momentary increments in caudate, but not nigral, dopamine were found to accompany the delivery of food pellets during the initial part of feeding periods. These results were interpreted as supporting the hypothesis that the nigrostriatal dopamine system is a component of the brain reinforcement network, and further taken to indicate that dopamine release is linked to the reinforcing stimulus properties of nutritive reinforcers rather than the motor activity associated with consumption. It was found, however, that increasing food deprivation beyond 24 hrs, to 48 hrs, attenuated the dopaminergic response to feeding, suggesting that other factors are also involved.

131.15

STRAIN DIFFERENCES IN ADAPTATION TO REPEATED STRESSORS: VARIATIONS OF THE INTAKE OF A PALATABLE DIET. J. Griffiths, N. Shanks and H. Anisman. Dept. of Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

Exposure to footshock over 14 days produced strain dependent differences in the intake of a palatable liquid diet. All strains of mice, however, showed some degree of adaptation, such that food intake following repeated stressor exposure equalled that of nonstressed animals. Exposure to a series of different stressors yielded more pronounced reductions in food intake. Moreover, while an adaptation was apparent in some strains, in other strains the decline of intake ordinarily produced by an acute stressor became progressively more pronounced or did not vary with continued stressor exposure. These data were related to strain differences in the variations of NE and DA utilization and levels associated with acute stressors, as well as the adaptation of amine activity associated with the chronic stressor regimen. Additionally, the data were related to the strain-specific alterations in responding for electrical brain stimulation (possibly reflecting an anhedonia), previously observed in this laboratory.

131.16

COPULATION INCREASES DOPAMINE ACTIVITY IN THE MEDIAL PREOPTIC AREA. R. C. Eaton, J. Moses and E. M. Hull. Department of Psychology, State University of New York at Buffalo, Amherst, NY. 14260

Previous studies, employing tissue punch techniques, have reported increased dopaminergic activity in the preoptic area following copulation in male rats (Ahlenius et al. 1987, Hoffman et al. 1987 & Mas et al. 1987). More recently, using in vivo microdialysis, Pfaus et al. (1990) and Pleim et al. (1990) have reported an increase in dopamine (DA) and its metabolites in the nucleus accumbens during copulation.

The MPOA, which has a small dopamine innervation, plays an important role in the regulation of copulatory behavior. Pfaus (1990), using in vivo voltametry, showed an increase of catecholamine (CA) activity in the MPOA during copulation. However, DA and norepinepherine (NE) oxidize at the same voltage and therefore are not dissociable with this technique.

We examined dopaminergic activity in the MPOA during copulation using microdialysis. Baseline levels were established in each of 15 male Long-Evans rats. A receptive female was then placed into the testing chamber and testing continued for 80 minutes. Samples were analyzed by HPLC-EC.

Animals that copulated and had the microdialysis probe in the MPOA ($n=7$) showed a significant increase in DOPAC ($p \leq 0.002$) and HVA ($p \leq 0.05$) levels. An additional animal with the correct probe placement, that did not copulate, failed to show the rise in DOPAC. Animals that copulated, but had incorrect probe placements, showed no increase in DOPAC or HVA.

We have demonstrated that endogenous levels of DOPAC and HVA in the MPOA are increased by copulation. Roth et al. (1976) suggest that short term changes in central DOPAC levels provide a useful index of activity of central dopaminergic neurons. These data support an increase of DA activity in the MPOA during copulation.

131.17

EFFECTS OF QUINELORANE ON YAWNING, PENILE ERECTION AND SEXUAL BEHAVIOR IN THE MALE RAT. P.C. Doherty P.A. Wisler* and M.M. Foreman. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

Quinelorane, a selective D_2 agonist, stimulates sexual activity in male rats and monkeys. Dopamine agonists also increase the occurence of erections in these species. The following experiment was performed to determine if quinelorane could induce erections in male rats, and to establish the relationship of such drug induced erections to the effects of this compound on sexual activity. Adult male Sprague-Dawley rats were given sub-cutaneous injections of saline vehicle or quinelorane (dose range: 0.1 - 100 µg/kg) just prior to a 30 min observation period for the display of yawning and erections. Quinelorane induced significant increases in both erections and yawns at the 3 µg/kg dose. Additional increases in erection were observed at 10 and 30 µg/kg with a slight decline occurring at 100 µg/kg. Yawning was sharply increased at 10 µg/kg and then decreased rapidly at 30 and 100 µg/kg. Quinelorane was more potent than quinpirole and apomorphine in inducing erections, and in reducing ejaculation latency (EL) in tests of copulatory behavior. However, quinelorane caused significant reductions in EL at doses lower than those needed to induce erections. These findings indicate that in addition to its stimulatory effect on sexual activity, quinelorane can also increase the occurrence of spontaneous erections in male rats.

DRUGS OF ABUSE—OPIOIDS: DOPAMINE AND DEPENDENCE

132.1

DEPRESSION OF BASAL DOPAMINE RELEASE AND SENSITIZATION TO MORPHINE-INDUCED STIMULATION IN THE VENTRAL STRIATUM DURING ABSTINENCE.

Acquas E.* Carboni E.* and Di Chiara G. Institute of Experimental Pharmacology and Toxicology, University of Cagliari, Italy.

In order to investigate the role of mesolimbic DA in opiate dependence, we studied by brain dialysis the changes in DA release in the mesolimbic ventral striatum occurring after morphine withdrawal in morphine dependent rats. Male rats (Wistar-Kioto, 300-350 g) were given s.c. (twice a day for 14 days) increasing doses (from 10 to 140 mg/kg) of morphine hydrochloride. At various times after withdrawal the rats were implanted with dialysis probes in the caudal nucleus accumbens-ventral striatum. Stabilized output of DA 1, 3 and 5 days after withdrawal from morphine was about one fifth than in saline controls. Seven days after withdrawal DA-output was still significantly lower (about 40%) than in controls. Administration of morphine (5.0 mg/kg s.c.) stimulated DA output maximally by about 35% in controls but failed to change it after one day of withdrawal. In contrast, 3, 5 and 7 days after withdrawal, morphine (5 mg/kg s.c.) stimulated DA-release more effectively than in controls. This effect was particularly marked after 3 days of withdrawal (max 250%). In view of the role assigned to the mesolimbic DA system in motivation, depression of DA transmission in the mesolimbic system and sensitization to the DA-releasing effect of morphine might play a role in the motivational mechanisms which sustain the self-administration of opiates in dependent subjects.

132.2

THE IDENTIFICATION OF OPPOSING TONICALLY ACTIVE ENDOGENOUS OPIOID SYSTEMS WHICH MODULATE THE MESOLIMBIC DOPAMINERGIC SYSTEM. R. Spanagel*, N. Brose, A. Herz* and T.S. Shippenberg. Department of Neuropharmacology, Max-Planck-Institute für Psychiatrie D-8033 Martinsried,F.R.G.

The mesolimbic dopaminergic (DA) pathway has been implicated in mediating the motivational properties of opioids and other drugs of abuse. However, the neuroanatomical sites of action of opioids and also the endogenous opioid systems which modulate this reward pathway remain unclear. These issues were examined by the use of in vivo microdialysis and administration of highly selective opioid ligands. The microinjection of DAMGO, a selective μ-agonist, into the ventral tegmental area (VTA) (the site of origin of A10 DA neurons) resulted in significant increases in DA release and metabolism in the nucleus accumbens (NAC)(the major projection site of A 10 DA neurons). However, DAMGO infusions into the NAC via the microdialysis probe did not affect DA release or its metabolites. The selective blockade of μ-receptors by CTOP within the VTA produced significant decreases in DA basal release and metabolism. In contrast, CTOP infusions into the NAC were without effect. The selective k-agonist U 69853 microinjected into the VTA was without any effect on DA overflow and metabolites. In contrast, U 69853 infusion into the NAC resulted in a dose dependent significant decrease in DA release and metabolism. Infusions of the k-antagonist nor-BNI into the NAC which selectively blocked k-receptors in this region dose dependently increased basal DA release within the NAC. These data demonstrate that tonic activation of μ- and k-receptors is required for the maintenance of basal DA release in the NAC. Such findings may have implications for the treatment of opiate dependence and disorders of mesolimbic DA function.

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132.3

VENTRAL PALLIDUM OPIATE AND GABA ANTAGONIST MICROINJECTIONS DISPLAY REGIONAL DIFFERENCES AS MEASURED BY INTRACRANICAL SELF-STIMULATION.

P. Johnson, A.D. Paul*, J.R. Stellar. Psychol. Dept., Northeastern Univ., Boston, MA. 02115

Ventral Pallidum (VP) microinjections of the mu opiate specific agonist, DAGO, and the $GABA_A$ specific antagonist, picrotoxin, have been shown to increase motor activity in a behavior activation paradigm (Austin et al. J. Pharm & Exper Ther, 252(3):1990, 1370-77). When tested using the self-stimulation rate-frequency paradigm, rostral VP injection of DAGO (1.23, 13.56 ug/0.5ul) produced dose-dependent reward and motor/performance decreases, while caudal VP injection produced reward and performance increases. Picrotoxin (0.03, 0.1 ug/0.5ul) appeared to have no effect on reward for any placement, but followed the DAGO regional pattern with regard to performance.

(Supported by the Whitehall Foundation)

132.4

EFFECTS OF REPEATED MORPHINE TREATMENT ON THE SENSITIVITY OF DOPAMINE RECEPTORS IN THE NUCLEUS ACCUMBENS.

M. Jeziorski and F.J. White, Wayne State University School of Medicine, Dept. of Psychiatry, Cellular and Clinical Neurobiology Program, Neuropsychopharmacology Lab, Lafayette Clinic, Detroit, MI 48207.

Acute administration of morphine induces increased locomotor activity in rats, a stimulation that is enhanced upon repeated application of morphine. Evidence suggests that the mesolimbic dopamine (DA) projection to the nucleus accumbens (NAc) may be implicated in the development and expression of this behavioral sensitization. Results reported from our laboratory indicate that a schedule of cocaine treatment that produces a similar pattern of sensitization increases the sensitivity of DA receptors on neurons in the NAc, an alteration that appears to be restricted to D1 DA receptors. To examine whether comparable changes in DA receptor sensitivity are associated with morphine-induced sensitization, single unit electrophysiological experiments were conducted on NAc neurons in morphine-treated rats (10 mg/kg/day i.p., 14 days). Rats were tested for sensitization prior to recording; ambulation counts were elevated five-fold on the 14th day of morphine treatment when compared to the first day. However, the ability of iontophoretic DA to inhibit the firing of NAc neurons was similar in vehicle-treated and morphine-treated rats, indicating no difference in DA receptor sensitivity. Further experiments will be conducted to investigate the individual responses of D1 and D2 receptors on NAc neurons after repeated morphine treatment. (Supported by USPHS grants DA-04093 and MH-40832 and by the State of Michigan.)

132.5

THE EFFECTS OF LESIONS OF THE HABENULAR NUCLEI ON THE DEVELOPMENT OF SENSITIZATION TO THE ACTIVATIONAL EFFECTS OF MORPHINE ADMINISTERED REPEATEDLY INTO THE VTA.

D. Funk and J. Stewart. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada, H3G1M8

Repeated intermittent injection of morphine into the cell body region of the midbrain dopamine (DA) neurons results in sensitization of its behavioral activating effects. The midbrain DA systems are regulated in part by an inhibitory projection from the habenular nuclei. To determine whether changes in this inhibitory projection from the habenular nuclei might participate in the development of sensitization to the behavioral activating effects of intra-ventral tegmental area (VTA) injections of morphine, groups of rats were given electrolytic lesions of the habenular nuclei and treated every third day for 6 days with bilateral injections of 5 μg/0.5 μl/side morphine or saline. Lesions did not affect the acute locomotor activation seen following intra-VTA morphine, nor the sensitization of locomotor activity seen when morphine was administered repeatedly. Intra-VTA morphine did, however, result in high levels of stereotyped gnawing and licking in lesioned animals. Interestingly, increases in these behaviors did not appear to result in the reduction of locomotor activity normally seen when levels of stereotypy are high. Though these findings confirm the idea that the habenular nuclei exert an inhibitory influence on the activity of the DA systems, they do not suggest that changes in the habenular influence underlie sensitization to the behavioral activating effects of morphine seen with repeated administration.

132.6

MICROINJECTIONS OF SELECTIVE μ AND ∂ OPIOID AGONISTS INTO THE VENTRAL TEGMENTUM INCREASE EXTRACELLULAR NUCLEUS ACCUMBENS DOPAMINE: AN IN VIVO MICRODIALYSIS STUDY.

D. P. Devine, P. Leone, D. Pocock, and R. A. Wise. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada H3G 1M8.

The putative involvement of ventral tegmental area (VTA) μ and ∂ opioid receptors in modulation of mesolimbic dopaminergic activity was assessed using in vivo microdialysis and HPLC with electrochemical detection. Independent groups of chloral hydrate anesthetized (400 mg/kg, supplemented when needed) male Long-Evans rats were given intracranial microinjections of selective μ and ∂ opioid agonists aimed at the VTA, and extracellular nucleus accumbens (NAS) dopamine (DA) and DOPAC were assayed. VTA microinjections of DAGO (selective μ agonist) or DPDPE (selective ∂ agonist) resulted in dose-dependent increases in NAS DA and DOPAC. VTA microinjections of CTOP (selective μ antagonist) also produced dose-dependent increases in NAS DA, suggesting the possibility that CTOP may exert a partial agonist activity following intracranial administration. Pretreatment with VTA CTOP antagonized DAGO-mediated increases in NAS DA, but failed to antagonize DPDPE-mediated increases in NAS DA. After CTOP pretreatment, NAS DOPAC increases were roughly equivalent following DAGO or DPDPE administration. These results suggest that VTA μ and ∂ opioid receptors may each be involved in opioid disinhibition of mesolimbic DA neurons. The specific roles played by VTA μ and ∂ opioid receptors in regulation of NAS DA release is being further examined using the selective ∂ antagonist naltrindole.

132.7

OPIOID EFFECTS IN THE VTA AND VENTRAL PALLIDUM: MEDIATION BY GABA AND MESOLIMBIC DOPAMINE.

M.A. Klitenick and P.W. Kalivas. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

A variety of evidence lends support to a circuit posited to be comprised of the VTA, nucleus accumbens (NAS) and ventral pallidum (VP) and that increased dopaminergic transmission within this circuit results in an increase in locomotor activity. The present experiments addressed two aspects of this circuit. In the first experiment the effects of morphine administration on extracellular levels of GABA was assessed using *in vivo* microdialysis in the VTA. The second experiment investigated the modulatory role of GABA in the VTA on the activity elicited by opioid injections into the VP. In the first experiment it was found that morphine, administered through the dialysis probe, into the VTA produced a significant decrease in extracellular levels of GABA. This is in agreement with the results of other studies suggesting that within the VTA, an indirect action by morphine via GABAergic afferents on dopamine neurons, or disinhibition, is the mechanism involved in the increased dopaminergic activity in the terminal fields and the resultant hyperactivity. In the second experiment, the increase in locomotor activity elicited by injections of DAMGO into the VP were blocked by intra-VTA injections of the $GABA_B$ agonist baclofen. Furthermore, disruption of dopaminergic transmission by 6-OHDA lesions of the NAS reversed this blockade. These results and those of other studies suggest that the locomotor activity elicited by activation of the VP may be under the tonic control of the mesocorticolimbic dopamine system. These data suggest that the tonic inhibitory influence of GABAergic afferents can be blocked by opioids, thus indirectly activating the dopaminergic system leading to an increase in locomotor activity.

132.8

ALTERATIONS IN THE MOTIVATIONAL EFFECTS OF OPIOIDS FOLLOWING PROLONGED PAIN: INVOLVEMENT OF THE MESOLIMBIC SYSTEM.

T.S. Shippenberg. Dept. of Neuropharmacology, Max-Planck-Institute for Psychiatry, D-8033 Martinsried, FRG.

Place conditioning and microdialysis were used to examine the influence of prolonged pain associated with Freund's adjuvant (FA)-induced inflammation on the motivational effects of opioids. μ-receptor agonists (Ag) functioned as appetitive reinforcers in naive rats, producing dose-related place preferences. κ-Ag produced place aversions. 6-hydroxydopamine lesions of the n. accumbens (NAC), but not other areas, abolished both effects of opioids. Microdialysis revealed that μ-Ag increased NAC dopamine (DA) release: κ-Ag decreased release. Rats injected with FA 7-days prior to conditioning showed place preferences in response to μ-Ag and an increase in NAC DA release. κ-Ag failed to produce aversive effects in FA-rats and did not alter DA release. These data demonstrate that κ-Ag lack aversive effects in subjects with prolonged pain and suggest that pain-induced changes in such effects result from alterations in endogenous κ-opioid systems which modulate mesolimbic DA activity.

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132.9

MORPHINE ADMINISTERED TO THE VENTRAL TEGMENTAL AREA PRODUCES PLACE PREFERENCE CONDITIONING IN THE NEONATAL RAT. G. Rossi and G. A. Barr. Biopsychology Doctoral Program, Dept. of Psych., Hunter College-CUNY, NY, NY 10021 and Dept. Devel. Psychobiol. New York State Psychiatric Inst., NY, NY 10032.

When given peripherally, morphine is known to produce a conditioned place preference (CPP) in adult and infant rats. In adults, morphine injected into the ventral tegmental area (VTA) is reinforcing and likely acts by activating mesolimbic dopamine neurons. Little is known of the neurobiological basis of reinforcement in the immature animal and whether or not similar reinforcement mechanisms are present throughout development. Therefore, we have begun to examine the neural mechanisms underlying the reinforcing properties of abused drugs in the infant rat. Pups were injected with one of 4 doses (.05, .15, .45, 1.35 µg) of morphine or the vehicle directly into the VTA and were immediately confined to an odor-cued environment for 30 minutes. They were then tested for a preference between the cued environment and unadulterated wood shavings. The low dose of morphine (.05 µg) injected into the VTA significantly increased duration of time spent in the conditioned environment, demonstrating a preference for the conditioned area over the unscented area; the most effective injection sites were directly into the VTA. Stimulation of structures just outside the VTA, and higher doses of morphine were ineffective. On the basis of these findings we conclude that the neural substrates of opiate reward in the neonate may be similar to those of the adult. (Supported in part by DA-06600)

132.10

CONDITIONED PLACE PREFERENCE (CPP) PRODUCED BY INTRANIGRAL MORPHINE. A.A. Baumeister, M. Hurry*, R. Leoni*, B.W. Curtis* and T. Chaney*. Department of Psychology, Louisiana State University, Baton Rouge, LA 30803.

Bilateral guide cannulae were implanted 1 mm above the caudal substantia nigra of male Sprague Dawley rats. One week later animals were allowed to explore the CPP apparatus (an alley having one white and one black compartment) for twenty minutes on two consecutive days. The amount of time spent in each side of the alley on day two (pretest) was recorded. On days three and five rats received intranigral injection of saline, then were confined to one side of the apparatus for twenty minutes. On days four and six they received intranigral morphine (10 nmol/0.5 µl/side) followed by confinement to the other side of the apparatus for twenty minutes. The side of the apparatus paired with morphine was independent of pretest scores and was counterbalanced. On day 7 animals were tested for place preference (posttest) during a 20 minute period. No injection was given before the posttest. The mean percent of time that the animals spent on the side paired with intranigral morphine was significantly increased, $t(21) = 2.84$, $p < 0.01$, on the posttest (63.2%) compared to the pretest (53.5%). No place preference was observed when morphine was injected 1 mm caudal to this site. This study suggests that the substantia nigra may play a role in morphine reward. (Supported by NIDA grant DA05907)

132.11

THE BEHAVIORAL AND PHARMACOKINETIC PROFILE OF HIGH DOSE BUPRENORPHINE ADMINISTERED SUBLINGUALLY IN HUMANS. S.L. Walsh, K.L. Preston*, M.L. Stitzer*, S.L. Dickerson*, E.J. Cone* and G.E. Bigelow* Dept. of Psychiatry, Johns Hopkins University School of Medicine and NIDA Addiction Research Center, Baltimore, MD 21224

Buprenorphine (BUP) is a mixed opioid agonist-antagonist which is currently being investigated as a treatment for opiate dependence. The purpose of this study was to evaluate the subjective and physiological actions and pharmacokinetics of BUP across a wide range of doses in non-dependent experienced opioid abusers (N=4). Sessions were conducted once weekly; sublingual BUP doses were 0, 1, 2, 4, 8, 16 and 32 mg. Physiological and subjective indices were monitored for 96 hours post-dosing; BUP blood levels were analyzed by radioimmunoassay.

BUP doses, 2 mg and higher, produced equivalent decreases in respiration rate and concomitant increases in heart rate with no effect on blood pressure. BUP produced significant and long-lasting pupillary constriction which was present for 48 hours or longer. The dose-effect function for subjective effects (e.g. magnitude and liking) was an inverted-U, with dose-related increases up to 8 mg and then a leveling off and downturn at 16 and 32 mg, respectively. In general, the pharmacokinetic data indicate that blood levels were dose- and time-related up to 32 mg. Clinically significant blood levels (3 ng/ml) were observed up to 96 hours following administration of 32 mg BUP. These data indicate that BUP's effects on subjective and physiological responses are not dose-dependent and may reach a ceiling at doses as low as 2 mg. These effects are not solely attributable to the pharmacokinetic actions of BUP.

132.12

SUBSTANCE P(1-7) INHIBITS WITHDRAWAL JUMPING BEHAVIOR IN MORPHINE-DEPENDENT MICE. J.S. Kreeger *and A.A. Larson. Department of Veterinary Biology, University of Minnesota, St. Paul MN 55108

Human patients who take morphine for treatment of chronic pain reportedly do not develop tolerance/dependence to the extent predicted. The N-terminal metabolite of the putative pain neurotransmitter SP, SP(1-7), has been demonstrated to have biological activity, specific binding sites and to interact with mu-selective opiate binding sites in the CNS. The purpose of this study was to determine the effects of SP(1-7) on the development of opiate dependence and the expression of withdrawal. Morphine-dependent, male, Swiss-Webster mice were injected with SP(1-7) and withdrawal jumping monitored over a 15 min period after a challenge with naloxone (s.c.). Doses of 0.5 and 1.0 nmol of SP(1-7) intrathecally (i.t.) 30 min prior to 20.0 mg/kg naloxone in mice receiving 25.0 µg of morphine sulfate (MS) i.t. once daily. for 3 days significantly decreased withdrawal jumping ($p<0.01$). SP(1-7) (1.0 nmol) coadministered i.t. with MS on days 1 and 2 or days 1, 2, and 3 of the dependence protocol had no effect on withdrawal jumping in response to naloxone. Coadministration of SP(1-7) with MS on day 3, however, caused a decreased withdrawal jumping ($p<0.05$), suggesting that SP(1-7) alters the expression of withdrawal rather than the development of dependence. Parenteral administration of 1.0 nmol of SP(1-7) with naloxone (i.p.) in MS-dependent mice also decreased withdrawal jumping ($p<0.01$). Spinal and supraspinal effects of SP(1-7) appear to differ as injection of 1.0 nmol of SP(1-7) into the right cerebral ventricle immediately prior to naloxone (s.c.) significantly increased, rather than decreased, jumping behaviors ($p<0.01$). From these data, it appears that the N-terminal portions of SP and SP metabolites may play a role in the expression of withdrawal in morphine-dependent mice. (NIDA 04090,04190,00124, and 07234)

132.13

RHYTHMIC FICTIVE SWALLOWING AS AN INDEX OF NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL IN THE RAT. D. Bieger, C.W. Loomis and I. Young, Basic Medical Sciences, Faculty of Medicine and School of Pharmacy, Memorial University, St. John's, Nfld., Canada A1B 3V6

Twitch-like repetitive activity of ventral neck musculature which occurs in the rat during naloxone-precipitated opioid withdrawal has been proposed as a quantitative measure of physical dependence. The present study was aimed at defining i) the physiological nature of this response and ii) its utility in evaluating the exposure of supraspinal structures after intrathecal opioid administration at the lumbar level. Male Sprague Dawley rats (300-400g) were implanted with intrathecal catheters for continuous infusion of morphine (5µg/h) or saline (1 µl/h) via mini-osmotic pumps for 4 d (groups A and B). Uncatheterized rats were given either s.c. morphine 25mg/kg bid. for 4 d (group C) or no treatment (group D). Tail flick latency and paw pressure withdrawal were determined once daily. On day 4, animals were anesthetized with urethane and fitted with intrapharyngeal and esophageal balloons for recording of respective intraluminal pressures. Baseline and i.v. naloxone (NAL) (0.5 or 1.0 mg/kg)-induced responses were determined over 3 to 4 h. Our results show that in opioid-treated, but not untreated rats, NAL induced rhythmic fictive swallowing (RFS). The number of swallows recorded in the first 5 min post injection was 40±24, 11±26, 1.3±1.5 and zero for groups C,A,B and D, respectively. The preliminary data indicate: 1. the previously reported twitch activity of ventral neck muscles during opioid withdrawal is a component of swallowing; 2. rate of NAL-induced RFS correlates with the total morphine dose and presumably with the degree of physical dependence; 3. lumbar intrathecal infusion of an antinociceptive dose of morphine causes supraspinal signs of physical dependence; 4. spinal catheterization and/or intrathecal saline infusion may lead to activation of supraspinal opioid mechanisms. Supported by MRC (Canada).

132.14

EXCITATORY AMINO ACID ANTAGONISTS DO NOT BLOCK MORPHINE WITHDRAWAL BEHAVIORS. G. Aston-Jones, C. Chiang, Y. Zhu, R. Valentino, and M. Page. Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, 19102.

Recent studies revealed that antagonism of excitatory amino acids (EAAs) in brain (Rasmussen and Aghajanian, 1989) or within LC (Akaoka et al., 1990) strongly attenuated morphine withdrawal-induced activation of LC neurons. As LC hyperactivity may play a role in opiate withdrawal, we studied the effects of several EAA antagonists on behavioral indices of morphine withdrawal. Rats were pretreated continuously for 6 days with morphine delivered from chronically implanted osmotic minipumps (34 mg/kg/day). Animals were then given an EAA antagonist or vehicle and subsequently administered naltrexone (1 mg/kg, ip) to precipitate withdrawal. Withdrawal behaviors scored included jumping, wet dog shakes, head shakes, teeth chattering, chewing, diarrhea, rhinorrhea, lacrimation, ptosis, and piloerection. None of the antagonists tested by icv (kynurenate, 11-33 µmoles, n=3; AP5, 20-35 nmoles, n=4; CNQX, 3.5 nmoles, n=2; or CGS19755, 1.2 nmoles, n=2) or by ip administration (CGS19755, 3.8 mg/kg, n=2) consistently decreased any withdrawal behavior. Jumping and wet dog shakes appeared to be increased by the EAA antagonists given icv. Higher doses for each agent induced profound ataxia, precluding withdrawal assessment.

These results indicate that EAA antagonists of either NMDA or non-NMDA receptors do not prevent naltrexone-precipitated morphine withdrawal. As LC withdrawal hyperactivity is attenuated by doses of kynurenate or CNQX that were ineffective on behaviors scored here, these components of the morphine withdrawal syndrome may not depend on LC hyperactivity. Alternatively, the effects of these antagonists on LC neurons may differ in conscious vs. anesthetized animals. Nonetheless, our results are consistent with observations (Britton et al., 1984) that lesions of the ascending NE projections from LC do not attenuate behavioral signs of opiate withdrawal. Although LC may not mediate these behavioral manifestations of opiate withdrawal, its hyperactivity may be involved in other withdrawal phenomena (e.g., related to mood, state or craving). Supported by PHS grant DA 06214.

132.15

EFFECTS OF ACUTE/CHRONIC MK-801 ON NALOXONE-PRECIPITATED JUMPING IN MORPHINE-DEPENDENT MICE. K.L. Marquis, M.J. Piesla*, E.A. Muth and C.A. Boast. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

It has been recently reported that chronic coadministration of the excitatory amino acid antagonist MK-801 with morphine retards the development of tolerance and reduces the degree of dependence produced by the opiate in rats (Trujillo and Akil, Science 251:85-87, 1991). The current study was conducted to examine the acute and chronic effects of MK-801 on a measure of opiate dependency, naloxone (NAL)-precipitated jumping, in mice. Male CF-1 mice (Charles River) were made dependent on morphine by either the implantation of a 75 mg morphine pellet (P) for 3 days or by the administration of morphine (10 mg/kg sc) by injection (I) twice daily for 9 days. In P-dependent mice, 0.05 and 0.1 mg/kg NAL (ip) produced an average (X) of 87.3 and 118.6 jumps in 10 minutes, respectively. The acute administration of 0.1 mg/kg ip MK-801 (30 min prior to NAL), did not alter the number of jumps precipitated by 0.1 mg/kg ip NAL (X=99.3) but significantly reduced the number of jumps which occurred following 0.05 mg/kg ip NAL (X=11.4) in P-dependent mice. Likewise, in I-dependent mice, in which a dose of 1.0 mg/kg ip NAL produced an average of 26 jumps, the acute administration of MK-801 (0.17 mg/kg ip) abolished jumping behavior. In contrast, the acute administration of 0.054 mg/kg ip MK-801 in I-dependent mice increased jumps (X=56), but not significantly. The chronic coadministration of MK-801 (0.054-0.17 mg/kg ip) with morphine (injection) did not significantly affect the average jumps precipitated by NAL (1 mg/kg ip). While the effects of chronic MK-801 on opiate dependency did not occur in the current study, a clear effect of acute administration of MK-801 on this measure was observed. This effect may depend on the intensity of the withdrawal reaction as controlled by the level of dependency and/or dose of naloxone.

132.16

qEEG CHARACTERISTICS OF TWO DOSAGE REGIMENS DURING BUPRENORPHINE MAINTENANCE AND WITHDRAWAL IN HEROIN-DEPENDENT SUBJECTS. R. Herning*, B. Koeppl*, W. Pickworth, R.E. Johnson*, P.J. Fudala, J.H. Jaffe* and N. Khazan. NIDA/Addiction Research Center, Baltimore, MD 21224.

In outpatient studies, chronic daily buprenorphine (BUP) administration to opioid-dependent subjects has been found to be an efficacious treatment associated with reduced illicit opioid use. In the present study quantitative EEG (qEEG) was used to compare BUP daily vs. alternate-day treatment. EEG data from 16 of 24 subjects enrolled in this study is reported. All subjects were rapidly inducted onto sublingual BUP (2, 4, and 8mg) over 3 days then maintained on 8mg through study day 18. For the next 18 days, Group 1 (n=8) continued to receive 8mg BUP daily, while Group 2 (n=8) received 8mg BUP or placebo on alternate days. Daily placebo was then administered for an additional 16 days. EEG was recorded from Fz, C3, C4 and Pz leads two and a half hours after drug administration and EEG power spectra were computed. Upon BUP withdrawal, a significant decrease in EEG total power and in alpha and theta power emerged associated with a higher alpha frequency; effects characteristic of BUP/Opioid abstinence (LUKAS et al., Clin. Pharmacol. Ther., 30: 127-132, 1984). The Pz lead, rich with alpha wave activity demonstrated the highest drop in EEG total power. While on alternate-day BUP, Group 2 showed no significant EEG correlates of opioid withdrawal on placebo days. However, self-reported increase in "urge" for an opioid; increase in subjective dysphoria scale score and pupillary dilatation in Group 2 were previously reported. These differences support the premise that BUP may produce a more effective opioid maintenance when administered daily. Nevertheless, the "urge" to use an opioid even on placebo days was not greater than that of Group 1 which exhibited little day to day fluctuation.

132.17

IgG FROM NEUROPEPTIDE FF ANTISERUM REVERSES MORPHINE TOLERANCE. J.R. Lake, M.V. Hammond*, R.C. Shaddox*, L.M. Hunsicker*, J.M. Cannoy-Searcy*, H.-Y.T. Yang[1] and D.H. Malin. Univ. of Houston-Clear Lake, Houston, TX 77058 & [1]NIMH Neuroscience Ctr. at St. Elizabeth's, Washington, D.C. 20032.

Neuropeptide FF (NPFF, F8Fa) appears to play a role in opiate dependence and abstinence syndrome (Malin et al., Peptides 11:969,1990). The present study assessed the role of NPFF in opiate tolerance. Twelve rats were rendered tolerant by 7 days s.c. infusion of 0.57 mg/kg/hr morphine via Alzet osmotic minipump. They were then tested for pain sensitivity (tail flick with 15 sec. cut-off) and injected i.c.v. with 22 µg IgG from NPFF antiserum or control serum. Sixty mins. later they were injected with 6 µg morphine i.c.v. and retested 5, 12 and 20 mins. later. The table shows their morphine analgesia as % of maximal possible effect. The morphine analgesia of 5 non-tolerant rats is also shown for purposes of comparison. IgG from NPFF antiserum virtually restored the morphine response to levels shown by non-tolerant rats.

% Max. Analgesic Response to 6 µg morphine i.c.v. (M±SEM)			
Non-tolerant/no IgG	56.2 ± 18.8	95.3 ± 4.7	100.0 ± 0.0
Tolerant/control IgG	7.9 ± 3.8	8.2 ± 4.6	16.8 ± 10.3
Tolerant/NPFF IgG	57.3 ± 13.5	90.7 ± 7.2	89.5 ± 7.8

In contrast, IgG from NPFF antiserum did not affect analgesic response to i.c.v. morphine in 12 opiate-naive rats. These results are consistent with the hypothesis that endogenous NPFF contributes to opiate tolerance.

132.18

INHIBITION OF MORPHINE TOLERANCE AND DEPENDENCE BY MK-801: BEHAVIORAL AND NEUROCHEMICAL STUDIES. K.A. Trujillo, D.M. Bronstein and H. Akil, Mental Health Research Institute, University of Michigan, Ann Arbor, MI.

We have previously reported that the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 inhibits the development of morphine tolerance and dependence. Since learning has been found to influence tolerance and dependence, and since MK-801 interferes with learning, it is possible that this drug inhibits tolerance and dependence primarily by interfering with learning processes. A series of studies was designed to explore this possibility. Learning cues were manipulated either 1) by repeatedly injecting animals in the presence or absence of specific environmental cues, or 2) by chronically treating animals using osmotic mini-pumps and morphine pellets. Preliminary results indicate that MK-801 interferes with tolerance and dependence even in the absence of associative cues, suggesting that the actions of this drug are not simply due to its effects on learning. MK-801 may therefore act more directly on the neural changes responsible for opiate tolerance and dependence.

In addition to studying the behavioral interactions between morphine and MK-801 we have also begun to examine the effects of these drugs on brain opioid peptides. Preliminary results suggest that MK-801 attenuates the ability of morphine to increase striatal prodynorphin peptides. Effects on ß-endorphin are currently being analyzed. These results suggest that MK-801 interferes not only with the development of opiate tolerance and dependence, but also with specific neurochemical changes associated with these phenomena.

This work was supported by NIDA NRSA DA05336 (K.A.T.), and NIDA DA02265 and NIMH MH422251 (H.A.).

132.19

THE ACOUSTIC STARTLE RESPONSE AS A MEASURE OF BEHAVIORAL DEPENDENCE IN RATS. R.S. Mansbach, L.H. Gold and L.S. Harris. Dept. of Pharmacology & Toxicology, Medical College of Virginia, Richmond VA 23298

There are at present few objective measures of behavioral disruption following chronic administration of abused drugs. Those measures which are available often are difficult to establish and fail to identify drugs that do not produce a classical withdrawal syndrome. The acoustic startle response (ASR) was examined in the present study as a potentially sensitive behavioral index during naloxone-precipitated morphine withdrawal. Groups of 6-8 male rats were implanted with 2 pellets (s.c.) containing 75 mg each of morphine sulfate or placebo. The ASR was elicited by 40-msec, 122 or 107 dB[A] noise bursts delivered at 30- or 60-sec intervals. Pre-implant startle tests revealed no differences between placebo and morphine groups. In Experiment 1, administration of 0.05-0.2 mg/kg s.c. naloxone 5 min prior to startle testing induced large, dose-dependent startle decreases in morphine-dependent rats but no change or increases in placebo rats also injected with naloxone. In Experiment 2, a modified procedure involving fewer startle trials and fewer test days revealed unmistakable and significant disruption of the ASR following 0.2 mg/kg naloxone. Startle responses in placebo-pellet rats were not significantly affected by naloxone. The ASR decreases in morphine rats were accompanied by body-weight loss in all subjects, but weights of placebo-pellet rats were unaffected. Evidence of conditioned abstinence in both startle and body weight measures was also observed. These data support the use of startle-response measures in examining behavioral and pharmacological factors leading to drug abuse relapse. Supported by PHS grant DA-00490.

132.20

NICOTINE TOLERANCE AND DEPENDENCE: A BEHAVIORAL ASSESSMENT USING SCHEDULE CONTROLLED RESPONDING, LOCOMOTOR ACTIVITY, AND SENSORIMOTOR REACTIVITY. D. HELTON, J. TIZZANO, D. MODLIN*, AND K. RASMUSSEN. Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN 46140.

Previous studies have demonstrated that chronic nicotine (NI) treatment produces tolerance and dependence. Sensitive behavioral measures have been used to describe the magnitude and time course of behavioral disruptions resulting from abrupt removal of NI. The present experiments extend previous findings using a battery of behavioral measures following abrupt NI cessation. NI (0, 10 or 20 mg/kg/day) was continuously administered for 12 days in rats (10/group) by surgically implanting Alzet osmotic mini-pumps subcutaneously. The first experiment evaluated effects using a light/dark discrimination task. There were no significant effects on percent correct responding, or rate of responding during NI administration, or for 5 days following removal of NI. The second experiment evaluated effects on locomotor activity and auditory startle responding. Chronic NI administration produced significant dose-dependent increases in locomotor activity during the first 5 days of exposure, but no significant alterations were seen in activity levels following NI removal (0-5 days). However, during NI withdrawal (1-5 days), significant increases were seen in startle amplitude in both NI groups compared to controls. These studies indicate that the expression of NI tolerance and dependence is dependent on the behavioral task employed.

133.1

[^{3}H]DTG BINDING IN RAT HIPPOCAMPUS: A SIGMA SITE? Duncan P. Taylor, Diana Marrero*, and Jennifer Defnet*. CNS Neuropharmacology, Bristol-Myers Squibb Co., Wallingford, CT 06492-7660.

The role of the σ binding site in brain function is unknown. Several compounds with affinity for this site, such as BMY 14802 (Taylor et al., 1990; Moon et al., 1990), have been found to be active in models of neuroprotection. Recently, Debonnel et al. (1990) showed that σ ligands, such as 1,3-di-o-tolylguanidine (DTG), potentiate NMDA-induced hippocampal neuron activation, and this effect could be blocked by haloperidol and BMY 14802. We investigated the pharmacologic specificity of in vitro [^{3}H]DTG binding in the rat hippocampus and compared it to our previous studies of σ sites using the same ligand in whole guinea pig brain as well as (+)-[^{3}H]-3-PPP in rat cortex. Some interesting differences were noted: stereoselectivity for enantiomers of BMY 14802, butaclamol, dextrorphan and dexoxadrol was lost, and reversed stereoselectively for the enantiomers of cyclazocine and N-allylnormetazocine was seen. In addition, phencyclidine was more potent in the rat hippocampus. Our data suggest not only that [^{3}H]DTG labels a σ type of binding site in rat hippocampus, but that this site may be a heterogeneous mixture of multiple subtypes of σ sites or that the rat hippocampus may contain a different subtype than the guinea pig brain or the rat cortex.

133.2

PD 128298, A NOVEL SELECTIVE LIGAND FOR SIGMA BINDING SITES: CHARACTERISTICS AND FUNCTIONAL ACTIVITY. T. A. Pugsley, Y. H. Shih*, S. Whetzel*, F. Ninteman* J. Wiley*, M. D. Davis, L. Meltzer, H. Tecle*, and T. Heffner. Dept. of Pharmacol. and Chem., Parke-Davis Pharmaceutical Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Although the functional role of sigma sites remains obscure, studies suggest a role of this site in: a) modulation of motor behavior, smooth muscle contraction and phosphoinositide metabolism; b) enhancement of norepinephrine release; c) inhibition of neuronal firing rates. This study describes the binding characteristics and functional activity of 3-phenyl-1-(1,2,3,6-tetrahydro-1-propyl-3-pyridinyl)-1-propanone oxime, monohydrochloride (PD 128298).

PD 128298 potently displaced [^{3}H] -(+)-3-PPP from guinea pig brain sigma binding sites with an IC_{50} value of 1.6 nM, similiar to that of haloperidol and lower than that of DTG; it displaced [^{3}H] -DTG binding with an IC_{50} of 19 nM. It was at least 400-fold selective for sigma versus other binding sites. In vitro PD 128298, like DTG and haloperidol, inhibited the contractions of the guinea pig ileum elicited by electrical stimulation. In vivo PD 128298 (5-20 mg/kg, s.c.) was active in a behavioral despair test and in potentiating the behavioral effects of methamphetamine in self-stimulating rats. No consistent effects of PD 128298 on locomotor activity in rodents was observed. Neurochemical studies indicated modest increases in the synthesis and extracellular levels of dopamine in rat striatum. No extrapyramidal-like effects were induced by PD 128298 in haloperidol sensitized monkeys. These findings with PD 128298 may aid in understanding the potential functional effects of sigma ligands.

133.3

STRUCTURAL CLASSES OF COMPOUNDS WITH POTENT AFFINITY FOR BRAIN SIGMA BINDING SITES B.K. Koe, C. B. Fox,* and L.A. Lebel. Central Research Division, Pfizer Inc., Groton, CT 06340

Interest in brain sigma binding sites stems from the potent affinity of several classes of therapeutic or potential therapeutic drugs for these sites, such as antipsychotics (haloperidol, rimcazole, BMY 14802); antidepressants (sertraline (5-HT uptake blocker), opipramol (tricyclic), clorgyline (MAO inhibitor)); antiischemic agents (ifenprodil, SL 82.0715). Moreover, a link to glutamate NMDA receptors is suggested by the effects of sigma ligands on NMDA induced activation of hippocampal neurons in vivo. These diverse compounds can be grouped into several structural types: 1-substituted piperidine (SC 50691); 1,3- (3-PPP, 3-PPPP) and 1,4- (haloperidol, ifenprodil, SL 82.0175) substituted piperidines; 1,2,6-substituted piperidine (lobeline); 1,2,3,4-substituted piperidines (SKF 10047, dextromethorphan, pentazocine,); and structures with phenylbutylamine-like (sertraline, clorgyline, opipramol, GBR-12909, haloperidol, BMY 14802, rimcazole, JO 1784), phenylpentylamine-like (U-50488, BD 737, proadifen, caramiphen), or phenyloctylamine-like (carbetapentane) separation between phenyl and a basic amine. We report on two new sigma ligands that potently inhibit (±)-[^{3}H]3-PPP binding in vitro to rat brain membranes and in vivo to brain of intact mice. Their structures fall within the classes described: E-2020 (IC_{50} 5.8 nM, ID_{50} 2.7 μmol/kg p.o.), an acetylcholinesterase inhibitor, and 3,4-dichlorobenzyloxymethyl-2-piperidine (IC_{50} 4.5 nM, ID_{50} 0.27 μmol/kg i.p.), a dexoxadrol derivative with little affinity for PCP binding sites.

133.4

BMY-14802, A SIGMA LIGAND AND POTENTIAL ANTIPSYCHOTIC DRUG, REVERSES AMPHETAMINE-INDUCED CHANGES IN STRIATAL SINGLE-UNIT ACTIVITY IN FREELY MOVING RATS. G.V.Rebec, Z.R.Wang*, and J.L.Haracz. Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

Preclinical evidence suggests that BMY-14802, which has a high affinity for sigma but not dopamine receptors, may be an effective neuroleptic (Taylor and Dekleva, Drug Develop. Res., 11:65, 1987). Virtually all neuroleptic drugs currently available for clinical use antagonize at least some of the actions of amphetamine. To determine the extent to which this action also applies to BMY-14802, we assessed the effects of this drug on amphetamine-induced changes in the activity of single striatal neurons in awake, behaving rats. Consistent with previous reports (Haracz et al., Brain Res., 489:365, 1989), 1.0 mg/kg d-amphetamine typically activated movement-related neurons (25 of 26) but suppressed the activity of cells whose firing rate was unrelated to movement (9 of 12). Subsequent administration of BMY-14802 (10-20 mg/kg) generally reversed the effects of amphetamine on both types of cells, but this effect was more apparent for movement-than non-movement-related cells (80% vs 40%, respectively). Behavioral analyses indicate that BMY-14802 also reversed amphetamine-induced behavioral activation. Taken together, these results suggest that although BMY-14802 antagonizes the behavioral effects of amphetamine, this potential neuroleptic does not act homogeneously in opposing the action of amphetamine on striatal neurons. [Supported by USPHS Grant, DA-02451]

133.5

PHENCYCLIDINE DISRUPTS SENSORY GATING IN THE RAT VIA A NORADRENERGIC MECHANISM. Christine L. Miller*[1], Robert Freedman[2], Herbert Nagamoto[2], and Paula Bickford-Wimer[1]. Depts. of Pharmacology[1] and Psychiatry[2], Univ. Colorado Health Sci. Ctr, and Vet. Admin. Med. Ctr, Denver, CO 80262

Various forms of psychosis in humans are accompanied by disruption of auditory sensory gating measured in the P50 wave response to paired clicks. Similar disturbances in auditory sensory gating in the rat are observed after administration of the psychotomimetic drug phencyclidine (PCP). PCP interacts with sigma receptors, and NMDA receptors, and can also act as an indirect catecholamine agonist. The present study addressed the question of which of these actions of PCP are important in the loss of sensory gating. Two equivalent tones, separated by 500 msec, were presented to chloral hydrate anesthetized Sprague-Dawley rats. An evoked potential of 30 to 50 msec latency was recorded in the CA3 region of the hippocampus in response to the first tone and was diminished in amplitude to the second (sensory gating). Administration of PCP (1 - 5 mg/kg, i.p.), and other sigma ligands, caused an increased ratio of the 2nd response to the 1st response (gating is lost). The NMDA antagonist effects of PCP do not appear to be significant in this paradigm, since the NMDA antagonist CPP has little effect on auditory gating at i.p. doses up to 16 mg/kg. Lesioning of noradrenergic input to the hippocampus, with DSP4, blocks the effects of PCP on auditory gating . It is possible that PCP exerts its gating effects by increasing noradrenergic activity through a sigma receptor mechanism. This work was supported by VA Medical Research Services and USPHS Grants DA-02429 and MH-38321.

133.6

IN VIVO AND *IN VITRO* BINDING ACTIVITIES OF A SERIES OF HALOGENATED ARYL-PIPERAZINES AT THE SIGMA SITE. D.J. Wilson[1]*, S.J. Vine[1]*, M. Nobbs[1]*, G.C. Ormandy[1], R. Ferris[2], D.R. Riddall[1]*, Wellcome Research Labs, Kent, U.K. and R.T.P., N.Carolina, USA[2].

The characteristics and functional role of the sigma receptor has received much attention recently. However, due to the lack of functional assays, the importance of the sigma receptor remains largely unknown although recent investigations have suggested it to be a target for the development of antipsychotic drugs. The development of novel selective sigma ligands would thus be of value in defining the role of the sigma receptor. We have investigated the structure-activity relationship for a series of halogenated aryl-piperazines. *In vitro* binding studies were carried out on guinea pig membranes incubated in 50mM Tris-HCl, pH7.7, 27°C with ^{3}H-DTG, ^{3}H-PPP, ^{3}H-dextromethorphan or ^{3}H-SKF10047. Our studies have revealed a number of highly potent compounds (pKi about 9.2) with at least 1000 fold selectivity compared with binding to PCP, dopamine D1, D2, noradrenaline α_1, α_2, β, acetylcholine muscarinic and serotonin $5HT_2$ receptors. A number of these compounds were then tested in binding studies *in vivo*. Compounds were administered intraperitoneally (ip) or orally (po) to mice to determine their ability to displace the specific binding of ^{3}H-SKF10047 (ip) to sigma sites in the brain. These compounds were potent displacers of specific ^{3}H-SKF10047 binding with ED_{50}'S of around 0.1. mg/kg ip or po, relative to haloperidol which has an ED_{50} of 0.8 mg/kg ip. Thus, these compounds may serve as important tools *in vitro* and *in vivo* for defining the functional role of the sigma site, and the therapeutic potential of ligands interacting with this receptor.

133.7

DuP 734 IS A NOVEL *SIGMA* RECEPTOR ANTAGONIST. S.W. Tam, G.F. Steinfels, P.J. Gilligan*, J.F. McElroy, V.J. DeNoble, A.L. Johnson*, and L. Cook*. Central Nervous System Diseases Research, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880-0400

The *sigma* receptor has been suggested to play a role in the etiology of schizophrenia, and *sigma* receptor antagonists may be useful as antipsychotics without the serious motor side effects of neuroleptics. DuP 734 (1-(cyclopropylmethyl)-4-(2'-4"-fluoroethyl)-2'-oxoethyl) piperidine HBr) is a novel compound with potent binding affinity for *sigma* (Ki = 10 nM) and 5-HT_2 (Ki = 15 nM) receptors and weak binding affinity for dopamine receptors (Ki>1000 nM), and 29 other receptors, ion channels and second messenger systems.

Since *sigma* receptors have been demonstrated to be associated with dopamine neurons of the substantia nigra, extracellular single unit recording techniques were used to study the DuP 734 antagonism of the effect of the selective *sigma* ligand (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine [(+)-3-PPP] on dopamine neuronal activity in the substantia nigra of the rat. DuP 734 dose-dependently antagonized the inhibition of dopamine neuronal activity produced by a single i.v. dose of 0.5 mg/kg of (+)-3-PPP with an ED90 of 0.99 mg/kg i.v..

The *sigma* receptor agonists (+)-SKF 10,047 and phencyclidine both induced turning behavior in rats with unilateral lesion of the substantia nigra. DuP 734 dose-dependently antagonized the turning induced by (+)-SKF 10,047 and phencyclidine with oral ED50s of 2.4 and 5.4 mg/kg, respectively.

In conclusion, DuP 734 is a potent orally active *sigma* receptor antagonist.

133.8

THE PHARMACOLOGY OF A *SIGMA* RECEPTOR ANTAGONIST ANTIPSYCHOTIC: DuP 734. L. Cook*, S. W. Tam, W. K. Schmidt,and K. W. Rohrbach. Central Nervous System Research, The Du Pont Merck Pharmaceutical Company, Wilmington DE 19880-0400

DuP 734 (1-(cyclopropylmethyl)-4-(2'-4"-flouroethyl)-2'-oxoethyl) piperidine HBr) presents a novel profile of activity in a battery of test procedures designed to distinguish antipsychotic therapeutic activity and neuroleptic side-effect potential. Its receptor binding profile shows affinity for *sigma* and 5HT2 and not to dopamine receptors.

Similar to haloperidol, DuP 734 potently antagonizes mescaline induced effects (ED50=0.35 mg/kg po) as well as isolation induced aggression (ED50=1.9 mg/kg po) in mice. However, unlike haloperidol, it only weakly antagonizes apomorphine induced climbing (ED50=14 mg/kg po) in mice and failed to inhibit conditioned avoidance (CAR) or escape (CER) behavior at 23 mg/kg po or produce catalepsy at 77 mg/kg po in rats. DuP 734 suppresses operant responding (VI60) and 5HTP induced head twitch in rats (ED50's of 6.6 and 1.8 mg/kg po respectively). The overall profile of DuP 734 indicates activity in some procedures reflecting antipsychotic activity (mescaline antagonism and aggression) while showing weak activity in a dopamine dependent procedure (apomorphine climbing) and no activity in procedures associated with neurological side-effect liability (catalepsy and CER).

Although DuP 734 itself does not antagonize CAR activity, it enhances the potency of haloperidol in CAR by 3X (the ED50 for haloperidol is shifted from 0.94 to 0.32 mg/kg po), while not effecting the ED50 of haloperidol for CER or catalepsy. This indicates a specific facilitation of the therapeutic activity of haloperidol. The combination treatment with the *sigma* receptor antagonist DuP 734 thus provides an improved antipsychotic margin of safety for haloperidol.

133.9

THE NOVEL ANTIPSYCHOTIC DuP 734 PREFERENTIALLY INCREASED DOPAMINE TURNOVER IN THE FRONTAL CORTEX IN RAT BRAINS. C. Rominger and S.W.Tam. Central Nervous System Diseases Research, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880-0400.

The effects of acute administration of the potent sigma and 5-HT_2 receptor antagonist DuP 734 on the turnover of dopamine and serotonin in discrete brain regions of the rat were studied ex vivo. Rats were euthanized by microwave irradiation focused on the brain. DuP 734 preferentially and significantly increased the level of dopamine and its metabolites, 3,4-dihydroxyphenyl acetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in the frontal cerebral cortex at 1 and 3 mg/kg, s.c.. At a higher dose of 9 mg/kg s.c., DuP 734 also significantly increased dopamine turnover in the nucleus accumbens, caudate putamen and hypothalamus but not in the substantia nigra and ventral tegmentum. In contrast, the haloperidol-induced increase in dopamine turnover was not selective between the frontal cerebral cortex, caudate putamen, and nucleus accumbens at 0.01-0.1 mg/kg s.c. DuP 734 at 1 and 3 mg/kg s.c. increased 5-HT turnover in the ventral tegmentum but not in the other brain regions studied. Haloperidol 0.01-0.1 mg/kg s.c. had no effect on 5-HT turnover in these brain regions. The results indicate that DuP 734 is relatively selective in modulating the dopamine neuronal activity in the mesocortical areas, and has a much weaker activity in affecting dopamine neuronal activity in the nigro-striatal pathway which mediates motor activity. These results support the hypothesis that DuP 734 is a novel antipsychotic with low potential for extrapyramidal symptoms.

133.10

[^{3}H]DuP 734: A RECEPTOR BINDING PROFILE IN BRAIN OF A HIGH-AFFINITY NOVEL *SIGMA* RECEPTOR ANTAGONIST. S. G. Culp, D. Rominger, S. W. Tam and E. B. De Souza. Central Nervous System Diseases Research, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880.

Schizophrenia is associated with alterations in the regional brain densities of *sigma* and D_2 dopamine receptors and it has been suggested that *sigma* receptor antagonists may represent novel antipsychotic agents. DuP 734 [1-(cyclopropylmethyl)-4-(2'-(4"-fluorophenyl)-2'-oxoethyl) piperidine], is a novel *sigma* receptor ligand which antagonizes the behavioral effects of known hallucinogens without binding to or antagoninzing D_2 dopamine receptors. The *in vitro* binding properties of [^{3}H]DuP 734 in homogenates of guinea pig brain were examined. Specific [^{3}H]DuP 734 binding (10 µM haloperidol-displaceable) in cerebellum was dependent on pH and membrane protein concentrations, saturable and of high affinity (K_D= 228 ± 34 pM; B_{max}= 3856 ± 340 fmol/mg protein). [^{3}H]DuP 734 binding was substantially reduced by treatment of the membrane with proteases and completely abolished by heat denaturation. The pharmacological characteristics of [^{3}H]DuP 734 binding in cerebellum were similar to those previously reported for *sigma* receptors in brain [DuP 734 > haloperidol > (+)-pentazocine > DTG > (+)-3-PPP > (-)-pentazocine > amitriptyline > (-)-butaclamol > (+)-SKF 10,047 > (-)-3-PPP > (+)-butaclamol > (-)-SKF 10,047 > PCP >> MK801]. [^{3}H]DuP 734-labeled *sigma* receptors were heterogenously distributed in CNS (pons/medulla ≡ hypothalamus > spinal cord > cerebellum > hippocampus ≡ thalamus ≡ cerebral cortex ≡ striatum). In summary, DuP 734 binds with high affinity to *sigma* receptors in brain and represents a potential antipsychotic agent.

133.11

[^{3}H]DuP 734-LABELED *SIGMA* RECEPTORS IN GUINEA PIG BRAIN: AUTORADIOGRAPHIC LOCALIZATION STUDIES. J. A. Heroux, S. W. Tam and E. B. De Souza. CNS Diseases Research, The Du Pont Merck Pharmaceutical Company., Wilmington, DE 19880-0400.

The psychotomimetic effects of certain cycloalkyls and benzomorphans that interact with *sigma* receptors has led to the hypothesis that these sites may be important in the etiology of schizophrenia. DuP 734 [1-(cyclopropylmethyl)-4-(2'-(4"-fluorophenyl) -2'-oxoethyl) piperidine] is a novel *sigma* receptor antagonist. The receptor binding specificity and neuroanatomical distribution of [^{3}H]DuP 734-labeled *sigma* receptors in guinea pig brain were examined using quantitative autoradiography. [^{3}H]DuP 734 binding to slide-mounted sections of guinea pig brain was saturable and of high affinity (K_I: 3.9 nM). Competition studies yielded the following rank order of potency: DuP 734 > haloperidol > (+)-pentazocine > (-)-butaclamol > DTG > (+)-SKF 10,047 > (+)-3-PPP > (-)-pentazocine > (+)-butaclamol > U50,488H > (-)-SKF 10,047 > cinanserin > PCP >>> MK801, sulpiride. High densities of [^{3}H]DuP 734 binding sites displaceable by haloperidol were present in the dorsal and ventral bands of Broca as well as in the ventral pallidum. The mammillary complex of the hypothalamus, central gray, red nucleus of the midbrain, pontine reticular nucleus, purkinje cell layer of the cerebellum and dorsal and ventral horns as well as the central gray matter of the spinal cord all showed enrichments of [^{3}H]DuP 734 binding sites. Lower levels of binding were present in the cerebral cortex and basal ganglia and negligible specific binding was present in the white matter tracts. The kinetic and pharmacological characteristics and distribution of [^{3}H]DuP 734 binding sites in brain are similar to those previously reported for *sigma* receptors.

133.12

ABSORPTION AND DISTRIBUTION OF A TRITIATED SIGMA ANTAGONIST (NPC 16377) AFTER ORAL ADMINISTRATION IN THE RAT AND MOUSE. Marie Rock*, Carolyn Fait*, Venkatoraman Balasubramanian, Theresa Hartman*, David B. Clissold, Michael Pontecorvo and John Ferkany. CNS Division, Nova Pharmaceutical Corporation, Baltimore, MD 21224.

An absorption and distribution study was conducted in rats and mice after oral administration (125 mg/kg) of tritiated NPC 16377. The radiolabeled compound (S.A. 48 Ci/mmole) was 99% pure by HPLC and TLC. Other studies have shown that pharmacological activity in the mouse occurs approximately 45 minutes after a single oral administration (125 mg/kg) of NPC 16377. However, the compound did not demonstrate pharmacological activity in the rat unless either a 475 mg/kg dose was given, when effects were seen at 1 hour, or repetitive dosing was done (125 mg/kg), with activity observed on the 5th daily dose. Organ distribution (brain, fat, stomach, liver, kidney and lung) was performed at 3 hours, 24 hours and 48 hours post dose. Blood levels were measured at 0.5, 1, 3, 6, 12, 24, 48 and 72 hours post dose. The results of this study showed a close correlation of blood and brain levels. The mouse demonstrated 18,000 DPM/gm brain tissue at 3 hours and 5000 DPM/gm brain tissue at 48 hours, whereas the rat did not achieve levels of 5000 DPM/gm until 48 hours. In rats, a lower dose (75 mg/kg) produced brain tissue levels which were significant and paralleled the blood levels.

133.13

NPC 16377, A POTENT, SELECTIVE SIGMA LIGAND: NEUROPROTECTIVE ACTIONS. B.E. Jones, D.B. Clissold, M.J. Pontecorvo, E.W. Karbon, M.E. Abreu, R. Erickson and J.W. Ferkany. CNS Research and Medicinal Chemistry, Nova Pharmaceutical Corporation, Baltimore, MD 21224.

Studies were undertaken to evaluate the neuroprotective efficacy of the sigma agent NPC 16377. Adult male gerbils (60-80g) anesthetized with halothane, were fitted with a loose silastic ligature placed around the carotid artery and passed through a double lumen cannula protruding out the nape of the neck. The day after surgery ischemic insult (duration 5 min) was accomplished by pulling tight the ligature. NPC 16377 was administered either i.p. (10 or 35 mg/kg) 30 and 10 min prior and 60 min post insult or p.o. (150 or 200 mg/kg) 60 min prior and 60 min post insult. Body temperature was maintained for at least three hour post-insult by a heating lamp. Gerbils were allowed to recover to 72 hrs. Neuroprotection was assessed by microscopic examination. A six point rating scale was applied (0 = no damage; 1 = 10% or less cells damaged; 2 = 10-25% damage; 3 = 25-50% damage; 4 = 50-75% damage; 5 = 75% or greater damage) to the CA1 region of the hippocampus. Significant neuroprotection was noted in the 35 mg/kg i.p. (0.6 ± 0.5) group compared to control (4.2 ± 0.7) and 150 mg/kg p.o. (2.8 ± 0.5) and 200 mg/kg p.o. (1.6 ± 0.5) groups compared to control (4.5 ± 0.4). The data indicates that NPC 16377 is an effective neuroprotectant following either i.p. or p.o. administration.

133.14

BEHAVIORAL PROFILE OF NPC 16377, A POTENT AND SELECTIVE SIGMA LIGAND. D.B. Clissold, T. Hartman*, H. Valentine, M.E. Abreu, R. Erickson, E.W. Karbon, M.J. Pontecorvo and J.W. Ferkany. CNS Research and Medicinal Chemistry, Nova Pharmaceutical Corporation, Baltimore, MD 21224.

NPC 16377 (NPC) has been described as a potent and selective ligand at sigma sites in brain membranes. Because sigma selective compounds may represent novel therapeutics for the treatment of psychoses, the effects of NPC were explored in a variety of tests predictive of antipsychotic or neuroleptic potential.

In rats, NPC (i.p. and p.o.) as well as clozapine (Clozaril, CLO, i.p., p.o.), BMY 14802 (BMY, i.p., p.o.) and Haloperidol (Haldol, HAL, i.p.) reduced avoidance but not escape behavior in the conditioned avoidance paradigm. In mice, NPC, HAL, CLO and BMY reversed d-Amphetamine-stimulated hyperlocomotion following p.o. administration, but only HAL and NPC did so at doses that did not reduce spontaneous locomotion. NPC (i.p.) also reversed d-Amphetamine stimulated hyperlocomotion in rats. NPC and CLO, in contrast to BMY and HAL, did not affect apomorphine-induced climbing in mice until locomotor suppressant doses were administered. Consistent with results from sigma binding studies, NPC effectively reversed the behavioral actions of (+)SKF 10047. At doses 10-fold greater than those eliciting behavioral responses in mice, NPC (p.o.) failed to impair rotorod performances or induce catalepsy; lethality was also absent until extreme doses were administered. The data are consistent with the suggestion that potent and selective sigma ligands, in particular NPC, may have antipsychotic properties.

133.15

NEUROCHEMICAL AND NEUROENDOCRINE EFFECTS OF NPC 16377 - A POTENT AND SELECTIVE SIGMA LIGAND. M.E. Abreu, L.A. Martin , R. Erickson , E.W. Karbon, M.J. Pontecorvo and J.W. Ferkany. CNS Research and Medicinal Chemistry, Nova Pharmaceutical Corporation, Baltimore, MD 21224.

A number of studies have suggested a relationship between compound affinity for the sigma receptor and antipsychotic/psychotomimetic activity. Based on the recent identification of NPC 16377 as a potent and selective sigma agent, studies were initiated to evaluate neurochemical and neuroendocrine effects of NPC 16377 as compared to typical and atypical antipsychotic agents as well as with other characterized sigma agents. Male Sprague-Dawley rats were injected (ip) with behaviorally-relevant doses of compounds and brain regions and plasma samples were obtained 60 minutes later. Dopamine (DA) and DOPAC levels were measured in striatum, frontal cortex and nucleus accumbens using HPLC with electrochemical analysis and plasma ACTH, corticosterone and prolactin were measured by RIA. Neither NPC 16377 nor ifenprodil (IFN) increased DA turnover (DOPAC/DA ratio), in any of the brain regions studied while BMY 14802 (BMY), clozapine (CLZ) and haloperidol (HAL) elicited significant dose-dependent increases in all brain regions. Except for IFN, NPC 16377 and other compounds increased plasma ACTH and corticosterone levels. Interestingly, NPC 16377 caused a significant suppression of plasma prolactin levels while all other compounds (HAL, IFN, BMY and CLZ) exhibited dose-dependent increases in prolactin. These results provide evidence for a unique neuroendocrine and neurochemical profile associated with acute administration of the sigma selective ligand, NPC 16377, as compared to other agents exhibiting activity at the sigma receptor.

133.16

IN VITRO AND *IN VIVO* BINDING PROPERTIES OF NPC 16377, A POTENT AND SELECTIVE LIGAND FOR SIGMA BINDING SITES. W. Karbon, M. Bailey*, S. Borosky, M. Abreu, L. Martin, R. Erickson, K. Natalie*, M. Pontecorvo and J. Ferkany. Nova Pharmaceutical Corporation, Baltimore, MD 21224-2788.

Several studies have implicated sigma binding sites in mediating the behavioral effects of several potential antipsychotic and cerebroprotective agents including rimcazole, BMY 14802 and ifenprodil. We have recently synthesized a series of potent sigma agents, exemplified by NPC 16377, which has been tested for its ability to interact with CNS binding sites both *in vitro* (Table 1) and *in vivo* .

TABLE 1 *IN VITRO* BINDING PROPERTIES OF SIGMA AGENTS

	IC_{50} (nM) versus [^{3}H]				
Compound	DTG	PPP	Sulpiride	Ketanserin	8-OH-DPAT
NPC 16377	36	43	2671	1184	9110
Haloperidol	7	4	0.7	24	18700
Ifenprodil	16	52	170	nt	nt
BMY14802	237	275	3030	419	837

NPC 16377 also inhibited [^{3}H] SKF 10,047 binding *in vivo*, having ID_{50} values of 1.4 and 2.9 mg/kg following i.p. and p.o. administration, respectively, but did not inhibit [^{3}H] raclopride binding at doses up to 50 mg/kg i.p. The results of these studies indicate that NPC 16377 is a potent and selective ligand for sigma binding sites.

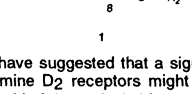
133.17

EFFECTS OF NPC 16377, A POTENT AND SELECTIVE SIGMA LIGAND, ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF MESENCEPHALIC DOPAMINE-CONTAINING NEURONS IN THE RAT. *P. D. Shepard and H. Romeyn*, MD Psychiatric Res. Cntr., Baltimore, MD 21228.*

The aminoalkoxychromone derivative NPC-16377 (NPC) is a highly potent and selective sigma ligand with a low affinity for the dopamine (DA) D_2 receptor (Erickson et al., Karbon et al., this meeting). Preclinical pharmacological studies have indicated that NPC exhibits many of the same biochemical and behavioral effects ascribed to antipsychotic drugs (APDs) possessing a reduced liability for producing extrapyramidal side effects (Abru et. al., Clissold et al., ibid). In an effort to determine whether NPC exhibits an electrophysiological profile characteristic of "atypical" APDs, extracellular single unit recording techniques are being used to assess the effects of this compound on the activity of DA-containing neurons in the substantia nigra (SNc) and ventral tegmental area (VTA) of the chloral hydrate anesthetized rat. Systemic administration of NPC (8 - 128 mg/kg, i.v.) produced a dose-dependent increase in the activity of SNc (ANCOVA $F(6,36) = 16.7$ $p < 0.001$) and VTA ($F(5,50) = 5.9$ $p < 0.01$) DA-containing neurons. Both the proportion of neurons excited by the drug as well as the magnitude of the excitation varied considerably between individual neurons. None of the cells sampled exhibited a significant change in firing rate in response to doses below 8 mg/kg. A single bolus injection of NPC (16 mg/kg, i.v.) failed to antagonize the rate-decreasing effects of an autoreceptor-selective dose of apomorphine (10 μg/kg, i.v., n=6). However, comparable doses of the drug were found to partially antagonize the inhibitory effects of d-amphetamine (0.8 mg/kg, i.v., n=10). These data are consistent with the results of recent behavioral studies in which NPC has been found to antagonize the locomotor stimulant effects of indirect but not direct-acting DA agonists (Clissold et al, this meeting).

133.18

AMINOALKOXYCHROMONES AS SELECTIVE SIGMA RECEPTOR BINDING AGENTS. R.H. Erickson, K.J. Natalie, Jr.*, W. Bock*, Z. Lu*, J. Clifton*, W.J. Rzesotarski*, D.J. Meloni*, F. Farzin*, R.J. Patch*, M.J. Pontecorvo, M.A. Bailey*, K. Naper*, and E.W. Karbon. CNS Division, Nova Pharmaceutical Corporation, Baltimore, MD 21224.



1

Recent reports have suggested that a sigma receptor antagonist lacking activity at dopamine D_2 receptors might possess antipsychotic activity yet would be devoid of the undesirable extrapyramidal side effects common to most antipsychotics currently in use. We have discovered that aminoalkoxychromones (**1**) are potent and selective sigma receptor ligands. We present the results of our structure-activity studies on (**1**) which led to the development of NPC 16377, a potent sigma ligand which exhibits selectivity versus not only dopamine D_2 receptors but also a variety of other neuroreceptors.

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SYMPOSIUM. TROPHIC AGENTS AND THE DEVELOPMENT AND MAINTENANCE OF NEURONS. R. W. Oppenheim, Wake Forest Univ. (Chairperson); Y-A.Barde, Max Planck Institute; Lars Olsen*, Karolinska Institute; M. V. Chao*, Cornell Univ. Med. Sch.; F. Collins, Synergen, Inc.; J. McManaman, Baylor College of Med.

This symposium is dedicated to the pioneering work of R. Levi-Montalcini and V. Hamburger on cell death and trophic interactions. In recent years substantial progress has been made in identifying new trophic agents and in characterizing the cellular and molecular mechanisms that mediate biological responses to neurotrophic molecules. Y.-A Barde and Lars Olsen will describe studies on the three members of the NGF family: NGF, BDNF and NT-3. New discoveries regarding trophic agent receptors and their actions will be summarized by M.V. Chao. F. Collins will discuss the role of CNTF in mediating both neuronal survival during development and the response to injury of peripheral neurons in adult animals. Finally, J. McManaman will describe studies on a new, putative trophic agent, CDF, that acts on developing motoneurons to affect their survival and differentiation.

138

SYMPOSIUM. NEURAL NETWORK MODELS OF VERTEBRATE SENSORIMOTOR SYSTEMS. B. Peterson, Northwestern Univ. (Chair); S. Grillner, Karolinska Inst.; D. Bullock*, Boston Univ.; J. Bower, Caltech; E. Fetz, Univ. of Washington

Neural network models have evolved to the point where investigators with a detailed working knowledge of neural pathways that mediate vertebrate sensorimotor behaviors can use them to gain insights into how these pathways may function. This symposium concentrates on such anatomically and physiologically based modeling efforts. Grillner will describe a realistic hybrid model of the lamprey locomotor system that simulates both biophysical and pharmacological properties of individual neurons and behavior of circuits of which they form a part. This network replicates the alternating swimming rhythm and its modulation by sensory, intersegmental and supraspinal mechanisms. Bullock will describe a model spinal cord network of Renshaw cells and Ia interneurons that achieves independent control of muscle length and joint stiffness in neuromuscular systems that conform to the size principle of motor unit recruitment and also generates realistic tri-phasic EMG bursts during movement. Bower will describe structurally realistic models of cerebellar cortex and olivo-cerebellar circuits intended to shed light on the role of the cerebellum in the motor control of sensory performance. Peterson will describe neural networks that model dynamic and adaptive properties of the vestibuloocular reflex (VOR) and that suggest where changes must occur in brainstem-cerebellar VOR circuits to produce experimentally observed adaptive changes in gain, phase and direction of the VOR. Fetz will discuss dynamic recurrent networks that simulate a step-tracking task by transforming changes in target position into appropriate patterns of motor unit activity; these networks provide new insights into the connections and response patterns of premotoneuronal cells recorded in monkeys. In addition to their functional relevance, these models are of theoretical interest since attempts to deal with realistic systems and actual observed data have forced the modelers to devise new approaches that do not always follow the paths taken by more abstract modelers.

EXCITATORY AMINO ACIDS: RECEPTORS II

139.1

COMPARISONS BETWEEN THE FUNCTIONAL PROPERTIES OF GLUTAMATE RECEPTORS NATIVE TO CA3 HIPPOCAMPAL NEURONS AND RECOMBINANT GLUTAMATE RECEPTORS. T.A. Verdoorn, N. Burnashev*, P. Jonas*, P.H. Seeburg*, and B. Sakmann* Max Planck Institut für medizinische Forschung and ZMBH Heidelberg, Germany.

We have compared the functional properties of non-NMDA receptors found on the soma of CA3 rat hippocampal pyramidal neurons with those of transiently expressed recombinant glutamate receptor (GluR) subunits. Currents induced by kainate, AMPA and glutamate were measured in outside-out macropatches taken from pyramidal cells of brain slices and in HEK cells transfected with various combinations of cloned subunits. Currents mediated by both native and recombinant GluRs showed rapid and profound desensitization in response to fast application of 300 μM AMPA and glutamate but not to 300 μM kainate. Steady state currents in native membranes exhibited current-voltage relationships with prominent outward rectification (chord conductance ratio for 300 μM kainate G(+80)/G(-80) = 2.66±0.16, n=5). Recombinant homomeric GluR-B channels as well as heteromeric combinations containing GluR-B also showed outward rectification (300 μM kainate G(+80)/G(-80) for GluR-B = 2.87±1.04, n=4; for GluR-A/B = 1.90±0.14, n=5). Combinations not containing GluR-B showed marked inward rectification (G(+80)/G(-80) ≈ 0). This suggests that native GluRs in CA3 pyramidal cells contain at least a GluR-B subunit. Single channel amplitudes were estimated by noise analysis and in each case the apparent conductance of channels activated by kainate was different from that of channels opened by glutamate or AMPA. In native membranes the kainate-activated conductance was 3.0±0.2 pS (n=9) whereas AMPA opened channels with an apparent conductance of 7.2±0.7 pS (n=9). The estimated condutances in recombinant GluR-A/B combinations were 0.26±0.05 pS (n=9) for kainate and 1.3±0.1 pS (n=10) for glutamate. AMPA conductances were similar to those produced by glutamate and the differences between glutamate and kainate conductances were also seen with other homo- and heteromeric subunit combinations. Comparisons of other properties such as desensitization rate may help define the possible subunit structure of these neuronal receptors more clearly.

139.2

MOLECULAR CLONING AND DEVELOPMENTAL ANALYSIS OF A THIRD ISOFORM OF RAT GLUR-4. V. Gallo, L.M. Upson*, W.P. Hayes and A. Buonanno, Unit on Mol. Neurobiol. and Section on Cell. Neurobiol., LDN, NICHD, NIH, Bethesda, MD 20892.

The glutamate receptor GluR-4 has been proposed to have two spliced isoforms (flip and flop; Sommer et al., Science 249, 1583, 1990). By screening a rat cerebellar library, we have isolated a cDNA obtained from a third transcript (GluR-4c) of the glutamate receptor gene GluR-4. This additional isoform of the flop version of GluR-4 encodes a protein that has a C-terminus segment of 36 amino acids different from the previously described GluR-4 flip/flop. Transcripts synthesized from the GluR-4c cDNA form kainate/AMPA-activated channels in oocytes (see Winters et al., Soc. Neurosci. Abs., 1991). Using oligonucleotide probes specific for the three isoforms, transcripts of 6.2, 4.2 and 3.0 kb derived from the GluR-4 gene were identified. Northern blots revealed that the levels of the three transcripts increase during cerebellar development, with a maximal increase between postnatal day 1 (P1) and 8 (P8). The 6.2 and 4.2 transcripts are present in cultured cerebellar granule cells and astrocytes, but the 3.0 kb transcript is present only in granule cells. *In situ* hybridization histochemistry revealed that the GluR-4c transcripts are preferentially expressed in cerebellar granule cells and Bergmann glial cells. In P1 and P8 cerebella, granule cells were labelled also in the external germinal layer, before their migration into the internal granule cell layer. GluR-4 flip- and flop-specific oligonucleotide probes also showed that the flip GluR-4 isoform labelled the granule cell layer only faintly, whereas both probes labelled Bergmann glial cells in the Purkinje cell layer.

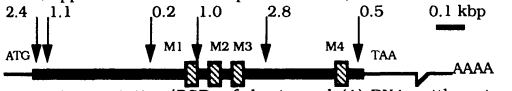
139.3

GLUTAMATE AND KAINATE RECEPTOR GENES AND SUBFAMILIES

P. Gregor, X. Yang*, V. I. Teichberg# and G. R. Uhl. Lab. of Mol. Neurobiol., Natl. Inst. on Drug Abuse /ARC, & Depts. of Neurol. and Nsci., JHUSM, Box 5180, Baltimore, MD 21224. #Dept. of Neurobiol., The Weizmann Inst. of Sci., 76100 Rehovot, Israel.

The chicken kainate (K) binding protein (KBP) is a 49 kDa polypeptide abundant in cerebellar Bergmann glia. Cloning of its cDNA has revealed that KBP displays features typical of ligand-gated ion channels and that it is encoded by a single copy gene producing two major transcripts of 4 and 6 kb.

We have now isolated the structural portion of the chicken KBP gene on a 16 kbp genomic fragment, and report here that its intron/exon organization is homologous to that of ligand-gated ion channels. The analysis shows at least 6 introns in the coding region (arrows; approximate sizes in kbp as indicated):



Reverse transcription/PCR of brain poly(A)-RNA with primers adjacent to the 1.1 kbp intron suggests the presence of several transcripts. We speculate that alternate splicing might also generate the 93 kDa polypeptide, which we have shown to be immunologically related to KBP.

We have also cloned two cDNA fragments encoding new GluR subunits, termed GluR6 and GluR7. These belong to a novel subfamily, together with the previously characterized, but unclassified, subunit GluR5 (77% nucleotide sequence identity).

These data confirm and extend the evidence for diversity of excitatory amino acid receptors.

Supported by NIDA (G.R.U.), and by ALS and Alzheimer's assoc.(V.I.T.)

139.4

DIVALENT ION PERMEABILITY AND PHARMACOLOGY OF CLONED KAINATE/AMPA RECEPTORS EXPRESSED IN OOCYTES. J.F. McGurk, R.S. Roginski, R.S. Zukin and M.V.L. Bennett. Dept. Neuroscience, Einstein Coll. Med., Bronx, NY 10461.

The GluR-1, -2, -3 and -4 glutamate receptor genes of rat brain encode channels activated by both kainate and AMPA. Each gene encodes RNAs that undergo alternate splicing to yield receptor variants termed "flip" and "flop". GluR-1 "flip" and "flop" and GluR-3 "flop" clones were transcribed and the cRNAs injected into *Xenopus* oocytes. Oocytes injected with either the GluR-1 or GluR-3 cRNAs generated large (>1500nA) inward currents when perfused with 500μM kainate and voltage-clamped at -100mV. Subunit cRNAs injected singly or GluR-1 "flip" and GluR-3 "flop" injected together directed translation of channels permeable to $Ca^{2+} > Sr^{2+} > Ba^{2+} > Co^{2+} = Mg^{2+}$. The current-voltage relation of these receptor channels in normal Ringer's solution showed inward rectification. This rectification was also evident in medium in which the only permeant cation was one of the divalent cations listed above. In GluR-1 "flip"-injected oocytes currents were elicited by L-glutamate (EC_{50} = 25μM; Hill coefficient (n) ~1), kainate (EC_{50} = 60μM; n ~1) and AMPA (EC_{50} = 7μM; n ~1). GluR-3 "flop"-injected oocytes showed a modest shift in the dose-response for kainate (EC_{50} ~100μM) with no change in n, as well as much reduced responses to L-glutamate and AMPA. Kainate and AMPA responses were blocked by the relatively selective antagonist CNQX (IC_{50} = 0.5μM at 100μM kainate and 3μM at 5μM AMPA). The IC_{50} for CNQX block in GluR-3 "flop"-injected oocytes is near 0.3μM (at 50μM kainate). Cloned kainate/AMPA receptors exhibit properties different from those of receptors expressed from rat brain mRNA.

139.5

CALCIUM MAY DIRECTLY PERMEATE KAINATE RECEPTORS IN CULTURED CEREBELLAR PURKINJE NEURONS R.J. Miller, J.R. Brorson, D. Bleakman and P.S. Chard. Department of Physiological and Pharmacological Sciences, The University of Chicago, Chicago IL 60637.

Glutamate receptors of the non-NMDA classes have been thought to have low permeablity to Ca^{2+}. However, a Ca^{2+}-permeable subtype of the kainate (KA) receptor has recently been described. We have examined the effects of KA upon cerebellar Purkinje neurons, which lack NMDA receptors. About one half of neurons cultured from E16 rat embryos were identified as Purkinje neurons by immunocytochemical labelling for calbindin D-28k. Most neurons lacked any direct response to NMDA (10-50μM) when synaptically mediated responses were blocked with TTX (1μM) and CNQX (10μM). We used whole cell voltage clamp recording and simultaneous amphotericin B perforated patch clamp/ fura-2 micro-fluorimetry to examine KA induced inward currents and $[Ca^{2+}]_i$ increases. In Na^+ free solutions, with the cerebellar neurons voltage clamped at -80mV, KA (10-50μM) induced inward currents and increases in somatic $[Ca^{2+}]_i$. Such responses could be blocked by CNQX (10μM). Removal of the extracellular Ca^{2+} abolished the $[Ca^{2+}]_i$ responses. KA-induced $[Ca^{2+}]_i$ increases were also seen in physiological (Na^+ containing) solutions. These results suggest that KA receptors in these neurons may have appreciable Ca^{2+} permeability.

139.6

HIGH AFFINITY ^{3}H-MK801 BINDING TO MOUSE BRAIN NEURONS IN CELL CULTURE IS SENSITIVE TO NMDA ANTAGONISTS. L.M. Nowak[+], R.G. Gurshman,[*+] C. Poulopoulou,[+] M.M. Salpeter[o] & G.A. Weiland.[+] Depts of Pharmacology[+] and Neurobiology & Behavior[o], Cornell Univ., Ithaca, NY 14853.

The uncompetitive NMDA antagonist MK801 binds with high affinity to activated NMDA channels. Under conditions where the NMDA channels close (e.g. addition of the competitive antagoinst APV), MK801 can be "trapped" at its binding site. Anticipating that by taking advantage of this capacity to trap MK801 we might be able to use ^{3}H-MK801 as a probe of NMDA channel distribution for high resolution autoradiography, we designed a set of conditions for obtaining specific ^{3}H-MK801 binding to NMDA channels in intact neurons. Forebrains from 15-17 day mouse embryos were grown in cell culture using conventional methods. Binding assays were performed on mixed neuron and glial cell cultures (7-10 days *in vitro*) or on glia cultures (2-3 mos. *in vitro*) at room temperature. Cultures were incubated for 60 min with ^{3}H-MK801 in a balanced salt solution containing (in mM): 150 NaCl, 2.5 KCl, 1.0 $CaCl_2$, 10 Hepes-Na (pH 7.5), 1.0 dithiothreitol, 3% sucrose and 300 nM tetrodotoxin. Saturation binding done in the presence of 10 μM L-GLU and 2 μM glycine gave evidence for two sites in mixed cultures and one site in glia cultures. The low affinity (apparent K_D ~ 300 nM to 2 μM) binding was APV insensitive and present on both glial cultures and in mixed cultures. Whether this site represents uptake remains unclear since saturation was not easily demonstrated. In contrast, high affinity binding (K_D ~ 0.5 to 5 nM; B_{max} 10-30 pM/35 mm culture dish) was saturable, inhibited by 3 mM APV, stimulated by GLU plus glycine, and only present in cultures containing neurons. This is consistent with high affinity binding being associated with NMDA receptor-channels. Supported by NS24467 to LMN and GM10422 to MMS.

139.7

Cloning, expression and gene structure of a G-protein coupled glutamate receptor. K.M. HOUAMED, W. ALMERS, Dept. of Physiol. and Biophys., Univ. of Washington, Seattle, WA 98195 and J. L. KUIJPER*, T. L. GILBERT*, B. A. HALDEMAN*, P. J. O'HARA*, E. R. MULVIHILL*, F. S. HAGEN*, Zymogenetics Inc., Seattle, WA 98105

Metabotropic glutamate receptors (Glu_GRs) couple to G-proteins rather than acting as ligand-gated ion channels. A cDNA for a Glu_GR was cloned from rat cerebellum. It encodes a large protein of 1199 amino acids (AAs). This protein may be divided into three domains: a large (577 AAs) N-terminal that is probably extracellular, a large (312 AAs) C-terminal that is probably intracellular, and a central region (310 AAs) with the 7 hydrophobic transmembrane sequences typical of other G-protein coupled receptors. The Glu_GR gene contains at least two introns which define the coding region of the central domain and separate it from the two others. The Glu_GR has no detectable sequence similarity with other known G-protein coupled receptors, and hence represents a prototype for a new receptor family. Its N-terminal has limited similarity to the egg peptide hormone receptor from sea urchins, a peptide receptor with guanylate cyclase activity. Agonists for the Glu_GR cause IP_3-driven, Ca-activated Cl current in Xenopus oocytes. The half-maximal concentrations are quisqualate (0.7 μM), glutamate (12 μM), Ibotenate (32 μM), and trans-ACPD (0.38 mM). Aspartate, kainate (1 mM), or NMDA, AMPA (0.1 mM) had no discernible effects. AP3 antagonized the responses, and pertussis toxin bloked the transduction pathway. This pharmacologic profile is expected for metabotropic GluRs. Supported by NIH # AR-17803.

139.8

ACTIVATION OF THE ACPD SUBTYPE OF EXCITATORY AMINO ACID RECEPTOR DECREASES SYNAPTIC INHIBITION IN HIPPOCAMPAL AREA CA1. M.A. Desai and P.J. Conn, Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322.

Glutamate-stimulated phosphoinositide hydrolysis is mediated by a novel subtype of excitatory amino acid receptor termed the ACPD receptor. To determine the physiological role of ACPD receptor activation, we examined the effects of *trans*-ACPD (a selective agonist at this site) on evoked extracellular field potentials from each of the three major regions of the hippocampus. We report that *trans*-ACPD increased population spike amplitude in areas CA1, and CA3, and induced formation of multiple population spikes in area CA1, but not area CA3. In contrast, *trans*-ACPD caused a decrease in population spike amplitude in the dentate gyrus. Evidence suggests that the effect of *trans*-ACPD in areas CA1 and CA3 are at least partially mediated by direct excitatory effects on pyramidal cells. We now report that *trans*-ACPD also decreases synaptic inhibition in area CA1. This was seen as a decrease in paired-pulse inhibition of extracellular field potentials and a decrease in the fast and slow components of intracellular IPSPs evoked by stimulation of Schaffer collateral afferents. This disinhibition is likely to contribute to induction of multiple population spikes, an effect of ACPD that is only seen in area CA1. Thus, *trans*-ACPD enhances excitatory synaptic responses in area CA1 by a combination of direct excitatory effects on pyramidal cells and by a decrease in synaptic inhibition. (Supported by N.I.H. Grant NS28405-01).

139.9

RESPONSE TO METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION IN CEREBELLAR PURKINJE CELLS

T. Knöpfel, I. Vranesic* and C. Staub*.
Brain Research Institute, University of Zürich, CH-8029 Zürich (Switzerland)

Exitatory amino acids (EAA) interact with receptors which operate ion channels (e.g. NMDA and AMPA receptors) as well as with receptors coupled to second messenger systems (i.e. metabotropic receptors). We have investigated the response to activation of metabotropic EAA receptors in slice cultured Purkinje cells which were voltage-clamped (-55 to -65 mV) and superfused with a balanced salt solution containing 0.5 uM TTX.

Bath application of AMPA (1 - 2 uM), quisqualate (0.25 uM) or t-ACPD (50 - 100 uM) induced an inward current. The AMPA-current was abolished by CNQX, while inward currents induced by quisqalate or t-ACPD were partially reduced or left unchanged, respectively, by this AMPA receptor antagonist. This pharmacological profile suggests that the CNQX insensitive inward current was mediated by a metabotropic EAA receptor.

This inward current was not blocked by Ba^{2+} (1 mM), Mg^{2+} (10 mM), TEA (10 mM) or Cs^+ (1 mM), thus does not appear to be generated by a block of potassium conductances as described in hippocampal pyramidal cells (Nature, 347:765-767). We demonstrated that the inward current was associated with a rise in $[Ca^{2+}]_i$ as measured with fura-2 and can be attributed to a Na^+/Ca^{2+} exchanger current following the release of Ca^{2+} from internal stores.

139.10

GLUTAMATE MEDIATED TRANSMISSION OF INSPIRATORY DRIVE TO PHRENIC MOTONEURONS IS INHIBITED BY AP4 AT PRESYNAPTIC SITE. J.L. Feldman, J.J. Greer, & J.C. Smith, Systems Neurobiology Lab., Dept. Kinesiology, UCLA, Los Angeles, CA 90024-1527.

Excitatory amino acids (EAAs) mediate inspiratory drive to phrenic motoneurons largely via monosynaptic bulbospinal projections. AP4 strongly inhibits inspiratory activity of phrenic motoneurons. The inhibition is not associated with a change in phrenic motoneuron input resistance or resting membrane potential. In addition AP4 does not block the depolarization induced by either quisqualate, kainate or glutamate. The present study was designed to answer two fundamental questions pertaining to these earlier findings: 1) What is the identity of EAAs mediating inspiratory drive to phrenic motoneurons? 2) Is the site of AP4 inhibitory action pre or postsynaptic?

Spontaneous respiratory drive was recorded from cranial nerve (X) and C4 (phrenic) ventral roots in *in vitro* neonatal rat brainstem-spinal cord preparations (n=6). A barrier at the spinomedullary junction allowed for the addition of drugs to bathing solutions surrounding either the spinal cord or brainstem. Three sets of experimental protocols were followed: i) 500 μM DHK (EAA uptake inhibitor) was added to the solution bathing the spinal cord while the preparation spontaneously generated respiratory activity for 30 minutes. The solution bathing the spinal cord (1 ml) was analyzed for the presence of amino acids by HPLC. ii) Protocol i was repeated with the descending bulbospinal respiratory drive abolished by the addition of 3.0 μM CNQX to the solution bathing the brainstem. iii) protocol i was repeated with the phrenic motoneuron activity depressed by the addition of 100 μM DL-AP4 to the solution bathing the spinal cord.

Removing inspiratory synaptic drive to spinal motoneurons (protocol ii) decreased the amount of glutamate to 24±7% of that collected in the presence of inspiratory drive (protocol i). The addition of AP4 in the presence of inspiratory drive (protocol iii) decreased the concentration of glutamate to 12±8% of control. Aspartate levels were unchanged in all cases. Thus, we conclude: 1) Glutamate is the primary EAA mediating synaptic transmission of inspiratory drive to phrenic motoneurons. 2) AP4 acts presynaptically to reduce the amount of glutamate released from bulbospinal terminals. Supported by American Lung Association, NIH grants HL02204 & NS27941.

139.11

DETERMINANTS OF THE AMPLITUDE AND TIME COURSE OF EXCITATORY SYNAPTIC EVENTS IN THE RAT HIPPOCAMPUS. CF Zorumski, LL Thio, GD Clark & DB Clifford. Washington Univ Med Sch, St Louis, MO 63110.

Quisqualate responses in vertebrate CNS neurons desensitize rapidly and profoundly. This rapid desensitization is blocked by the lectin wheat germ agglutinin (WGA) (Neuron, **5**, 61, 1990). We examined the effect of WGA on evoked excitatory postsynaptic currents (epscs) in pairs of cultured postnatal rat hippocampal neurons to determine whether desensitization regulates excitatory synaptic transmission. Evoked epscs mediated by non-NMDA receptors decayed with a time constant of 6.5 ± 0.3ms (± SEM) at -70mV which is comparable to the 100μM quisqualate channel burst duration of 6.4 ± 0.2ms at -80mV. Evoked epscs increased in amplitude by 100 ± 27% and decayed 42 ± 11% more slowly after treatment with 580nM WGA. The time constant of decay increased from 5.8 ± 0.6ms to 7.9 ± 0.5ms. Similarly, spontaneous miniature epscs recorded in 1μM TTX increased in amplitude by 53 ± 11% and decayed 48 ± 23% more slowly after WGA treatment. These changes were not accompanied by a change in mepsc frequency. Combined with the WGA-induced increase in quisqualate-gated channel burst length (Soc Neurosci Abstr, **16**, 547, 1990), these results suggest that the decay of evoked epscs is determined by channel burst length and that desensitization regulates excitatory synaptic strength.

LEARNING AND MEMORY—ANATOMY: ANIMAL STUDIES

140.1

VISUALIZATION OF THE TURTLE EYE-BLINK REFLEX ARC WITH THE ACTIVITY-DEPENDENT DYE SULFORHODAMINE. J.C. Houk and J. Keifer. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

To examine mechanisms underlying adaptive plasticity in the cerebellorubral circuits, we have used the *in vitro* turtle brainstem-cerebellum in which to study the conditioned eye-blink reflex (Keifer & Houk, *Soc. Neurosci. Abs.* 16: 763, 1990). One technique to study the spatial distribution of neuronal activity specific to the unconditioned and conditioned reflex responses is the application of the dye sulforhodamine 101. This dye, when applied to the bath containing the *in vitro* preparation, appears to be taken up in an activity-dependent manner by neuronal cell bodies and dendrites that are activated by an electrical stimulus (Houk et al., *Soc. Neurosci. Abs.* 15: 612, 1989). We report here the visualization of the turtle eye-blink reflex arc during physiological monitoring of the unconditioned reflex response.

The neural correlate of the eye-blink reflex was evoked by a single shock to the trigeminal nerve in the *in vitro* turtle brainstem-cerebellum and recorded from the abducens nerve as a burst of action potentials. During physiological recording of the reflex, a 0.1% solution of sulforhodamine 101 was added to the bath. The dye was applied for 50 min while the reflex was activated once every 30 s. The preparation was then washed for 20 min in normal saline and immersion fixed in 4% paraformaldehyde. Using rhodamine epifluorescence, well-labeled cell bodies were observed unilaterally in the trigeminal, abducens, and accessory abducens nuclei. Neurons in the superior and magnocellular reticular formation, as well as the raphe, were also labeled. In two cases the reflex arc was clearly visualized as the axonal projections from the trigeminal nucleus to abducens and accessory abducens were well labeled. Bath application of CNQX, which blocks the reflex response, during sulforhodamine application results in no label in the trigeminal, abducens or accessory abducens nuclei. These results support the hypothesis that uptake of sulforhodamine is activity-dependent since the sites labeled in these experiments are specific to the reflex under study. Further, this dye will provide a useful tool to study active regions in the nervous system involved in the acquisition of the conditioned eye-blink response.

140.2

FUNCTIONAL BRAIN SYSTEMS FOR DIFFERENTIAL CONDITIONING OF TONE-SIGNALED REWARD DEMONSTRATED USING 2-DEOXY-2-FLUORO-D-GLUCOSE AUTORADIOGRAPHY. F. Gonzalez-Lima[1], F.J. Helmstetter[2], and J. Agudo[3]. [1]Dept Psychol and Inst Neurosci, Univ of Texas at Austin, Austin, TX 78712; [2]Dept Psychol, Univ of Wisconsin at Milwaukee, Wisc 53201; [3]Dept Cell Biol, Univ of Valladolid, Spain.

Five groups of rats were injected with 2-DG and exposed to two FM tones of low and high frequencies. In group 1 the low tone was paired with water reward (CS+) and in group 2 the high tone was paired. In group 3 stimuli were explicitly unpaired and in group 4, randomly presented. Group 5 was conditioned but satiated prior to 2-DG testing. Two kinds of changes were observed in the auditory system: 1) enhanced responses of the brainstem nuclei such as cochlear were always dependent on the learned signal value of CS+ vs. CS- in thirsty and satiated rats; 2) at higher levels such as the inferior colliculus, increases in activity to CS+ were dependent on the reinforcing value, i.e. if CS+ was presented to satiated rats, no enhanced responses were observed. Nonauditory structures with learning-related 2-DG increases during conditioning with two CSs or one CS included caudal caudate-putamen and intralaminar thalamus. Additional structures changed during CS+/CS- differentiation of drinking response included reticular thalamus, flocculus and facial nucleus, all with suppression of 2-DG uptake. This metabolic suppression of neural substrates with the two CSs, provide for the first time a neuroanatomical basis for the behavioral process of response inhibition described by Pavlov during CS+/CS- differentiation. (R01 MH43353)

140.3

THE ROLE OF AMYGDALA CENTRAL NUCLEUS IN ASSOCIATIVE LEARNING. M. Gallagher & P. C. Holland* Department of Psychology, University of North Carolina, Chapel Hill, NC 27599 and Department of Psychology, Duke University, Durham, NC 27706.

A common view of the learning function served by the amygdala central nucleus (CN) is that of associating the emotional significance of events with relevant cues for those events. For example, animals with CN damage are viewed as deficient in the ability to acquire conditioned fear. Our results indicate another function for CN that emphasizes its role in attentional processing.

Our research using appetitive Pavlovian procedures revealed that rats with neurotoxic damage of the central nucleus (CN) are deficient in conditioned but not unconditioned orienting behavior. This suggests that CN damage may produce impairment in other tasks that require attention to the conditioned stimulus (CS). Although animals normally ignore a redundant CS that is added to a predictive CS (blocking), either increasing or decreasing reinforcement magnitude can direct attention to a redundant CS and produce learning (unblocking). Indeed, unblocking caused by a downshift in reinforcement magnitude uniquely requires an attentional process to account for conditioning to the redundant CS. Rats with CN damage exhibited unblocking in response to an increase, but not in response to a decrease, in reinforcement magnitude. These, and other data, support a role for CN in attentional (CS) processing.

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140.4

EFFECTS OF RHINAL CORTICAL OR LIMBIC LESIONS ON FEAR REACTIONS IN RHESUS MONKEYS. M. Meunier, J. Bachevalier, E.A. Murray, P.M. Merjanian, and R. Richardson, Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

The fear reactions of monkeys with ablation of the rhinal cortex (N=3) were compared to those of monkeys with damage to the amygdala either alone (N=3) or in combination with the hippocampus (N=3) and of unoperated controls (N=4). The stimuli used were a toy snake, a taxidermic monkey head, a human wearing a mask, and an object covering food. The 4 stimuli were shown for 20 sec each on each of 3 days. Monkeys with lesions of the rhinal cortex showed more freezing behavior than the normal controls, particularly in the presence of the masked human, combined, paradoxically, with an absence both of facial expression and of submissive behavior. Animals with amygdalectomy, by contrast, displayed a total loss of fearful reactions, fewer aggressive responses than normals, and an increased tendency to examine objects, often orally. These data suggest that the rhinal cortex interacts complexly with the amygdala in the mediation of fear.

140.5

EFFECTS OF ANTERIOR RHINAL CORTICAL LESIONS ON DELAYED NONMATCHING-TO-SAMPLE IN RHESUS MONKEYS. E.A. Gaffan* and E.A. Murray. Dept. Psych., Reading Univ., Reading, U.K. and Lab. Neuropsychol., NIMH, Bethesda, MD 20892.

Prior work had indicated that rhesus monkeys with aspiration lesions of the amygdaloid complex (A) plus subjacent portion of the anterior entorhinal cortex were impaired on a version of delayed nonmatching-to-sample (DNMS) employing a single pr. of repeatedly used objects (Mishkin and Oubre, 1976, Soc. Neurosci. Abstr. 2:1127), and the deficit was attributed to the A damage. Because complete removals of the rhinal cortex (comprised of both the entorhinal cortex and perirhinal cortex) alone have been found recently to yield severe impairments on DNMS, we tested whether damage to the anterior rhinal cortex (Ant Rh) is responsible for the deficit on DNMS with a single pr. Naive rhesus monkeys were trained on versions of DNMS with both 20 prs. and a single pr., received either Ant Rh lesions, neurotoxic lesions of A, or were kept as unoperated controls, and were retrained postoperatively on both versions of DNMS. Preliminary results indicate that monkeys with Ant Rh lesions re-learned DNMS with 20 prs. as rapidly as the controls, but they took significantly longer than controls to relearn the single-pr. version (Ant Rh, $\bar{X}$=310 trials and 82 errors; Con, $\bar{X}$=0 trials and 0 errors). The deficit on single-pr. DNMS reported earlier to follow A removal by aspiration is due, at least in part, to Ant Rh damage.

140.6

LONG-TERM EFFECTS OF NEONATAL LIMBIC LESIONS ON TACTILE RECOGNITION IN RHESUS MONKEYS. L. Malkova, J. Bachevalier, and M. Mishkin. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Four animals with neonatal amygdalo-hippocampal lesions (AH), 4 with neonatal cortical area TE lesions (TE), and 6 normal controls (N) were trained in tactile delayed nonmatching-to-sample (DNMS) at the age of 6-7 years. The animals in Group TE were unimpaired, reaching criterion on 10-sec DNMS in 510 trials and averaging 77% on 30-120 sec delays, as compared to scores of 430 trials and 83%, respectively, in the normal controls. By contrast, none of the animals in Group AH reached the criterion in 1000 trials, though two did so with additional correction training. On 30-120 sec delays, these two animals averaged 68%. Despite their impairment in tactile recognition, Group AH animals performed as well as the normal controls in a tactile discrimination task. These data, together with the long-lasting visual recognition loss found earlier in Group AH, indicate that early damage to limbic structures results in a permanent, global, sensory memory loss.

140.7

EFFECTS OF NEONATAL LESIONS OF THE AMYGDALOID COMPLEX OR HIPPOCAMPAL FORMATION ON THE DEVELOPMENT OF VISUAL RECOGNITION MEMORY. J. Bachevalier and M. Mishkin. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Assessment of the effects of early combined amygdalo-hippocampal lesions on the development of cognitive functions in monkeys pointed to a severe, long-lasting impairment in visual recognition memory, but sparing of the ability to form visual discrimination habits (Bachevalier and Mishkin, Soc. Neurosci., 14:1, 1988). Here we investigated the separate contributions of the amygdaloid complex and hippocampal formation to the development of recognition memory. Six monkeys with neonatal damage to the amygdaloid complex and rostral entorhinal cortex (A+) and 6 with neonatal damage to the hippocampal formation, caudal entorhinal cortex, and parahippocampal gyrus (H+) were tested at 10 months of age in delayed nonmatching-to-sample, with trial-unique objects. Delay intervals ranged up to 2 min and list lengths up to 10 objects. The H+ group did not differ from normal controls, whereas the A+ group was significantly impaired, though their impairment was not as severe as that of the earlier group with combined lesions. The data suggest that, in the monkey, either the amygdala, the rostral entorhinal cortex, or both contribute more than the hippocampal formation to the development of recognition memory.

140.8

NEUROPATHOLOGICAL AND BEHAVIORAL FINDINGS IN A MODEL OF GLOBAL ISCHEMIA IN THE MONKEY. N.L. Rempel, R.P. Clower, D.G. Amaral, S. Zola-Morgan, L.R. Squire. Depts. of Neuroscience and Psychiatry, UCSD, La Jolla, CA 92093, and V.A. Med. Center, San Diego, CA.

Patient R.B. became amnesic following an ischemic episode that resulted in a bilateral lesion of the entire CA1 field of the hippocampus. We have developed a model of global ischemia in the monkey (S.N. Abs. 15, 341, 1989). Four monkeys subjected to 15 min of ischemia (ISC) were tested on a battery of amnesia-sensitive tasks, including delayed nonmatching to sample (DNMS). We have now completed a thorough neurohistological examination of the brains from these animals.

Neuropathology: CA1 pyramidal cells were counted in Nissl-stained coronal sections in four quadrants (rostral to caudal) of the hippocampus. The results were compared to similar counts from 4 normal monkeys of approximately the same age and weight. Cell loss was more prominent in the caudal than in the rostral extent of the hippocampus. Specifically, in 3 animals, cell loss averaged 10% in the rostral 3 quadrants, and 30% in the caudal-most quadrant. Losses were even more striking in the fourth monkey, which had 70% cell loss in the rostral 3 quadrants and nearly 100% in the caudal quadrant. Total volume of the CA1 field was also reduced (~30% on average), suggesting that ischemia produces substantial losses in overall CA1 pyramidal cell number. Preliminary examination reveals no significant damage in other brain structures that have been implicated in memory function.

Behavior: ISC monkeys were as impaired on DNMS as monkeys with lesions of the hippocampal formation and adjacent cortex (H^+). The impairment was still detectable 6-8 months after surgery. However, the ISC lesions produced significantly less impairment than H^+ lesions on other memory tasks.

These findings suggest that ischemia consistently produces CA1 cell loss in monkeys, and that this loss produces enduring deficits in memory. The finding that the ISC lesion produced overall less memory impairment than H^+ lesions suggests that the ISC animals (and by extension, patient R.B.) did not have widespread neuropathology affecting memory that was undetected by histological examination.

140.9

RECOGNITION MEMORY IMPAIRMENT IN MONKEYS WITH SELECTIVE HIPPOCAMPAL LESIONS. R.P. Clower, P. Alvarez-Royo, S. Zola-Morgan, and L.R. Squire. UCSD Depts. of Neuroscience and Psychiatry, La Jolla, CA 92093 and V.A. Medical Center, La Jolla, CA 92161.

Work with an animal model of human amnesia in the monkey has shown that the hippocampal formation and the neuroanatomically related perirhinal and parahippocampal cortices are critical components of the medial temporal lobe memory system (Zola-Morgan and Squire, 1990). Findings from patient R.B. suggested that ischemic damage limited to the hippocampus can produce clinically significant amnesia. In the monkey, however, identification of the separate contributions to memory of the hippocampus itself has not been possible because a direct surgical approach necessarily damages underlying cortex (i.e., posterior entorhinal and parahippocampal cortex). One important question is whether surgical lesions limited to the hippocampus produce detectable memory impairment.

Using magnetic resonance imaging to help establish stereotaxic coordinates, we have successfully produced circumscribed, bilateral lesions of the hippocampus (the H lesion), including the CA fields, the dentate gyrus, and the subiculum, but sparing parahippocampal, perirhinal, and entorhinal cortex (Alvarez-Royo et al., S.N. Abs., 1990). Four monkeys with the H lesion were compared to seven normal animals and three monkeys with lesions that included the hippocampus, parahippocampal cortex, and posterior entorhinal cortex (the H^+ lesion) on delayed nonmatching to sample, a benchmark task of recognition memory in the monkey. At a delay interval of 10 minutes, H monkeys were as impaired as animals with the H^+ lesion. Preliminary results from two additional memory tasks, delayed retention of easy object discriminations and concurrent object discrimination, indicate that H animals perform as well as normal animals and better than H^+ animals. Together, these findings suggest that lesions limited to the hippocampus are sufficient to produce a memory impairment, but one that is overall less severe than the impairment associated with lesions that also damage adjacent cortex.

140.10

POSTOPERATIVE ACQUISITION OF DELAYED NONMATCHING TO SAMPLE WITH SHORT (0.5 SEC) DELAYS IS NOT IMPAIRED BY HIPPOCAMPAL FORMATION LESIONS IN MONKEYS. P. Alvarez-Royo, S. Zola-Morgan, and L. R. Squire. UCSD Depts. of Neuroscience and Psychiatry, La Jolla, Ca 92093, and V.A. Medical Center, San Diego, Ca.

The delayed nonmatching to sample (DNMS) task is widely used to evaluate recognition memory in monkeys. In most studies, monkeys are initially trained using a delay interval of 8-10 seconds. Monkeys with large medial temporal lobe lesions that include the hippocampus, amygdala, and the perirhinal, entorhinal and parahippocampal cortices (H^+A^+ lesions) as well as monkeys with lesions of the hippocampal formation that include parahippocampal cortex (H^+ lesions) can achieve high levels of performance at these delays (90% correct). However, they require significantly more trials than normal animals to reach this performance level. This impairment raises two possibilities: the learning of the nonmatching rule itself may be impaired, or, alternatively, the 8 second delay may be sufficiently long that memory-impaired animals have difficulty bridging it.

One way to distinguish between these two possibilities is to train monkeys using very short delays, thus decreasing the memory requirements of the task. Overman et al. (1990) trained monkeys at a 1-sec. delay and showed that H^+A^+ lesions did not impair postoperative relearning of the nonmatching rule. The question remains, however, as to how well animals with medial temporal lobe lesions would perform without the benefit of preoperative training. We prepared a group of monkeys with H^+ lesions and trained them postoperatively on the DNMS task along with a group of normal monkeys, using a very short delay (0.5 secs). The animals with H^+ lesions learned the task normally at this delay. However, they then performed significantly worse than normal animals at longer delays of 1 and 3 minutes. These data suggest that the impairment in learning the DNMS task commonly observed after lesions involving medial temporal lobe structures is not due to an impaired capacity to learn the nonmatching rule, but rather to a difficulty in bridging the 8-10 sec delays used for training the animals. The data also provide evidence that immediate (short-term) memory is independent of the medial temporal lobe memory system.

140.11

LESIONS OF THE PERIRHINAL AND PARAHIPPOCAMPAL CORTICES IN MONKEYS PRODUCE A MODALITY GENERAL AND LONG LASTING MEMORY IMPAIRMENT. W.A. Suzuki, S. Zola-Morgan, L.R. Squire, and D.G. Amaral. Group in Neurosciences and Dept. of Psychiatry, UCSD, La Jolla, CA 92093, V.A. Medical Center, San Diego, CA, 92161, and The Salk Institute, San Diego, CA 92138.

The perirhinal and parahippocampal cortices provide about 70% of the direct cortical input to the hippocampal formation, a structure long associated with memory function. We have recently shown that bilateral lesions limited to the perirhinal and parahippocampal cortices (PRPH I group) in the monkey produce a severe visual memory deficit on three amnesia-sensitive tasks, including the delayed non-matching to sample (DNMS) task (Zola-Morgan et. al, 1989). We now present evidence from a second group of monkeys with PRPH lesions (PRPH II group) that the memory deficit produced by this lesion occurs in both the visual and tactual modalities, and endures for several years. The presence of a modality-general and enduring memory impairment is an important characteristic of human amnesia. The PRPH II group, like the PRPH I group, was severely impaired on the visual DNMS task. The PRPH II group was also impaired on a newly developed tactual version of the DNMS task. The lesioned animals required more trials than normal animals to learn the task (PRPH II = 1991, Normals = 995), and performed worse than normal animals when the delay intervals were increased (Mean of 3 longest delays PRPH II = 63% Normals = 75%). When the PRPH II group was retested on the visual version of DNMS two years after surgery, they still exhibited a severe memory deficit.

In summary, as in human amnesia, the PRPH lesion produces a memory impairment that is modality general and long lasting. These findings emphasize the importance of the perirhinal and parahippocampal cortices for normal memory function and suggest that damage to these areas may underlie at least part of the memory deficit exhibited in amnesic patients such as patient H.M.

CARDIOVASCULAR REGULATION I

141.1

LOCALIZATION OF SPINAL CORD INTERNEURONS WITH PUTATIVE MONOSYNAPTIC CONNECTIONS TO SYMPATHETIC PREGANGLIONIC NEURONS: A TRANSNEURONAL TRANSPORT STUDY USING WHEAT GERM AGGLUTININ. J. Cabot, J. Carroll*, and V. Alessi*, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794-5230.

In vivo and *in vitro* physiological studies have conclusively established that sympathetic preganglionic neurons (SPNs) receive segmental and intersegmental spinal inputs. The anatomical locations of the spinal cells of origin of such inputs are largely unknown. This study provides light microscopic data which suggest that neurons in the base of dorsal horn and in the intermediate spinal gray of the rat thoracic spinal cord provide segmental, presumably monosynaptic, input to SPNs. These findings are consistent with other recent observations in another vertebrate (pigeon) using a different trans-synaptically, transneuronally transported marker, Fragment C of tetanus toxin (Cabot et al., *Neurosci.* **40**: 805).

Male Sprague-Dawley rats were sacrificed 3-6 days after injections of either wheat germ agglutinin (WGA) or a combination of WGA and cholera toxin B subunit(CTB) into the superior cervical ganglion. Following intracardiac perfusion, the last cervical spinal cord segment(C8) and the first 3 thoracic spinal cord segments(T1-3) were sectioned in the transverse plane, incubated in anti-WGA, anti-CTB or in a combination of anti-WGA and anti-CTB, and then reacted using standard ABC immunohistochemical protocols.

SPNs were retrogradely labeled with WGA and/or CTB in all sympathetic nuclei (II_f, II_p, IC, CA) in T1 and rostral T2; in II_f and II_p in caudal C8; in II_p, IC and CA in caudal T2 and rostral T3. Transneuronally labeled neurons were immunopositive for WGA only and exhibited perinuclear, somal and proximal dendritic accumulations of small WGA-positive granules. Transneuronally WGA-labeled neurons were found: (1) throughout the lateral portions of the reticulated region of lamina V, but were most concentrated dorsolaterally in this general region; (2) in the lateralmost region of lamina IV in continuity with the dorsolateral border with lamina V; and (3) in the lateral aspects of dorsal lamina VII. Transneuronally labeled neurons were observed to be ipsilateral to labeled SPNs and to be most prevalent within the T1 and rostral T2 spinal segments. (Support in part by HL24103).

141.2

HEMODYNAMIC FLUCTUATIONS AND SPINAL VASOMOTOR TONE IN BRAIN DEATH. J. Ishise, Y. Kita* and S. Murakami* Section of Emerg. Med. and Intensive Care Unit, Kanazawa Univ. Sch. of Med. Kanazawa, Japan, 920.

Although the brain stem including Medulla oblongata (MO) is no longer functioning in brain death, slow periodic perturbation of blood pressure (BP) and heart rate (HR) are occasionally seen. The mechanism of these autonomic functions is investigated by a new analysis method for HR variability in combination with neuropathological examinations.

Ten patients fulfilled the criteria of brain death, arterial BP, ECG and respiratory activities were continuously recorded. A power spectrum analysis of HR fluctuations was performed with a fast Fourier transform algorithm and the power (%) of three different frequency spectra of 0.00-0.05Hz, 0.05-0.15Hz and 0.15-0.5Hz was calculated. MO, the cervical and upper thoracic spinal cord were taken out at autopsy performed at least four days after brain death, each specimen was processed for light microscopic examinations, stained with HE, KB, LFB-PAS and Bodian.

Both systolic and diastolic pressure showed irregular up and down changes ranging from 5 to 50mmHg, of which frequency was 8-16/hour depending on patients. Changes corresponding to the mechanical ventilation were seen but no changes correlating to Mayer wave (0.05-0.15Hz) were observed. HR showed slow fluctuations (0.00-0.05Hz), which was supposed to represent a vasomotor tone. Light microscopy showed complete autolysis in MO and medullo-cervical junctions in all cases and the gray matter including neurons and fibers projecting into the white matter of both anterior and posterior horn i.e. components of the reflex arc remained almost intact below the level of C3/4. Neurons of Nucleus intermediolateralis of the thoracic spinal cord also remained intact.

In conclusion, these results strongly suggest that slow periodic perturbation of BP and HR in brain-dead patients derive from a vasomotor tone originating from sympathetic neurons of the thoracic spinal cord.

141.3

Responses of Splanchnic Sympathetic Preganglionic Neurons to Stimulation in the Caudal Raphe Nuclei. S.F. Morrison, Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

Single stimuli applied to the rostral ventrolateral medulla (RVLM) have previously been shown to excite splanchnic SPNs in one of four characteristic patterns with response latencies that appear to be related to the conduction velocities of RVLM-spinal sympathoexcitatory neurons. The present study was undertaken to determine the response patterns of splanchnic SPNs to similar stimulation in vasomotor regions of the caudal raphe nuclei (raphe pallidus and raphe obscurus; midline, from -.5 to 3 mm rostral to the calamus scriptorius (CS)) and to compare, for the same SPN, the patterns evoked by raphe and RVLM stimulation. Rats were anesthetized with urethane and chloralose and in some the brainstem was transected caudal to the inferior colliculus. Stimulation in the raphe obscurus 1 mm rostral to the CS inhibited SPNs (onset: <15 ms, nadir: 70-80 ms). Stimuli in the raphe pallidus at the level of the RVLM excited SPNs at either a short (30-50 ms) or a long (110-150 ms) latency. For individual SPNs, the excitatory responses evoked by raphe pallidus stimulation were similar in character and latency to those produced by RVLM stimulation. These results support the hypothesis that individual SPNs are selectively innervated by supraspinal inputs in a manner that may be related to their functional role. A potential interaction between the pathways underlying raphe and RVLM stimulus-evoked excitations of SPNs is also suggested. Supported by NIH HL-47196.

141.4

BARORECEPTOR ACTIONS IN BRAIN STEM CARDIO-RESPIRATORY NEURAL NETWORKS. B. G. Lindsey, A. Arata, K. F. Morris, Y. M. Hernandez and R. Shannon, Dept. Physiol. & Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

We studied the effects of baroreceptor stimulation on brain stem cardiorespiratory related neurons with *ongoing* network interactions identified by correlation analysis of their simultaneously recorded spike trains. Baroreceptors were stimulated in 24 anesthetized, vagotomized, artificially ventilated cats by inflation of an embolectomy catheter in the descending aorta or local pressure changes in the carotid sinus. Spike trains were recorded in parallel from the regions of n. tractus solitarius, rostral ventrolateral medulla (RVLM), n. ambiguus - lateral reticular n., and the medullary raphe n. Respiratory and cardiac related neurons were identified by cycle triggered histograms. Of 361 neurons tested, the firing rates of 78 increased, 115 decreased, and 168 showed no change. Responsive cells were elements of 185 pairs of neurons that exhibited short-time scale correlations. We detected baroreceptor modulation of distributed neural assemblies with up to 13 concurrent functional relationships among the constituent neurons. The data provide evidence for many network interactions, including: (a) baroreceptor mediated inhibition of ventral respiratory group inspiratory (I) neurons and concurrent excitation of their I modulated raphe targets; (b) inhibition of RVLM neurons by baro-excited raphe neurons; (c) inhibition of baro-inhibited raphe neurons by baro-inhibited RVLM neurons; (d) convergent excitation of raphe neurons by baro-excited raphe and caudal lateral medullary neurons. This approach allows analysis of the parallel processing of baroreceptor information and network dynamics. Supported by NS19814 & BRSG S07 RR05749.

141.5

EVIDENCE FOR A KYNURENATE-INSENSITIVE GLUTAMATE RECEPTOR IN THE NUCLEUS TRACTUS SOLITARIUS (NTS). Corinn M. Pawloski* and Frank J. Gordon. Dept. of Pharm., Emory Univ. Sch. of Med., Atlanta, GA 30322. Previous studies from this laboratory (JPET 250:953-961, 1989) and others (Talman, Neurosci. Lett. 102:247-252, 1989) have questioned whether L-glutamate (GLU) is a neurotransmitter of baroreceptor information in the NTS. Microinjections into the NTS of the excitatory amino acid (EAA) receptor antagonist, kynurenate (KYN) abolish baroreceptor reflexes, but fail to affect cardiovascular responses evoked by injections of GLU into the NTS. One possible explanation for these results is that exogenously-administered GLU might act at receptors that are not blocked by KYN and are inaccessible to synaptically released GLU. One candidate for this KYN-insensitive receptor is the metabotropic EAA receptor that is selectively activated by the agonist trans-D,L-1-amino-1,3-cyclopentanedicarboxylate (ACPD). The purpose of the present studies was to determine if KYN-insensitive ACPD receptors are present in the NTS. Rats were anesthetized with urethane, paralyzed and artificially respirated. Multibarrel glass pipettes were placed into the NTS which was functionally identified by microinjection (50 nl) of GLU (74 pmol). Microinjections of KYN (2 nmol) into the NTS abolished aortic baroreflexes and markedly reduced depressor responses evoked by subsequent microinjections of N-methyl-D-aspartate (NMDA; 2.6 pmol) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA; 0.1 pmol). Microinjections of ACPD (7.4 to 74 pmol) into the NTS produced dose-related depressor responses that were not reduced after KYN-blockade. Depressor responses produced by GLU were potentiated by KYN. These data demonstrate the presence of a KYN-insensitive ACPD EAA receptor in the NTS. Moreover, they suggest a potential explanation for previous observations where cardiovascular responses evoked by microinjections of GLU into the central nervous system are unaffected by KYN pretreatment. In addition, these results suggest that GLU remains a viable candidate neurotransmitter of baroreflex information in the NTS.

141.6

PROCESSING OF CARDIOPULMONARY AFFERENT INPUT WITHIN NUCLEUS TRACTUS SOLITARII (NTS) INVOLVES ACTIVATION OF SOLUBLE GUANYLATE CYCLASE (GC). S.J. Lewis, H. Ohta, B. Machado, J.N. Bates* and W.T. Talman. Depts. Pharmacol., Neurol. and Anesth. & Cardiovasc. Ctr., Univ. of Iowa and VAMC, Iowa City, IA 52242.

Microinjection of the nitrosothiol S-nitrosocysteine (SNC) into the NTS of anesthetized rats produces dose-dependent hypotension and bradycardia. This study examined (1) whether actions of SNC, glutamate or N-methyl-D-aspartate (NMDA) within NTS involve activation of soluble GC and (2) whether inhibition of GC activation within NTS modifies vagal cardiopulmonary afferent reflexes elicited by i.v. 5-HT. Unilateral microinjection of methylene blue (MB, 250 pmole) into the NTS of rats anesthetized with chloralose significantly inhibited hypotensive and bradycardiac effects of SNC and NMDA, but not glutamate, injected at the same site in NTS. 5-HT-induced reductions in arterial pressure and heart rate were attenuated 1 min after bilateral microinjection of MB (250 pmole/NTS) (pre vs post, -46±6 vs -8±2 mmHg and -188±12 vs -58±9 bpm; $p<.05$). These results suggest that actions of SNC and NMDA and processing of cardiopulmonary afferent input into NTS involve activation of soluble GC. (Support: HL-14388, HL-32205, NS-24621, and VA Merit Review)

141.7

BLOCKADE OF NMDA RECEPTORS IN NUCLEUS TRACTUS SOLITARII INHIBITS BARO- AND CHEMORECEPTOR REFLEX RESPONSES TO STIMULATION OF CAROTID SINUS NERVE IN RAT. H. Ohta and W.T. Talman. Department of Neurology, University of Iowa and VAMC, Iowa City, IA 52242.

The carotid sinus (CSN) and aortic depressor nerves (ADN) terminate in the nucleus tractus solitarii (NTS). Our previous studies have suggested that NMDA receptors in NTS are integral to transmission of baroreceptor afferent signals of ADN. In the present study we have sought to define the cardiovascular reflex responses elicited by stimulation of CSN in rat and to determine if NMDA receptors in NTS mediate those responses. The left CSN was isolated through a lateral incision in the neck of Sprague Dawley rats anesthetized with chloralose. Stimulation (200 μA, 2 ms, 20 sec) of CSN produced frequency-dependent (<50 Hz) depressor and bradycardiac responses that followed transient increases in arterial pressure and respiratory rate. These responses were blocked by ipsilateral injection into NTS of 2% lidocaine or 10 mM MK-801 (50 nl). The injection of MK-801 selectively blocked responses to microinjection of NMDA, but not other excitatory amino acid agonists, into NTS. These results suggest that CSN carries functional baro- and chemoreceptor afferents and that cardiovascular responses to stimulation of CSN, as is the case with ADN, are mediated through NMDA receptors in NTS. Support: VA Merit Review; NIH HL32205, HL14388, and NS24621.

141.8

INHIBITION OF CHEMORECEPTOR (CR) INPUT TO NUCLEUS TRACTUS SOLITARIUS (NTS) NEURONS DURING BARORECEPTOR (BR) STIMULATION. S. W. Mifflin. Dept. of Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78284.

Previous studies of NTS neurons have found no evidence for convergence of inhibitory BR and excitatory CR synaptic inputs which might explain the inhibitory interactions between BR and CR reflexes in the regulation of sympathetic nerve discharge. The possibility that presynaptic inhibitory interactions occur between BR and CR inputs to NTS neurons remains. To examine this, in pentobarbital anesthetized, mechanically ventilated and paralyzed cats, extracellular recordings were obtained from 16 NTS neurons activated by lingual artery injection of <100μl of CO_2 saturated bicarbonate and not by increases in arterial pressure (AP) evoked by inflation of a balloon-tipped catheter in the abdominal aorta. All values are mean ± SD. Post-stimulus time histograms of discharge evoked by electrical stimulation of the carotid sinus nerve (CSN) were obtained at control levels of AP (136 ± 27 mmHg) and during elevations in AP (173 ± 25 mmHg). In 6 of the 16 cells, CSN evoked discharge was reduced 42% ± 9% (range = 30-52%) ($p<.05$, Wilcoxon signed rank test) during an increase in AP. In the remaining 10 cells, elevations in AP had no discernible effect on CSN evoked discharge. During increases in AP, the action potential showed no consistent changes in amplitude, duration or A-B break which might suggest postsynaptic inhibition. Inhibition of CSN evoked discharge during an increase in AP was attenuated by bilateral vagotomy and section of the contralateral CSN in 2 cells tested. These results suggest that excitatory CR inputs to some NTS neurons are inhibited by BR activation and this inhibition might be presynaptic in origin. (Supported by NIH grant HL-41894)

141.9

CENTRAL PATHWAY OF THE VON BEZOLD-JARISCH (B-J) REFLEX IN THE RAT. A.J.M. Verberne and P.G. Guyenet. Dept. of Pharmacol., Univ. of Virginia, Charlottesville, VA 22908.

The central pathway involved in the sympathoinhibitory and hypotensive response of the B-J reflex elicited by iv 5-HT or phenylbiguanide (PBG) was studied in halothane-anesthetized, paralysed rats. 5-HT (2.5 ug/kg) or PBG (5 ug/kg) produced marked, transient reductions in lumbar sympathetic nerve discharge and modest reductions of blood pressure. These effects were blocked by the 5-HT3 receptor antagonist MDL 72222 (100 ug/kg, i.v.). The sympathoinhibitory responses to PBG and 5-HT were blocked by bilateral microinjection of kynurenic acid (KYN, glutamate receptor antagonist; 5 nmol/100 nl) into the caudal ventrolateral medulla (CVL) but not the rostral ventrolateral medulla (RVL). B-J reflex was also inhibited by bilateral microinjection of bicuculline methiodide (BIC, GABA receptor antagonist; 100 pmol/50nl) into RVL but not CVL. Bilateral KYN (1.25 nmol/25 nl) into the solitary tract nucleus also inhibited the B-J reflex. Xanthurenic acid, a KYN analogue with no glutamate receptor antagonist activity, was ineffective. Barosensitive neurons in the RVL were inhibited by B-J reflex activation (47/57 units) while some (10/57 units) underwent an excitation/inhibition sequence. This inhibition was blocked by iontophoretic BIC (n=12 cells) but not by strychnine (glycine receptor antagonist) (n=8 cells). These findings underline the similarity between the medullary pathways mediating the B-J and arterial baroreceptor reflexes. (HL 28785)

141.10

ROLE OF THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) IN THE MEDIATION OF HYPOTHALAMIC AND SOMATO-SYMPATHETIC PRESSOR RESPONSES. James M. Kiely and Frank J. Gordon, Dept. of Pharmacology, Emory Univ. School of Medicine, Atlanta, Ga. 30322

These studies investigated the relative role of synaptic vs. axonal neural transmission in the RVLM in the production of centrally-mediated pressor responses. Rats were anesthetized with urethane, bilaterally vagotomized, paralyzed and respirated. Hypothalamic pressor responses of 30-40 mmHg were produced by monopolar electrical stimulation (150 μA, 100 Hz, 1.0 msec pulse duration) of the perifornical (PFH) and paraventricular (PVN) hypothalamic nuclei. Somatosympathetic pressor reflexes of 30-40 mmHg were evoked by electrical stimulation (300 μA, 20 Hz, 1 msec pulse duration) of sciatic nerve afferents. All pressor responses could be abolished by ganglionic blockade with chlorisondamine (5 mg/Kg, i.v.). Multibarrel glass pipettes were placed bilaterally into the RVLM which was functionally identified by microinjections of L-glutamate. All pressor responses could be reversibly abolished after blockade of both synaptic and axonal transmission in the RVLM was produced by injection (50 nl) of 20% lidocaine. After recovery of the pressor responses, synaptic transmission in the RVLM was interrupted by medullary injections of a neurotoxic dose of kainic acid (1 nmol/50 nl). Kainate-induced inactivation of cell somata in the RVLM abolished somato-sympathetic pressor reflexes, but attenuated PFH pressor responses by only 35%. Pressor responses evoked from the PVN were not affected by injections of kainate into the RVLM. These results indicate that both synaptic and axonal transmission within the RVLM participate in the production of centrally-mediated pressor responses. (Supported by NIH Grant HL 36907)

141.11

INHIBITION OF 5-HT UNIT FIRING AND SYMPATHETIC DISCHARGE BY 8-OH-DPAT IN THE BARORECEPTOR DENERVATED CAT. K.A. King*, R.B. McCall and J.M. McCall, Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, Michigan 49001

It has previously been demonstrated that, in the baroreceptor intact cat, the 5-HT_{1A} agonist 8-OH-DPAT produces inhibition of midline medullary 5-HT cells at i.v. doses lower than those required to produce sympathoinhibition. It is possible that this difference is observed because at low doses, the hypotension produced by 8-OH-DPAT elicits a reflex increase in sympathetic nerve discharge (SND), thereby masking a small inhibitory effect of the drug. Midline 5-HT neurons do not receive baroreceptor inputs, and therefore exhibit the effect of the drug at all doses. The purpose of this study was to determine if reflex baroreceptor activation is responsible for the apparent difference in sensitivity to the effects of 8-OH-DPAT. 8-OH-DPAT (1 to 100 ug/kg, i.v.) was administered to 11 baroreceptor denervated cats while recording SND and 5-HT unit discharge simultaneously. 5-HT unit firing was inhibited by 50% following 1 ug/kg of 8-OH-DPAT, and was completely inhibited by a 3 ug/kg dose. SND was inhibited by 37% following 10 ug/kg, and a dose of 100 ug/kg was required to completely inhibit SND. These results were not different from those observed in sham operated animals (N=6). The difference in the sensitivity of midline medullary 5-HT unit firing and SND to the inhibitory effects of 8-OH-DPAT is therefore not due to compensation by the baroreceptor reflex.

141.12

COMPARISON OF THE EFFECTS OF CLONIDINE AND 8-OH DPAT ON SYMPATHOEXCITATORY NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA. R.B. McCall and M.E. Clement. The Upjohn Co., Kalamazoo, MI 49001.

The effects of intravenous and iontophoretic clonidine were determined on the firing rates of sympathoexcitatory neurons in the rostral ventrolateral medulla of the cat. Individual neurons were initially identified using spike triggered mid-signal average of sympathetic activity and responses to baroreceptor activation. Twenty six neurons displayed activity which were temporally related to sympathetic nerve discharge, were inhibited during baroreceptor activation and were antidromically activated from the intermediolateral cell column. Individual neurons had firing rates ranging from 0.75 to 4.1 Hz (mean=1.67 Hz) and axonal conduction velocities of 1.4 to 3.9 m/s. As reported in the rat, we found that sympathoexcitatory neurons could be differentiated based on their sensitivity to clonidine. Approximately 50% of the neurons were inhibited by clonidine. There was only a weak correlation between the inhibition of unit activity and whole sympathetic nerve activity. The discharge rates of the remaining neurons were either not altered or were increased by clonidine. Unlike the rat, these two groups of neurons could not be further differentiated on the basis of axonal conduction velocity or discharge frequency. In contrast, intravenous 8-OH DPAT inhibited all sympathoexcitatory neurons. There was a remarkable correlation between the inhibition of unit activity and whole sympathetic activity. The central antihypertensive mechanism of these drugs will be discussed.

CALCIUM CHANNELS: PHYSIOLOGY AND PHARMACOLOGY II

142.1

ANTI-w-CONOTOXIN GVIA ANTIBODIES: A VALUABLE TOOL FOR THE IDENTIFICATION OF THE w-CONOTOXIN RECEPTOR OF RAT BRAIN. A.M. Snowman, M.W. McEnery, A.H. Sharp, and S.H. Snyder. Dept. of Neuroscience, The Johns Hopkins Univ. School of Medicine, Baltimore, MD

[125]I-w-conotoxin GVIA (w-CTX), a peptide toxin from the venom of the carnivorous cone snail *Conus geographus*, binds with picomolar affinity to the pre-synaptic N-type calcium channel of neurons. To date, the w-CTX receptor has been refractory to isolation. We have produced anti-w-CTX antisera that are specific for the w-CTX peptide and do not cross-react with other toxins which compete for [125]I-w-CTX binding to the receptor. The anti-w-CTX antibodies have been immobilized on a Protein A Sepharose column and loaded with excess ligand. Under these conditions, the w-CTX receptor is retained on the column. When used in tandem with photoaffinity cross-linking experiments , we identify a 240 kDa protein that specifically incorporates derivatized w-CTX and is greatly enriched in our receptor preparation.

142.2

ω-AGA-IVA, A NOVEL PEPTIDE ANTAGONIST FROM *AGELENOPSIS APERTA* SPIDER VENOM, BLOCKS RAT BRAIN SYNAPTOSOMAL CALCIUM CHANNELS. M. E. Adams and V. J. Venema*. Department of Entomology, University of California, Riverside, CA 92521.

We have identified a novel peptide toxin from venom of the funnel web spider, *Agelenopsis aperta*, which antagonizes rat brain calcium channels with high potency and selectivity. This toxin, ω-Aga-IVA, constitutes about 1% of total protein in the venom and belongs to a family of peptides known as "Type IV" ω-agatoxins. The biochemical properties and pharmacological selectivity of ω-Aga-IVA are strikingly different from previously described ω-agatoxins and ω-conotoxins. ω-Aga-IVA is composed of 48 amino acids, including 8 cysteines. Its amino acid sequence is unrelated to known calcium channel antagonists. ω-Aga-IVA, unlike ω-Aga-IIA and ω-Aga-IIIA, does not inhibit high affinity binding of ^{125}I-ω-conotoxin GVIA to rat brain synaptosomal membranes, suggesting a different selectivity for this toxin. In accordance with this interpretation, ω-Aga-IVA blocks depolarization induced ^{45}Ca entry into rat brain synaptosomes (EC_{50} = 15 nM), whereas ω-Aga-IIIA or ω-conotoxin GVIA are inactive when tested at micomolar concentrations. Further evidence for the unique selectivity of ω-Aga-IVA is provided by electrophysiological studies showing that the toxin blocks high threshold Ca current in rat Purkinje neurons (see Mintz *et al.*, next abstract). Type IV ω-agatoxins thus offer novel pharmacological tools for biochemical and functional analyses of Ca channels in the mammalian brain.

Supported by NIH grant NS24472.

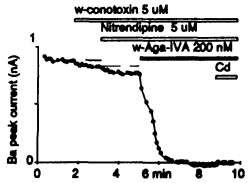
142.3

SELECTIVE BLOCK OF THE P- TYPE CHANNEL IN CEREBELLAR PURKINJE NEURONS BY THE SPIDER NEUROTOXIN ω-AGA-IVA. I. M. Mintz, V. J. Venema*, M.E. Adams, and B. P. Bean. Dept. of Neurobiology, Harvard Med. Sch., Boston MA 02115 and Dept. of Entomology, Univ. California, Riverside CA 92521.

The peptide ω-Aga-IVA isolated from *Agelenopsis aperta* venom was tested on voltage-gated Ca channels in freshly dissociated rat (P7-P15) cerebellar Purkinje neurons, using whole cell recordings of Ba (5 mM) currents.

The major component of the high-threshold Ca current in rat Purkinje cells is unaffected by ω-conotoxin and nitrendipine (Regan et. al., Neuron 6:269, 1991). This "P-type" current is completely and potently blocked by ω-Aga-IVA (Kd<7 nM). Reversal of block was extremely slow if cells were washed while held at -90 mV but was accelerated >1000 fold by voltage-steps to +70mV.

In contrast, even 100 nM ω-AgaIVA had no effect on T-type currents or Bay K 8644-enhanced L-type currents in rat DRG neurons. L -type currents in rat cardiac muscle cells were also untouched.



In rat CA1 hippocampal neurons, rat DRG neurons and frog sympathetic neurons, 100 nM ω-AgaIVA slowly blocked a small fraction of high-threshold current, suggesting that other channel types may be weakly sensitive to the toxin.

142.4

BLOCKADE OF VOLTAGE-SENSITIVE CALCIUM CHANNELS BY ARYLAMINE TOXINS FROM SPIDER VENOM. EF Nemeth, LD Artman*, TN Parks, H Jackson, Natural Product Sciences, Inc., 420 Chipeta Way, Salt Lake City, UT 84108.

The venom of the spider *Agelenopsis aperta* contains two main classes of toxins: low molecular weight arylamines and peptides of 5-12 kD. The former toxins block glutamate receptors whereas the latter affect Na^+ or Ca^{2+} channels. We have examined the ability of arylamines to act on Ca^{2+} channels by assessing their effects on influx of extracellular Ca^{2+} monitored by fura-2 in two cell types: primary cultures of neurons (rat cerebellar granule cells; RCGCs) and a cell line of vascular smooth muscle (A7r5; VSM). In both cell types, depolarizing concentrations of K^+ caused rapid and sustained increases in $[Ca^{2+}]_i$ which were abolished in the absence of extracellular Ca^{2+} and inhibited by nifedipine (1 μM). Ca^{2+} influx in these cells thus occurs largely through "L-type" Ca^{2+} channels. The arylamine toxins (α-agatoxins) AG 489 and AG 505, the most abundant α-agatoxins in this venom, blocked increases in $[Ca^{2+}]_i$ evoked by K^+-depolarization in each cell type. The IC_{50} for AG 489 in RCGCs and VSM was 44 and 158 μM, respectively. AG 505 blocked increases in $[Ca^{2+}]_i$ with IC_{50}s of 10 and 26 μM in RCGCs and VSM, respectively. The synthetic toxin FTX was ineffective in RCGCs at concentrations up to 300 μM. AG 489 and AG 505 did not block Ca^{2+} influx through receptor-operated, voltage-insensitive Ca^{2+} channels in parathyroid cells when tested at concentrations as high as 300 μM. These two arylamine toxins thus block voltage-sensitive Ca^{2+} channels in diverse cell types. They are, however, about 100-fold more potent as blockers of NMDA receptor-mediated increases of $[Ca^{2+}]_i$ in RCGCs. Nonetheless the results demonstrate that at concentrations above 1 μM, α–agatoxins from *Agelenopsis aperta* inhibit various Ca^{2+} influx pathways in excitable tissues. Perhaps there are some common structural features between the ion channel associated with the NMDA receptor and that of voltage-sensitive Ca^{2+} channels that allow arylamine toxins to block Ca^{2+} influx through both channel types.

142.5

FTX INHIBITS TRANSIENT Ca^{2+}-CURRENT OF RAT NEUROHYPOPHYSIAL NERVE TERMINALS. J.R. Lemos and G. Wang. Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545

Funnel-web spider toxin (FTX) specifically blocks a novel Ca^{2+}-conductance ("P") in Purkinje cells (Llinas, et al. 1989; *Proc. Natl. Acad Sci.* 86:1689). In the intact neurohypophysis, FTX reduced the magnitude of the calcium spike (Salzberg, et al., 1990; *Biophys. J. Abstr.* 57:305a). Here we examined the effects of FTX on the Ca^{2+}-currents (I_{Ca}) which presumably underlie the Ca^{2+} spike of isolated rat neurohypophysial nerve terminals.

Using the whole-cell patch clamp technique, the nerve terminal I_{Ca} was elicited with either 10 mM Ca^{2+} or Ba^{2+}. The I_{Ca}, with both transient and long-lasting components, was recorded by depolarizing from -90 mV to +10 mV for 1 second. 50 μM Cd^{2+} reversely eliminated both components of I_{Ca}. In the presence of FTX (0.5-5 μl/ml) in the bath solution, however, only the transient component of I_{Ca} was reduced in a dose-dependent manner. 0.5 μl/ml FTX decreased this component by 52.1%, whereas 5 μl/ml inhibited it by 67%. No shift in the I-V curves for either component was observed. Furthermore, there are no significant differences between the effects on the Ca^{2+}-mediated and the Ba^{2+}-mediated current. The inhibition of the current by FTX was reversed by washing. In contrast, the toxin had minimal effects on the long-lasting component of I_{Ca}, elicited from either -90 mV or -40 mV, of the nerve terminals. In the same preparation, however, FTX strongly inhibited the long-lasting I_{Ca} of the *pars intermedia* cells.

These results suggest that the portion of neurohypophysial I_{Ca} that is sensitive to FTX could belong to the P-channel family. The P current is also considered to be responsible for presynaptic calcium spikes in the squid. The properties and functions of the FTX-blocked I_{Ca} in the nerve terminals will have to be further investigated. FTX was a kind gift of Drs. Cherksey and Llinas. Supported by NSF and NIH grants to J.R.L.

142.6

POLYAMINE BLOCK OF P-TYPE CALCIUM CHANNELS. B. Cherksey*, R. Goodnow, M. Sugimori*, K. Nakanishi and R. Llinas*. Dept. of Physiology and Biophysics*, NYU Medical Center, New York, N.Y. 10016 and Dept. of Chemistry, Columbia University, New York, N.Y. 10027

FTX, the specific blocker of the neuronal P-type calcium channel isolated from the venom of funnel-web spiders has been reported to be a low molecular weight, non-aromatic polyamine. In previous studies, we had synthesized and tested the mixed condensation product of spermidine with the amino acid arginine and found that the mixture had FTX-like activity (Cherksey, B., *et al.* 1989 Biol. Bull., *177*, 321). We have now prepared well-characterized synthetic FTX analogues and have tested them for their electrophysiological activity in guinea pig cerebellar slices.

Three arginine-polyamine adducts were prepared for these studies: FTX(4:3), FTX(3:4) and FTX(3:3), where the numbers 3 and 4 indicate the number of methylene groups separating the amino groups in the polyamine. Other polyamines tested included ones in which lysine was substituted for arginine and polyamines in which the triamine was replaced by diamines with carbon numbers from 8 to 12.

Electrophysiological assay of the activity of the FTX's was performed in the current clamped guinea pig Purkinje cell. In this assay system, FTX(3:3) and FTX (4:3) were found to block the P-type calcium channel in micromolar concentrations while FTX(3:4) was found to be inactive. FTX(3:3) exhibited the greatest potency being approximately 10 times as active as FTX(4:3). When elevated concentrations of the compounds were tested (into the millimolar level) no block of sodium or potassium conductances was seen.

Substitution of lysine for the arginine in the synthetic FTX's resulted in a loss of P-channel blocking activity. The amino acid - diamine conjugates were found to exhibit potassium channel blocking activity when tested against brain homogenate and non-neuronal tissue in the lipid bilayer system.

Our results suggest that simple polyamines and polyamine-amino acid conjugates exert substantial, specific effects on ionic channels and may prove to be important natural global channel modulators manufactured by the body and found in the brain.

142.7

ELEMENTARY PROPERTIES OF CALCIUM CHANNELS IN CEREBELLAR PURKINJE CELLS. M.M. Usowicz, M. Sugimori & R.R. Llinás. Dept. of Physiology and Biophysics, New York University Medical Center, New York, NY 10016.

Recent experiments have indicated that cerebellar Purkinje cells possess P-type Ca channels that are pharmacologically distinct from T-, N- and L-type Ca channels. We have examined the elementary properties of Ca channels in Purkinje cells. Cell-attached patch recordings were made from adult Purkinje cells in thin cerebellar slices of the guinea-pig (Edwards et al, Pflügers Archiv 414:600), with patch-pipettes filled with (in mM): 110 $BaCl_2$, 10 HEPES, 0.5 μM TTX, pH 7.4 with TEA-OH. Currents were recorded in two different regions of the cell; either from the soma or from the dendrite, usually at a bifurcation 30 - 100 μm from the soma.

The threshold of activation for single-channel Ba currents was -30 to -15 mV from a holding potential of -90 mV, consistent with the absence of low voltage-activated Ca channels from adult Purkinje cells. Currents arising from a small population of channels were maximal at about 20 mV, when elicited in multi-channel patches by a depolarizing ramp change in command potential. (All potentials have been calculated for a resting potential of -60 mV). Channel openings were to a main conductance level of about 17 pS (slope conductance) and, less frequently, to a lower conductance level of about 10 pS.

Supported by NINCDS grant NS13742 (RRL), MRC (Great Britain) Travelling Fellowship (MMU) and SERC/NATO Fellowship (MMU).

142.8

CALCIUM CHANNELS IN RAT CEREBELLAR NEURONS Daniela Pietrobon*, Gabriella Calderini* and Lia Forti*
C.N.R. Center Physiology of Mitochondria, University of Padova; *Fidia Research, Abano Terme, Italy

Single channel recordings from rat cerebellar granule cells frequently show two types of calcium channels which are neither L- nor T-type calcium channels. These channels have conductances of 15 and 20 pS respectively. In both types of channels steady state inactivation occurs at relatively negative voltages, both channels being totally inactive at a holding potential of -40 mV. Both types of channels do not inactivate during a 700 msec long test pulse. However they activate at different voltages. With 90 mM barium ions as charge carriers the 15 pS channel starts to activate around -30 mV while the 20 pS channel starts to activate around 0 mV. Less frequently we have observed two other types of calcium channels, of 19 and 7 pS respectively, which are neither L- nor T-type. The L-type channels in these neurons, like those in cardiac cells (Pietrobon & Hess 1990 Nature 346:651), have two different gating modes: besides the typical short-opening mode they have a mode of activity characterized by much longer open times. Both the probability of entering the long opening mode and the mean time the channel spends in this mode are voltage dependent. At negative voltages the average duration of the long-opening mode is much longer in cerebellar than in cardiac cells. Moreover, the voltage dependence of the rate of exit from the long opening mode is less pronounced in neurons than in myocytes.

142.9

AN UNCONVENTIONAL TRANSIENT Ca CURRENT IN GABA-ergic NEURONS OF RAT THALAMIC RETICULAR NUCLEUS J.R. Huguenard and D.A. Prince. Dept. of Neurology & Neurological Sciences, Stanford Univ. Medical Center, Stanford, CA 94305.

Both thalamocortical relay neurons (TCs) and the GABAergic cells of the nucleus reticularis (nRt) possess transient Ca^{2+} currents (I_T) that underlie bursts of fast Na^+-mediated spikes, however both the morphology and the proposed function of these bursts are different. Because burst duration is relatively long in nRt, during such bursting its efferents produce prolonged inhibition in relay nuclei. The resultant hyperpolarization in TCs contributes to deinactivation of a powerful I_T, causing them to switch from tonic to phasic firing pattern, and thus influences the coherence of sensory information relayed to the cortex.

To test the hypothesis that these differences in burst generation reflect fundamental differences in the underlying T currents, we used patch clamp techniques to study whole-cell currents in acutely isolated rat nRt cells. These neurons generated a slow transient Ca^{2+} current (I_{Ts}) that had similarities to I_T recorded in TCs, including steady-state inactivation properties, and sensitivity to blockade by divalent cations (Ni^{2+}, Cd^{2+}), amiloride, and anticonvulsants. However, I_{Ts} inactivated relatively slowly and in a nearly voltage-independent manner, with a time constant (τ_h) near 90 ms between -60 and -30 mV at 23°C. By contrast, τ_h for I_T varies between 50 and 20 ms in the same voltage range. Furthermore, I_{Ts} was activated with a higher threshold (-55 mV) compared to I_T (-65 mV), and activation of I_{Ts} occurred over a broader voltage range. Finally, deinactivation was slower for I_{Ts} (τ = 520 ms at -90 mV) compared to I_T (τ = 320 ms).

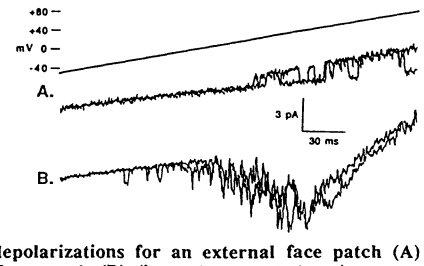
We conclude that neurons in nRt possess a different form of T channels than those in TCs. Because I_{Ts} inactivates relatively slowly, and the rate of inactivation is not markedly increased by depolarization, burst firing in nRt is not terminated as rapidly as in TCs. Prolonged barrages of ipsps onto TCs result, with consequent emergence of burst firing in thalamocortical efferents. Supported by NIH grants NS06477 and NS12151.

142.10

UNITARY AND CLUSTERED SINGLE CALCIUM CHANNELS ON A CHOLINERGIC NERVE TERMINAL E.F. Stanley, LB NINCDS, Bld. 9 Rm 1E124, NIH, Bethesda MD 20892.

We have previously characterized inward Ca current in a presynaptic nerve terminal using the sheet-like calyx terminal of the chick ciliary ganglion (J. Neurosci. 11:985). In this study single channel currents were recorded from both the external, non-synaptic aspect of the calyx and the internal, transmitter release face.

The external face of the calyx exhibited only outward single channels while single calcium channels were recorded from the internal, release face of the nerve terminal. These calcium channels were recruited at -30 mV, had a conductance of 11 to 13 pS in 110 Ba and were often highly clustered.



Ramp depolarizations for an external face patch (A) and a release-face patch (B) (inward current down).

142.11

EVIDENCE FOR L- AND TWO MODES OF T-TYPE CURRENT IN INSULIN SECRETING CELLS. T.G.Hales & B.Ribalet. Dept's of Physiol. and Anesthes., Center Health Sci. UCLA. LA. CA. 90024.

Tail current analysis was used to characterize Ca currents in the insulin secreting cells, HIT and RIN. Ca currents were recorded using the whole-cell patch-clamp technique. The pipette contained an NMDG based solution supplemented with BAPTA and ATP. The bath contained an NaCl based saline with Ca^{2+} (3 mM) and TTX. Tail currents evoked by repolarizing the membrane to -100 mV following a depolarizing pulse of 8 ms were fitted by the sum of three exponentials, τ_1=0.2, τ_2=2.2 and τ_3=17 ms. The thresholds of activation for τ_1, τ_2 and τ_3 components were -40, -60, and -40 mV and their ½ maximal amplitudes occurred at 0, -28, 14 mV, respectively. Consistent with τ_1 being deactivation of L-type current this component ran down during the course of most experiments, was inhibited by 36% by nifedipine (1 μM) and increased by 94% by substituting Ba^{2+} for external Ca^{2+}. The slower deactivating τ_2 and τ_3 components had common properties. Their amplitudes were stable throughout most experiments, relatively insensitive to nifedipine (1 μM) and reduced in the presence of external Ba^{2+} by approximately 28%. Also, replacing external Na^+ by Tris had no effect on τ_1, but inhibited τ_2 and τ_3 by 70% and 60%. Together, these observations suggest that τ_1 and τ_2 are the time constants of deactivation of L- and T-type currents. The similar properties of τ_2 and τ_3 components, led to our proposal that τ_3 is the time constant of deactivation of a second mode of T-type channels. With increased length of depolarization the amplitude of τ_2 decayed (75 s^{-1}). For pulses to >-20 mV, the decay of τ_2 was accompanied by a simultaneous increase (75 s^{-1}) of τ_3 amplitude, suggesting a transition between two modes of T-type channels. At less depolarized potentials the transition of T-type channels from mode 1 to mode 2 may explain the apparent inactivation of Ca currents, mode 2 channels being predominantly closed below -20 mV. The existence of L- and two modes of T-type Ca channels may determine the shape of voltage-activated Ca currents in HIT and RIN cells.

142.12

PARVALBUMIN IS AN INTRACELLULAR NEURONAL Ca^{2+} BUFFERING PROTEIN. P.S. Chard, D. Bleakman and R. J. Miller Dept. of Pharmacol. and Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Cytosolic high affinity Ca^{2+} binding proteins such as parvalbumin and calbindin are thought to regulate the free $[Ca^{2+}]_i$ through rapid sequestration. Interest in these proteins and their role in Ca^{2+} buffering has increased with the finding that their concentrations are altered in certain CNS dysfunctions (e.g, status epilepticus and Alzheimer's disease). Introduction of these proteins into neurons would aid in elucidating their role in $[Ca^{2+}]_i$ homeostasis. Using combined whole cell patch clamp/Fura-2 based microfluorimetry we have examined the effects of intracellular introduction of the Ca^{2+} binding protein parvalbumin on the buffering of $[Ca^{2+}]_i$ in cultured dorsal root ganglion neurons (DRG). 5-12 day old DRG neurons in culture were replated 1 hour prior to the experiment. These cells stained negatively for parvalbumin. I_{Ca}'s and subsequent increases in $[Ca^{2+}]_i$ were evoked at 0mV from a holding potential of -80mV for either 80ms or 320ms duration (whole cell patch clamp with , in mM; 140CsCl, 1Mg, 10HEPES, 3.6 ATP and an ATP regenerating system in the pipette and 140TEACl, 2Ca in the bathing solution). Cells of similar size on the same coverslip were examined in the absence or presence of parvalbumin (250μM - 1mM). Buffering of the rise in $[Ca^{2+}]_i$ was more effective in the parvalbumin containing cells than in control cells. The observed rise in $[Ca^{2+}]_i$ which was initially large, decreased during the timecourse of the experiment in a manner disproportionate to the size of the I_{Ca}. In some cells with a recorded peak I_{Ca} of 300-600pA the rise in $[Ca^{2+}]_i$ was virtually abolished. In 4 pairs of cells where the recorded I_{Ca} and corresponding rise in $[Ca^{2+}]_i$ was normalized to the cell size, parvalbumin reduced the peak rise in the $[Ca^{2+}]_i$ by 42 ± 23% ($P<0.001$, paired t-test). There was no observed change in the inactivation kinetics of the macroscopic I_{Ca} in contrast to changes observed with 1mM BAPTA in the patch pipette. These results demonstrate that parvalbumin, in a concentration range which occurs in neurons, can effectively contribute to intracellular Ca^{2+} buffering.

RETINA AND PHOTORECEPTORS: GANGLION CELLS

143.1

SIZE, STRATIFICATION AND MORPHOLOGY OF THE MIDGET, PARASOL AND SMALL BISTRATIFIED GANGLION CELLS OF THE HUMAN RETINA. D.M. Dacey and M. R. Petersen. Depts. of Biological Structure and Ophthalmology, The University of Washington, Seattle, WA 98195.

We have intracellularly injected ganglion cells in wholemounts of the human retina maintained *in vitro*. Over 1000 HRP-filled cells provide the first detailed picture of the diversity of human ganglion cell types. Here we focus on the midget and parasol cells and compare them with a third, clearly identified type termed the *small bistratified* cell. Human midget (n = 300) and parasol cells (n=250) show a larger mean dendritic field diameter than their counterparts in the macaque monkey, this difference being greatest (about 2 fold) in the central retina. Radial sections through midget and parasol dendritic trees show that, as in the macaque, these cells can be divided into a pair of types that occupy distinct inner and outer strata of the inner plexiform layer (ipl). The midget cells stratify broadly to completely overlap and extend beyond a more narrow parasol cell strata. The inner and outer types can also be distinguished morphologically: inner cells have larger cell bodies and larger and more densely branched dendritic trees than outer cells.

The small bistratified cell (n=46) can be uniquely distinguished by its small and distinctly bistratified tree. Dendritic field diameter is the same as, or slightly larger than that for parasol cells at all eccentricites sampled, but somal diameter is small, about the same as for a midget cell. The two dendritic strata are narrow and occupy the same depth in the ipl as the parasol cells. The parasol-like dendritic tree size combined with the midget-like soma size suggests that the small bistratified cells would show broad-band type receptive field size but be relatively rarely sampled by a recording electrode. A good candidate, also consistent with the cell's bistratified morphology, is the non-concentric, color opponent cell (DeMonasterio and Gouras, *J. Physiol.*, 251:157, 1975).

143.2

NEUROPEPTIDE Y IMMUNOREACTIVITY IDENTIFIES A SUBGROUP OF GAMMA TYPE GANGLION CELLS IN THE CAT RETINA. J. J. Hutsler, C. A. White & L. M. Chalupa. Dept. Psychology & Center for Neurobiology, University of California, Davis, CA 95616.

Retinal ganglion cells (RGCs) in the cat have been traditionally classified on the basis of their morphological and functional properties. Recently, it has been discovered that alpha ganglion cells can be subdivided on the basis of their immunoreactivity for somatostatin (White & Chalupa, 1991). We now report that neuropeptide Y (NPY) identifies a different subgroup of cat RGCs. In the adult retina about 1,800 neurons distributed throughout the ganglion cell layer are immunoreactive for NPY. Their highest density (about 50 cells/mm^2) is at the area centralis, with a decline in cell density towards the periphery (10-12 per mm^2,10 mm from AC). The somas of these neurons are typically round, although many appear elliptical or spindle shaped. Somal size remains relatively constant across the retinal surface, ranging in diameter from 14 μm at the AC to 16 μm near the periphery. Colchicine pretreatment revealed 2 to 5 sparsely branching primary dendrites. In many cases a presumptive axon could be followed for over 500 μm into the nerve fiber layer. These morphological properties of the NPY immunoreactive neurons are consistent with what has been described for small to medium sized gamma cells (Stone & Clarke, 1980; Kolb et al., 1981). In addition, deposits of rhodamine latex microspheres into the LGN and SC resulted in retrograde labeling of NPY immunoreactive cells. Immunoreactive neurons not labeled by the tracer as well as those without an axon entering the fiber layer may be displaced amacrine cells. The precise proportion of ganglion cells within this population as well as their projection patterns are being investigated. (Supported by EY-03991 from the NEI).

143.3

HOMOCYSTEIC ACID (HCA)-EVOKED CURRENT IN RAT RETINAL GANGLION CELLS. Dongxian Zhang and Stuart A. Lipton. Dept. of Neurology, Children's Hospital & Progr in Neuroscience, Harvard Med Schl, Boston, MA 02115.

HCA, one of the sulfur-containing amino acids, is an endogenous excitatory amino acid in the retina. HCA has been proposed to be a neurotransmitter released by bipolar cells (Neal & Cunningham, *Neurosci. Lett.* 1989;102:114-9). Also, in the rat retina HCA immunoreactivity has been observed in the inner nuclear and ganglion cell layers as well as in the outer and inner plexiform layers (Ortega et al., *Eur. J. Neurosci.* Suppl. 1990;3:119). To begin to explore the potential role of HCA as a transmitter impinging on retinal ganglion cells, whole-cell recordings with patch electrodes were performed on isolated rat retinal ganglion cells in culture. With $[Mg^{2+}]_o$ = 0, HCA (200 μM) induced inward currents when ganglion cells were held at V_H = -60 mV. HCA-evoked currents were of relatively small amplitude (10-40 pA) and displayed a characteristic increase in noise, similar to that observed previously in NMDA-activated currents of rat retinal ganglion cells (Aizenman, Frosch & Lipton, *J. Physiol.* 1988:396:75-91). Furthermore, HCA-evoked currents were completely blocked by the NMDA receptor antagonist APV (200 μM). These observations suggest that HCA activates the NMDA receptor of retinal ganglion cells. Thus, HCA should be considered as a neurotransmitter candidate in the rat retina.

143.4

CHARACTERIZATION OF NICOTINIC ACETYLCHOLINE RECEPTORS ON ISOLATED GOLDFISH RETINAL GANGLION CELLS. B.E. Yazejian and G.L. Fain. Department of Ophthalmology, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024-7008.

Although vertebrate retinal ganglion cells have been shown to give nicotinic responses to applied acetylcholine (ACh), the properties of the receptors have not yet been well characterized. We have recorded whole-cell membrane currents from enzymatically isolated goldfish retinal ganglion cells while applying drugs through a U-tube. ACh, as well as nicotine and DMPP, evoked cation-selective currents which were rapidly desensitizing and highly inwardly rectifying. No outward currents were seen at positive potentials for E_{Na}s≥0mV although small outward currents were seen above E_{Na} when E_{Na} was shifted to negative potentials by substituting some of the external Na (Na_o) with glucose. Replacing 70% of the Na_o with NMDG completely blocked the response; this effect was at least in part pharmacologic since NMDG at only 1mM reversibly blocked 58±19% of the response to 30μM ACh (n=2). Responses to 30μM ACh were also reversibly blocked: 63±7% by 300μM curare (n=8), 61±2% by 300μM hexamethonium Cl^- (n=4) and in 3 of the 6 cells tested, 50±2% by 100μg/ml α-BTX. At least some of the block of the ACh response by mecamylamine (MEC) was use-independent since a 2s pre-application of 300μM MEC irreversibly blocked the response to 30μM ACh tested 60s later.

143.5

GLYCINE RECEPTORS MAY LIE IN THE SURROUND PATHWAY OF MOUSE RETINAL GANGLION CELLS. Charlene Stone and Lawrence Pinto, Northwestern Univ., Neurobiology Dept., Evanston IL 60208

We investigated the receptive field properties of retinal ganglion cells in isolated, superfused retinas of *spastic* mutant mice; normal littermates were used as controls. Extracellular recordings of responses to centered circular and annular stimuli were used to construct peri-stimulus-time histograms. In the mutant retina, annular stimuli did not elicit the surround-type response observed in the control; instead, a center-type response was recorded. However, in both phenotypes responses to large centered circular stimuli were smaller than to stimuli of intermediate size, demonstrating an antagonistic influence of the surround mechanism. These results suggest that a glycine receptor may normally be required for the transmission of excitatory signals from the surround mechanism. Spatial frequency tuning curves, obtained with sinusoidally-modulated gratings, were dominated by the center mechanism and therefore were not affected by the spastic mutation. Finally, we examined the effect of strychnine on the response to centered circular stimuli. Very low concentrations of strychnine attenuated the light response in mutant retinas (apparent $K_I = 8.1 \times 10^{-13}$M). In control retinas, the light response was also attenuated by strychnine but the apparent K_I was much higher (1.0×10^{-7}M). Perhaps the expression of a novel retinal form of the glycine receptor, with a higher affinity for strychnine, is unmasked by the *spastic* mutation. EY01221.

143.6

SYNAPTIC CONDUCTANCE FOR LINEAR OPERATION OF ON-ALPHA GANGLION CELL Michael A. Freed, Ralph Nelson, Peter Sterling, and Robert G. Smith LNP, NINDS, NIH, Bethesda, MD 20892 and Dept. Anat., U. PA Sch. Med., Phila. PA 19104

A ganglion cell polarizes in proportion to stimulus contrast in the range below half-saturation. We investigated the On-alpha ganglion cell of cat retina to learn how synaptic conductances sum to produce this linear voltage response.

On-alpha somas (n=3), located in the area centralis (AC) of an eyecup preparation, were impaled with 100 MΩ microelectrodes. A cell was injected with current and stimulated with half-saturating contrasts (0.2 mm bar for 1 s, 647 nm, ~6 log[q μm-2 s]). Slope conductance (dI/dV) in the dark was 8-9 nS. The light-evoked conductance increase was ~1 nS. If all 1600 synapses on an On-alpha cell released a quanta within the cell's integration time, each causing a 0.3 nS conductance, a total of 380 nS would result. This is 38-fold more than measured conductances. This suggests that less than 3% of the synapses release quanta per integration time during a half-saturating stimulus.

We implemented a compartmental model of a reconstructed On-alpha cell from the AC (R_m = 9500 Ω-cm2). The simulated On-alpha cell summed synaptic conductances in a linear fashion only when individual conductances were quantal and spread out over the dendritic arbor. Larger conductances, as might occur when multiple quanta impinge on a single dendritic branch, caused saturation of the synapse's driving force and significant nonlinearity. This suggests that On-alpha cell responses to low-contrast stimuli are due to linear summation of intermittent and spatially distributed quantal synaptic conductances.

143.7

FUNCTIONAL ROLE OF PUSH-PULL BIPOLAR CELLS IN UNIFIED MODEL OF X AND Y RETINAL GANGLION CELLS. P. Gaudiano, Dept. of Cognitive & Neural Systems, Boston University, Boston, MA 02215.

The feed-forward shunting network has been shown to possess properties that make it well suited for spatial information processing in the retina. These properties include adaptive gain control, relative contrast processing, and suppression of uniform backgrounds (Grossberg, J. Theor. Biol., 27:291, 1970; Sperling, Perc. & Psych., 8:143, 1970). However, time-domain analysis shows that those properties that lead to good spatial processing can result in poor temporal processing. This problem is solved by addition of a push-pull preprocessing layer. The anatomical interpretation of the resulting model is structurally analogous to known retinal circuitry as described by McGuire and Sterling (J. Neurosc., 6:907, 1986): light impinging on cones depolarizes one type (ON) and hyperpolarizes a second type (OFF) of bipolar cell. At a given location, both bipolar cell types converge upon individual ganglion cells: the ON bipolar excites ON-center ganglion cells, while the OFF bipolar inhibits them. A complementary arrangement exists for OFF-center ganglion cells. The model shows that X and Y cells may consist of the same formal mechanism acting in different parametric regimes. In agreement with morphological and physiological data, an increase in receptive field center (RF) size changes the model's response from X-like to Y-like (Saito, J. Comp. Neurol., 221:279, 1983). Specifically, simulated X cells exhibit sustained responses; approximately linear spatial summation; null responses to modulated gratings. Simulated Y cells exhibit transient responses; spatial phase-independent and spatial frequency-dependent on-off (nonlinear) responses to modulated gratings. This duality results from mathematical properties of the push-pull network, which can selectively enhance sustained or transient response components on the basis of RF morphology.

143.8

A SILICON NETWORK FOR MOTION DISCRIMINATION THAT USES SPATIO-TEMPORAL INTERPOLATION. T. Delbrück, *Computation and Neural Systems Program 139-74, Caltech, Pasadena CA 91125.*

I have designed and tested a silicon motion discrimination network. An integrated one-dimensional circuit receives visual input from adaptive high-gain receptors. The receptor output capacitively couples into a single first order unidirectional delay line running along the receptor array. The delay line interpolates and predicts a motion signal such that the delay between stages is the delay of one element. Hence an input motion signal piles up on the delay line in a wave-like fashion; if the input is moving at the speed of the delay line the piling is maximal. Motion in the opposite direction does not pile up; a series of small pulses are the only result. An impulse response traveling along the unidirectional delay line does not change its integrated area, instead the pulse becomes smaller and more spread out in time. Hence, unidirectionality is not itself enough for motion discrimination; a nonlinearity is required. An expansive, even, nonlinearity locally transforms the signal on the linear delay line into a motion discrimination that is sign-of-contrast invariant. The temporal resolution and tuning for velocity of a particular stage are initially broad but become sharper the longer the motion has continued. This model most resembles the Adelson and Bergen energy model for motion discrimination; the main difference is that the silicon model is wire-efficient: a single delay line encodes the entire spatial dimension for one direction of motion.

143.9

INDEPENDENT ON AND OFF COMPUTATIONS OF RETINAL DIRECTIONAL SELECTIVITY IN RABBIT. N.M. Grzywacz[1] and F.R. Amthor[2]. [1]Smith-Kettlewell Inst., 2232 Webster St., San Francisco, CA 94115, and [2]Dept. of Psychol. and NRC, Univ. of Alabama at Birmingham.

It has been hypothesized that the bistratification of the dendritic trees of ON-OFF directionally selective ganglion cells of the rabbit retina could provide a substrate for direct ON and OFF excitatory inputs. This is consistent, for example, with the good match between the extents of the receptive-field regions in which ON and OFF responses are elicited and the dendritic ramifications in the inner and outer sublamina of the inner plexiform layer. However, it is not clear whether ON and OFF inputs remain segregated or interact via the facilitatory or inhibitory mechanisms underlying retinal directional selectivity.

We used an apparent motion paradigm that simulate moving edges to investigate facilitatory and inhibitory ON-OFF interactions. The data show that an ON stimulus does not produce significant preferred-direction facilitation of the response to an OFF stimulus. Similar results are found for OFF-ON interactions and for null-direction inhibition. Hence, facilitation and inhibition are probably not due to a general effect on the ganglion cell, but appear to be specifically related to directional selectivity. Further support for this conclusion comes from the spatio-temporal profile of facilitation and inhibition. The spatial distribution of facilitation is narrower than the receptive-field center. Therefore, it is unlikely that facilitation arises from a general elevation of the excitatory input to the ganglion cell. Likewise, the time courses of the inhibition in the receptive-field center and in the surround are different. Thus, the mechanism of the inhibition underlying directional selectivity is distinct from the mechanism mediating the general surround inhibition.

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143.10

MULTINEURONAL SIGNALLING BY RETINAL GANGLION CELLS. M. Meister*, L. Lagnado*, and D. A. Baylor, Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

How do the optic nerve fibers encode the visual information transmitted to the brain? We would like to understand how the aggregate response of the retinal ganglion cell population to a given visual stimulus should be interpreted. Is it completely determined by the receptive fields of the individual neurons? Or do different neurons join in signalling certain features, such that the firing of one cell acquires different meaning depending on the activity of another?

Using a multielectrode array to record from the isolated retina, we have been able to monitor simultaneously the action potentials of up to 100 ganglion cells within a region of 0.5 mm diameter. The photoreceptor layer is stimulated with a "white noise" display: a checkerboard pattern in which each pixel flickers randomly among different colors. By correlating the spike train of each neuron with the visual stimulus at different times and spatial locations, one can compute each cell's spatial receptive field, response time course, and spectral sensitivity. Studies of the salamander retina have revealed receptive fields of the type known from single-cell recording. We are now in a position to extend the receptive field concept to the combined firing of several cells, and to test whether ganglion cells act independently or in concerted fashion as they encode visual information.

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143.11

CONTRAST DEPENDENCE OF DISPLACEMENT DETECTION: PSYCHOPHYSICS AND PHYSIOLOGY

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Thresholds were obtained for the just-detectable displacement of a bright block stimulus seen against a uniform background. By varying the luminances of the block and the background, contrast could be controlled. Experiments were performed in the near periphery of the retina, 6.7 deg. from the fovea. When contrast is reduced from near unity to about .2 there is little change in threshold, but further reduction in contrast produces a progressive increase in threshold.

Physiological experiments were performed in the monkey; discharges were recorded from P- and M- ganglion cells with receptive fields at retinal eccentricities comparable to the psychophysical experiments. Contrast dependence of M-cell responses to target displacement was plotted and could be well matched to the psychophysical results with identical stimulus conditions and retinal position. P-cell responses were weak and bore little realtion to the psychophysical results. The signals processed for the detection of small displacements are thus carried by the M-pathway.

143.12

SPATIAL PRECISION OF MACAQUE GANGLION CELL RESPONSES TO MOVING STIMULI. B.B. Lee, C.F. Wehrhahn, J. Kremers* and G. Westheimer. Max Planck. Inst. f. Biophys. Chem., 3400 Göttingen, FRG.

Psychophysical performance on vernier tasks depends on contrast. For a briefly flashed offset edge target, thresholds increase exponentially below a Michelson contrast of 0.2. We measured sensitivity to location of flashed edges and response variability in M- and P-cells of the macaque retina as a function of contrast, and shown it is likely that the M-cell signal provides a substrate for the central mechanism for vernier localization (ARVO, 1991).

We now extend this analysis to moving achromatic and chromatic borders, measuring responses to these stimuli as a function of contrast. To estimate the precision of the ganglion cell signal we used a cross-correlation measure between individual responses. This measure indicated a much higher degree of coherence and hence spatial precision in the M-cell compared to the P-cell signals, especially at low achromatic contrasts. The data do not provide a model for vernier performance, but such models must be constrained by the retinal input. M-cells seem likely to provide the signal for this type of performance; the P-cell signal is too noisy.

143.13

CONTRAST GAIN CONTROL IN THE PRIMATE RETINA. E.A.Benardete*, E.Kaplan, and B.W.Knight*. Lab. of Biophysics, The Rockefeller University, New York, NY 10021.

Contrast provides the major signal for image processing in vision. Work on the cat retina has shown that a nonlinear feedback mechanism, the contrast gain control, adjusts the temporal response of retinal ganglion cells according to the unsigned average of contrast over a large region of the retina. Here we report the existence of similar processes in the primate retina.

Retinal ganglion cell activity was monitored as synaptic (S) potentials in the LGN of anesthetized and paralyzed macaque monkeys. The contrast of sinusoidal gratings produced on a CRT was temporally modulated with a sum of 8 sinusoids in order to calculate temporal transfer functions. The data fit a model (Victor, '87) that describes both the dynamics of photoreceptors and the contrast gain control.

We found that increasing stimulus contrast changes the temporal frequency response of magnocellular-projecting (M) cells, but not of parvocellular-projecting (P) cells. M cells show a nonlinear response to contrast characterized by a selective attenuation of low temporal frequencies that can be attributed to a change in the time constant of the contrast gain control. P cells, on the other hand, respond linearly to contrast across a wide range of temporal frequencies. This difference in dynamics suggests that M cells receive nonlinear input from retinal elements which do not influence the activity of P cells. This similarity between the monkey M population and cat retinal ganglion cells further supports the view that P cells represent a new specialization not found in lower mammals.

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NEUROENDOCRINE REGULATION I

144.1

GROWTH HORMONE (GH) STIMULATES SOMATOSTATIN (SRIF) RELEASE AND mRNA LEVELS IN THE RAT HYPOTHALAMUS. M.C. Aguila and S.M. McCann. Department of Physiology, Neuropeptide Division, UT Southwestern Medical Center, Dallas, TX 75235-9040.

GH suppresses its own secretion by stimulating SRIF release. Thus, the possible regulation of GH releasing factor (GRF), SRIF release and its mRNA by GH was studied in the hypothalamus of male rats in vitro. The median eminences (ME's) were incubated in buffer containing 10^{-7} to 10^{-11}M GH for 30 min. SRIF and GRF released into the medium were quantitated by RIA. The release of SRIF from ME fragments was significantly increased ($p<0.001$) by 10^{-9}M GH. However, 10^{-9}M GH inhibited ($p<0.01$) GRF release from the ME. To determine the effect of GH on SRIF mRNA levels, PVN explants were cultured during 6 hrs in medium with 10^{-7} to 10^{-11}M GH. Levels of SRIF mRNA (determined by a S_1 nuclease protection assay) were significantly elevated ($p<0.01$) by 10^{-9}M GH. Likewise, 10^{-9}M GH significantly stimulated the release of SRIF and GRF from PVN explants at 30 min and 6 hrs. These results demonstrate a dual action of GH on GRF release and indicate the presence of an inhibitory feedback system within the hypothalamus on the regulation of GH secretion which is mediated by increased release and transcription of SRIF and decreased release of GRF from the ME.

144.2

GROWTH HORMONE (GH)-RELEASING HEXAPEPTIDE GHRP STIMULATES GH RELEASE VIA CENTRAL GH-RELEASING FACTOR (GRF) PATHWAYS. G.S. Tannenbaum and C.Y.Bowers*. Depts. of Neurology & Neurosurgery and Pediatrics, McGill University, Montreal, Quebec H3H 1P3; and Tulane University Medical School, New Orleans, LA.

The synthetic hexapeptide GHRP is a potent secretagogue of GH, however its mechanism of action remains unknown. In the present study, we examined the temporal pattern of GH responsiveness to varying doses (1, 5, 10 and 20 μg) of GHRP and GRF(1-29)NH_2 alone and in combination, and assessed the possible involvement of endogenous hypothalamic GRF. Six-hour (1000-1600 h) GH secretory profiles were obtained from free-moving adult male rats iv administered GHRP and GRF at times of spontaneous peaks (1100 h) and troughs (1300 h) in GH secretion. At peak times, GHRP stimulated GH release in a dose-dependent manner ($r = 0.89$; $p<0.02$); the magnitude of the response was similar to that induced by GRF, except at the 1 μg dose. Moreover, the GH response to GHRP was cyclical with GHRP-induced GH release significantly greater during peak compared to trough periods (459.5 ± 77.1 vs 213.9 ± 46.3 ng/ml at 5 μg dose, $P<0.01$; 704.3 ± 134.7 vs 219.6 ± 42.5 ng/ml at 20 μg dose, $P<0.05$), similar to that observed with GRF, the latter known to be due to the cyclic increased release of endogenous somatostatin. Comparison of the mean GH response when GRF (1 μg) and GHRP (5 μg) were administered simultaneously (931.8 ± 77.3 ng/ml) with the additive effect of the GH responses to GRF (1 μg) and GHRP (5 μg) administered separately (1077.9 ± 112.3 ng/ml) revealed no significant differences. Passive immunization with a specific GRF antiserum virtually obliterated the GH response to 5 μg GHRP compared to normal sheep serum controls given the same dose of GHRP (19.3 ± 7.6 vs 293.1 ± 21.2 ng/ml; $P<0.001$). These results demonstrate that 1) GHRP is a potent GH secretagogue but it does not act synergistically with GRF in the adult male rat; 2) GHRP does not disrupt the cyclical release of endogenous somatostatin; and 3) the GH response to GHRP is dependent on central GRF pathways.

144.3

GROWTH HORMONE REBOUND SECRETION FOLLOWING SOMATOSTATIN WITHDRAWAL IS CAUSED BY CALCIUM INFLUX.
MB McFarlane*, TO Bruhn and IMD Jackson. Division of Endocrinology, Brown University/Rhode Island Hospital, Providence, RI 02903.

It is well established that somatostatin (SRIF) is a potent inhibitor of growth hormone (GH) secretion, and that prior exposure to SRIF enhances basal and growth hormone-releasing factor (GRF)-induced GH secretion *in vivo* and *in vitro*. While the intracellular mechanisms involved in the action of SRIF have been described in detail, little is known about the events causing a burst of GH secretion following SRIF termination. We used a perifusion system to evaluate the post-SRIF rebound release of GH. Anterior pituitaries from male SD rats were gently dispersed with neutral protease and placed in perifusion columns on a bed of Sephadex G-10. After perifusion with Minimum Essential Medium (MEM) to evaluate basal GH release, 10 nM SRIF was perifused for 40 minutes, suppressing GH levels by 54.5±3.6% ($p<0.01$). The SRIF treatment was followed by a return to either normal MEM, Ca^{2+}-free MEM with 1.5 mM EGTA or normal MEM with 2 mM cobalt chloride (Co^{2+}). Upon return to normal MEM, GH levels increased to 186.9 ±15% ($p<0.05$) over basal release with peak levels reaching 261.4±25.4% ($p<0.01$). By either blocking calcium channels with Co^{2+} or removing Ca^{2+} from the perifusion medium we were able to completely abolish the GH rebound event. Moreover, we could produce a GH rebound secretory event by treatment with Co^{2+}-containing or Ca^{2+}-free media followed by normal MEM. Our findings suggest that the post-SRIF GH rebound secretion requires Ca^{2+} influx, an event which can be mimicked by simply interfering with Ca^{2+} balance. Because prolonged exposure to SRIF is known to prevent Ca^{2+} uptake by somatotrophs, it seems likely that termination of SRIF will cause GH rebound release due to a sudden increase in intracellular calcium.

144.4

HYPOTHALAMIC PRE-PROSOMATOSTATIN mRNA EXPRESSION IN TRANSGENIC MICE WITH EXCESS OR DEFICIENT GROWTH HORMONE.
D.L. Hurley and C.J. Phelps. Department of Anatomy, Tulane University School of Medicine, New Orleans, LA 70112.

In two spontaneous mutations causing hypopituitary dwarfism in mice (Snell and Ames), concomitant deficits in hypothalamic immunoreactive somatostatin (SST) levels have been observed. The present studies were undertaken to determine the effect of specific transgenically engineered GH deficiency or excess on CNS expression of somatostatin mRNA. Transgenic dwarf mice produced by selective destruction of endogenous GH cells via expression of a genomic insert of rat GH-diphtheria toxin A chain construct ([Tg (GH, DT-A+Mt-1, GHRF) Bri 78], Behringer *et al.*, **Genes Develop. 2**: 453, 1988) were examined for CNS expression of SST by *in situ* hybridization. Transgenic GH-excess (giant) mice ([Tg (Mt-1, GHRF) Bri 11], Hammer *et al.*, **Nature 315:** 413, 1985), which harbor metallothionein (MT)-hGHRH inserts, were also studied. An ^{35}S-labelled RNA probe (R. Goodman, New England Med. Ctr.) complementary to the 520 nt preproSST mRNA was hybridized to tissues under standard conditions. SST mRNA in hypothalamic anterior periventricular neurons was comparable for control animals and transgenic dwarfs. Signals in non-GH regulatory regions such as cortex were also comparable. SST mRNA signals were elevated in anterior periventricular neurons of the giant mice compared with brains of normal controls. Non-GH regulatory regions displayed levels of SST mRNA similar to those of normal animals. Thus, feedback effects of pituitary hormones upon hypophysiotrophic neurons may occur at a transcriptional level. However, while increased GH levels result in increased transcriptional activity in hypothalamic SST neurons, the absence of GH of transgenic dwarfs does not result in decreased steady-state levels of SST mRNA. Supported by PHS grant NS25987 (CJP).

144.5

IONIC CURRENTS IN PRIMARY CULTURES OF DISPERSED GOLDFISH PITUITARY CELLS.
J.P. Chang, C.J. Price, R.M. Jobin* and J.I. Goldberg. Dept. of Zool., Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E9.

Gonadotropin releasing hormone (GnRH) stimulation of gonadotropin and growth hormone secretion from goldfish pituitary cells appears to involve calcium influx through voltage-gated calcium channels. These secretion responses to GnRH are attenuated by phenylalkylamine and dihydropyridine calcium channel antagonists. Furthermore, both GnRH and potassium induce elevations in intracellular calcium in an extracellular calcium-dependent manner as measured by Fura-2 fluorescence. As an initial step in investigating the role of voltage-dependent ion channels in mediating hormone release from goldfish pituitary cells, we characterized the excitable membrane properties of these cells using tight-seal whole-cell recordings. Current clamp recordings revealed that these cells are electrically excitable. Single action potentials were evoked by depolarizing current pulses. Voltage clamp recordings revealed a fast inward sodium current and both a fast transient, and a sustained outward potassium current. After sodium and potassium currents were eliminated by ion substitution, a high-voltage activated calcium current was uncovered ($V_{holding}$: -70 mV). This current showed little inactivation over 100 ms, and was reduced 30-50% by verapamil or nifedipine. In preliminary experiments under total-current recording conditions, bath application of GnRH elicited a transient 50 pA inward current. These results are consistent with the involvement of voltage-gated ion channels in stimulation of hormone release.

Supported by NSERC Canada.

144.6

EVIDENCE FOR SEX SPECIFIC EXPRESSION OF PROTRH PEPTIDES IN CULTURED ANTERIOR PITUITARY CELLS.
T.O. Bruhn, T.G. Bolduc*, D.B. MacLean and I.M.D. Jackson. Div. Endocrinology, Brown Univ./ R I Hospital, Providence, RI 02903.

We have recently demonstrated that thyrotropin-releasing hormone (TRH) and another ProTRH peptide, $PreProTRH_{25-50}$, are synthesized by anterior pituitary (AP) cells kept in monolayer culture for at least 7 days. In contrast, TRH was undetectable during the first 3 days of culture or when AP tissue was directly extracted following removal. The objective of this study was to determine whether the synthesis of TRH by cultured AP cells is influenced by gender. Pituitaries from male (m) and female (f) 15 day old rats were separately dispersed and cultured for up to 18 days. Levels of TRH in "female" cultures were 736 ± 40 compared with 2741 ± 165 fmol/well ($p<0.01$) in "male" cultures. Marked differences in TSH and GH (m 1.8 and 2.2 x greater than f, $p<0.01$) and LH and FSH contents (f 1.4 and 1.5 x greater than m, $p<0.05$) but not PRL and ACTH were evident at 3 days in culture. Sex differences in GH contents (m 1.5 x greater than f, $p<0.05$) persisted throughout the time course of culture while TSH, LH, FSH, PRL, ACTH contents were not significantly different at 18 days. Immunohistochemical studies using antisera directed against 4 different epitopes of the ProTRH molecule revealed positive staining in approximately 10% of the cell population. While these cells had the appearance of gonadotrophs, thyrotrophs or somatotrophs, further studies are needed to determine the cell type expressing ProTRH peptides. In conclusion, we have found a marked sex difference in TRH expression by cultured AP cells. We are currently exploring this further by investigating the role of gonadal steroids administered *in vivo* on subsequent TRH expression by AP cells *in vitro*.

144.7

GALANIN (GAL) CONTROL OF PROLACTIN (PRL) RELEASE IN THE RAT.
V. Rettori*, A.G.Reznikov and S.M. McCann. Department of Physiology, UT Southwestern Medical Center, Dallas, TX 75235-9040.

The action of GAL injected intraventricularly (3V) was compared in ovariectomized (OVX) versus OVX EB-primed (P) (50 μg estradiol benzoate (EB) s.c. 72 hrs before) conscious rats. GAL induced a release of PRL in OVX animals; however, in the EB-P rats it had no effect. GAL had no effect on PRL release from hemipituitaries (APs) of castrated males, but the response to TRH (25 ng/ml) was augmented. GAL (10^{-10}-10^{-6}M) had a suppressive effect on AP PRL release in females, but enhanced increased release from TRH. In APs of EB-P females basal release of PRL was suppressed by 10^{-8}-10^{-6}M GAL. The dramatically enhanced response of EB-P APs to TRH was not altered by GAL. In the OVX rat GAL stimulates PRL release by hypothalamic action since GAL suppressed release of PRL by APs incubated in vitro. The lack of response to GAL in the EB-P animals may be via high levels in the AP causing lack of response to the small quantity of GAL injected. 3V injection of GAL antiserum had no effect in OVX rats, but augmented the surge of PRL in OVX-EB+progesterone-primed animals indicating a physiologically significant inhibitory effect on PRL release. (Supported by HD09988 and DK10073.)

144.8

SMS 201-995 INHIBITS ESTROGEN-INDUCED PITUITARY TUMOR FORMATION: EFFECTS ON GALANIN AND PROLACTIN
J.F. Hyde, Dept. of Anatomy and Neurobiology, University of Kentucky College of Medicine, Lexington, KY 40536.

Estrogen (E) induces prolactinoma formation in Fischer 344 rats. In addition to its effects on prolactin (PRL), E also increases galanin gene expression within the anterior pituitary (AP). We have shown that galanin and PRL secretion from AP cells are inhibited by somatostatin. The objectives of this study were to examine if the somatostatin analog SMS 201-995 (Sandostatin) affects 1) E-induced pituitary tumor growth, and 2) galanin or PRL levels in the AP. Ovariectomized F344 rats (n=4-5/group) were implanted with 17β-estradiol-containing or empty capsules (sc), and Alzet osmotic pumps containing SMS 201-995 (Sandoz; 1.5 mg/pump) or saline. After 2 weeks, the AP were analyzed for hormone content by RIA. E treatment increased AP size 2.5-fold, and this effect was completely antagonized by SMS 201-995. E increased plasma PRL levels compared to controls (794.5 ± 56.0 vs. 15.6 ± 1.5 ng/ml). SMS 201-995 reduced the elevated PRL levels (32.6 ± 2.7 ng/ml). Galanin levels in the AP were increased by E (310.3 ± 28.4 vs. controls 1.9 ± 0.1 ng/mg protein). SMS 201-995 alone decreased galanin levels in the AP (1.3 ± 0.1 ng/mg protein). Galanin levels were increased with E plus SMS 201-995 (406.4 ± 43.6 ng/mg protein). AP PRL levels paralleled galanin content. Conclusions: SMS 201-995 1) prevents E-induced pituitary tumors in F344 rats, and 2) increases the AP contents of galanin and PRL in E-exposed rats, possibly by inhibiting hormone release. These studies suggest that SMS 201-995 may be useful in the treatment of some prolactinomas. (Supported by American Cancer Society #IN-163)

144.9

COMPARISON OF BIOLOGICAL ACTIVITIES OF ENDOTHELIN-1, -3, SARAFOTOXIN S6b AND S6c IN RAT PITUITARY CELL CULTURE. B. Kanyicska and M.E. Freeman. Dept. of Biol.Sci., Florida State Univ., Tallahassee, FL 32306.

Natural isopeptides of the endothelin/sarafotoxin peptide family were used to study structure-activity relationship and to characterize the endothelin receptors in the pituitary gland. In order to establish the time- and concentration dependency of the responses of pituitary cells in culture, the effect of endothelins (ET-1, ET-3) and sarafotoxins (S6b and S6c) on anterior pituitary hormone secretion was investigated over a wide range of concentrations (from 10^{-15} to 10^{-6} M) and incubation times (from 1 to 48 hours). ET-1, ET-3, S6b and S6c elicited an inhibition of prolactin (PRL) secretion and stimulated the release of luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) from primary monolayer cultures of anterior pituitary cells obtained from female rats. None of the peptides effected growth hormone (GH) secretion, regardless of concentration and incubation period tested. The incubation time neccessary to elicit maximal responses varied greatly from 3 h to 48 h, depending either on the peptide applied or on the hormone assayed. The concentration of peptides necessary for the half maximal response (EC_{50}) was calculated using non-linear regression analysis of dose-response curves. ET-1 was the most potent inhibitor of PRL secretion (ED_{50}:0.78 pM) and also the most potent releaser of LH (0.11 nM), FSH (0.45 nM) and TSH (0.6 nM). Interestingly, the relative rank order of potency of ET-like peptides were similar for all four pituitary hormones: ET-1>S6b>ET-3>>>S6c. This suggests, that pituitary lactotroph, gonadotroph and thyrotroph cells possess the same or very similar set of ET-receptor(s). These data also indicate the relative importance of certain structural elements to ensure persistence and/or potency of ET-like peptides. Supported by NIH, NICHHD, HD-11669.

144.10

AROMATIC L-AMINO ACID DECARBOXYLASE (AADC) ACTIVITY IN THE HYPOTHALAMUS (HT) DURING THE ESTROUS CYCLE: EFFECTS OF AGING. P.S. Mohankumar, S. Thyagarajan and S.K. Quadri. Neuroendocrine Research Laboratory, Kansas State University, Manhattan, KS 66506.

A surge in luteinizing hormone (LH) occurs during the afternoon of proestrus (PE) in young (Y; 4-5 months) rats. This LH surge is reduced in amplitude and its onset is delayed in middle-aged (MA; 8-10 months) rats. Several studies have indicated that neurotransmitters, especially the catecholamines (CA), play an important role in the regulation of the LH surge. However, very few studies have examined the activity of the enzymes involved in the synthesis of the CA. In this study, we investigated the activity of AADC, which synthesizes dopamine (DA) from l-dopa, in the medial preoptic area (MPA) and the arcuate nucleus (ARC) of the HT. Groups of Y animals were sacrificed at 1000, 1200, 1400, 1600, 1800 and 2000 hrs on the day of PE. Groups of MA and old (O; 18-22 months) animals were sacrificed at 1400, 1600, 1800 and 2000 hrs on the day of PE and during pseudopregnancy, respectively. The MPA and ARC were punched out from brain sections and stored in 0.32 M sucrose at -70°C. The punches were homogenized at the time of assay, and the homogenates were incubated with the substrate and cofactors at 37°C for 30 min. DA was isolated using a cation exchange resin and quantified using high performance liquid chromatography. In the MPA of Y animals, AADC activity increased significantly from 246.4±37.6 pg/ug protein at 1000 hrs to 533.1±188.4 pg/ug protein at 1400 hrs ($p<0.05$). In MA and O animals, there were no significant changes in AADC activity, though there was a trend to increase at 1600 hrs in MA animals. In the ARC of Y animals, AADC activity was high at 1000 hrs (407.7±114.4 pg/ug protein) and decreased significantly at 2000 hrs (73.8±24.7 pg/ug protein, $p<0.05$). In MA and O animals, AADC activity showed no signifcant changes. These changes in AADC, through its effects on CA synthesis, might contribute to the age-related changes observed in the LH profile. (Supported by NIH grant AG05980)

144.11

STRUCTURE ELUCIDATION OF A PINEAL DECAPEPTIDE WITH ANTIGONADOTROPIC AND DOPAMINE RELEASING ACTIVITY. B. Benson and I. Ebels*. Dept. of Anatomy, Univ. of Arizona, Tucson, AZ 85724.

An antigonadotropic peptide was purified from more than ten kilograms of defatted bovine pineal glands. The peptide was extracted with 0.2 N acetic acid and partially purified by serial ultrafiltration and Sephadex G-25 chromatography. Low M_r peptides were further separated by serial HPLC on semipreparative C-8 columns with aqueous binary and ternary gradient mobile phases consisting of acetonitrile (ACN) and trifluoroacetic acid (TFA). Final isolation was achieved by HPLC on a 1.0 X 25 cm, C-8 60 Å column with ternary gradient mobile phases containing ACN, TFA, methanol and $NH_4C_2H_3C_2$ as ion-pairing reagent. Automated amino acid analysis of the homogenous antigonadotropic peptide yielded Thr, Phe, Pro, Tyr and Ser; automated microsequence analysis revealed the primary structure:

NH_2-Ser-Phe-Pro-Thr-Thr-Lys-Thr-Tyr-Phe-Pro-COOH

When injected intravenously into unanesthetized male rats either the natural or a synthetic decapeptide of identical structure reduced serum levels of LH and PRL, probably via stimulatory effects on hypothalamic dopamine release. (Supported by N.I.H. grant #HD 19521)

TRANSPLANTATION: ANIMAL MODELS OF PARKINSON'S DISEASE I

145.1

EFFECTS OF SUSBTANTIA NIGRA GRAFTS ON THALAMIC NUCLEI AND BASAL GANGLIA OUTPUT. A. Zainos-Rosales*, R.Aguilar-Roblero, J.L.Mendoza-Ramirez* and R. Drucker-Colin. Instituto de Fisiologia Celular, UNAM. Apa. Pos. 70- 600 México, D.F. 04510 México.

Turning behavior induced by apomorphine in rats with a previous unilateral lesion of mesostriatal dopaminergic pathway with 6-OHDA, is reduced by intrastriatal or intraventricular fetal susbtantia nigra grafts (FSNG). Several studies have shown that this type of graft induces a recovery of supersensitive dopamine receptors. No studies have shown a functional influence of the graft beyond the denervated striatum. The aim of this study was to determine the effects of fetal susbtantia nigra grafts on the striatal output nuclei:susbtantia nigra reticulata (SNR),globus pallidus (GP) and entopeduncular nucleus (EP) by means of the 2-deoxyglucose autoradiographic method. Male Wistar rats were lesioned unilaterally with 6-OHDA and turning behavior was tested. These animals received FSNG (15-17 days) and turning was tested again in all animals for 8 weeks. At the end of this period, rats received an intravenous administration of [14C]-2-deoxyglucose (10 uCi/100gr).The rats were divided as follows Group A:intact rats plus saline;Group B:intact rats plus apomorphine; Groupc C: 6-OHDA-lesioned rats plus saline; Group D: 6-OHDA-lesioned rats; Group E:6OHDA-lesioned rats plus FSNG with decrease in turning behavior and Group F:6-OHDA-lesioned rats plus non-dopaminergic graft. The results show an asymmetrical change in glucose consumption in SNR and EP but not in GP,in groups D,E, and F, between left and right sides. However in Group E this asymmetrical change is decreased in SNR because of an enhancement in glucose consumption in the non-lesioned side. This effect was not found in EP in Groups D, E and F.On other hand, we found a bilateral enhancement in the thalamic nuclei (TN) only in Group E (around 50% with regard to Group B).Our results suggest that FSNG has a moderate influence on striatal output nuclei,but has a much greater effect on TN. Lesioning TN has been used as a therapeutic method for parkinsonian patients.This study presents some evidence for the participation of TN in a rodent model of Parkinson's disease.

145.2

EFFECTS OF REFRIGERATION AND TISSUE CULTURE ON DOPAMINE PRODUCTION AND TRANSPLANT SURVIVAL OF FETAL MESENCEPHALIC DOPAMINE CELLS. J.X. Qi, P. Patino, E. Kriek, C. Kruse, C. Hutt, and C.R. Freed, Depts. Med., Pharm., Surg., Univ. Colo. Sch. Med., Denver, CO 80262.

Because of delays between recovery and transplant of fetal dopamine cells into patients with Parkinson's disease, we have studied the transplant survival of fetal cells after short term refrigeration or tissue culture. Effects of 24 refrigeration (8-10 degrees C) in high K+ (30 mM) or high Na+ (150 mM) buffer were examined. Survival in tissue culture was estimated by the accumulation of homovanillic acid (HVA). Results showed that transplants of fresh tissue or tissue refrigerated either in high Na+ or high K+ buffer showed high quality transplants both behaviorally (>95% decrease in ipsilateral circling to methamphetamine 5 mg/kg i.p.) and histologically. Cells held in tissue culture for one week showed less good behavioral effects (72% decrease in methamphetamine circling) and in histologic appearance. Refrigeration in Na+ or K+ buffers prior to tissue culture did not reduce the rate of production of HVA in tissue culture over one week. We conclude that 24 hr refrigeration of mesencephalic dopamine cells does not hurt transplant survival of rat mesencephalic dopamine cells while one week in tissue culture prior to transplant produces lower quality transplants.

145.3

EFFECTS OF NIGRAL GRAFT ON SUBSTANCE P AND ENKEPHALIN EXPRESSION IN THE GLOBUS PALLIDUS AND SUBSTANTIA NIGRA IN 6-OHDA-LESIONED RATS. D. Gaudin, B. Lavoie, A. Parent and P.J. Bédard. Centre de Recherche en Neurobiologie., Hôp. Enfant-Jésus, Depts Pharmacology and Anatomy, Laval Univ., Québec, (QC), CANADA G1K 7P4.

Studies by Young et al, 1986 and more recently by Gerfen et al., 1991, using *in situ* hybridization, have shown that the degeneration of dopaminergic nigrostriatal pathway resulted in a differential modulation of the neuropeptides substance P (SP) and enkephalin (ENK) in the striatum. In this perspective, we have investigated two structures receiving important striatal inputs: the substantia nigra pars reticulata (SNr) and the globus pallidus (GP). The effects of dopaminergic depletion and intrastriatal fetal dopaminergic graft on the expression of SP and ENK have been immunohistochemically studied in the rat bearing a 6-OHDA-lesion. Eight female rats were lesioned with 6-OHDA in the left substantia nigra and 4 untreated animals were used as control. The animals were tested for circling with amphetamine (2.5mg/kg) and apomorphine (0.25mg/kg). Four rats received a striatal graft of 1.5 x 10^6 cells taken from the ventral mesencephalon of 13-14 day old rat embryos. The results analysed by a computarized image-analysis system, showed a decrease of SP in SNr and an increase of ENK in GP in lesioned rats. These changes were normalized in animals which received striatal grafts. [Supported by MRC du CANADA, Network of Excellence on Neural Regeneration and Functional Recovery and FCAR]

145.4

NORMALIZATION OF SPONTANEOUS ACTIVITY OF SUBSTANTIA NIGRA PARS RETICULATA CELLS, MODIFIED AFTER 6-OHDA-LESION, BY NIGRAL GRAFTS INTO THE STRIATUM. L. Tremblay, D. Gaudin., H. Richard* and P.J. Bédard. Centre de Recherche en Neurobiologie, Hôp. Enfant-Jésus, Depts Pharmacology and Physiology, Laval Univ., Québec, (QC), CANADA G1K 7P4.

The results of several studies have shown that the interruption of the nigrostriatal pathway causes an increase of firing rate of the substantia nigra pars reticulata (SNr) cells. Recently, we have shown that the intrastriatal graft of fetal dopaminergic cells corrected the 6-OHDA-induced changes of D-1 receptors that appear to be localized on striatonigral neurons. This study was performed to evaluate the effects of a graft implanted into the striatum on neuronal activity of SNr cells modified by the degeneration. Female rats were lesioned with 6-OHDA in the left substantia nigra. The animals were tested for circling with amphetamine (2.5mg/kg) and apomorphine (0.25mg/kg). The rats which displayed circling with these two drugs were then divided into two groups. One group received a graft of 1.5 x 10^6 cells taken from the ventral mesencephalon of 13-14 day old rat embryos. The other group was not grafted. 6 months after the graft we performed single cell recordings of nigral reticulata neurons under ketamine anesthesia.

The analysis confirmed the increase of the firing rate on the side ipsilateral to the lesion and showed that the frequency is also elevated on the contralateral side. The graft of fetal nigral neurons normalized the firing pattern of the SNr on both sides. This study is the first to demonstrate, with an electrophysiological approach, that nigral grafts placed in the striatum have a transsynaptic action. [Supported by MRC of CANADA, Network of Excellence on Neural Regeneration and Functional Recovery and FCAR].

145.5

MORPHOLOGICAL AND BEHAVIORAL DRAWBACKS OF PHASEOLUS VULGARIS LEUCOAGGLUTININ-LABELING OF A FETAL DOPAMINERGIC CELL SUSPENSION PRIOR TO GRAFTING IN THE DENERVATED RAT CAUDATE-PUTAMEN. H.W.M. Steinbusch, M.J. Dolleman- Van Der Weel* and A. Nijssen*. Dept. Pharmacology, Faculty of Medicine, Free University, Amsterdam, The Netherlands

Unilaterally 6-hydroxydopamine (6-OHDA) lesioned rats were allocated into three groups, which all received an injection of a fetal dopaminergic cell suspension in the denervated caudate-putamen. The first group (T-Pha) received dopaminergic cells, which were incubated with Phaseolus vulgaris leucoagglutinin, the second group (T-saline) received cells submitted to the same incubation procedure, but without Pha-L and the third group (T) received simply dissociated cells. Upon 8 weeks survival morphological analysis showed a punctate Pha-L staining of fiber particles and some pale Pha-L stained spots, presumably cell bodies, inside the grafts of group T-Pha. Pha-L labeled grafts were significantly decreased in graft volume and contained markedly less dopamine-immunoreactive (DA_i) cells in comparison to the grafts of groups T-saline and T. The ratio DA_i cell type I (cell with $3 \leq$ processes) / DA_i cell type II (cell with ≥ 4 processes) was in group T-saline and group T approximately 8 and in group T-Pha 3. Thus, the primary effect of Pha-L was a selective neurotoxicity to DA_i cell type I neurons. Concerning our behavioral data the labeled grafts did not cause a recovery from lesion induced motor asymmetries in the d-amphetamine induced rotations. On the contrary, upon 7 weeks survival an increased number of amphetamine induced rotations was found in group T-Pha while in group T-saline and group T a (restricted) decrease of rotational behavior occurred. In apomorphine induced rotations there was no difference between the three groups. In conclusion, Pha-L can not be used successfully as a marker for host-graft interactions, using fetal dopaminergic cells in long term survival experiments.

145.6

ADRENAL MEDULLA GRAFTS RESTORE STRIATAL SYMMETRY. E.I. Curran and I.B. Becker. Neuroscience Program and Department of Psychology, The University of Michigan, Ann Arbor, MI 48109.

Intraventricular adrenal medulla grafts decrease the rotational behavior induced by amphetamine (AMPH) or apomorphine (APO). We have previously shown that adrenal medulla grafts differentially effect these two behaviors. Some animals express a decrease in both AMPH- and APO-induced turning, but others exhibit a decreased response exclusively to AMPH or APO. One mechanism that may distinguish animals that express a decreased behavioral response to AMPH from those that show a decreased response to APO may involve a decrease in the asymmetry in DA release between the intact and the denervated striatum. To examine this hypothesis, we used *in vivo* microdialysis techniques to determine basal and AMPH-stimulated DA release within the intact and the denervated striatum of rats with adrenal medulla grafts that resulted in a decrease in AMPH-induced turning, APO-induced turning, or were noneffective.

The symmetry in AMPH-stimulated DA release is restored in animals with a graft-induced decrease in AMPH-induced turning. This effect is significantly greater than that observed in rats with adrenal medulla grafts that decreased APO-induced turning and animals with no recovery. This increase in striatal symmetry was associated with significantly higher basal and AMPH-stimulated DA release within the denervated striatum. These results suggest that: (1) A decrease in AMPH-induced turning involves changes in extracellular DA concentrations within the denervated striatum that contribute to an increase in striatal symmetry, and (2) Graft-induced recovery in the behavioral response to APO does not appear to involve this mechanism. (Supported by NS22157).

145.7

PERIPHERAL NERVE SEGMENTS PROVIDE A SUBSTRATUM FOR RELIABLE AND EXTENDED SURVIVAL OF ADRENAL MEDULLA TRANSPLANTS IN THE BRAIN. L.C. Doering. Department of Biomedical Sciences, Division of Anatomy, McMaster University, Hamilton, Ontario, CANADA L8N 3Z5.

Solid grafts of the adrenal medulla (AM) can survive for up to 1 year in the sciatic nerves of adult rats. This finding prompted the intracerebral grafting of peripheral nerves as a local environment to promote the survival of chromaffin cells within the CNS.

Small aspiration cavities were initially made over the striatum in adult Lewis rats. After 2-4 weeks a 1.0 cm length of sciatic nerve was inserted into the cavity. After an additional 2-4 weeks, grafts (0.5-1.0 mm^3) of adult AM were finally implanted into the core of the nerves.

The peripheral nerve-AM complexes were studied by electron microscopy and immunohistochemistry. Antibodies against tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) were used to identify the AM, and Schwann cells were labeled with the antibodies 192-IgG and Rat-401. All the AM transplants consisted of 1.0-2.0 x 10^3 chromaffin cells positive for TH and DBH after 6 months of survival (longest time point studied).

In conclusion, intracerebral sciatic nerve segments provide an excellent tissue environment for the consistent long term viability of chromaffin cells and open new avenues to truly examine the efficacy of the AM transplant. **(Supported by The Parkinson Foundation of Canada)**

145.8

Recovery in hemiparkinsonian rats after intracaudal implantation of IL-1 containing pellets. J.Wang*, R.J.Plunkett*, J.G.Sheng*, E.H. Oldfield* and K.S. Bankiewicz. CNS Implantation Unit, Surgical Neurology, NINDS, National Institutes of Health, Bethesda, MD 20892.

Solid tissue implantation and/or surgical trauma to the MPTP-lesioned caudate nucleus in hemiparkinsonian monkeys leads to behavioral improvement, the mechanism of which may be ingrowth of remaining host dopaminergic fibers. The mechanism for stimulation of such fiber growth is under investigation. One possibility is a secretory product of microglia cells which are present at the site of implant or trauma. In a previous experiment we demonstrated that neonatal microglia, which release interleukins, when implanted into 6-OHDA-lesioned hemiparkinsonian rats leads to behavioral recovery which is associated with the sprouting of host dopaminergic fibers. In the current experiment, slow release polymer pellets loaded with IL-1 were stereotaxically implanted into the lesioned caudate of hemiparkinsonian rats.

27 ganglionectomized male Sprague-Dawley rats were rendered hemiparkinsonian either by intra-nigral (partially lesioned-PL) or intra-bundle (totally lesioned-TL) injections of 6-OHDA. 6 groups were studied: unimplanted PL (n=5) and TL (n=4) rats, placebo implanted PL (n=4) and TL (n=4) rats, IL-1 implanted PL (n=4) and TL (n=6) rats. Amphetamine-induced rotation was tested weekly for 4 weeks after the lesion and 8 weeks after implantation.

There was no change of turning in any of the studied groups except the IL-1-implanted PL rats. In this group turning was reduced by 85% ($p<0.001$) between 4-8 weeks after implantation. In all animals with decreased turning there were tyrosine hydroxylase immuno-positive fibers in the medial and lateral caudate. We also observed significant gliosis around the implanted pellets, as well as in the entire caudate. Biochemical analysis of the implanted and sprouted areas revealed increased tissue levels of dopamine on medial part of the caudate nucleus only in the IL-1 implanted PL rats. Secretory products (trophic factors?) that are released from the host IL-1-stimulated astrocytes may be responsible for dopaminergic fiber sprouting and behavioral recovery.

145.9

A REAL-COLOR AND DIGITAL IMAGE ANALYSIS OF XENOIMPLANTED RABBIT DOPAMINERGIC NEURONS. J.J. López-Lozano and B. Brera. Neurobiology Lab., Dept. of Exp. Surgery. Clínica Puerta de Hierro, 28035 Madrid, Spain

In earlier works, we have shown that for rabbit ventral mesencephalic neurons (RVMN) of different postconceptional ages to survive in the unilaterally denervated striatum of adult rats, the animals need to be immunosuppressed. Failure in this respect (ie, via administration or individual variability in CyA levels) or lack of immunosuppression cause the implanted cells to be rejected, onset of which occurs one week later. In contrast, RVMN implanted into the striatum of rats with therapeutic CyA levels were able to survive for long periods of time (6 mo), integrate and develop to a mature TH-phenotype. As an extension of these studies, this report applies image analysis to assess and quantify morphologically and morphometrically the implanted cells and their relationships with the host. Rabbit embryos of 16-18 days were decapitated and their ventral mesencephalon was denervated by and enzymatic-mechanical procedure as described (Brain Res 1989;86:351). Then, a suspension of cells was stereotaxically injected into 6-OHDA denervated striatum of CyA-treated rats (10 mg/kg ip). At intervals of 1-6 mo, their striata were morphologically and morphometrically analyzed by TH and GFAP-ICC and Nissl-stain under an Olympus microscope interfaced to an IMCO-10 (Kontron)-MIP operative system (Micron). This study will evaluate in depth: 1) the morphological and morphometric values of VMN (area, perimeter, diameter, polarity, type of cells, etc.); 2) quantitative information regarding the a) location, migration and percentage of implanted cells according to the implantation site; b) the measurement of neural projections and the relationship with the host (innervation area, spatial location, etc.). Supported by CACYT 86/0461 and Severo Ochoa-Fundacion Ferrer award to JJLL.

145.10

EFFECTS OF DOPAMINE D1 AND D2 RECEPTOR SELECTIVE AGENTS ON DOPAMINE RELEASE AS MONITORED BY *IN VIVO* VOLTAMMETRY IN INTRASTRIATAL GRAFTS OF DOPAMINERGIC NEURONS.

Moukhles H., Forni C., Salin P., Nieoullon A. and Daszuta A., Neurochemistry Unit., LNF, CNRS, 13402 Marseille, France.

Intrastriatal grafts of fetal tissue containing dopamine (DA) neurons have been shown to induce functional recovery in 6-OHDA lesioned rats. In order to evaluate the integration of the transplanted dopaminergic neurons in the host circuitry, we have investigated the effect of dopamine D1 and D2 receptor selective drugs on DA release measured by in vivo voltammetry, in both grafted striatum and contralateral control striatum. Previous data obtained in the laboratory by using this method have shown that the development of grafted cells could be related to changes in tl e amplitude of the signal given by the multifiber carbon electrode (Forni et al., Expl. Brain Res., 76, 75-87, 1989). In similar conditions, after stabilization of the signal observed between 3 and 7 weeks post-grafting, the effect of the D2 antagonist raclopride, dopamine D1 agonist SKF 38393 and D1 antagonist SCH 23390, were tested following i.p. or s.c. injections in freely moving grafted and normal rats. Raclopride (0.5 mg/Kg) was without effect on DA release, SCH 23390 (0.1 mg/Kg) induced a small (20%) increase, while the opposite effect could be observed in the voltammetric signal following SKF 38393 injection (10mg/Kg). Similar data were obtained from both sides of the grafted animals and in the normal control group. Administration of mixed agonist/antagonist will be used to further examine the possible occurence of a "local" functional "striato-nigral feedback loop" in the grafted striata.

SECOND MESSENGERS IV

146.1

NITRIC OXIDE MEDIATES GLUTAMATE-INDUCED ENDOGENOUS ADP-RIBOSYLATION IN CEREBELLAR GRANULE CELLS J. T. Wroblewski, R. Raulli and E. Costa. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Sch. of Med., Washington, DC 20007.

In primary cultures of cerebellar granule cells the stimulation of ionotropic *N*-methyl-D-aspartate (NMDA)-sensitive glutamate receptors leads to a Ca^{2+}-dependent activation of nitric oxide (NO) synthase and enhanced NO formation. Sodium nitroprusside (SNP) which releases NO has been shown to enhance the activity of endogenous ADP-ribosyltransferases causing in granule cell homogenates the incorporation of ^{32}P label from [^{32}P]NAD into a 45 kD protein band. This effect was also produced in a dose-dependent manner by the direct application of NO to granule cell homogenates confirming that the action of SNP is mediated by NO. The incubation of intact granule cells with glutamate caused a decrease in the ability of SNP to induce ADP-ribosylation in cell homogenates, suggesting that glutamate receptor stimulation may enhance the activity of endogenous ADP-ribosyltransferases. The effect of glutamate was abolished by the NO synthase inhibitor N^G-monomethyl-L-arginine indicating that it is mediated by NO formation. This effect was also inhibited by CPP, the selective antagonist of NMDA-sensitive glutamate receptors. Among other glutamate receptor agonists an even stronger decrease of the radioactive band was obtained with NMDA and a partial decrease with kainate, while AMPA was inactive. In contrast, pretreatmant of granule cells with quisqualate, the agonist of the metabolotropic glutamate receptor caused an enhancement of the ability of SNP to cause ADP-ribosylation in cell homogenates. These results suggest that the activation of glutamate receptors may affect the activity of endogenous ADP-ribosyltransferases resulting in ADP-ribosylation of GTP-binding proteins and thereby in the regulation of neurotransmitter receptor action at the level of recognition site-effector coupling

146.2

NITRIC OXIDE SYNTHASE: MOLECULAR CHARACTERIZATION AND FUNCTIONAL EXPRESSION OF THE CLONED GENE D. S. Bredt, P. M. Hwang, C. Glatt, T. M. Dawson, M. Fotuhi, V. Dawson, C. Lowenstein, C. D. Ferris, R. R. Reed, and S. H. Snyder. Johns Hopkins U. Sch. of Med. Baltimore

Nitric oxide (NO), first identified as endothelial derived relaxing factor, is now recognized as a major neuronal messenger molecule. Nitric oxide is generated in the brain and vasculature from arginine and NADPH by a calcium dependant enzyme. Our discovery that NO production also requires calmodulin enabled us to purify nitric oxide synthase (NOS) to homogeneity. Tryptic peptides of NOS were sequenced and used to isolate the cDNA from rat brain. The clones isolated included a 4.6 kb open reading frame which codes for a 160 kD polypeptide similar to the apparent mass of purified NOS. The carboxy-terminal half of the amino acid sequence shares high similarity with cytochrome P450 reductase, while the amino-terminal half is unlike any known protein. The cDNA codes for functional NOS as expression in kidney cells yield high levels of NOS protein and enzyme activity. Mapping of NOS protein and mRNA throughout the body reveals discrete neuronal localizations and an absolute overlap with neuronal NADPH diaphorase. Cells transfected with NOS cDNA histochemically stain for NADPH diaphorase establishing that NOS catalytic activity accounts for diaphorase staining of neurons in brain. NADPH diaphorase positive neurons are uniquely resistant to degenerative, ischemic, and excitotoxic insults. Transfection of NOS cDNA into neuronal cells should help clarify the role of NO as a mediator of neurotoxicity.

146.3

INHIBITION OF *N*-METHYL-D-ASPARTATE-INDUCED CALCIUM INFLUX IN CEREBELLAR NEURONS BY SODIUM NITROPRUSSIDE IS NOT MEDIATED BY NITRIC OXIDE. L. Kiedrowski, E. Costa and J. T. Wroblewski. Fidia-Georgetown Institute for the Neurosciences, Georgetown University School of Medicine, Washington, DC 20007.

In primary cultures of cerebellar granule cells sodium nitroprusside (SNP) acting through the release of nitric oxide (NO) stimulates guanylate cyclase and causes cGMP accumulation. SNP also inhibited, in a dose-dependent manner (IC_{50}= 8.2 μM), the $^{45}Ca^{2+}$ influx enhanced by *N*-methyl-D-aspartate (NMDA). This SNP effect was time-dependent, reached a maximum if SNP was added 10 min prior to NMDA, and disappeared when measured 10 min after SNP withdrawal. SNP also inhibited the NMDA-induced NO synthase activation, as measured by the conversion of [^{3}H]arginine to [^{3}H]citrulline. However, SNP failed to inhibit NO synthase activation induced by the Ca^{2+} ionophore A23187 suggesting that SNP had no direct effects on NO synthase activity but decreased Ca^{2+} availability to this Ca^{2+}/calmodulin-dependent synthase. The action of SNP was selective for $^{45}Ca^{2+}$ influx induced by the activation of NMDA receptors and failed to decrease the action of kainate. SNP also failed to inhibit the rate of $^{45}Ca^{2+}$ efflux from preloaded cells suggesting that its action is due to decreased Ca^{2+} entry, rather than enhanced Ca^{2+} extrusion. The fact that SNP was more potent and less rapid in inhibiting $^{45}Ca^{2+}$ influx than in producing the NO-mediated stimulation of cGMP formation suggested that its action may be produced not by the released NO but by $[Fe(CN)_5NO]^{2-}$ or $[Fe(CN)_5H_2O]^{2-}$ ions. In fact, the photolysis of SNP yielded a compound that inhibited NMDA-induced $^{45}Ca^{2+}$ influx but failed to enhance cGMP formation. The inhibitory action of SNP was also mimicked by $[Fe(CN)_6]^{4-}$ but not by $[Fe(CN)_6]^{3-}$, $[CN]^-$, Fe^{2+}, Fe^{3+} or $[NO_2]^-$ ions.

146.4

PLATELET-ACTIVATING FACTOR IS A MEDIATOR OF *fos* EXPRESSION INDUCED BY A SINGLE SEIZURE IN RAT HIPPOCAMPUS. **V.L. Marcheselli, J.P. Doucet,* and N.G. Bazan. LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.**

Electroconvulsive shock (ECS) and convulsant drugs (e.g. pentylenetetrazol) induce a rapid increase of *fos* expression in brain. At the same time, a rapid activation of phospholipase A_2 occurs (*BBA* 218:1, 1970), and platelet-activating factor (PAF), as well as free polyunsaturated fatty acids, accumulate. We have shown that a PAF antagonist, BN-50730, injected intraperitoneally, partially inhibits the *fos* mRNA expression induced by ECS (*Soc Neurosci Abs* 16:629, 1990). To ensure access of the PAF receptor antagonist to the brain, we injected, 30 min prior to the experiment, BN-50730 solubilized in DMSO (2.5 mg/270-300 g body weight rats) intracerebroventricularly. Under these conditions we found 90% inhibition ($p<0.001$, n=6) of *fos* mRNA induction in hippocampus by single ECS. Control animals were rats similarly injected with vehicle alone or sham-operated ones. The accessibility of the drug to the sites of action may play a role in this effect. BN-50730 is a potent PAF antagonist of the high-affinity intracellular binding sites (*J Biol Chem* 265:9140, 1990) but not of the sites located on the synaptic plasma membranes (*Trans Am Soc Neurochem* 22:187, 1991). BN-52021, a weak noncompetitive PAF antagonist of intracellular binding sites, is ineffective in decreasing ECS-stimulated *fos* expression. These results suggest that a) PAF *in vivo* may play a role as a mediator of c-*fos* expression in brain, and b) that intracellular PAF-binding sites may be involved in the activation of immediate-early gene expression during seizures. **Supported by NINDS NS23002.**

146.5

WITHDRAWN

146.6

SEROTONIN TRANSLOCATES Ca^{2+}-ACTIVATED PKCS WITHOUT TRANSLOCATING Ca^{2+}-INDEPENDENT PKCS IN THE NERVOUS SYSTEM OF *APLYSIA*. W. S. Sossin and J. H. Schwartz. Center for Neurobiology and Behavior, HHMI, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

Ca^{2+}-activated and Ca^{2+}-independent PKCs are present in the nervous system of the marine mollusk *Aplysia californica* (Kruger et al., J. Neurosci. in press). Sensitizing stimuli or application of the facilitatory transmitter, serotonin, to intact isolated ganglia produce the presynaptic facilitation of sensory-to-motor neuron synapses that underlies behavioral sensitization, which is a simple form of learning. Activation of PKC can also produce this presynaptic facilitation (Braha et al., *Proc Natl Acad. of Sci.* **87**, 2040-2044 1990). To determine which type of PKC is activated, we developed a sensitive and selective assay to measure both Ca^{2+}-activated and Ca^{2+}-independent PKC activities in crude supernatant and membrane fractions of nervous tissue. This assay is based on the specific binding of the Ca^{2+}-activated PKCs to phosphatidylserine micelles in the presence of Ca^{2+} and makes use of a novel synthetic peptide with sequences conforming to phylogenetically conserved pseudosubstrate regions of the Ca^{2+}-independent kinases. We provide evidence that the presynaptic facilitation is produced by a Ca^{2+}-activated isoform: application of serotonin increases the amount of the Ca^{2+}-activated PKC translocated to membrane. Under these conditions, no Ca^{2+}-independent kinase activity is translocated.

146.7

DISTRIBUTION OF PROTEIN KINASE Cδ IN THE RAT BRAIN. I. Merchenthaler, A. Negro-Vilar and W. Wetsel. LMIN, NIEHS, NIH, Research Triangle Park, NC 27709

Protein Kinase C (PKC) represents a family of isoenzymes that consists of at least nine members with clearly related but distinct structures. PKCα,-β_1,-β_2, and -τ are characterized by stringent dependencies on Ca^{2+}, phospholipid and diacylglycerol. By contrast, PKCδ,-ε,-ε',-ζ and -η exhibit Ca^{2+}-independent activities. We have selected the PKCδ isoenzyme for these studies and report, for the first time, its distribution in the rat brain. When vibratome sections are stained with polyclonal PKCδ antisera and the PAP technique, we have found that, while PKCδ is distributed throughout the brain, it is confined to specific nuclei and fiber tracts. **In the telencephalon**, PKCδ-immunopositive (PKCδ-i) perikarya were present in the lateral septum, the central nucleus of the amygdala, the diagonal band, and the basal caudate-putamen. **In the diencephalon**, the majority of PKCδ-i perikarya were seen in the thalamus and the medial geniculate bodies. **In the hypothalamus**, the magnocellular subdivisions of the hypothalamic paraventricular nucleus, the supraoptic nucleus, and the accessory magnocellular nuclei were distinctly stained. Perikarya in the arcuate and periventricular preoptic nuclei were lightly stained. **In the brainstem**, immunopositive perikarya were observed in the substantia nigra, the medial vestibular nuclei, the inferior cerebellar peduncle, the inferior olive, and the nucleus of the solitary tract. **In the cerebellum**, some of the Purkinje cells appeared to be immunopositive, and some of them seemed to be heavily innervated by PKCδ-i climbing fibers. PKC-i fibers and nerve terminals were seen in the cerebral cortex, the lateral septum, the internal zone of the median eminence and the neural lobe, the inferior cerebellar peduncle, and the cerebellar nuclei. These morphological observations indicate that PKCδ is associated with a variety of functions in the central nervous system.

146.8

CALCIUM INDUCED CALICIUM RELEASE IS DEMONSTRATED IN RAT AND CHICKEN BRAIN MICROSOMES. David J. Hirsch, Ajay Verma, and Solomon H. Snyder. Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Using autoradiographic techniques, we have shown that endoplasmic reticular (ER) Ca2+ is selectively concentrated in different brain areas. Release of Ca2+ by IP3 also varies markedly in different brain regions (1). Regulation of the ER Ca2+ pools resistant to IP3 in different brain regions is unclear. In the present study we directly demonstrate Ca2+ induced Ca2+ release (CICR) in rat and chicken brain microsomes. To demonstrate CICR in rat brain microsomes we loaded ER Ca2+ pools over a range of free Ca2+ concentrations. In rat brain microsomes 45Ca2+ accumulation increases with elevated Ca2+ levels plateauing between 1-10 uM Ca2+ with half maximal 45Ca2+ uptake being apparent at 0.25 uM. ER 45Ca2+ uptake was reduced between 10-100 uM free Ca2+ resulting in approximately three fold less uptake at 100 uM than at 1 uM. Reduced net accumulation of 45Ca2+ at the higher free Ca2+ levels appears to reflect opening of the Ca2+ induced Ca2+ release (CICR) channel as it was sensitive to inhibitors of CICR (2). Thus, this release is prevented by 5 mM magnesium and the local anaesthetics tetracaine and procaine. The maximal effect of tetracaine is observed at approximately 3 mM. Adenine nucleotides, on the other hand, stimulate CICR. 10 mM ATP allows release of Ca2+ to be triggered by lower free Ca2+ concentrations.

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146.9

FLUCTUATION OF RAT BRAIN CA2+ POOLS IN KAINIC ACID INDUCED LIMBIC SEIZURE STATUS. Ajay Verma, David J. Hirsch, Ted M. Dawson, Charles Glatt, and Solomon H. Snyder. Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Microinjection of kainic acid into a unilateral amygdala in rats elicits limbic seizure status involving predominantly unilateral limbic structures. Such seizure activity markedly increases Ca2+ influx (1) and local cerebral glucose utilization (2) in limbic structures on the kainic acid-injected side. Elevated metabolic demand and endoplasmic reticulum (ER) modifications (3) may reflect neuronal efforts to correct excessive cytoplasmic Ca2+ levels via calcium ATPases. Using in vitro 45Ca2+ autoradiography, we have examined ATP dependent ER Ca2+ uptake in fresh-frozen 20 uM sections of rat brain during limbic seizure status. Two hours after kainic acid injection into the left amygdala ER 45Ca2+ uptake was markedly stimulated in the ipsilateral amygdala, cerebral cortex, hippocampus, and discrete thalamic nuclei as well as the contralateral cerebellar hemisphere and vermis. This pattern reflects the activation of neuronal structures involved in seizure propagation. Mechanisms underlying modulation of these calcium pools, as well as their pharmacological and 2nd messenger sensitivity will be presented.

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146.10

ISOLATION OF A FRACTION OF ENDOPLASMIC RETICULUM (ER) FROM BOVINE ADRENAL MEDULLA, HIGHLY ENRICHED IN A THAPSIGARGIN-SENSITIVE 45-CALCIUM UPTAKE ACTIVITY. D.Mathiasen*, L.M.Røssum* and M.Treiman. Institute of Medical Physiology C, Biotechnology Center for Signal Peptide Research, Panum Institute, Blegdamsvej 3C, DK-2200 N, Denmark.

Regulation of the intracellular calcium (Ca) in excitable cells is in part determined by energy-dependent Ca uptake into non-mitochondrial Ca-stores. In order to study the properties of this Ca uptake, a fraction of ER from bovine adrenal medulla was isolated with only minor contamination by plasma membranes, mitochondria and chromaffin granules. Following centrifugation at 380g x 20min, the supernatant was centrifuged at 28,000g x 20min, and the resulting supernatant at 105,000 g x 1h. The microsomal pellet, enriched in cytochrome C-reductase (RSA=4.7), was further fractionated on a sucrose density gradient (15-30%), 95,000g x 16h. A light fraction (15-16% sucrose) was identified containing 4.6% of the total gradient protein, and 2.6%, 0.6% and 0% of marker activities for ER, plasma membranes and mitochondria, respectively. ATP-dependent 45-Ca uptake activity in this fraction was 4-5 nmoles/mg protein (plateau at 30min) at 50nM free Ca. 80% of this uptake was inhibited by thapsigargin 100nM.

146.11

VASOPRESSIN-INDUCED INTRACELLULAR CALCIUM RESPONSES IN SINGLE RAT CORTICOTROPES. B.J.Canny* and D.A.Leong, Dept. of Int. Med., University of Virginia, Charlottesville VA 22908.

In a previous study we have shown that increasing concentrations of vasopressin (AVP) increase the number of cells that secrete corticotropin (ACTH) while having little effect on the amount of ACTH secreted by each cell. Since the cytoplasmic free calcium ion concentration ($[Ca^{2+}]_i$) is important in stimulus-secretion coupling in corticotropes, we have used fura-2 videomicroscopy to examine the role of $[Ca^{2+}]_i$ in regulating this phenomenon in single rat corticotropes post-identified using a specific ACTH reverse hemolytic plaque assay. Three distinct $[Ca^{2+}]_i$ profiles were observed in response to a range of AVP concentrations; (i) no change in $[Ca^{2+}]_i$, (ii) a low amplitude increase in $[Ca^{2+}]_i$ (step increase), and (iii) a high amplitude increase in $[Ca^{2+}]_i$ followed by a fall a to new level above baseline (spike/plateau response). The number of corticotropes exhibiting each of the 3 responses to increasing concentrations of AVP is shown below.

[AVP]	No change	Step	Spike/Plateau
10^{-12} M	6	4	-
10^{-10} M	-	4	4
10^{-8} M	1	-	6

With increasing AVP concentrations the proportion of cells responding with a spike/plateau of $[Ca^{2+}]_i$ increased; this was associated with a corresponding decrease in the proportion demonstrating either no change or a step increase. As the secretion of ACTH from a single corticotrope in response to AVP is either on or off (i.e. the amount of ACTH secreted from each cell is not a function of AVP concentration), we propose that secretion may be associated with spike/plateau responses and not step increases in $[Ca^{2+}]_i$.

ALZHEIMER'S DISEASE: NEUROPATHOLOGY I

147.1

SELECTIVE DISTRIBUTION OF ALZHEIMER'S DISEASE LESIONS IN THE VISUAL CORTEX. R.K. Tikoo and R.O. Kuljis. Department of Neurology, The University of Iowa College of Medicine, Iowa City, Iowa 52242-1053.

In Alzheimer's disease (AD), lesions in the primary visual cortex consist primarily of senile plaques (SP) and amyloid angiopathy (AA). Virtually nothing is known, however, about the mechanisms leading to these lesions, and about their effect - if any - on cortical function. In order to begin to address these issues, we analyzed their three-dimensional spatial distribution to test several hypotheses on their role in the pathophysiology of AD. Series of transverse and tangential sections of the primary visual (striate) cortex from five histologically confirmed patients with Alzheimer's disease were stained using the Nissl, thioflavin-S and Bielschowsky methods. Over 130,000 SP were arbitrarily classified into 4 types: "diffuse," "classical," "amyloid (core)" and "(predominantly) neuritic." Plaques and AA were then plotted in each section and their distribution reconstructed in three dimensions using computers. There are several clearcut, consistent patterns to the distribution of SP and AA. Among the most striking: AA appears to have a predilection for layer IVC in all sections examined. A high density of SP was noted in layers II-IVA and in the boundary between layers IVC and V. Plaque density was assessed: (a) per type of lesion (all layers); diffuse=45%, classic=15%, amyloid=2.6%, neuritic=36%; (b) per layer (all types); I=0.2%, II-IVA=23%, IVB=1%, IVC=23%, V=1%, VI=17%. A detailed breakdown per type of lesion and layer will be presented, together with three-dimensional reconstructions and analyses of the spatial distribution of these lesions. Our findings indicate that AD lesions have a predilection for well-defined compartments in the striate cortex. These patterns of distribution implicate cortico-cortical connections and local neuronal circuits, but not thalamo-cortical connections in the pathophysiology of AD.

147.2

ABNORMALITIES IN OLFACTORY BIOPSIES OF ALZHEIMER'S PATIENTS. Edward W. Johnson*, Bruce W. Jafek*, Pamela M. Eller*, Christopher M. Filley*, Mary Chapman*, and John C. Kinnamon, Rocky Mountain Taste & Smell Center and UCHSC, Denver, CO 80262.

Abnormalities in human olfactory epithelium can be associated with certain pathological states which involve loss of smell. In light of this and in conjunction with a project in the Depts. of Neurology and Psychiatry at the Univ. of Colorado Health Sciences Center involving patients with Alzheimer's Disease (AD), we have tested and biopsied eight AD patients and two age-matched controls. So far all of the AD patients have tested hyposmic or anosmic. Biopsies from three of these patients have shown that the olfactory epithelium had an abnormal appearance reminiscent of dysfunctional epithelium. Concentrated above the olfactory tissue were scattered particles. Using a Kevex 8000 X-ray Microanalytical Unit in conjunction with a JEOL 2000EX Electron Microscope, we determined that these particles were silicon crystals. In this preliminary screening these crystals were not observed over small areas of the respiratory epithelium of these patients. At the light microscopic level no similar particulate matter has been seen over the nasal epithelia of control subjects. Efforts are continuing to test and obtain biopsies from additional AD patients and age- and environment-matched controls to determine if there are consistent changes in the ultrastructure of the olfactory epithelium of AD patients and to see if these preliminary findings of silicon crystals over the olfactory epithelium of only the AD patients is a consistent observation.

147.3

TANGLE-ASSOCIATED NEURITIC CLUSTERS (TANCs): A NEW LESION IN ALZHEIMER'S DISEASE (AD) AND AGING. D.G. Munoz & D. Wang. Department of Pathology, University of Western Ontario, London, Canada.

Senile plaques and neurofibrillary tangles (NFT) are the two major tissue lesions in AD. Mature senile plaques epitomize aberrant sprouting, the process of excessive proliferation of neuronal processes. The sprouting-inducing factor is thought by many to be β/A4 protein. Many sprouted, dystrophic neurites in the crown of senile plaques are loaded with chromogranin A_4, a soluble protein of large dense core synaptic vesicles. NFTs have not been associated with neuritic sprouting, but using double label immunocytochemistry we show that some extracellular NFTs are associated with clusters of chromogranin A-labelled dystrophic neurites. These tangle-associated neuritic clusters (TANCs) are present in the hippocampus of all 14 AD patients and in 10 of 23 normal elderly controls, as well as in the nucleus basalis of Meynert of AD brains. We postulate that TANCs represent aberrant sprouting induced by a factor bound to certain extracellular NFTs. Since the tangles in TANCs are no recognized by antibodies to native or synthetic β/A4 peptide, the latter may not be the sprouting-inducing factor, at least in TANCs.

147.4

IMMUNOHISTOCHEMICAL STUDY OF ALZHEIMER PATHOLOGY IN PATIENTS WITH KNOWN SEVERITY AND DURATION OF ILLNESS. P. V. Arriagada, J. H. Growdon, E.T. Hedley-Whyte, B.T.Hyman, Mass General Hospital, Harvard Medical School. Boston, MA 02114.

Our recent analysis of the relation between the number and distribution of neurofibrillary tangles (NFT) and senile plaques (SP) and the severity of cognitive impairment and duration of Alzheimer's disease (AD) suggested a positive correlation between NFT in association cortex and both Blessed Dementia Scale and duration of illness. Surprisingly, no correlation between clinical parameters and SP was found. As an extension of this study, we further examined the brains of 10 individuals with the clinical and pathological diagnosis of AD who had a Blessed Dementia Scale (BDS) recorded within 15 months prior to death. Preparations from the hippocampal formation, entorhinal cortex, amygdala, neocortical areas 8, 9, 20, 21, 17 and 18, and raphe nucleus and locus coeruleus were stained with thioflavine S (thio S) and also immunohistochemically with both Alz-50 (P.Davies, Albert Einstein) and anti-ß /A4 amyloid (D. Schenk, Athena Neuroscience) antibodies. The BDS correlated best with the number of NFT in association cortices (R=0.67, p=0.032). In 2 patients with BDS score less than 30, NFT were restricted mainly to limbic areas and rarely found in neocortical association areas, whereas individuals with a higher (worse) BDS score had increasing numbers of thio S or Alz-50 positive NFT in association cortex. Total SP or regional SP counts (thioS) were not correlated with duration or severity of illness. Neither the number of ß/A4 and Alz-50 positive SP in association cortex (area20) and in hippocampus (CA1) nor the ratio of Alz-50/ßA4 SP in these areas correlated with BDS or duration. Our results suggest that NFT rather than SP are most strongly correlated with clinical symptoms in Alzheimer's disease. Supported by NIA P50 AG05134, AG08487, and the Brookdale Foundation.

147.5

Transmitter specific abnormal neurites are present in amyloid positive plaques prior to the appearance of paired helical filament (PHF) containing processes. W.C.Benzing, E.J. Mufson@, and D.M. Armstrong. FGIN, Georgetown Univ., Washington, D.C. 20007, @Rush Preb., St. Luke Hosp., Chicago, IL 60612.

Immunocytochemical methods were used to examine the relationship between neurotransmitter containing swollen neurites, beta-amyloid deposition, and PHF containing neurites in the amygdala of post-mortem brain tissue from a group of patients with Alzheimer's disease (AD), age matched controls (AMC) and age matched non-demented subjects which had considerable numbers of senile plaques (i.e. high plaque (HPC) controls). The HPC may represent a preclinical stage of AD. Using Thioflavin-S as a histological marker for senile plaques (SP), the amygdala of AD and HPC cases could be divided into regions of low and high plaque density. The regions of low plaque density were characterized by few, if any, SP in HPC and moderate numbers of SP in AD. In contrast, regions of high plaque density contained numerous SP in HPC and an even greater number in AD. In all regions, beta-amyloid immunostaining revealed a much greater number of SP than could be visualized by Thioflavin-S. In the "low plaque" regions of HPC, swollen neurites immunostained with antibodies to various neurotransmitters were observed in many of the beta-amyloid-immunoreactive (BA-IR) plaques. Few if any PHF-immunoreactive (PHF-IR) processes could be found in these regions, except in the most severely affected HPC cases. However, when these same "low plaque" regions were examined in AD cases, both transmitter- and PHF-immunostained swollen processes were found within many BA-IR plaques. High plaque regions of both HPC and AD cases contained dense accumulations of BA-IR plaques, PHF-IR plaques and transmitter containing swollen neurites. These data suggest beta-amyloid deposition and the formation of transmitter-containing swollen neuritic processes occur early in AD and that these events occur prior to the formation of PHF containing SP.

147.6

MAPPING DEGENERATING PROCESS IN ALZHEIMER BRAINS: DEMONSTRATION OF A STRONG HETEROGENEITY IN SPORADIC CASES. P. Vermersch*, B. Frigard*, A. Delacourte; U156 INSERM, 59045 Lille, FRANCE

Alzheimer's disease (AD) pathology is known to be prominent in associative cortical areas and in the hippocampus, but most previous studies have examined histologic characteristics of AD in a limited range of brain regions.

We have described a reliable and early marker of the degenerating process in AD: a triplet of abnormally phosphorylated Tau proteins named Tau 55, 64 and 69, accumulates in neurons of AD brains (Delacourte et al, Acta Neuropathol., 1990, 80:111-117), its presence and distribution being strongly correlated with both symptoms of dementia and distribution of tangles, respectively. Here we present a semi-quantitative evaluation of these proteins in all cortical areas according to Brodmann's classification.

Five patients were diagnosed antemortem as probable AD according to NINCDS-ADRDA criteria and subsequently confirmed at autopsy to have definite AD. Tissue samples from all cortical areas were homogenized in the Laemmli sample buffer (1:10) and heat treated. Proteins were resolved on 10-20% SDS gel gradients, transferred on nitrocellulose and immunodetected with our anti-PHF which specifically detects Tau 55,64,69. For each sample, a semi-quantification of the immunodetection intensity for Tau 55,64,69 was independently performed by three of us (scale from 0 to 10). The final score was the mean of each observer's score for each Brodmann's area. The good level of interobserver agreement was confirmed by Kappa test using the Fleiss'method.

Our study reveals that all Brodmann's areas were implicated in the neurodegenerating process. In all patients, all temporal and most associative areas of the neocortex were dramatically impaired. Primary areas, especially visual areas 17 were relatively well spared. **However, a strong topographical heterogeneity was evident, as reflected by the differential distribution of the neurobiological markers of the disease, respectively to the postero-anterior asymetry.**

147.7

THE EARLIEST SYMPTOMS OF ALZHEIMER DISEASE: ANATOMIC CORRELATES. B.T. Hyman, P.V. Arriagada, A. McKee, J. Ghika, S. Corkin and J.H. Growdon Neurology and Neuropathology, Mass. General Hospital, Boston, MA 02114 and Dept. of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge MA 02139.

An articulate 82 year old physicist, still active in his work, came to the Mass. General Hospital Memory Disorders Unit because he and his family detected declining memory and work performance during the past 9 months. The neurological examination was normal. The Blessed Dementia Scale score was 4 (just outside the normal range). On a short series of cognitive tests, his performance resembled that of age-matched controls. Based on the clinical history, the diagnostic impression was early Alzheimer's disease. The patient died of a myocardial infarction 7 weeks later. Autopsy showed marked Alzheimer neuropathological changes. Forty cytoarchitectural areas were surveyed using thioflavine S, and Alz-50 and anti-ß/A4 immunostains. Neurofibrillary tangles (NFT) were most prominent (>10/mm2) in the entorhinal cortex, hippocampus, amygdala, inferior temporal gyrus, and the dorsal raphe, and were rare in other cortical areas. Senile plaques (SP) were prominent (>20/mm2) in widespread association areas and in the amygdala, and were less numerous in primary sensory areas. Alz-50 positive SP were present mainly in limbic areas, and were far less common than ß/A4 SP.

These results emphasize that Alzheimer patients with extremely mild cognitive impairment may already have substantial neuropathological changes, and that the entorhinal cortex, hippocampus, and amygdala contain NFT early in the disease. We suggest that clinical symptoms emerge as multiple sites of distributed neural systems become affected, and as compensatory mechanisms fail. Supported by the Brookdale Foundation, NIA P50 AG05134, AG08487 and RR-00088. We thank P Davies (Einstein) and D. Schenk (Athena), for Alz-50 and anti-ß/A4 antibodies.

147.8

BRAIN-BEHAVIOR CORRELATIONS FOR AUDITION AND VISION IN A VERY MILD CASE OF ALZHEIMER'S DISEASE (AD). S. Corkin, D.D. Kurylo, R. Dolan, B.T. Hyman, P.V. Arriagada, A. McKee, J. Ghika, and J.H. Growdon. Dept. of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139, and Neurology and Neuropathology Services, Mass. General Hospital, Boston, MA 02114.

It is difficult to specify precisely the relation between behavioral impairments in AD and the underlying neuropathology for two reasons: (1) Test performance in advanced AD is globally affected by the severity of dementia and may not reveal selective deficits, and (2) the interval between last testing and death is usually protracted so that the state of the brain at autopsy differs from that at testing. The present case study was free of these concerns: The patient was only mildly demented, and died 2 days after the last test. The 82-year old man (see abstract by B.T. Hyman et al.) performed auditory tests (sound localization, perception of complex tones, phoneme and timbre discrimination, and tonal memory) and visual tests (texture and color discrimination and flicker and motion detection). At autopsy, we examined auditory and visual cortices, optic tract, LGN, and superior colliculus. For audition, test performance was normal; the number of SPs were ranked as follows: primary auditory cortex <auditory association cortex < multisensory cortex. For vision, test performance was impaired in most conditions. The optic tract and LGN appeared normal and the superior colliculus showed mild gliosis. The number of SPs increased as follows: area 17 < 18, 20, 21 < 38. NFTs were rare in primary auditory and visual cortices but were present in high-order association cortex. Sensitive markers for visual dysfunction clearly demonstrated deficits in this very mild AD patient that were not due to overall cognitive decline. We conclude that visual disorders may appear early in AD in relation to abnormalities of high-order visual areas.

147.9

HUMAN OLFACTORY EPITHELIUM (OE) IN AGING AND DISEASE JQ Trojanowski, PD Newman*, WD Hill, and VM-Y Lee*, Dept. of Path. & Lab. Med., Univ. of Penn. Sch. of Med., Phila., PA 19104-4283.

We characterized human OE cells and dystrophic OE neurites. Keratin 8 was present in each class of OE cell. Sustentacular cells lacked other cell type specific polypeptides, and differed from neurons and basal cells, both of which expressed neural cell adhesion molecules (N-CAMs) and microtubule associated proteins. Basal cells expressed NGF receptors (NGFr) while OE neurons did not. Unlike their perikarya, olfactory axons expressed NGFr, vimentin and GAP-43, but OE neurons and axons were negative for peripherin and neurofilament (NF) proteins. Olfactory nerves differed from other nerves which were positive for all 3 NF subunits and peripherin, in addition to vimentin and GAP-43. Dystrophic OE neurites were GAP-43 positive, connected to olfactory neurons, and contained proteins not found in normal OE nerves. These neurites were present in 11/11 Alzheimer disease patients, 11/14 subjects with other neurodegenerative diseases, 6/8 normal adults, but not in 9/9 fetal and neonatal cases.

The molecular phenotype of different human OE cells is distinct, and dystrophic OE neurites are frequent in adults with and without a neurological disease.

149.10

STAGEING OF ALZHEIMER`S DISEASE: NEUROPATHOLOGICAL CONSIDERATIONS. H.Braak and E.Braak. Dept. of Anatomy, J.W.Goethe University, D-6000 Frankfurt/Main 70, Germany,

Series of sections through entire hemispheres of seventy five autopsy brains from both non-demented individuals and cases of presenile and senile dementia of the Alzheimer type (AD and SDAT) were examined for presence of A4-amyloid deposits and neurofibrillary changes. Differentiation of stages could not be based upon features of the distribution pattern of A4-amyloid deposits. Neurofibrillary tangles and neuropil threads (but not neuritic plaques), in contrast, showed - with increasing severity of affection - a consistent sequence of changes in their pattern of distribution rendering differentiation of six stages of Alzheimer`s disease possible. Recognition of these stages required qualitative evaluation of only a few key preparations. Speed and simplicity turned out to be particular advantages of the proposed morphological stageing of Alzheimer`s disease (supported by the Deutsche Forschungsgemeinschaft).

147.11

TEMPORO-FRONTAL DIFFERENCES IN ALZHEIMER'S DISEASE ASSOCIATED PROTEIN - A BIOCHEMICAL STUDY. R. Ravid, J.J. Van Heerikhuize*, D.F. Swaab*, W. Kamphorst*, H.A. Ghanbari. Netherlands Institute for Brain Research, Amsterdam; Pathological Institute, Free University, Amsterdam, The Netherlands; Abbott Laboratories, Illinois, USA.

Using a recently developed enzyme linked immunoassay (Alz-EIA), levels of Alzheimer's disease associated protein (ADAP) were measured in homogenates of freshly frozen frontal or temporal cortex samples of human brain from thirty rapid autopsies (post mortem interval 2-4 hours). Brain tissue samples included 3 groups of specimens: Alzheimer's disease (AD); controls without neurological or psychiatric disease (NC); dementias other than AD (NAD), i.e. cases of Parkinson's disease and Pick's disease. The AD group had substantial ADAP levels in frontal and temporal lobe specimens, which were significantly higher than either the normal or the NAD group. It is apparent from these data that the ALZ-EIA clearly distinguishes ADAP levels in AD brain from those of controls and other NAD brains. In addition a regional difference was present between frontal and temporal lobe specimens. The biochemical ALZ-EIA offers a rapid, easily performed and quantitative diagnostic test which may serve as a valuable aid to the clinico-pathological diagnosis of Alzheimer disease. We would like to acknowledge the Netherlands Brain Bank for supplying human brain specimens.

NEUROBIOLOGY OF SCHIZOPHRENIA

148.1

BRAIN MORPHOLOGY AND SCHIZOPHRENIA. A. Breier, R.W. Buchanan,* R. Munson,* A. Elkashef,* F. Gellad.* Maryland Psychiatric Research Center, Baltimore, MD 21228

Previous antemortem and postmortem studies have yielded inconsistent data regarding morphologic abnormalities of specific brain structures in schizophrenia. We have used magnetic resonance imaging (MRI) to examine the morphologic characteristics of specific brain regions in 19 healthy volunteers and 44 chronic schizophrenic patients. Schizophrenic patients, in comparison to healthy controls, had significant reductions in right and left amygdala/hippocampus volumes; right prefrontal volume and trend reductions in left prefrontal cortex; and no significant differences in right and left caudate volumes. A secondary analysis revealed reductions in right amygdala, and trend reductions in the left amygdala and left hippocampus. In addition, prefrontal white matter but not gray matter was reduced in the schizophrenic patients. Moreover, schizophrenic right white matter volume was significantly related to right amygdala/hippocampus volume (r=.34, p=.03) which is data that provides preliminary support for a hypothesis of abnormal cortico-limbic connection in schizophrenia. Furthermore, performance on the Stroop color-word conflict test, a neurocognitive task, was significantly related to left (r=.44, p=.01) and right (r=.42, p=.02) amygdala/hippocampus volume in the schizophrenic patients. The implications of these data for the pathophysiology of schizophrenia are discussed.

148.2

ABNORMAL EXPRESSION OF 2 MICROTUBULE-ASSOCIATED PROTEINS IN THE HIPPOCAMPAL FORMATION IN SCHIZOPHRENIA. S.E.ARNOLD, V.M-Y. LEE*, R.E. GUR, J.Q. TROJANOWSKI, University of Pennsylvania School of Medicine, Phila., PA 19104-4283.

Immunohistochemistry with a panel of 15 monoclonal antibodies was used to study the expression of neuronal cytoskeletal proteins in the hippocampal formations of 6 patients with schizophrenia, 6 normal controls and 6 with neurodegenerative disorders. In 5 of the 6 subjects with schizophrenia, prominent and specific alterations were found in the distribution of 2 microtubule-associated proteins (MAPs), MAP2 and MAP5, which were anatomically selective for the subiculum and entorhinal cortex. In contrast, the immunoreactivity of other cytoskeletal proteins (i.e., tau, tubulins and selected neurofilament protein phosphoisoforms) was similar for all subjects. Defects in the expression of MAP2 and MAP5, both of which contribute to the establishment and maintenance of neuronal polarity, could underlie some of the cytoarchitectural abnormalities described in schizophrenia and impair signal transduction in the affected dendrites. The subiculum and entorhinal cortex interconnect the hippocampal formation with widespread cortices and subcortical nuclei and play important roles in higher cognitive functions. Hence, novel pathologic lesions that distort the polarized geometry of neurons could play a role in the emergence of aberrant behavior in schizophrenia.

148.3

INCREASED GABA-A RECEPTOR BINDING IN SUPERFICIAL LAYERS OF SCHIZOPHRENIC CORTEX. F.M. Benes, S.L. Vincent, G. Alsterberg, E.D. Bird, J.P. SanGiovanni. Dept. of Psychiatry and Program in Neuroscience, Harvard Medical School; Mailman Research Center, McLean Hospital, Belmont, MA 02178.

A recent investigation has demonstrated decreased numbers of interneurons in the anterior cingulate and prefrontal areas of post- mortem schizophrenic brain. Because there were reductions of interneurons in layers II-VI, it was hypothesized that the missing neurons might be GABAergic basket cells and that their loss would result in a compensatory upregulation of the GABA-A receptor. To test this hypothesis, a high resolution emulsion technique for localizing the GABA-A receptor was applied to normal (N = 8) and schizophrenic (N = 6) specimens of anterior cingulate cortex. The results of a computer-assisted quantitation of autoradiographic grains indicate a preferential increase of bicuculline-sensitive ^{3}H-muscimol binding on neuronal cells bodies of layers II (84%) and III (74%), but not V and VI, of the schizophrenic cases. There were no differences in the average size of neuronal cell bodies in any of the layers in control and schizophrenic cingulate cortex. The neuropil in layer I also showed significantly greater GABA-A binding in the patient group. The differences in the schizophrenic group did not appear to be due to confounding effects because both young and old schizophrenic cases showed increased GABA-A binding, as did a neuroleptic-naive patient. Since superficial cortical layers mediate cortico-cortical integration, the data reported here are consistent with the possibility that schizophrenia may involve a deficit in inhibitory activity regulating associative information processing in the cingulate region. Supported by MH00423, MH42261 and the Scottish Rite Foundation.

148.4

BORNA DISEASE VIRUS SEROLOGY IN SCHIZOPHRENIA. R. W. Waltrip, R. W. Buchanan,* A. Breier, W. T. Carpenter, A. Summerfelt,* K. M. Carbone. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228.

Preliminary data is presented from an ongoing study comparing Borna disease virus (BDV) seropositive (S+) and seronegative (S-) patients with schizophrenia (Sz). BDV is an incompletely characterized RNA virus that naturally causes an immunologically-mediated mononuclear encephalitis in horses and sheep in central Europe. Experimentally, BDV effects can range from asymptomatic infection through social behavioral abnormalities to fatal meningoencephalitis. Antibody (Ab) studies in humans suggest an association with neuropsychiatric disease with reported rates varying from 2-3% in normals to 6.8% in a mixed psychiatric cohort. We have detected 13(16%) S+ in 81 patients with Sz and 1(5.6%) S+ in 18 normal volunteers using indirect immunofluorescence (IFA). Antibodies from S+ localize to the same intracellular antigen as antibodies from a BDV infected rabbit by double-antibody IFA. A 60kD BDV-specific protein was radioimmunoprecipitated by S+, but not S-, human sera from each of two independently developed persistently BDV infected cell lines. Virus neutralization titers, magnetic resonance imaging (MRI) volumetric data, and MRI findings regarding CNS inflammation will be presented.

148.5

ELEVATED STRIATAL DOPA DECARBOXYLASE ACTIVITY IN VIVO IN PSYCHOSIS. J. Reith, H. Kuwabara*, C. Benkelfat*, G. Savard*, F. Andermann*,G. Chouinard*, A. Sherwin*, S. Dyve*, P. Cumming*, D. Jolly*, S. Lal*, S. Bachneff*, & A. Gjedde. Positron Imaging Laboratories, Montreal Neurological Institute, McGill University, Canada H3A 2B4.

To test the hypothesis that an abnormality of dopaminergic neurotransmission may contribute to psychosis, we determined regional cerebral DOPA decarboxylase activity in 4 patients with intractable complex partial seizures (CPS) with psychosis, 8 patients with intractable CPS without mental disturbances, 4 schizophrenic patients and 12 healthy volunteers, using [^{18}F]fluoro-L-DOPA and positron tomography. The enzyme activity was calculated on the basis of a kinetic model, yielding the rate of conversion of FDOPA to FDOPAMINE [Gjedde et al., PNAS (USA) 88:2721, 1991]. Patients were scanned by a Scanditronix PC2048 15 plane PE tomograph after i.v. injection of 3-6 mCi [^{18}F]fluoro-L-DOPA. All patients were neuroleptic-naive, or received neuroleptics for brief periods only at least one year prior to the scan. The estimates of decarboxylation rates in units of h^{-1} were (±SEM),

Region	Controls(12)	CPS(8)	CPS+Psychosis(4)	Schizophrenia(4)
L Caudate	4.7±0.3	4.3±0.3	6.1±0.4*	6.6±0.7**
R Caudate	4.9±0.2	4.8±0.4	6.8±1.8	7.0±0.6***
L Putamen	4.6±0.4	4.3±0.3	5.5±0.3	6.3±0.5*
R Putamen	4.8±0.3	4.4±0.3	5.2±0.2	5.8±0.7

***$P< 0.001$,**$P< 0.02$,*$P< 0.05$

The estimates indicate a significant increase of in vivo dopa decarboxylase activity in neostriatum in patients with psychotic episodes.

148.6

MAZINDOL AUGMENTATION OF TYPICAL ANTIPSYCHOTICS IN NEGATIVE SYMPTOM SCHIZOPHRENICS. J. Seibyl, J. Krystal, R. Johnson*, L. Brenner*,G. Heninger, D. Charney. Dept of Psychiatry, VA Medical Center and Yale Univ., West Haven, CT 06516.

Mesofrontal dopamine (DA) deficits have been implicated in negative schizophrenic symptoms including affective flattening, withdrawal, and alogia. Consistent with this, most schizophrenics experience minimal negative symptom improvement or even worsening with standard neuroleptic treatment. Mazindol is a long-acting agent that blocks DA reuptake at the dopamine transporter site. We tested the responses of positive and negative symptoms to mazindol augmentation of neuroleptic in partially-refractory, stable outpatient schizophrenics. METHODS: In an ongoing study, outpatients stabilized on neuroleptic medication were enrolled in a double-blind, placebo controlled trial of mazindol (2 mg/day) augmentation of typical neuroleptic agents. Weekly Brief Psychiatric Rating Scale (BPRS), Positive and Negative Symptom Scale (PANSS), AIMS, Webster EPS ratings, and fasting prolactin and HVA were obtained for four weeks prior to mazindol/placebo augmentation and for six-eight weeks after randomization. RESULTS: The first six patients receiving active mazindol demonstrated a 30-40% reduction of BPRS and PANSS negative symptom ratings. Increases in positive symptoms were noted in one patient who received a pilot dose of mazindol 8 mg/day. No other increases in positive symptoms were seen in patients treated with mazindol 2 mg/day. There was a modest reduction of extrapyramidal side effects and 1/6 patients showed worsening of tardive dyskinesia with mazindol. Subjectively, 5/6 patients experienced increased mood, anergy, and affective reactivity and correctly guessed the identity of the randomized medication. CONCLUSIONS: Preliminary data suggests mazindol may be a useful adjunct to standard neuroleptic medication for treating refractory negative symptoms in otherwise stable outpatient schizophrenics.

148.7

A POSITRON EMISSION TOMOGRAPHY STUDY OF REGIONAL CEREBRAL BLOOD FLOW DURING PROBLEM SOLVING IN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA. K.F. Berman, E.F. Torrey*, A. Abi-Dargham, C. Randolph, P.J. Andreason*, D.R. Weinberger. NIMH, Clinical Brain Disorders Branch, Neuroscience Center at St. Elizabeth's, Washington, D.C. 20032.

To explore further the previously described finding of hypofunction of the prefrontal cortex ("hypofrontality") in schizophrenia we used the oxygen-15 water method for measuring regional cerebral blood flow (rCBF) with positron emisison tomography in five pairs of monozygotic twins (two female and three male pairs, mean age 34) who were discordant for the illness. rCBF was measured during four cognitive conditions: the Wisconsin Card Sorting Test (WCS), Raven's Progressive Matrices (RPM), and two sensorimotor control tasks (one each for the WCS and RPM). Data were collected on the Scanditronix PC2048-15B brain tomograph which produces 15 slices with reconstructed resolution of 6-6.5 mm in three planes. rCBF for each pixel was normalized (i.e. expressed as a percentage of the whole brain mean).

In each of the five pairs the affected twin was more hypofrontal than the well co-twin during both WCS and RPM. The statistical difference between dorsolateral prefrontal cortex rCBF of well and ill co-twins was significant (for WCS: mean(±SD) left 141.6±8.2 vs. 127.8±8.4 P<.003, right 146.8±12.6 vs. 129.7±11.2 P<.007; for RPM: left 145.5±14.3 vs. 129.6±9.1 P<.03, right 152.1±13.5 vs. 136.7±11.9 P<.03; Paired T Test). Differences during the sensorimotor control tasks were also observed, but were less consistent and less robust (for WCS Control: left P<.08, right P< .20; for RPM Control: left P<.05, right P<.08). These data suggest that hypofrontality can be demonstrated in most, if not all, patients with schizophrenia if adequate control groups are used. These results also indicate that non-genetic factors are important determinants of hypofrontality in schizophrenia.

148.8

FRONTO-TEMPORAL RIGHT-LEFT ASYMMETRY IN NORMALS BUT NOT IN SCHIZOPHRENIC PATIENTS PERFORMING A CONTINUOUS VISUAL GRAY SCALE DISCRIMINATION TASK. H.H. Holcomb, B. Gordon, H. Loats, T. Gastineau, N. Cascella*, D. Ross*, R.F. Dannals, H. Rayvert, A. Wilson, and C.T. Tamminga. Maryland Psychiatric Research Center, Univ. Maryland; Cognitive Neurology, and Nuclear Medicine Division, Johns Hopkins Medical Institutes, Baltimore, Maryland 21205.

Many research groups have found the right hemisphere, especially the frontal and parietal lobes, to be important for attentional tasks. In this pilot study we used a series of visual continuous discrimination tasks in normal volunteers and schizophrenic patients, to better understand right-left hemisphere relationships in people with normal and impaired attentional resources. Activity patterns were assessed with 18F-2DG positron emission tomography, volumetrically registered with magnetic resonance image set.

Four normal volunteers (ages 22-34) and 3 schizophrenic patients (ages 25-32) were trained on a visual discrimination task. Each trial required the subject, right or left button press, to indicate his decision as to whether the square presented on a computer monitor was black or gray. By reducing contrast and brightness in preset increments the investigator effectively assured a constant accuracy level across subjects, 85 - 90% correct.

Metabolic activity patterns measured 8 mm above the AC-PC plane revealed marked (20%) metabolic asymmetry in the frontal and temporal lobes of normal volunteers. This was completely absent in two out of three schizophrenic patients. The inability to generate physiologically appropriate metabolic activity patterns in the presence of "normal" behavior, may be an important component of the schizophrenic syndrome.

148.9

RITANSERIN ANTAGONISM OF MCPP EFFECTS IN NEUROLEPTIC-FREE SCHIZOPHRENIC PATIENTS. J.H. Krystal, J.P. Seibyl, M.L. Wong, G.R. Heninger, and D.S. Charney. Schizophrenia Res. Cntr., Yale U. Sch. of Med., West Haven VA Med. Cntr, West Haven, CT 06516

The serotonin (5-HT) partial agonist m-Chlorophenyl-piperazine (MCPP) produces an exacerbation in the positive symptoms of schizophrenia in unmedicated patients. Behavioral or endocrine effects of MCPP appear to be blocked by clozapine, but not haloperidol treatment. In order to identify receptors relevant to the behavioral effects of MCPP, the capacity of ritanserin, an antagonist of 5-HT_{1C} and 5-HT_2 receptors, to block MCPP effects was assessed in schizophrenic patients. METHODS: In an ongoing study, schizophrenic patients off neuroleptics for at least two weeks (N=4), completed four test days over two weeks in a randomized double-blind design. Medications included MCPP (0.1 mg/kg, i.v. over 20 min.), MCPP following ritanserin (10 mg., p.o.), ritanserin, or placebo. Behavioral, physiologic, and plasma hormone levels were evaluated prior to medication and for 3 hours post-infusion on each test day. Findings in a larger sample of patients will be presented at the meeting. IMPLICATIONS: The ability of ritanserin to block the behavioral or biochemical effects of MCPP may provide useful information concerning the contributions of brain 5-HT systems to psychosis or the mechanisms of action of clozapine.

148.10

OCULOMOTOR DELAYED RESPONSE TASKS IN SCHIZOPHRENIA: EVIDENCE FOR PREFRONTAL CORTEX DYSFUNCTION. D.W. Hommer, A.D. Radant and C. Peng. GRECC, Seattle VAMC and Dept. of Psychiatry and Behav. Sci., Univ. of Washington.

A growing body of evidence suggests that schizophrenia can be characterized by dysfunction in the prefrontal cortex and/or its associated basal ganglia thalamocortical circuits. We used infra-red oculography to examine the saccades of 15 schizophrenics, 11 alcoholics and 32 normals. A delayed saccade task was employed to test working memory and ability to suppress context inappropriate responses. The accuracy and latency of internally guided saccades that followed a 1.2 second delay were considered measures of working memory. Internally guided saccades that interrupted fixation during the delay period and were directed towards the cued, future location of the target provided a measure of context inappropriate responses. We also measured the accuracy and latency of visually guided saccades as well as the number of intrusive saccades that broke fixation during a task that did not require use of working memory. Neither the latency, accuracy or number of intrusive saccades differed among the groups during this task. During the delayed saccade task schizophrenics made only slightly less accurate memory guided saccades following the delay; the latency of these saccades did not differ among the groups. However, during the delay period, when fixation should have been maintained, schizophrenics made over twice as many intrusive saccades as normals or alcoholics (p<.001). The number of these context inappropriate saccades correlated significantly with clinical ratings of positive symptoms of schizophrenia (r=.58). These results are consistent with a failure of the prefrontal cortex to suppress interfering responses.

149.1

IDENTIFICATION AND CHARACTERIZATION OF IDAZOXAN TYPE IMIDAZOLE RECEPTORS IN BOVINE ADRENAL CHROMAFFIN CELLS. S. Regunathan, M.P. Meeley and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Clonidine and related imidazolines bind to α_2-adrenergic as well as imidazole receptors (IR) in brain and peripheral tissues. We sought to identify and characterize IR in bovine adrenal chromaffin cells (ACC). The binding of ^{3}H-idazoxan to membranes (P_2) prepared from ACC was measured. Idazoxan binds with high affinity (K_D 5nM, B_{max} 680 fmole/mg protein) and other drugs inhibited the binding with the rank order of potency: cirazoline>idazoxan>clonidine>amelroide>detomedine>phentolamine> rilmenidine. Compounds that did not inhibit the binding (K_i>10 μM) were epinephrine, p-iodoclonidine, SKF86466, cimetidine and imidazole-4-acetic acid, thus compatible with idazoxan type IRs. Both endogenous clonidine-displacing substance (CDS) and 4-aminopyridine, a K^+ channel blocker, inhibited the binding with an IC_{50} of 7 units and 10 μM, respectively. The inhibition of binding by CDS or cirazoline was not modified by $Gpp_{(NH)}p$, a GTP analogue. The ^{3}H-idazoxan binding sites were identified both in plasma and mitochondrial membranes of chromaffin cells. In cultured chromaffin cells all agents that bind to IR increased the influx of ^{45}Ca and released catecholamines, CDS being most potent. We conclude that in ACC (a) idazoxan type IRs with high affinity for CDS, but not α_2-adrenergic receptors, are present in both mitochondrial and plasma membranes; (b) it is not a G-protein coupled receptor; and (c) IRs may be involved in release of catecholamine by modulating Ca^{++} influx.

149.2

COMPARATIVE MOLECULAR MODELING OF ADRENERGIC RECEPTOR SUBTYPES

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Energy optimised explicit atomic models have been constructed of the main subtypes of adrenergic receptors, α1, α2 and β1, β2, β3. Hypothetical interactions are suggested to account for the pharmacological selectivities of a range of agonists (phenylephrine, clonidine, dobutamine, salbutamol) and antagonists (prazosin, yohimbine, practolol, butoxamine). In particular, a role is proposed for (i) transmembrane helix 7 (position 3; Phenylalanine/Asparagine) in the subdivision of α/β receptors, and (ii) transmembrane helix 4 (position 15; Proline, position 12; Serine) in the interaction of the β-hydroxyl group of adrenergic ligands. The basic usefulness of receptor modelling studies in by-passing problematic experimentation (such as mutagenesis-testing of chimaeric constructs which may have modified conformational stability), and in the future development of therapeutic drugs will be discussed. (We thank Dr. Ole Olsen, Novo-Nordisk for help in video preparation).

149.3

CHARACTERIZATION OF α_2-ADRENOCEPTORS IN THE SHEEP SPINAL CORD BY AUTORADIOGRAPHY. B.A. Bucklin*, J.C. Eisenach, R.M. Booze. Section on Obstetric Anesthesia and Department of Physiology and Pharmacology, Wake Forest University Medical Center, Winston-Salem, NC 27157-1009.

α_2-Adrenergic receptors have been shown to play an important role in nociceptive processing. Quantification and localization of these receptors and their subtypes will be important in further characterization of nociceptive processing and drug development. Therefore, we characterized the α_2-binding conditions of p-[^{125}I] iodoclonidine with sheep spinal cord tissue sections by high-resolution autoradiographic techniques. Four adult sheep were anesthetized and thoracic spinal cord was removed. Serial sections of 25 microns thickness were thaw-mounted onto chrome-alum/gelatin-subbed slides, incubated in 50mM Tris-HCl/10mM $MgCl_2$ (pH7.5) for 30 min at room temperature and then incubated at varying concentrations of p-[^{125}I] iodoclonidine (.05nM to 1.5nM) for 90 min at room temperature. Non-specific binding was defined as binding which was not inhibited by 100 μM phentolamine. Analysis of the saturation experiments was performed using EBDA, and saturability of binding was determined by LIGAND. p-[^{125}I] iodoclonidine bound to a single population of binding sites over the concentration range studied. Non-regression linear analysis determined the binding to be saturable and of high affinity (K_d = .5nM). The amount of non-specific binding was low. Localization of α_2-receptors in the sheep spinal cord was performed using p-[^{125}I] iodoclonidine (.5nM) as the ligand. Non-specific binding was defined as mentioned previously. Films were developed 3 days after exposure. Images showed localization of α_2-adrenoceptors to the superficial dorsal horn and intermediolateral cell column of the spinal cord. These studies define the binding conditions necessary for localization of α_2-adrenergic receptors and demonstrate a high density of specific binding sites in the dorsal horn and intermediolateral cell column of the thoracic spinal cord. This information will be useful for further studies of α_2-adrenoceptor subtypes at sites of hemodynamic control and analgesia.

149.4

DIFFERENTIAL EXPRESSION OF α_2-ADRENERGIC RECEPTOR SUBTYPE GENES IN HUMAN TISSUES. M. Scheinin, M. Perälä*, H. Hirvonen*, H. Kalimo* and K.E.O. Åkerman*. Åbo Akademi and Univ. of Turku, Turku, Finland.

Genetic subtypes of α_2-adrenergic receptors may mediate distinct physiological functions and are therefore possible targets for the development of subtype-selective drugs. We have investigated the tissue distribution of the expression of two human neuronal α_2-adrenoceptor subtype genes, C4 and C10.

The plasmids pSP65α_2-C4 and pSP64α_2-C10 (Kobilka et al., Science 238, 650; Regan et al., PNAS 85, 6301) were used to generate antisense cRNA probes with ^{32}P-UTP as the label. After solution hybridization, single-stranded RNA was digested with RNase treatment.

In human fetal tissues, both receptor subtype mRNAs were abundantly expressed in all investigated major brain regions. In addition, expression of C10 was detected in spleen, kidney, adrenals, and skin, whereas C4 mRNA was detectable only in kidney and skin. Most regions of the adult brain also expressed both subtypes, but with marked quantitative differences. For example, cerebral cortex contained almost solely C10-mRNA, whereas the caudate nucleus expressed predominantly C4-mRNA. This is in accordance with the known distribution of the two receptor subtypes in human brain, based on pharmacological criteria. In peripheral tissues, C10 expression was most abundant in spleen and renal cortex, and expression of C4 was strongest in renal cortex and renal medulla.

These differences in the expression patterns of the two neuronal α_2-adrenergic receptor genes warrant further investigation, and may provide a basis for the development of new, subtype-selective pharmacological agents with more targeted actions compared to currently used α_2-adrenoceptor agonists and antagonists.

149.5

α_2-ADRENERGIC RECEPTOR ACTIVATION POTENTIATES DBcAMP-STIMULATED RAT PINEAL SEROTONIN N-ACETYLTRANSFERASE (E.C. 2.3.1.87) ACTIVITY. N.C. Schaad and D.C. Klein*, Sect. Neuroendocrinology, Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda 20892.

α_2-Adrenergic receptors in the rat pineal gland were investigated. A single high affinity (Kd = 4 nM), low density (Bmax = 60 fmoles/mg protein) binding site was apparent from studies using the selective ligand ^{3}H-yohimbine. A possible role for these receptors was further investigated using biochemical techniques.

Activation of α_2-adrenergic receptors did not influence cyclic AMP formation in the intact gland in the presence of isobutylmethylxanthine (IBMX), nor did it alter basal or forskolin-stimulated adenylate cyclase in membrane preparations. Alone, the α_2-adrenergic agonist clonidine (1 to 30 μM) had no effect on the stimulation of serotonin N-acetyltransferase activity (E.C. 2.3.1.87; NAT). However, clonidine potentiated the stimulation of NAT activity induced by N^6-2'-O-dibutyryladenosine 3:5'-cyclic monophosphate (50 to 100 μM) or IBMX (100 to 500 μM). The EC_{50} for this effect of clonidine was 0.3 μM. Clonidine potentiation was inhibited by yohimbine, but not by prazosin, indicating an involvment of α_2-adrenergic receptors. Similar potentiation was observed with two other α_2-adrenergic agonists, oxymetazoline (1 μM) and UK 14304 (1 μM). These data suggest that α_2-adrenergic receptors play a role in the regulation of rat pineal NAT activity, apparently by a mechanism which does not involve changes in cyclic AMP production.

149.6

THE D3 DOPAMINE RECEPTOR COUPLES TO ION CHANNELS VIA A PTX-SENSITIVE G-PROTEIN WHEN EXPRESSED IN GH_4/C_1 CELLS. J. Rendt-[1]*, P. Falardeau-[2], B. Giros-[3]*, J-C. Schwartz-[3]*, M.G. Caron-[2], and G.S. Oxford-[1]. [1]Dept. of Physiology, Univ. of N.C., Chapel Hill, NC 27599; [2]Dept. of Cell Biology, Duke Univ. Med. Cntr., Durham, NC 27710; [3]Unité de Neurobiol. et Pharmacol., INSERM, Paris, France.

Recently a novel form of dopamine receptor (D3R) was cloned from a rat brain genomic library which was screened with a probe coding for a D2 receptor sequence. Initial functional studies of this receptor expressed in COS-7 and CHO cells were negative as regards changes in cAMP levels and guanine-nucleotide dependence (*Nature* 347:146, 1990). We have reexamined the functionality of this receptor subtype in GH_4/C_1 cells transfected with a cDNA encoding the D3R. These cells do not normally express dopamine receptors. Utilizing whole-cell and nystatin-perforated patch clamp methods we have examined the ability of D3R agonists to alter resting and action potentials, and ionic conductances in transfected cells. Both dopamine (1μM) and quinpirole (100-400nM) applied to individual cells via a U-tube caused the cessation of spontaneous action potentials accompanied in most cases by a membrane hyperpolarization (<10mV). Under voltage clamp conditions these agonists induced an outward current from a holding potential of -40mV. I-V curves for agonist-induced currents were assessed with voltage ramps and reversed direction near E_K and were sensitive to changes in $[K_o]$. Sulpiride (10μM) blocked these effects as did pretreatment of cultures with PTX (350ng/ml, 6hrs). We conclude that in these cells the D3R couples to a K channel (normally activated by somatostatin receptors) via a PTX-sensitive G-protein to mediate the inhibitory responses. (Supported NS18788 to G.O. and NS19576 to M.G.C.).

149.7

COUPLING OF THE RAT D3 DOPAMINE RECEPTOR TO ADENYLYL CYCLASE IN GH4C1 CELLS. P. Falardeau, N. Godinot*, B. Giros, J.C. Schwartz*, and M. G. Caron, OGM, CHUL, Laval Univ., Québec, Canada, G1V 4G2, Dept Cell Biology, Duke Univ. Med. Ctr, Durham, NC, 27710

The gene for the D3 dopamine receptor was recently cloned and described as being homologous to the D2 receptor although the distribution of its mRNA in the central nervous system and its pharmacology are distinct from those of D2 receptors. When expressed in CHO cells, the D3 receptor lacks the high affinity component of agonist binding as well as its coupling to a signalling system, suggesting the absence of a suitable G-protein. Expression of D2 receptors in these cells leads to a guanine nucleotide-dependent coupling of this receptor to inhibition of cAMP. In order to determine which transduction system is coupled to this new dopamine receptor, a cDNA for the rat D3 receptor was transfected permanently into prolactin-secreting GH4 cells which lack the D2 receptor. The levels of expression of the D3 receptor, as defined by [3H] spiperone binding is 1,2pmol /mg of protein. Dopamine itself as well as other agonists, compete [3H] spiperone binding in a biphasic fashion (for NPA, KDhigh=0,37nM; 55%: KDlow=49,3nM; 45%). In the presence of Gpp(NH)p, a guanylnucleotide, the agonist competition curves are monophasic, indicating the presence of a G-protein interaction with this receptor. Moreover, inhibition of forskolin stimulated cAMP was assessed in this system. Stimulation of D3 receptors with quinpirole, an agonist selective for a D2 class of receptor, induces a decrease of intracellular cAMP stimulated with forskolin (83%) in a dose-dependent fashion (IC50= 15nM for quinpirole). This decrease can be specifically reversed in presence of specific D2/D3 antagonists. These results show that the newly cloned D3 Dopamine receptor can be coupled to inhibition of adenylyl cyclase through a specific G-protein not present in CHO cells. These results further suggest that receptors may be the determinant of receptor-G protein specificity.

149.8

SITE-SPECIFIC SYNTHETIC Gs PEPTIDES EVOKE HIGH AFFINITY BINDING TO β-ADRENERGIC RECEPTORS IN PERMEABLE C6 GLIOMA CELLS. M. Watanabe, M. B. Lazarevic*, H. Hamm and M. M. Rasenick, Department of Physiology & Biophysics and the Committee on Neuroscience, University of Illinois College of Medicine, Chicago, IL 60680, U.S.A.

A newly devised method for receptor binding assays in saponin permeable C6 glioma cells permits investigation of β-adrenergic receptor behavior under conditions where this receptor appears tightly coupled to Gs to effect activation of adenylyl cyclase. Although β-adrenergic agonists must be present to activate adenylyl cyclase, (i.e. GTP or GTP analogs have no effect alone), these compounds reduce the affinity of β-adrenergic receptors for agonists in permeable C6 cells. Since the carboxyl terminal domain of the G protein α subunit has been suggested to recognize the receptor, we attempted to ascertain whether peptides corresponding to this region would affect agonist binding affinity using the method of isoproterenol competition of [^{125}I] pindolol binding. Synthetic site-specific αs peptides (15-29, 354-372, 384-394) and αi2 peptides (8-22, 315-324, 345-355) were tested. Two αs peptides (354-372, 384-394), which correspond to carboxyl terminal part of αs subunit, increased agonist binding affinity for the β-adrenergic receptor, (i.e. left shifted the isoproterenol displacement curve for iodopindolol). Other synthetic peptides had no effect on receptor affinity. Since the receptor-G protein complex is thought to represent the high affinity state for agonists and free receptor corresponds to low affinity state, it is likely that the Gs C-terminal peptides may bind to the β-adrenergic receptor and promote high-affinity agonist binding.

149.9

A SYNTHETIC C-TERMINAL PEPTIDE OF Gs BLOCKS G PROTEIN-β ADRENERGIC RECEPTOR INTERACTION IN PERMEABLE C6 GLIOMA CELLS. M.B.Lazarevic*, M. Watanabe, M. M. Rasenick and H.Hamm, Department of Physiology & Biophysics and the Committee on Neuroscience, University of Illinois College of Medicine, Chicago, IL 60680, U.S.A.

G protein-β adrenergic receptor coupling was studied in saponin-permeable C6 glioma cells by introducing synthetic peptides which correspond to various domains of the G proteins, αs and αi. Effects upon adenylyl cyclase activity were measured. The C-terminal peptide of αs, αs384-394, decreased isoproterenol stimulation of adenylyl cyclase in a dose dependent fashion, suggesting that it blocked activation of Gsα by the isoproterenol-activated β adrenergic receptor. Synthetic N-terminal peptides and an inversed sequence "nonsense" peptide, did not elicit these effects. This peptide did not change the activity of adenylyl cyclase in the presence of forskolin or sodium fluoride. Activity of adenylyl cyclase catalytic unit extracted from membranes was not affected by peptide αs384-394.

These data suggest that this synthetic C-terminal peptide of αs, αs 384-394, binds to the G protein association domain on the β adrenoceptor and blocks the binding of the G protein to that site.

149.10

IMMUNOCYTOCHEMICAL DEMONSTRATION OF LIGAND-REGULATED INTERNALIZATION AND RECYCLING OF BETA ADRENERGIC RECEPTORS. M.v.Zastrow*, C.J. Evans and B.K. Kobilka*. Dept. of Mol. and Cell. Physiology, HHMI, Stanford and Dept. of Psychiatry, UCLA.

We have examined agonist-induced redistribution of human β_2 adrenergic receptors in human 293 cells using a receptor-specific antiserum and immunocytochemical localization. Isoproterenol induces rapid and substantial loss of receptors from the cell surface and their accumulation in intracellular vesicles. This redistribution occurs with similar kinetics as receptor sequestration measured pharmacologically, with a $t_{1/2}$ of 2-5 minutes, and it occurs long before any detectable receptor down regulation is observed. Agonist-induced intracellular accumulation of receptors is blocked by the adrenergic antagonist alprenolol, and internal receptors are returned to the cell surface following removal of agonist. Neither receptor internalization nor its reversal is blocked by 95% inhibition of cellular protein synthesis with cycloheximide. These data provide direct evidence of rapid, ligand-regulated internalization and recycling of adrenergic receptors.

149.11

IMPORTANCE OF THE β_2-ADRENERGIC RECEPTOR PALMYTOYLATION IN THE DESENSITISATION PROCESS. **S. Moffett,*B. Mouillac,*H. Bonin*and M. Bouvier Dept. of biochemistry, Université de Montréal, Montreal, Canada, H3C 3J7.**

Prolonged agonist stimulation of the β_2-adrenergic receptor (β_2AR) leads to its functional uncoupling from the adenylyl cyclase (AC) stimulation pathway. Phosphorylation of the β_2AR by the cAMP dependent protein kinase and the β_2AR kinase contribute to this desensitization process. Recently, the palmytoylation of the cystein^{341} (cys^{341}) of the β_2AR has been proposed as an important factor in the coupling of the receptor to the AC system. Thus, to evaluate the role of the receptor palmytoylation in the desensitization process, a mutant receptor in which the cys^{341} is replaced by a glycine (gly$^{341}\beta_2$AR) was constructed by site directed mutagenesis. Chinese hamster fibroblasts stably expressing the wild type (WT) β_2AR or the gly$^{341}\beta_2$AR were then generated and used for the study. A 20' exposure of cells expressing the WT human β_2AR to the agonist isoproterenol (1 μm) induces a 33±6% reduction of the receptor's capacity to maximaly stimulate the AC. In the cells expressing the gly$^{341}\beta_2$AR, the same treatment induces a 13±4% desensitization of the β_2AR-stimulated AC. This significantly decreased desensitization of gly$^{341}\beta_2$AR is accompanied by a reduced agonist-mediated uncoupling of the receptor as assesed by its agonist binding properties. Indeed, whereas the agonist treatment completely abolishes the GTP sensitive component of the agonist binding in wt β_2AR expressing cells, this was not the case in the cells expressing gly$^{341}\beta_2$AR. In contrast to the desensitization, the agonist induced phosphorylation of the receptor is not affected by the cys$^{341}\rightarrow$gly^{341} mutation. Incubation of the wt- and gly$^{341}\beta_2$AR expressing cells to isoproterenol leads to a 2 fold increase in the phosphorylation level of both receptors. Taken together these results suggest that uncoupling of the receptor from the AC stimulatory pathway not only involves phosphorylation but is also influenced by the palmytoylation status of the receptor.

149.12

PRODUCTION OF SUPERSENSITIZED D2 AND/OR D3 RECEPTORS IN RATS. R.M. Kostrzewa, R. Brus*, and J. Guo*. Depts. of Pharmacology, College of Medicine, East Tennessee State Univ., Johnson City, TN 37614 and Silesian Academy of Medicine, 41-808, Zabrze, Poland

In an attempt to produce supersensitization of D2 receptors, rats were treated daily for the first 28 d from birth with quinpirole (2.6 mg/kg/d, i.p.). Starting at the 19th until the 32nd day, the acute quinpirole injection was associated with jumping behavior that could be attenuated by spiperone. In adulthood, acute quinpirole, in a dose as low as 25 μg/kg, increased the yawning response to about 2-fold the level of controls. Similarly, the antinociceptive action of quinpirole was enhanced in these rats in the hot plate test of analgesia. This effect was attenuated by spiperone. These findings indicate that ontogenic treatments with quinpirole will supersensitize specific effects of this agonist in rats that are studied as adults. The B_{max} and K_d for striatal D2 receptors was not changed by these treatments. Since extremely low doses of quinpirole elicit yawning behavior, it may be that D3, as well as D2 receptors, become sensitized for prolonged periods by this dosing regimen. This animal model may be useful for studying long-lived D2 receptor supersensitization. (Supported by the Scottish Rite Schizophrenia Research Foundation and Fogarty International Exchange Program)

150.1

ONTOGENY OF IMMEDIATE EARLY GENE RESPONSE TO COCAINE. Barry E. Kosofsky, Neil W. Kowall, and Steven E. Hyman, Departments of Neurology and Psychiatry, Massachusetts General Hospital, Boston, Massachusetts, 02114.

To investigate the mechanisms by which drugs of abuse interfere with normal brain development we have administered cocaine (40 mg/kg ip) to rat pups at various stages of maturation (P8, P15, P28, Adult). 45 minutes post-injection of cocaine the pups were sacrificed and regional brain analyses (cortex, striatum, cerebellum) performed to quantitate drug lnduced changes in immediate early gene (IEG) mRNA expression (proto-oncogenes zif-268, c-fos, c-jun). At the earliest age examined (P8) IEG induction was limited to striatum, where a 3 to 4-fold increase in c-fos, and a 2-fold increase in zif-268 was evident. At ages P15, P28, and adult, cocaine induced all 3 IEG's (c-fos, c-jun, and zif-268) in striatum and cortex. In cerebellum, only c-fos was induced, 1.5-fold at P15 and P28, and 5-fold in the adult. The regional, cellular, and stage-specific selectivity of drug induced action suggests a regional and developmental continuum of CNS vulnerability consequent to maternal drug abuse during human brain maturation.

150.2

INDUCTION AND SUPPRESSION OF STRIATAL PROTO-ONCOGENE EXPRESSION AFTER TREATMENT WITH COCAINE. J. Gu* and M.J. Iadarola. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

The c-fos proto-oncogene is induced in a variety of CNS regions by pharmacological, physiological and pathophysiological stimuli. Previous studies have shown that after metrazol or ECS seizures, c-fos mRNA is rapidly elevated but subsequently is resistant to re-induction for a variable length of time. One treatment that we have shown to markedly increase c-fos expression in rat striatum is injection of the indirect dopamine agonist, cocaine. Here we demonstrate that cocaine also causes a period of suppression of re-induction, the degree of which is dependent upon the frequency of administration. A single i.p. injection of 30 mg/kg of cocaine produced a peak increase in c-fos mRNA within 30 min. If, after the first injection and an interval of one hr, a second injection was given, the mRNA level was less than that following a single injection. The reset period requires at least a 2 hr interval. In contrast to the single acute injection, if multiple injections are given over a single day (4 X, 2 hrs apart) full re-induction is suppressed for more than 24 hr. Transcripts coding for Fos-related antigen 1 (Fra-1), another member of the Fos family, was not induced, even after repeated injections. However, mRNA coding for NGF-IA, a zinc-finger-containing transcription factor, was induced in striatum and showed a similar suppression of induction as did c-fos. Multiple injections also caused induction of c-fos and NGF-IA mRNAs (but not Fra-1 mRNA) in hippocamus as well as striatum. The present data suggest that a set of feedback controls is activated in striatum by dopaminergic stimulation to modulate the expression of several genes that code for transcriptonal regulatory proteins.

150.3

Effects of Chronic Cocaine on *c-fos* and Other Immediate Early Genes and on AP1 Binding in Rat Nucleus Accumbens. B.T. Hope and E.J. Nestler, Lab of Molecular Psychiatry, Yale University, New Haven, CT, 06508.

Chronic abuse of cocaine has long-term effects on both brain and behavior, many of which remain long after the acute effects of cocaine have dissipated. These long-term effects could involve alterations in neuronal gene expression. We have examined the effects of chronic cocaine (15 mg/kg, twice daily) on mRNA levels of the immediate early genes (IEG), *c-fos, c-jun, zif, fosB, junB*, in rat nucleus accumbens (NAc), a brain region implicated in drug reward. The protein products of these genes regulate gene expression. *c-fos, c-jun* and *zif* mRNA levels are increased 60 min after acute cocaine. Chronic cocaine treatment desensitizes the acute drug effect in that these mRNA levels no longer increase.

We have employed the gel shift assay to examine the effects of chronic cocaine on AP-1 binding in rat NAc. The AP-1 complex, composed of Fos, Jun, and related proteins, binds DNA and thereby regulates gene expression. AP-1 binding levels are increased 2 1/2 hours after acute cocaine. However, unlike the effects on IEG mRNA, AP-1 binding levels remain increased after chronic plus acute cocaine treatment. Indeed, 18 hours after the last injection of chronic cocaine, AP-1 binding levels are still increased. We suggest there is a change in the composition of the AP-1 complex and its regulatory function that contributes to the long-term addictive actions of chronic cocaine abuse.

150.4

COCAINE-INDUCED ELEVATION OF DYNORPHIN, FOS, AND PKC IMMUNOREACTIVITY IN RAT STRIATUM AND HIPPOCAMPUS. J.B. Daunais, T.E. Helton, W.T. Bohler, and J.F. McGinty. Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, N.C. 27858.

Fos protein, Fos-related antigens (FRA), dynorphin (DYN), and PKC-alpha immunoreactivity (ir) are elevated by repeated administration of the indirect-acting dopaminergic agonist, cocaine. Cocaine HCl (Sigma) or saline was administered i.p. at 10, 20, or 30 mg/kg once daily X 10 days or 40 mg/kg i.p. X 3 days. The behavior of the rats was rated for one hour following treatment. Two hours following the final injection, the rats were transcardially perfused with 4% buffered paraformaldehyde, and 50 μm serial sections were collected through the striatum and hippocampus (HPC) for immunocytochemistry (ICC).

DYN-ir was increased in patches and matrix in the dorsal and ventral striatum at the 30 and 40 mg/kg doses. DYN-ir was particularly increased in striatal patches and in the shell of the accumbens as the dose of cocaine increased.

FOS-ir and FRA-ir increased in a dose-dependent fashion, but had differing patterns of expression. In brains treated with 30 or 40 mg/kg, FRA-ir was moderately to heavily expressed in anterior cingulate, medial prefrontal, and piriform cortices, dorsomedial shell and the rostral-most anterior nucleus accumbens (NAc). Expression was extremely heavy throughout the dorsal striatum. In contrast, FOS-ir was low to moderate in the above cortical areas, and in the rostral NAc and dorsomedial shell of the NAc and in a patch-like pattern in the central medial dorsal striatum.

Cocaine appeared to have a biphasic effect on the expression of PKC in the striatum and hippocampus. At 10 mg/kg, PKC-α was intense in large, possible cholinergic or somatostatinergic, neurons in the striatum and in hilar neurons and CA3-2 pyramidal neurons in the hippocampus. These changes in PKC-α-ir decreased as the dose of cocaine increased. No FOS/FRA-ir induction or alteration in DYN-ir was observed in HPC after cocaine at any dose. These data indicate widespread neurochemical changes after repeated high dose cocaine exposure. Supported by DA 03982.

150.5

COCAINE-INDUCED SEIZURES ALTER FOS AND DYNORPHIN IMMUNOREACTIVITY IN RAT T.E. Helton, J.B. Daunais and J.F. McGinty Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858.

A toxic side effect of chronic, high doses of cocaine is the generation of seizures and sudden death. However, little is known about the neurochemical sequelae of cocaine-induced seizures. In this study rats were treated with escalating doses of cocaine over 14 days (Stripling and Ellinwood Pharmacol, Biochem. and Behav. 6, 571-579, 1977) to "kindle" seizures. Rats were perfused and their brains examined histochemically for changes in immediate early gene and peptide expression. Rats were handled for 2 days prior to the initiation of cocaine treatment to lower stress induced by handling. Sixteen rats were treated with increasing doses of cocaine: days 1-7, 55 mg/kg, days 8-12, 60 mg/kg, days 13-14, 65 mg/kg. Two rats died as a result of seizures and four rats were anesthetized and perfused within 90 mins of cocaine injection, after experiencing life threatening seizures on day 1, 4, 8, and 10. The remaining rats continued to receive daily injections and 40% of those eventually displayed seizures but the time to onset and the number of seizures were highly variable. Cocaine-induced seizures did not kindle gradually, but appeared to be all or none clonic convulsions with head bobbing and loss of righting reflex. Surviving rats were perfused with 4% buffered paraformaldehyde 1 or 3 hours after their last injection. Analysis to date only includes those rats that experienced multiple seizure events. Fifty µm thick sections were cut throughout the forebrain and collected for immunocytochemistry. An increase in Fos-immunoreactivity(ir) was observed in the dentate granule cells of the hippocampal formation, striatum, piriform cortex, entorhinal cortex, neocortex and amygdala. A decrease in dynorphin-ir was observed in the hippocampal mossy fibers. Analysis of somatostatin and enkephalin-ir will also be reported. These data indicate widespread neurochemical changes in limbic forebrain following cocaine-induced seizures. Supported by DA03982.

150.6

CHANGES IN TYROSINE HYDROXYLASE mRNA LEVELS IN THE A10 REGION OF COCAINE-SENSITIZED RATS. B.A. Sorg, J.D. Steketee, R. Reeves*†, and P.W. Kalivas. Department of VCAPP and Program in Genetics and Cell Biology†, Washington State University, Pullman, Washington 99164-6520.

Levels of the mRNA coding for tyrosine hydroxylase (TH) were measured in the A10 region of cocaine-sensitized rats. Animals were given one of three treatments: 1) control group = 7 days of saline treatment (1 ml/kg, i.p.); 2) acute cocaine group = 6 days of saline followed by cocaine treatment on day 7 (15 mg/kg, i.p.); and 3) cocaine-sensitized group = 7 days of cocaine (15 mg/kg, i.p.). Locomotor activity was measured following treatment on days 1 and 7. Twenty-four hr after the last injection, animals were sacrificed and micropunches of the A10 region were removed for subsequent Northern blot analysis of TH mRNA levels. No changes were detected in TH mRNA levels 24 hr after acute cocaine treatment. In contrast, the cocaine-sensitized group showed a strong linear relationship ($r = -0.951$) between levels of TH mRNA 24 hr after the last cocaine treatment and the levels of horizontal motor activity following the first day of cocaine treatment. A weaker correlation existed ($r = -0.749$) between TH mRNA levels and locomotor activity in response to the last cocaine injection. Thus, the higher the levels of locomotor activity in response to the cocaine treatments, the lower the levels of TH mRNA following the cocaine sensitizing treatment. These results suggest that there may be a compensatory response in A10 dopamine cells such that TH mRNA levels are regulated by the degree of cocaine-induced locomotor activity.

150.7

COCAINE-INDUCED MOTOR ACTIVITY IN MICE WITH GENETICALLY MANIPULATED MESOTELENCEPHALIC DOPAMINE SYSTEM. C. Vadasz[1,2], I.Laszlovszky[1], A. Fleischer[1], and I. Vadasz[1]. [1]N.S.Kline Institute for Psychiatric Res., Orangeburg, N.Y. 10962 and [2]New York University Medical Center, 550 First Avenue, New York, N.Y. 10016.

The mesotelencephalic dopamine system was manipulated by genetic selection for high and low expression of mesencephalic tyrosine hydroxylase (TH/MES). This resulted in developing congenic mouse lines with significant differences in TH/MES. Since some of the cocaine-induced behaviors are thought to be mediated by dopaminergic mesostriatal systems, we tested the hypothesis that a common set of genes affect the mesotelencephalic DA system and cocaine induced running. Congenic mice with high (B6.C) and low (B6.I) TH/MES received i.p. injections of saline or cocaine (10,20, or 30 mg/kg). B6.C animals exhibited significantly lower motor activity than B6.I mice at 10 mg/kg ($p<0.003$) and 20 mg/kg ($p<0.0007$). Scatchard analysis of [^{3}H]WIN 35,428 binding in B6.C and B6.I striata demonstrated no significant differences in B_{max} and K_d. This suggests that common genes are implicated in the control of TH/MES and cocaine-induced running, however, these genes do not affect the density and affinity of the dopamine transporter in the striatum.

150.8

GENETIC DIFFERENCES IN DRUG SELF-ADMINISTRATION. J.M. Carney, M.S. Cheng*, J.F. Wu*, W.R. Landrum* and T.W. Seale. Dept. of Pharmacology, UK Med. Ctr., Lexington, KY, 40536 and Dept. of Ped., OUHSC, Oklahoma City, OK, 73190.

A wide variety of drugs have significant human abuse potential. These drugs have generally been demonstrated to function as positive reinforcers in animal tests. The present study was designed to characterize a mouse model of chronic drug self-administration and to demonstrate genetic differences in self-administration. Adult male C57BL/6J mice were used in the first study. Mice were implanted with chronic external jugular catheters and placed in individual resident cages. An operant lever and stimulus light was located at one end of the chamber. Mice were given access to response contingent (FR1) saline injection for 5 days prior to drug testing. Different groups of 3 mice each were given access to morphine (0.1 mg/kg/injection), cocaine (0.1 mg/kg/inj), methamphetamine (0.01 mg/kg/inj) and pentobarbital (0.32 mg/kg/inj). All mice initiated and maintained drug self-administration reliably above saline. In the second study, C57BL/6J and DBA/2J were tested at two cocaine doses (0.1 and 1.0 mg/kg/inj) C57 reliably self-administered cocaine. In contrast, DBA mice failed to initiate cocaine self-administration. At either dose, these data demonstrated that there are strong genetic determinants of the reinforcing properties of cocaine. Supported in part by NIDA grant 04028 and NIDA contract 87-42108.

150.9

DOPAMINE D_2 RECEPTOR ALLELES IN SUBSTANCE ABUSERS. B. O'Hara, S. Smith*@, A. Persico*, S. Farmer*, G. Cutting*#, D. Newlin*@, D. Gorelick+, and G. Uhl. Labs. of Mol. Neurobiol., Tx.+ & Etiol.@, NIDA/ARC, Depts. Neurol., Neurosci., #Peds., JHU Sch. Med., Box 5180, Baltimore, MD 21224.

The A1 allele of the dopamine D_2 receptor gene has been reported to be more prevalent in alcoholics than in nonalcoholics. These results have spurred attempts to replicate this initial finding, and have led us to examine whether subjects with substantial self-reported drug, alcohol or nicotine use also display elevated A1 allelic frequencies.

No robust association of the A1 allele and heavy substance use was found. However, a consistent trend toward higher A1 frequencies was present in heavy substance users of largely European descent (whites), but not in those of primarily African descent (blacks). Examination of allelic frequencies with respect to race revealed highly significant differences. A1 was present in 60% of 203 blacks and 33% of 121 whites. A1:A2:A3 allelic frequencies were .37:.60:.03 and .18:.82:.00, respectively. The racial differences in A1 and A3 frequencies suggest the possibility that further differences might be found among distinct white ethnic groups and underscore the need for caution in interpreting allelic associations when careful matching of ethnicity has not been performed.

150.10

Buprenorphine Fully Suppresses Spontaneous Activity in the Locus Coeruleus. D.A. Highfield, G. Sonti*, S.J. Grant Dept. Psychology and Prog. in Neuroscience, Univ. Delaware, Newark, DE. 19716.

Buprenorphine is a synthetic opioid proposed as a potential treatment for cocaine craving. But little is known of buprenorphine's cellular mechanism of action. Commonly described as a partial opioid agonist, buprenorphine is now known to act differentially as an agonist at mu receptors and as an antagonist at kappa receptors.

Noradrenergic neurons in the locus coeruleus (LC) are known to contain mu opioid receptors, and morphine has a prominent inhibitory effect on LC neurons spontaneous activity. Therefore, the ability of buprenorphine to suppress LC activity would indicate whether buprenorphine acts as a full or partial agonist. Since hyperactivity of the LC neurons contributes to expression of the classic opiate withdrawal syndrome, buprenorphine's effect on LC neurons would be highly relevant to its clinical application.

Extracellular single unit activity was recorded from 50 LC neurons of chloral hydrate anesthetized rats using standard physiological and anatomical criteria. Buprenorphine (12-400 µg/kg, i.v.) suppressed LC activity in a dose dependent manner, with full suppression at 50-100 µg/kg. This suppression had a remarkably long duration of action, and could not be reversed by either higher doses of buprenorphine (up to 1 mg/kg) or by the opioid antagonists naloxone or naltrexone (up to 10 mg/kg). However, buprenorphine was blocked by pre-treatment with naltrexone (160-500 µg/kg) or the mu_1 selective antagonist naloxonazine (160 µg/kg).

These results are consistent with the hypothesis that buprenorphine is a full agonist at the mu receptors on LC neurons. Since buprenorphine appears to act identically to morphine in the LC, the apparent lack of spontaneous or precipitated withdrawal after chronic treatment with buprenorphine may be due to its long duration of action and resistance to reversal by antagonists.

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150.11

ACUTE AND CHRONIC EFFECTS OF COCAINE ON DISCHARGE CHARACTERISTICS OF NORADRENERGIC LOCUS COERULEUS (LC) NEURONS: STUDIES IN ANESTHETIZED AND UNANESTHETIZED RATS. A.L. Curtis, E. Conti*, and R.J. Valentino. Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA 19102, U.S.A.

Cocaine blocks monoamine reuptake and decreases LC spontaneous discharge rates. The present study characterizes acute and chronic cocaine effects on LC spontaneous discharge, sensory-evoked discharge, activation by stress, and activation by corticotropin-releasing factor (CRF) in anesthetized and unanesthetized rats. In anesthetized rats, cocaine (0.1-3.0 mg/kg, i.v.) decreased LC spontaneous discharge and LC discharge elicited by repeated sciatic nerve stimulation. The ratio of evoked/tonic discharge rate (signal-to-noise) was not significantly decreased by cocaine. Similarly, chronic cocaine (40 mg/kg/day for 14 days) administered by osmotic minipumps, decreased both LC spontaneous and sensory-evoked discharge, but not the signal-to-noise ratio. Neither acute or chronic cocaine blocked LC activation by i.c.v. CRF or by hemodynamic stress induced by nitroprusside infusion, indicating that cocaine does not attenuate all inputs that activate LC. These effects of cocaine are in contrast to those of monoamine reuptake blockers that are antidepressants, i.e., desmethlyimipramine (at norepinephrine terminals) and sertraline (at serotonin terminals). Chronic antidepressant administration either increased the LC signal-to-noise ratio, or attenuated LC activation by hemodynamic stress. Acute cocaine had similar effects on LC responses in unanesthetized (auditory stimulation) as in anesthetized rats, but was one-third as potent. In conclusion, acute and chronic cocaine decreased LC spontaneous discharge and sensory-evoked discharge, but neither stress- or CRF-elicited LC activation suggesting that cocaine attenuates LC activation by specific stimuli. PHS Grants MH40008, 00840, 42796, NARSAD to A.L.C.

150.12

CHRONIC BUPRENORPHINE ATTENUATES COCAINE PLACE PREFERENCE T. A. Kosten, PhD, D. W. Marby, BA,* & E. J. Nestler, MD, PhD. Dept Psychiatry, Yale Univ School Medicine, New Haven, CT 06519.

Previous research has shown that chronic buprenorphine (B) reduces cocaine (C) use among opiate addicts and suppresses C self-administration in primates. The present study investigates the effects of chronic B administration on the development of C conditioned place preference (CPP). CPP is another drug abuse model in which rats are exposed to C in a distinctive place and show enhanced preference for this place after training. Rats were injected with B (0.5 mg/kg s.c. 2x/day) or vehicle for 1 wk prior to and during CPP training. After baseline assessments, rats were trained by pairing either saline (S) or C injections (15 mg/kg i.p.) with side 1 for 4 days. CPP was assessed by comparing time spent on this side after training to baseline (change in min/30 min). Vehicle treated rats (n=12) trained with C showed an 8.6 $\pm$ 1.0 min CPP compared to the 0.7 $\pm$ 0.6 min shown by those rats (n=9) trained with saline. B treated rats (n=8) trained with C showed significantly less CPP at 3.9 $\pm$ 0.8 min. The B treated rats trained with S (n=6) showed a 1.9 $\pm$ 1.1 min CPP. Training with B alone without chronic treatment did not result in CPP (B: -1.6 $\pm$ 1.6 vs S: 1.0 $\pm$ 1.5 min). Because B is a mixed opiate agonist-antagonist, this effect may be dose-dependent. The effects of chronic treatment with other doses of B are currently being investigated.

Supported by NIDA grant #P50-DA04050.

151.1

EFFECTS OF TWO DOSES OF ETHANOL ON EEG ACTIVITY IN HEALTHY MALES H.L. Cohen, B. Porjesz*, and H. Begleiter, Dept. of Psychiatry, S.U.N.Y. Hlth Sci Ctr., Bklyn, Brooklyn, New York 11203

The present investigation examined the effects of placebo (P), low dose (LD) and high dose (HD) ethanol on EEG activity in 15 males (X=23.1 years) at low risk for the development of alcoholism. Five EEG recordings using the entire 10/20 system were made under each condition. Only one condition was presented per day and condition order was randomized. Blood alcohol levels were measured both prior to and at intervals of ca 28,66,94 and 132 minutes after P, LD or HD administation. The Fast Fourier Transform was used to calculate Power Spectral Densities for each EEG recording. Measures of the relative areas under the power spectral curve were made for each combination of the following frequency bands: slow alpha (SA, 7.5-10Hz), fast alpha (FA, 10.5-13.0Hz), slow beta (SB, 13.5-19.5Hz) and fast beta (FB, 20-26Hz) and electrodes: F3, F4, C3, C4, O1, O2, P3 and P4. Repeated measures MANOVA revealed that ethanol had significant effects in association cortex, i.e. at central leads C3 and C4 and frontal leads F3 and F4, rather than in sensorimotor cortex. These effects were dose-dependent and occurred primarily in the SA frequency band and suggest that there is differential sensitivity of both cortical region and EEG frequency band to the effects of ethanol.

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151.2

FRONTAL GLUCOSE HYPOMETABOLISM IN CHRONIC ALCOHOLISM. M.H. Dao-Castellana*, Y. Samson, F. Legault*, J.L. Martinot*, A. Holler*, H.J. Aubin*, A. Feline*, A. Syrota*. Service Hospitalier Frederic Joliot, DRIPP, CEA, Orsay, France.

Neuropsychological and neuropathological data suggest that chronic alcoholism may result in frontal damage. Therefore, we measured cerebral glucose utilization (CMRGlu) with positron emission tomography (PET) and [18F]-fluorodeoxyglucose in 10 alcoholic subjects selected according to DSMIIIR criteria. PET studies were performed after 1-3 weeks of alcohol withdrawal. Frontal function was assessed by Stroop test and verbal fluency, and memory by Wechsler memory scale. Using magnetic resonance imaging studies performed at the same levels than PET images, three large lobar regions (frontal, parietal, and temporal), and four smaller areas (medial prefrontal, dorsolateral prefrontal, orbito-frontal, and internal temporal cortices) were defined. Regional CMRGlu values were normalized to mean cortical values. Compared to control values, a significant bilateral hypometabolism was found in the lobar frontal ($p=.01$), medial prefrontal ($p=.002$), and dorsolateral prefrontal ($p=.005$) cortices. The degree of medial prefrontal hypometabolism correlated with frontal tests (verbal fluency, $p=.001$; Stroop test time score, $p=.02$), but correlation with Wechsler memory scale did not reach statistical significance ($p=.07$). The degree of dorsolateral prefrontal hypometabolism correlated with the number of errors on Stroop test ($p=.016$). No other correlations were found with any other brain areas. Thus, the inner face of the frontal lobe, which includes the cingulate gyrus, and the dorsolateral prefrontal cortex may be specifically impaired by chronic alcoholism.

151.3

CLASSICAL GENETIC ANALYSES OF RESPONSES TO SEDATIVE-HYPNOTIC AGENTS IN CROSSES DERIVED FROM LONG-SLEEP AND SHORT SLEEP MICE. C.M. de Fiebre, N.E. Colley, J.M. Wehner and A.C. Collins. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309-0447.

A classical (Mendelian) genetic analysis of responses to eight sedative-hypnotic compounds (ethanol, urethane, trifluoroethanol, chloral hydrate, barbital, paraldehyde, methyprylon, pentobarbital) was conducted in crosses derived from mouse lines which were selectively bred for differential duration of loss of the righting reflex ("sleep-time") following ethanol. The sleep-time responses of these mice, the long-sleep (LS) and short-sleep (SS) mouse lines, and the derived F1, F2 and backcross (F1 X LS, F1 X SS) generations were measured for these eight agents. Generally, differences in responses among the generations were greater for water soluble compounds than were differences for more lipid soluble compounds. Also, the inheritance pattern seen for water soluble compounds could be explained primarily by additive genetic effects while the modes of inheritance for lipid soluble compounds were more complex. Furthermore, the genetic correlation between ethanol responsiveness and responsiveness to the other agents decreased with increasing lipophilicity. These results suggest that the selection of the LS-SS mouse lines was specific for water soluble anesthetic agents and was not a generalized selection for duration of anesthesia. Elucidation of the differential mechanisms by which water and lipid soluble agents act may lead to an improved understanding of the mechanisms of anesthesia in general as well as an improved understanding of the unique anesthetic actions of ethanol.
Supported by AA-03527, MH-16880, HD-07289 and DA-00116.

151.4

GENOTYPIC VARIATIONS IN ETHANOL'S PHARMACOLOGIC PROFILE AND ITS RELATION TO INHIBITORY AND EXCITATORY NEUROTRANSMITTERS S. Liljequist, Dept of Drug Dependence Research, Karolinska Institute, PO Box 60500, S-10401 Stockholm, Sweden.

Behavioral effects of ethanol were examined in CBA, C57, and NMRI mice. In NMRI animals, increasing doses of ethanol had a biphasic action on locomotor activity (stimulation followed by inhibition) whereas only inhibition was seen in C57 and CBA mice. Ethanol produced a similar dose-dependent and preferential increase in the DOPAC/DA (dopamine) ratio in the limbic forebrain as compared to striatum in all animals suggesting no direct relationship between ethanol-produced enhancement of DA release and ethanol-induced locomotor stimulation. C57, and especially CBA mice were more sensitive to the sedative actions (sleep time) of ethanol than NMRI mice. CBA mice were more sensitive to the convulsant effects of the GABA antagonist picrotoxin whereas no such differences were noted after administration of the specific GABA receptor antagonist bicuculline. In receptor binding studies the modulation of 3H-flunitrazepam binding by GABA and pentobarbital was similar in the forebrain of CBA and C57 mice. Further behavioral studies revealed that CBA mice differed in their response to non-competitive and competitive NMDA receptor antagonists as compared to C57 and NMRI mice. In summary, these data suggest that genotypic differences are present in the GABA and glutamate receptor systems and that a complex interplay between various inhibitory and excitatory neurotransmitter systems may determine the pharmacologic profile of ethanol.

151.5

CHRONIC ETHANOL INTOXICATION: EFFECTS ON $GABA_A$ AND NMDA RECEPTOR FUNCTION E. Sanna, M. Serra*, A. Cossu*, P. Follesa*, A. Concas and G. Biggio Dept Exper. Biology, Chair of Pharmacol., Univ. of Cagliari, 09123 Cagliari, Italy

Rats were intoxicated by forced ethanol (EtOH) administrations for 6 days and tested while still intoxicated and during withdrawal (WD). Three hours after the last EtOH ingestion, ^{35}S-TBPS binding to cortical membranes of EtOH-dependent rats was higher (25%) compared to control rats. However, both ^{35}S-TBPS binding and $^{36}Cl^-$ uptake measured in cortical membrane preparations were unchanged 12-24 hours after the last EtOH ingestion. Vice versa, EtOH-dependent rats tested during EtOH WD showed an increased sensitivity to the convulsant action of isoniazid. Moreover, EtOH abstinent rats were more susceptible also to the seizures elicited by kainic acid and other excitatory amino acids but not to those induced by strychinine. This effect was paralleled by an increase of ^{3}H-MK 801 binding to hippocampal membranes. The pharmacological effects were no longer seen at 3 and 6 days of EtOH WD, while at 6 days we found a decrease of ^{3}H-MK 801 to hippocampal membranes of EtOH-treated rats. These results indicate that, in contrast to the acute administration, chronic EtOH treatment may lead to a reduction in the function of $GABA_A$ receptor complex. On the other hand, the $GABA_A$ receptor complex may not be involved directly during EtOH WD. The role of excitatory neurotransmission in the expression of EtOH WD syndrome will be discussed.

151.6

MULTIPLE WITHDRAWALS FROM CHRONIC ETHANOL ALTERS INFERIOR COLLICULAR SEIZURE SENSITIVITY. T.J. McCown and G.R. Breese. Brain and Dev. Res. Ctr., Univ. of North Carolina, Chapel Hill, NC 27599

In rats, multiple withdrawals from chronic ethanol(ETOH) treatment significantly facilitates kindling in the inferior collicular cortex, but attenuates kindling in the amygdala (McCown and Breese, Alcoholism: Clin. Exp. Res. 14:394,1990). Since the change in seizure susceptibility appears to be permanent, studies were designed to assess changes in parameters of seizure genesis. Rats received 6 cycles of a chronic ETOH/withdrawal treatment (ETOH liquid diet for 5 days followed by a 1 day withdrawal), or control liquid diet over the same period. Six days after the last withdrawal, the stimulation frequency for seizure genesis was significantly decreased in the multiple withdrawal group. No change was found in the control liquid diet group. Because local application of NMDA or bicuculline in the stimulation site also decreases the effective stimulation frequency, an increase in excitation or a decrease in inhibition could account for these findings. (Supported by NS 26595).

151.7

ENDOGENOUS OPIOIDS ARE INVOLVED IN THE HIGH ETHANOL PREFERENCE OF C57BL/6J MICE. S.R. George, G. Ng* and C. Naranjo*. Addiction Research Fdn. and Depts. Med. and Pharmacol., Univ of Toronto, Toronto, Ont., CANADA M5S 1A8

We have examined the role of opioid peptides in a model of high ethanol preference and consumption, using C57BL/6J mice (C57). Animals were housed in temperature and humidity controlled environmental rooms with a 12-hr light-dark cycle, and received tap water or 10% ethanol in a two-tube, free choice paradigm. C57 animals consumed an average of 10-15 gm EtOH/kg/day compared to 1-1.5 gm EtOH/kg/day in control DBA/2 mice. Ethanol preference in C57 was 50-60% of total daily fluid intake. Steady state concentrations of proenkephalin, Met-enkephalin or preproenkephalin mRNA were not significantly different between ethanol-naive C57 and DBA/2 mice. C57 had a significantly lower pain threshold that was increased by a mu opioid agonist, which also decreased ethanol consumption. Attempts to prevent endogenous opioid degradation by bestatin and captopril did not alter ethanol drinking in C57. Administration of kelatorphan decreased ethanol consumption, as did administration of kyotorphin. Thus, manoeuvres that increased the concentrations of opioid transsynaptically served to decrease ethanol consumption in the model of C57Bl/6J mice that demonstrate a high preference for ethanol.

151.8

REGIONAL DIFFERENCES IN FUNCTIONAL BRAIN ACTIVITY BETWEEN ALCOHOL PREFERRING (P) AND NON-PREFERRING (NP) RATS. M.J.Lewis, T.-K.Li, L.Lumeng, L.J. Porrino. Howard Univ., Wash.,DC 20059; Indiana Univ. Sch.Med.,Ind.,IN & VAMC; & Bowman Gray Sch.Med., Winston-Salem, NC.

Research has shown that there are major differences in the content of monoamines between rats genetically bred for preference and non-preference for alcohol (A) intake. P rats have lower levels of serotonin and dopamine in selected brain regions in comparison to NP rats. To investigate further differences in brain activity of P and NP rats, the quantitative 2-[^{14}C)-deoxyglucose (2-DG) method was used to map the distribution of rates of cerebral glucose in both lines. Quantitative autoradiographic analysis of 40 brain regions showed that levels of functional activity were higher in the olfactory tubercle (OT) and lower in the CA3 portion of hippocampus of P rats in comparison to NP rats. No significant differences in other brain regions were found. Previous studies with unselected rats has shown that A injection selectivlely increases metabolic activity in the OT. These data suggest the importance of the OT in the behavioral effects of A. (Supported by NIAAA grants AA06263, RR08016, and AA07611)

151.9

AN ALLOCORTICO-LIMBIC LINK MODULATES ETHANOL-SEEKING BEHAVIOR IN THE RAT. L. Pulvirenti#, S. Rassnick and G.F. Koob Dept. of Neuropharmacol., Res. Inst. Scripps Clinic, La Jolla, Ca 92037 and #Bioch. Psychopharmacol. Unit, Dept. of Neurol., Univ. of Pavia, Italy

Ethanol (EtOH) is a widely abused drug in humans and is self-administered by animals. The neural substrates and the intimate biochemical mechanism mediating its reinforcing action, however, remain still obscure. Electrophysiological and neurochemical evidence indicates that EtOH may activate the mesolimbic dopamine system, particularly within the NAC. To assess the functional significance of such interaction, we studied the effect of microinfusion of fluphenazine, a dopamine receptor antagonist, within the NAC, in rats trained to self-administer EtOH orally. Low doses of fluphenazine (2 and 4 µg/side) significantly reduced EtOH self-administration, without a significant effect on water responding in a two-lever choice paradigm. Since the integrated functional output of the NAC seems to be modulated by glutamate afferents arising from allocortical structures such as the amygdaloid complex and the hippocampal formation, we also evaluated the effect of pharmacological blockade of NAC glutamate receptors on EtOH self-administration. Intra-NAC microinjection of amino phosphonovaleric acid (AP-5, a NMDA receptor antagonist) at the dose of 1.5 and 3 µg/side significantly and selectively reduced responding for EtOH, while blockade of NAC quisqualate receptors was ineffective. These data indicate that a glutamate-dopamine link within the NAC may be part of the neural substrates that mediate EtOH-seeking behavior in the rat.

151.10

EFFECT OF CHRONIC ETHANOL INTAKE AT LOW DOSES ON BRAIN DOPAMINE RECEPTORS. E.Daniele*, S.Govoni*, M.D.Lograno*, F.Matteo*, V.Olgiati#, R.Cagiano*^ V.Cuomo*^, Pharmacobiol. Dept and ^Ins.Pharmacol. Univ of Bari ; #Pierrel Res. Labs. Milano, Italy.

Brain dopaminergic (DA) circuits have been shown to be involved in ethanol (E)-induced tolerance, dependence and withdrawal. On the other hand, there are few studies exploring the effect of prolonged exposure to low levels of ethanol mimiking the pattern of consumption of the subsance observed in southern part of Europe where alcohol can be considered a diet component. Along this line the present investigation studied, in the rat, the effect of a 8 week long treatment with 3% E in the drinking water. Striatal dopamine receptors were evaluated 2 and 24 hrs following E withdrawal by measuring the binding of tritiated spiperone and SCH 23390. The treated animals had an increased number of D1 receptors (respectively 130% and 108% at 2 and 24 hours). Kd values were superimposable in all groups.The biochemical data were in agreement with behavioral data in the same animals showing an enhanced motor response to amphetamine administration.The present results indicate that low levels of E for long periods of time are able to induce a significant increase of DA receptors. It should be stressed that the animals used in the present study did not show tolerance to acute E injection. This observation indicates that the changes of DA receptors may be dissociated from tolerance although it cannot be excluded that they may be important for the development of dependence.

151.11

CHRONIC ETHANOL ADMINISTRATION INCREASES GABA$_A$ RECEPTOR α6 SUBUNIT mRNA LEVELS IN THE RAT CEREBELLUM. A. L. Morrow[1], J. S. Herbert*[1] and P. Montpied[2]. [1]Center for Alcohol Studies, UNC Sch. of Med., Chapel Hill, NC 27599 and [2]NIMH, Bethesda, MD 20892.

Chronic ethanol exposure alters the function of GABA$_A$ receptor-gated Cl$^-$ channels in the CNS. We have recently shown that prolonged ethanol inhalation reduces the expression of GABA$_A$ receptor α1 and α2 subunit mRNAs in the rat cerebral cortex, with no effect on the level of α3 subunit transcripts [Alcohol 7:237-244 (1990); Mol. Pharm. 39: 157-163 (1991)]. In the present study, rats were administered alcohol by liquid diet for 2 weeks using a pair fed design. The rats drank 8-10 g/kg/day of 5-7.5% ethanol diet (BEC = 248±35 mg/dL) and exhibited tremors (100%) and audiogenic seizures (50%) following ethanol withdrawal. GABA$_A$ receptor α subunit mRNA levels were quantified by Northern analysis using subunit specific oligonucleotide and cRNA probes. Cerebral cortical GABA$_A$ receptor α1 subunit mRNA levels were altered as with chronic ethanol inhalation. In the cerebellum, chronic ethanol administration reduced the levels of GABA$_A$ receptor α1 subunit mRNAs (4.8 and 4.4 kb) by 30-40% and increased the levels of GABA$_A$ receptor α6 subunit mRNA (2.7 kb) by 30% ($p<0.05$, n=16). β-actin mRNA levels were also reduced by 29%, whereas poly(A)+ RNA levels were not significantly altered following chronic ethanol exposure by liquid diet. These data suggest that chronic ethanol exposure regulates GABA$_A$ receptor gene expression by differential effects on the synthesis of specific subunits of GABA$_A$ receptors.

151.12

OMEGA-CONOTOXIN AND DIHYDROPYRIDINE LABELLED CALCIUM CHANNELS ARE DIFFERENTIALLY AFFECTED BY ETHANOL IN NG 108-15 CELLS. S.Bergamaschi*, C.Lopez*, F.Battaini#, M.Parenti^, S.Govoni*, M.Trabucchi*#. Inst. Pharmacol. Sci. , ^Dept. Pharmacol.Tox. and Ther. Univ. of Milan, #Dept. Exp.Med. Bioch.Sci. IInd Univ. of Rome, Italy.

Literature data indicate that ethanol (E) affects calcium homeostasis. To get insight into the mechanisms by which E alters calcium movements the present study investigated the effect of E treatment on omega-conotoxin (CgTx) and dihydropyridine (DHP) labelled calcium channels in NG 108-15 cells. Cells were differentiated with dibutyryl cAMP (1mM, 4 days). Both undifferentiated and differentiated cells were exposed to 200 mM E for 72 hrs. Iodinated CgTx and tritiated PN 200-110 were used to label calcium channels. Displacement experiments showed that the two ligands label two distinct binding sites. In undifferentiated cells ethanol exposure induced a large increase in DHP binding sites (Bmax: Control: 24±3.2, E: 42±5.3 fmol/mg prot.). In contrast CgTx binding sites were not modified by the treatment, suggesting that the two channels may be independently regulated and display a differential sensitivity to E. This view was further strenghtened by experiments in differentiated cells where E induced a significant decrease in CgTx binding (Bmax:Control: 33±5.5, E: 19±3.5 fmol/mg prot.).Therefore E appears to disrupt the balance between the two channels favoring the expression of those labelled by DHP while leaving unaffected or inhibiting CgTx sensitive ones. The unbalance between the two channels may be relevant to the changes in neuronal activity which have been observed following ethanol intake.

152.1

ISOLATION AND IDENTIFICATION OF THE ZETA (ζ) RECEPTOR BINDING SITE. I.S. Zagon, S.R. Goodman, G. Allison*, and P.J. McLaughlin. Dept. of Neuroscience and Anatomy, Penn State Univ. College of Medicine, Hershey, PA 17033.

An opioid growth factor (OGF), [Met^5]-enkephalin, interacts with the ζ receptor to regulate growth of normal and abnormal tissues and cells, including those of the nervous system. To identify the cellular and molecular nature of the ζ receptor, homogenates of S20Y neuroblastoma (NB) cells were electrophoresed, electrotransferred and ligand blotted with radiolabeled [Met^5]-enkephalin. Four polypeptides were identified: 32, 30, 17, and 16 Kd. [Met^5]-enkephalin and (-) naloxone, but not (+) naloxone, blocked the appearance of these polypeptides. Subcellular fractionation studies revealed these polypeptides to be located in the nuclear fraction; receptor binding assays confirmed these findings. Two-dimensional gel electrophoresis and ligand blotting were used to identify the 17 and 16 Kd polypeptides, and antibodies were made to these gel purified proteins. Western blotting with antiserum to either the 17 or 16 Kd proteins reacted with 4 polypeptides of 32, 30, 17 and 16 Kd, suggesting a relationship between these binding sites.

152.2

MOLECULAR MODELING OF *SIGMA* RECEPTOR LIGANDS: A MODEL OF BINDING BASED ON CONFORMATIONAL AND ELECTROSTATIC CONSIDERATIONS. T.-P. Su, K. Shukla* and T.M. Gund*, Neuropharmacology Laboratory, NIDA Addiction Res. Ctr., Baltimore, MD 21224 and Dept. of Chemistry, Chem. Engineering & Environmental Sci., New Jersey Inst. Tech., Newark, NJ 07102.

Sigma receptor ligands encompass many structurally dissimilar classes of substances. This report describes conformational and electrostatic potential (ESP) studies on several representative *sigma* ligands to derive a model pharmacophore. The ligands include haloperidol (HAL), (+)3-(3-hydroxyphenyl)-N-(1-propyl)-piperine (PPP), (+)pentazocine (PENT) and progesterone (PRO). Low energy conformations of these compounds were generated using the conformation generator program, RCG5. Quantum mechanical methods were used initially to calculate net atomic charges, which were then used to calculate the ESP on the Van der Waals surface using the ARCHEM program. The four molecules were superimposed to achieve the best fit. Thus, a pharmacophore model was produced as a triangle fitted onto the superimposed molecules. Fitting position (FP) 1 constitutes the nitrogens (N) of HAL, PPP and PENT and C-8 of PRO. FP 2 includes C-7 of PENT, C-2 of PPP, the center of the fluorphenyl ring of HAL and the center of ring B in PRO. FP 3 constitutes the lone pair of electrons on the N for PENT, PPP, HAL and the lone pair of electrons on the C-20 carbonyl oxygen in PRO. Consistency in the position of the ligand charges were also observed from the ESP calculations. The pharmacophore obtained from this study may help advance our understanding of the *sigma* receptor and assist in designing novel antipsychotic and neuroprotective agents.

152.3

LONG-TERM EFFECTS OF MORPHINE ON MU-OPIOID RECEPTOR REGULATED ADENYLATE CYCLASE ACTIVITY AND NORADRENALINE RELEASE IN RAT BRAIN. A.N.M. Schoffelmeer, B.J. Van Vliet*, T.J. De Vries* and A.H. Mulder*. Dept. Pharmacol., Free Univ., Med. Fac., 1081 BT Amsterdam, The Netherlands.

In striatal slices from rat foetuses (E21) of morphine-dependent dams D-1 dopamine receptor-stimulated cAMP production, unlike that induced by forskolin, was markedly enhanced. However, the inhibitory effect of the mu-opioid receptor agonist DAMGO on the stimulated adenylate cyclase activity remained completely unchanged. Interestingly, identical effects were observed in primary neuronal cultures treated chronically with morphine. Similarly, chronic morphine treatment in vivo induced a profound increase of the electrically evoked ^{3}H-noradrenaline release from fetal cortex slices without affecting the presynaptic inhibitory effect of DAMGO. Release induced by the calcium ionophore A23187 was unchanged in morphine-tolerant cortex slices.

These and other data indicate that tolerance to morphine may be due to adaptive changes in stimulatory effector proteins, such as Gs proteins and voltage-sensitive calcium channels, rather than to mu-opioid receptor desensitization, which at the same time may underly morphine withdrawal effects.

152.4

CHRONIC D-AMPHETAMINE INHIBITS OPIOID ANTAGONIST-INDUCED SUPERSENSITIVITY. A. Duttaroy*, B. Billings*, J. Candido* and B.C. Yoburn. College of Pharmacy, St. John's University Queens, NY 11439.

Chronic opioid antagonist treatment (naltrexone: NTX) increases opioid receptor density and agonist potency. Since opioid abusers may adminster nonopioids during treatment, we examined the effect of chronic d-amphetamine (AMP) on NTX-induced upregulation and supersensitivity.

Mice were implanted s.c. with a 15mg NTX or placebo (PLA) pellet for 8 days. Mice were injected with saline (SAL) or AMP (7.5 or 5.0mg/kg/day, s.c.) for 7 days beginning 24hr following implantation. Pellets were removed on the 8th day, and 24hr later mice were tested for morphine analgesia (tail flick) or brains were removed and binding studies conducted. Chronic NTX significantly enhanced the analgesic potency of morphine in SAL treated mice but not in mice treated with 5.0mg/kg AMP (ED_{50}=2.67, 1.47, 2.30, 2.32mg/kg; PLA-SAL, NTX-SAL, PLA-AMP, NTX-AMP; respectively). Similar results were observed for 7.5mg/kg AMP. In saturation studies, NTX increased [^{3}H]DAMGO B_{max} 60-70% without altering K_d in both SAL and AMP treated mice. Studies with 2nm [^{3}H]DPDPE were similar. These studies indicate that daily AMP can limit the expression of NTX-induced supersensitivity but not receptor upregulation. The robust nature of receptor density increases contrasts with the more sensitive functional characteristics of opioid receptors (supported by NIDA DA 04185).

152.5

(+)-BENZOMORPHAN SIGMA LIGANDS STIMULATE STRIATAL DOPAMINE SYNTHESIS. R.G. Booth and R.J. Baldessarini. Div. of Medicinal Chemistry, Sch. of Pharmacy, Univ. of North Carolina, Chapel Hill, NC 27599, & Harvard Medical Sch. & McLean Hosp., Belmont, MA.

Dopamine (DA) synthesis in minced rat corpus striatum was assessed by measuring the activity of tyrosine hydroxylase (TH) with [1-^{14}C]-L-tyrosine. At 0.1 μM, the (+)-enantiomers of the 6,7-benzomorphan *N*-allylnormetazocine (NANM) and pentazocine (but not [+]-cyclazocine) significantly increased TH activity over basal levels by 15% and 20%, respectively. The (–)-benzomorphans were inactive at 0.1 μM, and inhibitory at higher concentrations. The effect of (+)-NANM was not additive with stimulation of TH activity by excess forskolin and was reversed by the DA autoreceptor agonist S(+)-*N*-*n*-propylnorapomorphine (NPA), suggesting a neuroregulatory mechanism involving cyclic-AMP. Stimulation of DA synthesis by (+)-NANM and (+)-pentazocine was fully blocked by the reported sigma "antagonist" BMY-14802 and by the (–)-benzomorphans, but not by the opiate antagonist naloxone. Ligands for phencyclidine, κ- and μ- opiate receptors had no significant stimulatory effect on TH activity. These results suggest that (+)-NANM and (+)-pentazocine may act as agonists at putative sigma receptors in striatum to modulate DA synthesis, perhaps by altering intracellular levels of cyclic-AMP. [Support: UNC-CH Research Grants 5-44339 & 6-69410, USPHS Grants MH-34006 & MH-47370, & Bruce Anderson Foundation]

152.6

INCREASED DOPAMINE RELEASE *IN VIVO* AND ORAL-FACIAL MOVEMENTS FOLLOWING INTRAPERITONEAL ADMINISTRATION OF TWO SIGMA LIGANDS J.M. Walker, S.L. Patrick, R.L. Patrick†. Schrier Research Laboratory, Dept. of Psychology, †Section of Neurobiology, Division of Biology and Medicine, Brown University, Providence, RI. 02912.

Several lines of evidence suggest a role of sigma receptors in the modulation of midbrain dopaminergic neurons: 1) sigma receptors are concentrated in the dopamine-rich substantia nigra pars compacta; 2) unilateral microinjections of potent sigma ligands into the substantia nigra produces contralateral circling behavior that is blocked by 6-hydroxydopamine lesions; and 3) low systemic doses of DTG produce marked increases in glucose utilization in the substantia nigra. We now report the effects of 1,3-di-o-tolylguanidine (DTG) and (+)-pentazocine on extracellular dopamine levels studied using microdialysis in freely moving rats. Before each experiment, a guide cannula was implanted in the right caudate nucleus under nembutal anesthesia. Following a three day recovery period, animals were placed in a clear plexiglass chamber, and perfusion buffer was collected (1.5 μl/min) before and after drug or vehicle injection. Simultaneously, the incidence of vacuous chewing movements and facial tremors was recorded. At the dose of 1 mg/kg i.p., DTG produced a 40% increase in extracellular dopamine levels (ANOVA: $F_{1,11}=15.9$; $P=0.0021$). (+)-Pentazocine produced a similar effect at 10 mg/kg ($F_{1,10}=8.7$; $P=0.01$). (+)-pentazocine is thus about equipotent to morphine in the caudate. Behavioral observations revealed concurrent increases in the frequency of vacuous chewing movements ($p<0.05$) and facial tremors ($p<0.05$) at doses of the drugs that increased dopamine release. These data show that two putative sigma ligands induce significant behavioral and neurochemical effects following intraperitoneal administration.

152.7

SIGMA RECEPTORS LABELED WITH (+)[^{3}H]-3-PPP AND [^{3}H]DTG ARE DIFFERENTIALLY REGULATED BY HALOPERIDOL AND GUANINE NUCLEOTIDES. I. Stein and Y. Itzhak. Depts. of Biochemistry & Molecular Biology & Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Recent studies from our laboratory and others suggest the existence of multiple sigma receptor binding sites in the CNS of rodents. In the present study we examined the effect of repeated exposure of rats to the neuroleptic, haloperidol, on the binding of (+)-3-(3-hydroxyphenyl)-N-1-(propyl)piperidine [(+)-3-PPP] and 1,3-di-o-tolyl-guanidine (DTG) to sigma binding sites in rat brain, and the responsiveness of these sites to guanine nucleotides. Repeated exposure of rats to haloperidol (4mg/kg/day for 14 days) resulted in a 75% decrease in the number of (+)[^{3}H]-3-PPP binding sites and abolished the sensitivity of these binding sites to GTP and Gpp(NH)p. A complete recovery of the total number of (+)[^{3}H]-3-PPP binding sites and the responsiveness to guanine nucleotides was regained 28 days after the treatment with haloperidol was ceased. In contrast, [^{3}H]DTG binding sites expressed neither sensitivity to the repeated exposure to haloperidol nor to guanine nucleotides. These findings further support the hypothesis that (+)-3-PPP and DTG label distinct "sigma" binding sites which are differentially regulated by haloperidol and guanine nucleotides.

Supported by the Theodore and Vada Stanley Foundation, National Alliance for the Mentally Ill, Arlington VA.

152.8

CLONING OF G-PROTEIN COUPLED RECEPTORS FROM SH-SY5Y NEUROBLASTOMA CELLS. L. Toll, C.J. Green* and C.M. Bitler*. SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025.

Based upon the method of Libert et al. (Science, 244, 569, 1989), G protein coupled receptors were cloned from the μ and δ opiate receptor-containing human neuroblastoma cell line SH-SY5Y. Primers from conserved regions in the third and sixth transmembrane spanning segments from G protein coupled receptors were synthesized. These primers were used in a polymerase chain reaction in conjunction with whole RNA from SH-SY5Y which was reverse transcribed, in order to amplify cDNA from G protein coupled receptors. Amplified cDNA was cloned into M13 and sequenced. Four distinct clones of putative G protein coupled receptors have been identified. One clone represents the human ß2 receptor sequence, one represents RDC4, a previously identified, but unknown receptor. The final two clones represent previously unidentified G protein coupled receptors. They have considerable homology with each other, and less homology with other known receptors. The amino acid sequences will be discussed.

152.9

CLONING OF G PROTEIN-COUPLED RECEPTORS FROM NG 108-15 CELLS BY POLYMERASE CHAIN REACTION. L.-Y. Liu-Chen, S. Li* and Y.-W. Chen*. Departments of Pharmacology and Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140.

There is considerable homology within transmembrane domains of G protein-coupled receptors. A method was developed to clone new G protein-coupled receptors by using two oligonucleotide primers, corresponding to highly homologous regions within transmembrane domains III and VI, and performing polymerase chain reaction (PCR) on cDNA from the tissue of interest (Libert et al., Science 244: 569-572, 1989). We took a similar approach to clone potentially unknown G protein-coupled receptors from NG 108-15 cells. NG 108-15 cells express many receptors, including δ opioid, α_2-adrenergic, muscarinic, B_2 bradykinin, substance K, 5-HT_3, P_2 purinergic, endothelin and cannabinoid receptors. mRNAs were isolated from confluent cells and reverse transcribed to cDNA. PCR was performed on cDNA with Taq polymerase and two primers with Sal I or EcoR I restriction site attached. PCR products, ranging from 300 to 1000 base pairs, were cloned into vector pGEM3Zf(+) and their nucleotide sequences were determined by the dideoxynucleotide chain termination method of Sanger. One clone representing partial sequence of a potentially new G protein-coupled receptor has been found. We are in the process of screening for more clones. These clones will be used to screen a cDNA library for full-length clones of G protein-coupled receptors.(Supported by NIDA grant 04745 and Sloan Foundation Fellowship)

152.10

EXPRESSION OF μ, δ, AND κ_3 BINDING SITES IN A HUMAN NEUROBLASTOMA CELL LINE. K.M. Standifer, J. Cheng, J.L. Biedler*, and G.W. Pasternak. Cotzias Laboratory of Neuro-Oncology and Laboratory of Cellular and Biochemical Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Continuously cultured neuroblastoma and neuroblastoma hybrid cell lines have played an important role in the study of opioid receptor pharmacology. We now report that a human neuroblastoma cell line (SK-N-BE(2)-C) expresses μ, δ, and κ_3 opioid receptors in levels similar to those found in rat brain. Total opioid binding was measured with the non-selective ligand ^{3}H-diprenorphine. Analysis of saturation data revealed a B_{max} of 490 fmol/mg protein and a K_D of 0.49 nM. Subtype selective assays were performed with ^{3}H-DAMGO (μ), ^{3}H-DPDPE (δ), ^{3}H-U69593 (κ_1), and ^{3}H-NalBzoH (κ_3). The total number of sites obtained by adding the B_{max} values from saturation studies of the selective assays closely approximated that seen with the non-selective ligand ^{3}H-diprenorphine and can be broken down as follows: 50% μ, 16% δ, and 34% κ_3. The μ binding comprised a combination of both μ_1 and μ_2 sites. We were unable to detect any specific κ_1 binding. Morphine (200 nM) inhibited forskolin stimulated cyclase by 30%, with a maximal inhibition of 60%. We are presently engaged in a more extensive characterization of these binding sites, as well as more functional aspects of the receptors.

152.11

Supraspinal antinociception produced by [D-Met^2]-FMRFamide (DMFa) in mice. R. B. Raffa and C. D. Connelly*. Drug Discovery Research, The R.W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477-0776

The ability of Phe-Met-Arg-Phe-NH_2 (FMRFamide) or [D-Met^2]-FMRFamide (DMFa) to produce antinociception in mice, or to block morphine-induced antinociception, was examined using the tail-flick and tail-immersion tests. DMFa dose-dependently produced antinociception following intracerebroventricular (i.c.v.) administration with ED_{50} values (95% confidence limits) of 5.0 (2.2-7.2) and 12.8 (8.1-19.9) µg in the tail-flick and tail-immersion (55°C) tests, respectively. In contrast, FMRFamide did not produce a maximal effect in the tail-flick test. Naloxone, administered s.c. 20 min prior to DMFa, dose-dependently attenuated DMFa-induced antinociception. The pA_2 value for naloxone was the same against i.c.v. morphine or DMFa and morphine-tolerant mice were cross-tolerant to the antinociceptive effect of DMFa. FMRFamide and DMFa produced rightward, parallel shifts of the morphine antinociceptive dose-response curve. These findings, that DMFa both elicited naloxone-sensitive antinociception and attenuated morphine-induced antinociception, are consistent with the view that FMRFamide-related peptides (FaRPs) are weak agonists at opioid receptors and, further, appear to reconcile the apparently chimeric agonist and antagonist properties of these peptides observed *in vivo*.

152.12

SINGLE-DOSE PHARMACOKINETICS AND ABSOLUTE ORAL BIOAVAILABILITY OF CI-977 IN BEAGLE DOGS. C.M. BARKSDALE, S.E. ROSE*, D.A. TURLUCK*, G.D. NORDBLOM*, and D.S. WRIGHT*. Pharmacokinetics/Drug Metabolism Department, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48105-2430.

CI-977, [5R-(5α,7α,8β)]-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]-dec-8-yl]-4-benzofuranacetamide, hydrochloride, is a novel kappa opioid receptor agonist being evaluated as an analgesic. To determine the pharmacokinetics and absolute oral bioavailability of CI-977, six beagle dogs (3M, 3F) received single 150-µg/kg PO and 15-µg/kg IV doses in a crossover paradigm on separate occasions with a period of 4 weeks between treatments.

Blood samples were drawn at selected intervals up to 24 hours postdose, and plasma was analyzed with a validated radioimmunoassay procedure.

Mean Cmax and tmax values after PO dosing were 3.44 ng/ml and 45.8 min, respectively. Harmonic mean elimination half-life and AUC(0-∞) values were 66 min and 357 ng·min/ml for IV dosing and 326 min and 1,140 ng·min/ml for PO administration, respectively. Mean absolute oral bioavailability was 7.4%.

153.1

DEVELOPMENTAL EXPRESSION OF 5-HT1C RECEPTOR mRNA IN RAT HYPOGLOSSAL NUCLEUS (n XII). W.H.Zhu*, J.T.Erickson, M.F.Czyzyk-Krzeska, E.E.Lawson and D.E.Millhorn. Dept. of Physiology, Univ. of N. Carolina, Chapel Hill, NC 27599.

nXII in rat is innervated by fibers that contain several neurotransmitters including serotonin (5-HT). The present study was undertaken to determine if nXII motor neurons express 5-HT receptor mRNA at different developmental ages (0,7,14,21&28 days). Tissue sections (10μm) through the entire extent of nXII were cut and mounted on gelatin-coated slides. A mixture of two ^{35}S-labeled oligonucleotide probes directed against sequences of mRNA that encode portions of the third cytoplasmic domain and carboxyl terminus of either the 5-HT1A or 5-HT1C receptor subtype were used for in situ hybridization. We found a high level of expression of 5-HT1C receptor in the caudal aspect of nXII in 0 day old rats. This signal became progressively weaker during the first postnatal month and was indistinguishable from background labeling by 28 days. A strong unchanging signal for 5-HT1C receptor mRNA was detected in the choroid plexus and in cells within the ventral aspects of the medulla throughout development. We failed to detect 5-HT1A receptor mRNA in nXII at any developmental age. The significance of transient expression of 5-HT1C in nXII is unknown but could be involved in regulation of the upper airway patency during prenatal and early postnatal development. (HL33831, HL34919, AHA, ALA)

153.2

EFFECTS OF NEONATAL 5,7-DHT TREATMENT ON FOREBRAIN 5HT2 RECEPTORS. J.H. Haring and D. Sawyer*. Dept. of Anatomy and Neurobiology, St. Louis Univ., St. Louis, MO 63104.

Palacios and colleagues (Brain Res. 346:231, 1985 and 524:139, 1990) have shown high concentrations of 5HT2 receptors to be present on the neurons of the neocortex and parts of the basal ganglia. The purpose of the present study was to examine the impact of neonatal 5HT reduction on the development of the forebrain 5HT2 receptor population in the rat.

Neonatal Sprague-Dawley rats of both sexes received subcutaneous injections of 5,7-DHT (100mg/kg in saline) or saline alone on PND0 (i.e. day of birth) and PND1. On PND21, rats were euthanized by sodium pentobarbital overdose. Their brains were removed and rapidly frozen. The status of forebrain 5HT2 receptors was assessed by ketanserin binding in 20um cryostat sections cut in the transverse plane. The distribution of 5HT2 receptors was studied by film autoradiography, while saturation analysis measured receptor numbers (Bmax) and binding affinity (Kd). 5,7-DHT treatment reduced 5HT uptake 50-60% in cortical synaptosomes by PND3. The reduction of 5HT innervation did not appear to change the previously described pattern of forebrain 5HT2 receptors (e.g. the dense band in layer Va). Binding affinity values were lower in treated rats but not statistically different from controls. However, a significant decrease in Bmax was detected in treated rats. These data suggest that 5HT innervation may not be necessary for the neuronal expression of 5HT2 receptors, but may serve to influence the number of receptors during development. Support: NS25752 and DE07734.

153.3

LONG-TERM SURVIVAL OF MONOAMINERGIC NEURONS IN REAGGREGATE TISSUE CULTURE. A. Heller, H.K. Choi, L. Won and P.C. Hoffmann. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

In three-dimensional reaggregate tissue culture, cells interact to permit recapitulation of their normal neurochemical and morphological development *in vivo* (Kotake et al., J. Neurosci. 2:1307-1315, 1982). We have succeeded in maintaining dopaminergic (DA) and serotonergic (5-HT) neurons in three-dimensional reaggregate tissue culture for periods up to 4 months. Dissociated cells from embryonic mice of mesencephalic tegmentum (containing DA and 5-HT neurons) and corpus striatum (a target area for monoaminergic neurons) were reaggregated in 3.5 ml of media containing serum which had been dialyzed to remove endogenous 5-HT. After 1 month in culture, the reaggregates were transferred to 30 ml of media. Following 4 months in culture, the reaggregates were analyzed for DA and 5-HT levels, tyrosine hydroxylase (TH)-immunocytochemistry and histofluorescence. These reaggregates show increasing endogenous levels of DA (118.8 ± 2.7 ng/mg protein; an approximately 3-fold increase over 3 week old cultures), while 5-HT levels (38.2 ± 2.7 ng/mg protein) appear to plateau at an earlier point in development (4 weeks). DA perikarya, processes and patches of punctate varicosities were observed by TH-immunocytochemistry and Falck-Hillarp histofluorescence. The ability to culture monoaminergic neurons over their normal developmental period *in vivo*, allows examination of neuronal function, as well as cell vulnerability to neurotoxic agents at various stages of development. Supported by MH28942.

153.4

CHARACTERIZATION OF ACUTELY DISSOCIATED AND CULTURED NEONATAL RAT OLFACTORY BULB NEURONS. R.L. Shoemaker, G. Bora*, and G. Kapatos. Dept. Psychiatry Wayne State Univ., Detroit MI 48235.

Periglomerular neurons (PGN) in the intact rat olfactory bulb (OB) exhibit both gamma-aminobutyric acid (GABA) and dopamine (DA) neurotransmitter phenotypes. Expression of the DA but not GABA phenotype is dependent upon olfactory receptor neuron (ORN) afferents. As a first step in elucidating the cellular interactions required for the maintenance of the PGN DA phenotype, we have established a culture system for neurons derived from the neonatal rat OB. In the hope of defining and isolating PGN OB neurons we have also examined the expression of voltage-sensitive calcium and sodium channels and glutamate receptors in acutely dissociated OB neurons. Survival of OB neurons in culture was greater with increasing plating density and required the presence of a feeder layer of OB astrocytes. The number of neurons, determined by cell counts after neuron specific enolase immunohistochemistry, was stable for at least 7 days in vitro (DIV). The neurons exhibited diverse morphologies. A majority of the neurons were immunoreactive for GABA, glycine, and glutamate. A small number of neurons, presumably PGN, continued to express tyrosine hydroxylase (TH) immunoreactivity, a marker for the DA phenotype, and their number increased with DIV. The number of TH positive neurons was not increased by nerve growth factor, forskolin or depolarization, suggesting that control of TH gene transcription in PGN by ORN may not involve transcriptional elements previously described for TH. Flow cytometric analysis of intracellular calcium and membrane potential demonstrated that virtually all OB neurons had L-type calcium channels. A subpopulation (35%) contained both L-type calcium channels and sodium channels. In addition, 35% of the total neurons expressed both L-type channels and glutamate receptors. Studies are currently underway to characterize these subpopulations with respect to neurotransmitter phenotype following sorting and culture. (Supported by NIH Grant NS26081).

153.5

CHARACTERIZATION OF SPECIFIC [^{3}H]NOREPINEPHRINE UPTAKE IN DISSOCIATED NORADRENERGIC CELL CULTURES FROM EMBRYONIC RAT RHOMBENCEPHALON. H.K. Raymon and F.M. Leslie. Dept. of Pharmacology, University of California, Irvine, CA. 92717.

Many epigenetic factors have been shown to influence brain development. In order to study the direct effects of these factors on the maturation of a chemically defined system, the noradrenergic system, an in vitro cell culture model was established. Rhombencephali were dissected from the brains of rats at embryonic day 14. Cells were dissociated and plated in either a serum-containing or serum-free fully defined medium. [^{3}H]Norepinephrine (NE) uptake was used as a marker for assessing the maturational stage of the noradrenergic cells. Specific uptake was defined as the difference in uptake in the absence and presence of 10 µM desmethylimipramine (DMI). [^{3}H]NE uptake was temperature and sodium dependent. There was a non-linear relationship between plating density and [^{3}H]NE uptake, with uptake plateauing at higher cell densities. Specific uptake was detectable by 4 days in vitro (DIV) and increased steadily up to 21 DIV. These data are consistent with the findings by Coyle and Axelrod, 1971 (*J. Neurochem.* **18**, 2061-2075), of specific, DMI-inhibitable [^{3}H]NE uptake into rat brain synaptosomes at 19 days gestation.

Previous studies have shown that treatment with KCl has no effect on [^{3}H]NE uptake in mouse locus coeruleus explant cultures. In order to confirm that our system was similar to this well characterized tissue culture system, we tested the effects of 4 and 20 mM KCl in serum-containing and in serum-free medium. No significant effect of KCl on [^{3}H]NE uptake was observed in either medium. The effects of cocaine and other drugs of abuse on the development of these noradrenergic cells are currently being examined.
Supported by NIH grants NS19319 and MH09737.

153.6

DOPAMINE D_1 AUTORECEPTOR FUNCTION: POSSIBLE EXPRESSION IN DEVELOPING RAT PREFRONTAL CORTEX AND STRIATUM. M. H. Teicher, A. L. Gallitano*, H. A. Gelbard*, H. K. Evans*, E. R. Marsh*, R. G. Booth and R. J. Baldessarini. Departments of Psychiatry and Neuroscience Program, Harvard Medical School, Mailman Laboratories for Psychiatric Research, McLean Hospital, Belmont MA 02178.

Dopamine (DA) receptors modulating DA synthesis are known to be either type D_2 or D_3. Attempts to detect D_1 autoreceptors in the adult rat striatum have failed to elicit any compelling evidence for their existence. However, the density of D_1 receptors in forebrain changes markedly with development. We hypothesized that the transient postnatal surge in D_1 density might include some autoreceptors. To test this hypothesis we studied 294 developing rats (15 days - adulthood) using the γ-butyrolactone (GBL) technique of Walters & Roth. The selective DA D_1 agonist SKF-38393 inhibited DA synthesis in the striatum and prefrontal cortex (PFCTX) of 15 and 22-day-old rats, but did not inhibit synthesis in adults. The effects of SKF-38393 in developing rats were blocked by the D_1 antagonist SCH-23390, and were mimicked by the non-catechol D_1 agonist CY-208-243, suggesting receptor mediation. The mixed D_2/D_3 agonist quinpirole attenuated DA synthesis in striatum of both 16-day-old and adult rats, but failed to inhibit the GBL-induced increase in DA synthesis in the developing PFCTX. These findings suggest that synthesis-modulating D_1 function may emerge in developing forebrain. In the adult striatum these functions appear to be subsumed by D_2 or D_3 receptors, whereas synthesis-modulating DA receptor function appears to be essentially lost with age in the PFCTX.

153.7

L-DOPA DOES NOT ALTER THE LEVELS OF DARPP-32 mRNA IN MOUSE BRAIN. R. M. Lewis and R. Perez. Dept. of NACS, Univ. Pittsburgh, Pittsburgh, PA 15261.

DARPP-32 (dopamine- and adenosine 3',5'-monophosphate-regulated phosphoprotein of M_r=32,000) is a cytosolic phosphoprotein phosphatase inhibitor which is phosphorylated by cAMP-dependent protein kinase in response to dopamine. DARPP-32 is enriched in regions of the brain which have D1 dopamine receptors. Previous studies indicate that neither expression of DARPP-32 mRNA during development of the brain, nor maintenance of DARPP-32 mRNA levels in the adult appears to require dopamine. We tested the possibility that excess dopamine could down-regulate the expression of DARPP-32 mRNA. Mice were injected daily with 100 or 200 mg/ml L-DOPA (i.p., 30' after 50 mg/ml Ro 4-4602, i.p.) for one to five days, and sacrificed six hours after the last injection. Levels of DARPP-32 mRNA were assessed by in situ hybridization in sagittal sections of brain. No changes in DARPP-32 mRNA levels were detected in any region of the brain compared to saline-injected animals. This evidence supports the conclusion that DARPP-32 gene expression is independent of dopamine input.

153.8

USE OF ^{3}H-GBR12935 TO MEASURE DOPAMINERGIC NERVE TERMINAL GROWTH INTO THE DEVELOPING RAT STRIATUM. W.Le', J.R.Bostwick, G.Crawford, and S.H.Appel. Dept. Neurology, Baylor Col. Med., Houston, TX. 77030

Various biochemical markers have been used to index dopaminergic innervation of the striatum during development. Earlier studies have shown that increases in tyrosine hydroxylase (TH) activity, high affinity dopamine (DA) uptake and DA content in the striatum are associated with nerve terminal formation. GBR12935, a potent and selective inhibitor of DA uptake, binds with high affinity to the presynaptic DA transporter. In this study we determined the temporal association between the appearance of the DA transporter, measured by ^{3}H-GBR12935 binding, and other biochemical markers of DA nerve terminal growth into the developing striatum.

The B_{max} and K_d of ^{3}H-GBR12935 specific binding in adult rat striatal tissue were 3.6 pmoles/mg protein and 2.8 nM, respectively. ^{3}H-GBR12935 binding was minimally detected in the rudimentary striatum of ED14 rat brains, increased to 23% of the adult level by birth, and reached the adult level during the fifth postnatal week. This finding contrasts with a slower developmental increase in ^{3}H-DA uptake, a functional measure of the transporter. TH activity levels followed a developmental curve similar to that of ^{3}H-GBR12935 binding but did not reach adult levels until the seventh postnatal week. DA content increased at a slower rate, being only 10% and 92% of the adult level at birth and postnatal week eight, respectively. These results indicate that the appearance of a structural, but not optimally functional, DA transporter may be the earliest biochemical index of dopaminergic nerve terminal growth into the striatum during development.

Supported by the American Parkinson's Disease Association and the Cullen Foundation.

153.9

QUINPIROLE-INDUCED ALTERATIONS IN K+-EVOKED DOPAMINE RELEASE IN THE NEOSTRIATUM OF DEVELOPING AND ADULT RATS. S.L. Andersen and R.A. Gazzara. Center for Developmental Psychobiology and Department of Psychology, SUNY-Binghamton, Binghamton, NY 13902-6000.

The effect of local administration of the D-2 agonist quinpirole, via the microdialysis probe, on K+-evoked dopamine (DA) release in the neostriatum was assessed in urethane-anesthetized pups aged 5, 10-11, 15-16, and 21-22 days, and adult rats. Samples were collected every 15 min and extracellular levels of DA, DOPAC, HVA, and 5-HIAA were measured with HPLC-ED.

A dose-response curve (0.1, 1, 10, and 100 uM) of (-)-quinpirole was conducted within animals to study the development of release-modulating D-2 autoreceptors. Quinpirole did not produce significant decreases of K+-evoked DA release at all ages at all doses. Additionally, a developmental trend was found in that the adult group showed the largest decrease in K+-evoked DA release at the highest dose of quinpirole (100 uM). The effect of quinpirole was blocked by the D-2 antagonist (-)-sulpiride. These data suggest that the DA D-2 autoreceptors are functional in the neostriatum by 5 days of age, but are not completely mature in their ability to regulate K+-evoked DA release.

Supported by NIH BRSG Grant S07RR07149-17.

153.10

CHARACTERIZATION OF NEOSTRIATAL DOPAMINE RELEASE AS MEASURED BY MICRODIALYSIS IN DEVELOPING AND ADULT RATS. R.A. Gazzara and S.L. Andersen. Center for Developmental Psychobiology and Dept. of Psychology, SUNY-Binghamton, Binghamton, NY 13902-6000.

We have used microdialysis to measure the extracellular levels of dopamine (DA) in the neostriatum of adult rats and 5-day-old pups. This study determined the neurogenic origin of the DA measured as defined by its calcium (CA) dependency and tetrodotoxin (TTX) sensitivity.

In urethane-anesthetized 5-day-old rat pups and adult rats, baseline levels of DA were measured during perfusion with a CA-containing dialysis solution. When the solution was switched to a CA-free solution, DA dropped to nondetectible levels in the rat pups, and to less than 20% of baseline levels in the adult rats. In a separate group of animals, when 1 µM TTX was added to the dialysate, DA dropped to nondetectible levels in both groups. The CA dependency of high K^+-evoked DA release was also determined in rat pups and adult rats. Baseline K^+-evoked DA levels were measured during perfusion with CA-containing dialysis solution. When the solution was switched to a CA-free solution, the K^+-evoked DA levels dropped to nondetectible levels in rat pups, and to less than 25% of baseline K^+-evoked DA levels in adult rats.

These results suggest that the extracellular DA measured in 5-day-old rat pups is derived from neurogenic release as determined by its CA dependency and TTX sensitivity.

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153.11

FREE RADICALS, MONOAMINE OXIDASE AND PARKINSON'S DISEASE. F.F. Oldfield, D.L. Cowan* and A.Y. Sun. Departments of Pharmacology and Physics*, University of Missouri, Columbia, MO 65212.

An electron spin resonance (ESR) spin trapping technique has been used to detect free radical formation during the enzymatic reaction of dopamine (DA) with either purified monoamine oxidase (MAO) or brain mitochondrial preparations. The ESR spectra were those of the hydroxyl adduct of the spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO). When ethanol is added, hydroxyethyl adducts are observed, confirming the presence of hydroxyl free radical ($^{\cdot}$OH). Quantitative analysis indicates that the number of hydroxyl radicals released to the solution is comparable with the number of dopamines oxidized. Hydroxyl adduct formation is eliminated by denaturing the MAO and decreased by addition of the MAO inhibitors Clorgyline, Pargyline and Deprenyl. Results with added Fe^{2+} and with the Fe chelators, Diethylenetriaminepentaacetic acid (DETAPAC) or Desferroxamine, implicate redox-cycled trace Fe in the $^{\cdot}$OH radical production. Addition of superoxide dismutase strongly inhibits radical formation. The free radical formation by DA was substantially greater than that produced in an incubation medium containing an equivalent amount of the other neurotransmitters, serotonin or norepinephrine. These results support the hypothesis that oxidative stress associated with DA metabolism plays a role in the tissue injury and cell dysfunction seen in the Parkinsonian brain.

(Supported by AA7585).

154.1

POSTNATAL CHANGES IN THE DISTRIBUTION OF ACETYLCHOLINESTERASE IN THE RABBIT VISUAL CORTEX. B. Ju* and E.H. Murphy, Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

The rabbit visual cortex contains an extensive network of acetylcholinesterase (AChE)-positive fibers. Since there is evidence that cholinergic innervation influences cortical development and plasticity, we have studied the postnatal development of AChE in the visual cortex of the rabbit. Eye opening in the rabbit occurs on day 11 and their cortex is mature by day 50. We studied animals aged 1-48 days using a modification of Koelle's histochemical method. Labeled axons are present in all layers of area 17. In layer I, there is little change in AChE-positive fiber density during the first two postnatal weeks. Between days 15 and 20, AChE-positive fiber density increases rapidly, followed by a more gradual increase to adult levels over the next several weeks. In contrast, AChE positive fibers in layers II and III show their greatest increase in staining density between days 10 and 15. In layers IV-VI, levels of AChE positive fibers increase steadily throughout development. Beginning at day 12, AChE positive cell bodies are present in layers V and VI. We have shown that the time course of the increase in AChE staining intensity varies for different laminae of area 17. In addition, our results show that the dramatic increase in AChE staining intensity in layers II and III is correlated with eye opening.

154.2

PRE-AND POSTNATAL DEVELOPMENT OF GLUTAMATE EXCITATORY AMINO ACID RECEPTORS IN MONKEY STRIATE CORTEX. #C. Shaw, +A. Hendrickson and +*A. Erickson. #Depts. of Ophthalmology and Physiology U. British Columbia, Vancouver Canada V6T1Z3, Canada, and +Depts. of Biological Structure and Ophthalmology, U. Washington, Seattle 98195.

Using conventional in vitro autoradiographic methods, we have used [3H] MK801, [3H] kainate, and [3H] CNQX to examine the distributions of glutamate receptor subtypes NMDA, kainate, and quisqualate respectively from fetal day (F) 72 until adulthood. **NMDA** receptors were found in the cortical plate at F72, but showed no laminar preference until F152 when a faint band of denser labelling was present in layer 4. By P4-7wk layers 4C and 6 had the densest binding. At P10mo layer 4C was darker than the supragranular layers, a pattern which persisted into adulthood. **Kainate** receptors were first detected at F72 in a band at the bottom of the cortical plate. At F126 two clear bands of label were observed in deep layer 6 and 4. Around birth, layer 6 was the most heavily labelled, the bottom of layer 4C showed a narrow band of label, and layer 4C was lighter than the supragranular layers. At P4-7wk, the narrow band in layer 4C was gone. Layer 6 was still the most heavily labelled and some label was present in layer 5. After P13mo into adulthood layers 6 and 5 alone were heavily labelled. **Quisqualate** receptors were first detected at F126 and were most dense in layer 6. By P2d most of the cortex was labelled with the exception of layer 4C. A thin narrow band of label centered on layer 4A appeared just before birth and persisted into adulthood. By P7wk layers 4C and 4B were quite light. From P20wk to adulthood layers 5 and 6 were densely labelled, layers 4C/4B were light and layers 1-3 were moderately labelled.

These results reveal differences in the age of first appearance for the various glutamate receptor subtypes and complex changes in their laminar distribution during development. The lack of a general overlap of receptor populations in the thalamic input layer 4 raises questions about the possible role of glutamate receptors in mediating geniculo-striate developmental interactions. The role of visual experience in shaping the characteristics of these receptors is under investigation. (EY01208; MRC PG-29; B.C. Medical Services Fnd. Grant)

154.3

NMDA RECEPTORS AND L-TYPE CALCIUM CHANNEL BINDING SITES RE-ORGANIZE DURING THE CRITICAL PERIOD FOR KITTEN VISUAL CORTEX PLASTICITY. M. Cynader, Y.L. Liu, W.G. Jia, V. Booth* and W. Jacobson†, Dept Ophthalmology, University of British Columbia, Vancouver, and †Div Reproductive Science, Toronto General Hospital, Toronto, Ontario, Canada.

Calcium entry into neurons plays an important role in many intracellular processes, including those associated with growth and activity-dependent plasticity. We compared the development of two calcium entry systems in the developing kitten visual cortex by using [^{125}I]MK-801 to label NMDA receptors, and [^{3}H]PN-200 to label L-type calcium channels.

Both binding sites showed a changing laminar distribution during the peak period for visual cortex plasticity. In young kittens (0-10 days of age) both ligands showed concentrations in cortical Layers IV and V). In older kittens (20 and 30 days of age), NMDA receptors were concentrated in the middle cortical layers (around Layer IV). By 40 days of age, their peak concentration was in the superficial layers (Layers I-III), and this distribution was maintained into adulthood. The PN-200 binding sites attained a final distribution similar to that of the NMDA binding sites, *i.e.*, concentrated in the superficial layers of the cortex, but they achieved this earlier in postnatal life than did the NMDA sites.

These results suggest that subtle differences in the spatial and temporal developmental pattern of these calcium entry systems play a role in critical period plasticity.

154.4

LOCALIZATION OF NERVE GROWTH FACTOR BINDING SITES IN DEVELOPING KITTEN VISUAL CORTEX. I. Cokgor, R.H. Dyck, M.S. Cynader, Department of Ophthalmology, University of British Columbia, Vancouver, B. C. Canada, V5Z 3N9

Nerve Growth Factor (NGF), is a target-derived neurotrophic protein that promotes the survival and growth of several specific populations of neurons. Extensive work now suggests that NGF, besides its actions on peripheral neurons, also plays a critical role in brain function and development. We studied NGF binding sites in developing cat visual cortex using the method of receptor autoradiography. Tissue sections from cats ranging in age from birth to adulthood were incubated for two hours in 100 pM [^{125}I] NGF (NEN Products). After incubation, the sections were washed for three minutes in three changes of Hepes buffer (20 mM) before they were exposed to X-ray film.

We found NGF binding sites to display strong concentrations in visual cortex only for the youngest kittens studied (0,10 days postnatal). These binding sites were concentrated in the most superficial cortical layers, where neurons are the least mature. In older kittens, no evidence for layer-specific concentrations of NGF binding sites was found in the visual cortex, although binding was found in nonvisual structures such as medial septum, and hippocampus. These results show that NGF may function as an endogenous neurotrophic factor in the developing cat visual cortex and is expressed only at younger ages.

154.5

ULTRASTRUCTURAL LOCALIZATION OF ZINC IN THE DEVELOPING CAT VISUAL CORTEX; . C. Beaulieu, R. Dyck, and M. Cynader, University of British Columbia, Dept. Ophthalmology, Vancouver, B.C. Canada.

Light microscopic studies have demonstrated that the distribution of the chelatable pool of zinc in the cerebral cortex and hippocampus appears to be developmentally regulated. In order to analyse the ultrastructural distribution of this pool of zinc, visual cortex of cats at postnatal days 0-1 (n=3), 15 (n=1), 52 (n=1), and adult (>6 months; n=3) were analysed. Zinc was revealed by using the selenide method developed by Danscher.

Zinc was found to be localized mostly in a population of terminals containing round vesicles and making asymmetrical synapses. In these structures, the reaction product was concentrated mostly over the synaptic vesicles. Some multivesicular bodies present in the cell cytoplasm were also found to contain zinc. This staining pattern is similar among the different ages studied. The staining intensity however varies among the different ages. The concentration of the silver grains over positive terminals is higher in adult animals than in young ones. This could be due to the greater number of synaptic vesicles present in terminals of adult cortex.

Since endogenous zinc has been shown to affect neuronal activity by modulating GABAergic and glutamatergic neurotransmission, we also analyzed the GABA- and glutamate-content of Zinc-positive terminals in adult cortex, by using a post-embedding immunogold technique. We found that no Zinc positive terminals evaluated to date were immunopositive for GABA. The glutamate content of the zinc-positive terminals is presently under investigation.

154.6

Postnatal development and laminar distribution of noradrenergic (NA) fibers in cat visual cortex

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In our previous studies, we have demonstrated that adrenergic receptors (α 1, α 2, ß 1 and ß 2) showed significant changes either in laminar distribution or in number during the postnatal development of kitten visual cortex . In order to further investigate the postnatal development of this neurotransmitter system in kitten visual cortex, especially in relation to the critical period, we used a polyclonal antibody against dopamine-ß-hydroxylase (DBH) to localize noradrenaline (NA)-containing afferents in visual cortex of kittens of various ages from birth to adulthood. In young kittens, less than two weeks of ages, NA fibers were sparse, short and randomly oriented, and were concentrated in layer I and in deep cortical layers V and VI. By postnatal day 40, the density of NA fibers was much higher than that of younger kittens. NA fibers were present throughout all cortical layers and exhibited higher densities in layers I, II, III, V and VI, with a band of lower staining in the layer IV. While tangential fibers predominated in layers I, V and VI, relatively straight radial fibers traversed layers II and III. After postnatal day 40, we did not find major changes either in the density or in the laminar distribution of NA fibers. This developmental laminar distribution pattern of NA fibers resembles that of the ß-adrenergic receptors that we and others have studied in kitten visual cortex.

154.7

NEUROGRANIN: DEVELOPMENTAL EXPRESSION OF A GAP-43 RELATED PKC SUBSTRATE IN CAT VISUAL CORTEX; Y.H.Yucel#, J. Baudier* And M.S. Cynader#. #Dept. Ophthalmology UBC Vancouver, CANADA,* Lab. de Biologie Moléculaire du Cycle Cellulaire, Grenoble, FRANCE.

The developmental expression of neurogranin (NG), a brain specific protein kinase C (PKC) substrate, was investigated in cat visual cortex with immunocytochemical techniques using a specific anti-neurogranin polyclonal antibody. NG and GAP-43, bind calmodulin in the absence of calcium and share a 17 amino acid sequence that correspond to the PKC phosphorylation site and to the calmodulin binding domain. Immunoreactivity was localized mainly in cell bodies and occasionally in dendrites which contrast with axonal localization of GAP-43. In adult visual cortex, NG immunoreactivity was distributed in a trilaminar pattern: Layers I and layer II, layer IVa and Layer VI. Layers I and II showed the highest number of cells labelled and intensity of NG immunoreactivity. Layers IVa and layer VI also demonstrated marked immunoreactivity relative to the other layers. Pyramidal cells were the main cells identified by NG in the superficial layers, in contrast to the stellate cells identified in the deeper layers. In kitten visual cortex, at day P0, P10, in contrast to the adult pattern, immunoreactivity was bilaminar and higher in the deep layers, primarily in the pyramidal cell bodies and apical processes. At P90 and P120, the distribution of NG reactive cells was similar to that seen in the adult visual cortex. NG immunoreactivity in the developing cat visual cortex seems to show an "inside first and outside last" spatiotemporal distribution, paralleling the known developmental process in this structure. Its developmental expression and resemblance to GAP-43 suggest a role for this substrate of PKC protein in visual cortex development.

154.8

DEVELOPMENTAL EXPRESSION OF THREE TRANSCRIPTION FACTORS IN KITTEN VISUAL CORTEX

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The developmental expression of three putative transcription factors, c-fos, jun-b and NGFI-A, was examined using immunocytochemistry. This was done in an attempt to identify genes that are important for the establishment of the critical period of plasticity in the development of kitten visual cortex. We found only low levels of neuronal expression of these molecules on the day of birth. The 10 day old kitten shows c-Fos staining in layer VI and JunB staining in all layers. Also, NGFI-A appears in layers II, III, and VI of area 17 and layer VI of area 18. At 20 days of age, c-Fos and JunB show staining in layers IV and VI of area 17 and lower levels of staining in layer VI of area 18. In contrast, NGFI-A appears in layers II, III, IV, and VI of area 17 and layers III and deep IV of area 18. Adult patterns of expression are established by 50 days of age. At this time, c-Fos and JunB show prominent staining bands in layers II, III and VI. NGFI-A-positive cells appear in layers II, III, very deep IV and VI. The identical nature of the c-Fos and JunB staining, with the exception of the day 10 animal, is consistent with their formation of heterodimers in order to bind to DNA. Staining of pyramidal and non-pyramidal cells is seen with all three transcription factors, and the localization of these proteins is restricted to the nuclei of expressing cells. The variation in expression of these molecules during the critical period suggests that they may be fundamently involved in this phenomenon.

154.9

C-FOS EXPRESSION IN VISUAL CORTICAL AREAS OF DARK-REARED KITTENS FOLLOWING EXPOSURE TO LIGHT.

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We have examined the possibility that expression of an immediate early gene (c-fos) may mediate or otherwise be linked to the substantial and rapid changes that occur in the mammalian central visual pathways in response to visual stimulation in early postnatal life. Thirteen kittens were placed with mothers in a darkroom within two days of birth. At 30 days of age, nine of them were introduced to an illuminated environment for either 1,2 or 6 hrs at which time they were immediately sacrificed and perfused. The remaining 4 kittens were sacrificed at the same age in the darkroom after receiving equivalent motor stimulation as their littermates that received exposure to light. Immunocytochemical methods were employed to examine expression of c-fos in the midbrain, thalamus and various visual cortical areas. The animals deprived of any visual input showed no evidence of c-fos expression in any visual structure. However, those kittens exposed to light for 1 hr or more exhibited dense labelling in cortical areas 17, 18 and 19 as well as other cortical visual areas. Sparser label was also evident in the superficial and deep layers of area 19 and the suprasylvian visual areas as well as the ventral (but not dorsal) LGN. In animals exposed to light for 1hr, labelled cells in areas 17 and 18 were distributed approximately uniformly across all layers. Following 2 hrs of visual exposure the labelled cells were concentrated more in superficial (II, III) and deep (VI) layers. This laminar distribution of labelled cells was even more apparent after 6 hrs of visual exposure and the overall number of cells was also very reduced.

154.10

LIGHT EXPOSURE INDUCES A DEVELOPMENTALLY SPECIFIC GENOMIC RESPONSE IN RODENT VISUAL CORTEX. P.F. Worley, R.V. Bhat and J.M. Baraban. Depts. of Neurology and Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Recent studies indicate that synaptic activity induces a rapid genomic response in adult neurons that may be involved in neuroplasticity. Activity plays a major role in postnatal development yet relatively little is known about genomic responses to activity in developing brain. To examine and compare activity-dependent genomic responses to physiological stimuli in developing and adult cortex we are using a rodent model of visual deprivation. The model is based on classical studies indicating that development of the visual cortex is delayed (or altered) in animals raised in the dark and that subsequent visual experience can reinstate the activity-dependent developmental processes. Rodents are used because of the potential utility of this model for future molecular biological studies. Rat pups raised in complete darkness to 3-5 weeks are placed in normally illuminated cages for defined intervals prior to sacrifice. Levels of mRNA and immunostaining for *c-fos*, *zif268*, and *jun*-B are rapidly increased in neurons of the visual cortex of these animals following light exposure. This response involves a broader array of transcription factors and is much more robust than the response to light in dark-adapted adult animals. Aspects of the developmental time course, anatomic specificity and pharmacology of the response will be presented.

154.11

SPECIFIC ELEVATION OF EXPRESSION OF THE mRNA FOR THE BETA ISOFORM OF PROTEIN KINASE C IN KITTEN VISUAL CORTEX DURING THE CRITICAL PERIOD. R.L. Neve and M.F. Bear. Dept. of Psychobiology, U. of Calif., Irvine, CA 92717; Center for Neural Science, Brown University, Providence, RI 02912.

The kitten striate cortex displays considerable synaptic plasticity during a critical period of postnatal development. We are exploring the possibility that cortex is rendered modifiable during this time by specific patterns of gene expression.

RNA isolated from visual cortex (A17), cortical area 6 (A6), and cerebellum of kittens at various postnatal ages was examined with Northern blots and quantitative slot blots to determine the developmental course of expression of genes encoding the protein kinase C (PKC) isoforms. Of particular interest is the developmental pattern of PKC beta, which is expressed at high levels in A17 (greater than 300% of postnatal day 84 [P84] value) during the critical period. The developmental profile of PKC beta expression in A6 is similar to that in A17 with the exception that it is maintained at a high level in A6 even at 12 weeks of age. The expression of PKC alpha rose slightly between P10 and P60, and dropped in both A17 and A6 by P84. PKC gamma and epsilon mRNAs varied among time points without displaying a developmental trend in both A17 and cerebellum. In situ RNA hybridization studies to define the laminar and cellular specificity of expression of PKC isoform mRNAs in the visual cortex are in progress.

154.12

LAMINAR DISTRIBUTION OF EXCITATORY AMINO ACID- AND CARBACHOL-STIMULATED PI TURNOVER IN THE KITTEN STRIATE CORTEX. S.M. Dudek, M. Catallozzi* and M.F. Bear, Center for Neural Science, Brown University, Providence, RI 02906.

Work in our laboratory has focused on the possible role of excitatory amino acid (EAA) receptor mechanisms in ocular dominance plasticity in the kitten visual cortex. We have found that the developmental expression of the metabotropic EAA receptor, in particular, correlates closely with the critical period for this type of synaptic plasticity. However, lack of selective, high-affinity ligands for this receptor or insoluble products of receptor-stimulated PI hydrolysis have thus far hindered antatomical studies localizing EAA-stimulated PI turnover. Recently, Hwang, et al (*Science* 249: 802) described a method to visualize the sites of PI turnover utilizing tritiated cytidine. ^{3}H-labeled cytidine diphosphate diacylglycerol is thought to accumulate in the tissue proportionately to levels of PI turnover. We used a variation of this technique to investigate the laminar distribution of EAA- and carbachol-stimulated PI turnover in the kitten visual cortex.

Trans-ACPD, an agonist of the metabotropic receptor, stimulated the accumulation of ^{3}H in kitten Area 17 (P35-50). All layers showed activity above background, but layer IV was the most densly labeled. In contrast, the muscarinic acetylcholine agonist carbachol stimulated labeling in superficial layers, layer V, and lower layer VI. Work is in progress to examine whether these laminar patterns change during development and to test the hypothesis that metabotropic EAA receptors are located on the geniculocortical axon terminals. Supported by NIH grant EY06929.

154.13

IDENTIFICATION OF PROTEINS DOWN-REGULATED DURING POSTNATAL DEVELOPMENT OF THE CAT VISUAL CORTEX. P. Kind, C. Blakemore and S. Hockfield. Sect. Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510

The visual system of the cat has been used extensively as a model system for studying developmental plasticity in the mammalian nervous system. While numerous techniques exist for studying physiological and anatomical changes through development, few exist which permit the study of biochemical changes that underlie these functional changes. The present study was designed to identify proteins that may play a role in the development of the mammalian visual system and govern the parameters and termination of developmental plasticity.

An immunosuppression strategy (Hockfield, 1987) was used to generate monoclonal antibodies to antigens present in the 5 wk. kitten visual cortex (VC) but which are reduced or absent in the adult cat VC. Six neonatal mice were tolerized to homogenized adult cat VC (areas 17 and 18); three mice received a high dose (HD: 9 mg/injection) and three received a lower dose (LD: 4.5 mg/injection). Two weeks after the tolerizing injections, the mice were immunized with 5 wk. kitten VC (50 ug/injection) in the rear foot pads. Hybridomas derived from lymphocytes from the popliteal and inguinal lymph nodes were screened for antibody production immunocytochemically on 5 wk. VC; positive clones were subsequently tested on both 5 wk. and 15 wk. VC for differential staining patterns.

A total of 18 antibodies showed either a change in cortical distribution or a decrease in the amount of staining between the 5 wk. and 15 wk. VC (12 from the HD mice and 6 from the LD mice). Of the 18 clones, 11 were frozen after the first or second subcloning and 7 were further subcloned and their distribution, biochemistry and developmental expression partially characterized.

These results demonstrate that the biochemical make-up of the kitten VC is distinct from that of the adult, suggesting that molecular changes underlie developmental plasticity and its termination. They also show that neonatal tolerization can be an effective method of producing antibodies to a select group of antigens which are not shared between two tissues. [Supported by NEI(SH) and McDonnell-Pew(PK)].

154.14

DEVELOPMENT OF CALBINDIN-D AND PARVALBUMIN IMMUNOREACTIVITY IN INTERNEURONS OF KITTEN VISUAL CORTEX. N.E.J. Berman and D. Hogan. Dept. of Anatomy and Cell Biology, Univ. of Kansas Medical Center, Kansas City, KS, 66103.

Calbindin-D (CalD) and parvalbumin (PV) are calcium binding proteins that have been shown to be markers of non-overlapping subpopulations of GABA-ergic interneurons in mammalian neocortex. We have examined the ontogeny of CalD and PV immunoreactive interneurons in five areas of kitten visual cortex from the day of birth (P0) through P101. During the first 3 postnatal weeks, PV-ir interneurons were dense in the more mature layers of cortex, but were not observed in the overlying cortical plate. At three weeks of age, when cortical lamination is mature, stained cells were found in all cortical layers except layer I. The density of PV-ir neurons within the mature layers did not change significantly with age. At all ages examined, area 17 contained twice the density of PV-ir neurons as did the most lateral visual areas. In addition, after 3 weeks of age, layers II/III of the cortex had a 30-50% higher density of PV-ir neurons than layers IV, V and VI in all five areas examined. By contrast, very few CalD-ir interneurons were seen during the first two weeks of life, when it is expressed transiently by pyramidal cells (see Hogan and Berman, '91). Those few observed were scattered in all layers of cortex, cortical plate, and white matter. The first increase in density of CalD-ir cells occurred in layer II of cortex around P14. In the mature cortex, the greatest density of CalD-ir cells remained in layer II, similar to the pattern of mature PV staining. In contrast to PV, interneurons in lateral areas express Cal-D sooner, but there was no difference in the distribution of CalD-ir neurons among visual areas at maturity.

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154.15

CALBINDIN-D IS TRANSIENTLY EXPRESSED IN PYRAMIDAL CELLS OF NEONATAL KITTENS IN AN AREA DEPENDENT PATTERN. D. Hogan and N.E.J. Berman. Dept. of Anatomy and Cell Biology, Univ. of Kansas Medical Center, Kansas City, KS, 66103.

Calbindin-D (CalD) is a 28 kD calcium binding protein that has been shown to colocalize with GABA-ergic interneurons in mammalian neocortex. We have examined the ontogeny of CalD in neonatal kitten cortex from the day of birth (P0) through maturation of the brain (P101). Transient staining of immature layer V pyramidal cells was seen in kittens younger than three weeks. This is unusual since pyramidal cells are never labeled neonatally with other markers of GABA-ergic neurons such as parvalbumin, neuropeptide-Y or the related PYY, cholecystokinin, somatostatin 28, or its derivatives SOM 14, or SOM 28(1-14). This transient staining of pyramidal cells was most intense and the stained neurons were densest in cingulate cortex. Apical dendrites of pyramids in cingulate cortex were prominently stained and could be followed to layer I, where they were seen to branch extensively. CalD stained pyramidal neurons were nearly continuous throughout layer V in coronal sections though rostral brain at approximate AP levels A18-A20. In caudal sections, levels A4-A8, staining was most notable in its absence from area 17 of primary visual cortex. This lack of staining in visual cortex appeared to be selective, since this pattern was opposite to the rostolateral to caudomedial progression of cortical maturation. Transient staining disappeared first from caudal and lateral areas, though it was lost first in the sulci and persisted longer in the gyri. Pyramidal neurons in the cingulate gyrus expressed CalD longest, but CalD expression by pyramidal neurons ceased by the third postnatal week in all areas of the brain. Supported by MH38399, BNS881997 and RCD8954894.

AGING PROCESSES II

155.1

AGE-RELATED Mg^{2+} EFFECTS ON PAIRED-PULSE FACILITATION AND LONG-TERM POTENTIATION IN CA1 HIPPOCAMPAL FIELD POTENTIALS. D.L. Deupree and D.A. Turner. Neurosurgery and Neurobiology, Duke Univ. Med. Ctr. and Durham VAMC, Durham, N.C. 27710.

The sensitivity of synaptic plasticity to bath Mg^{2+} was investigated in hippocampal slices from young (2 months) and old (24 months) male F344 rats. The concentration of Mg^{2+} in the ACSF was altered to study presynaptic and circuitry influences of Mg^{2+}, as well as the effects upon NMDA receptor involvement, in brief and long-lasting forms of synaptic enhancement.

Hippocampal slices (n = 5 for each trial) were prepared and extracellular population spike field potentials (50% maximal amplitude) were recorded from the CA1 region. The experiments included paired-pulse facilitation and long-term potentiation (LTP: 10 pulses, 50 μsec each, at 100 Hz).

When bathed in 4.0 Mm Mg^{2+} slices from young rats showed increased paired-pulse facilitation at 50 msec (compared to slices in either 1.0 or 2.4 Mm Mg^{2+}: 4.0 mM 294 $\pm$ 30% baseline, 2.4 mM 200 $\pm$ 12%; $p<0.05$). Slices from aged rats showed no changes with manipulation of Mg^{2+} during paired-pulse experiments. Slices from both age groups showed equal decreases in LTP with increasing Mg^{2+} concentrations. However, at any given Mg^{2+} level, the degree of LTP in slices from aged rats was less than that in slices from young rats (in 2.4 mM Mg^{2+}: aged 128 $\pm$ 13%, young 166 $\pm$ 6.7%; $p<0.05$).

The paired-pulse results suggest either circuitry changes or a deficit in Ca^{+} handling in aged rats. The LTP results may indicate an age-related decrease in either NMDA receptor function or linkage to Ca^{2+} dependent biochemical processes underlying LTP. Overall, these experiments suggest that brain aging is associated with altered synaptic plasticity, which may underlie age-related memory deficits. Supported by the VAMC Research Service and ADRDA.

155.2

ALTERED ELECTROPHYSIOLOGICAL RESPONSIVENESS OF CAUDATE NEURONS IN AGED CATS TO EXCITATORY AMINO ACIDS RECEPTOR AGONISTS AND DOPAMINE. M.S. Levine, C. Cepeda, Z. Radisavljevic, M. Bertolucci. MRRC, UCLA Los Angeles, CA 90024.

We have demonstrated decreased excitation in neostriatal neurons in cats and rats. Extracellular recordings in cat caudate (Cd) suggested that changes in excitation may be mediated by glutamate (GLU) receptors. The present study assessed the responsiveness of aged Cd neurons in slices (400 μm) to application of excitatory amino acids (EAAs) and dopamine (DA). Results have been obtained from 5 aged (10-16 yrs) and 3 adult (3 yrs) cats. Average resting membrane potentials (74$\pm$6.5 (S.D.) mV (N=25) aged vs 71$\pm$6.5 mV (N=12) young), action potential amplitudes (68$\pm$6.5 mV (N=25) aged vs 67$\pm$5.3 mV (N=12) young) and input resistances (20.2$\pm$4.3 MΩ (N=25) aged vs 18.9$\pm$6.0 MΩ (N=12) young) were similar. In young Cd, GLU (N=8) or N-methyl-D-aspartate (NMDA) (N=11) produced a rapid depolarization accompanied by a train of action potentials. A population of aged Cd cells had higher thresholds for these responses (10/19 cells, current $\geq$290 nA). In aged Cd GLU evoked depolarizations without spikes (3/19) and both GLU (4/19) and NMDA (9/24) induced depolarizations with prepotentials, responses never observed in young Cd. DA modulated responses to GLU and NMDA. Aged cells had a higher threshold current (196$\pm$95 nA (N=16) vs 113$\pm$35 nA (N=8)). DA's ability to modulate synaptically responses was also decreased. In young Cd, DA decreased the amplitude of responses at low bath concentrations. In aged Cd, DA decreased amplitudes in 2/5 tests but at high concentrations (100μM). These findings indicate that in aged cats decreased excitation may be mediated by alterations in EAA receptors and the ability of DA to modulate responses is impaired. Supported by USPHS AG7462.

155.3

MK801 BINDING SITES DO NOT CHANGE SIGNIFICANTLY WITH AGE IN MOST CORTICAL REGIONS, IN CONTRAST TO NMDA BINDING SITES K.R. Magnusson and C.W. Cotman[1]. Dept. of Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 50623; [1]Dept. of Psychobiology, University of California-Irvine, Irvine, CA 92717.

We have previously reported that binding to N-Methyl-D-aspartate (NMDA) sites in the brains of BALB/c and C57Bl mice is decreased with increasing age (Soc. Neurosci. Abstr. 16:19.10). The present study was performed to determine whether other components of the NMDA receptor complex, specifically the channel protein, also exhibit similar declines during the aging process. Quantitative autoradiography was performed on horizontal sections which were incubated with either 10nM ^{3}H-MK801 in the presence of 10μM each of glutamate and glycine (for the channel protein) or 100nM ^{3}H-L-glutamate and 100μM each of kainate, AMPA and SITS (for NMDA sites). Significant differences between age groups (3,10,and 30 months) and strains (BALB/c and C57Bl) for each brain region analyzed were determined by ANOVA of fmol/mg protein values. In both strains of mice, MK801 binding was only significantly decreased in old (30 months) mice, as compared with the 3 month old mice, in a few cortical subregions of frontal and parietal cortex. This is in contrast to binding to NMDA sites which was decreased in almost all cortical subregions analyzed in old C57Bl and BALB/c mice, as compared with 3 month old mice. These results suggest that either different components of the NMDA receptor complex are differentially regulated or that NMDA receptors are not lost with increasing age, but a subpopulation becomes "inactivated". Supported by Physician Scientist Award AG00329.

155.4

AGE-RELATED CHANGES IN GLUTAMATERGIC RECEPTOR BINDING IN RHESUS MONKEY BRAIN. M.V. Wagster, L.C. Cork and D.L. Price. Neuropathol. Lab., The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

N-methyl-D-aspartate (NMDA) receptor binding was examined in the prefrontal cortex, striatum, thalamus and substantia nigra of 18 rhesus monkeys (*Macaca mulatta*) ranging from 8-34 years of age. *In vitro* receptor autoradiography using [^{3}H] glutamate (GLUT) in the presence of kainate and quisqualate was employed to visualize NMDA sites. In the regions examined, [^{3}H] GLUT (NMDA) binding concentration was highest in superficial layers of neocortex and in paraventricular nucleus of thalamus. Moderate levels were seen in deep neocortical layers, in medial dorsal and lateral dorsal nuclei of thalamus, and in striatum. Low levels were detected in the remainder of the thalamic nuclei and in substantia nigra. The [^{3}H] GLUT (NMDA) binding concentration decreased with age in medial dorsal nucleus of thalamus; a slight decrease in binding with age was noted in prefrontal cortex. Neither striatum nor substantia nigra showed any consistent decrease in NMDA receptor binding with age.

155.5

TREATMENT OF HIPPOCAMPAL SLICES FROM AGED RATS WITH AN A_1 ADENOSINE RECEPTOR ANTAGONIST REVEALED AN NMDA RECEPTOR-MEDIATED SYNAPTIC RESPONSE COMPONENT. V.K. Gribkoff and L.A. Bauman. Neuropharmacology, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492.

We have found that evoked population synaptic responses recorded in area CA_1 of hippocampal slices from aged (24+ mo Fischer 344) rats were smaller in amplitude than those potentials comparably recorded from slices obtained from young (2-4 mo Fischer 344) rats. In addition, in some slices from aged rats the response to Schaffer-collateral stimulation, while smaller, was more complex, with multiple peaks generated at high stimulus currents. This response profile was not observed in slices from young rats. Application of the A_1 adenosine receptor antagonist 8-cyclopentyltheophylline (8-CPT; 1 nM-2 uM)produced a significantly greater increase in synaptic responses in slices obtained from aged rats; only a small enhancement was observed in slices from young rats in response to incubation in 8-CPT.In addition, in slices from aged rats only, 8-CPT greatly increased the later components (multiple peaks), producing a hyperexcitable response profile.

In the presence of 8-CPT (1 uM), application of the N-methyl-D-aspartate (NMDA) excitatory amino acid receptor antagonists MK-801 (2-20 uM) or AP5 (20-50 uM)greatly reduced the later components of the population synaptic responses in slices from aged rats. A small reduction was also observed when NMDA receptor antagonists were applied to slices from aged rats incubated in control medium. No effect of NMDA antagonists was observed on responses evoked in slices from young rats under either experimental condition.

155.6

IN VIVO EVALUATION OF HIPPOCAMPAL CHOLINERGIC FUNCTION THROUGH EAA RECEPTORS IN THE AGING BRAIN - EFFECT OF ACETYL-L-CARNITINE

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Autoradiographic studies have evidenced a rich distribution of excitatory amino acid (EAA) receptors in the hippocampus. The aim of this study was to elucidate the role of quisqualate (QA) and N-methyl-D-aspartate (NMDA) receptors on ACh release in the hippocampus of 5 - and 24 - month - old Fischer rats.

Perfusion with QA 10^{-6} M or NMDA 10^{-6}M enhanced ACh release in the hippocampus of young but not of old rats. Moreover, (3H) CGS 19755, a selective and competitive NMDA antagonist, showed a slight decrease of NMDA receptors in the hippocampus of old rats. Six-month treatment (100 mg/kg/day in drinking water) with Acetyl-L-Carnitine (ALCAR) significantly increased NMDA binding capacity in the hippocampus of 24-month-old rats, compared with their age-matched controls. These results suggest that ALCAR, besides improving behavior and expressing a neurotrophic activity, may also be used in age-dependent brain disorders in which the glutamatergic system may be involved.

155.7

AGING INCREASES DIZOCILPINE-INDUCED LEARNING IMPAIRMENT AND DECREASES HIPPOCAMPAL AND STRIATAL NMDA RECEPTOR CONCENTRATIONS IN RATS D.K. Ingram[1], P. Garofalo[1], E.L. Spangler[1], C. Mantione[2], I. Odano[2], and E.D. London[2]. [1]Gerontol. Res. Ctr., NIA, NIH, and [2]Addiction Res. Ctr. NIDA, ADAMHA, Baltimore, MD 21224.

Age-related declines in the densities of N-methyl-D-aspartate receptor (NMDA-r) in rat and monkey brain have been reported previously (G. Wenk *et al. Neurobiol Aging* 12:93, 1991). We have expanded these findings to show that aging increases the sensitivity to dizocilpine (MK-801), a non- competitive antagonist of NMDA-r, in a maze test that has produced robust evidence of age-related decline in learning performance (D. Ingram, *Neurobiol Aging*, 9:475, 1988). MK-801 (0.02 or 0.04 mg/kg) or saline was administered s.c. to 3-mo and 24-mo old male F-344 rats 20 min before training (15 trials) in a 14-unit T-maze. This shock-motivated task requires the rat during each trial to locomote through 5 segments each within 10 sec to avoid footshock (0.8 mA). Whereas MK-801 had no effects on maze performance in young rats at these low doses, aged rats were cognitively impaired at both doses as measured by errors committed. These rats were sacrificed about 1 week later, and brain tissue extracted for analysis of NMDA-r using [^{3}H] glutamate as the radioligand in the presence of 20 μM quisqualate. Results revealed an age-related decline in NMDA-r in hippocampus and striatum but not in frontoparietal cortex or basal forebrain. These findings support the observation that aged rats are more sensitive to behavioral effects of antagonism of NMDA-r than are young rats and suggest that deficits in this system might underlie age-related loss in memory processing.

155.8

EFFECTS OF AGING ON THE MESOSTRIATAL DOPAMINE SYSTEM: FOCUS ON THE STRIATUM. R. Burwell, I. Whealin, and M. Gallagher. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599.

The effects of age on pre- and post-synaptic markers for the mesostriatal dopamine (DA) system were investigated by quantitative receptor autoradiography in behaviorally characterized aged and young rats.Twenty male Long-Evans rats completed a longitudinal study of reaction-time performance. At the end of the study, the aged rats combined with a cohort of 10 young rats were also tested for spatial learning in the Morris water maze.

Density of DA D1 receptor binding in the substantia nigra (SN) was significantly lower in the aged subjects. This pattern was observed in lateral, mediolateral, and medial areas of the SN. Analysis of D1 binding in a series of anterior caudate sections revealed no reliable age differences. However, the D1 binding in the SN was significantly correlated with D1 binding in this region of the caudate for aged animals. Age-related differences in striatal patch and matrix compartments in the caudate were also assessed. Mu opiate receptor patches were localized in sections adjacent to those used to localize D1 receptor sites. Analysis revealed a non-significant trend toward higher density of D1 binding in the matrix compartment. Additional autoradiograms will be analyzed for age-related changes in lateral/medial and rostral/caudal gradients throughout the striatum.

Similar analyses for D2 receptor and DA uptake sites in the same brains are currently underway. Interrelationships among these markers as well as behavioral measures will be discussed.

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155.9

EFFECTS OF AGING ON MUSCARINIC BINDING IN THE STRIATUM OF BEHAVIORALLY CHARACTERIZED RATS. A. H. Nagahara†, T. M. Gill, E. A. Liles*, J.A. Willner, and M. Gallagher. Curriculum in Neurobiology† and Department of Psychology, University of North Carolina, Chapel Hill, NC 27599.

Age-related changes have been observed in the cholinergic basal forebrain/hippocampus that correlate with spatial learning ability. The present experiment investigated the effects of aging on the cholinergic system within the striatum using in vitro autoradiography. Total muscarinic, M1, and M2 binding were examined using [^{3}H]-quinuclidinyl benzilate (QNB) alone and [^{3}H]-QNB in the presence of the M1 receptor antagonist, pirenzepine. In addition, muscarinic binding in the striatal patch and matrix compartments was examined using adjacent brain sections labelled with [^{3}H]-DAGO. Aged animals were separated into impaired and unimpaired subgroups based on spatial learning ability in the Morris water maze task.

Preliminary analyses revealed different topographical gradients for the muscarinic receptor subtypes in the striatum. The density of M1 binding was higher in the dorsal region of striatum than in the ventral region. M1 binding was also higher in lateral areas. In contrast, M2 binding was higher in medial regions of the striatum. The density of M2 binding was significantly lower in the matrix than in the patch compartment, while the density of M1 binding appears was comparable in both compartments. These patterns were similar in young and aged brains. Further analyses are in progress on these and additional autoradiograms to examine the effects of aging and the relationship to behavioral impairments. Supported by MH14277 to AHN, RSDA to MG (KO2-MH00405) and grants MH39180 and BNS 87-19881.

155.10

EFFECTS OF AGING ON MUSCARINIC RECEPTOR SUBTYPES IN BEHAVIORALLY CHARACTERIZED RATS. T.M. Gill & M. Gallagher. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599.

The effect of aging on muscarinic binding within the medial septal region (MS), vertical limb of the diagonal band (VDB), hippocampus, laterodorsal tegmental nucleus (LDT), and pedunculopontine tegmental nucleus (PPT) was investigated using in vitro autoradiography. Tritiated Quinuclidinyl benzilate (QNB) alone and [^{3}H] QNB in the presence of the M1 receptor antagonist, pirenzepine, were used to determine total muscarinic, M1, and M2 binding in young and aged brains. Based on spatial learning ability, the aged rats were separated into impaired and unimpaired subgroups.

A modest but significant decrease in total muscarinic binding was observed in the aged brains within the MS, VDB, dentate gyrus of the hippocampus, and LDT. The pattern of change for muscarinic receptor subtypes depended on brain region. Decreases for both subtypes were evident in the MS, VDB, and LDT, but within the dentate gyrus of the hippocampus there was an increase in M2 and a decrease in M1 binding. In all regions the most pronounced changes were observed in the aged impaired subgroup.

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155.11

REORGANIZATION IN THE MOLECULAR LAYER OF THE DENTATE GYRUS IN AGED LEARNING-IMPAIRED RATS. M.M. Nicolle†, A.H.Nagahara†, J.A. Willner, & M. Gallagher. Curriculum in Neurobiology† and Department of Psychology, University of North Carolina, Chapel Hill, NC 27599

Lesion of the perforant path (PP) input onto dentate granule cells causes sprouting of other inputs into the denervated zone of the molecular layer (outer 2/3rd). One marker used to detect this reorganization is autoradiographic analysis of kainic acid binding which is normally confined to the inner 1/3rd of the molecular layer but expands outward in response to PP denervation. We compared the topography of [^{3}H]-kainic acid binding in the dentate gyrus of young and aged rats that were characterized for spatial learning ability. The aged rats (N=14) included a subgroup that was impaired in the spatial task (N=7) and another subgroup that was unimpaired (N=7).

The peak density of [^{3}H]-kainic acid binding in the molecular layer did not differ between young and aged rats. However, analysis of the topography of kainate binding in the same area (superior blade in the dorsal hippocampus) revealed a significant age-related increase in width of labeling over the molecular layer, an effect that was confined to the impaired aged subgroup. This change in the topography of kainate binding may reflect a reorganization of inputs to the molecular layer in response to decreased innervation from entorhinal cortex in learning-impaired aged rats.

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155.12

EFFECT OF AGE ON ^{3}H-NISOXETINE BINDING TO UPTAKE SITES FOR NOREPINEPHRINE (NE) IN THE LOCUS COERULEUS (LC) OF HUMANS. S.M. Tejani-Butt[1] and G.A. Ordway[2]. [1]Dept. Psych., Univ. of Pa Sch. of Med. & Dept. Vet. Affairs Med. Ctr., Phila., PA 19104, [2]Dept. Psych., Case West. Res. Univ., Metro Health M.C., Cleveland, OH 44109.

The presynaptic uptake system for NE is receiving considerable attention in the overall assessment of monoamines in neurodegenerative and psychiatric disorders. We have shown recently that ^{3}H-nisoxetine (^{3}H-NIS) is a useful radioligand for mapping of sites associated with NE uptake in rat brain by quantitative autoradiography (Neurosci. Abstr. 16:524, 1990). In the present study, we have used ^{3}H-NIS as a marker for the NE transporter to investigate age-related changes in noradrenergic innervation in the LC of humans. Sections (20μ) were taken from the LC (caudal region) from subjects who died from natural causes ranging in age from 20 to 80 years. Regression analysis of the data demonstrated an inverse correlation between age and the binding of ^{3}H-NIS ($r=-0.6$; $p<0.01$) to uptake sites for NE in the LC. When the subjects were divided into two groups (<50 yr and >50 yr), a significant ($p<0.01$) age-related reduction in the binding of ^{3}H-NIS was seen. A 45% decrease in the density of uptake sites for NE was observed in the group that was >50 yr as compared to the group that was <50 yr of age. Since considerable cell loss (40-60%) has been reported to occur with age in the LC, the lower binding of ^{3}H-NIS most likely reflects loss of LC cells rather than a down-regulation of these sites. (Research funds from the Dept. of Vet. Affairs, NARSAD and USPHS grant MH 45472).

155.13

EFFECTS OF DEPRENYL ON BRAIN NEUROTRANSMITTERS (NT) IN OLD MALE RATS. S. Thyagarajan and S.K. Quadri. Neuroendocrine Research Laboratory, Kansas State University, Manhattan, KS 66506.

Deprenyl, an irreversible monoamine oxidase-B inhibitor, is reported to increase the life span, correct certain age-related behavioral deficits and have beneficial effects in Parkinsons' disease patients. Catecholamines (CA) and indoleamines (IA) in the hypothalamus (HT) are known to influence the aging process. However, it is not known how the metabolism of these NT, especially the IA, is affected in the HT of old male rats after long-term treatment with deprenyl. In this study, Sprague-Dawley male rats (18-23 mo.) were injected s.c. with 0.25 mg/kg (low dose; LD) or 2.5 mg/kg (high dose; HD) of deprenyl or the vehicle (saline; control). The animals were treated daily for 24 weeks. The concentrations of NT were determined in the HT, striatum (ST), and the hippocampus (HI) at the end of treatment by HPLC-EC. Serotonin (5-HT) concentration in the HT of the HD rats (62.5±3.4 pg/ug protein) was higher ($p<0.0001$) than that in the control rats (13.1±1.9 pg/ug protein) and LD rats (19.3±1.7 pg/ug protein). 5-HT concentration in the ST was 13.2±1.7 pg/ug protein in the control rats and 50.9±6.2 pg/ug protein ($p<0.03$) in the HD rats. Dihydroxyphenylacetic acid (DOPAC) concentration in the HT significantly ($p<0.05$) decreased in the HD rats. DOPAC concentration in the ST of the HD rats (7.3±3.1 pg/ug protein) was significantly ($p<0.01$) lower than that in the control rats (66.3±5.3 pg/ug protein) and LD rats (63.0±13.7 pg/ug protein). Dopamine concentration in the ST increased significantly ($p<0.007$) in the LD rats. Norepinephrine concentration in the HI was significantly ($p<0.0001$) higher in the HD rats (60.1±5.5 pg/ug protein) than that in the control rats (13.0±2.9 pg/ug protein) and LD rats (19.6±3.3 pg/ug protein). These results suggest that long-term treatment of old male rats with deprenyl significantly alters the synthesis and metabolism of the CA and IA not only in the striatum, but also in the hypothalamus and hippocampus. (Supported by NIH grant AG05980)

155.14

PRESERVATION OF THE DENSITY OF THE DOPAMINE UPTAKE COMPLEX IN AGING RAT BRAIN. Inglefield, J. R. and E. K. Richfield. Depts. of Neurobiology and Anatomy and Neurology, University of Rochester, Monroe Community Hospital, Rochester, NY 14620.

This aging study was performed to determine if there are selective regional or global changes in the density or pharmacology of the dopamine uptake complex (DAUC) of aged rodent. Fifteen regions of the Fischer 344 rat (aged 4, 12 and 24 months) central nervous system were analyzed for the density of the DAUC using [^{3}H]GBR 12935 and *in vitro* quantitative autoradiography. Additionally, cocaine competitions were performed in the striatum of all of the animals. A 26-fold variation in the DAUC density was found in the regions sampled; however no significant age-related changes were identified. Intra-striatal analysis of the DAUC density revealed binding heterogeneities: decreasing lateral to medial and decreasing dorsal to ventral gradients. No significant effect of aging on striatal quadrants was observed. Also, the proportion of high and low affinity sites for cocaine was unchanged in the three age groups. Taken together, these findings suggest a stability of this dopamine neuron pre-synaptic marker in aging rat central nervous system.

155.15

THE EFFECTS OF AGING ON DOPAMINE DIFFUSION AND CLEARANCE IN THE STRIATUM OF THE FISCHER 344 RAT.

M.N. Friedemann and G.A. Gerhardt. Departments of Pharmacology and Psychiatry, University of Colorado Health Sciences Center, Denver, CO 80262.

Previous studies have shown that there are age-related changes in dopamine (DA) uptake sites (B_{max}) and rates (V_{max}). In order to further investigate the effects of aging on DA uptake processes, the present study combines *in vivo* chronoamperometric recordings and local drug application techniques which allow direct measurement of DA diffusion and clearance from the extracellular space. Male Fischer 344 rats, 6 and 24 months old were anesthetized with urethane and surgically prepared for *in situ* recording. DA (5-60 pmol) was pressure ejected 240 to 350 μm away from a Nafion-coated carbon fiber electrode at sites within the dorsal or ventral striatum. No significant differences between the age groups for either the amplitude or the clearance time of the DA ejections were found. There were also no significant differences between dorsal and ventral striatum. In addition, the application of the uptake inhibitor, nomifensine, (20-200 pmol) just prior (30 sec) to the DA application resulted in significant increases in the amplitude and time course in dorsal striatum. However, in ventral striatum, nomifensine significantly increased the amplitude of the DA signal only in the 6 month old rats, while the time courses of the signals were significantly increased in both age groups. The inability of nomifensine to modulate the amplitude of the DA signal in the ventral striatum of aged rats suggests that these nerve terminals may be differentially effected by the aging process. (Supported by USPHS grants AG06434 and AG00441 to GAG and Pharmaceutical Manufacturers Association Foundation Advanced Predoctoral Fellowship to MNF).

155.16

GABAERGIC SYSTEMS IN AGEING MOUSE BRAIN. Pirjo Saransaari and S.S. Oja. Tampere Brain Res. Ctr, Dept. Biomed. Sci., Univ. Tampere, Finland.

GABAergic transmitter systems are most likely involved in age-related alterations in physiological functions of the central nervous system. We studied in different brain areas the uptake, release and binding of GABA in mice of various ages from 3 to 24 months. The resting and potassium-stimulated (50 mM) release of GABA was measured with superfused tissue slices, uptake with isolated synaptosomes and binding to both A and B sites with purified synaptic membrane preparations. Potassium stimulation enhanced more than 15-fold the release of preloaded GABA from cerebral cortical slices in 3-month-old mice. This response decreased but the release of endogenous GABA increased with age in the same preparations. In some other areas the changes in the release of endogeous and exogenous GABA were also strikingly dissimilar but in the striatum the release of both exogenous and endogenous GABA significantly diminished during ageing. There must occur alterations in the relative sizes of the releasable GABA pools, since the total GABA concentrations did not change very dramatically during ageing. The uptake of GABA by cortical synaptosomal preparations was not either markedly affected by age, consisting always of two saturable components, high- and low-affinity. The maximal binding capacity of GABA to A sites decreased and the binding constant significantly increased between 3 and 18 months of age, suggesting that both the number of available receptors and their affinity for GABA diminished. The results clearly show how variable are the studied GABAergic parameters in different brain areas during normal ageing. (Supported by the Emil Aaltonen Foundation, Finland.)

155.17

Basal forebrain cholinergic anatomy in behaviorally characterized aged rats. D.M. Armstrong, R. Sheffield, G. Buzsaki, K. Chen, and F. H. Gage. Fidia Georgetown Institute for the Neurosciences, Georgetown University, Washington, D.C. 20007.

In the rat, cognitive impairments are often associated with deficiencies in cholinergic neurotransmission. In the present study we employed immunocytochemical techniques in order to quantitatively assay the age-related alterations of basal forebrain cholinergic neurons in three populations of behaviorally characterized female Fisher 344 rats: 6 months (n = 10), 27 months (n = 8) and 33 months (n = 8). As a group the three populations of animals were indistinquishable from each other with respect to the size and number of choline aceytltransferase (ChAT) positive neurons within the basal forebrain. However, when rats were categorized according to their performance on the Morris water maze the following observations were made: (1) in the medial septum (MS) and nucleus of the diagonal band ChAT-positive neurons were larger in the 27 month old non-impaired group compared to 27 and 33 month old behaviorally impaired animals and compared to young controls. We hypothesize that the neuronal swelling observed in the 27 month old non-impaired animals may represent a compensatory response which correlates with the intact functional status of the animal. (2) 33 month old impaired rats also exhibited a decrease in the number of ChAT-positive neurons in the MS compared to young controls and 27 month non-impaired rats. When this loss of cholinergic immunoreactivity was further examined using selected adjacent sections immunolabeled for nerve growth factor receptor (NGFr) we observed no apparent loss of NGFr-positive cells.

155.18

RECOGNITION MEMORY DEFICITS IN A SUB-POPULATION OF AGED MONKEYS RESEMBLE THE EFFECTS OF MEDIAL TEMPORAL LOBE DAMAGE.

P. R. Rapp and D. G. Amaral. The Salk Institute, La Jolla, California, 92037.

Individual differences in recognition memory function in the aged monkey were assessed using a delayed nonmatching-to-sample (DNMS) procedure that critically depends on the functional integrity of the medial temporal lobe. Four young (9-11 years) and 10 aged (22-33 years) rhesus monkeys *(Macaca mulatta)* were initially trained to a learning criterion of 90% correct on the DNMS task using a 10 second delay between the sample and recognition phase of each trial. The memory demands of the task were then increased by gradually extending the retention interval from 15 seconds to 10 minutes. Three of the aged monkeys performed as well as the young subjects at all delays. The remaining aged monkeys performed well at the shortest delays (15 and 30 seconds), but progressively greater impairments emerged at delays of 60 seconds, 2 minutes, and 10 minutes (p's < .05). The results suggest that recognition memory is compromised in only a subpopulation of aged monkeys. Moreover, aged monkeys that are impaired in the DNMS task exhibit the same delay-dependent pattern of impairment that is the hallmark feature of memory dysfunction resulting from medial temporal lobe damage.

155.19

ALTERATIONS OF CHOLINERGIC CELL NUMBER AND SIZE IN THE MEDIAL SEPTAL NUCLEUS OF AGED RHESUS MONKEYS. H. M. Stroessner-Johnson, P.R. Rapp and D.G. Amaral. The Salk Institute, La Jolla, CA 92037; UCSD, Group in Neurosciences, San Diego, CA 92037.

The number and size of cholinergic neurons in the medial septal nucleus (MS) was quantified in four aged (23-27 years) and four young (10-12 years) behaviorally characterized rhesus monkeys. Four sections at anatomically matched levels (300µm intervals) were immunohistochemically processed using a monoclonal antibody (AB8) against choline acetyltransferase (ChAT). The number of ChAT positive cells in which there was a clearly defined nucleus was determined using a computer-aided digitizing system. Cross-sectional area of labeled cells was measured from camera lucida drawings (final magnification 1250x) using a digitzing tablet.

Across the four rostro-caudal levels, there was a 24% decrease in the average number of ChAT positive cells in the aged group. This effect was regionally selective and predominately confined to the caudal two sampled sections of the MS where there was a 42% decrease in average number of cells in the aged group.

A somewhat surprising finding was that the average cross-sectional area of cholinergic cells was significantly greater in the aged monkeys ($p<.05$). At caudal levels this effect was at least partly attributable to an apparent loss of small to medium sized ChAT positive cells in the aged group. However, the increase in average cell size at rostral levels of the MS occurred in the absence of significant cell loss. These results indicate that cholinergic cells in the MS of the nonhuman primate may undergo a variety of morphological alterations as a consequence of aging.

155.20

Attenuation of the age-related decline in hippocampal muscarinic receptor density through daily exercise or underfeeding. R.P. Farrar[1,2,3] D.E. Fordyce[1,3] and J.W. Starnes [2,3] Institute for Neuroscience, [2]Department of Pharmacology and [3]Department of Kinesiology, The University of Texas at Austin, Austin, TX.

It was the intent of the present investigation to compare the effects of food restriction and exercise, on cognition associated hippocampal muscarinic receptor density during aging. Male F344 rats were subdivided into the following groups where numbers indicate months of age: sedentary groups: (10S, 12S, 24S, 27S). Sedentary groups (10S, 12S, 24S, 27S), rats underfed or exercised from 10 to 24 months of age (24U and 24E, respectively), rats underfed or exercised from 3 to 12 or 27 months of age (12U, 27U, 12E, 27E, respectively). Age resulted in significant reductions in muscarinic density in both sets of rats (24S vs. 10S and 27S vs. 12S). Underfeeding from 3 months to 12 months of age significantly increased muscarinic density (12U vs. 12S) and resulted in significantly greater muscarinic density compared to sedentary controls at old age (24U vs. 24S, 27U vs. 27S). Exercise appeared to maintain muscarinic receptor density in old animals to a level similar to that of middle-age sedentary controls, whether exercise is initiated at 3 months or 10 months (24E vs. 10S, 27E vs. 12S). In the present investigation, the physiological treatments of underfeeding and exercise both maintained hippocampal muscarinic receptor binding during the aging process in F344 rats. Underfeeding appeared to be more effective than exercise when the protocols were initiated at 3 months or 10 months of age.

156.1

AN INEXPENSIVE MICROCOMPUTER-BASED MAPPING SYSTEM FOR PLOTTING THE SPATIAL DISTRIBUTION OF LABELED CELLS AND FIBERS IN TISSUE. W.G. Tourtellotte and G.W. Van Hoesen. Departments of Anatomy and Neurology, University of Iowa, Iowa City, IA 52242.

Plotting the spatial distribution of labeled cells or fibers in tissue is a valuable method for reducing the data generated by anterograde or retrograde tract tracing experiments, immunohistochemistry, in situ hybridization, or regional pathological analyses. We have developed a Computerized Charting System (CCS) to supersede conventional mapping techniques that use mechanical X-Y recorders for cell mapping and the camera lucida for high-resolution axon tracing.

The CCS is based upon an inexpensive and easy to configure microprocessor-based motorized microscope stage. The electronics are consolidated onto a circuit board that plugs directly into an IBM-PC compatible microcomputer. The circuitry controls the motorized stage movement and a joystick for remote positioning.

The CCS provides features that encode the stage position for tissue section contouring, mapping the distribution and enumerating up to 20 separate cell populations or other discreet markers, and plotting the distribution of labeled fibers. It provides other interactive features including scaling, windowed data entry, a photographic interface, position adjustments, and high-resolution plotting of either the entire tissue section or enlarged portions.

The CCS can provide an important technical contribution to mapping the location of labeled fibers and cells revealed by a variety of modern experimental techniques. We have used it in our laboratory for: (1) retrograde and anterograde neuroanatomical tract tracing using horseradish peroxidase, fluorescent dyes, PHA-L, and ^{3}H-labeled amino acids; (2) mapping the distribution of cells identified with immunohistochemistry; (3) mapping the distribution of silver grain-positive cells using in situ hybridization; (4) mapping the spatial distribution of neurofibrillary tangles and neuritic plaques in Alzheimer's disease; and (5) mapping the pattern of congophilic angeopathy in human brain. (Supported in part by: NS 14944).

156.2

COMPUTER ASSISTED ANALYSIS OF MORPHOLOGICALLY DEFINED CORTICAL COLUMNS. D.Buxhoeveden and E. Armstrong. A.F.I.P., Washington, D.C. 20306

A method for analyzing linearity is important if cortical structure is to be understood. During ontogeny the originally uniform vertical arrangement of neurons becomes more varied. Consequently a morphometric analysis of columns that can objectively and reproducibly measure the amount of deviation from a straight line, offers a biologically based model by which cortical structures can be compared between areas, ages and species.

Nissl stained sections from temporal cortex (Tpt and AI) were digitized at 100X for adult brains and 200X for fetal brains to test the model. One program identified the center of horizontal neuronal clusters and was used to segregate cell columns. A second set of algorithms analyzed the linearity of columns by measuring total path lengths, nearest neighbor ratios, and linear regressions. Formulas and ratios of the data define characteristics for classifying columns by types. Columnar types can then be compared across areas, species and ages.

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156.3

FEATURE-BASED MAPPING OF MAJOR CELLULAR REGIONS IN THE POSTERIOR CINGULATE GYRUS. E. Armstrong,[1] R.L. Becker, Jr.*[1] and M. Carlotto*[2], A.F.I.P.[1] Washington, D.C., 20306; TASC,[2] Reading, MA.

Information from molecular, cellular and physiological studies come from different levels of resolution. Their integration demands the mapping of common features and imaging techniques have the potential of providing objective maps automatically and reproducibly.

To begin refining the techniques we have digitized Nissl stained sections of the posterior cingulate cortex. Cytoarchitecturally defined regions were classified by using differences in optical densities perpendicular to the medial axis, after the binary image of the cortex had been segmented without holes and resampled into a series of vectors of fixed length.

Optical densities were smoothed and laminar structures and contrasts between textured regions were enhanced by filtering techniques based on anisotropic diffusion. Both geometric properties of the cortex, e.g., sulci, and fractal dimensions for measuring features like roughness and homogeneity were examined. Cellular regions were successfully discriminated. Signature vectors for the regions have shown that the clusters developed by these methods are unimodal.

Supported by NSF 8820485 (EA)

156.4

THE Kr-Ar ION LASER: INCREASED SENSITIVITY FOR MULTICOLOR LASER SCANNING CONFOCAL MICROSCOPY. T.C. Brelje*, R.L.Sorenson*, M.W. Wessendorf, and R. Elde. Dept. Cell Biol. and Neuroanat., Univ. Minnesota, Minneapolis, MN 55455

The usefulness of laser confocal microscopy in multi-color fluorescence studies has been constrained by the limited range of excitation wavelengths available on an air-cooled argon (Ar) ion laser. The present study examined a krypton-argon (Kr-Ar) ion laser as an alternate light source.

A 15 mW Kr-Ar ion laser was obtained with simultaneous emission of 4-5 mW at 488 nm (blue), 568 nm (yellow), and 647 nm (red). Interference filters and dichroic mirrors were obtained that allowed visualization of green emission (using blue excitation), orange-red emission (using yellow excitation), and deep red emission (using red excitation). The characteristics of this laser were compared to that of an Ar ion laser with lines at 488 nm (blue) and 514 nm (blue-green).

A comparison was made between the abilities of the two lasers to excite Lucifer Yellow, BODIPY, fluorescein, tetramethylrhodamine, cyanine 3.18, Texas Red, Lissamine rhodamine, allophycocyanin, Ultralight T680, and cyanine 5.18. It was found that using the Ar ion laser, fluorescein, BODIPY, Lucifer Yellow, cyanine 3.18, and tetramethylrhodamine could be excited efficiently or moderately efficiently. In addition, Lissamine rhodamine and Texas Red could be excited, although with considerably reduced efficiency. However, with neither the 488 nor 514 nm "lines" could allophycocyanin, Ultralight T680, or cyanine 5.18 be visualized. In contrast, all of the fluorophores could be excited highly efficiently using the Kr-Ar laser.

It is concluded that the Kr-Ar ion laser is a more sensitive and versatile means of visualizing many fluorophores and that the Kr-Ar laser may be preferable for studies involving combined fluorescent probes. Supported by DA 05466, DA 06299, and DK 33655.

156.5

SPECIFIC THREE-COLOR IMMUNOFLUORESCENCE BY LASER SCANNING CONFOCAL MICROSCOPY USING A Kr-Ar ION LASER. M. Wessendorf, T.C. Brelje*, W. Wu, R. Sorenson*, and R. Elde. Dept. Cell Biol. and Neuroanat., Univ. Minnesota, Minneapolis, MN 55455

Efforts to visualize two or more fluorophores in a single piece of tissue using laser scanning confocal microscopy have been hampered by the poor efficiency with which an argon (Ar) ion laser excites many fluorophores. Furthermore, in many circumstances an Ar ion laser is unable to excite more than one fluorophore specifically. For instance, in doubly labeled samples the red component of fluorescein (FL) emission is indistinguishable from the red emission of rhodamine or Texas Red. Both of these problems result from the limited range of excitation wavelengths available using an Ar ion laser. Thus we examined the usefulness of the krypton-argon (Kr-Ar) ion laser described in the previous abstract for visualizing multicolor immunofluorescent staining.

The ability of the microscope to visualize various fluorophores specifically was examined using brightly stained tissue. FL, tetramethylrhodamine, Lissamine rhodamine (LR), cyanine 3.18, Texas Red, allophycocyanin, Ultralight T680 and cyanine 5.18 (Cy5) were tested. It was found that FL, LR, and Cy5 could be visualized specifically using the filters for blue, yellow, and red excitation, respectively. To test their applicability to 3-color fluorescence immunocytochemistry in the nervous system, sections of rat spinal cord were triply stained for serotonin, TRH, and substance P using secondary antibodies labeled with FL, LR, and Cy5, respectively (Jackson ImmunoResearch). Numerous triply labeled processes were observed in the ventral horn and intermediate gray matter.

It is concluded that specific 3-color immunofluoresce employing a laser scanning confocal microscope is feasible using a Kr-Ar laser.

Supported by DA 05466, DA 06299, and DK 33655.

156.6

CONFOCAL SCANNING MICROSCOPY REVEALS LEARNING-ASSOCIATED CHANGES IN PKC CONCENTRATION IN HERMISSENDA PHOTORECEPTORS. S. IMPEY, J. FARLEY, and Y. CHOOI ODLE.* Program in Neural Sciences, Dept. of Biology, and Institute for Cell and Molecular Biology. Indiana University, Bloomington, Indiana 47405.

Previous results implicate PKC activation as playing a crucial role in the induction and maintenance of persistent learning-associated changes in K^+ currents in Hermissenda (Hc) Type B photoreceptors (Nature, 1986; 319:220; PNAS, 1991; 88). Confocal laser scanning microscopy, image analysis, and fluorescent phorbol ester derivatives were used to monitor PKC in living whole-mounted Hc nervous systems. When incubated in uM concentrations of NDB-TPA, large numbers of neurons within the pedal and cerebropleural ganglia were fluorescently-labelled. Pre-incubation with unlabelled TPA decreased the fluorescence of NDB-TPA stained neurons in a dose-dependent manner. Double-label images of cells stained with NDB-TPA and rhodamine-conjugated phalloidin suggest a microfillament involvement in translocation. Quantitation of NDB-TPA fluorescence in optical sections of Type B photoreceptors from associatively-trained animals revealed a 2-3 fold increase in intensity, 2-5 days following training, relative to the same cells from untrained animals. These results suggest that associative learning results in increased PKC concentration in Type B photoreceptor plasmalemma membranes, which may contribute to the persistent suppression of K^+ channel activity in these cells.

156.7

GOLGI-LIKE LABELING OF NEURONS WITH FLUORESCENT DEXTRANS VISUALIZED WITH CONFOCAL SCANNING LASER MICROSCOPY.

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Small mol. wt. dextrans conjugated to fluorscein or tetramethylrhodamine are taken up by neuronal cell bodies and transported in an anterograde direction to produce intense terminal labeling following central injections (Nance & Burns, *Brain Res. Bull.*, **25**: 139, 1990). Since both fluorophors are compatible with argon lasers, the terminal labeling and the injection sites were examined with confocal scanning laser microscopy (CSLM). The dextrans were injected into various brain areas of rats and 5-14 day later, animals were perfused and the brains sectioned at 40-100 µm and visualized with a Sarastro CSLM. Confocal images were produced with Phoibos 1000 software running on a Silicon Graphics Personal Iris computer and displayed on a high resolution color monitor. In addition to focus-through images, stereo pairs and volume rendered (surface shaded) projections were generated. Three dimensional renderings of both terminal fields and injection sites provided detailed images of cell bodies and their dendrites as well as their terminal fields. Images of these extracellularly labeled neurons could be rotated and viewed at any angle and appeared qualitatively comparable to optimally stained golgi material. Thus, in addition to being sensitive anterograde tract tracers, these dextran conjugates can provide detailed morphological information about neurons labeled at the injection site when combined with CSLM. Supported by Medical Research Council of Canada and the Health Sciences Centre Research Foundation, University of Manitoba.

156.8

SPATIAL AND TEMPORAL IMAGING OF CALCIUM TRANSIENTS IN CA1 HIPPOCAMPAL PYRAMIDAL NEURONS. S.R.Young, J.Ambati*, X.Liu*, C.Scheffey, R.K.S.Wong. Dept. of Pharmacology, SUNY Health Science Center, Brooklyn, NY 11203.

Whole cell patch clamp recordings (Hamill et al. *Pflugers Arch* 391:85, 1981) were made from acutely isolated pyramidal neurons of guinea pig hippocampus (Kay & Wong. *J Neurosci Meth* 16:227, 1986). The extracellular solution contained (in mM) NaCl (140), KCl (5), HEPES (10), $CaCl_2$ (1), $MgCl_2$ (1), Dextrose (25). The pipettes contained (in mM) Trizma base (115), HEPES (10), BAPTA (10), ATP (4), Leupeptin (0.1), cAMP (0.01), Phosphocreatine (10), Fura-2 (0.1), Creatine phosphokinase (50 U/ml). Distribution of fura-2 in the cells reached equilibrium within 5 minutes. Optical recordings of fura-2 fluorescence were made with a Photometrics CC200 CCD camera controlled by a host computer that recorded simultaneous current data from the patch clamp amplifier (Lasser-Ross et al. *Soc Neurosci Abst* 15:572, 1989).

Voltage clamp commands from a holding potential of -50 mV to -90 mV (250 ms), then to -10 mV (up to 750 ms) activated a calcium current with rapidly decaying and sustained components (Kay & Wong. *J Physiol* 392:603, 1987). Ratio images at 340 nM and 380 nM (Grynkiewicz et al. *J Biol Chem* 260:3440, 1985) show that $[Ca^{++}]_i$ continues to rise throughout depolarizations of up to 750 ms. The spatial pattern of $[Ca^{++}]_i$ increase activated by depolarization suggests that voltage dependent Ca^{++} channels are present on the somata of pyramidal cells as well as on the dendrites.

156.9

SIMULTANEOUS KINETIC IMAGING OF CALCIUM AND pH IN SINGLE LIVING CELLS. Stephen J. Morris†, Diane M. Beatty°, Thomas B. Wiegmann†*, Larry W. Welling‡* and Bibie M. Chronwall°. †Mol. Biology and Biochem., and °Cell Biology and Biophys., SBLS, UMKC, Kansas City, MO 64110-2499; ‡Renal Sect. and ‡Research Service, VA Med. Ctr., Kansas City, MO 64128.

There is a growing interest in resolving multiple images of "ratio" fluorophores like indo or SNARF™ or the emission from multiple dyes placed in the same cell system. For rapid kinetic studies, the problems of photodynamic damage and photobleaching on one hand and the need for good spatial and temporal resolution on the other, press the resolution of the instrumentation. Rapid resolution of multiple probes at multiple wavelengths presents a third set of problems. These are solved by a new design of ultra low light fluorescence video microscope for simultaneous, real-time capture of intensified images of cells containing dual wavelength "ratio" dyes or multiple dyes at video frame rates (30 frames/sec or faster) (BioTechniques **8**:296-308 (1990)). This instrument has been improved to simultaneously acquire up to four images. This allows capturing intensified images of up to four dyes contained in the same cell system. These can be two dual-emission wavelength "ratio" dyes or multiple dyes.

We present three examples of simultaneous multi-parameter imaging. (1) Synchronous observations of calcium (with indo-1) and pH distribution (using SNARF-1™) in kidney epithelial cells, show differing kinetics in response to ionomycin treatment. Intracellular calcium increases rapidly when the bath Ca^{2+} is raised. The pH remains stable for several seconds, then suddenly collapses. (2) During fusion of human red blood cells (RBC) to fibroblasts expressing influenza hemagglutinin, movement of soluble and membrane-bound dyes follow different kinetics, depending on the molecular weight of the soluble dye. This implies that the fusion involves the formation of small metastable pores. Furthermore, the swelling of the RBC occurs after the onset of fusion, and therefore cannot provide the driving force. (3) Results from neuro-intermediate lobe cells in primary culture will also be presented.

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156.10

DIFFERENTIAL EFFECTS OF EXTRACELLULAR ATP ON IONIC CURRENTS IN EXCITABLE AND NON-EXCITABLE CELLS. *A. Jesurum, *M. Diverse-Pierluissi, E.W. Westhead and *D.J. Gross. Program in Molecular and Cellular Biology and Department of Biochemistry. University of Massachusetts. Amherst, MA. 01003.

Chromaffin cells derived from the neural crest have been shown to respond to externally applied ATP by either enhancing or inhibiting catecholamine secretion. Using electrophysiological techniques, we have shown that under voltage clamp whole-cell configuration, 20% of the chromaffin cells respond to a 100 µM ATP stimulation with an increase in inward current. The response occurs within 50 msec and the cells rapidly desensitize in the continuous presence of the agonist. The response recovers after 5 minutes in the absence of ATP. By using the voltage sensitive fluorescent indicator di-8-ANEPPS, we have measured a ~35mV depolarization in response to ATP. The video imaging data correlates with the voltage clamp data. Pretreatment of the cells with cholera toxin prolongs the ATP evoked depolarization by slowing the rate of desensitization.

ATP has been previously shown to act as a mitogen in A431 cells which are nonexcitable cells derived from a human epidermoid carcinoma. We show that in 80% of the cells tested, ATP mediates an increase in inward current (1-2 nA) which seems to be carried dominantly by calcium. The onset of the response shows a delay after stimulus, with 67% of the cells tested having a lag time of 4 seconds. As in chromaffin cells, the current is rapidly desensitized in the continuous presence of the agonist. Subsequent stimulations fail to yield responses of the same magnitude as the initial response and require longer exposure to ATP before responding. Di-8-ANEPPS fluorescence imaging of membrane potential in these cells indicates a rapid hyperpolarization. We believe that the imaging data reflects an outward Ca^{2+}-activated K^+ current proposed to exist in these cells. Experiments performed using current clamp will be used to determine changes in membrane voltage evoked by ATP.

156.11

INCORPORATION OF FLUORESCENT PHOSPHATIDYLCHOLINE INTO RAT HIPPOCAMPAL SLICES.

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Fluorescently labelled phospholipid, NBD-phosphatidlycholine (NBD-PC) was incorporated into small unilamellar vesicles composed predominantly of phosphatidlycholine (bovine brain). Hippocampal slices (400 µm) were incubated in HEPES buffer saline under aerating conditions for a period of up to 1 hour. Slices were washed and visualized with x20 and x40 long working distance objectives The labelled lipid was concentrated in specific regions of the hippocampus. Visualization at higher powers showed that pyramidal cell bodies were consistently labelled in CA1, CA3 and the dentate gyrus; particular axons and fiber tracts were labelled throughout the hippocampus. This would suggest that this fluorescent phosphatidylcholine analog is incorporated into specific phospholipid pools. However, when another fluorescent lipid probe, Bodipy-phorbol ester, was used at various concentrations, the label was distributed with no specific localization. In *in vitro* assays, the PLA_2 probe changes its fluorescent properties (peak wavelength and fluorescent intensity) upon cleavage of the labelled fatty acid. We are evaluating whether these properties are observed upon activation of PLA_2 in intact slices.

156.12

APPLICATION OF A MURINE EMBRYO CULTURE SYSTEM TO VISUALIZE EXPRESSION OF THE NEURAL CELL ADHESION MOLECULE (N-CAM) DURING EARLY MAMMALIAN EMBRYOGENESIS.

Poorni Iyer*, J.E. Martin*+, T. Rick Irvin* and J.K. Daniloff.+ Institute of Environmental Studies, +Dept. of Veterinary Anatomy and Fine Structure, Louisiana State University, Baton Rouge, La 70803.

We have demonstrated the expression of the neural cell adhesion molecule, N-CAM, during early mammalian embryonic development, employing double-labelling immunofluorescent microscopy in murine embryos grown in vitro. A monoclonal antibody to mouse N-CAM and indirect immunofluorescence with biotinylated and streptavidin-FITC conjugated antibodies were utilized to identify N-CAM expression in mouse blastocysts at the 72 hour culture stage. Embryos exhibited marked levels of fluorescence with localization specifically in the inner cell mass region. These findings demonstrate the presence of N-CAM in the mouse blastocyst after 72 hours in culture, and support the use of this embryo culture system to study localization and distribution of N-CAM at various timepoints during mammalian early embryogenesis.

Supported by NIH grant R29 N525102 and International Diagnostic Technologies 135206131.

156.13

ASSESSMENT OF NEURONAL ADHESION/SURVIVAL IN CULTURE IS FACILITATED BY LABELLING NEURONS WITH FLUORESCEIN DIACETATE R.E. Petroski, V.Q. Jenab, C. Gardner* and H.M. Geller
Dept. of Pharmacology, UMDNJ-Robert Wood Johnson Medical School and The Graduate School, Rutgers University, Piscataway, NJ 08854.

Neuronal survival and differentiation is dependent on a complex set of interactions with glial cells in the mammalian central nervous system. In order to examine the contribution made by astrocytes on neuronal adhesion, survival and process outgrowth, reliable methods for quantitating neurons growing on a glial substrate must be developed. The use of fluorescein diacetate (FDA) to determine neuronal viability in conjunction with propidium iodide has been previously described (Jones & Senft, J. Histochem. Cytochem., 1985). We report the use of FDA to selectively label living neurons but not astrocytes grown in cell culture.

Embryonic rat hypothalamic neurons were plated on a substrate of confluent cortical astrocytes. Cultures were treated with 10 μM FDA in DMEM/FCS for 30 min. in a humidified 5% CO_2 incubator. Live cultures were examined using fluorescein optics using a Zeiss Axioplan or Axovert microscope. Neurons displayed brilliant green somata as well as intensely labelled processes. The glial monolayer showed only a dim level of fluorescence which can be virtually eliminated by washing out the FDA-containing medium for 10 min. The fluorescent label lasts for several hours after wash out. The dye was used in conjunction with a laser cytometer (ACAS, Meridian Instruments) to enable automated cell counting. The dye was found not to harm neurons. Supported by NS 24168.

156.14

STORAGE PHOSPHORS AS AN ALTERNATIVE TO PHOTOGRAPHIC EMULSION FOR RECORDING AND QUANTIFYING AUTORADIOGRAMS. N.M. Appel, S.A. Mathews*, G.M. Storti* and M.J. Kuhar. NIDA Addiction Research Center, Baltimore, MD 21224; Quantex Corporation, Rockville, MD 20850.

Autoradiograms are often produced by apposing radiolabeled specimens (tissues, gels etc.) to photographic emulsion. There are deficiencies in this approach, however. Film optical density is not linearly related to exposure duration, the dynamic range of film is limited (saturation) and long exposure times may be necessary. Storage phosphors are materials that produce a trapped electron population that is proportional to radiation input. The electrons are trapped for long periods of time and are released when stimulated by light or heat. A characteristic visible light emission is produced when the trapped electrons are released. The emitted light can be monitored with a detector, recorded and stored on a computer for subsequent display and analysis. Autoradiograms were produced by apposing methylmethacrylate [^{125}I] standards (Amersham) to storage phosphor-coated acrylic screens and compared to autoradiograms produced when apposed to Ultrofilm ^{3}H (Cambridge). The relationship between resulting optical density and radioactivity of or exposure duration to [^{125}I] was linear over a much longer range for the screens than for film. In addition, the dynamic range of screens was greater than film. Using screens autoradiograms were apparent after shorter exposures than with film or with lower radioactivity. Moreover, the screens are reusable and darkroom facilities (chemicals, plumbing, dedicated space) obviated. These features demonstrate the advantages of this technique as an alternative to film for generating autoradiograms.

156.15

HIGH RESOLUTION AND IMPROVED SPEED FOR FLUORESCENT MICROSCOPY USING KODAK TECHNICAL PAN FILM T.C. Waters *, G.E. Hoffman, and S.L. Small. Depts. Physiology and Neurology, Univ. Pittsburgh, School of Med., Pittsburgh, PA 15261

In the photomicrographic evaluation of fluorescence, short exposures (which reduce the fading of labile fluorophores) and minimal grain size (which improves image resolution) are both desired. Often a compromise of one or the other is necessary with standard film/development combinations. This abstract illustrates a method of developing Technical Pan (Kodak 2514) film providing high resolution and an effective speed equal to or higher than that commonly achieved with Kodak Tri-X, a standard high speed film. Brain sections processed for Texas Red immunofluorescence were used to evaluate negatives using Kodak's Technical Pan (TP) or Tri-X film over a wide range of exposure times. The following developers for TP were compared: D19 (68°F-4 min), HC 110-1:31 (68°F-6.0 min), and HC 110-1:10 (68°F 6.5 min); D76-1:1 (68°F-9.5 min) was used for Tri-X. Contrast range, granularity and speed were compared. D19 produced the highest contrast; both HC-developed negatives produced intermediate contrast ranges; Tri-X negatives were of very low contrast. Grain size for all TP negatives was minimal; Tri-X grain was large and irregular. TP developed in D19 and HC 110-1:31 produced excellent exposures of Texas Red stained cells (mag.100X) ranging from 3-4 sec; optimal exposure of HC 110-1:10 was 1.31 sec; Tri-X, yielded usable exposures over a wide range (21.38-1.54 sec). The use of HC 110-1:10 provided increased speed over other developers tested, little change in contrast and excellent resolution.
Supported by NIH NS 28477, NS28730 and NSF BNS 8919953.

NEUROGLIA AND MYELIN II

157.1

EXPRESSION OF PROTEIN KINASE C (PKC) GENES IN NEURONAL AND GLIAL CELLS DERIVED FROM PRIMARY HUMAN BRAIN CULTURES.
N.P. Dooley, J. Nalbantoglu*, and V.W. Yong. Neuroimmunolgy Unit, Montréal Neurological Institute, Montréal, Québec, CANADA, H3A 2B4.

PKC is encoded by a family of genes and to date seven isoforms of this enzyme have been identified. Our laboratory has shown that PKC is involved in glial cell proliferation and in oligodendrocyte (OL) process extension. We investigated the *in vitro* expression of four PKC isoforms in neuronal and glial cells obtained from adult human brain surgical specimens and from legal curettage abortions. Total RNA was isolated using the guanidinium thiocyanate-phenol-chloroform method from cultures of neuronal and glial cells of greater than 90% purity. Isoform expression was then determined following analysis of the radiolabelled products of the reverse transcriptase-polymerase chain reaction on non-denaturing polyacrylamide gels. In adults, primer pairs for $PKC_{\beta 1}$, $PKC_{\beta 2}$, and PKC_{α} produced bands of predicted molecular weight in OLs, astrocytes (ASTs) and microglia. The same pattern of expression was observed in fetal ASTs and neuronal cultures. The PKC_{γ} band was observed only in RNA extracts from intact adult whole brain. Currently, we are also investigating PKC expression in dedifferentiated glial cells (gliomas). The localization of PKC isoforms to specific neuronal and glial cells in the human central nervous system may aid in the elucidation of their possible distinct functions within each cell type.

157.2

EXCITABILITY PROPERTIES OF DORSAL COLUMN AXONS IN THE MYELIN-DEFICIENT (MD) RAT SPINAL CORD. D. Utzschneider, J.A. Black and J.D.Kocsis Dept. of Neurology, Neuroscience Program, and Sect. of Neurobiology, Yale Univ. Sch. Med., New Haven, CT; VAMC, West Haven, CT 06516

The md rat mutant virtually lacks CNS myelin due to a point mutation in the proteolipid gene product which results in a marked inhibition of oligodendrocyte development. We used in vitro electrophysiological methods to compare conduction properties of md rat spinal cord axons to control age-matched rats (18-26 days) and rats that received transplantations of myelin-forming precuror cells. Latency measurements of compound action potential (CAP) recordings obtained in vitro at room temperature with glass microelectrodes in the dorsal columns indicate that conduction velocity of md rat spinal cord axons is about 25% of age-matched controls (0.6 m/s vs 2.4 m/s). Both groups were able to follow high frequency stimulation (up to 400 Hz) for several hundred msec. Only at very prolonged stimulation presentations did the md spinal cord axons show greater conduction decrement as compared to controls. Both groups of axons are blocked by TTX and show variable changes in waveform following 4-aminopyridine application. GABA application resulted in reduced amplitude of the CAP and conduction slowing for both groups. Injections of fetal and neonatal brain suspensions containing myelin-forming precursor cells resulted in varible amounts of myelination within the dorsal columns. There was a higher frequency of isolated early field potential components in the transplant group as compared to controls.

These results indicate that amyelinated md rat axons, while compromised with respect to conduction velocity, can sustain high frequency impulse firing for hundreds of msec. Additionally, the md axons have Na^+ and K^+ channels and $GABA_A$ receptors as do controls. Transplanation of brain suspensions leads to anatomically defined myelination, and increased probability of early discrete field potential components. Supported by the NMSS and MSTP.

157.3

MORPHOMETRIC ANALYSIS OF NORMAL, MUTANT AND TRANSGENIC CNS: CORRELATION OF MBP EXPRESSION TO MYELINOGENESIS. H.D. Shine, C. Readhead*, B. Popko*, L. Hood*, and R. L. Sidman. Department of Neurosurgery, Baylor College of Medicine, Houston, TX 77030.

The neurological mutant mice *shiverer* (*shi*) and *myelin deficient* (*shi*^{mld}) lack a functional gene for the Myelin Basic Proteins (MBP), have virtually no mycin in their CNS, shiver, seize, and die early. Mutant mice homozygous for an MBP transgene have MBP mRNA and MBP in net amounts approximately 25% of normal, have compact myelin, do not shiver or seize, and live normal life spans. Various combinations of the normal, transgenic, *shi* and *shi*^{mld} genes yield mice that express MBP mRNA at levels of 0%, 5%, 12.5%, 17.5%, 50%, 62.5%, and 100% of normal. MBP protein content correlated with predicted MBP gene expression. Immunocytochemical staining localized MBP to white matter in normal and transgenic *shi* mice with an intensity of staining comparable to the degree of MBP gene expression. An increase in the percentage of myelinated axons and the thickness of myelin correlated with increased gene expression up to 50% of normal and then remained constant at expression levels greater than 50%. Mean axon circumference of myelinated axons was greater than axon circumference of unmyelinated axons at each level of gene expression -- further evidence that oligodendroglial cells preferentially myelinate axons of larger caliber. These data suggest that oligodendroglial function is governed in part by the degree of MBP expression. Supported by NIH grants HD18655, NS20820, and AG07687 and the Retina Research Foundation.

157.4

DISTRIBUTION OF THE MYELIN-ASSOCIATED GLYCOPROTEIN IN MURINE OLIGODENDROCYTES IN VITRO. L.L. Bambrick. CNS Dept. University Hospital, London, Ontario, N6A 5A5.

The myelin-associated glycoprotein (MAG) is an adhesion molecule and the principal glycoprotein of myelin. It has been implicated in oligodendrocyte (OGD)-axon and OGD-OGD adhesion. We have previously shown that MAG has an association with the cytoskeleton in cultured rat OGD. In cultured murine OGD, antibodies to galactocerebroside (GC, an OGD glycolipid) induce the patching of GC into domains. These domains overlie patches of myelin basic protein and are demarcated by veins containing the cytoskeleton proteins actin and tubulin. It has been proposed that these domains correspond to areas of compacted and non-compacted myelin, respectively. In vivo, MAG is principally found in non-compacted myelin. To pursue the question of MAG localization in cells with respect to the cytoskeleton, we have investigated the localization of MAG in murine OGD membrane sheets in vitro using immunofluorescence in cells where domain patching has been initiated with anti-GC antibodies. We report some staining for MAG in unorganized sheets. In areas of GC patching, MAG staining is observed in the veins around the GC-positive domains. Probing for actin using a rhodamine-conjugated phalloidin shows that MAG is colocalized in these veins with the cytoskeleton protein actin. LLB is a Raymond C. Raymond fellow, the support of Drs. P.E. Braun and G.P.A. Rice is acknowledged.

157.5

REMYELINATIVE CAPACITY OF QUIESCENT OLIGODENDROCYTES FROM OPTIC NERVES FOLLOWING LONG TERM WALLERIAN DEGENERATION. Dr. S.K. Ludwin, Queen's University, Kingston Ontario.

We have previously shown that following Wallerian Degeneration (WD) in the rat optic nerve, some oligodendrocytes identified by antibodies to carbonic anhydrase and myelin oligodendrocyte glycoprotein could survive for periods of up to 2 years in the absence of axons.

We have now tested the remyelinative and regenerative capacity of these quiescent oligodendrocytes. Fragments undergoing WD for periods from 13 months to 2 years were implanted into the brains of neonatal Shiverer mice which were studied 4 weeks later using antisera to myelin basic protein (MBP). Myelination was demonstrated outside and within the implant and the presence of MBP indicated that it was of donor origin. In addition, numerous oligodendrocytes, both within and outside the graft, displayed MBP positive cytoplasm, reminiscent of the pattern seen in development and remyelination. Silver stains demonstrated ingrowth of axons from the host tissue into the graft, and may have provided the stimulus for the production of MBP in the graft. Some of these axons were subsequently remyelinated by the oligodendrocytes remaining in the graft.

These results indicate a potential for recovery and remyelination by oligodendrocytes even when deprived of axonal stimulus for long periods, and their subsequent in vivo responsiveness to the presence of axons.

157.6

INCREASED MYELINATION IN JIMPY MICE EXPRESSING BOTH PLP AND DM-20 TRANSGENES. N. Nadon, H. Arnheiter*, S. Wells* and L. Hudson*. Biology Dept., Univ. of Tulsa, Tulsa, OK 74104 and Lab. of Viral and Mol. Path., NINDS, NIH, Bethesda, MD 20892.

Proteolipid protein (PLP) is the major structural protein in myelin of the mammalian central nervous system (CNS). Adult mice express two isoforms of PLP, through the use of alternative splice sites in exon three. The less abundant isoform, DM20, lacks 35 amino acids in an extracellular domain, but is otherwise identical to PLP. Loss of PLP/DM20 function in the X-linked jimpy mouse mutant results in the development of tremors and premature death. Jimpy mice exhibit a reduction in the number of mature oligodendrocytes and severe hypomyelination of the CNS (<1% of the axons are myelinated). We have used a transgenic mouse system to analyze the roles of PLP and DM20 in the development of myelination. Previously, we reported that jimpy mice carrying a PLP transgene did not show any moderation of the dysmyelination, in spite of the high level of expression of the PLP transgene in wild-type mice (~70% of endogenous PLP/DM20 RNA levels). Transgenic mice have now been produced that express only the DM20 isoform. The DM20 lines examined to date express the transgene at ~10-30% of endogenous DM20 mRNA levels. DM20 transgenes have been bred into the jimpy background, alone and in combination with the PLP transgene. The level of myelination in jimpy mice expressing the DM20 transgene alone was no greater than in jimpy mice without the transgene. However, jimpy mice expressing both PLP and DM20 transgenes exhibited an increase in the number of axons myelinated, suggesting that both isoforms of PLP may be required for oligodendrocyte development. Further characterization of the PLP/DM20 transgenic jimpy mice will include analysis of the expression of the endogenous myelin proteins and their mRNAs, and the affect of the transgenes on oligodendrocyte survival and the lifespan of the jimpy mice.

157.7

IN VIVO COMPARISON BETWEEN TWO MURINE MYELIN BASIC PROTEIN RESPONSIVE T_H CELL TYPES. E. Barbarese*, H.D. Soares, and R. Clark*. Dept of Neurology, Univ of CT Health Cntr., Farmington, CT 06030.

Experimental autoimmune encephalomyelitis (EAE) is an animal model disorder for multiple sclerosis, a human demyelinating disease. Genetically susceptible animals develop EAE following injections of either central nervous system (CNS) homogenates, myelin basic protein (MBP), or MBP responsive T helper (T_H) cell lines or clones. In mice, a $CD4^+CD8^-$, class II restricted, IL-2 dependent T helper cell elicits EAE symptoms (i.e. hindlimb paralysis and sometimes death). One approach towards characterizing EAE's pathological sequelae has focused upon MBP responsive T_H cell homing capabilities. The present experiment utilizes two types of MBP responsive T_H cells to examine CNS homing characteristics. One of the MBP responsive T_H lines, LNC8, causes EAE symptoms 6 days following injection. The other MBP responsive T_H cell clone, B48, fails to induce any of the disease's symptoms. Mice received i.p. injections of fluorescently labelled (DiI) LNC8 or B48 cells. Animals were sacrificed at 3 or 4 days following injections. The number of LNC8 T cells in brain and spinal cord rose from approximately 20 cells/cm^2 on day 3 to 150 cells/cm^2 on day 4. Conversely, the number of B48 cells in brain and spinal cord fell from approximately 50 cells/cm^2 on day 3 to basal levels on day 4. Histological analysis demonstrated lesions and astrogliosis in the CNS of LNC8 injected mice. Little neuropathological damage was observed in B48 injected animals. Both MBP responsive T_H cell types pass through CNS tissue, however, the disease causing LNC8 cells appear to accumulate in brain and spinal cord on day 4 while the B48 cells do not. Supported by NINCDS #19943.

157.8

ASTROCYTIC REACTION TO OLIGODENDROCYTE AND NEURONAL DEGENERATION. N.R. Bhat, Dept. Biochem. and Sanders-Brown Center on Aging, Univ. of KY, Lexington, KY.

Reactive gliosis is a common neuropathological feature of several neurodegenerative disorders. In this study, two primary culture models i.e., mixed glia and hippocampal cultures derived from newborn rat brain have been used to characterize astrocytic reaction to oligodendrocyte (OL) and neuronal degeneration resp. A week-old mixed glial cultures containing astrocytes, OL progenitors and microglia were treated with the monoclonal antibody A2B5 along with complement to lyse OL progenitors. Western blot and Northern analysis revealed an increased expression of GFAP after 2 days of immunocytolysis. There was also an increase in glutamine synthetase activity, another astrocytic marker. In the 2nd set of experiments, 10-12 day old hippocampal cultures containing both neurons and glia were exposed to either kianate or glutamate. A majority of the neurons succumbed to this excitotoxic insult within 24 h. Immunoblot analysis of the culture extract showed an increase in GFAP. Pure glial cultures did not respond the same way. Addition of purified microglia to hippocampal cultures resulted in an enhanced astrocytic response to neuronal death. The culture models described here should prove useful in further analysis of the intercellular signals involved in astrocytosis. (Funded in part by Center on Aging).

157.9

A TRANSGENIC TAG FOR TRACKING TRANSPLANTED GLIAL CELLS
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LVMP, NINDS, NIH Bldg 36/5D04, Bethesda MD 20892

Transplanted oligodendrocytes and their precursor cells can migrate and remyelinate focal demyelinating lesions produced by lysolecithin in *shiverer* mice (Gout et al., Neuroscience Lett. 87:195-199,1988). However in these experiments the endogenous *shiverer* oligodendrocytes, which have a deletion for the myelin basic protein (MBP) gene, could not be readily distinguished from transplanted oligodendrocytes, which expressed MBP only transiently in their cell bodies. To investigate mechanisms of remyelination by grafted glial cells in normal mice, we have generated a constant supply of genetically marked oligodendrocytes. The marker was β-galactosidase, and the glial-restricted expression was specified by the selection of the promoter for the most abundantly expressed CNS myelin gene, proteolipid protein (PLP). Detection of enzymatically active β-galactosidase in a human fetal glial line transfected with a PLP promoter(1.2 kb) fused to the E. coli *lacZ* plasmid indicated that the construct was suitable for glial expression in transgenic mice. Several transgenic founder lines containing the PLP promoter-*lacZ* construct are being analyzed histochemically to determine the extent and the onset of β-galactosidase expression under the PLP promoter. Since the PLP gene is apparently activated before the actual onset of myelination, the transgene may likewise be turned on before final oligodendrocyte differentiation. This may then allow us to follow transgenic grafted cells during the process of migration and myelination.

157.10

CEREBELLAR SLICES: A SYSTEM FOR STUDYING MYELINATION.
J.W. Kasckow, P.N. Bullock*, S. Malek-Hedayat*, and L.H. Rome*. Dept. Biological Chem., UCLA Med. Ctr., Los Angeles, Ca. 90024.

Myelination in vitro has been studied using various systems including organotypic cultures (Silberberg et al., J Neurochem 19:11, 1972), mixtures of oligodendrocytes or Schwann cells added to dorsal root ganglia (Wood & Bunge, Nature 320:756, 1986) as well as glass microfibers coated with astroglial matrix (Bullock & Rome, J Neurosci Res 27:383, 1990). We have modified and extended a previous method using cerebellar slices to enable the study of in vitro myelination. Cerebella from 1 day old rat pups were cut into 300 μm sections. Slices were cultured onto 12 well plates of collagen coated petriperm in an enriched medium containing insulin, fetal calf serum and horse serum. Slices were metabolically labeled with radioactive sulfate and then disrupted with a chaotropic salt solution allowing efficient extraction of sulfolipids. Myelin biosynthesis as assessed by sulfolipid incorporation increased linearly up to 32 days in vitro. Slices were also examined by electron microscopy. As early as day 17, myelination around axons could be seen exhibiting the characteristic alternation of major dense and intraperiod lines.
Supported by HD 06576 and NIMH 5T22 MH 17140-08..

157.11

GLIAL CELLS IN THE DEVELOPING OPTIC NERVE OF THE FROG *HYLA MOOREI*.
D.E. Playford and S.A. Dunlop, Department of Psychology, University of Western Australia.

In the frog, numbers of retinal ganglion cell and axons increase throughout life. We have recently shown that, in fully mature *Hyla moorei* of 9 cm length (2+ years), 2% of optic axons are myelinated. Comparable numbers of myelinated axons are found behind the eye, at the foramen and at the chiasm. However, this mature pattern is achieved only gradually over 1-2 years by the differential addition of myelin along the length of the nerve. Thus, in tadpole stages when myelination is initiated, most myelinated axons are found at the foramen and fewer at the eye and chiasm. In young adults of 5 and 7cm length, there is a gradient along the nerve with most myelinated axons at the chiasm. We have examined changes in the glial cell population at the three levels in the nerve from tadpole stages to adulthood using ultrathin sections on formvar grids. Astrocytes were pale and contained neurofilaments; oligodendrocytes were dark and had microtubules. Immature glial cells were also observed. Glial cell numbers increased throughout life but, at all stages, astrocytes were predominant. However, the proportion that oligodendrocytes represented of the total glial cell population consistently mirrored the numbers of myelinated axons. Thus, in tadpole stages, the highest proportions of oligodendrocytes were found at the foramen; in young adults the highest proportions were found at the chiasm and in fully mature adults there were equal prortions at all three levels. Our data suggest that the differential patterns of myelination in the developing frog optic nerve are underpinned by changes in the proportions of oligodendrocytes.
Funded by the National Health & Medical Research Council of Australia.

157.12

IDENTIFICATION OF A RAT cDNA CLONE FOR MYELIN/OLIGODENDROCYTE GLYCOPROTEIN:
M.V. Gardinier, P. Amiguet*, C. Linington*, and J.-M. Matthieu. Labo. de Neurochimie, CHUV, CH-1011 Lausanne, Switzerland

The myelin/oligodendrocyte glycoprotein (MOG; 26 and 28 kDa) is expressed specifically in central nervous system myelin. Our group and others have shown that: 1) MOG is localized on the external surface of the myelin sheath; and 2) anti-MOG antibodies can induce demyelination both in vivo and in vitro. Thus, MOG may represent an ideal target antigen involved in demyelinating disease. Protein sequence analysis of purified MOG yielded a sequence of 26 amino acids (aa). A rat oligodendrocyte-enriched cDNA library (≈8 x 10^5 clones) was screened with a mixture of 3 MOG-specific monoclonal antibodies, and 3 putative MOG cDNA clones were isolated. These clones appear to contain identical inserts (≈1.1 kb). Initial sequence analysis reveals an open reading frame containing a Met codon, a possible signal peptide region (encoding 26 aa), followed by sequence encoding the amino acids identified by N-terminal sequence analysis. The nucleotide sequence revealed 2 aa changes (out of 26 aa), perhaps due to species differences (rat vs. mouse). A 1.5 kb MOG mRNA is detected on a Northern blot of brain polyA+ RNA.

157.13

ONCOGENE-SPECIFIC DOWN-REGULATION OF MOUSE MYELIN P_2 EXPRESSION.
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Myelin P_2 protein is a small (14,800 Da) basic protein that belongs to a family of proteins involved in fatty acid binding. In rodents, P_2 protein is found in myelin and in the cytosol of Schwann cells, while in other species, the protein is also found in oligodendrocytes. To study the regulation of this protein in the nervous system, we cloned the mouse P_2 gene and determined the transcription start sites by primer extension analysis. The 5'-flanking sequences contained a TATA box (-31), two CAAT boxes (-194, -232), and two AP-1 binding sites (-53, -391). To identify the transcriptional regulatory effects of these AP-1 sites, Schwann cell lines (MT_4H1) were co-transfected with the chloramphenicol acetyltransferase (Cat) reporter gene under the control of the P_2 promoter with expression plasmids for c-jun, jun B, jun D, c-fos, and fra-1. Cat activity levels indicated that co-transfection of the P_2 promoter, along with a c-jun- and/or c-fos-containing plasmid, down-regulates myelin P_2 expression in Schwann cells. jun B, jun D, and fra-1 did not alter P_2 Cat activity. Results suggest that the presence of the AP-1 binding sites on the mouse myelin P_2 promoter inhibits the expression of this gene in Schwann cells. Furthermore, this down-regulation of myelin P_2, after co-transfection with c-jun, is reversed by forskolin at a concentration of 10 μM. (Work supported by a grant from N.I.H. R01-NS 21700).

157.14

COMPARISON OF OLIOGODENDROCYTE MARKERS IN IMMUNOHISTOCHEMISTRY.
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The investigation of glial cells in the nervous system is increasingly important as their interaction with other neural cell types is still not well understood. Markers to distinguish glial cells, especially oligodendrocytes (oligos) and Schwann cells (both of which produce myelin) are proving to be valuable tools in the study of nervous system. Antibodies, functioning as discriminating markers, can be used in a variety of techniques, such as immunohistochemistry (IHC) and immunoblotting. In the present study, we describe the comparison of several oligo-specific antibodies in IHC of cultured cells, sectioned brain tissue and immunoblotting.

We present data using three monoclonal antibodies anti-CNPase (2",3'-cyclic nucleotide 3' phosphodiesterase), anti-GalC (galactocerebroside) and anti-MBP (myelin basic protein). The IHC labeling by each is compared in fixed, rat oligo cultures from cerebral cortex. Anti-CNPase and anti-GalC yield similar labeling in these cultures. Labeling patterns of the three antibodies in fixed sections of rat brain are also compared. The antibodies are used in immunodetection of Western blot transfer of delipidated rat brain extracts. The relative advantages and limitations of these antibodies are presented.

157.15

HUMAN OLIGODENDROCYTES ARE NOT SUSCEPTIBLE TO LYSIS BY NON-MHC RESTRICTED CD4+ LYMPHOCYTES. T.C.G.Ruijs, E.A. Brown* and J.P.Antel. Montreal Neurological Institute, McGill University, Montreal Quebec, Canada H3A 2B4.

In the putative autoimmune disease multiple sclerosis, CD4+ T-lymphocytes reactive to myelin antigens may play a role in injury to oligodendrocytes (hOL). CD4+ lymphocytes classically recognize only cells expressing Major Histocompatibility Complex (MHC) class II molecules, but hOL appear to lack expression of MHC class II. CD4+ lymphocytes can, under selected culture conditions, acquire non-MHC restricted cytolytic capability. We investigated the relative susceptibility of hOL to non-MHC restricted cytolysis mediated by enriched CD4+ lymphocytes activated with the lectin phytohemagglutinin (PHA). Following activation for 3-30 days, 84% (± 3%) of T-lymphocytes were CD4+. These lymphocytes were cytotoxic to the control target cell line U937 in a 18-hour ^{51}Cr release assay, in the presence of lectin to increase effector-target cell contact (25.8 ± 1.9% lysis at 10:1 effector to target ratio). CD4+ cells did not lyse hOL (8.9 ± 2.3% lysis). In contrast, U937 and hOL were both lysed by non-MHC restricted PHA-activated lymphocytes of the CD8+ subset (24.0 ± 5.6% and 28.3 ± 5.8% lysis respectively). Supernatants of cultures did not induce significant lysis of target cells.

157.16

CHROMOSOMAL ASSIGNMENT OF A HUMAN OLIGODENDROCYTE-SCHWANN CELL MARKER GENE BY PCR AND SOUTHERN BLOTTING

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Antibodies to the myelin-associated enzyme 2', 3'-cyclic nucleotide 3'-phosphohydrolase (EC 3.1.4.37, CNPase) have been useful to distinguish oligodendrocytes from astrocytes and neurons in brain and in cell culture. Although the CNS enzyme is capable of hyrolyzing over 4,000 umol/min/mg protein of 2',3'-cAMP to 2'-AMP, the physiological role of this enzyme is not known. The mouse genes are located on two different chromosomes and one may be a pseudogene. In cases where chromosomal assignments are to two or more chromosomes, it is highly desirable to use non-coding flanking or intron relatively non-conserved for an unambiguous assignment. Pure intron human CNPase sequences were amplified in various somatic cell hybrid (hamster-human) DNAs in which human chromosomes 1-22, X and Y were each represented at least once in each panel. Two DNA panels were used and the PCR results were further confirmed by Southern blots on each panel as somatic and control DNA EcoR1 digests. The primer pairs were selected for amplification did *not* amplify the host (hamster) DNA CNPase gene(s). We conclude that the human 2', 3'-cyclic nucleotide 3'-phosphohydrolase gene containing intron 3 is located on chromosome 17.

157.17

RADIATION-INDUCED SCHWANN CELLS IN VENTRAL SPINAL CORD. S.A. Gilmore, N. Phillips*, P. White* and T.J. Sims. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205-7199

Our earlier studies established that x-rays can be used to induce a predictable pattern of Schwann cell development in lumbosacral cords of immature rats. These intraspinal Schwann cells occur initially near the dorsal root entry zones between two and three weeks post-irradiation (P-I). Although intraspinal in location they undergo division, form myelin, and spread throughout the dorsal funiculi and into the dorsal gray matter. The area occupied by these cells is attained by 45 days P-I, and further spread is not evident by 60 days P-I, the longest interval studied in detail. Rarely, small clusters of cells resembling the dorsally situated intraspinal Schwann cells, but separated from them by clearly measurable distances, were noted in the ventral cord at 45 days P-I. This raised the question: do Schwann cells develop regularly but at a later post-irradiation interval in the ventral cord? This report summarizes findings from 53 rats perfused/fixed at intervals from 30 days to 7 months P-I. Interrupted, serial, transverse sections of lumbosacral cord were stained neurohistologically or immunohistochemically. Light microscopic evaluation revealed the presence of ventrally located intraspinal Schwann cells in 21 (40%) of the 53 rats. The distribution of these cells, however, was markedly different from that observed dorsally. In general, they formed small, isolated clusters which occurred more frequently in gray than in the white matter and appeared often to be closely associated with blood vessels. In a few cases a continuum of cells could be traced from the ventral root entry region, through the ventral white matter into the ventral gray matter, the usual pattern observed dorsally. Although these clusters were quite small in rats killed at the shorter intervals, they did not increase in size with a lengthening of the post-irradiation period. In summary, radiation-induced Schwann cells do occur in the ventral spinal cord but differ in many features from those noted dorsally. (Supported by NIH Grant NS-04761).

157.18

SCHWANN CELL DIFFERENTIATION IN VITRO: DIFFERENCES BETWEEN CELLS FROM ADULT RAT AND HUMAN NERVE.

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We recently described (Morrissey et al., J. Neurosci. in press) a method to isolate pure populations of adult derived Schwann cells (ASCs) from peripheral nerve. Rat (R-)ASCs proliferated in response to chemical and axonal mitogens and exhibited fully differentiated function, including support of neurite growth, basal lamina (BL) formation and myelination. We now report that, unexpectedly, similar culture conditions fail to support human (H-)ASC differentiation. In medium containing 10% serum, R-ASCs and monkey (M)-ASCs proliferated in response to axonal contact whereas H-ASCs did not. Coculture for 96h with either human or rat sensory axons stimulated proliferation of R- and M-ASCs (BrdU incorporation indices: 25% to 70%); neither axon stimulated H-ASC proliferation. When serum was elevated to 15% and ascorbate (0.05mg/ml) added, R-ASCs formed BL and ensheathed and myelinated both rat and human sensory axons. M-ASCs associated with neurites and formed BL, but failed to ensheathe or myelinate. H-ASCs associated poorly with neurites and failed to myelinate under these culture conditions. Exploring this species variability may offer insights into the interactions between axons and Schwann cells. The conditions necessary for stimulation of full H-ASC functionality must be determined before such populations can be used clinically for transplantation into injured nerves.

Supported by NS19923 and The Miami Project to Cure Paralysis.

157.19

AXOPLASMIC PROTEINS OF NEURONAL AND GLIAL ORIGIN. S.L. Tanner*, E.E. Storm*, and G.D. Bittner. Dept. of Zoology, University of Texas, Austin, TX 78712.

When severed from its cell body, the distal (anucleate) stump of a medial giant axon (MGA) from the crayfish, *Procambarus clarkii*, continues to generate action potentials, retains an intact axolemma and cytoskeleton, and maintains axoplasmic proteins at a level consistent with that in an intact MGA. We are examining two mechanisms which could account for protein maintenance in an anucleate MGA: (1) axoplasmic proteins which are synthesized in the cell body and transported to the axon could have unusually long half-lives, (2) axoplasmic proteins may be renewed by proteins synthesized in glia and transferred to the axon.

To determine the relative contribution of mechanism #1 to axonal maintenance, we radiolabel MGA cell bodies and analyze the proteins in fast axonal transport within individual MGAs by SDS-PAGE/fluorography. To determine the contribution of mechanism #2, we incubate anucleate MGAs in radiolabelled amino acids and analyze by SDS-PAGE/fluorography. Although we have not yet determined turnover times of axonally transported proteins, our data suggests that glial cells are capable of synthesizing many of the proteins supplied to the MGA by axonal transport. Previous work in our lab has indicated that these proteins are tranferred to the axon. Supported by NSF and TATP grants to GDB.

157.20

A POSSIBLE ROLE FOR GLIA AND STRESS PROTEINS IN THE MAINTENANCE OF AXOPLASMIC PROTEINS. R.A. Sheller[1], R. M. Grossfeld[2], and G.D. Bittner[1]. [1]Dept. of Zoology, University of Texas, Austin, TX 78712 and [2]Dept. of Zoology, North Carolina State University, Raleigh, NC 27695

In the crayfish, *Procambarus clarkii*, the distal stumps of severed MGAs (anucleate MGAs) exhibit long term survival (LTS) for 50-200 days as measured by morphological, electrophysiological, and biochemical criteria (G.D. Bittner, TINS 14:188, 1991). MGAs are surrounded by several layers of glia which undergo hypertrophy during LTS of anucleate MGAs.

When intact or anucleate MGAs are incubated in ^{35}S-methionine, radiolabelled proteins are detected in axoplasm collected by perfusing the MGAs with an intracellular saline. Our data from SDS-PAGE analyses suggest that many radiolabelled proteins are synthesized in glia and transferred to the axoplasm of intact and anucelate MGAs. Radiolabelled actin is one such transferred protein identified with a monoclonal antibody on Western blots. Analyses of Western transfers also show that a 70 kD stress protein (SP70) may be synthesized in glia and transferred to axoplasm. Axoplasmic samples possess more SP70 immunoreactivity (N27F3-4 antibody) than glial sheath samples. The intercellular transfer of this protein may occur at a greater rate in heat shocked MGAs versus MGAs kept at room temperature. Stress proteins transferred from glia to axoplasm could be involved in the LTS of anucleate MGAs by stabilizing axoplasmic proteins. Supported by NSF and TAT grants to GDB.

157.21

BEHAVIOUR OF LABELED SCHWANN CELLS TRANSPLANTED IN A MYELIN LESION OF THE ADULT MOUSE SPINAL CORD. Baron-Van Evercooren A., Gansmuller A., Clerin E., Gumpel M. INSERM, U134, Hopital Salpetrière, Paris, France.

In demyelination of the central nervous system (CNS), Schwann cells (Sc) participate with oligodendrocytes (O) in myelin repair. However, the modalities of intrusion of Sc into the CNS are largely unknown. The present study was designed to analyze the behaviour of transplanted Sc in response to a myelin lesion of the spinal cord. Purified rat Sc (RSc) and immortalized mouse Sc (MSc 80) were labeled prior transplantation with bisbenzimide (Hoechst 33342). Cells were transplanted in or at 8mm, of a 1% lysolecithin induced lesion of the shiverer mouse spinal cord. Codetection of Hoechst+ (H) RSc nuclei and P0+ peripheral myelin on cryostat sections, allowed to demonstrate that RSc transplanted in the lesion, formed myelin around host axons 7 d. post-transplantation (p.t.). Fourty d. p.t., the extent of repair had considerably increased. E.M. analysis of adjacent sections revealed that H+ RSc were associated in a 1:1 relationship with axons and were in process or had formed myelin. They were surrounded by a basement membrane and their myelin was compacted. Codetection of H+ RSc and P0+ myelin and their precise identification by E.M. on adjacent sections, allowed to demonstrate inequivocally, that the myelin of the lesion is formed by transplanted RSc and not by host ones. MSc 80 cells transplanted in the lesion are able to remyelinate demyelinated axons, to the same extent that RSc do. In addition, when transplanted at 8mm from a lesion, H+ MSc 80 were found 20 d. p.t., associated with P0+ myelin in the lesion. MSc 80 cells are thus recruited by a myelin lesion and participate, in competition with host O, to its repair. This experimental model will allow to evidence migration pathways of Sc underway towards a lesion. Supported by MSS RG 1773-B2, INSERM.

CYTOSKELETON, TRANSPORT, MEMBRANE TARGETTING II

158.1

EXPRESSION OF PARVALBUMIN AFTER TRANSFECTION OF NEURONAL AND NON NEURONAL CELLS. Chr. Andressen, V. Gotzos and M.R. Celio. Institute of Histology, University of Fribourg, CH-1700 Fribourg.

Ca^{2+} in the brain plays a major role in the control of axoplasmic transport, neurotransmitter release, and neuronal excitability. Most of these phenomena are mediated or modulated by calcium binding-proteins such as parvalbumin. To understand the physiological function of parvalbumin we have manipulated its concentration in cultured cells. For this we transfected several parvalbumin-negative neuronal (PC12/Neuroblastoma) and non-neuronal (ovarian adenocarcinoma) cell lines with different constructs, leading to the expression of parvalbumin. We fused either the SV 40 promotor, the chicken ß actin promotor or the inducible human metallothionein promotor with a full length cDNA of PV. To obtain a splice signal we used the SV 40 late viral protein gene 19 s splice donor/splice acceptor sequence. Transfection experiments were performed by using the $CaPO_4$ method and selection by G418. Cells in culture were filmed and the expression of parvalbumin was confirmed by immunohistochemistry. Transfected cells exhibited dramatic changes in their shape and in their motility as well as in the duration of their cell cycle. These results suggest that parvalbumin may be preferentially involved in the control of cytoskeletal events.

158.2

IMMUNOCHEMICAL AND GENETIC ANALYSIS OF LAMPREY NEUROFILAMENTS. A. Jacobs, V. Lee, D. Lurie, J. Kamholz, D. Pijak, J. Garbern, M. Selzer. Dept. of Neurology & David Mahoney Institute of Neuroscience, Univ. of PA, Philadelphia, PA 19104

Unlike mammalian CNS axons, transected spinal axons of sea lampreys regenerate and their growth cones are packed with neurofilaments (NF). To study the role of lamprey NF in axonal regeneration, we are comparing them immunologically and genetically to their mammalian counterparts. Mammalian NF are heteropolymers of low-, middle- and high-molecular-weight subunits (NFL, NFM, and NFH), while lamprey NF are assembled from a single 180 kDa subunit (NF180). Immunologic evidence that lamprey NF180 is closely related to other members of the mammalian intermediate filament (IFA) family was first provided by the staining with anti-IFA mAbs of both the NF180 band on separations of lamprey spinal cord homogenates and neurons in tissue sections (Pleasure et al, 1989). To further investigate this relationship, we raised mAbs to lamprey cytoskeleton and found several which label NF180 on Western blots and stain axons in immunohistochemical sections. Thus far, two of these mAbs (LCM-3 & LCM-16) have been studied. On Western blots, LCM-3 binds NF180 and also putative cytokeratin bands of M_r 48 and 52k. This staining pattern is similar to that seen with anti-IFA. In tissue sections, LCM-3 labels large, intermediate and small axons, as well as some large dendrites. These staining characteristics lead us to believe that LCM-3 recognizes a core epitope of the lamprey NF protein. In contrast, LCM-16 does not stain the cytokeratin bands and in tissue sections, labels only large and intermediate size axons. cDNA probes for each of the mammalian NF genes were hybridized to Southern blots of lamprey genomic DNA. One to three unique hybridization bands were seen for each of the mammalian NF probes under conditions of lowered stringency, suggesting sequence similarities between the gene for lamprey NF180 and genes for each of the three mammalian NF subunits. Isolation and characterization of genomic and cDNA clones of lamprey NF gene(s) will enable us to study changes in expression of NF180 during regeneration.

158.3

DIFFERENTIAL DISTRIBUTION OF THE HIGH MOLECULAR WEIGHT NEUROFILAMENT PROTEIN, NF-H, IN NEURONS OF DEVELOPING RAT BRAIN. M. Perazzolo[a*], M.H. Lee[a], V. M-Y. Lee[b*], H.M. Wisniewski[a,c*] & D. Soifer[a,c]. [a]Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314; [b]University of Pennsylvania, Phila., PA 19104; [c]CSI/IBR Center for Developmental Neuroscience, S.I., NY 10314.

Sequential changes in the synthesis and processing of individual cytoskeletal proteins can serve as indicators of the maturation of specific groups of neurons. The neurofilament protein, NF-H appears relatively late in the development of retinal ganglion cells and spinal cord neurons and is differentially processed in different cytoplasmic domains. Western blot analysis of neurofilament proteins from tissue samples suggest that there are differential rates of appearance of the highly phosphorylated form of NF-H in different regions of developing mouse brain. Immunohistochemical analysis of serial sections of developing rat brain using phosphate dependent (RMO24.9) and independent (RMDO9.5) monoclonal antibodies to NF-H allows the generation of a high resolution map of the developmental appearance of dephospho- and phospho-epitopes of NF-H. Discrete groups of neurons are labelled as early as embryonic day 18 (E18) while others do not express NF-H in their adult distribution until postnatal day 21 (P21). Our data allow the construction of a developmental map and database of the time course of expression and modification of NF-H during development and demonstrate that the NF-H gene is not homogeneously expressed in rat brain neurons during development and that NF-H protein is differentially phosphorylated in the axon as compared to perikaryon and dendrites of those cells expressing the protein.

158.4

BOTH HIGH AND LOW MOLECULAR WEIGHT SQUID NEUROFILAMENT PROTEINS ARE EXPRESSED FROM A SINGLE GENE BY ALTERNATIVE RNA SPLICING. James Way*, Ben Szaro[1], Harish Pant, Philip Grant, Harold Gainer, and James Battey. Lab. of Neurochem., NINDS, NIH, Bethesda MD 20892, and [1]Dept. of Biol., SUNY Albany, Albany NY 12222.

Previous studies have shown that two low molecular weight neurofilament proteins (NF 60 and NF 70) from the squid, *Loligo Pealei*, are generated from distinct mRNAs transcribed from a single gene (Szaro et al., submitted). In this study, we have isolated cDNA clones encoding a high molecular weight neurofilament protein (putative NF 220) from a stellate ganglion cDNA library by low stringency screening using NF 60 rod domain cDNA probes. Structural analysis of these cDNAs reveals that this neurofilament protein is also generated by alternative processing of the primary transcript from the same gene that encodes the NF 60 and NF 70 proteins. All three proteins are identical in the structure of their amino terminal, lamin-like rod domains. They differ in the structure of their carboxy terminal tail sequences. In contrast to the relatively short tail segments of the NF 60 and 70 proteins, the longer tail domain of the high molecular weight neurofilament protein has multiple copies of a tandemly repeated sequence motif containing Lys-Ser-Pro (KSP). This repeated motif is similar to the tail domains of mammalian NF-M and NF-H proteins, where these serine residues are often extensively phosphorylated. Hence, diversity in neurofilament protein structure is generated in the squid by alternative RNA processing from a single gene, in contrast to mammals where it is derived from three distinct genes.

158.5

PHOSPHORYLATION, CALMODULIN BINDING AND CALPAIN-MEDIATED DEGRADATION OF INDIVIDUAL NEUROFILAMENT PROTEINS. J.A. Greenwood, J.C. Troncoso, A.C. Costello* and G.V.W. Johnson. Dept. of Psychiatry, Univ. of Alabama at Birmingham, Birmingham, AL 35294 and Depts. of Pathology and Neurology, The Johns Hopkins Univ., Baltimore, MD 21205.

Protein phosphorylation has been hypothesized to regulate neurofilament metabolism. Neurofilaments consist of three phosphoproteins with apparent molecular masses of 70 kD (NF-L), 150 kD (NF-M) and 200 kD (NF-H). Both NF-H and NF-M have extensively phosphorylated COOH-terminal domains that are highly conserved across species.

In this study, quantitative immunoblot analysis, calmodulin affinity chromatography and blot overlay techniques were used to examine the role of phosphorylation and calmodulin binding on the in vitro calpain-mediated degradation of the individual neurofilament proteins.

Our results indicate that the phosphorylation state of NF-M directly modulates its susceptibility to calpain hydrolysis; dephosphorylated NF-M was proteolyzed significantly faster than native NF-M. In contrast, native and dephosphorylated NF-H were apparently proteolyzed at similar rates. However, the presence of calmodulin significantly inhibited the calpain-induced degradation of native NF-H. The proteolysis of NF-M and NF-L were unaffected by the presence of calmodulin. In the presence of Ca^{++}, calmodulin selectively bound to NF-H and this association appears to be modulated by the phosphorylation state of NF-H. In a neurofilament-enriched fraction with all three subunits present, calmodulin retained its selective binding to NF-H. These results suggest that protein phosphorylation may act either directly or indirectly to regulate the in vivo metabolism of the two higher molecular weight neurofilament subunit proteins.

Supported by NIH grants NS27538 and NS25369 and a grant from the American Health Assistance Foundation.

158.6

DISTRIBUTION OF PLECTIN, AN INTERMEDIATE FILAMENT BINDING PROTEIN, IN THE RAT NERVOUS SYSTEM. L. Errante[1], R. Foisner*[2], G. Wiche*[2] and G. Shaw[1]. [1]Dept. of Neuroscience, Univ. of Florida College of Medicine, Gainesville, FL 32610 and [2]Institute for Biochemistry, Univ.of Vienna, A-1090 Vienna IX, Austria.

Plectin is a 300kDa protein that was originally identified and characterized as a major component of the cytoskeleton in the rat glioma C6 cell line (Pytella and Wiche, 1980). Recent work in our laboratory has shown that a similar 300KDa protein may be a cytoskeletal component of the bovine and rat spinal cord. To further elucidate the presence of the plectin in neural tissue, mono- and polyclonal antibodies against plectin have been used to localize plectin within the rat nervous system. Preliminary results show that plectin is localized to: ependymal cells lining the ventricles, choroid plexus cells; tanycytes of the hypothalamus; Bergmann glia cells in the cerebellum; radial glial cells in the spinal cord; a subset of astrocytes in white matter; and a subset of motoneurons within the brainstem and spinal cord. Double-label immunocytochemistry shows that plectin and vimentin are often detected in the same cell, but their distribution is distinct; plectin staining tends to be punctate whereas vimentin is filamentous. In addition plectin is concentrated in cells and cell processes lining the ventricles and close to the pia surface, suggesting a possible role in the maintenance of the blood-brain and blood-CSF barrier.

158.7

BRAIN TISSUE CONTAINS TWO ISOFORMS OF THE SPECTRIN BINDING PROTEIN AMELIN. W. E. Zimmer*, L. A. Casoria*, I. S. Zagon and S. R. Goodman. Dept. of Structural and Cellular Biology, Univ. of South Alabama, Mobile, AL 36688 and Dept. of Anatomy, The Milton S. Hershey Medical Center, Hershey, PA 17033.

Amelin is a 93 kDa spectrin binding protein found in mammalian neural cells. It was first identified as an antigenic analog of RBC protein 4.1 found in the cell bodies and dendrites of neurons, as well as in the cell bodies of certain glial cells. We have produced an antisera to the brain protein isolated from 2-D gel analysis of total brain homogenates. Our antibody demonstrated specific binding to the 93 kDa amelin protein in total brain homogenates which comigrates with purified protein, however, this antibody does not recognize RBC 4.1. The brain amelin antibody demonstrates binding with a 97 kDa protein in embryonic brain tissue which is diminished in content during mouse brain development. Additionally, a 93 kDa protein recognized by this antibody is initially expressed during the second postnatal week of development increasing in content with maximal expression found in adult brain tissue. In contrast, the same homogenates probed with anti-RBC 4.1 demonstrated significant quantities of the 93 kDa protein in embryonic brain which decreases in content during development. Moreover, our brain amelin antibody demonstrated specific antigen localization in axons within the medullary layer of adult mouse cerebellum. These data, in addition to 2-dimensional peptide mapping analyses, demonstrate that these are two isoforms of the spectrin-binding protein amelin in mammalian brain cells; one which is antigenetically similar to RBC 4.1 located in soma and dendrites, and a second which is distributed within axons of mammalian brain. This work has been supported by NIH grant #RO1 NS26536-04.

158.8

EVIDENCE ON THE ASSOCIATION OF cAMP PROTEIN KINASE WITH COLD-STABLE MICROTUBULES IN RAT CEREBRAL CORTEX. D. Tinelli*, J. Perez*, C. Cagnoli*, P. Pecin*, L. Steardo and G. Racagni. Center of Neuropharmacology, Inst. Pharmacol. Sci., Univ. of Milan, Italy.

In the central nervous system cAMP can affect microtubule functions through its effect on a cAMP protein kinase (cAMP pk, type II), bound to the microtubule associated protein 2 (MAP2). The effects of phosphorylation are well described only in the fraction that undergoes depolymerization at cold temperatures (cold-labile), during temperature dependent polymerization-depolymerization cycles.

We investigated the possible association of cAMP pk and its substrates to cold-stable microtubules from rat cerebral cortex. cAMP binding has been evaluated by photoaffinity labeling with 8-azido-[^{32}P]-cAMP. A cAMP receptor with apparent M_W of 52 KDa was present in cold-stable preparations. This cAMP receptor (pk R subunit) is in the inactive holoenzyme form since endogenous phosphorylation (with 5 μM cAMP) shows an increase in ^{32}P incorporation in different protein bands (M_W=52, 70, 280 KDa). This effect is mediated by the activation of catalytic subunit of pk since it is completely inhibited by a specific pk inhibitor. The binding of the complex 8-azido-[^{32}P] cAMP/RII with nitrocellulose blotted proteins of cold-stable microtubules identifies bands at 52, 70 and 280 KDa as possible substrates able to bind R subunit.

In conclusion, the results demonstrate the presence of cAMP pk in cold-stable microtubules in rat cerebral cortex.

158.9

CULTURED RETINAL NEURITES IN A HYPOTONIC ENVIRONMENT. H.Takahashi[1], S.Akiya[1]*, T.Ogata[2]* and H.Horie[3]. [1]Dept. of Ophthalmol., UOEH, Kitakyushu [2]Dept.of Ophthalmol., Keio Univ., Tokyo and [3]Dept.of Physiology, Yokohama City Univ., Yokohama, Japan.

New glaucoma hypothesis that a hypotonic environment affects the axonal transport and that the optic nerve swells in the lamina cribrosa was reported in vivo. Cultured mammalian neuronal cell bodies and neurites can adapt to a hypotonic environment (Horie, et al, Brain Res 477:233, 1989:Takahashi, et al, Soc Neurosci Abstr 15:159, 1989). In this study, retinal neurons from newborn mice were cultured for 2-3days and their neurites were analyzed by a phase contrast microscope equipped with a video-system. When a half osmolal (1/2 Na+) concentration of Tyrode solution was applied to neurites, they partially swelled and then recovered to the initial sizes. These swellings were strongly enhanced after 1hr treatment with $1x10^{-5}$ M colchicine. Ultrastructural studies revealed that microtubule density was lower in the swollen than unswollen portions, and that abundant organella, especially mitochondria and vesicles, were in the swollen portions. These suggest that the distribution of microtubules might not be homogeneous in neurites, and that there might be such specific portions in the lamina cribrosa.

158.10

PHOSPHORYLATION OF τ PROTEIN IN τ-TRANSFECTED 3T3 FIBROBLASTS. **L. Sygowski*, A.W.Fieles*, M.M.S.Lo, A.I. Salama, V.M.-Y.Lee & C.B.Caputo. Pharm Dept., ICI Americas Inc., Wilm. DE 19897 & U of Pa Schl of Med., Phila., PA 19104.**

τ phosphorylation was assessed in 2 clones of 3T3 fibroblasts stably transfected with the gene for 3 repeat human τ. Western blots of cell extracts were probed with antibodies tau-14 & tau-46. Both clones expressed τ which migrated in 2 bands that were slightly higher than the band for the same τ expressed in E.coli. No expressed τ was detected in extracts from untransfected 3T3 cells. Only the upper τ band reacted with T3P, an antibody which reacts with a phosphorylated τ epitope, while both bands reacted with tau-1, an antibody which reacts with only a non-phosphorylated τ epitope. After treating blots of cell extracts with alkaline phosphatase, the intensity of the T3P-reactive band decreased while that of both tau-1 bands increased, suggesting that some τ in the upper band is phosphorylated at both the T3P & tau-1 sites. Autoradiographs of extracts of cells labeled with ^{32}P-H_3PO_4 showed the higher tau-14 reactive band to be heavily labeled. Adding 8-Br-cAMP, phorbol dibutyrate and okadaic acid resulted in a small increase in the radiolabel, with 2393 dpm incorporated into τ vs. 1715 dpm for vehicle-treated cells. Thus τ-transfected 3T3 cells express τ protein, which is phosphorylated in cultured cells. This phosphorylation is enhanced slightly by adding kinase cofactors and a phosphatase inhibitor.

158.11

DIFFERENTIAL SUBCELLULAR DISTRIBUTION OF PARTICULAR mRNAs DURING THE DEVELOPMENT OF HIPPOCAMPAL NEURONS IN CULTURE

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Previous studies have demonstrated a differential subcellular distribution of particular mRNAs within hippocampal neurons in culture. The mRNAs encoding actin, tubulin, and GAP-43 are confined to the cell body, whereas the message encoding the dendritic protein MAP2 is found far into the dendrites. (Kleiman et. al., Neuron, 5:821-830, 1990). This study evaluates when during neuronal development the selective sorting of RNA into cell body or somatodendritic compartments begins.

The intracellular distribution of mRNA encoding actin, tubulin, GAP-43 and MAP2 was evaluated by *in situ* hybridization with ^{35}S-labeled cRNA probes. Neurons were fixed after 1, 2, 3, 5, 7, 10, or 14 days in culture and stored in 70% ethanol until hybridization.

Actin, tubulin, and GAP-43 mRNA was detectable in the cell bodies of cultured neurons at all developmental stages examined. These mRNAs were never detected in dendrites. Only low levels of MAP2 mRNA was detectable in neurons that were fixed at 2 or 3 days in culture and at these times there was no detectable labeling in dendrites. The level of MAP2 mRNA expression increased as a function of time in culture. Beginning at approximately 5 days, MAP2 mRNA was detected in the dendrites of a few neurons. At later time points, a larger percentage of the neurons exhibited dendritic labeling with the MAP2 probe. By 10 days in culture, most neurons exhibited significant dendritic labeling.

It is of interest that MAP2 mRNA first becomes detectable in dendrites at approximately 5 days in culture, which coincides with the beginning of significant dendritic outgrowth in these neurons. Supported by NIH NS23094 to GB and OS. RJK received a predoctoral fellowship from NIH HDO7323.

158.12

SYNAPTOSOMAL RNA: ASSESSMENT OF CONTAMINATION BY GLIA AND COMPARISON WITH TOTAL RNA.

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Previous studies suggest that synaptosomes include dendritic fragments which contain RNA, amongst which are mRNAs that encode synaptic proteins (Rao and Steward, J. Neurosci., in press). Thus, synaptosomes may be a source for the population of mRNAs that are localized in dendrites (Chicurel et al, 1990). However, synaptosomes may be contaminated by fragments of glia. In the present study we evaluate: 1) the degree of contamination of synaptosomal RNA by mRNA from glia. 2) the overall complement of synaptosomal mRNA compared to mRNA from cell body fractions or from whole brain.

Total RNA was isolated from synaptosome and cell body (P1) fractions from young rats, and from whole forebrains. Levels of GFAP mRNA (which is specific to astrocytes) were assessed by Northern and dot blot hybridization. Levels of mRNA for the alpha subunit of CAM kinase2 were assessed as a positive control, since this mRNA is known to be present in dendrites (Burgin et al, 1990, J. Neurosci., 10,1788). Overall mRNA complexity was evaluated using a reticulocyte lysate system, and comparing the pattern of labeled polypeptides using PAGE-fluorography. The mRNA for CAM kinase was detected in all three samples, as was the mRNA for GFAP. These results suggest that mRNA from astrocytes is a significant contaminant of synaptosomal RNA. Fluorographic analysis of the translation products revealed fewer labeled bands in synaptosomes compared to whole brain and differences between the three samples in the pattern of labelled bands. These results support previous suggestions that synaptosomes contain a complement of mRNAs that is different from that found in total tissue RNA. Supported by NIH NS12333 to O.S.

REGULATION OF GENE EXPRESSION II

159.1

PARAMETERS AFFECTING THE USE OF HUMAN POSTMORTEM BRAIN FOR MOLECULAR BIOLOGICAL STUDIES.

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The isolation of RNA from human postmortem brain tissue is a necessary procedure for the study of gene expression and preparation of cDNA libraries. We have evaluated the competence of RNA, isolated from human brain stored for various periods at -70^{o}C, for use in molecular biological studies. mRNA was quantitated by two separate methods: Northern blot hybridization and quantitative analysis of polymerase chain reaction products. Biological activity was assessed both by cDNA synthesis and in vitro translation. Neither postmortem interval nor holding of tissue on wet ice for short periods affect the quality of the RNA. However, results suggest that prolonged freezer storage of postmortem human brain will preclude its use for library construction or in vitro expression, but not for isolation of nucleotide sequences or evaluation of gene expression by PCR.

159.2

mRNA AT THE SYNAPSE: ANALYSIS OF A PREPARATION ENRICHED IN HIPPOCAMPAL DENDRITIC SPINE mRNA.

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Synaptic transmission and its regulation involves multiple neurotransmitters/neuromodulators, ligand receptors, ion channels and intracellular second messengers. The nature, function and regulation of another kind of synapse-associated molecule—mRNA—is only beginning to be appreciated. In addition to the cell body, mRNA is found in dendrites and astrocytic processes and may be specifically targeted to these locations to direct local protein synthesis. We have found that a specific mRNA population is closely associated with a synapse in the vertebrate CNS. Specifically, our anatomical data indicate that the branched spines of the mossy fiber (MF)-CA3 hippocampal synapse contain a large number of polyribosomes. Because of their unusual association with the MF boutons, the branched spines can be isolated as part of a synaptosomal complex that includes the MF boutons and the fine astrocytic processes associated with this synapse. We have been able to prepare and analyze intact mRNA from this synaptosome preparation. The results indicate that certain mRNAs (mRNAs coding for CaM kinase II and MAP-2, for example) are enriched in the synaptosomal complex preparation as compared to the total hippocampus; other mRNAs are less prevalent or altogether absent (i.e., mRNAs coding for synaptophysin, the Glu-RK1 kainate receptor, and tubulin). A subtractive hybridization protocol was designed to identify and clone mRNAs localized in the dendritic spines (rather than the astrocytic processes) and these are now being analyzed. In summary, our results suggest that a *localized* translational regulation of gene expression may be important in establishing and modulating synaptic function.
Supported by the NIH. MEC is a Howard Hughes Predoctoral fellow.

159.3

ACTIN AND TUBULIN ARE COMPONENTS OF A HETEROGENEOUS mRNA POPULATION PRESENT IN THE SQUID GIANT AXON.

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Previously we have reported that the squid giant axon contains a biologically active population of poly(A)$^{+}$mRNAs and polyribosomes (see Giuditta et al. 1991 J. Neurosci. Res. 28:18). The sequence complexity of this RNA population is sufficient to encode ca. 100-200 different mRNA species. To initiate characterization of the axonal mRNA population, a cDNA library was constructed in a λ-Zap vector using a modification of the Gubler-Hoffman method. The library contained ca. 1.6 X 10^5 pfu with insert sizes ranging from 600-4000 bp in length. Dideoxy sequence analysis of several relatively abundant clones yielded cDNAs encoding β-actin and β-tubulin. The actin and tubulin cDNAs were ca. 1.8 and 2.0 kb in length, respectively and contained the entire coding portions of the cognate mRNAs. The predicted amino acid sequence of squid actin and tubulin share ≥ 95% sequence identity to the mammalian forms of the protein. Results of in situ hybridization analyses provide additional evidence for the axonal localization of these mRNAs. Taken together, these findings (1) provide direct evidence for the existence of mRNAs in the squid giant axon, (2) establish the identity of two components of the axonal mRNA population, and (3) raise the possibility that key elements of the cytoskeleton are synthesized locally in the squid giant axon.

159.4

MULTIPLE MRNA SPECIES OF THE TYPE I CORTICOSTEROID RECEPTOR EXIST IN THE RAT HIPPOCAMPUS.

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Cloning of the type I, mineralocorticoid receptor cDNA from the rat hippocampus (Patel et al. Mol. Endo. 3:1877, '89) revealed that the 5' untranslated region (5'UT) of this clone (β) varied significantly from the 5'UT of the species present in human kidney (α) described by Arriza et al. (Science, 237:268, '87). The β UT exon was subsequently found 2Kb upstream of the α exon on the rat MR gene (Patel et al. Neurosci. Abst.1989). Results from RNase protection studies indicate that these two species account for approximately 60% of the total type I receptor mRNA pool, suggesting that other 5' variants of the mRNA exist.

We have employed "rapid amplification of cDNA ends" (RACE) reaction (Frohman et al., PNAS 85:8998, '88) to identify other 5'UT variants of the type I receptor mRNA in the hippocampus. Oligonucleotides complementary to a region within the open reading frame (common to all variants) were used to generate a cDNA pool. After a second round of amplification by polymerase chain reaction, we isolated a cDNA variant of the type I receptor mRNA which contained an unique 5'UT of 130bp. The new 5'UT (γ) exon was localized ~500bp upstream of the β exon on the type I receptor gene by southern blot analysis. RNase protection assay revealed that this variant was rarely expressed (<5% of the total type I mRNA) in normal animals. We are currently isolating other 5'UT variants of the type I receptor mRNA in the hippocampus.

159.5

TWO NOVEL POU SEQUENCES ARE PRESENT IN THE DEVELOPING RAT CNS AND PERIPHERY. W.S. Young, III, C. Le Moine, and D.J. Bradley. Lab. of Cell Biology, NIMH, Bethesda, MD 20892

The family of POU transacting factors present in the CNS was expanded by He et al. (Nature 340:35) who used PCR to clone 4 new sequences and suggested the presence of many more. We used primers based on the sequence at the 5' and 3' ends, either exactly for the SCIP (Tst-1) POU-domain or degenerate (courtesy Dr. S. Sato, NIH) for the class III POU domain. We isolated two clones whose identities are different from previously describe class III POU genes. Over the POU domains, RHS-1 differs in only one amino acid each from human Brn-1 and Brn-2, whereas RHS-2 differs by 6 and 4, respectively. They differ from each other and SCIP at the nucleotide level by about 20-25%. Northern analyses of total rat brain RNA using ^{32}P-labeled oligonucleotide probes detected bands of 3.9 and 3.3kb for RHS-1 and RHS-2, respectively.

The sequences were inserted into pGEM vectors from which ^{35}S-riboprobes were generated for hybridization histochemistry. RHS-1 and RHS-2 probes detected mRNA in rat neuroectoderm as early as embryonic days 9.5 (especially cranial) and 11.5, respectively. Transcripts were detected throughout the rat CNS into adulthood. RHS-1, to a much greater degree than RHS-2, also detected mRNA in developing peripheral tissues, especially in the kidney and developing bone. Both probes labeled mRNA in different locations about the vibrissae anlagen. These probes are unlikely to cross-hybridize to each other under our conditions, but they may detect other POU sequences. These concerns and functional studies will be better studied with full-length clones.

159.6

SEQUENCE ANALYSIS OF VASOPRESSIN mRNAs IN THE HOMOZYGOUS BRATTLEBORO RAT WHICH CODE FOR GLYCOPEPTIDE IMMUNOREACTIVITY. [1]A.P. Evans*, [2]R. Ivell*, [3]F.W. Van Leeuwen*, [3]M. Corner and [1]J.P.H. Burbach*. [1]Rudolf Magnus Institute, University of Utrecht, Utrecht, The Netherlands, and [2]Institute for Hormone and Fertility Research, Hamburg, F.R.G., and [3]Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

A single base deletion in the vasopressin (VP) gene is the cause of diabetes insipidus (di) in the homozygous (di/di) Brattleboro rat. The di/di rat expresses a mutant VP precursor with an altered C-terminus. A striking feature of the di/di rat is the appearance of apparently normal VP gene products together with the mutant precursor protein in solitary hypothalamic neurons. The di/+ phenotype of these VP neurons in the di/di rat suggests the existence of VP mRNAs which code for the normal VP precursor protein (Van Leeuwen et al. PNAS 1989: 86, 6417). The aim of the present study was to elucidate the nucleotide sequence of these VP mRNAs of the di/di rat. The strategy used included specific amplification of VP cDNAs by PCR, followed by cloning of VP cDNAs in an expression vector. Clones containing VP cDNAs with restored reading frame were selected by immunoscreening using a rat glycopeptide antiserum. Sequence data, obtained so far on a limited number of clones, indicate that single nucleotide insertions in the mutated VP mRNA are present. These preliminary findings can explain the presence of normally immunoreactive VP gene products in the di/di rat.

159.7

ESCAPE FROM TRANSLATIONAL DEGRADATION: THE NEURONAL GROWTH-ASSOCIATED ALPHA-TUBULIN mRNA, Tα1, IS PREFERENTIALLY STABILIZED IN PC12 CELLS. J.G. Toma and F.D. Miller. Dept. of Anat. and Cell Biol., Univ. of Alberta, Edmonton, Alberta, CANADA.

Both α- and β-tubulin mRNAs are posttranscriptionally regulated: as the intracellular tubulin monomer:polymer ratio increases the partially-translated mRNA is degraded on the ribosome (Cleveland D.W. TIBS, 13:339,1988). We have previously demonstrated that two α-tubulin mRNAs encoding virtually identical proteins are differentially regulated in mammalian neurons. Tα1 α-tubulin mRNA is expressed at high levels during the growth of developing and mature neurons, while T26 mRNA is constitutively expressed. In this study, we have examined the post-transcriptional regulation of these two mRNAs in C6 glioma and PC12 cells. Northern blot analysis indicates that, in C6 glioma cells, agents that increase the monomer:polymer tubulin ratio, such as colchicine and nocodazole, lead to decreased steady-state levels of both Tα1 and T26 mRNAs. In contrast, in PC12 cells, treatment with these same two drugs decreases the levels of T26 mRNA, but does not affect the levels of Tα1 mRNA. The preferential stabilization of Tα1 α-tubulin mRNA occurs in the presence or absence of NGF. Thus, this one particular tubulin mRNA escapes translational degradation in a cell-type specific manner, possibly as a cellular mechanism for maintaining high levels of tubulin synthesis during neuronal growth. Since the nucleotide sequences of Tα1 and T26 mRNAs are virtually identical over the entire coding region, we are currently swapping the 5' and 3' untranslated regions, in an attempt to map the sequences that confer this "escape".

159.8

EFFECT OF DOPAMINERGIC TREATMENTS ON PREPROENKEPHALIN (ppENK) mRNA EXPRESSION: ADRENAL VS. STRIATUM.
J.D. DeCristofaro, Y. Sharon and E.F. La Gamma. Dept of Peds and Neurobiology, SUNY at Stony Brook, NY.

Dopaminergic pathways are known to regulate expression of ppENK mRNA in the striatum. Treatments with D1 antagonists or D2 agonists result in enhanced expression (TINS 13:244, 1990). We sought to determine whether there was a synergistic effect when both D1 antagonist and D2 agonist were given simultaneously and whether there was any effect on ppENK mRNA in the adrenal medulla which also has dopaminergic receptors. Eight adult male Sprague-Dawley rats were divided into four groups: Vehicle treated, D1 antagonist SCH 23390 (50 ug/kg/dose), D2 agonist LY171555 (1 mg/kg/dose), or both D1 and D2 treated. Rats were treated twice daily for 7 days subcutaneously. Treatment with the D1 antagonist alone or D2 agonist alone resulted in significant increases in striatal ppENK mRNA levels as previously reported, but little change in adrenal ppENK levels. When given together striatal ppENK mRNA levels also increased, but no more than with either agent alone. The combined effect on the adrenal was more dramatic, decreasing ppENK mRNA levels to undetectable, without influencing adrenal TH mRNA. This suggests that dopaminergic signal transduction mechanisms differ in these tissues and that transmitter regulation of transplanted adrenal tissue into the striatal region may be unpredictable.

159.9

THE DIFFERENTIAL REGULATION OF GLIAL-SPECIFIC GENE EXPRESSION BY IL-6 AND RETINOIC ACID IN C6 GLIOMA CELLS. W.A. Schreier and J. de Vellis. UCLA Mental Retardation Research Center, Los Angeles, CA 90024, USA.

We have studied the effects of two differentiating agents, Interleukin-6 (IL-6) and retinoic acid (RA), on the regulation of the expression of three glial-specific genes in the C6 rat glioma cell line. Glutamine synthetase (GS, E.C.6.3.1.2) and glycerol phosphate dehydrogenase (GPDH, E.C.1.1.1.8) are differentially regulated by IL-6 and RA. A 24 hour treatment of confluent C6 cultures with 50 U/ml IL-6 induces a 40-60% increase in GS enzymatic activity, and causes a 20-40% decrease in GPDH activity. IL-6 treatment also causes a 40% decrease in GPDH mRNA levels. In contrast, RA treatment causes a 20-60% decrease in GS activity, and induces a 60-100% increase in GPDH activity. RA produces a slight (20%) increase in GPDH mRNA levels, which is enhanced 80% by cycloheximide (10 μg/ml). IL-6 causes a 2-fold increase in the astrocyte-specific intermediate filament protein, GFAP, as measured by immunoblotting, and a 2-fold increase in GFAP mRNA which is completely blocked by cycloheximide. RA produces no detectable increase in GFAP protein, but does cause a 30% increase in GFAP mRNA which is also blocked by cycloheximide.

The elucidation of the mechanisms by which these factors differentially regulate astrocyte and oligodendrocyte specific genes will hopefully yield some insight into normal gliogenesis and cell differentiation.

Supported by DOE contract DE-FC03-87-ER60615 and NIH grant HD-06576.

159.10

REGULATION OF VASOACTIVE INTESTINAL POLYPEPTIDE GENE EXPRESSION BY CALCIUM INFLUX. E.M. Adler* and J.S. Fink. Molecular Neurobiology Lab, Massachusetts General Hospital, Charlestown, MA 02129.

Second messenger regulation of gene expression provides a means by which cells can transduce environmental stimuli into long-term changes in phenotype. Synthesis of vasoactive intestinal peptide (VIP) is stimulated by activation of 2nd messenger pathways, including cAMP, protein kinase C and calcium (Ca). cAMP and kinase C activation enhance VIP gene transcription through a 17-bp DNA sequence termed the cAMP-responsive element (CRE, JBC 262:8743, PNAS 85:6662). To determine if Ca also activates VIP transcription, confluent cells of the VIPergic neuroblastoma cell line SH-SY5Y were exposed to 60 mM KCl or 5 uM A23187. VIP mRNA levels, measured by Northern blot using a VIP cRNA probe, increased 2-fold to KCl and 20-fold to A23187, demonstrating that Ca stimuli enhance VIP transcription. Ca and cAMP also interacted to regulate VIP gene expression: VIP mRNA was induced 9-fold by forskolin (10 uM) and isobutylmethylxanthine (IBMX, 0.5 mM) alone, 12-fold by forskolin and IBMX together with 60 mM KCl, and 22-fold by forskolin, IBMX and A23187. To determine if the CRE could mediate transcriptional activation by Ca, PC12 cells stably transfected with the CRE linked to the reporter gene chloramphenicol acetyltransferase (CAT) were studied. Treatment with 30 or 60 mM KCl or 5 uM A23187 alone had no effect on CAT activity, either in undifferentiated cells or when cells were differentiated for 3 days by exposure to 50 ng/ml nerve growth factor, but Ca stimuli (30 mM KCl or 5 uM A23187) markedly enhanced the effect of 10 uM forskolin. These data demonstrate that Ca influx activates VIP gene transcription and that Ca and cAMP stimuli interact at the CRE to activate transcription. The ability of a Ca stimulus alone to activate VIP gene transcription in SH-SY5Y cells may reflect a 2nd Ca-responsive element outside the CRE, indirect elevation of cAMP by Ca in this line, or differences in basal levels of kinase/phosphatase activity or transcription factor expression in different cell lines.

159.11

c-AMP REGULATES GAD_{67} GENE EXPRESSION IN C6 CELLS. J. Segovia, N.J.K. Tillakaratne and A.J. Tobin. Department of Biology, Brain Research Institute, and Molecular Biology Institute, University of California, Los Angeles, CA 90024.

Dopamine deafferentation with 6-OHDA leads to an increase, in the ipsilateral striatum, of the mRNA encoding GAD_{67}, one of the two forms of glutamate decarboxylase, with parallel changes in GAD activity. We are now testing the hypothesis that alterations in GAD_{67} mRNA depend on changes in cAMP. We have employed the C6 rat cell line, which expresses GAD_{67} mRNA and possesses GAD activity. Our preliminary results indicate that GAD activity and GAD mRNA levels can be regulated by increasing cAMP levels. Both the addition of dibutyryl cAMP (1 mM) into the culture media and forskolin (50 μM) lead to a marked decrease of GAD activity. Northern blot analysis shows comparable decreases in GAD_{67} mRNA levels after these treatments. (Supported by NIH grants NS20356 & NS22256)

159.12

Induction of c-fos mRNA following electroconvulsive shocks in rat cerebellum. Y.S.Kim[1]*,J.B.Park[2]*,C.D.Bae[2]*,J.Kim[3] and P.G.Suh[4]* Dept.of Psychiatry[1], Biochemistry[2] and Physiology[3], Seoul Natl.Univ.College of Med., Seoul, Korea, 110 and Dept. of Life Sci[4], POSTECH, Pohang, Korea 790.

We examined the possible refractory period or down-regulation on the induction of c-fos mRNA following electroconvulsive shocks(ECS) in rat cerebellum by Northern analysis with total RNA.

We confirmed that single ECS induces the expression of c-fos mRNA. When we administered two consecutive ECS spaced 2hr, the second ECS could induce the expression of c-fos mRNA. This suggests that the first ECS does not result in an apparent refractory period to the second ECS on the induction of c-fos mRNA. And when we examined the effect of chronic ECS on the induction of c-fos mRNA, we were able to observe the induction of c-fos mRNA even after 5 and 10 daily ECS. According to our observations with chronic ECS, there were no apparent down-regulation on the c-fos expression by multiple daily induction of the gene.

159.13

DOPAMINE RECEPTOR AGONISTS INDUCED THE EXPRESSION OF DYNORPHIN AND c-Fos IN PRIMARY CELL CULTURES OF RAT X-P HE* and J.S. Hong LMIN, NIEHS/NIH, Research Triangle Park, NC 27709

The effects of dopamine receptor agonists on the expression of dynorphin(DYN) and Fos or Fos-related antigens (FRA) were studied in primary striatal cultures from rats. Cultures were established on poly-L-lysine-coated plastic 4-chamber slides from 7-day-old pups. Levels of DYN, c-Fos or FRA immunoreactivities and their mRNAs were investigated using immunocytochemical and in situ hybridization techniques(ISH). Activation of DA receptors by apomorphine, a mixed D1 and D2 agonist, at 10 mM, bid, (3 daily administration for peptide and 1 day administration for mRNA) resulted in an increase in number of DYN^+ cells in striatal cultures. Apomorphine treatment also induced c-Fos and FRA staining, and its maximal effect appeared about 4 hours after administration. ISH experiments showed the maximal induction of c-Fos mRNA 2.5 h after treatment. The specific D1 dopamine receptor agonist, SKF 38393, but not the specific D2 dopamine receptor agonist, quinpirole, increased the level of striatal c-Fos message. In order to further understand the cellular mechanisms underlying the regulation of DYN expression by the dopamine system, the effects of forskolin, a cAMP activator, on DYN and c-Fos were examined. Forskolin induced an increase in number of both DYN^+ and $c\text{-}Fos^+$ cells , indicating that cAMP may be involved in the regulation of c-Fos and DYN expression.

159.14

c-FOS IS SPONTANEOUSLY INDUCED IN THE RAT BRAIN IN THE ACTIVITY PERIOD OF THE CIRCADIAN CYCLE.

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A rapid and transient induction of c-fos in response to several different stimuli has been reported on neuronal cell cultures, as well as *in vivo* in the CNS. Altogether these findings have pointed to c-fos as a high resolution marker of neuronal activity, and suggest that c-fos could also monitor physiological states of CNS activity. The brain undergoes a spontaneous alternation of activity and rest cycles, coincident in part with the sleep-wakefulness cycle. We investigated the possible correspondence between these circadian oscillations and c-fos mRNA levels in different brain areas. Preliminary findings, based on Northern blot hybridization with a ^{32}P labelled v-fos cDNA probe, indicate a spontaneous induction of c-fos in various CNS areas at 9 pm, 1 am, and 5 am, corresponding in the rat to states of prevalent alertness and wakefulness. The expression of c-fos is low or absent during the light hours (9 am, 1 pm, 5 pm). The highest expression appears at 9 pm, when the behavioural observation reveals awakening and intense motor activity. The signal is higher in the brainstem and cerebellum, and lower in cerebral cortex, thalamus and hyppocampus. Previous studies on this aspect were based on c-fos induction in the suprachiasmatic nucleus by the photic stimulation. We here report for the first time a spontaneous induction of c-fos in several CNS areas, coincident with the period of activity of the rat. Immunohistochemical and *in situ* hybridization studies are now in progress.

LONG-TERM POTENTIATION: PROTEIN KINASES AND SECOND MESSENGERS

160.1

DETECTION OF THE THR^{286}- OR THR^{287}-AUTOPHOSPHORYLATED Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II BY A SPECIFIC ANTIBODY. T. Suzuki[1], K. Okumura-Noji*[1], A. Ogura*[2], Y. Kudo*[2], & R. Tanaka*[1] [1]Dept. of Biochem., Nagoya City Univ. Med. Sch., Nagoya 467, Japan, [2] Mitsubishi Kasei Inst. of Life Sci., Tokyo 194, Japan

The antibody specific to the Ca^{2+}/calmodulin-dependent protein kinase II (CaM KII) which had been autophosphorylated only at the site of Thr^{286} of the α-subunit was prepared as follows. The peptide Y-66 with a sequence of Met^{281} to Cys^{289} of the α-subunit was synthesized and its Thr residue was phosphorylated by the CaM KII. The phosphorylated Y-66 (PY-66) was purified, and used as an immunogen after coupling to hemocyanine. IgG was purified after removal of anti-hemocyanine and anti-Y-66. ELISA proved that the IgG obtained reacted specifically with PY-66. Western blot showed that the antibody reacted specifically to the autophosphorylated CaM KII both in purified and PSD-bound form. Immunocytochemistry showed clearly the CaM KII autophosphorylation in the cultured hippocampal neurons treated with NMDA.

160.2

LTP INDUCED BY TETRAETHYL AMMONIUM INCREASES SYNAPSIN I PHOSPHORYLATION AT ITS CAM KINASE II SITES. E.M. Dudek, C. Moore*, K. Miller* and M.D. Browning, *Dept. of Pharmacology, Univ. of Colorado Hlth. Sci. Cntr., Denver, CO 80262*

Long-term potentiation (LTP) is a form of synaptic plasticity that is widely thought to underlie certain forms of learning. Clear evidence has shown that LTP involves increases in transmitter release, but little is known about the molecular mechanisms which underlie this enhanced release. Protein phosphorylation is widely recognized as the primary molecular mechanism for regulation of protein function and a family of phosphoproteins known as the synapsins have been shown to regulate transmitter release. We have tested the possibility that synapsin phosphorylation might play a role in the increased transmitter release seen with LTP. We have focussed in these studies on LTP that is induced by tetraethyl ammonium (TEA) (Aniksztejn and Ben-Ari, Nature 349:67-69, 1991) and have confirmed that TEA produces long-lasting potentiation of the field epsp in CA1 mini-slices. In parallel experiments, CA1 slices were incubated in the absence or presence of 25 mM TEA and then homogenized in 5 mM zinc acetate. The synapsin proteins were then extracted and assayed in a modification of the back phosphorylation assay of Forn and Greengard (PNAS 75:5195, 1978). Ca^{2+}/calmodulin kinase II (CAM kinase II) is inactive in the extracts used for the Forn and Greengard assay. This is likely to be due to residual zinc in the extracts, as we have found that zinc is a potent inhibitor of CAM kinase II (K_i ~4 μM). Therefore we have exhaustively dialyzed the extracts before the back phosphorylation assay. Using this modification of the assay we have found that the basal level of synapsin phosphorylation in hippocampal slices is quite low but that TEA incubation leads to a significant increase in the phosphorylation state of the CAM kinase II sites on synapsin I. Supported by PHS grant NS26377.

160.3

ACTIVATION OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II AND PROTEIN KINASE C BY GLUTAMATE IN CULTURED HIPPOCAMPAL NEURONS.

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The role of Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) and protein kinase C (PKC) has been implicated in the induction of long-term potentiation (LTP) in the CA1 region of the hippocampus. Furthermore, the persistent activation of the protein kinases has been reported to be important for the maintenance of LTP. We have investigated the activation mechanisms of CaM kinase II and PKC by glutamate in cultured hippocampal neurons. Glutamate did elevate the Ca^{2+}-independent activity of CaM kinase II. The response was potentiated by the addition of 1μM glycine and by the removal of extracellular Mg^{2+}, and blocked by the specific antagonists of NMDA receptor, indicating the involvement of the NMDA receptor. In addition, glutamate stimulated the translocation of PKC from the cytosol to the membrane fraction. The effect was not blocked by NMDA receptor antagonists and partially blocked by DL-2-amino-3-phosphonopropionate, and quisqualate or (trans)-1-amino-cyclopentyl-1,3-dicarboxylate produced a similar effect on the translocation of PKC, showing the action of the metabotropic quisqualate receptor. These results suggest that glutamate can activate CaM kinase II and PKC through the ionotropic NMDA receptor and the metabotropic quisqualate receptor, respectively, and induce the LTP in the hippocampal neurons.

160.4

TOWARD A FUNCTION FOR THE RC3 PROTEIN; A POST SYNAPTIC, CALMODULIN BINDING, PROTEIN KINASE C SUBSTRATE WHICH IS A PUTATIVE COMPONENT OF LTP.

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The RC3 mRNA, isolated by subtractive hybridization, encodes a 78 AA protein, highly conserved among mouse, rat and cow, which has a region of homology with GAP-43/b50/F1. Like GAP-43, the RC3 protein (independently identified by Baudier and colleagues as neurogranin) is a substrate for protein kinase C and binds calmodulin in the absence of Ca2+. RC3 exhibits a postnatal onset and is significantly enriched in the cerebral cortex, striatum and hippocampus where it is primarily localized to dendrites in association with post-synaptic densities. These qualities suggest the hypothesis that it plays a postsynaptic role with respect to LTP, memory and associative learning.

We have expressed RC3 in bacteria and purified it to homogeneity. The recombinant protein binds to calmodulin in the absence of Ca2+ and serves as a substrate for protein kinase C in vitro. This preparation is suitable for structural (NMR and X-ray crystallography) and electro-physiological (microinjection into hippocampal brain slice neurons) studies.

Studies are also in progress to create transgenic mouse lines which display a spectrum of RC3 expression. To date, we have generated one mouse line which contains, integrated into it's genome, an anti-RC3 ribozyme under the control of the neuron specific enolase promotor. Experiments are currently underway to determine whether this ribozyme is expressed and is able to depress RC3 expression. If our hypothesis is correct, mutant mice are expected to display memory and/or learning deficits.

160.5

INDUCTION OF LONG-TERM POTENTIATION (LTP) IN THE RAT SUPERIOR CERVICAL GANGLIA (SCG) BY THE PRESYNAPTIC ACTIVATION OF PROTEIN KINASE C (PKC).

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The action of phorbol 12,13 dibutyrate (PDBu) was examined on the isolated SCG of adult Sprague-Dawley rats using extracellular and intracellular recording techniques. PDBu increased the postsynaptic compound action potential (CAP) amplitude for nearly 2 hours. Inactive phorbols did not activate LTP and pretreatment with H7 blocked the induction of LTP by PDBu. The concentration of Mg^{2+} required to block the CAP amplitude at 50% of control was significantly increased in PDBu. Intracellular recordings from SCG neurons showed an increase in the average amplitude of unitary EPSPs to 263 + 39% (n=7) of control after PDBu addition. An increase in quantal content without significant changes in the input resistance, membrane potential, threshold and the amplitude of the ACh-induced potentials, suggests that LTP is mediated by the activation of PKC in presynaptic terminals. This work supported by NIH grant#NS 27319 to JFF and MRC #MT-2475 to CP.

160.6

PREFERENTIAL INVOLVEMENT OF PKC(α) IN THE LTP MODEL OF SYNAPTIC PLASTICITY.

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Phosphorylation of ion channels, receptors, cytoskeletal proteins, and other proteins by PKC has been suggested to play an important role in CNS synaptic plasticity. PKC exists in at least 8 isoforms, four Ca^{2+}-dependent and four Ca^{2+}-independent enzymes. This study will attempt to delineate which of four Ca^{2+}-dependent PKC isoforms (α, βI, βII, and γ) are involved in hippocampal LTP expression. Asymptotic levels of LTP were produced in 5 freely-moving rats with 4 episodes of high frequency stimulation to the lateral perforant path (ten trains of ten 400 Hz pulses repeated at 3, 24 and 27 hours). Low frequency stimulation was given to 5 control animals (100 pulses, one per 15 sec). Hippocampi were removed one hour after the last stimulation episode. PKC concentration and distribution were determined by Western blotting using affinity purified polyclonal antibodies against peptides specific for each isozyme. We found a reduction in PKC(α) in the particulate fraction from the LTP-induced animals when compared to the control group receiving only low frequency stimulation. Our data suggest involvement of PKC(α) in this modification of synaptic transmission. Immunohistochemical localization of the various isoforms is under study as is the time course of PKC alteration during the course of LTP expression.

160.7

LTP IS ASSOCIATED WITH A PERSISTENTLY ACTIVATED FORM OF PKC PRESENT IN THE CYTOSOLIC FRACTION.

E. Klann, S.J. Chen and J.D. Sweatt, Div. of Neuroscience, Baylor College of Medicine, Houston, Texas, 77030.

We have previously reported that the maintenance phase of long-term potentiation (LTP) in area CA1 is associated with an NMDA receptor-mediated, persistent increase in protein kinase activity (Soc. Neurosci. Abstr. 16:144, 1990). We have further investigated this phenomenon by measuring the phosphorylation of selective synthetic peptide substrates for protein kinase C (PKC) and the Ca^{2+}/calmodulin-dependent protein kinase (CaMKII). LTP of the Schaffer collateral input into area CA1 of rat hippocampal slices was studied. Slices were frozen, dissected and homogenized in buffer containing 50 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 1 mM EGTA and 1 mM EDTA. Kinase reaction mixtures contained homogenate, 1 mM Na^+ pyrophosphate, 100 μM ^{32}P-ATP and either 7.5 μM $S6_{(229-249)}$, a selective substrate for PKC, or 8.3 μM [Arg^3-$GS_{(1-10)}$], a selective substrate for CaMKII. We detected an LTP-associated increase in phosphorylation of $S6_{(229-249)}$ (259±38% of control, n=10), that was blocked by the addition of 1 μM $PKC_{(19-36)}$, a selective peptide inhibitor of PKC (- $PKC_{(19-36)}$, 343±69% of control; + $PKC_{(19-36)}$, 184±78% of control, n=4). In contrast, we detected only a slight increase in [Arg^3-$GS_{(1-10)}$] peptide phosphorylation (141±26% of control, n=5). These results suggest that PKC activity rather than CaMKII activity is elevated. To localize the elevated PKC activity, control and LTP homogenates were centrifuged at 133,000 g for 45 minutes and separated into pellet and soluble fractions. Fractions were assayed using either $S6_{(229-249)}$ or myosin light chain as the substrate. Homogenates exhibited an LTP-associated increase in substrate phosphorylation (244±42% of control, n=6) as did the soluble fraction (237±69% of control). The pellet fraction showed no increase in substrate phosphorylation (118±13% of control). These findings suggest that a novel, persistently active, cytosolic form of PKC is associated with the maintenance phase of LTP. This work was supported by the McKnight and Klingenstein Foundations.

160.8

INDUCTION OF CEREBELLAR LONG-TERM DEPRESSION IN CULTURE REQUIRES PKC ACTIVATION.

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Long-term depression (LTD) of the parallel fiber-Purkinje neuron (PF-PN) synapse may be induced in vivo by co-activation of climbing fibers (CFs) and PFs. In cultured mouse PNs we have demonstrated that a similar depression may be induced when iontophoretic glutamate pulses and PN depolarization are substituted for PF and CF stimulation, respectively, and that this form of LTD is dependent upon activation of metabotropic quis receptors (Linden et al. Neuron, in press). As one consequence of metabotropic quis receptor activation is PKC activation via diacylglycerol liberation, we chose to apply PKC inhibitors and activators to determine their effects on LTD induction in culture. Inhibitors which act at both the catalytic site (Ro-31-8220) and the regulatory site (calphostin C) of PKC blocked LTD induced by glutamate/depolarization conjunction when applied during the conjunctive stimulus, but had no effect when applied 10 min after the conjunctive stimulus. Conversely, application of PDAc, a PKC activator, induced a depression of AMPA, but not NMDA test pulses. This selectivity was also seen when LTD was induced by glutamate/depolarization conjunction. In addition, preliminary evidence indicates that depression induced by PDAc and glutamate/depolarization conjunction are non-additive, suggesting that they share common mechanisms. These observations confirm and extend a previous report that LTD in vivo may be induced by phorbol esters (Crepel and Krupa, Brain Res. 458:397).

160.9

B-50 PHOSPHORYLATION FOLLOWING DIFFERENT PATTERNS OF ELECTRICAL STIMULATION IN RAT HIPPOCAMPAL SLICES. C. Gianotti*, M.G. Nunzi, B. Bacci, F.W. Gispen*[1] and R. Corradetti*[2]. Fidia Research Laboratories, 35031 Abano Terme (PD), Italy, [1]Rudolf Magnus Institute, Utrecht University, 3521 GD Utrecht, Netherlands and [2]Dept. Preclin. and Clin. Pharmacology, Florence University, 50134 Florence, Italy

We studied B-50 (GAP43/F1) phosphorylation by quantitative immunoprecipitation, using a polyclonal anti-B-50 antiserum in ^{32}Pi-prelabelled rat hippocampal slices (400 um) incubated in an interface-type chamber. From each animal, one slice was left unstimulated while two others were stimulated (0.017 Hz) in the stratum radiatum; the responses were extracellularly recorded in the CA1 region. To elicit long-term potentiation (LTP), a train of high-frequency stimuli (100 Hz, 1s) was used. In potentiated slices B-50 phosphorylation was significantly ($p<0.05$) higher (146±8%, n=7) than in unstimulated controls or in low frequency stimulated slices (107±7%, n=7). These data are consistent with the hypothesis that an increase in neurotransmitter release, probably due to a PKC-dependent phosphorylation of protein B-50, occurs during LTP. In view of the role of B50 in synaptic plasticity, we are exploring whether age-induced alterations in LTP expression may be related to changes in B-50 phosphorylation.

160.10

PROTEIN KINASE C EXPRESSION AND FUNCTION IN NEURONS OF THE HIPPOCAMPUS. K.Kruger, H.Boswell, & S.A.DeRiemer. Dept. Biological Sciences,Columbia Univ., New York, N.Y. 10027

We are investigating the physiological roles of isozymes of protein kinase c (PKC) in the rat hippocampus. The hippocampus was chosen because PKC activity is highest in this area of the brain and the activation of PKC has been shown to enhance synaptic transmission in CA1 pyramidal neurons (Baraban et al.1985). PKC appears also to be important for the induction and expression of long term potentiation in the hippopcampus. There are seven isozymes of PKC that have been identified by cDNA cloning and sequencing. We are studying the alpha, beta, and gamma isoforms which are members of the class that require calcium as well as diacylglycerol for activation. We examined the localization of the isozymes in rat embryonic hippocampal neurons in cell culture and in brain slices with antibodies to regions that are highly variable between isoforms (Makowski et al. 1988). The isozymes are coexpressed in all the neurons in cultures from day 19 embryos. Expression of the enzyme is significantly lower in glial cells. The intensity of staining varied between the cytoplasm, perikaryon, and the dendrites for the different isozymes. Marked staining of varicosities and growth cones was seen with the gamma isozyme. Immunohistochemical analysis of cryostat sections from the brain of young animals showed these isozymes to be coexpressed. The gamma isozyme is the most widely distributed, seen in CA1 and CA3 pyramidal cells and dendate granule cells. We are using the whole cell patch technique to look at the effects of PKC isozymes on the electrical properties of hippocampal neurons. Research supported by NIH grant# NS-08614 to KK.

160.11

TYROSINE KINASE INHIBITORS BLOCK LONG-TERM POTENTIATION IN THE CA1 REGION OF THE HIPPOCAMPUS. T.J. O'Dell, E.R. Kandel, and S.G.N. Grant*. Center for Neurobiology and Behavior, HHMI, College of Physicians and Surgeons, Columbia University, NY, NY 10032.

High levels of protein tyrosine kinase activity are expressed in the hippocampus and cerebellum, two brain regions important in learning and memory. We therefore investigated whether tyrosine kinase activity is required for long-term potentiation (LTP), a form of synaptic plasticity thought to contribute to memory formation. Using in vitro assays of protein kinase activity we found that two compounds, genistein and lavendustin A, selectively inhibited pp60$^{c\text{-}src(+)}$, a tyrosine kinase present in hippocampal neurons. These inhibitors blocked LTP in the hippocampal slice when bath applied prior to tetanic stimulation but had no effect on established LTP, on normal synaptic transmission, or on neurotransmitter actions mediated by PKA or PKC. LTP was also blocked when lavendustin A was injected into the postsynaptic CA1 pyramidal cells via an intracellular recording electrode. These data suggest that tyrosine kinase activity is required postsynaptically for the induction of LTP. Since CamKII and PKC also seem to be essential our data suggest that the induction of LTP requires a network of protein kinase activity that includes tyrosine as well as serine/threonine kinases.

160.12

LOW CONCENTRATIONS OF ARACHIDONIC ACID PRODUCE AN ACTIVITY-DEPENDENT ENHANCEMENT OF SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES PRELOADED WITH A DIACYLGLYCEROL ANALOG. C.R. Bramham*, D.S. Lester*, D.L. Alkon. Section on Neural Systems, NIH, Bethesda, MD 20892.

Liberation of arachidonic acid (AA) and activation of protein kinase C are both implicated in long-term potentiation. AA, when applied at 50 uM and combined with weak afferent stimulation, leads to an increase in synaptic transmission. Recently it was found that AA and diacylglycerol, both lipid activators of protein kinase C, work synergistically to activate this enzyme in a model membrane bilayer system, and in a cellular model of associative learning (see Etcheberrigaray et al. this meeting).

Population EPSPs evoked by Schaffer-collateral stimulation were recorded in stratum radiatum of rat hippocampal slices perfused with standard ACSF. Averaged EPSPs (6 test pulses at 0.1 hz) were obtained at regular intervals. Oleoylacetylglycerol (OAG 5 ug/ml), a diacylglycerol analog, was perfused for 15 min followed by 15 min of AA (5 uM) perfusion during which time stimulus pulses were applied every 4 s. This procedure consistently led to an increase in EPSP amplitude which reached a stable plateau after about 30 min. The combination of AA and repetitive afferent stimulation was ineffective without OAG pretreatment, as was sequential perfusion of OAG/AA without afferent stimulation. These results suggest that arachidonic acid-induced potentiation depends not only repetitive synaptic activity but also on the membrane concentration of diacylglycerol.

160.13

ARACHIDONIC ACID AND 1-OLEOYL-2-ACETYL GLYCEROL INDUCE LONG-TERM POTENTIATION IN HIPPOCAMPAL CA1 NEURONS IN LOW MAGNESIUM SOLUTION. K.Kato[1], K.Uruno[2], K.Saito[2], H.Kato[2], C.F.Zorumski[1]. [1]Dep. Psychiatry, Washington Univ. Sch. Med., St. Louis, Mo 63110, U.S.A., [2]Dep. Physiol., Yamagata Univ. Sch. Med., Yamagata 990-23, JAPAN.

To evaluate the role of protein kinase C (PKC) and arachidonic acid (AA) on the maintenance of long-term potentiation (LTP), we examined the effects of phospholipase blockers on tetanus-induced LTP and of diacylglycerol (DG) and AA on synaptic transmission in CA1 neurons of guinea pig hippocampal slices.

Neomycin (1 mM) or 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC, 0.1 mM), blockers of phospholipase, suppressed the maintenance of tetanus-induced LTP. Perfusion of 1-oleoyl-2-acetyl-glycerol (OAG, 100 μg/ml) or AA (100 μM) produced a temporary increase in both the amplitude of the population spike (PS) and the slope of the field excitatory postsynaptic potentials (EPSPs) but failed to produce LTP. However, administration of OAG or AA in low-Mg^{2+} (0.1 mM) solution induced LTP. Both OAG- and AA-induced LTP were blocked by dl-2-amino-phosphonopentanoic acid (AP5; 50 μM). The administration of a potent PKC activator, phorbol-12,13-dibutyrate (PDBu), in low-Mg^{2+} (0.1 mM) solution enhanced the PS and EPSPs for two or three hrs, but this enhancement was not sustained. Coapplication of PDBu with AA had no effect on the magnitude of AA-induced potentiation in low Mg^{2+} solutions.

These results suggest that PKC activation itself is not as critical as AA for the maintenance of LTP and that DG and AA coupled with the influx of Ca^{2+} through NMDA receptor-gated channels play important roles in the prolonged nature of LTP.

160.14

PHOSPHOINOSITIDE HYDROLYSIS INDUCED BY HIGH FREQUENCY ELECTRICAL STIMULATION IN THE DENTATE GYRUS OF RAT HIPPOCAMPAL SLICE. M.J. Bonner, E.C. Burgard and J.M. Sarvey. Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

High frequency electrical stimulation in the hippocampus results in long-term potentiation (LTP), a form of synaptic plasticity. Activation of second messenger systems is implicated in the induction and maintenance of LTP. Stimulation of phosphoinositide (PI) hydrolysis by a high frequency train (HFT; 100Hz, 2 sec) to the perforant path in the dentate gyrus was studied in vitro in hippocampal slices. HFT increased total PI hydrolysis 1 min post-stimulation by as much as 200% (n=15 slices/gp) of non-HFT exposed (controls) slices. The PI metabolism induced by HFT was antagonized by the NMDA-antagonist CPP (10 µM). Increased PI turnover was detected over a time course from less than 1 min up to 60 min after HFT. PI turnover increases were also reflected in increases in IP1, IP2, and IP3 at 1 min and 30 min post-HFT. HFT that was subthreshold for LTP did not significantly affect PI hydrolysis. The membrane-bound portion of the PI cycle was affected similarly. This study shows that in vitro HFT stimulation of the perforant path in the dentate gyrus of the rat hippocampal slice can induce sustained increases in PI hydrolysis. These results suggest that PI hydrolysis is involved in the expression of LTP in the dentate gyrus.

160.15

NMDA RECEPTORS CAUSE Ca^{2+}-DEPENDENT INCREASES IN cAMP LEVELS AND Ca^{2+}-CHANNEL ACTIVITY. D.M. Chetkovich, R. Gray, D. Johnston and J.D. Sweatt. Div. Neurosci., Baylor Coll. of Med., Houston, TX.

We have previously reported that LTP-inducing tetanic stimulation in area CA1 of rat hippocampus leads to an APV-sensitive increase in cAMP. We now have found that a 10 min bath application of NMDA to hippocampal slices produced concentration-dependent increases in cAMP in area CA1, with maximal stimulation at 1 mM NMDA and an EC_{50} of 55 μM. The effect of NMDA (100 μM) was blocked by the NMDA receptor antagonist, APV (50 μM) (327 ± 38% of control cAMP without APV, n=5; 87 ± 15% of control cAMP with APV, n=3). IBMX (100 μM) did not attenuate the effect of NMDA (400 μM) (393 ± 46% of control without IBMX, n=9; 352 ± 24% of control with IBMX, n=6), suggesting that the effect of NMDA on cAMP is not due to an alteration in phosphodiesterase activity. We examined whether NMDA receptors couple to adenylyl cyclase (AC) directly via a G-protein. AC activity in membranes prepared from area CA1 was stimulated by both forskolin (1 μM) (1314 ± 45% control AC activity, n=5) and GTP (10 μM) (146 ± 7% control AC activity, n=4); however, NMDA did not affect membrane AC either in the presence or absence of GTP (n=4). Thus NMDA receptors apparently do not couple directly to AC via G-protein activation. NMDA receptor activation is known to cause Ca^{2+} influx. Removal of extracellular Ca^{2+} (1 mM EGTA, 0 Ca^{2+}) blocked the NMDA effect (98 ± 11% control cAMP with 100 μM NMDA, no Ca^{2+}, n=4). We have previously reported that brief coapplication of NMDA (1 mM) and Ca^{2+} (2 mM) to CA1 pyramidal cells (bathed in high K^+ and 0 Ca^{2+}) increased the activity of voltage-gated Ca^{2+} channels. This effect could be mimicked with 8-bromo-cAMP, but not with NMDA or Ca^{2+} alone. These results, taken together, suggest that NMDA receptor-mediated increases in cAMP occur secondary to Ca^{2+} influx, perhaps through Ca^{2+}/CaM stimulation of AC. Supported by a C. Bell Pearce Award (D.C.), USPHA RR-05425 (D.S. and R.G.), MH 44754 (D.J.), AFAR and McKnight (D.S.).

160.16

CYCLIC AMP AS THE RESPONSE REGULATOR FOR LONG- AND SHORT-TERM POTENTIATION OF SECRETION. B.H. Morimoto* and D.E. Koshland, Jr. Dept. Molecular and Cell Biology, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720

The HT4 neural cell line serves as a model for memory by exhibiting short- and long-term potentiation of neurotransmitter secretion. Membrane depolarization elicits secretion and this response can be potentiated. The extent of potentiation correlates with the elevation of cAMP levels. The direct elevation of cAMP by forskolin, or the addition of dibutyryl cAMP, induces potentiation, suggesting cAMP levels are responsible for potentiation. The temporal relationship between potentiation and cAMP levels provide further evidence that cAMP is the response regulator for potentiation. Additionally, adrenergic and adenosine receptors which are coupled to adenylate cyclase can also induce potentiation. Long-term potentiation appears to require that cAMP levels exceed a threshold, which is prevented by the adaptation of the β-adrenergic receptor and achieved by other non-adapting stimuli.

160.17

CYCLIC AMP CAUSES SHORT-LASTING DEPRESSION AND LONG-LASTING POTENTIATION IN CA1. S. Pockett and J.R. Slack*. Dept. of Physiology, University of Auckland, Private Bag, Auckland, New Zealand.

The effect on synaptic transmission of superfusing hippocampal slices with membrane-permeable analogues of cyclic AMP or with the adenylate cyclase activator forskolin was studied, using extracellular recording techniques. The Schaffer collateral/commissural pathway was stimulated at 0.05Hz and population spike amplitude measured. The population spike in area CA1 was affected in a biphasic manner by addition of dibutyrl cyclic AMP: during the presence of dbcAMP the population spike was depressed and after wash-out of dbcAMP the population spike was potentiated. The depression lasted only as long as the dbcAMP was in the bath and the potentiation lasted unchanged for at least 3 hours after wash-out of dbcAMP. With 200μM dbcAMP the depression was to 22% ± 11% S.E.M. (n=7) of the baseline population spike amplitude and the potentiation was to 137% ± 8% (n=7) of baseline. Similar results were obtained with 8-bromo cyclic AMP and forskolin. The presence of picrotoxin (10μM) in the recording chamber had no significant effect on the depression caused by 200μM dbcAMP but significantly decreased the potentiation to 116% ± 6% (n=7) of baseline. These findings suggest that cyclic AMP may play a role in long-term potentiation.

160.18

MECHANISM OF SHORT-LASTING DEPRESSION CAUSED BY CYCLIC AMP IN CA1. J.R.Slack*, S.Pockett and R.Milne. Dept of Physiology, University of Auckland, Private Bag, Auckland, New Zealand.

Bath application of 200μM dibutyryl cyclic AMP to hippocampal slices caused a depression of CA1 population spike amplitude in response to 0.05Hz stimulation. The depression lasted only as long as dbcAMP was present and was followed by long-lasting potentiation after washout of dbcAMP. We hypothesized that the depression was caused by action of dbcAMP on adenosine receptors. Many adenosine receptor blockers have numerous intracellular actions in addition to their receptor blockade, but 8-p-sulfophenyltheophylline (8PSPT) does not enter cells and hence has only adenosine receptor-blocking actions (Daly, J.W., in Purines in Cellular Signalling, Springer-Verlag 1989). Bath application of 10μM 8PSPT caused a large increase in population spike amplitude, which was completely reversible on washout. This implies that endogenous adenosine in the slice preparation is tonically active to depress population spike amplitude. Application of 200μM dbcAMP during the presence of 8PSPT caused no depression. The long-lasting potentiation caused by dbcAMP was still seen, however, when the dbcAMP and 8PSPT were washed out. Thus the short-lasting depression caused by dbcAMP is probably due to its action on adenosine receptors.

160.19

CYCLIC GMP POTENTIATION OF NICOTINIC TRANSMISSION IN THE RAT SUPERIOR CERVICAL GANGLION (SCG). Clark A. Briggs, Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064.

Nicotinic transmission in the SCG is potentiated by 1 mM 8-bromo-cyclic GMP (8-Br-cGMP; Briggs *et al.* (1988), *Br.J.Pharmac.* **93**: 399). However, the lack of effect of dibutyryl-cGMP and the ability of dibutyryl- as well as 8-Br-cAMP to potentiate nicotinic transmission questioned the specific involvement of a cGMP-mediated process. This hypothesis and the potential involvement of nitric oxide (NO), an agent that can stimulate the synthesis of cGMP, was further investigated. The methods were similar to those previously described. Briefly, rat SCG were isolated and superfused *in vitro* at ambient temperature (25°C) with oxygenated Locke's solution, and the postganglionic compound action potential response to stimulation of the preganglionic nerve at a slow rate (1/60 seconds) was used as a measure of nicotinic synaptic efficacy.

At low concentrations (0.3 μM - 100 μM applied for 30 minutes), 8-Br-cGMP caused dose-dependent potentiations of 5% - 80%. While 8-Br-cAMP also caused a potentiation of ganglionic transmission, it appeared to be about 5x *less* potent than 8-Br-cGMP. Nitroprusside and azide potentiated ganglionic transmission at concentrations known to stimulate cGMP formation in the rat SCG (0.1 μM - 1 mM applied for 30 minutes). Nitroprusside caused a 24 ± 2 % potentiation at 10 μM (n=10), and was more effective than azide (11 ± 2 % potentiation at 10 μM, n=3). Ferricyanide (10 μM) had no effect, as would be expected if nitroprusside acted through the release of NO. Simultaneous addition of 8-Br-cGMP and 8-Br-cAMP did not cause a supra-additive potentiation. The data are consistent with the concept that cGMP may mediate a potentiation of nicotinic transmission that may be related to NO formation. However, additional studies are needed to evaluate the possibility of a physiological role for an 'EDRF'/NO system regulating synaptic transmission in the SCG.

161.1

COMPUTER SIMULATIONS OF E-S POTENTIATION IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS. J.C. Wathey, S. Chattarji, W.W. Lytton, J.M. Jester and T.J. Sejnowski. The Salk Institute, La Jolla, CA 92037.

Long-term potentiation (LTP) of hippocampal excitatory synapses is often accompanied by E-S potentiation (i.e., an increase in the probability of spiking to an EPSP of fixed strength). We used computer simulations of a CA1 pyramidal cell to test the plausibility of the hypothesis that changes in dendritic excitability contribute to E-S potentiation. These changes were simulated by adding "hot spots" of voltage-sensitive Ca^{++} conductance to various dendritic compartments. This typically caused spiking in response to previously subthreshold synaptic inputs. The magnitude of the simulated E-S potentiation depended on the passive electrical properties of the cell, the excitability of the soma, and the relative locations on the dendrites of the synaptic inputs and hot spots. The specificity of the simulated E-S potentiation was quantified by co-localizing the hot spots with a subset (40/80) of the synaptic contacts, denoted "tetanized", and then comparing the effects of the hot spots on these and the remaining (untetanized) synaptic contacts. The simulated E-S potentiation tended to be specific to the tetanized input if the untetanized contacts were, on average, electrically closer to the soma than the tetanized contacts. Specificity was also high if the two inputs were segregated to different primary dendrites. The results also predict, however, that E-S potentiation by this mechanism will appear to be nonspecific (i.e., heterosynaptic) if the synapses of the untetanized input are sufficiently far from the soma relative to the tetanized synapses.

To test this hypothesis we recorded field EPSP and population spikes from area CA1 in rat hippocampal slices. Tetanization of a proximal Schaffer collateral input induced LTP at that input and potentiated population spikes evoked via a more distal untetanized perforant path input. This suggests that postsynaptic excitability changes can contribute to E-S potentiation.

161.2

QUANTAL ANALYSIS OF SUPERIMPOSED EXCITATORY POSTSYNAPTIC POTENTIALS FROM MULTIPLE SYNAPSES. Paul C. Bush, Shaolin Li* and Terrence J. Sejnowski Salk Institute, La Jolla, CA 92138-9216 USA.

Extracellular stimulating electrodes in hippocampal slices typically activate multiple synaptic boutons, even at minimal levels of stimulation, each of which could have a different quantal size and release probability. The superposition of these EPSPs make conventional quantal analysis problematic. We have developed a method for analysing such data that is capable of separating a small number of release sites. This method relies on the differences in the time courses of EPSPs from different locations in the dendritic tree as measured at the soma.

Our method begins with an estimation of the attenuation factor of the dendritic tree for each synapse by applying a maximum likelihood estimator to the Fourier transform of individual EPSP traces. This produces a time integral of the voltage of the EPSP at the synapse. An inverse filter is then used to produce histograms of quantal amplitudes for each synapse. We have tested our method on randomly generated multiple-synaptic quantal amplitude histograms, generated from α-function EPSPs with noise added at the level observed in microelectrode recording (approximately 3:1 signal:noise power ratio). The algorithm accurately recovered the individual quantal amplitude histograms, from which parameters for the appropriate statistical model are easy to extract.

161.3

Quantal Analysis and Model Discrimination A.C. Greenwood[1], E.M. Landaw[2], E.W. Kairiss[3], T.H. Brown[3]. Depts. of Physiology[1] & Psychology[3], Yale Univ., New Haven, CT 06520; Dept. of Biomathematics[2], U. of Calif. Los Angeles, CA 90024.

There is growing interest in the quantal basis of synaptic plasticity and modulation in the CNS - including hippocampal long-term potentiation (LTP). A fairly general quantal model includes (i) either a Poisson or binomial distribution to describe quantal release; (ii) a Gaussian or gamma density function describing the single-quantal amplitude distribution; and (iii) Gaussian noise. In this model, plasticity affects quantal size and/or release parameters. We developed a maximum likelihood (ML) estimation procedure to fit two sets of model parameters to a pair of response data sets (from before and after synapse modification). One can fix any parameter to a measured or hypothetical value. Likelihood ratio tests are used to test hypotheses about quantal parameters or mechanisms of synaptic plasticity. Standard errors and confidence intervals of estimated parameters are derived from the likelihood surface and from Monte Carlo (MC) simulations. For various conditions, we estimated the *a priori* probability that an experiment will permit the rejection of a hypothesized locus of plasticity. The chance of success was found to depend on the potentiation factor (F) and the ratio of pre-LTP quantal size to noise (Q/N). Initial work assumed 200 samples before and 200 after LTP induction. Plots of mean^2/variance vs mean (M^2/V) are also used to discriminate among possible loci of LTP. We explored this method analytically and in MC studies, and compared it to our ML method of model discrimination. The results suggest the use of both methods and the optimization of Q/N and F. MC simulations based on independent estimates of Q/N and noise can endow the M^2/V method with estimates of confidence. Both methods are unreliable for Q/N = 1.25 and F =1.3. For any F, reliability decreases with Q/N. When either method is used near the limit of its reliability, an increase in the size of each quantum can be identified more confidently than can an increase in the number released. For F = 1.3 and Q/N = 20, the M^2/V method performs the latter task less reliably than the ML method. We are studying nonstationarity, to which the M^2/V method is most easily adapted. (Supported by a NSF graduate research fellowship and NIH #54645)

161.4

TEMPORAL SEQUENCES ENCODED AND RECOGNIZED USING LTP INDUCTION AND EXPRESSION RULES. J.Larson, J.Whitson*, R.Granger, & G.Lynch. CNLM, Univ. of Calif., Irvine, CA 92717.

If LTP represents a memory storage mechanism, its induction and expression characteristics may constitute rules governing encoding and read-out of memory in cortical circuitry. Stimulation patterns based on the 4-7 Hz theta EEG rhythm have been shown to be ideally suited for producing robust and stable LTP (Larson & Lynch, Science, 232: 985, 1986); these repetitive stimulation cycles have been shown to give rise to unique learning and recognition rules in computational simulations (Ambros-Ingerson, Granger & Lynch, Science, 247: 1344, 1990). Sensory cues often consist of sequential elements, raising the question of how LTP induction and expression rules relate to encoding and retrieval of temporal sequences. It has been shown that the sequence in which synapses are stimulated determines the degree to which they potentiate (Larson & Lynch, Brain Res., 489: 49, 1989), thereby yielding a physiological rule for LTP induction using temporal sequences: the greatest LTP is induced in the earliest input and smallest LTP in the last input.

Physiological simulations of synaptic responses predicted an LTP expression rule for temporal sequences; this was supported by physiological tests using sequential stimulation of two afferents (S1 and S2) in field CA1 of the hippocampal slice. Before potentiation, the sequences S1-S2 and S2-S1 gave equal responses; after potentiation of S1, the sequence S1-S2 yielded a significantly larger response than the reverse sequence. Thus, the optimal sequence for depolarization of the postsynaptic cell is the sequence in which synapses are activated from strongest to weakest. The functional consequences of these findings were investigated with a network simulation, using the LTP induction rule to train on cues consisting of temporal sequences, and testing for recognition of the cues using the LTP expression rule. The network showed a high capacity for encoding and accurately recognizing temporally-patterned cue sequences, e.g., 10,000 cues in a network of 1,000 cells. (Supported by ONR N00014-89-J-1255 and N00014-89-J-3179).

161.5

INDIVIDUAL DIFFERENCES IN EMERGENCE NEOPHOBIA PREDICT HIPPOCAMPAL LONG-TERM POTENTIATION (LTP). D. Mitchell, K. Patel, and S. Maren. Department of Psychology and Neurosciences Program, University of Southern California, Los Angeles, CA 90089-1061.

Rats show a great deal of individual variability in behavioral neophobia (avoidance of novelty) that can powerfully influence both associative and nonassociative learning. Long-term potentiation in the hippocampus, which is thought to modulate learning, also shows considerable within and between strain variability. We compared individual differences in reluctance to explore a novel environment with individual differences in *in vivo* hippocampal LTP.

Fourteen male Long-Evans rats individually housed in a start box with ad lib food and water for 24 hrs were subsequently permitted to explore an adjacent novel alley during a 6 hr videotaped emergence test. Two to four weeks following behavioral testing the rats were anesthetized and implanted stereotaxically with a recording electrode in the hilus of the dentate gyrus and a bipolar stimulating electrode in the medial perforant pathway. Each animal was administered 5 consecutive stimulation trains separated by 20 min (10 40 ms bursts at 5 Hz; intraburst frequencies 25, 50, 100, 200, and 400 Hz). LTP of the excitatory postsynaptic potential (EPSP) slope and population spike (PS) amplitude was assessed 30 min following the last train from input/output functions generated at the beginning and end of the experiment. The animals were ordinally ranked for both emergence (time spent exploring alley) and LTP scores. LTP of EPSP slope, but not PS amplitude, was negatively correlated (r = -.87, p < .0001) with behavioral emergence. This correlation could not be attributed to differences in baseline magnitude of the perforant path evoked response as there were no significant differences between neophobic and non-neophobic animals on this measure. These data suggest that individual differences in behavioral habituation that are frequently used to quantify neophobia may reflect individual differences in hippocampal plasticity.

161.6

LONG-TERM POTENTIATION OF PERFORANT PATH AND MOSSY FIBER INPUT TO CA3 PYRAMIDAL CELLS: AN *in vivo* COMPARISON OF OPTIMAL TETANIZATION PARAMETERS. Mark F. Yeckel and Theodore W. Berger, *Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

Pyramidal cells of the hippocampal CA3 region receive direct monosynaptic excitatory input from entorhinal cortical cells, as well as an indirect disynaptic input via mossy fiber axons of dentate granule cells. We have recently demonstrated, *in vivo*, that a given subpopulation of CA3 pyramidal cells can express different forms of long-term potentiation (LTP) for different subsets of synapses: perforant path LTP is NMDA dependent and specific to the tetanized fibers (i.e., homosynaptic); mossy fiber LTP is not dependent upon NMDA receptor activation and LTP is also expressed for nontetanized commissural input (heterosynaptic). We have investigated further the expression of LTP by these convergent pathways , more specifically, whether or not their optimal LTP induction parameters may differ.

Stimulating electrodes were placed in 1-3 afferent pathways of halothane anesthetized rabbits: the ipsilateral angular bundle, the ipsilateral hilus, and the contralateral CA3 cell layer. Tetanizing stimuli of 400 Hz (10 trains of 10 impulses; 1 train/10s), followed 45 min later by 100 Hz (1-3 trains of 100 impulses; 1 train/10 s), or 100 Hz followed by 400 Hz, were delivered to the either the perforant path or to mossy fibers. Analysis of input/output functions for monosynaptic population responses recorded in the CA3 pyramidal cell region revealed that 100 Hz delivered to the mossy fibers induced a greater magnitude increase in the number of cells activated than 400 Hz, irrespective of the order in which the tetanizing stimuli were given (n=7). In contrast, the number of CA3 cells evoked by perforant path input was greater after LTP was induced with 400 Hz vs. 100 Hz (n=10). Because it appears that the optimal induction parameters may differ for these pathways, the possibility exists that different patterns of afferent activity could lead to the selective induction of homosynaptic or heterosynaptic LTP (in different pathways), and thus have fundamentally different consequences for global functional properties of the hippocampal system. Supported by ONR, AFOSR, MH45156, MH18273, and MH00343.

161.7

NONLINEAR RESPONSE PROPERTIES OF HIPPOCAMPAL GRANULE CELL POPULATION EPSP's BEFORE AND AFTER THE INDUCTION OF LONG-TERM POTENTIATION. Jeffrey R. Balzer, Robert J. Sclabassi and Theodore W. Berger. *Depts. of Behavioral Neuroscience, Neurosurgery and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.*

In a previous series of experiments, using an *in vivo* rabbit preparation, nonlinear systems analysis was used to characterize the transformational properties of hippocampal dentate granule cell responses to perforant path input before and after the induction of long-term potentiation (LTP). Perforant path fibers were stimulated with a train of impulses having randomly varying inter-impulse intervals and first, second and third order kernels were computed by cross-correlating the intervals of the random impulse train with amplitudes of the evoked granule cell population spikes. Results showed that second and third order nonlinearities decreased significantly after the induction of LTP. That is, the magnitude of granule cell responses were less dependent on the temporal characteristics of perforant path input after the induction of LTP.

In the experiments reported here we investigated the effect of LTP on the nonlinear response properties of granule cell synaptic potentials as measured using the population EPSP. Prior to the induction of LTP, nonlinear response properties of the EPSP were highly nonlinear, i.e., the slope and magnitude of the EPSP depended significantly on inter-impulse interval. Unlike for the population spike, however, the induction of LTP did not alter either second or third order nonlinear response properties of the EPSP. These results show that LTP-induced changes in nonlinear response properties of granule cell output cannot be accounted for on the basis of changes in perforant path synaptic input. Supported by ONR, AFOSR, MH45156 and MH00343.

161.8

Isolated NMDA receptor-mediated synaptic responses in hippocampus can express both LTP and LTD. X. Xie, T.W. Berger and G. Barrionuevo. *Departments of Behavioral Neuroscience & Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

Tetanic stimulation of hippocampal glutamatergic pathways can result in either long-term potentiation (LTP) or long-term depression (LTD) of AMPA receptor-mediated responses. However, there are conflicting reports as to whether NMDA receptor-mediated activity expresses LTP, and it is not known if NMDA receptors express LTD. Here we demonstrate both LTP and LTD of pharmacologically (CNQX 10μM in medium) isolated NMDA receptor-mediated EPSPs recorded from granule cells of the hippocampal dentate gyrus. Brief 50-Hz tetanic activation (3 bursts of 20 pulses with 5 sec interval) of perforant path afferent fibers resulted in substantial LTP of NMDA receptor-mediated EPSPs, either when slices were perfused with low Mg^{2+} (100μM) medium or when impaled granle cells were depolarized to -50mV in normal Mg^{2+} (2mM). The average amplitude increase in both normal (n=8) or low Mg^{2+} (n=10) was 75% and 60% respectively measured 30 min posttetanus. Hyperpolarization (-100mV) of the postsynaptic cell during delivery of the same tetani reversibly blocked the induction of LTP (-6%, n=6). Furthermore, by hyperpolarizing the postsynaptic cell during 10-Hz tetanic stimulation of the afferent fibers, we demonstrate that the level of postsynaptic voltage determines whether the same tetanic stimulation induces LTP or LTD: pairing of four 10-Hz tetani with hyperpolarization elicited LTD of the EPSPs (-34%, n=8). When delivered alone, the 10-Hz tetani induced robust LTP (+43%, n=3). Taken together, these findings provide evidence that the induction of use-dependent, NMDA receptor-mediated synaptic plasticity is under the control of postsynaptic mechanisms. Supported by AFOSR, NS01196, NS24288, MH45156 and MH00343.

161.9

DRAMATIC DIFFERENCES IN THE MAGNITUDE AND TEMPORAL DEVELOPMENT OF LONG-TERM POTENTIATION (LTP) IN RAT HIPPOCAMPAL AREA CA1 AT POSTNATAL DAYS 11 AND 15.
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The ontogeny of long-term potentiation (LTP) is largely unstudied (Harris and Telyer, 1984). We examined the magnitude, temporal development and duration of LTP in hippocampal slices from 11 and 15 day old rats. Remarkable differences were found in the LTP seen at these two ages. In addition, the LTP at both immature ages differed from the LTP that has been reported from adult animals.

Electrophysiological recordings of the field EPSP were made with extracellular electrodes placed in s. radium of hippocampal field CA1 while the Schaffer collaterals were regularly stimulated (1-2/min). LTP was induced with tetanic stimulation (2X @ 100Hz for 1 sec) and recordings were continued for 3-20 hours. LTP in slices from day 15 animals increased in the first 30 min post-tetanus, plateaued for 2-3 hours, and then declined over the next hour (n=7). LTP then remained constant at this lower, but still potentiated level for as long as the slices survived. LTP in slices from day 11 animals was maximal immediately post-tetanus and gradually declined to baseline over the next ~2.5 hrs (n=7). In slices which were retetanized following return to baseline, LTP could be reinduced (n=3). We also confirmed that LTP is APV sensitive at both of these immature ages.

Thus the expression of LTP in immature animals contrasts with the adult in a number of regards: at day 11 LTP is not persistent but can be reevoked, and at day 15 there is an exaggerated early phase of the LTP. By correlating biochemical and physiological development during this dynamic period, we may be able to understand the different components which underlie the adult response. Alternatively, understanding LTP in the immature animal may shed light on processes involved in synapse formation and selection which are not present in the adult animal.

161.10

EVIDENCE FOR DIFFERING MECHANISMS OF SHORT- AND LONG-TERM POTENTIATION EXPRESSION. *P.E. Schulz & D. Johnston*, Department of Neurology & Division of Neuroscience, Baylor College of Medicine, Houston, Tx 77030.

It has been suggested that tetanus induced potentiation has several components including: a brief APV-insensitive post-tetanic potentiation, a decremental component of about 10 minutes duration, and a sustained component of greater than 15 minutes duration. The decremental and sustained components, which we will operationally term short- and long-term potentiation (STP and LTP), have not previously been shown to be expressed differently. We tested the hypothesis that STP and LTP are expressed via the same mechanism reasoning that if they are: (1) saturating LTP should decrease STP, and (2) STP and LTP should vary similarly under different induction conditions. Rat hippocampal slices, 375μm thick, were perfused with saline containing (in mM): NaCl 120, KCl 3, $MgCl_2$ 1.2, $NaHCO_3$ 23, $CaCl_2$ 1.5, and dextrose 11. Field recordings of pEPSPs were made in CA1. Slices were repeatedly tetanized at stimulus intensities initially yielding pEPSPs of 1.3mV, 2.6mV, or in an additional group 2.6mV in the presence of 10μM picrotoxin and altered divalent cation concentrations. Four slices that had potentiated the most by LTP saturation were examined from each of the three groups reasoning that these slices would show the greatest decrease in STP. The findings were: (1) within each group the magnitude of STP remained similar for each tetanus from the first through post LTP saturation; (2) across the three groups the STP magnitude was different despite the induction of similar LTP magnitudes; and, (3) STP was APV-sensitive both for tetanus one and after LTP saturation. These findings suggest different mechanisms for STP and LTP expression despite a similar induction requirement of NMDA receptor activation. (AG00432, AG08664, MH44754)

161.11

DURATION OF LTP IN PIRIFORM CORTEX IN VIVO IS INCREASED AFTER EPILEPTIFORM BURSTING. A. Kapur and L.B. Haberly, Neuroscience Training Program and Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

Stimulation with trains of 4 or 5 shocks repeated at 200 ms intervals (theta burst stimulation) induces long term potentiation (LTP) at afferent fiber synapses in the piriform cortex slice (Jung et al, Synapse 6: 279, 1990; Kanter & Haberly, Brain Res. 525: 175, 1990). In the present study, it was found that theta burst stimulation also induces LTP at afferent fiber synapses in urethane anesthetized rats (1.4-1.8 g/kg, IP). However, in contrast to findings in the slice where LTP persisted for the duration of experiments (up to 4 hours), in intact animals LTP decreased by 80 % or more within 1-2.5 hr in 7/11 animals. Induction of LTP was blocked by APV (1-3.5mM, topically applied) or MK-801 (0.5-1mg/kg), indicating NMDA dependence as in the slice.

When theta burst stimulation was delivered to afferent fibers in the lateral olfactory tract (LOT) after recovery from a period of epileptiform bursting, LTP was induced that persisted with little or no decrement for the duration of experiments (up to 6 hours) (n=5/5). In these experiments, interictal-like bursts were evoked at 0.2 - 1 Hz by LOT shocks following topical application of a drop of saturated picrotoxin solution; theta burst stimulation was applied after responses had returned to normal waveform. To determine if this effect was: (a) due to residual disinhibition at the time of train delivery, or (b) the result of a change induced by epileptiform activity, theta burst stimulation was applied during disinhibition restricted to the vicinity of the recording site that did not induce epileptiform activity. This local disinhibition was accomplished by diffusion or iontophoresis of bicuculline (10mM) through the recording micropipette. Tip diameters (10-16 μm) and iontophoretic parameters (0-50 nA) were adjusted so that the number of action potentials evoked by stimuli was markedly increased, but no epileptiform (all-or-none) field potential components were evoked. In the presence of local disinhibition, LTP was decremental (n=7/8) as in control experiments, suggesting that the increased duration after disinhibition was a consequence of a change induced by the epileptiform activity. Supported by grant NS 19865 from NINDS.

161.12

ASSOCIATIVE LONG-TERM POTENTIATION IN PIRIFORM CORTEX SLICES REQUIRES $GABA_A$ BLOCKADE. E.D. Kanter and L.B. Haberly, Neurosci. Training Prog. and Dept. of Anat., Univ. of Wisc., Madison, WI 53706

Pyramidal neurons in piriform cortex receive two excitatory inputs on adjacent segments of their apical dendrites: afferent fibers from the lateral olfactory tract synapse distally in layer Ia and intrinsic association fibers synapse more proximally in layer Ib. This anatomical configuration suggests the possibility of associative interactions based on coincident inputs from the two fiber systems. Potentiating stimuli (4-pulse trains repeated at theta frequency) were delivered to either Ia afferents or Ib association fibers and single weak shocks were delivered to the other pathway. Neither trains to the first pathway alone nor weak shocks to the second pathway alone produced potentiation in the second pathway. LTP was produced only when the stimuli were given simultaneously and only when the $GABA_A$ antagonist bicuculline, which blocks the fast, Cl^--mediated IPSP, was used to block inhibition. Initially, bicuculline (2-5 μM) was added to a bathing medium in which Ca^{2+} was raised to 10 mM to prevent epileptiform (all-or-none) responses. In later experiments focal, iontophoretic application of bicuculline from either the recording pipette or from a second pipette inserted near the recording site allowed the use of standard medium. Using this method robust associative LTP was consistently observed without epileptiform activity. The magnitude of associative LTP in each pathway was similar to that of homosynaptically induced LTP (approx. 15% in Ia and 40% in Ib; Kanter and Haberly, Brain Res. 525:175, 1990). Bicuculline did not increase the magnitude of homosynaptically induced LTP in either pathway. The $GABA_B$ antagonist phaclofen, at a concentration that completely blocks the slow, K^+-mediated IPSP in piriform cortex, did not facilitate the induction of associative LTP. Induction in both directions was blocked by 15 μM D-APV, indicating that the potentiation is dependent on NMDA receptors. These results suggest that $GABA_A$ receptor blockade increases the spread of depolarization along pyramidal dendrites sufficiently to allow induction of associative LTP. Supported by NIH grant NS 19865.

161.13

Lesions of the medial septum which produce deficits in working/spatial memory do not impair long-term potentiation in the rat hippocampus.
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Long-term potentiation (LTP) of hippocampal synaptic transmission is of interest as a possible mechanism for memory and learning in the mammalian brain. We have investigated whether impairment of working/spatial memory through medial septum (MS) lesions influences the ability to induce LTP in rat hippocampus in vivo and in vitro.

MS lesions (1 mA, dc, 40s) were made under Equithesin anaesthesia. After recovery, the working/spatial memory of lesioned and control rats was assessed as the number of correct arm entries made in an 8-arm radial maze. After at least 15 training sessions, the mean score obtained by lesioned rats was 5.8 ± 0.1 ($n=8$), which was significantly lower than the mean score obtained by control rats (7.84 ± 0.05, $n=11$, $p < 0.001$).

The rats were then anaesthetized with urethane, and LTP inducibility examined in the fimbria to CA3 and mossy fibre to CA3 pathways in vivo. High-frequency (HF) stimulation induced LTP of the evoked CA3 field response in each pathway. There was no significant difference in the magnitude of potentation observed in control and lesioned rats, although in both groups the LTP induced by HF fimbrial stimulation was greater than that induced by mossy fibre HF stimulation. LTP could also be induced in the CA1 region of hippocampal slices prepared from lesioned rats.

Acetylcholinesterase activity was severely reduced in the hippocampi of lesioned rats, indicating that the septal cholinergic input to the hippocampus was effectively destroyed. The results demonstrate that the induction of hippocampal LTP is not affected by MS lesions which impair working/spatial memory, and severely disrupt the septal cholinergic input to the hippocampus. Supported by the DFG and SFB 220

161.14

SEPTAL LESIONS IMPAIR HIPPOCAMPAL PRIMED BURST POTENTIATION AND LTP.
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Acetylcholine (ACh) has long been implicated in memory function. However, the mechanism by which ACh acts to influence memory is unknown. Hippocampal connections show long lasting use dependent changes in strength; it has been suggested that such alterations represent a mechanism for memory encoding. Since lesions of the ACh-containing afferents to the hippocampus profoundly disrupt many types of learning, we decided to investigate the effects of cholinergic deafferentation upon hippocampal synaptic plasticity. Cholinergic deafferentation of the hippocampus was accomplished by making bilateral electrolytic lesions of the medial septum/diagonal band complex under pentobarbital anesthesia. Five to 30 days later, the rats were reanesthetized and prepared for acute recording. Field potential responses to commissural stimulation were recorded in the CA1 pyramidal cell layer of the dorsal hippocampus. A 1 + 4 patterned stimulus train was used to induce PB potentiation. Twenty minutes later, a 100 Hz/1 sec train was used to induce LTP. Septal lesions reduced the magnitude of both PB potentiation and LTP by $\approx 40\%$. The relationship between PB potentiation and LTP was unaltered. AChE histochemistry verified a major reduction in cholinergic input following septal lesions, suggesting that reductions in ACh could affect hippocampal plasticity. However, more specific manipulations of hippocampal cholinergic pharmacology will be required to verify this possibility.

161.15

THE EFFECT OF LOCALLY APPLIED CHOLINERGIC DRUGS ON PRIMED BURST POTENTIATION.
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Both acetylcholine (ACh) and the hippocampus have been implicated in learning and memory processes. Hence, ACh might be expected to modulate hippocampal synaptic plasticity (a physiological memory model). Primed burst potentiation (PB), a threshold form of LTP, was challenged with locally applied cholinergic drugs in area CA1 of the hippocampus. Drugs were administered via pressure micro-ejection from multibarrel glass micropipettes. The PB train, consisting of a priming pulse followed 170 msec later by a burst of 4 pulses at 200 Hz, was given concurrent with the peak excitation of a CA1 pyramidal cell induced by a locally applied agonist (within 5-10 sec of onset of drug application), or after a 3 min application of an antagonist. PB was defined as the mean percent change from baseline population spike amplitude, 11-20 min post-train. MANOVA of all drug treatments revealed a significant overall drug effect ($F_{(9,42)} = 2.65$, $p = 0.016$); *a posteriori* analysis showed that PB was attenuated in nicotine- and scopolamine-treated groups compared to saline controls; muscarine had no effect on PB. Nicotine's attenuation of PB was reversed in the presence of mecamylamine and hexamethonium. In summary, locally applied cholinergic drugs affect threshold hippocampal plasticity. However, it appears that muscarine and nicotine exert qualitatively different actions.

161.16

Inverted-U Relationship Between Corticosterone and Hippocampal Primed Burst Potentiation in Urethane-Treated Rats.
D.M. Diamond, M.C. Bennett, J. Meltzer, M. Fleshner and G.M. Rose. Dept. of Pharmacology, UCHSC and VAMC, Denver, Colorado

Stress blocks hippocampal LTP *in vivo* (*Psychobiol.*, 18:273, 1990) and *in vitro* (*BNB.*, 48:138, 1987). The stress effect may result, in part, from an inhibitory effect of elevated levels of corticosterone (CORT) on hippocampal plasticity. For example, we recently reported that there is a negative linear correlation between primed burst potentiation (PB-LTP), a low threshold form of LTP, and elevated levels of serum CORT (*Psychobiol.*, in press). In the present report, we characterize the relationship between PB-LTP and a broad range of CORT levels in urethane-treated animals.

We recorded CA1 population spikes from acute and chronically-prepared rats. Urethane produced a dose-dependent increase in CORT levels; doses of 0.1-1.8 g/kg produced CORT levels of 5-93 μg/dl. There was an "inverted-U" relationship between CORT and PB-LTP: The peak magnitude of PB-LTP occurred at 9-20 μg/dl; the magnitude of PB-LTP was smaller in animals with lower (0-8 μg/dl) and higher (21-93 μg/dl) levels of CORT. Adrenalectomized rats with CORT pellet implants exhibited a similar inverted-U relationship.

These data indicate that CORT exerts a concentration-dependent modulation of hippocampal plasticity. The findings may provide a neuroendocrine basis for the well-described inverted-U relationship between arousal and learning.

161.17

HETEROSYNAPTIC LTP IN THE RAT SUPERIOR CERVICAL GANGLION.
Rulin Wu* and Donald A. McAfee, Dept. of Pharmacology, University of California, Irvine, CA 92717

Brief tetanic preganglionic stimulation results in potentiation of cholinergic transmission for hours. The mechanism is presynaptic in origin, producing a long-term potentiation (LTP) of evoked acetylcholine release. We now have evidence that tetany in one group of presynaptic terminals can cause LTP in a smaller population of non-tetanized terminals.

Sympathetic ganglia were isolated and superfused in vitro (24°C). The preganglionic nerve was dissected into two bundles and each sucked into separate bipolar stimulating electrodes. Single test stimuli (1/min) were delivered to each bundle, and the amplitude of the compound action potentials in the postganglionic nerve was measured. Care was taken to prevent spread of the stimulus current from one bundle to the other. When, in 10 of 13 preparations, a brief tetany (20Hz/20sec) was delivered to one bundle, the test responses (homosynaptic) in that bundle exhibited PTP followed by LTP. Test stimuli in the non-tetanized bundle (heterosynaptic) exhibited only LTP. Homosynaptic LTP was about 2.5 fold greater than heterosynaptic LTP. Perhaps an endogenous factor released during tetany feeds back to homosynaptic and nearby heterosynaptic terminals to activate a mechanism that induces LTP. (This work was supported by PHS grant NS-22470.)

161.18

EVIDENCE THAT THE MATRIX RECOGNITION MOLECULE F55 IS PRESENT IN SYNAPSES AND CONTRIBUTES TO THE STABILIZATION OF LTP.
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Matrix recognition molecules organize cell-substratum connections given their localization to regions of contact. Recent work (Bahr *et al.*, *NeuroReport* 2:13, 1991) identified a candidate molecule (F55) possibly involved in the focal contacts responsible for synapses, the dominant class of intercellular junctions in brain. Assembly of adhesive contacts via newly exposed matrix receptors conceivably could stabilize synaptic shapes and hence properties. Accordingly, we tested whether matrix recognition is necessary for the stable expression of long-term potentiation (LTP), a lasting increase in synaptic strength in hippocampal CA1. To do this, slices of hippocampus were bathed in solutions containing Arg-Gly-Asp (RGD) peptides known to block members of the integrin superfamily of receptors which mediate a broad spectrum of adhesive interactions. Though the peptides produced no detectable difference in the amount of LTP expressed 1 to 2 min after induction, RGD-specific peptides did produce a reversible, dose dependent reduction in LTP over a period of 40 min. To screen for synaptic adhesion molecules perhaps involved in LTP, synaptic plasma membranes (SPMs) were seeded onto microtitre plates coated with the matrix protein fibronectin (Fn). Hippocampal SPMs consistently displayed 40 to 50% more SPM-Fn adhesion over that displayed by SPMs isolated from whole brain. This adhesive interaction was reduced either by the presence of RGD peptides or by pre-coating the plates with a modified Fn lacking the RGD sequence. Solubilized SPM proteins that were eluted from Fn-agarose with RGD peptides consisted of a 55 / 51 kDa doublet. The doublet bound to a second 'fresh' Fn-agarose column and was the only species RGD-eluted as evident on double silver-stained PAGE. The eluted material ran as a 50 kDa species on a size exclusion column, thus suggesting it is monomeric in nature. This RGD-binding protein was identified as the F55 molecule due to its immunological similarity with a subclass of integrins. This study implicates unique adhesion molecules in the stabilization of LTP. (AFOSR #89-0383 supported.)

161.19

RELEASE OF ASPARTATE AND GLUTAMATE FROM HIPPOCAMPAL SLICES: implications for the expression of long-term potentiation.

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We investigated the possible co-release of aspartate (Asp) and glutamate (Glu) to determine whether LTP could result from an increase in the release of Glu relative to Asp. Both amino acids are released from hippocampal area CA1 in a Ca^{2+}-dependent manner and are potent agonists at excitatory amino acid receptors with different affinities; Asp primarily activates NMDA receptors while Glu activates both NMDA and non-NMDA receptors. A decrease in the ratio of transmitter Asp/Glu could account for LTP by increasing non-NMDA receptor activation with little or no change in NMDA receptor activation. Potassium-evoked amino acid release was measured by HPLC with fluorometric detection from hippocampal slices perfused with glutamine (0.5 mM) containing, normal-glucose (10 mM) or low-glucose (0.2 mM) medium. The ratio of evoked Asp/Glu increased from 0.35±0.1 in normal medium to 0.67±0.2 in low-glucose medium ($p<0.01$). In separate slices, reducing glucose in the perfusion medium resulted in the persistent decrease in initial slope and peak amplitude of Schaffer-CA1 population EPSPs after approximately 30-minute delay. Such a change in EPSP shape is consistent with a shift in the relative activation of NMDA and non-NMDA receptors. These results are consistent with the view that Asp and Glu are co-released from Schaffer collateral/ commissural terminals but act on disparate populations of post-synaptic receptors. Supported by NS01196, NS24288, and an Andrew Mellon Predoctoral fellowship.

161.20

INDUCTION OF LONG-TERM POTENTIATION IN VIVO IS ACCOMPANIED BY CHANGES IN THE INCORPORATION OF ^{35}S-METHIONINE INTO SPECIFIC PROTEINS:

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High-frequency stimulation of perforant path fibres induces an increase in synaptic transmission, sustained for several hours. The longevity of the effect indicates that changes in gene expression and protein synthesis may be involved. We have tested this hypothesis by analyzing the in vivo incorporation of ^{35}S-methionine into hippocampal proteins following the induction of LTP in the anaesthetized rat dentate gyrus.

Urethane anaesthetized rats were implanted with stimulating and recording electrodes in the angular bundle and the dorsal dentate cell body layer respectively. A cannula was then inserted into the third ventricle through the midline, through which 500μCi of ^{35}S-methionine was infused at 1μl/min. LTP was induced unilaterally (250Hz, 200msec), one hour after the start of infusion and was followed for one and three hours. Dorsal hippocampi were then removed and homogenized in O'Farrell sample buffer. Labelled proteins were separated by two-dimensinal electrophoresis and the resulting gels were subjected to autoradiography. Quantitative analysis of the autoradiograms using the PdQuest system (Protein Databases Inc) revealed reductions in the incorporation of 3 proteins one hour after LTP. These were (approximate kD and pI): 4204 (28, 5.8), 1104 (13, 5.2) and 13 (13, 5). At three hours after induction these were no longer significant. However, two other proteins (5307 (20, pI 6.4) and 1606 (75, pI 5.1)) were found to have increased and decreased incorporation, respectively. These and other observed changes are currently being investigated.

ACETYLCHOLINE RECEPTORS: MUSCARINIC I

162.1

NOVEL AMINO TERMINALLY MODIFIED DERIVATIVES OF OXOTREMORINE ARE MORE EFFICACIOUS AGONISTS AT CORTICAL MUSCARINIC RECEPTORS OF THE M-1 SUBTYPE.

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In recent years there has been considerable interest in the development of centrally active agents capable of enhancing cortical cholinergic neurotransmission since such agents may act as a palliative therapy for the amelioration of the symptoms of dementia associated with Alzheimer's disease. Although a number of muscarinic receptor subtypes have been identified in the brain via molecular biological approaches, it is still believed that the pirenzepine-sensitive M-1 site in the cortex, stimulation of which is coupled to phosphatidylinositol (PI) hydrolysis, is that which is most clearly associated with cognitive function. Other central effects linked to muscarinic receptor activation such as tremor and hypothermia are believed to be M-2 receptor mediated.

Oxotremorine, long known as a potent centrally active muscarinic agonist, is a weak partial agonist at the cortical M-1 receptor (14±4% agonist response of carbachol). Efforts to enhance the M-1 agonist efficacy of this compound have resulted in the discovery of a series of novel differentially constrained amino-terminally modified analogs of oxotremorine which incorporate 2-substituted pyrrolidine or piperidine rings. The (R)-enantiomer of the pyrrolidine modification was found to be most efficacious producing a maximal PI response of 43±4% that of carbachol. The *in vitro* pharmacologic profiles of these novel muscarinic agonists will be presented.

162.2

AF150 AND AF151: NOVEL EFFICACIOUS M1 MUSCARINIC AGONISTS.

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Two new rigid oxazoline-piperidine analogs, AF150 and AF151 (Fisher et al, US Pat Appl, April, 1990), are selective and efficacious agonists for M1AChRs in rat cortex (CT) vs cerebellum (CER) {binding of ^{3}H-pirenzepine ± GppNHp (Gp) vs ^{3}H-QNB, respectively; e.g. for AF150 in CT: K_{H-Gp}=0.11uM (21%), K_{L-Gp}=11uM; K_{L+Gp}=19uM (100%); in CER: K_H=5.1uM (62%), K_L=81uM}. In CHO cells transfected with m1AChR, AF150 and AF151 are full agonists in stimulating PI hydrolysis, yet partial agonists (20% vs carbachol, CCh) in elevation of cAMP levels. AF150 and AF151 are partial agonists (25% vs CCh) in CHO cells transfected with m3AChR and in SK-N-SH cells (>m3AChRs), (PI hydrolysis), but full agonists in PC12 cells (>m4AChR), (inhibition of forskolin-stimulated cAMP levels). In CA1 neurons of rat hippocampal slices AF150 appears to be an M1>M2 agonist, but is more efective than AF102B (an M1 agonist). AF150 produced a depolarization and a reduction of the slow AHP, M1 effects, but had little effects on EPSPs or on input resistance. In AF64A (3nmole/2ul/side, icv)-treated rats, AF150 (0.1-1mg/kg,ip) restored cognitive impairments in a passive avoidance test without side-effects at low doses. Due to their unique profile at m1AChRs and activation of only distinct G protein subset(s), AF150 and AF151 can be considered as candidate drugs for Alzheimer's disease.

162.3

SELECTIVE SIGNAL TRANSDUCTION BY THE M1 AGONIST, AF102B, IN CELLS EXPRESSING VARIOUS mAChR SUBTYPES.

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AF102B, a rigid M1-selective cholinergic agonist, is currently evaluated for treatment of Alzheimer's disease (AD). AF102B stimulated PI hydrolysis (25% vs. CCh) in CHO cells transfected with the m1AChR subtype; this was atropine-sensitive. Unlike CCh, AF102B failed to stimulate PI hydrolysis in CHO cells transfected with the m3AChR subtype, or in SK-N-SH human neuroblastoma cells (mostly m3AChR). CCh selectively increased both basal and forskolin-stimulated cAMP accumulation in m1AChR but not m3AChR-transfected cells. In contrast, AF102B attenuated forskolin-induced cAMP accumulation in m1AChR-transfected cells. In PC12 cells (mostly m4AChR) CCh attenuated forskolin-stimulated cAMP accumulation; AF102B mimicked CCh (40% vs. CCh). However, following treatment of PC12 cells with pertussis toxin, which did not affect receptor expression, CCh increased forskolin-induced cAMP accumulation, yet AF102B had no effect on cAMP levels. Thus, the M1 selectivity of AF102B (m1 and m4 AChRs) may reach beyond the mAChRs ligand recognition site, imposing activation of distinct G protein subset(s); this is probably dictated by the rigidity of AF102B. This may contribute to the potential value of AF102B for AD patients. Supported by Snow Brand, Japan.

162.4

FUNCTIONAL CHARACTERIZATION (PI-TURNOVER) OF SDZ ENS 163, A MUSCARINIC AGENT WITH A NOVEL PHARMACOLOGICAL PROFILE.

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SDZ ENS 163, a pilocarpine derivative, is a drug with a favourable pharmacological profile, presently in clinical development for the symptomatic treatment of Alzheimer's Disease. It acts both in vitro and in vivo as a postsynaptic agonist (m1/m3) and as a presynaptic antagonists (m2) at muscarinic receptors. The selectivity of this drug for m1 and m3 receptors was investigated by binding and functional studies in vitro. Using CHO cells, expressing either m1 or m3 receptors, and ^{3}H-NMS as ligand, no selectivity was found (k_i 1.8 and 3.2 μM). Using A9L cells expressing either receptor and measuring PI-turnover, SDZ ENS 163 was a relatively unselective, partial agonists of both m1 and m3 receptors (pD_2 5.5 and 5.1, efficacy 45% and 7%, carbachol = 100%). SDZ ENS 163 acts more like an agonist in the rat brain with an accelerated PI-turnover, as measured in vivo non-invasively by ^{31}P magnetic resonance spectroscopy and post-mortem by gas chromatography. These findings are compatible with the presynaptic antagonistic and postsynaptic agonistic properties of this muscarinic drug.

162.5

ACTIVITY AND STEREOSELECTIVITY OF AGONIST QUINUCLIDINE ANALOGS IN PROPOSED IN VIVO MODELS FOR MUSCARINIC RECEPTORS. E. P. Bymaster, C. H. Mitch, D. O Calligaro, H. E. Shannon*, and J. S. Ward*. Lilly Research Laboratories, Eli Lilly and Company., Indianapolis, IN 46285.

The enantiomers of the muscarinic (M) agonist aceclidine (AC, 3-acetoxy-quinuclidine) and ± 3-(3-methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.2]octane (LY231844, L658903, L) were studied on models for M1, M2, and M3 receptors in vivo in rats. The IC50's for in vitro 3H-oxotremorine-M binding for S-AC and R-AC were 110 and 1050 nM, thus showing 10 fold stereoselectivity. L, with the ester bioisostere-oxadiazole, had an IC50 of 11 nM. The ED50's for ex vivo binding of L, S-AC and R-AC were 0.24, >50, >50 mg/kg sc. The t1/2 was 2.7 and 0.25 hr for L and S-AC. In the proposed M1 model, striatal dihydroxy-phenylacetic acid (DOPAC) levels were increased by L, S-AC, R-AC with ED50's of 0.14, 10, >50 mg/kg sc, presumably via M1 heteroreceptors on dopamine terminals. L, S-AC, and R-AC decreased M2 acetylcholine (ACH) release with ED50,s of 0.05, 13 and >50mg/kg. The ED50 for M3 salivation was 0.05, 5, and >50 mg/kg for L, S-AC, and R-AC. Scopolamine blocked all L- and S-AC-induced changes; whereas, n-methylscopolamine blocked only salivation. Trihexyphenidyl (0.3mg/kg) and pirenzepine (100mg/kg) partially blocked L on DOPAC, but not on ACH. S-AC was more potent than the R-AC in vitro and in vivo models and was non-selective. The analog of aceclidine, L, was quite efficacious, longer-lived, and non-selective.

162.6

EFFECTS OF BM-5 ON REGIONAL ACETYLCHOLINE TURNOVER AND MUSCARINIC RECEPTOR BINDING IN RAT BRAIN. H.M. VARGAS and T. CHU*. Department of Pharmacology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1735.

The muscarinic agent BM-5 (N-methyl-N-(1-methyl-4-pyrrolidino-2-butynyl)acetamide) has attracted interest because of its presynaptic antagonist-postsynaptic agonist profile *in vitro* and its ability to produce site selective effects within brain *in vivo*. This study assessed the effects of BM-5 on acetylcholine (ACh) turnover and [^{3}H]N-methylscopolamine binding in three brain areas. Endogenous (unlabelled) ACh levels and [$^2H_4$]ACh specific activity (i.e., turnover) were determined one minute after i.v. tracer [$^2H_4$]choline injection. In saline controls, ACh levels (nmol/g) in the striatum, cortex and hippocampus were 86 ± 2, 20 ± 1, 33 ± 2, respectively; corresponding mole ratios ($x10^{-4}$) of [$^2H_4$]ACh/ACh_{TOTAL} were 92 ± 2, 279 ± 21, 165 ± 15, respectively. Fifteen minutes after administration, BM-5 (1, 50 µmol/kg, s.c.) did not elevate or decrease ACh levels or [$^2H_4$]ACh ratios in any area. However, an intermediate dose (5 µmol/kg) did selectively reduce striatal ACh content by 23% and increase specific activity 37%, thus behaving as an antagonist. In comparison, oxotremorine (0.5 µmol/kg, s.c.) raised regional ACh levels (44, 18, 32%, respectively) and decreased [$^2H_4$]ACh mole ratios (12, 68, 60%, respectively). Competition binding studies ([^{3}H]NMS, 0.3 nM) indicated that BM-5 had K_i values of 87, 57 and 55 nM in the three regions, respectively. The results suggest that BM-5 exhibits comparable affinity for muscarinic receptors in these regions, but displays differential presynaptic activity in these same areas. The pharmacological agonist/antagonist profile of BM-5, therefore, will vary according to dose and brain region. (Supported by GM37816 and MH17691).

162.7

ACTIVITY AND SELECTIVITY OF BM 5 ENANTIOMERS FOR MUSCARINIC RECEPTOR SUBTYPES IN RAT BRAIN. W.S. Messer, Jr, L.A. Dokas, U. Hacksell*, P.G. Dunbar* and W. Hoss. Department of Medicinal & Biological Chemistry, College of Pharmacy, Univ. of Toledo, Toledo, OH 43606; Department of Biochemistry, Medical College of Ohio, Toledo, OH 43699; Uppsala Biomedicinska Centrum, Biomedicum, S-75223 Uppsala, Sweden.

The activities of the enantiomers of BM 5 were examined using several paradigms designed to measure muscarinic cholinergic activity in the central nervous system. Receptor autoradiographic methods were utilized to localize muscarinic receptors with a high affinity for each enantiomer. The enantiomers also were examined for their ability to inhibit adenylate cyclase in the cerebral cortex and neostriatum.

In autoradiographic studies, the inhibition of [^{3}H]-l-quinuclidinyl benzilate ([^{3}H]-*l*-QNB) binding to muscarinic receptors in rat brain sections was measured for each enantiomer. R-(+) BM 5 inhibited [^{3}H]-*l*-QNB binding at concentrations below 1.0 µM, while much higher concentrations of S-(-) BM 5 were required to produce a comparable level of inhibition. Analysis of the autoradiograms indicated that both stereoisomers had similar binding patterns with high affinity for receptors in the midbrain and brainstem and lower affinity for the cerebral cortex and hippocampus.

The ability of the enantiomers of BM 5 to inhibit adenylate cyclase activity was examined in the cerebral cortex and the neostriatum. In the cerebral cortex, R-(+) BM 5 was more potent inhibitor of adenylate cyclase than S-(-) BM 5. S-(-) BM 5 was approximately 100-fold less active than the R-(+) enantiomer. The differential effects of the enantiomers of BM 5 were more readily apparent in the cerebral cortex than in the neostriatum. It should be noted that the oxalate salts of BM 5 were utilized and that oxalate alone inhibited adenylate cyclase at higher concentrations.

In summary, R-(+) BM 5 is a potent muscarinic agonist in the adenylate cyclase assay, particularly in the cerebral cortex. The selectivity of R-(+) BM 5 for muscarinic receptors appears to be comparable to that found for other muscarinic agonists. In addition, the 100-fold difference in activity of the two enantiomers indicates a remarkable stereochemical selectivity for muscarinic receptors in both receptor binding and adenylate cyclase activity. The data provide a strong foundation for further studies of the molecular requirements for muscarinic receptor activity. Supported by NS 23929, NS 25765, and NS 23598.

162.8

ANTAGONISM OF MUSCARINIC M1 RECEPTOR MEDIATED CALCIUM RELEASE BY McN-A-343 AND THE NOVEL ANALOG, BN228.

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Activation of muscarinic receptors by carbachol causes Ca release from internal stores in N1E-115 neuroblastoma cells. Ca release was measured using fura-2 following exposure to 1 mM carbachol. The response is blocked by pirenzepine, a high affinity M1 receptor antagonist. McN-A-343 and its novel analog, BN228 (4-[N-(4-chlorophenyl)carbamoxyloxy]-pent-2-ynyltrimethylammonium Br; B. Nielsson, Uppsala) inhibit Ca release in response to carbachol. The IC_{50}'s for McN-A-343 and BN228 as M1 antagonists are 9 and 4 µM, respectively. Although McN-A-343 has been called a "selective M1 agonist", neither McN-A-343 nor BN228 elicit Ca release when applied in the absence of carbachol. Our results demonstrate that McN-A-343 does not act as an agonist but as an M1 antagonist in N1E-115 cells.[Supported by NSF BNS-9021217 to SHT and NIH GM317816].

162.9

CARAMIPHEN, IODOCARAMIPHEN AND NITROCARAMIPHEN ARE COMPETITIVE M_1 SELECTIVE COMPOUNDS. Robert L. Hudkins[1], James F. Stubbins*,[1] and Diane L. DeHaven-Hudkins[2]. [1]Department of Medicinal Chemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0540 and [2]Department of Enzymology and Receptor Biochemistry, Sterling Research Group, Malvern, PA 19355-1314.

Caramiphen is an antimuscarinic agent which is a more effective antidote to organophosphate acetylcholinesterase poisoning than atropine. Because of the structural similarity of caramiphen to the M_1 selective ligand dicyclomine, we examined the M_1/M_2 selectivity of this compound in receptor binding assays and report that caramiphen binds with high affinity (K_i = 1.2 nM) and selectivity (26-fold) for the M_1 subtype. An examination of the effect of aromatic substituent parameters (i.e. σ, π) on the binding affinity and receptor subtype selectivity led to the discovery of the M_1 selectivity of the p-iodo and p-nitro caramiphen derivatives. Iodocaramiphen binds with high affinity at the M_1 site (K_i = 2.1 nM) and displays a 58-fold greater preference for the M_1 than the M_2 site. Nitrocaramiphen binds with equally high affinity at M_1 sites (K_i = 5.5 nM) but with a 71-fold M_1 selectivity. All three compounds interacted with the M_1 binding site in a competitive manner. The nitro and iodocaramiphen derivatives were as potent and showed a greater selectivity of M_1 over M_2 binding than the prototypical agent pirenzepine (M_1 K_i = 5.2 nM, 51-fold selectivity).

162.10

"m1-TOXIN": ISOLATION, CHARACTERIZATION AND SELECTIVITY FOR m1 MUSCARINIC RECEPTORS. S.I. Max, J-S. Liang* and L.T. Potter. Molecular and Cellular Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Adem et al. (Biochim. Biophys. Acta 968, 340) observed that toxins from the venom of the green mamba, *Dendroaspis angusticeps*, could block 55% of the binding sites for ^{3}H-QNB in rat brain synaptosomal membranes. We purified the principal antimuscarinic component from this venom by gel filtration on Sephadex G-50, and reverse-phase HPLC. The isolated protein has a formula weight of about 7700 D, no alanine or proline residues, and a unique sequence. Its specificity was tested using CHO cells expressing m1-m5 muscarinic receptors individually. One hundred ng/ml toxin blocks 50% of m1 receptors, and full blockade is observed with 200 ng/ml. Concentrations 100-fold higher block 50% of m4 sites but have no effect on m2, m3 or m5 receptors. Hence, we have named this toxin, "m1-toxin". The toxin is selective in fresh or frozen tissue, membranes or solution. It blocks 40-60% of the ^{3}H-NMS sites in the hippocampus, cortex, striatum and whole brain, about 10% of the sites in glands, and less than 5% in heart or brainstem. Furthermore, it blocks 75% of the agonist-activated phosphoinositide hydrolysis in rat hippocampal slices. m1-Toxin is clearly the antagonist of choice for studies of m1 receptors.

162.11

USE OF m1-TOXIN TO FACILITATE THE STUDY OF m1 AND m4 MUSCARINIC RECEPTORS IN THE STRIATUM. S.L. Purkerson*, J-S. Liang*, S.I. Max and L.T. Potter. Molecular and Cellular Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

The striatum expresses mRNA primarily for m1 and m4 receptors, with much less for m2 receptors. Both the m1 and m4 subtypes are blocked by anticholinergic therapy for Parkinson's disease, which may be counterproductive, since m1 receptors are coupled to phosphoinositide hydrolysis and cellular excitation, whereas m4 receptors are coupled to decreased cAMP and inhibition. Little is known about the numbers, locations and functions of m1 and m4 receptors in the striatum, because existing ligands are not very selective; e.g. the present gold standard for m1 receptors, pirenzepine, shows only 5-6 fold higher affinity for m1 than m4. To go further, we have fully blocked m1 sites in the rat striatum with 0.8 μg/ml m1-toxin, with no effect on m4 sites. To judge from binding curves (toxin vs. ^{3}H-QNB or ^{3}H-pirenzepine; pirenzepine and other antagonists vs. ^{3}H-NMS before and after toxin), 40% of the striatal muscarinic receptors are m1, 55% are m4 and 5% are m2. Competition curves between agonists and ^{3}H-NMS show the affinities and K_i/K_H ratios for different agonists at m4 receptors coupled to their native G-proteins. It is therefore possible to use binding assays to screen new m4 agonists and antagonists for functional studies and as potential anti-Parkinson agents. Autoradiographs demonstrate the different distribution of m1 and m4 receptors. Physiological studies of toxin-spared m4 receptors are now possible. It is evident that m1-toxin represents a powerful new tool for studying the striatum.

162.12

β-HYDROXYETHYLAPROPHEN: A METABOLITE OF APROPHEN WITH ANTIMUSCARINIC ACTIVITIES.

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β-Hydroxyethylaprophen is a metabolite found in the urine of rats after administration of the antimuscarinic drug aprophen. Synthetic β-hydroxyethylaprophen also possessed potent antimuscarinic activities in (1) blocking the contraction of guinea pig ileum with a $K_B = 2.5 \times 10^{-7}$ M, (2) inhibiting the release of α-amylase from pancreatic acinar cells stimulated by carbachol ($K_i = 1.6 \times 10^{-9}$ M), and (3) competing for the binding of [^{3}H-N-methyl]scopolamine to muscarinic receptors of N4TG1 neuroblastoma cells ($K_i = 4.5 \times 10^{-8}$ M). The inhibition constants (M) for aprophen were $K_B = 3.1 \times 10^{-9}$, $K_i = 1.7 \times 10^{-9}$, $K_i = 5.1 \times 10^{-8}$, and atropine were $K_B = 2.0 \times 10^{-9}$, $K_i = 1.6 \times 10^{-9}$, $K_i = 2.4 \times 10^{-9}$, respectively. Thus, β-hydroxyethylaprophen was approximately 80-fold less potent an antimuscarinic compound in the ileum contraction assay than either the parent compound, aprophen, or atropine. However, all three compounds exhibited about the same potency in the pancreas assay. In the binding assay, β-hydroxyethylaprophen was equipotent to aprophen, and both compounds were 10-fold less potent than atropine. Because β-hydroxyethylaprophen is less hydrophobic than aprophen, it may be of interesting therapeutic value for organophosphate poisoning.

162.13

Point-mutated muscarinic M1 receptors bind the M2-selective antagonist methoctramine with high-affinity. T.-V. Dam, P. Bouchard*, P. Payette, M. Dennis* and F. Gossard*. National Research Council of Canada, Biotechnology Research Institute, Montréal, Québec, CANADA. H4P 2R2.

Five genes coding for acetylcholine muscarinic receptors have been found so far by cloning techniques. Pharmacological methods have defined three subtypes of muscarinic receptors that can be discriminated by their binding affinities for specific antagonists. The muscarinic receptor of the subtype 2 (M2) has high affinity, among others, for compounds of the polyethylene tetraamines family of which methoctramine is the prime representative. These antagonists are bound with lower affinity by other members of the muscarinic receptor family in particular by the subtype 1 (M1). Methoctramine has been proposed to bind to M2 by ionic interactions between the charged amine functions and the negatively charged amino acids in the second extracellular loop of the receptor. These charged amino acids are not found in M1. In order to test this model, we have introduced by site-directed mutagenesis, one to three negatively charged amino acids in the second external loop of the M1 receptor. The human M1 gene (Hm1) was introduced in an expression vector downstream of the SV40 late promoter. The construct was then modified using a mutagenesis technique on double-stranded DNA. Wild-type HM1 and mutant genes were introduced into CHO cells and stable cell lines established. The binding characteristics especially for methoctramine of these receptors were determined as well as the intracellular response by the binding of agonists.

162.14

A NOVEL M2-SELECTIVE MUSCARINIC ANTAGONIST. M.S. Gitler, V.I.Cohen, B. Jin, R.C.Reba, W.J.Rzeszotarski, and J. Baumgold. Dept. of Radiology, The George Washington University Medical Center, Washington, DC 20037.

Although several m2-selective antagonists have been described, they are not particularly potent. Thus, the development of potent m2-selective compounds remains an important goal. We now report that a bio-isoster of AQ-RA 741, is one order of magnitude more potent than previously described compounds and is slightly more selective. DIBA, the di-benzo derivative of AQ-RA 741 in which the pyridine of the tricycle was replaced with a benzene, had Ki values of 4, 0.3, 11 and 2 nM at m1 through m4 receptors, respectively. These values were from competition studies with [^{3}H]NMS in membranes from transfected A9 L cells (m1 and m3), rat heart (m2) and NG108-15 cells (m4). AQ-RA 741 had Ki values of 34,4,86 and 15 nM at each of these receptors. The autoradiographic distribution of DIBA binding sites was determined by diplacement of [^{3}H]NMS in rat brain. At low concentration, DIBA displaced [^{3}H]NMS from superior colliculi, thalamus, hypothalamus and pontine nuclei, but not appreciably from caudate, superficial cortex, or CA1 and CA2, consistent with its binding to m2 receptors. These data indicate that DIBA is the most potent, m2-selective muscarinic antagonist yet described. DIBA should therefore become a useful probe in future studies of muscarinic function.

162.15

CHARACTERIZATION OF THE EFFECTS OF CHOLINERGIC AGENTS ON EITHER IN VIVO ^{3}H-QNB BINDING OR EX VIVO HAChT C.E. Tedford, V.B. Ruperto*, R.A. Duffy* and R.D. McQuade, Schering-Plough Research, Bloomfield, New Jersey, 07003.

Two measurements of cholinergic activity were used to characterize the in vivo muscarinic actions of several standard agents. The ability of a compound to displace the in vivo binding of ^{3}H-QNB to rat cerebral cortex was investigated as a marker of central muscarinic receptor activity. Furthermore, ex vivo modulation of rat hippocampal high affinity choline transport (HAChT) was studied as a potential marker of presynaptic M_2 muscarinic receptor activity.

Rats were administered (sc)10 μCi of ^{3}H-QNB at various times prior to sacrifice. ^{3}H-QNB produced specific binding to cortical muscarinic receptors which was maximal at 1 hr. The centrally acting muscarinic antagonists, scopolamine (IC_{50} = 0.3 mg/kg) and atropine (IC_{50} = 17.5 mg/kg) produced a dose-dependent inhibition of ^{3}H-QNB binding, when coadministered (sc) with ^{3}H-QNB.

HAChT was measured in rat hippocampal synaptasomes following in vivo sc administration of various cholinergic agents. A dose-dependent reduction in HAChT was observed following treatment with various centrally-acting muscarinic agonists; arecoline, oxotremorine and Merck's (L-658,903). In contrast, the quaternary amine agonists, carbachol and oxotremorine-M did not reduce hippocampal HAChT. Antagonists such as atropine and scopolamine did not increase HAChT alone, but did block agonist-induced inhibition of HAChT. Finally, putative M1 receptor agonists (AF102B, SR 95639A) and ACh releasers (DuP 996) were ineffective in inhibiting HAChT, suggesting that presynaptic modulation of HAChT is primarily through M2 receptors.

In conclusion, two different approaches have been taken to demonstrate centrally mediated actions of known and putative muscarinic agents. These tests have proven useful in describing the possible mechanism of action of muscarinic agents as well as their ability to enter the central nervous system.

162.16

CHIMERIC m2/m5 MUSCARINIC RECEPTORS: IDENTIFICATION OF RECEPTOR DOMAINS CONFERRING ANTAGONIST BINDING SELECTIVITY. J. Wess, D. Gdula*, and Mark R. Brann*. National Institute of Neurological Disorders and Stroke, Lab. of Mol. Biology, Bethesda, MD 20892.

The five muscarinic receptors (m1-m5) can be discriminated by the use of several subtype-selective antagonists. We have previously shown that various tricyclic compounds including the AF-DX derivative AQ-RA 741 and the alkaloid himbacine show up to 200-fold higher affinities for m2 and m4 than for m5 receptors (Dörje et al., J. Pharamacol. Exp. Ther. 256, 727, 1991). To explore the structural basis underlying this selectivity, we have created a variety of chimeric m2/m5 muscarinic receptors in which regions of the m5 receptor were sytematically replaced by the homologous regions of the m2 receptor. Following their transient expression in COS-7 cells, the antagonist binding properties of the various receptors were studied. The affinity profiles of AQ-RA 741 and himbacine suggest that multiple receptor domains contribute to their higher affinity for m2 receptors. A completely different pattern was observed for the pirenzepine derivative UH-AH 37, which binds to m5 receptors with about 10-fold higher affinity than for m2 receptors. The subtype-selectivity of this antagonist is almost entirely dependent on a short receptor domain comprising most of transmembrane region VI and the third extracellular loop. Our data indicate that different structural epitopes are involved in conferring binding selectivity on different selective muscarinic antagonists.

162.17

ANTAGONISM OF MUSCARINIC AGONIST STIMULATED PHOSPHATIDYL-INOSITOL HYDROLYSIS BY 1-METHYL-(5-ALKOXY-4-ALKYLOXAZOL-2-YL)-1,2,5,6-TETRAHYDRO-PYRIDINES. Charles H. Mitch, David Calligaro, Frank Bymaster, Harlan Shannon, Barry Sawyer, Steve Quimby. Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285.

Blockade of muscarinic agonist induced stimulation of phosphatidyl-inositol hydrolysis was used to evaluate antagonist potencies of a series of 1-methyl-(5-alkoxy-4-alkyloxazol-2-yl)-1,2,5,6-tetrahydropyridines. Inhibition of 100uM acetyl choline in cortical slices was measured to determine M1 antagonist activity. Inhibition of 1mM carbachol in SK-N-SH cells was measured to determine M3 antagonist activity. Receptor affinity was determined using 3H-pirenzepine and 3H-oxotremorine-M as radioligands. As representative examples 1-Methyl-(5-propoxy-4-(sec-butyl)-oxazol-2-yl)-1,2,5,6-tetrahydropyridine was found to have IC50's of 81.0 nM and 340 nM respectively for antagonism of agonist stimulated PI turnover in cortical slices and SK-N-SH cells respectively, while it had receptor binding IC50's of 1 nM and 26 nM for 3H-PZ and 3H-Oxo-M respectivley. 1-Methyl-(5-(2-propoxy)-4-(iso-butyl)-oxazol-2-yl)-1,2,5,6-tetrahydro-pyridine was found to have IC50's of 5630 nM and 2320 nM for agonist stimulated PI turnover in cortical slices and SK-N-SH cells respectively, while it had receptor binding IC50's of 56 nM and 472 nM for 3H-PZ and 3H-Oxo-M respectively. Selected compounds were also evaluated for their ability to block agonist activity in smooth muscle preparations such as the rabbit vas deferens.

162.18

PHARMACOLOGICAL CHARACTERIZATION OF THE M4 MUSCARINIC RECEPTOR SUBTYPE. M.A. Buck*, W. Billard*, H. Binch III* and R.D. McQuade. Schering-Plough Research, Bloomfield, New Jersey, 07003.

Pharmacologically distinguishable subtypes of the muscarinic acetylcholine receptor have been classified into M1, M2, and M3 primarily on the basis of differential selectivity of the antagonists, pirenzepine (PZ) and AFDX-116. Transfection and stable expression of individual cloned muscarinic receptor genes (m1-m5) into HeLa cells lacking such receptors have allowed for a more detailed comparison of their pharmacological and biochemical properties. The binding and second messenger profiles of the m4 receptor were compared with those for the m1 and m2 receptors. The cloned human m4 muscarinic receptor displays saturable high affinity binding of ^{3}H-PZ (K_D=11.1 nM), similar to m1 receptors, albeit 10-fold less potent (K_D =1.5 nM). In contrast, m2 receptors display low affinity for PZ (K_i=241 nM). Competition studies with atropine demonstrate its equivalent potency at m4 and m1 sites (K_i =0.15 nM). At m2 receptors, however, atropine was 8-fold less potent. Conversely, competition displacement with AFDX-116 demonstrates the similarity between the m4 and m2 receptors; both exhibit K_i's of approximately 40 nM, while the drug was 10-fold less potent at m1 sites. Analysis of second messenger coupling indicates that the m4 receptor is similar to the m2 subtype; both inhibit adenylate cyclase and thymidine incorporation in the transfected HeLa cells.

Characterization of rat cortical tissues indicates the presence of M4 and M1 sites, with little evidence of M2 receptors. Saturation studies of ^{3}H-PZ reveal two high affinity sites, with K_D values of 1 and 11 nM, which correspond to the K_D values obtained for cloned m1 and m4 receptors, respectively. Atropine exhibits a single class of sites with high affinity , indicating the absence of m2 receptors. These data support the existence of an M4 pharmacological subtype characterized by high affinity for PZ, AFDX-116 and atropine and negative coupling to adenylate cyclase and thymidine incorporation.

EXCITATORY AMINO ACIDS: PHARMACOLOGY III

163.1

RILUZOLE INHIBITS RELEASE OF L-GLU AND L-ASP FROM SLICES OF HIPPOCAMPAL AREA CA1. D.Martin, M.A.Thompson* and J.V.Nadler. Depts. Pharmacol. and Neurobiol., Duke Univ. Med. Ctr., Durham, NC 27710.

Riluzole possesses anticonvulsant and neuroprotective activity in animal models. It is believed to exert its effects by reducing glutamate release. This study examined the effects of riluzole on the release of glutamate (L-glu) and aspartate (L-asp) from the Schaffer collateral-commissural-ipsilateral associational (SCCIA) pathway in area CA1 of the rat hippocampus. Transmitter release was evoked from superfused slices of the CA1 area (excluding stratum lacunosum-moleculare) by exposing the slices to 1-min pulses of 50 mM K^+. Under these conditions nearly all the release of L-glu, L-asp and GABA is Ca^{2+}-dependent and it originates predominatly from SCCIA pathway.

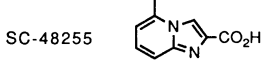
Riluzole (10-100 μM), in the absence or presence of 1.2 mM Mg^{2+} significantly inhibited the release of both L-glu and L-asp in a concentration-dependent manner. It depressed the release of these amino acids about equally and by as much as 40%. Only the highest concentration of riluzole inhibited GABA release. TTX (0.1 μM) reduced the K^+-evoked release of L-glu and L-asp by about 40%, suggesting that a portion of the release depended on the generation of action potentials as the K^+-concentration within the tissue was rising. The action of riluzole (30 μM) was not confined to the TTX-sensitive component of the release. In general, the actions of riluzole resembled those of NMDA receptor antagonists and inhibitors of arachidonic acid metabolism that act upon the lipoxygenase pathways.

Therefore the anticonvulsant and neuroprotective properties of riluzole may be at least partly explained by its ability to inhibit L-glu/L-asp release from synaptic terminals. One possibility is that riluzole either inhibits the breakdown of arachidonic acid by lipoxygenases or interferes with the up regulation of L-glu/L-asp release by lipoxygenase products. (Supported by NIH grant NS16064).

163.2

NMDA SELECTIVE EXCITATORY AMINO ACID ANTAGONISTS FROM ACID FUNCTIONALIZED IMIDAZOPYRIDINES. Don W. Hansen Jr., Karen B. Peterson, Sofya Tsymbalov, Joseph B. Monahan, William F. Hood, Robert P. Compton, Thomas H. Lanthorn, Diane H. Ragan, and Gerald B. Watson. Searle Research and Development, CNS Diseases Research Department, 4901 Searle Parkway, Skokie, IL 60077.

Our efforts to design and synthesize antagonists selective for the NMDA site on the EAA receptor complex has led us to explore the use of carboxy-imidazoles as the nucleus for NMDA antagonists. We have now developed phosphonate-carboxylate functionalized imidazopyridine based NMDA antagonists. This poster will describe the synthetic exploitation, the in-vitro pharmacology, and the neuro protective properties in this series. A significant compound in the area, SC-48255, bound selectively to the NMDA site with a Ki of 2 μM, was determined to be an antagonist by modulation of TCP binding, antagonized NMDA induced inward currents, and was found to be neuro protective in a gerbil ischemia assay.



SC-48255

163.3

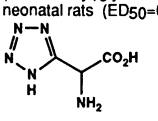
FPL 12495, A METABOLITE OF REMACEMIDE, IS A NONCOMPETITIVE NMDA ANTAGONIST. R. Ray*, R. Julien*, J. Gordon & J. Blosser. Fisons Pharmaceuticals, P.O. Box 1710, Roch., NY 14623

Remacemide (2-amino-N-[1-methyl-1,2-diphenylethyl] acetamide) has been identified as an anticonvulsant and antiischemic agent in various animal models of epilepsy and cerebral ischemia. The major metabolite of remacemide in rat is a desglycine analog (FPL 12495) which structurally resembles known noncompetitive NMDA receptor antagonists. In vitro biochemical receptor binding studies using the noncompetitive antagonist [3H]MK801 demonstrated that remacemide had low affinity for NMDA receptors while FPL 12495 had submicromolar affinity. The affinity of FPL 12495 was not affected by glycine, glutamate or by spermine suggesting that it interacted at the NMDA channel (MK801/PCP) site. FPL 12495 and remacemide demonstrated weak activity at other receptor sites to which non-competitive antagonists have been reported to interact. Thus, 10 to 200 times higher concentrations of FPL 12495 were required to antagonize nicotinic and sigma receptors and only weak inhibition (IC50=10uM) of norepinephrine and dopamine transporters was seen. Potent antagonism of the latter site by phencyclidine has been implicated in its psychotomimetic effects. Taken together, these results suggest that FPL 12495 has selective affinity for the non-competitive NMDA binding site. This activity may explain in part the antiischemic properties of remacemide.

163.4

IN VITRO AND IN VIVO PHARMACOLOGY OF D,L-(TETRAZOL-5 YL)GLYCINE: A HIGHLY POTENT AND STRUCTURALLY NOVEL NMDA RECEPTOR AGONIST. W.H. Lunn, D. Lodge*, J.D. Leander, A.I. Saccan, and D.D. Schoepp. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285 and Royal Veterinary College, Univ. of London, United Kingdom.

This work describes the pharmacological activity of D,L-(tetrazol-5-yl)glycine (LY285265), a structurally novel and highly potent agonist at the NMDA subtype of excitatory amino acid receptor. D,L-(tetrazol-5-yl)glycine potently displaced rat brain membrane [^{3}H]CGS19755 (10 nM) binding (IC_{50} = 98 ± 7 nM) and NMDA-specific ^{3}H-glutamate (10nM) binding (IC_{50} =36 ± 18 nM), but did not appreciably affect the binding of [^{3}H]AMPA , [^{3}H]kainate, or [^{3}H]glycine (IC_{50}s > 30,000 nM). D,L-(Tetrazol-5-yl)glycine is more potent than NMDA or *cis*-methanoglutamate as a depolarizing agent in the rat cortical slice, and unlike these other agents induced rapid receptor-mediated neurotoxicity. Depolarization by D,L-(tetrazol-5-yl)glycine can be antagonized by LY233053, a selective NMDA receptor antagonist. D,L-(tetrazol-5-yl)glycine is a very potent convulsant when administered to neonatal rats (ED_{50}=0.071 mg/kg i.p.). Convulsions in neonatal rats or lethality in mice induced by D,L-(tetrazol-5-yl)glycine were selectively antagonized by competitive and non-competitive NMDA receptor antagonists. D,L-(tetrazol-5-yl)glycine is a highly potent and selective NMDA receptor agonist, that could be used to further probe NMDA receptors *in vitro* and *in vivo*.

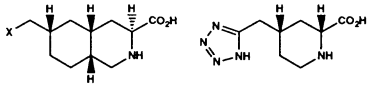


LY285265

163.5

CHARACTERIZATION OF (-)-LY235959, (-)-LY202157 AND (-)-LY235723 AS THE ACTIVE ISOMERS OF THE RACEMIC COMPETITIVE NMDA ANTAGONISTS LY274614, LY233536 AND LY233053, RESPECTIVELY. P.L. Ornstein, M.B. Arnold*, N.K. Augenstein*, J.D. Leander, D. Lodge, and D.D. Schoepp. Lilly Research Labs, Eli Lilly and Co., Indianapolis, IN 46285 and Royal Veterinary College, London, NW10TU, UK.

Competitive NMDA antagonists show promise as novel therapeutic agents for the treatment of acute and chronic neurodegenerative disorders such as cerebral ischemia, Huntington's Chorea, Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's disease. We recently described the characterization of LY274614, LY233536 and LY233053 as potent and selective competitive NMDA antagonists. We subsequently resolved these phosphonate and tetrazole substituted amino acids and found that the activity of the racemates resides with the (-)-isomer. (-)-LY235959, (-)-LY202157 and (-)-LY235723 selectively displace [^{3}H]CGS19755 binding with IC_{50}'s of 25 ± 11, 415 ± 122 and 67 ± 6 nM, respectively. At relatively high concentrations, these compounds did not appreciably inhibit [^{3}H]AMPA and [^{3}H]kainic acid receptor binding (IC_{50}'s >10,000 nM). These compounds also potently block NMDA-induced lethality in mice, with minimum effective doses of 0.625, 1.25 and 2.5 mg/kg (i.p.), respectively.



LY235959, X = PO_3H_2
LY202157, X = Tetrazole

LY235723

163.6

INHIBITION OF [^{3}H]-MK801 BINDING AND PROTECTION AGAINST NMDA-INDUCED LETHALITY WITH A SERIES OF IMIPRAMINE ANALOGS. L. A. McQuaid, J. D. Leander, L. G. Mendelsohn, N. R. Mason, R. R. Lawson* and E. C. R. Smith. Central Nervous System Research, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

We have evaluated a series of imipramine analogs to further explore the structure-activity relationships involved in their inhibition of [^{3}H]-MK801 binding and their ability to prevent N-methyl-D-apartate (NMDA) induced lethality in mice. The [^{3}H]-MK801 binding assay was carried out using well washed rat forebrain membranes in the presence of glycine (1μM). The SAR for inhibition of [^{3}H]-MK801 binding indicated that primary amines on short tethers were optimum. The primary amine on a two carbon tether was found to be the most potent imipramine analog with respect to inhibition of [^{3}H]-MK801 binding with an IC_{50} of 0.36±0.03 μM. The ring nitrogen-carbon portion of imipramine could be replaced with either a carbon-sulfur linkage or a carbon-carbon double bond. The minimally effective dose (MED) was determined for each of the imipramine analogs as that concentration which protected against NMDA lethality (200 mg/kg, i.p.) in greater than 50% of the mice tested. For protection against NMDA lethality, compounds containing a carbon-carbon double bond linkage to a cyclic amine were the most efficacious tricyclics tested. Possible explanations for the lack of correlation between the two assays will be discussed.

163.7

INFLUENCE OF VOLATILE ANESTHETICS ON NMDA-STIMULATED ^{45}CA UPTAKE IN RAT BRAIN. D.C. Martin, R.L. Dennsion, M.K. Baumgartner and R. S. Aronstam, Departments of Anesthesiology and Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA.

Volatile anesthetics inhibit glutamate-stimulated [^{3}H]MK-801 binding to the ionophore of NMDA receptor complexes in rat brain (*FASEB J.* 5:A408, 1991). In the present study, we examined the influence of several anesthetics on NMDA-stimulated ^{45}Ca uptake in rat brain. Calcium flux measurements were performed using a microvesicle preparation containing synaptosomes and membrane fragments which reseal and maintain electrical and ionic gradients. To measure ^{45}Ca influx, 50 μl of microvesicles (≈100 μg protein) was added to a tube containing 800 μl of a physiological salt solution and 50 μl ^{45}Ca (≈50 nM, 90-100,000 cpm) at 37°C. Uptake was initiated by addition of NMDA. After an appropriate incubation (typically 30 sec), uptake was terminated by filtration through glass fiber filters, and the radioactivity content of the filters was determined by liquid scintillation counting. Nonspecific uptake was measured in the absence of NMDA.

NMDA stimulated ^{45}Ca uptake (30 sec) by rat brain microvesicles by 48 ± 9% (mean ± S.D.; N = 6) with an EC50 of 1.4 ± 0.5 μM. The NMDA-stimulated ^{45}Ca uptake was inhibited by MK-801 with an IC50 of ≈ 10 μM. Uptake stimulated by 100 μM NMDA was inhibited 60-85% by enflurane, isoflurane, and halothane with IC50s of 0.1-2.1 mM. Thus, significant inhibition of NMDA receptor-mediated Ca^{2+} flux was produced by anesthetics at concentrations achieved during routine clinical use. Preincubation of brain microvesicles with NMDA resulted in a rapid desensitization of NMDA-stimulated 45Ca uptake, with a $t_{1/2}$ of less than 1 min. Each of the anesthetics diminished both the extent and rate of development of this desensitization. These findings further support the notion that anesthetics interfere with neurotransmission at NMDA receptor complexes. (Supported by USPHS grant AA-07698).

163.8

ANTICONVULSANT EFFECTS OF MEMANTINE AND MK-801 IN GUINEA PIG HIPPOCAMPAL NEURONS. J.P. Apland and F.J. Cann*. Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

Memantine (1-amino-3,5-dimethyladamantane) is used in treatment of Parkinsonism and movement disorders. Recent work has demonstrated that memantine (Mem) displaces MK-801 from its binding sites (Kornhuber et al., 1989), blocks NMDA receptor channels as potently as MK-801 (Bormann, 1989), and possesses neuroprotective properties (el Nasr et al., 1990). We compared the anticonvulsant properties of Mem to MK-801 and to centrally acting antitussives.

Extracellular recordings were obtained from area CA1 of guinea pig hippocampal slices in a total submersion chamber at 32° C in normal oxygenated artificial cerebrospinal fluid (ACSF). Drugs were applied by bath perfusion. Evoked responses were elicited by stimulation of the Schaffer collaterals. Pretreatment of slices for 16-20 min with 100 μM Mem blocked epileptiform afterdischarges following evoked responses in both Mg^{2+}-free ACSF and NMDA-containing ACSF. At this concentration Mem alone had no discernible effect on evoked responses when perfused for up to 50 min. Epileptiform afterdischarges in response to reapplication of NMDA did not fully recover after over 2 hr of washing with normal ACSF. MK-801 was effective at 10 μM and required application for over 1 hr for full suppression of afterdischarges. Mem also reversed epileptiform activity when applied after such activity was induced by NMDA or Mg^{2+}-free ACSF, but was more effective against afterdischarges induced by NMDA.

163.9

INHIBITION OF HIPPOCAMPAL PATHWAYS BY A CYCLOBUTYLENE ANALOGUE OF 2-AMINO-4-PHOSPHONOBUTANOIC ACID (AP4). J.F. Koerner, N.L. Peterson, H.B. Kroona, R.L. Johnson. Dept. of Biochem., Dept. of Med. Chem. and Neurosci. Grad. Program, Univ. of Minnesota, Minneapolis, MN 55455.

Previously, *E*- and *Z*-2,3-methano-4-phosphonobutanoic acid, two cyclopropyl analogues of 2-amino-4-phosphonobutanoic acid (AP4), were synthesized and tested for bioactivity at L-AP4 receptors. Modelling studies indicate that the biologically active conformations of these analogues, and therefore L-AP4, are in an extended form. In an attempt to further define this bioactive conformation, we synthesized the extended, rigid AP4 analogue 1-amino-3-(phosphonomethylene)-cyclobutane-1-carboxylic acid. This cyclobutylene compound was tested for its ability to inhibit evoked responses in the lateral perforant path (LPP) and medial perforant path (MPP) of the hippocampal slice with extracellular techniques. Concentration-response data were collected and IC_{50}'s determined. The cyclobutylene analogue showed pathway specificity giving an IC_{50} of 41μM in the LPP and 215 μM in the MPP. Paired pulse potentiation (PPP) studies in the LPP suggest that the analogue may have both pre- and postsynaptic activity (the PPP was increased 1.26 times the normal compared to L-AP4 which was 1.43 times the normal). This mixed response could be due to the analogue acting at postsynaptic glutamate receptors in the LPP in addition to the presynaptic L-AP4 receptors. However, much of the inhibition can be attributed to a presynaptic mechanism. Therefore, we conclude that this analogue is acting at the presynaptic L-AP4 receptor. Moreover, its relative potency in the LPP compared to other synthetic L-AP4 analogues is further evidence that L-AP4 assumes an extended conformation at the L-AP4 receptor of the LPP. [During our study, Allan, R. D. *et al* described a synthesis and tested the cyclobutylene analogue at NMDA receptors (*J. Med. Chem.* **1990**. *33*, 2905-2915).] Supported by NIH NS17944.

163.10

CHARACTERIZATION OF RETINAL AND HIPPOCAMPAL L-AP4 RECEPTORS USING CONFORMATIONALLY CONSTRAINED AP4 ANALOGUES. N.L. Peterson, W.B. Thoreson, R.L. Johnson, J.F. Koerner, R. F. Miller. Dept. of Biochem., Dept. of Physiol., Dept. of Med. Chem. and Neurosci. Grad. Program, Univ. of Minnesota, Minneapolis, MN 55455.

Several conformationally constrained AP4 analogues (*E*- and *Z*-1-amino-3-phosphonocyclopentanecarboxylic acid, *E*- and *Z*-1-amino-3-phosphono-cyclohexanecarboxylic acid, and *E*- and *Z*-2-amino-2,3-methano-4-phosphonobutanoic acid) made it possible to study and compare four different systems which are sensitive to L-AP4: two retinal (mudpuppy, rabbit) and two hippocampal slice (rat lateral perforant path; guinea pig mossy fiber-CA3) preparations. In addition, the pharmacology of three KAIN/AMPA pathways and one NMDA pathway in the hippocampus was examined. All experiments were done using extracellular recording techniques. Concentration-response data were collected and IC_{50}'s determined (noted in parentheses). The rank order potency of the L-AP4 sensitive systems was similar, though not identical. In these systems, the cyclopropyl analogues and L-AP4 were the most potent (1-35 μM), followed by the cyclopentyl analogues (130-960 μM), with the cyclohexyl analogues being the least potent (1735-5800 μM). The non-L-AP4 systems did not follow this general rank order potency. The KAIN/AMPA systems differed most notably in the potency of L-AP4 and the E-cylopentyl analogue where E-cyclopentyl AP4 was more potent than L-AP4 in two of the three systems and nearly equipotent in the third. The NMDA system pharmacology differed from both the L-AP4 systems and the KAIN/AMPA systems. The constrained analogues may be able to probe subtle differences in tertiary structure of the four L-AP4 sensitive receptor proteins. Therefore, although the rank order potencies are not identical, the similarities are consistent with the possibility that the L-AP4 receptors may comprise a family of receptor proteins. Supported by NIH NS17944, EY03014, and EY06213.

163.11

JSTX(JORO SPIDER TOXIN) SELECTIVELY BLOCKS QUISQUALATE(QUIS)-INDUCED SEIZURE IN RATS:BEHAVIORAL AND ELECTROPHYSIOLOGICAL ASSESSMENTS.
H.Kanai*,N.Ishida*,A.Masui,M.Sadamatsu*,T.Ueno*, T.Nakajima* and N.Kato. Dept. of Psychiatry, Shiga Univ. Med. Science, Otsu 520-21, Japan.

JSTX inhibits QUIS-evoked responses in the rat hippocampus in vitro. QUIS/AMPA receptors are rich in the hippocampus and the seizure by icv QUIS administration is similar to the temporal lobe epilepsy in humans. Anticonvulsant effects of JSTX were evaluated in freely-moving rats against QUIS/AMPA-induced seizures and compared with the effects of CPP, a NMDA antagonist,on the seizures induced by quinolinate(QUIN), a NMDA agonist. Rats were implanted with the electrode into dorsal hippocampus and the icv cannula. QUIS (30μg) elicited spiking EEG with mean latency time(LT) 7.7 min and evoked generalized seizures (GS) with 16 min LT. Mortality rate was 67%. JSTX (20μg) prolonged LT to spiking discharge to 102 min and completely blocked GS in 80% rats. None of the rats pretreated with JSTX died. JSTX failed to antagonize QUIN, while CPP blocked QUIN-induced seizures as expected. Though preliminary, JSTX seemed to suppress AMPA-seizures. CNQX appeared to be effective as well, but to act differently from JSTX.

163.12

GLYCINE ANTAGONIST, (+)HA-966, BLOCKS STRESS-INDUCED RISE IN DOPAMINE METABOLISM IN THE RAT MEDIAL PREFRONTAL CORTEX.
B.A. Morrow*, W.A. Clark, and R.H. Roth, Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Restraint stress selectively activates the dopamine (DA) neurons in the prefrontal cortex (PFC) and nucleus accumbens septi (NAS), compared to the striatum (CP), as assessed by postmortem tissue measurements of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) (Roth et al. *Ann. N.Y. Acad. Sci.* 537:138-147, 1988). Kalivas et al. proposed the A10 DA neurons terminating in the PFC have N-methyl-D-aspartate (NMDA) receptors while those terminating in the NAS are regulated by a different glutamate receptor (*J. Pharmacol. Exp. Ther.* 251:378-387,1989). The current study examines the involvement of glycine's allosteric agonist site on the NMDA receptor in the response of midbrain DA neurons to restraint stress. Rats, treated i.p. with 15 mg/kg (+)HA-966 (+1-hydroxy-3-amino-pyrrolidinone-2), a glycine antagonist, or saline, were restrained in wire cages for 30 min, then decapitated and the brains removed and dissected. Brain samples were homogenized, purified over alumina columns and assayed for DOPAC and DA using high performance liquid chromatography with electrochemical detection. (+)HA-966 completely blocked the stress-induced rise in DA metabolism in the PFC, but not the NAS. These data suggest that NMDA receptors are involved in the stress induced metabolic activation of DA neurons in the PFC and not the NAS.

DOPAC/DA Ratios, in percent control ± SEM; n=8 or 9

	SALINE		(+)HA-966	
	Control	Stress	Control	Stress
PFC	100 ± 11	143 ± 13*	102 ± 11	100 ± 7
NAS	100 ± 4	115 ± 3*	102 ± 3	116 ± 4*
CP	100 ± 7	99 ± 5	100 ± 3	104 ± 3

*=p<0.05 vs control

Supported in part by MH-14092 & MH-14276.

163.13

INHIBITION OF THE GLUTAMATE TRANSPORTER IN ASTROCYTES BY L-trans-2,4-PYRROLIDINE DICARBOXYLATE.
R.J. Bridges, S.N. Shim* and A.R. Chamberlin, Depts of Neurol. and Chem., Univ. of CA., Irvine, CA 92717.

Transport of L-glutamate into astrocytes and neurons is thought to be a key step in the termination of the excitatory signal and the prevention of excitotoxic damage. In previous studies, we synthesized L-trans-2,4-pyrrolidine dicarboxylate (L-trans-2,4-PDC) and identified it as a potent competitive inhibitor of the Na-dependent synaptosomal glutamate uptake system. In the present study we examined the ability of L-trans-2,4-PDC to inhibit this transport system in cultures of rat astrocytes. Type I astrocytes were prepared from the cortices of 4 day old rats. Oligodendrocytes and type II astrocytes were removed by shaking and the purified astrocytes were replated into 6 and 12 well plates, where they were allowed to reach confluency (14-21 days) before being used in the uptake studies. The activity of the Na-dependent transport system was assayed by quantitating the uptake of ^{3}H-D-ASP into the astrocytes for 3 min at 30°C. L-trans-2,4-PDC was found to inhibit the uptake ^{3}H-D-ASP in a competitive manner with a Ki value comparable to that of the Km of D-asparate (~ 50 μM). The findings demonstrate that L-trans-2,4-PDC is a potent inhibitor of both the synaptosomal and astrocytic uptake systems. Considering that the inhibitor does not appear to potently bind to any of the EAA receptors, the results suggest that L-trans-2,4-PDC will be useful in examing the role of glutamate uptake in complex preparations (e.g., co-cultures, tissue slices, *in vivo*) that contain both neuronal and glial components.

163.14

AMITRIPTYLINE PROTECTS AGAINST NMDA-INDUCED TOXICITY AND AUGMENTS KAINATE AND QUISQUALATE-INDUCED RELEASE OF GLUTAMATE IN NEURONAL CULTURES
P.P. McCaslin and X. Z. Yu*, Dept. Pharmacol. & Toxicol., UMC, Jackson, MS, 39216.

It has recently been reported that tricyclic antidepressants inhibit the binding of MK-801 to the N-methyl-D-aspartate (NMDA) receptor complex, cause a voltage-dependent equilibrium block of the NMDA receptor responses, and prevent NMDA-induced convulsions in vivo. In this report, the effect of amitriptyline on the excitatory amino acid (EAA)-induced toxicity and the EAA-induced release of several amino acids were determined in cerebellar granule neurons. Amitriptyline (25 uM) prevented the rapid toxicity induced by 100 uM NMDA in high calcium concentrations (10mM) but had no protective effect against the kainate-induced toxicity in these neurons even when amitriptyline was administered in much higher dosages. In fact, in higher dosages amitriptyline, alone, was toxic to the neurons. Quisqualate, kainate, or NMDA all produced an increase in the release of several amino acids (especially glutamate and taurine) when these EAA were incubated with neurons for one hour. Amitriptyline augmented the kainate- and quisqualate-induced release of glutamate and taurine but had no significant effect on the NMDA-induced release of these amino acids. In conclusion, these findings confirm the reports of others that tricyclic antidepressants have effects on the NMDA receptor and extend that work by showing that amitriptyline also has a selective interaction with kainate/quisqualate in augmenting the EAA-induced release of glutamate and taurine from cerebellar granule neurons. Supported by NIDA grant DA 64841.

163.15

THE KAPPA OPIATE AGONIST CI-977 DOES NOT BLOCK GLUTAMATE-OR HYPOXIA-INDUCED LDH RELEASE AND $^{45}CA^{++}$ UPTAKE IN CULTURED CORTICAL NEURONS.
G. Campbell, A. Probert, D. Rock, and F.W. Marcoux, Dept. of Pharmacology, Parke Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.

CI-977 is an agonist at opiate receptors with selectivity for the kappa subtype. It has been shown to be analgesic in animal models as well as neuroprotective in animal models of focal ischemia. The following studies were done to evaluate the effect of CI-977 on glutamate-and hypoxia-induced neuronal cell death or $^{45}Ca^{++}$ flux in cultured cortical neurons to determine if the neuroprotective effects of CI-977 might be due to an interaction with the NMDA receptor ion-channel complex.

CI-977 (10-100nM) caused a slight (10%) reduction in glutamate-induced neuronal death (LDH release). These effects were neither concentration-dependent nor reversed by the addition of 1 μM naloxone. CI-977 was also evaluated as an inhibitor of combined hypoxia/hypoglycemia-induced increases in intracellular Ca^{++} ($^{45}[Ca^{++}]$) and neuronal neuroprotective effects. At higher concentrations, there was a naloxone-insensitive decrease in the response. The competitive NMDA antagonist CPP was completely effective in both assays blocking LDH release and $^{45}Ca^{++}$ influx.

These studies indicate that CI-977 blocks neither glutamate-nor hypoxia-induced neuronal cell death in cultured neurons and suggests that the neuroprotective effects of CI-977 seen in animal models may not be accounted for by a direct interaction with the NMDA receptor complex.

163.16

ON CENTRAL MUSCLE RELAXANTS, GLYCINE RECEPTORS AND TWO OLD DRUGS: ZOXAZOLAMINE AND HA-966.
B.A. McMillen, H. L. Williams*, H.L. Romeyn* and P.D. Shepard, Dept. of Pharmacol., Sch. of Med., E. Carolina Univ., Greenville, NC 27858; Maryland Psych. Res. Ctr., Baltimore, MD 21228.

Zoxazolamine (Zox) is in the centrally acting muscle relaxant class of drugs, which reportedly act by decreasing CNS interneuronal activity. These drugs, but not anxiolytics, decreased dopaminergic (DA) turnover and induced a 'pacemaker' discharge pattern in DA neurons. A mechanism for these effects was not found. We observed that (+)-HA-966, an inhibitor of the glycine modulatory site on the NMDA receptor, has a similar effect on DA impulse flow, but (-)-HA-966 completely inhibits DA impulse flow. Binding of 20 nM [3H]-glycine to cortical synaptosomal membranes was inhibited by (+)-HA-966, IC50 = 3.16 uM, but only poorly by ZOX, IC50 = 474 uM, and chlorzoxazone, a related drug, caused no displacement. Neither 50 mg/kg Zox nor 30 mg/kg (+)-HA-966 prevented amphetamine (0.1 mM/kg plus 10 mg/kg iprindole) depletion of striatal DA, but 3.0 mg/kg of MK-801, a non-competitive NMDA receptor antagonist, did protect DA content. Since baclofen induces a regular firing rate in DA neurons, Zox and (+)-HA-966 were tested for displacement of 10 nM [3H]-l-baclofen from cortical synaptosomal GABA-b receptors, but were ineffective. Thus, the effects of these muscle relaxants on DA neurons are mediated by a mechanism other than glycine or GABA-b receptors.

163.17

DIPYRONE ACTIVATES N-METHYL-D-ASPARTATE (NMDA) RECEPTORS ON HIPPOCAMPAL NEURONS. A.M.N. Costa*, Y. Aracava, E.S. Rocha and E.X. Albuquerque. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ, Brazil, 21941; Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD, 21201.

It has been shown that NMDA receptors may be implicated in the transmission of nociceptive inputs in mammalian central nervous system (*TIPS*, 11:307, 1990). Recently, we have demonstrated that pyrazole is able to activate NMDA receptors on hippocampal neurons (*Neurosci. Abst.*, 16:86, 1990). In light of these findings, we decided to evaluate the actions of dipyrone, a pyrazolon derivative with analgesic properties, on NMDA receptor activation. Single channel currents were recorded from outside-out patches excised from rat hippocampal neurons kept in cultures for 5-15 days. When the outside-out configuration was achieved, the patch was placed inside a glass mini-pipe connected to a perfusion system. Nominally-Mg^{2+} free external solution containing the drugs to be tested was delivered via this system. Dipyrone (1-50 μM) activated single channel currents similar to those activated by NMDA. These currents were blocked by APV, a competitive antagonist of NMDA receptor. Single channel conductance was about 50 pS, a value similar to that reported for NMDA- and pyrazole-activated currents. Open-channel currents fluctuated between on and off states giving rise to a burst-like activity. Hyperpolarization increased the number of openings per burst and decreased the intraburst open times. No sign of channel blockade was observed with dipyrone up to 50 μM. The activation of NMDA receptors by dipyrone suggests that these receptors could be involved in the analgesic action of dipyrone. Support: U.S. Army Med. Res. & Devel. Comm. Contr. DAMD-17-88-C-8119, FINEP/UMAB Mol. Pharmacol. Training Program, CNPq & Capes.

163.18

THE ANTIHYPERTENSIVE DRUGS NITROPRUSSIDE AND NITROGLYCERIN INHIBIT EXCITATORY AMINO ACID (EAA) RESPONSES. Peter K. Kaiser, Sizheng Z. Lei, Sanjay Aggarwal and Stuart A. Lipton. Dept. of Neurology, Children's Hosp & Progr in Neurosci, Harvard Med Sch, Boston, MA

NMDA receptor-mediated increases in $[Ca^{2+}]_i$ were monitored with digital imaging techniques using the dye fura-2 in primary cultures of rat neonatal cortical neurons. Bath application of nitroglycerin (NTG; 10-100 μM) or nitroprusside (NP; 1-100 μM) inhibited NMDA (50 μM)-evoked responses. Since the active species of each of these drugs is nitric oxide (NO), we suspected that NO might, at least in part, down-regulate NMDA responses by oxidation of the redox modulatory site of the NMDA receptor-channel complex (Aizenman, Lipton & Loring, *Neuron* 1989;2:1257). In fact, consistent with this notion, the reducing agent dithiothreitol (DTT; 2 mM) reversed the effects by ~90%. However, in some cultures part of the inhibitory effect reversed spontaneously with several min of continued washout. Such reversibility would not be expected of a sulfhydryl oxidizing reagent until introduction of a chemical reductant. Further, NP (but not NTG) inhibited $[Ca^{2+}]_i$ responses to K^+ (50 mM) or kainate (50 μM) in the presence of 1 μM TTX; after pre-treatment with the strong oxidant DTNB (500 μM), NTG or NP produced an additional degree of blockade of the NMDA response. These effects would not have been expected of an agent acting solely at the redox modulatory site. Thus, NTG/NP may possibly act at the redox modulatory site but other effects are also implicated and will require further study. Meanwhile, these clinically-tested agents are being evaluated for the treatment of EAA receptor-mediated neurotoxicity.

163.19

PREFERENTIAL INHIBITORY EFFECTS OF ARYLAMINE SPIDER TOXINS ON NMDA RECEPTOR-MEDIATED INCREASES IN CYTOSOLIC CALCIUM. L.D. Artman*, T.N. Parks, H. Jackson and E.F. Nemeth. Natural Product Sciences, Inc., 420 Chipeta Way, Salt Lake City, UT 84108.

Low molecular weight arylamine spider toxins such as Joro spider toxin (JSTX) and argiotoxin-636 (Arg636) have been reported to be effective glutamate antagonists in a variety of invertebrate and vertebrate preparations. The ability of several synthetic arylamines to block NMDA- and non-NMDA-receptor-mediated increases in intracellular calcium ($[Ca^{2+}]_i$) was examined in cultures of neonatal rat cerebellar granule cells (RCGCs) loaded with fura-2. Rapid and sustained increases in $[Ca^{2+}]_i$ were elicited by NMDA (50 μM) plus glycine (1 μM) in 6-8 day old cultures, or by AMPA (30 μM) or kainate (KA, 100 μM) in 5 day old cultures. These increases in $[Ca^{2+}]_i$ were abolished in the absence of extracellular Ca^{2+}. Responses to NMDA/gly (100-300 nM increase over basal $[Ca^{2+}]_i$) were completely blocked by the addition of 1 mM Mg^{2+}. Responses to KA (100-300 nM increase over basal $[Ca^{2+}]_i$) or AMPA (20-30 nM increase over basal $[Ca^{2+}]_i$) were obtained in the presence of 1 mM Mg^{2+} and 1 μM nifedipine. CNQX (2 μM) produced a 50% blockade of KA- or AMPA-induced increases in $[Ca^{2+}]_i$. The synthetic arylamine toxins AG489 and AG505 (α-agatoxins from *Agelenopsis aperta*) blocked increases in $[Ca^{2+}]_i$ evoked by NMDA/glycine, AMPA or KA with widely varying potencies. IC_{50} values for AG489 were 245 nM, 38 μM, and 201 μM, respectively. IC_{50} values for AG505 were 229 nM, 14 μM, and 20 μM, respectively. Other arylamine toxins such as Arg636 and JSTX3 showed a 10-100 fold selectivity for NMDA- vs. non-NMDA-receptor-mediated increases in $[Ca^{2+}]_i$. These data show that arylamine spider toxins are preferential antagonists of NMDA receptor-mediated responses in RCGCs and perhaps thoughout the mammalian CNS.

163.20

POTENTIATION OF DEPRESSED NMDA-MEDIATED TRANSMISSION BY THE PARTIAL GLYCINE AGONIST, D-CYCLOSERINE. P.L. Wood, M. Li* and R. Ryan*. Biological Research, Hoechst-Roussel Pharmaceuticals, Somerville, NJ 08876.

Partial agonists of the NMDA-associated glycine receptor are potential clinical candidates for improving cognitive function. To assess this potential further, we examined the actions of the partial glycine agonist, D-cycloserine on pharmacologically depressed NMDA-mediated neurotransmission in the mouse cerebellum, as assessed by changes in the second messenger, cGMP.

Using this paradigm, D-cycloserine was found to reverse the depressant actions of the competitive NMDA antagonist, CPP. In contrast, the depressant actions of the non-competitive NMDA antagonists, MK-801, PCP and magnesium, were not altered by pretreatment with D-cycloserine.

These data therefore support the potential clinical utility of partial glycine agonists in augmenting NMDA-mediated transmission except under conditions where a non-competitive blockade of the receptor complex is in effect.

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION I

164.1

NON-RADIOACTIVE SOLUTION HYBRIDIZATION OF BRAIN mRNA. E. Robbins, M.E. Lewis, E.M. Reilly*, and F. Baldino, Jr., Cephalon, Inc., 145 Brandywine Pkwy., W. Chester, PA 19380.

We have developed a method for the rapid non-radioactive detection of hybridization of specific cRNA probes to their target mRNA using hapten-conjugated probes. In order to achieve maximum sensitivity, this method employs solution rather than solid phase hybridization. A digoxigenin-labeled cRNA probe complementary to preproenkephalin mRNA and the sense complement of this probe were hybridized to striatal RNA in our solution hybridization assay.

Total striatal RNA (1-2 ug) was aliquoted into a sterile, RNase-free tube to which 2x TES hybridization buffer containing probe was added. After a 4-hour incubation at 75°C, RNase buffer was added to each sample to digest unhybridized probe. Hybrids were then collected on a Whatman GF/C glass fiber filter. After blocking filters in blotto, detection of probe hybridized to striatal mRNA was carried out enzymatically following complex formation with anti-digoxigenin alkaline phosphatase (AP) conjugated antibody at a concentration of 1:1000 in 1x TBS. Unbound antibody was removed by successive washes in TBS, and AP substrate containing 1 mg/ml *p*-nitrophenyl phosphate in diethanolamine buffer was added. Color development was allowed to proceed in the dark until staining could be detected by eye in background samples (10-15 mins). Color intensity was determined by spectophotometric analysis of optical density at 405-420 nm. While nearly undetectable (background) amounts of sense RNA probe hybridized to cellular mRNA, substantial specific hybridization was observed using the preproenkephalin-specific cRNA probe.

This new method should provide a valuable tool for the study of other RNA species, such as those related to the study of altered gene expression in development, pathology or due to physiological or pharmacological manipulations.

164.2

LOCALIZATION OF ENKEPHALIN-SYNTHESIZING CELLS IN RAT, GUINEA PIG AND CHINCHILLA BRAINSTEM BY *IN SITU* HYBRIDIZATION. DW Hoffman, PD Gardner and WM Waldron. Departments of Psychiatry, Pharmacology and Biochemistry, Dartmouth Medical School, Hanover, NH 03756.

In situ hybridization has been used to map the distribution of mRNAs encoding enkephalin precursor in superior olivary and periolivary cells of the rat, guinea pig and chinchilla auditory brainstem which innervate auditory nerve dendrites at cochlear inner hair cells. Sense and antisense cRNA probes (935 bases) corresponding to the entire coding region of the preproenkephalin gene (gift of Dr. Steven Sabol) are generated using the SP6 RNA polymerase promoter. Brainstem sections are exposed to Hyperfilm after hybridization, and the density of 35-S-labelled probe binding in the autoradiograms is digitized and quantified using an image analysis system and grain counting program. More recently, probes synthesized with digoxigenin-UTP have been used, with anti-digoxigenin-alkaline phosphatase visualization of localization. Hybridization is seen in the rat in dorsal tegmental nucleus, dorsal parabrachial area, raphe nucleus and lateral superior olivary region, especially in the lateral nucleus of the trapezoid body . Enkephalin cell bodies are seen in the periolivary region in the rat, in comparison to their localization within the lateral superior olive in the guinea pig, as has been reported using immunochemical techniques. The distribution of enkephalin-synthesizing cell bodies in the auditory brainstem of the chinchilla more closely resembles that of the guinea pig than the rat. [Supported by the Henry Heyl Fund of the Hitchcock Foundation].

164.3

EFFECT OF PGE_2 ON THE SECRETION OF MET^5-ENKEPHALIN (ME) AND THE EXPRESSION OF PROENKEPHALIN A (PROENK) GENE IN BOVINE ADRENAL MEDULLARY (BAM) CELLS. H.H.Suh, K. Pennypacker, X-P. He*, M. McMillian, R. Fannin*, E-C. Mar*, P. Hudson, and J-S. Hong, LMIN/NIEHS/NIH. RESEARCH TRIANGLE PARK, NC. 27709.

We previously reported that long-term (24 hrs) stimulation of BAM cells with PGE_2 increased secretion of ME as well as proENK gene expression. The primary goal of the present study was to further characterize the effect of PGE_2 on regulation of proENK gene expression in BAM cells. The onset of secretion of ME and expression of proENK gene induced by PGE_2 began 3 and 6 hrs, respectively, after the treatment. Cycloheximide, a protein synthesis inhibitor inhibited both the secretion of ME as well as the expression of proENK gene induced by PGE_2. The increases in secretion of ME and expression of proENK gene induced by PGE_2 were effectively inhibited by nitrendipine and nimodipine, selective L-type calcium channel blockers but not by ω-conotoxin, a N-type calcium channel blocker. In addition, calmidazolium, a calmodulin antagonist, effectively inhibited both secretion of ME and expression of proENK gene induced by PGE_2. FURA-2 studies showed that PGE_2 produced a prolonged increase intracellular free Ca^{2+} level. In the gel shift DNA binding assays PGE_2 increased the AP-1 binding activity up to 3 hrs. Our results indicate that increases in secretion of ME and proENK gene expression induced by long-term PGE_2 in BAM cells may require the de novo synthesis of proteins. PGE_2-induced increase of Ca_i^{2+} through L-type calcium channels may lead to increased formation of Ca^{2+}/calmodulin complex which may increase AP-1 binding activity mediating the increasing in proENK gene expression. Utilizing a nuclear run-on assay, we are currently examining the effect of PGE_2 on the rate of transcription of the proENK gene.

164.4

DEVELOPMENTAL DECREASE IN PROENKEPHALIN:Met-ENKEPHALIN RATIO IN THE RAT ADRENAL. **P.M.Hudson, M.K.McMillian, D.Y.Lee, L.Thai and J.S.Hong LMIN/NIEHS/NIH RTP NC 27709**

Enkephalins are co-localized with catecholamines in adrenal chromaffin cells. Although enkephalin levels are generally co-regulated with catecholamine levels, denervation results in a pronounced and increase in preproenkephalin (PPE) mRNA and opiate peptide levels in rat adrenals, while catecholamine-synthesizing enzymes do not increase. The predominant opiate peptide which accumulates after denervation is proenkephalin. We have examined developmental changes in the levels of immunoreactive Met-enkephalin (ME) and proenkephalin (ME-RF). The rat adrenal becomes functionally innervated postnatally, and we hypothesized a fall in enkephalin levels and ME-RF:ME ratio would occur due to developmental increases in splanchnic nerve activity. ME content per mg protein decreased 2-fold between days 10 and 21. There was a more striking 4-fold decrease in ME-RF during the same period, as the ME-RF:ME ratio fell from 2.0 to the adult level of 1.0. Repeated nicotine injections, mimicing increased splanchnic nerve activity, produced a precocious fall in the ME-RF:ME ratio by day 10. In situ hybridization studies indicate that PPE mRNA levels are higher in adult than in 10 day old rat adrenal, suggesting that the higher proenkephalin levels observed in the 10 day old are associated with deficient processing to ME rather than increased opiate peptide production.

164.5

PERFORANT PATH DEAFFERENTATION ALTERS HIPPOCAMPAL DYNORPHIN AND ENKEPHALIN CONTENT IN RATS. L. Thai, J. S. Hong, and M. Gallagher, NIEHS/NIH, RTP, NC, 27709, Curriculum in Neurobiology, Univ. of N. Carolina, Chapel Hill, NC, 27599.

The perforant path (PP) innervates dentate granule cells which contain dynorphin and enkephalin. Stimulation of this pathway has been shown to inhibit dynrophin peptide levels and mRNA expression in the hippocampus of young rats. In contrast, basal hippocampal dynorphin content and prodynorphin mRNA are increased in the aged rat brain (PNAS, 86, 2948, 1989). We examined whether loss of PP input to dynorphin-containing cells in the dentate gyrus of young rats would mimic the effects of aging.

Radioimmunoassays (RIA) showed that a bilateral knife cut of the PP at the level of the angular bundle reliably increased dynorphin (15%) and decreased Met-enkephalin (15%) in the whole hippocampus of young adult Long-Evans rats. In a second study, the dissected dentate gyrus was assayed for high-affinity glutamate uptake (HAGU) and the CA3 region of hippocampus was assayed for dynorphin immunoreactivity. Bilateral PP lesions significantly decreased HAGU and produced a parallel increase in CA3 dynorphin content. Immunocytochemistry also revealed enhanced dynorphin immunoreactivity in the mossy fibers one month after lesioning. The effect of PP lesions on dynorphin mRNA is currently under study using in-situ hybridization. We conclude that removal of the PP elevates dynorphin in the hippocampus and may model the effects of aging on the regulation of this peptide.

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164.6

CHARACTERIZATION OF cAMP-RESPONSE ELEMENT-BINDING PROTEINS FROM NG108-15 CELLS CHRONICALLY EXPOSED TO MORPHINE AND ETHANOL. N.Miki, T.Osugi, M.Ikemoto and H.Taniura. Dept. of Pharmacology, Osaka Univ. Med. Sch., Suita, Osaka 565, Japan.

The gel retardation assay with a single stranded oligo-DNA of cAMP-response element (CRE) was employed to examine the possibility of transcriptional regulation of cAMP-inducible genes by chronic morphine or ethanol treatment of NG108-15 cells. When the nuclear extracts from the cells treated with morphine or ethanol for several days were provided for the assay, the amounts of DNA-protein complex were decreased about 30-40% of the control and recovered by 1-2 days after morphine withdrawal. Naloxone prevented the reduction of the DNA-protein complex by morphine. One hour treatment did not change the amount of the DNA-protein complex. The nuclear factors that specifically bind to a single stranded CRE and change the binding ability to CRE by morphine or ethanol treatment are being partially purified and characterized. Morphine and ethanol may modulate the expression of cAMP-inducible genes through which tolerance and dependence may develop.

164.7

MASS SPECTROMETRIC MEASUREMENT OF B-ENDORPHIN AND METHIONINE ENKEPHALIN IN HUMAN PITUITARIES. D.M. Desiderio, J.L. Lovelace*, J.J. Kusmierz* and C.Dass*. University of Tennessee, Memphis, Tennessee.

Two opioid neuropeptides, b-endorphin (BE) and methionine enkephalin (ME) were quantified with fast atom bombardment mass spectrometry (FAB-MS) in individual human pituitaries (post-mortem) and in tumor pituitaries (post surgery) in a study to clarify the molecular processes that occur in tumor formation. FAB-MS in the multiple reaction monitoring (MRM) mode was used to link the precursor ion (the protonated molecular ion, MH^+) of the peptide with a fragment ion that was unique to each neuropeptide. ME was quantified as the intact pentapeptide, and BE_{1-31} was quantified via its tryptic fragment BE_{20-24} (NAIIK). Two corresponding stable isotope-incorporated peptides. $[^2H_5\text{-}^4Phe]$-ME and $[^2H_4\text{-}^{22}Ile]$-BE_{1-31}, human, respectively, were used as internal standards. The amount of each neuropeptide quantified in control post-mortem pituitaries (n=8) was 75.2±29.6 (s.e.m.) pmol ME mg^{-1} protein and 132.5±22.3 (s.e.m.) pmol BE mg^{-1} protein, and in the pituitary tumor samples (n=5), 25.0±7.6 pmol ME mg^{-1} protein and 36.0±14.8 pmol BE mg^{-1} protein. The difference in the BE content between the control and tumor pituitaries was significant ($p=0.004$), and reflected an aberrant metabolism of the POMC system in those human pituitary tumor tissues. The measurements from FAB-MS methods (MH^+ ion monitoring and MRM mode) were compared to measurements from radioreceptor assay and RIA by analyzing the amount of endogenous ME extracted from five human post-mortem pituitary samples. ME-like receptoractivity (ME-LR) was 116±27 pmol mg^{-1} protein (s.e.m.); ME-like immunoreactivity (ME-LI) was 18.3±9.5 pmol mg^{-1} protein: MH^+ data were 42.9±9.5 pmol ME mg^{-1} protein; and the MRM data were 47.7±12.7 pmol ME mg^{-1} protein.

164.8

PRO-OPIOMELANOCORTIN-DERIVED PEPTIDES AND FEEDING BEHAVIOR OF DUNGENESS CRABS. C.W. Wilkinson, E.A. Colasurdo*, S.A. Galt*, and D.D. Jorgensen*. GRECC, VA Med. Ctr., Tacoma, WA 98493; Biology Dept., Univ. of Puget Sound, Tacoma, WA 98416; and Dept. of Psychiatry and Behav. Sci., Univ. of Washington, Seattle, WA 98195.

Microinjections of peptides derived from pro-opiomelanocortin (POMC) affect feeding behavior in mammals, and opiate receptor agonists and antagonists alter food intake in several classes of invertebrates. However, it is not known whether a POMC-like precursor is prevalent in invertebrate nervous systems, whether POMC-derived peptides play physiological roles in the regulation of food intake, or whether any such actions are evolutionarily conserved. We used gel chromatography, high pressure liquid chromatography (HPLC), and radioimmunoassays to determine if peptides similar in size and immunoreactivity to mammalian POMC-derived peptides exist in the nervous system of the Dungeness crab (*Cancer magister*). We also determined whether concentrations and molecular forms of these peptides were altered by the feeding state of the crab. Immunoreactive forms of endorphins, α-MSH, and ACTH were found in extracts of the nervous system of the Dungeness crab. Some of these peptides co-eluted with mammalian forms, and some were apparently novel forms. The relative proportions of immunoreactive peptides in extracts from food-deprived and satiated crabs differed markedly. In general, ACTH- and MSH-like forms were decreased and endorphin-like forms were increased by food deprivation.

164.9

CHARACTERIZATION OF POMC-DERIVED PEPTIDES IN RAT HEART. V.R. Evans*[1], L.J. Forman[2] and W.R. Millington[1]. [1]Sch. of Basic Life Sciences, Univ. Missouri-Kansas City, Kansas City, MO 64108. [2]Univ. Medicine and Dentistry of NJ-SOM, Camden, NJ 08103.

Pro-opiomelanocortin (POMC) is post-translationally processed to several bioactive peptides, including ß-endorphin (ß-END), ß-lipotropin (ß-LPH), α-MSH and ACTH, not only in the brain and pituitary, but in a variety of peripheral tissues as well. Previous studies have localized ß-END as well as other opioid peptides, in rat heart, which is consistent with the known effects of opiates on cardiac function. In the present study, we identified the individual molecular forms of ß-END in rat heart extracts and further demonstrated that other POMC-derived peptides, ACTH and α-MSH, are also present. Gel filtration analysis revealed that ß-LPH and ACTH were almost entirely cleaved to ß-END and α-MSH; ratios of ß-END: ß-LPH and α-MSH:ACTH were 4.6:1 and 6.1:1, respectively. Further analysis of ß-END sized peptides by ion exchange HPLC revealed that ß-END-1-31 was the predominant form, constituting 49.6% of total immunoreactivity. N-acetylated (N-Ac) and/or C-terminally shortened forms, including N-Ac-ß-END-1-31 (15.1%), N-Ac-ß-END-1-27 (8.4%), N-Ac-ß-END-1-26 (4.7%), ß-END-1-27 (14.3%) and ß-END-1-26 (7.9%), were also present, albeit in smaller proportional amounts. These results confirm the presence of ß-END in rat heart and further show that other POMC-derived peptides are also expressed. ß-END undergoes extensive post-translational processing to non-opioid forms in the heart although opioid active ß-END-1-31 is the predominant form.

164.10

PROOPIOMELANOCORTIN (POMC) PROCESSING AND CELLULAR ORIGINS IN RAT SPINAL CORD HB Gutstein, DM Bronstein, and H Akil. Dept. of Anes. and MHRI, U. of Mich., Ann Arbor, MI

While enkephalin and dynorphin peptides have been well characterized in the spinal cord, the anatomic localization of ß-endorphin (ßE) and the processing of POMC to ßE and other non-opioid peptides in the cord has not been extensively investigated. This study characterizes POMC processing in the spinal cord and suggests the presence of an intrinsic POMC/endorphinergic neuronal system in the spinal cord.

Eight rats were sacrificed, and spinal cords sectioned and frozen on dry ice. ßE, N-acetyl ßE, ACTH, and α-MSH IR was determined by RIA. To look for intrinsic POMC cells, eight rats anesthetized with chloral hydrate underwent complete excision of the spinal cord at two upper thoracic segments. Three days after surgery, the animals were sacrificed, lesions were verified in situ, and the cords were immediately dissected and frozen on dry ice. ßE IR was determined above and below the level of the lesions by RIA. Control and sham-operated groups were also studied.

Our data show that the proportion of acetylated ßE appears to increase caudally, while the degree of processing appears greater in the cervical cord than in other areas. After spinal cord lesioning, we found persistence of about 1/3 of the total ßE IR below the level of the lesions. Molecular sieving of this ßE IR revealed high relative proportions of POMC and ß-LPH to ßE. The ßE peak consisted almost exclusively of the highly opioid active ßE 1-31 form. Preliminary studies also show persistence of a somewhat higher proportion of ACTH-IR (1/2-2/3 of control levels) below the level of the lesion. Our data suggest that the spinal cord has a different POMC processing pattern from that observed in other areas of the CNS. As one progresses caudally, the degree of processing appears to decrease while the proportion of acetylation appears to increase. This complex pattern may result from a combination of two sources of POMC in the cord - caudal projections of NTS POMC cells, and, as our data suggest, intrinsic POMC cells in the spinal cord. Additional studies will be necessary to further define this intrinsic system and its possible physiologic functions.

164.11

EFFECTS OF CHRONIC NALTREXONE TREATMENT ON β-ENDORPHIN PEPTIDE LEVELS AND POMC mRNA IN RAT CNS. D.M. Bronstein, N.C. Day, H.B. Gutstein, K.A. Trujillo and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

An understanding of how the endogenous opioid peptide β-endorphin (βE) is regulated is critical for determining its functional significance. For example, we recently found that chronic morphine pelleting caused brain region-specific and time-dependent changes in βE-ir peptides and in mRNA levels coding for its precursor protein pro-opiomelanocortin (POMC) (Bronstein et al., *Brain Res. 519*:102, 1990), suggesting a possible involvement of βE in the expression of opiate tolerance and/or dependence. In the present studies, we investigated the effects of chronic opiate antagonist treatment on different parameters of βE biosynthesis in discrete regions of the CNS. Following 8 days of pelleting with either 10 or 30 mg naltrexone pellets, concentrations of total βE-ir decreased by 20-50% in the arcuate nucleus, thalamus, hypothalamus, midbrain, and septum compared to placebo animals. At the same time, the ratio of full length βE (βE_{1-31}) to more processed βE-ir forms (e.g., βE_{1-27}, βE_{1-26}) increased in a dose-dependent manner. In caudal POMC systems (i.e., the NTS and spinal cord), βE-ir peptide levels did not change. POMC mRNA levels in the arcuate nucleus were elevated in naltrexone-treated animals compared to placebo controls. These data have been interpreted as evidence of up-regulation of the rostral POMC system-- peptide levels decrease as a result of increased release and mRNA levels increase due to a compensatory induction of POMC biosynthesis. The finding that naltrexone altered the relative amounts of opioid-active (i.e., βE1-31) and opioid-antagonistic (i.e., βE_{1-27}) peptides suggests another way in which POMC neurons can regulate the signal transmitted across a synapse. (Supported by NIDA DA02265.)

164.12

METHYLAZOXYMETHANOL (MAM) INHIBITS PRO-OPIO-MELANOCORTIN (POMC) TRANSCRIPTION AND SECRETION OF POMC-DERIVED PEPTIDES IN AtT-20 CELLS. R. G. Allen, J. Parker* and P. S. Spencer. Center for Research on Occupational and Environmental Toxicology, and Departments of Biochemistry and Molecular Biology, and Neurology, Oregon Health Sciences University, Portland, OR 97201.

MAM, the aglycone of cycasin, is a potent cytotoxin with neurotoxic potential. By alkylating DNA, RNA, and protein, MAM is potentially able to interfere with a variety of cell functions. Vulnerable sites were studied in pituitary-derived AtT-20 neuroendocrine cell lines treated with increasing concentrations of MAM-acetate (0-500nM) for 48 hr. Treated cells were harvested and POMC mRNA quantitated by Northern analysis followed by densitometry. In parallel experiments, secretion of ß-endorphin, a POMC-derived opioid peptide, was quantitated by radioimmunoassay. MAM (100-250 nM) inhibited basal POMC transcription (in a concentration-dependent manner) to 20% of control values. ß-Endorphin secretion was reduced to 50% in the same time period, at the same concentration of MAM. Preliminary studies indicate these effects are reversible. We conclude that MAM is a potent inhibitor of actively transcribed genes and also interferes with secretion of peptide hormones. Studies are in progress to understand the mechanisms of these effects. Other studies will determine whether MAM alters post-translational processing of POMC-derived peptides and interferes with regulation of POMC gene expression by glucocorticoids, neurotransmitters and G-protein coupled processes. [Supported by NS 19611]

164.13

ALTERED PITUITARY PRO-ACTH/ENDORPHIN EXPRESSION IN HYPERACTIVE AND HYPERTENSIVE RATS. K.M. Braas, E.D. Hendley, G.S. Wand*, and V. May. Departments of Anatomy and Neurobiology and Physiology and Biophysics, University of Vermont College of Medicine, Burlington, VT 05405, and Endocrine Division, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

The hypothalamic-pituitary-adrenal axis, including altered pro-ACTH/endorphin (PAE) expression, has been implicated as one of the processes initiating and sustaining the hypertensive process. PAE expression is altered in pituitary gland from spontaneously hypertensive rats (SHR) compared with normotensive Wistar Kyoto (WKY) animals. To determine whether altered PAE expression is associated with the hypertensive or hyperactive trait of the SHR, we examined the WKHT strain, which is hypertensive but not hyperactive, and the WKHA strain, which is hyperactive but normotensive. Neurointermediate pituitary lobe β-endorphin and αMSH levels were elevated 150 to 200% in the SHR and WKHA strains compared with WKY, but not in the WKHT strain, suggesting a specific association with hyperactivity (WKY αMSH ≈ 890 pmol/lobe; SHR and WKHA significantly different than WKY by ANOVA, Newman-Keuls, $p<0.05$). In contrast, anterior pituitary lobe β-endorphin and ACTH content was decreased 30-50% in the SHR and WKHT strains (WKY ACTH ≈ 320 pmol/lobe; $p<0.05$), suggesting an association with the hypertensive trait. Morphological studies will help define the cellular basis for these tissue-specific alterations in PAE expression associated with the hypertensive and hyperactivity traits. Supported by PHS F07RR05429, NS26390, and NSF9010044.

164.14

CREATION OF TRANSGENIC MICE TO STUDY REGULATION OF THE PRODYNORPHIN PROMOTER. S.L. Sanders*[1], C.L. Christensen*[1], J. Douglass*[2], D.P. Wolf*[1], and M.H. Melner[1]. Div. of Neuroscience and Reproductive Biology[1], Oregon Regional Primate Research Center, Beaverton, OR 97006, and Vollum Institute[2], Portland, OR 97201.

Dynorphin has important regulatory effects in the CNS and reproductive system; however, little is known about the mechanisms which regulate its expression. The goals of this study were to create transgenic mouse lines expressing the reporter gene chloramphenicol acetyltransferase (CAT) under control of the rat prodynorphin (DYN) promoter, and to examine regulation of this promoter *in vivo*. Pronuclear stage embryos of B6D2 mice were injected with a DYN-CAT gene construct containing 1.7 kb of 5' flanking sequence and 151 bp of exon 1 (-1700 to +151). Of the resulting pups (100), 31% were identified as founder lines by incorporation of the DYN-CAT gene as analyzed by PCR. Initial screening of 4 of these lines for expression of the CAT enzyme indicated one positive line. Thin-layer chromatographic analysis of a CAT assay performed on tissue extracts of this DYN-CAT founder mouse showed high levels of CAT activity in the placenta, and to a lesser extent, in the uterus and cerebral cortex. CAT activity was undetectable in other tissues, although this may be due to the expression level being below the sensitivity of the assay. An unexpected finding in this analysis was the relatively high expression in the placenta and the uterus. Northern blot analysis of normal mice and rats for prodynorphin mRNA did not indicate high levels of expression in these tissues. This may indicate aberrant expression in this line or integration near a placental-specific element. However, this line could have appropriate expression in the brain as indicated by expression in the cortical region. Screening of the remaining mouse lines for expression of the CAT enzyme is continuing in order to identify other lines for comparison and verification of the results. With the relatively large number of founder mice, there is a high probability that additional expressing lines will be identified. Further studies are also being carried out to determine regional localization of expression in the brain. Supported in part by NIH DK41035, RR00163-30, and T32 HD07133.

164.15

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF PROTEIN KINASE C-ß AND C-FOS IN DIFFERENTIATED AND UNDIFFERENTIATED SH-SY5Y CELLS. M. M. Garcia and R. E. Harlan, Dept. of Anatomy, Tulane Univ. School of Med., New Orleans, LA 70112.

Of neural crest origin, the SH-SY5Y neuroblastoma cell line can be induced to differentiate by retinoic acid (RA) into a cholinergic phenotype and by phorbol ester (TPA) into an adrenergic phenotype. These cells also express the μ opiate receptor, with receptor density increased by RA and decreased by PMA. Activation of this receptor has been reported to increase c-Fos in the CNS, by an intracellular mechanism which is unclear. As the c-*fos* promoter has a TPA response element, we studied differentiation-induced changes in PKC and c-Fos in SH-SY5Y cells with immunocytochemistry.

Compared to undifferentiated controls, both RA- and PMA-treated cells show increased levels of PKC ß I and ß II immunoreactivity (IR). Both treatments also increase basal levels of c-Fos IR. Cells treated with the PKC inhibitor staurosporine (ST) show an altered phenotype vs. both RA and PMA, along with decreased PKC ß I IR and markedly decreased c-Fos IR. Cells treated with chronic morphine (MOR) or with the PKC inhibitor H-7 show similar phenotypic changes and PKC IR, but the MOR treated cells show increased c-Fos IR and the H-7 treated cells show a decrease. In response to acute MOR, the RA treated cells showed decreased c-Fos IR, while the PMA treated cells showed an increase. The ST and H-7 treated cells showed no change in c-Fos IR in response to MOR. RA + ST or H-7 did not block the MOR induced decrease in c-Fos IR, while treatment with PMA+ST or H-7 prevented the MOR-induced rise in c-Fos IR. We conclude that the regulation of c-Fos by MOR is cell specific and may be mediated by both PKC-dependent and independent pathways. (Supported by NS-24148, DA-06194 [REH], DA-05411, IN-133J, and the PMA Foundation [MMG].)

164.16

TWO NPY-LIKE PEPTIDES IN THE RIVER LAMPREY MAY CORRESPOND TO NPY AND PYY. C. Söderberg*, J. Dahlstrand*#, L. Brodin*#, G. Andersson, and D. Larhammar. Department of Medical Genetics, Uppsala University, Box 589, S-751 23 Uppsala, Sweden #) Department of Neurophysiology, Karolinska Institute, Stockholm, Sweden.

Neuropeptide Y (NPY) is an abundant and widely distributed neuropeptide in the CNS and PNS of all mammals investigated. NPY increases blood pressure and food intake and influences several other physiological parameters. NPY is a member of a peptide family which also includes the gut peptide PYY, pancreatic polypeptide (PP), and the fish pancreatic peptide PY.

We have previously isolated DNA clones encoding NPY from rat, chicken, goldfish, ray, and shark. Sequence comparisons show that NPY is one of the most highly conserved neuroendocrine peptides known (see Larhammar, Söderberg and Blomqvist, in "Neuropeptide Y and Related Peptides", Humana Press, in press).

By low-stringency hybridization with a shark NPY probe we have now isolated two cDNA clones with distinct NPY-like sequences from a library made from river lamprey *(Lampetra fluviatilis)* brain mRNA. Studies are in progress to see whether the expression patterns of these two NPY-like peptides correlate with the two previously identified cell populations in lamprey brain stem and spinal cord which exhibit distinct NPY-like immunoreactivities (L. Brodin et al., J. Neuroscience 9, 3428-3442, 1989). The complete sequences of the lamprey peptide precursors and their genes may clarify their relationships to mammalian NPY and PYY.

164.17

DETECTION OF NEUROPEPTIDE Y (NPY) IN VERTEBRATES AND INVERTEBRATES BY USING THE POLYMERASE CHAIN REACTION (PCR). A. G. Blomqvist and D. Larhammar. Dept. of Medical Genetics, Uppsala University, S-751 23 Uppsala, Sweden.

The NPY family consists of several 36-amino-acid peptides including also peptide YY (PYY) and pancreatic polypeptide (PP) as well as the fish pancreatic peptide PY. NPY in higher vertebrates is involved in a number of functions such as food intake, sexual behaviour, and cardiovascular mechanisms. In order to investigate NPY's functions in an evolutionary perspective, we are in the process of deducing the NPY sequences of a number of vertebrate and invertebrate species. Through DNA cloning, we have already shown that NPY is present and extremely well conserved in several vertebrates (see Evolution of the Neuropeptide Y family of Peptides. In "Neuropeptide Y and Related Peptides", Contemporary Neuroscience Series, Ed.: C.Wahlestedt and W.F. Colmers, Humana Press, in press). We are now extending these studies by using the polymerase chain reaction (PCR).

Two degenerate oligonucleotides have been constructed based on known NPY and PY nucleotide and peptide sequences from several species of fish and lamprey (see abstract by C. Söderberg et al.). Total genomic DNA and cDNA libraries were used as DNA templates. PCR gave a product of expected size, 121 bp, in cayman, salmon, anglerfish, and hagfish. The obtained products will be sequenced to confirm their identities and for evolutionary comparisons. Preliminary results show products of similar size also in different groups of invertebrates.

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION II

165.1

IN SITU HYBRIDIZATION USING OLIGONUCLEOTIDE PROBES SPECIFIC FOR THE BETA AND GAMMA FORMS OF PREPROTACHYKININ mRNA. J.E. Marchand, C.S. Connelly, and R.M Kream. Anesth. Res. Lab., Tufts University School of Medicine, Boston, MA 02111.

The primary transcript of the gene, PPT 1, which codes for substance P and related tachykinins, is alternatively spliced to yield three different mRNA species, alpha, beta, and gamma, which differ in their protein coding regions. In the rat CNS, beta and gamma are the predominant forms of PPT 1 mRNA, as determined by solution hybridization analyses. We have developed oligonucleotide probes which are complementary to and which bind specifically to the beta and gamma PPT mRNA. The beta probe is a 36 mer complementary to bases 224-259 of the rat beta PPT mRNA, corresponding to the major portion of exon 4. The gamma probe is a 30 mer complementary to bases 205-220 and 266-279 of the beta mRNA which spans the splice site comprising contingent regions of exon 3 and exon 5. The specificity of the hybridization analysis was determined by using selective absorptions with several non-radioactive oligonucleotides. In general, in situ hybridization patterns using the gamma probe yielded patterns of labeling consistent with previous studies. Consistent with solution hybridization analyses, in situ labeling using the beta probe was markedly lower in intensity than gamma labeling. Neuronal labeling patterns suggest coexpression of both forms of PPT 1 mRNA in all regions of the CNS examined.

165.2

EFFECTS OF HERPES SIMPLEX VIRUS (HSV) INFECTION ON THE NEUROPEPTIDES CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND GALANIN (GAL) IN MOUSE DORSAL ROOT GANGLIA (DRG). D.B. Henken & J.R. Martin, NIH, NINDS, Lab. of Exp. Neuropathology, Bethesda, MD, USA, 20892.

HSV is neurotropic and shows a preference for infecting and estabishing latency in neurons of sensory ganglia. In a time course study, we examine how HSV-2 infection affects host neuropeptide expression in mouse DRG. The right hind footpads of anaesthetized female BALB/c mice were inoculated with 10 μl of MS strain HSV-2 (9.3×10^6 pfu/ml). Five, 14, 21 or 28 days later, groups (n=4) of infected and sham-inoculated mice were perfusion-fixed. Spinal columns were decalcified and adjacent paraffin sections were immunoreacted to detect HSV, CGRP or GAL antigen. Somal areas for all labelled and unlabelled neurons in the infected and the contralateral uninfected DRG in each mouse were compared. HSV-positive neurons were small. HSV antigen was present in neurons at D 5, by D 14 the antigen had disappeared. GAL positivity was not seen until D 14, remained high at D 21 and was decreasing by D 28. The mean soma size of the labelled population was small to medium. GAL antigen was not seen in DRG at any interval following sham inoculation. At all times after infection, equal numbers of CRGP-positive neurons were seen in infected and uninfected ganglia and in sham-operated mice. These results suggest that HSV-2 infection differentially affects host neuropeptide production.

165.3

EFFECTS OF CAPSAICIN AND RESINIFERATOXIN ON PEPTIDERGIC NEURONS IN CULTURED DORSAL ROOT GANGLION. F. Liu, S. K. Jeftinija*, L. Urban and S. Jeftinija. Department of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011, USA. Sandoz Institute for Medical Research, Gower Place, London WC1E 6BN, UK

The neurotoxic effect of capsaicin has been shown te be selective on a subpopulation of C-type dorsal root ganglion neurons in newborn animals. The aim of this study was to provide evidence of the effect of capsaicin and its ultrapotent analog RTX on sensory peptidergic neurons maintained in organotypic cultures. Effects of two irritants were examined on neurons that contain substance P (SP) and calcitonin gene-related peptide (CGRP). Exposure of the cultures to 10μM capsaicin and 10nM RTX for period of 2 days or longer resulted in complete elimination of SP-immunoreactive (IR) neurites and reduction, but not elimination, of CGRP-IR neurites. In addition, both 10μM capsaicin and 10nM RTX significantly reduced the number of SP- and CGRP-IR cell bodies within DRG explants. Capsaicin in 100μM and RTX in 100nM concentration produced complete elimination of SP-IR fibers, greater decrease of number of CGRP-IR fibers but failed to completely eliminate IR cell bodies. Exposure of the cultures to irritants for 90 min did not produce a noticable effect on SP- or CGRP-IR neurons. It is important to establish that the effect of capsaicin and RTX on cultured neurons is of long duration (longer than 4 days) and is therefore different from depletion of peptides.

These findings demonstrate that SP-IR processes of cultured sensory neurons are much more sensitive to irritants than cell bodies. Furthermore, our results show that SP-IR neuronal elements are more sensitive to capsaicin than CGRP-IR elements. These data suggest that relatively early in development cultured sensory neurons express functional properties of differentiated pain sensory neurons. Work was supported by NIH grant NS27751 and USDA grant PL95-113.

165.4

THE EFFECTS OF ELECTRICAL FIELD STIMULATION AND INFLAMMATORY SUBSTANCES ON THE RELEASE OF CGRP FROM THE RAT TRACHEA

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Calcitonin gene-related peptides (CGRP) exists in the peripheral terminals of sensory C-fibers in the trachea. The present study is to investigate the effect of electrical field stimulation (EFS) and some inflammatory mediators e.g. bradykinin, serotonin and histamine, which are known to activate or sensitize a population of primary afferents, on the release of CGRP. The rat trachea (below larynx to carina) was dissected and perfused intralumenally with Krebs buffer (0.2ml/min). After lyophilization, the perfusates were subjected to RIA for determining CGRP. Under baseline condition, a resting release of CGRP was observed: 14±4 fmol/fraction. EFS (0.2 to 20Hz, 10V, 1ms, 10min) caused a frequency-dependent increase of CGRP release, e.g. 0.2Hz: 64±19; 5Hz: 175±36; 20Hz: 450±158 fmol/fraction. High frequency (20Hz) EFS desensitized the release evoked by a second stimulation. Bradykinin (10^{-6} to 10^{-4}M) induced a clear-cut release of CGRP in a concentration-dependent manner, while histamine or serotonin did not cause any release of the peptide. Serotonin 10^{-6}M significantly enhanced CGRP efflux evoked by capsaicin 10^{-6}M. In summary, CGRP in the rat trachea can be released upon EFS. The terminals from which the depolarization evoked release of CGRP occurs, is subject to local influence by agents known to excite or facilitate C-fiber activity. (This work is supported by Tobacco Related Disease Program, University of California.)

165.5

THYROTROPIN-RELEASING HORMONE (TRH) mRNA IS INCREASED IN SPECIFIC LIMBIC SUBREGIONS FOLLOWING ONE, TWO, OR THREE ELECTROCONVULSIVE SEIZURES (ECS). S.M. Knoblach, S.R. Keim and M.J. Kubek. Depts. of Anatomy, Psychiatry and Program in Medical Neurobiology, Indiana Univ. & VA Med. Ctrs., Indianapolis, IN 46202.

Prepro-TRH mRNA is significantly increased in specific limbic regions after 3 ECS. We examined whether TRH mRNA may increase after only 1 ECS and if further augmentations are produced by multiple treatments. Rats received either 1, 2 or 3 ECS or sham ECS. Six hours after the last treatment brains were removed and sectioned. A 35S-CTP labelled 548bp riboprobe transcribed from a prepro-TRH cDNA was incubated with brain sections for 20 Hrs at 60°C. X-ray film was evaluated via computer image analysis. Average grey values were increased in the hippocampal dentate gyrus (ventral 53+/-3, dorsal 43+/-6), amygdala (26+/-4), and pyriform (41+/-3) and entorhinal (26+/-6) cortices (each region vs. corresponding sham, $p<0.05$) after 1 ECS. TRH mRNA was undetectable in these same regions in sham controls. In the ventral hippocampus, an additional increase in TRH mRNA occurred from 1 to 2 seizures (2x ECS 81+/-5, $p<0.05$). The third ECS resulted in no further elevation. In conclusion, a single generalized seizure is sufficient to augment TRH mRNA in limbic regions where we have previously observed increases in TRH. Certain areas respond progressively to additional seizures. Supported by NS25661 & VA.

165.6

FORSKOLIN AND PHORBOL ESTER INDUCTION OF NEUROPEPTIDE Y (NPY) PRODUCTION BY AGGREGATING FETAL BRAIN CELLS IN CULTURE: A PROCESS REQUIRING INFLUX OF EXTRACELLULAR CALCIUM. Paolo Magni* and Ayalla Barnea. Depts. OB-GYN & Physiology, UT Southwestern, Dallas, TX 75235

NPY is one of the most abundant peptides in the brain. We addressed the question: Does activation of the cAMP or protein kinase C (PKC) pathway induce production of NPY and is there cross-talk between the two pathways? Aggregate cultures, formed from dissociated cells obtained from the hypothalamus-olfactory tubercle area of 17-day-old fetuses, were cultured in serum-free medium for 12 days. A 24 h exposure to forskolin (F; 10 μM) or phorbol 12-myristate 13-acetate (PMA; 20 nM) resulted in a 2-3-fold increase in the total NPY content (aggregate + medium) and exposure to both F + PMA resulted in an additive response. Inhibition of protein synthesis by cycloheximide (75 μM) dramatically reduced NPY content of the controls and prevented F and PMA stimulation of NPY accumulation. A 24-h inhibition of RNA synthesis by actinomycin D (5 μg/ml) did not alter the NPY content of control cultures but it totally abolished F/PMA induced accumulation of NPY; however, a shorter period of inhibition of RNA synthesis (12 h) did not affect F/PMA induction of NPY accumulation. Moreover, inhibition of influx of extracellular calcium by verapamil (100 μM) did not alter NPY content of control cultures but it drastically inhibited F and PMA action (by 100 and ≈ 60%, respectively). These results indicate that: i) NPY is synthesized by the cultured aggregates; ii) NPY-mRNA has a relatively long half-life; iii) F and PMA both induce synthesis of NPY by acting at a transcriptional and post-transcriptional level; iv) there is no evidence for cross-talk between the cAMP and PKC pathways and v) influx of extracellular Ca^{+2} is required for F/PMA induction of NPY synthesis.

165.7

DIFFERENTIAL EFFECTS OF CLOZAPINE AND HALOPERIDOL ON STRIATAL NEUROTENSIN SYSTEMS IN RATS. S.P. Gygi*, L.G. Bush*, J.W. Gibb and G.R. Hanson. Dept. Pharmacol. and Toxicol., Univ. of Utah, Salt Lake City, UT 84112.

Clozapine (CLOZ) and Haloperidol (HAL) are anti-psychotic drugs with unique therapeutical features. CLOZ is an atypical neuroleptic whereas HAL is a typical neuroleptic. We compared the effects of HAL and CLOZ on striatal neurotensin (NT) systems because they are selectively mediated by dopamine (DA) receptors. HAL and CLOZ were given alone and in combination with methamphetamine (METH), and the NT responses were assessed. In whole caudate HAL, CLOZ, and METH alone caused 278, 340, and 260% increases, respectively. HAL and METH in combination interacted synergistically to increase NT levels ten-fold. In contrast, CLOZ and METH in combination caused increases in NT like CLOZ or METH alone (307%). In preliminary experiments, there were substantial differences in NT responses to these drugs alone and in combination when comparing medial-lateral and rostral-caudal segments of the caudate nucleus. These data suggest that NT systems respond differentially to HAL and CLOZ which may account for some of clozapine's unique clinical properties. (Supported by USPHS grants DA 00869 and DA 04222).

165.8

TTX-BLOCKADE OF THE NEURONAL ACTIVITY CAUSES TEMPORAL INCREASE OF GALANIN EXPRESSION IN CHOLINERGIC NEURONS.

D.V. Agoston[1], S. Komoly*[2] Å. Rökeaus*[3], R.D. Fields[1] and M. Palkovits*[4]. LDN, NICHD,NIH[1]; LENP, NINDS,NIH[2]; Karolinska Institutet, Stockholm, Sweden[3] and LCB, NIMH, NIH[4], Bethesda, MD 20892.

Neuronal activity regulates the establishment as well as maintanance of neurotransmitter phenotype including galanin (GAL) (J. Neurosci. Res. 28:140, 1991; Science, 236:1268, 1987; Endocrinology, 127: 3096, 1990). Galanin co-exists with choline acetyltransferase (ChAT) in the septum-basal forebrain (Brain Res. 360: 130, 1985). The goal of our studies was to test the involvement of neuronal activity in the altered GAL expression of the basal cholinergic system originally observed following axotomy (P.N.A.S. 87: 7742, 1990).Transection of the diagonal band resulted in a transient (max. at 4th p.o. day) accumulation of GAL IR in numerous neuronal perikarya in the nucleus of the diagonal band. In a second series of experiments, neuronal activity was blocked by injecting tetrodotoxin (TTX) into the vertical limb of the nucleus of the diagonal band. Control animals received identical volume (0.1 ul) injections of the vehicle solution, while a third group received intrathecal colchicine. In some experiments, TTX was co-injected with the retrograde tracer, cholera-toxin β-subunit, to assess the adequate locations of injections. Animals were sacrificed at different intervals between 1 and 14 day post-injections and processed for GAL and ChAT immunohistochemistry, in situ hybridization, radioimmuneassay and enzyme activity. TTX-induced blockade of neuronal activity dramatically increased GAL expression in neurons of nucleus of tractus diagonalis, whereas no change was observed in ChAT immunohistochemistry up to 14 day. This increase in GAL expression was first seen at day 1 post-injection and found to be maximal at day 4 post-injection followed by a complete return to control levels by day 7. Unilateral injection caused ipsilateral changes, whereas bilateral injection induced bilateral increases in GAL expression. These changes mimicked the effect of the transsection of the diagonal band. We conclude that, perturbance of the neuronal activity alone can lead to temporal up-regulation of GAL expression in the basal forebrain cholinergic system.

165.9

VASOPRESSIN AND 7B2 ARE ABSENT BUT SPONTANEOUSLY REAPPEAR IN POST-MITOTIC SOLITARY HYPOTHALAMIC NEURONS OF THE HOMOZYGOUS BRATTLEBORO RAT F.W. Van Leeuwen, B.A.Th.F. Gabreëls and N.G. Seidah*. Neth. Inst. for Brain Res., Amsterdam, The Netherlands, and *Clin. Res. Inst. of Montreal, Canada.

The homozygous Brattleboro rat (di/di) synthesizes a vasopressin (VP) precursor with a different C-terminus, of which the passage from the endoplasmic reticulum (ER) towards the Golgi apparatus and further to neurosecretory granules is impaired. In addition, the phenotypic expression of coexisting peptides is disturbed (angiotensin II (Ang II), F8F amide (F8Fa) and secretory protein 7B2) or not affected (dynorphin, NPY and galanin).

During postnatal life a small but increasing number of solitary hypothalamic VP neurons of the di/di rat undergoes a switch to a genuine heterozygous phenotype. They also express Ang II. We report here on the reappearance of 7B2 in these heterozygous cells, indicating that also for 7B2 expression a normal VP precursor is required. The impaired passage of VP precursor in di/di rat VP cells may also disturb that of 7B2 transport. In heterozygous cells VP and 7B2 passage may be partly recovered. It is proposed that within the ER a compartmentalization of precursor synthesis takes place, i.e. within VP cells some precursors (VP, Ang II, F8Fa and 7B2) may be synthesized at different sites of the ER than others (dynorphin, NPY and galanin). Thus the ER may also be one of the sites where the specific contents of a secretory granule, which may change under different physiological conditions, can be regulated.

165.10

VASOPRESSIN CONTENT AND MESSAGE EXPRESSION IN DISCRETE AREAS OF THE RAT HYPOTHALAMUS FOLLOWING REMOVAL OF THE NEUROINTERMEDIATE PITUITARY LOBE. C. A. Johnston, C. D. Refsdal*[1], J. B. Gelineau-van Waes* and D. M. Dorsa[1]. School of Pharmacy, Univ. of Montana, Missoula, MT 59812-1075 and [1]GRECC, VA Med. Ctr., Seattle, WA 98108.

The influence of the neurointermediate pituitary (NIL) on anterior pituitary hormone secretion is well documented. Our laboratory has demonstrated that surgical removal of the NIL (NIL-X) differentially affects arginine vasopressin (AVP) and oxytocin (OXY) neurons in the rat brain (Neurosci. Lett. 120: 256, 1990). Central AVP neurons play an important role in the neuroendocrine regulation of a variety of anterior pituitary hormones whose secretion is affected by NIL-X, raising the possibility that some of the NIL-X induced hormonal changes may result from alterations in the synthesis and release of central neuropeptides. The present study evaluated AVP concentrations and mRNA levels in discrete areas of the male rat brain 13 days following NIL-X or visualization (SHAM) of the NIL. Following decapitation, brains were either frozen at -80 C until analyzed by in situ hybridization for AVP mRNA or were sliced and processed by micropunch analysis and radioimmunoassay for AVP content. The data demonstrate that removal of the NIL results in a decreased expression of AVP message and content in discrete areas of the brain containing AVP cell bodies at a time when AVP concentrations in the hypophyseal portal blood and daily water intake have normalized. This work was supported by MH48228 (CAJ) and NS 20311 (DMD).

165.11

Vassopressin mRNA and Neurophysin-Related Cell-Surface Antigen (NRSA) of Small-Cell Carcinoma (SCCL). William G. North and XiaoMing Yu*, Department of Physiology, Dartmouth Medical School, Hanover, NH 03756.

We have previously shown immunoreactive vasopressin and neurophysin are produced by SCCL, and that some neurophysin-related protein become associated with the cell membrane as NRSA. We now report on the expression of an abnormal vasopressin mRNA by a long-term culture of SCCL, by a tumor removed from a patient at biopsy, and by small-cell tumors raised in athymic mice. This mRNA is 900 bases in size (human vasopressin mRNA of neurons is 750 bases) and hybridizes with cDNA probes representing the exon A and exon B regions of the vasopressin gene, but fails to hybridize with cDNA probes representing the exon C region of the gene.

Protein extracts of SCCL were subjected to SDS-electrophoresis followed by Western blot analysis using mouse monoclonal and rabbit polyclonal antibodies to vasopressin-associated human neurophysin. This evaluation revealed the presence of two immunoreactive proteins with molecular weights of 30,000 daltons and 20,000 daltons in extracts from all three SCCL sources. Both of these proteins appear to be glycosylated and both are present in the cell membrane fraction of tumor cells. Our data therefore demonstrate that NRSA of small-cell tumors is translated by an abnormal vasopressin mRNA and most probably comprises two neurophysin-related proteins of 30,000 daltons and 20,000 daltons.

165.12

MEASUREMENT OF OXYTOCIN AND OXYTOCIN MESSENGER RNA IN A SINGLE MICRODISSECTED HYPOTHALAMIC NUCLEUS OF THE LACTATING RAT. DK Sundberg*, Clara Thore, Miklos Palkovits and RE Eskay*. Department of Physiology and Pharmacology, Wake Forest University, Bowman Gray School of Medicine, Winston Salem NC, 27103 and the National Institute of Health (NIMH and NIAAA), Bethesda, MD.

The neuropeptide, oxytocin (OT), is synthesized in certain discrete hypothalamic nuclei and transported to the neurohypophysis where it is released into peripheral blood. In addition, oxytocin neurons from the paraventricular nucleus (PVN) appear to project to areas throughout the brain. Plasma levels of this peptide increase during lactation and late pregnancy, however, the brain areas that contribute to these changes are not entirely understood.

In the following study we took advantage of the specific solubilities of the peptide and mRNA in alcohol to measure changes in both of these parameters in the same microdissected nucleus. Tissues were microdissected from early pregnant (seven days to avoid estrous cyclicity) and lactating female Sprague-Dawley rats. Tissues were extracted with acidified 98% methanol and the supernatant aliquoted and dried for measurement of OT by RIA. The alcohol insoluble pellet was extracted with 5M guanidium isothiocyanate for OT mRNA which was blotted on Nitran filters and hybridized to a ribonucleic acid probe to exon-C of the OT gene.

Results showed a significant increase in OT mRNA in the PVN, but not in the supraoptic nucleus (SON), the accessory hypothalamic nuclei (AN, anterior commissural nucleus and nucleus circularis) or the neurohypophysis during lactation. OT content, as measured by RIA, however, was found to be decreased in the neurohypophysis and SON but was unchanged in the PVN and AN. Peptide levels were also measured in spinal cord and brain stem areas and showed a general increase associated with lactation. (Supported in part by NIH grant NS 24723).

165.13

DIETARY SODIUM DEPLETION DECREASES THE LEVEL OF THE OXYTOCIN mRNA IN THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI OF THE RAT BRAIN. F. Riftina, R. Sakai, B.S. McEwen. Lab.of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

The present study examined the role of oxytocin in arousal of salt appetite in the rat by dietary sodium depletion. The level of oxytocin mRNA in the PVN and SON was determined by quantitative in situ hybridization with ^{125}I-labeled oligonucleotide probe. Male Sprague-Dawley rats were injected with diuretic furosemide and placed on a sodium deficient diet for 3 days. Control rats recieved injections of 0.9% NaCl and were maintained on a regular diet. Water intake was recorded daily in both groups. On the fourth day the brains were removed and immediately placed on dry ice for detection of oxytocin mRNA. Trunk blood was collected for a determination of serum sodium concentration and osmolality. Rats maintained on sodium restricted diet consumed on average 10 ml less water than control animals and appeared to be hyperosmotic. Serum sodium concentration remained within normal range. The level of the oxytocin mRNA in the magnocellular neurons of the PVN and SON decreased by 25%. The in situ hybridization data indicate that inhibition of oxytocin biosynthesis occurs during dietary sodium depletion and may contribute to the expression of salt appetite in rat. (NIMH grant MH43787).

165.14

LOCAL EFFECTS OF AN ANTIESTROGEN AND TESTOSTERONE ON THE EXPRESSION OF VASOPRESSIN mRNA IN THE BED NUCLEUS OF THE STRIA TERMINALIS. M.D. Brot and D.M. Dorsa, Departments of Psychology, Pharmacology and Medicine, Univ. of Washington, and GRECC, VA Medical Ctr., Seattle, WA 98108.

The expression of vasopressin (VP) neurons and mRNA in the bed nucleus of the stria terminalis (BNST) has been demonstrated to be androgen dependent. Male rats have a greater density of VP fibers and higher levels of VP mRNA than female or castrated male rats. Thus, plasma testosterone (T), and in the case of fiber density, central T as well, has been shown to regulate this system.

In this study we used microimplants to investigate the role of central T on VP message expression in the BNST, in addition to trying to block circulating T's effects by local implantation of an antiestrogen. Male rats were castrated and then T or sham replaced peripherally. Unilateral cannula implants containing T, the antiestrogen Keoxifene (K), or nothing were stereotaxically placed in the brain targeting the BNST. All animals were sacrificed after 10 days and their brains were frozen and later sectioned. Using in situ hybridization methodology, the slides were processed, emulsion coated, developed, and then analyzed for the number of labeled cells in the BNST for each animal over a series of 4 atlas-matched sections.

The results indicate that the removal of T by castration was effective in reducing the number of VP mRNA expressing cells in the BNST as compared to the T-replaced group. Conversely, the castrated, central T implanted group showed an increased number of hybridization-positive cells in the BNST on the side that contained the T when compared with the side that had no implant. Centrally administered K in T-replaced rats significantly reduced the number of cells in the BNST in relation to the T-replaced rats with sham implants, although some reduction of cell number was also noted on the opposite side with no implant. These data suggest that T, through conversion to E_2, may act locally within the BNST to induce expression of the VP gene.

165.15

ADRENAL GLUCOCORTICOIDS REGULATE THE ANGIOTENSINOGEN GENE EXPRESSION IN THE RAT BRAIN: IN VIVO AND IN SITU HYBRIDIZATION STUDIES. J. Angulo, F. Riftina, M. Ledoux*, and B.S. McEwen. Lab. of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

Renin-Angiotensin system is present in the brain and participates in regulation of electrolyte-fluid homeostasis. Three groups of male Sprague-Dawley rats were studied: adrenalectomized (ADX); ADX given Type II selective glucocorticoid agonist RU 28362; and sham-operated rats. The level of Angiotensinogen (ANG) mRNA was detected by quantitative autoradiography after in situ hybridization with a synthetic ^{32}P-labeled oligonucleotide probe. Six days postADX ANG mRNA in the preoptic area (POA) and OVLT in the ADX rats decreased by 50-60%. RU 28362 (10ug/h in Alzet pumps for 6 days) elevated the level of the ANG mRNA to that of the sham-operated rats. The data indicate that the expression of the ANG gene in the POA is under control of adrenal glucocorticoids. (MH43787).

165.16

EFFECTS OF DECENTRALIZATION AND AXOTOMY ON VASOACTIVE INTESTINAL PEPTIDE (VIP)- AND NEUROPEPTIDE Y (NPY)-LIKE IMMUNOREACTIVITY (IR) IN THE SUPERIOR CERVICAL GANGLION (SCG). H. Hyatt-Sachs, T.W. McKeon, R. Schreiber*, S. Piszczkiewicz, Y. Sun, M.R. Bachoo* and R.E. Zigmond. Dept Neurosci, Case West Res U, Cleveland, OH

The adult rat SCG contains low levels of VIP-IR detected by radioimmunoassay (RIA) and some immunoreactive neural processes, but very few immunoreactive neuronal cell bodies, detected by immunohistochemistry. The level of VIP-IR and the number of VIP-IR fibers was increased by about 2-fold at both 2 and 7 days after the preganglionic cervical sympathetic trunk (CST) was cut (Hyatt-Sachs et al., Soc. Neurosci. Abstr. 16: 1029, 1990). In contrast, we find that the levels of NPY-IR did not change 2 days after CST section and decreased by 30% after 7 days. To determine whether preganglionic nerve stimulation could block the increase in VIP-IR produced by preganglionic deafferentation, the CST was stimulated unilaterally with 40 Hz trains (250 msec on, 500 msec off) for 90 min immediately after the two CST were cut. Stimulation did not prevent the increase in VIP-IR measured 2 days later.

After postganglionic nerve section, VIP- and NPY-IR also changed, but the changes differed qualitatively and quantitatively from those reported above. Two days after the internal carotid nerve (ICN) was cut, VIP-IR increased in the ganglion 12-fold and there was a large increase in the number of immunostained neuronal cell bodies and fibers. NPY-IR increased 2-fold. There was no change in total ganglion protein. When the CST, ICN, and external carotid nerve were all cut, VIP-IR increased 25-fold and there were large increases in immunostained cells and fibers. NPY-IR only increased 2-fold and, again, total protein was unchanged.

These results indicate that adult sympathetic neurons that do not normally express VIP can do so after axotomy. Whether the changes in VIP-IR after axotomy differ from those after decentralization only quantitatively or whether different mechanisms are involved remains to be determined. (NS12651 and MH00162)

165.17

INCREASES IN VASOACTIVE INTESTINAL PEPTIDE (VIP)- AND PEPTIDE HISTIDINE ISOLEUCINE AMIDE (PHI)-LIKE IMMUNOREACTIVITY (IR) AND VIP/PHI mRNA IN THE ADULT SUPERIOR CERVICAL GANGLION (SCG) MAINTAINED IN ORGAN CULTURE R.E. Zigmond, H. Hyatt-Sachs, C. Baldwin*, X.M. Qu*, T.W. McKeon, R. Schreiber*, and U. Vaidyanathan*. Dept. of Neurosci., Case Western Reserve University, School of Medicine, Cleveland, OH 44106

The adult rat SCG contains ca. 10 pg of VIP-IR. Immunohistochemically, the ganglion has neural processes exhibiting VIP-IR and IR for the related peptide PHI, but very few immunostained cell bodies. After placing the SCG in organ culture for 24 or 48 h with BGJ_b medium supplemented with fetal calf serum, VIP-IR increased by 6 and 30-fold, respectively. This IR coeluted with synthetic VIP on HPLC. There was also a dramatic increase in the number of neuronal cell bodies and processes showing VIP-IR and PHI-IR. However, cultured ganglia exhibited no change in tyrosine hydroxylase activity or in total ganglion protein. Levels of NPY-IR were found to increase 2.5-fold after 24 h in culture.

The increase in VIP-IR measured after 24 h in culture could be completely blocked by including actinomycin D (1 μg/ml) or anisomycin (100 μM) in the culture medium. The increase in NPY-IR was totally blocked by anisomycin, but was blocked only by about 50% by actinomycin D. Northern blot analysis using a riboprobe complementary to rat VIP/PHI mRNA showed that, although only a faint band was seen in blots of RNA from SCG that had not been cultured, dark bands corresponding to the size of VIP/PHI mRNA were seen in blots of RNA from ganglia after 24 or 48 h in culture.

We have previously shown that VIP- and PHI-IR are present in a population of preganglionic sympathetic but only in very few postganglionic neurons in the SCG. The number of postganglionic neurons expressing VIP and PHI-IR increases dramatically in organ culture due to an increase in the level of VIP/PHI mRNA.

165.18

MODULATION OF THE INCREASE IN VASOACTIVE INTESTINAL PEPTIDE-LIKE IMMUNOREACTIVITY (VIP-IR) IN ADULT SUPERIOR CERVICAL GANGLIA (SCG) MAINTAINED IN ORGAN CULTURE. Y. Sun, H. Hyatt-Sachs, X.M. Qu*, and R.E. Zigmond. Dept. of Neurosci., Case Western Reserve University, School of Medicine, Cleveland, OH 44106

Maintaining adult SCG in organ culture for 24 or 48 h in BGJ_b medium with fetal calf serum produces a 6- and 30-fold increase, respectively, in their content of VIP-IR (Zigmond et al., this volume). Placing ganglia in organ culture causes 3 major changes. The neurons are (1) placed in a different humoral milieu than found in vivo, (2) deafferented, and (3) axotomized. Since a similar increase in VIP-IR was seen when SCG were cultured in a defined medium, stimulatory factors in serum are not required for this change. Inclusion of dexamethasone (10^{-7} M) inhibited the increase in VIP-IR by about 40%, raising the possibility that glucocorticoids in vivo might suppress the expression of this IR. One effect of deafferentation is to eliminate, or at least greatly reduce, action potential firing in postganglionic neurons. Interestingly, depolarization of the cultured ganglia with veratridine (5×10^{-5} M) completely blocked the normal increase in VIP-IR, and this effect was reversed by tetrodotoxin (10^{-7} M). While these data raise the possibility that the principal reason expression of VIP-IR is suppressed in vivo is impulse activity, our finding that cutting the cervical sympathetic trunk produced only a 2-fold increase in VIP-IR in vivo makes this hypothesis unlikely. Axotomy produces a larger increase in VIP-IR in vivo and a large increase in the number of immunostained principal neurons (Hyatt-Sachs et al., this volume). Since NGF is not normally included in the culture medium and since both cultured ganglia and axotomized ganglia would be deprived of target-derived NGF, we examined the effect of including 100 ng/ml NGF in our medium. Ganglia cultured in the presence of NGF had about a 2-fold larger increase in VIP-IR than did ganglia cultured in its absence. Thus, deprivation of NGF seems unlikely to cause the increase in VIP-IR either in culture or after axotomy.

165.19

AN APPROACH TO IDENTIFY PEPTIDE MARKERS IN NEUROENDOCRINE TUMORS. R. Benoit[1], S. *Lavielle[2] and N. Ling[3]. Dept. of Medicine, McGill Univ., Montreal, Canada; Lab. of Organic and Biol. Chemistry, Univ. P. and M. Curie, Paris, France[2] and the Whittier Institute, San Diego, CA[3].

We are looking for specific markers present in high concentration in secretory granules of neuroendocrine tumors, namely basic peptides between 1 000 and 8 000 mol. wt. We think that these peptides can also be found in neurons in normal tissue. Our initial studies were performed with 2.2 G of an acid extract of the most frequent neuroendocrine tumor of the gut in humans, the carcinoid tumor. Sephadex G-75 gel filtration was performed first and five pools of fractions were made based on Substance P and Neurokinin A-like immunoreactivity peaks measured in the column eluate. One pool contained the largest amount of both protachykinin-derived peptides and showed minimal absorbance at 280 nm. It was fractionated by chromatography on carboxymethyl cellulose (CMC). Fractions which contained most of the Substance P immunoreactivity were selected first and further purification was done by reversed-phase HPLC on C18.

Three preponderant peptides contained in the Substance P zone observed on HPLC were purified to homogeneity and sequenced. So far, the most abundant one is a peptide derived from the C-terminal end of human Secretogranin-I precisely after cleavage at LYS^{566} and ARG^{567} of the protein. Conclusion: our purification sheme can lead rapidly to identification of neuroendocrine peptides.

165.20

STOICHIOMETRY AND IDENTIFICATION OF BIOTINYLATED RESIDUES IN NEUROPEPTIDES AND PROTEINS. **S. L. Knock, J. S. Smith*, B. T. Miller, and A. Kurosky*. Marine Biomed. Inst., Depts. of Human Biol. Chem. & Genetics and of Anat. & Neurosci., Univ. Texas Med. Br., Galveston, TX 77550**

The widespread application of the biotin-avidin system and its increasing use has directed more inquiry toward a better understanding of the chemical properties of biotinylating reagents and their reactions. Using neuropeptide Y and lysozyme as models, we have developed a method for the determination of the stoichiometry of biotinylation of peptides and proteins after reaction with an N-hydroxysuccinimide ester of biotin containing the extended spacer arm 6-aminohexanoic acid. The method of analysis, based on the quantification of phenylthiocarbamyl derivatives of 6-aminohexanoic acid, is able to measure low picomolar amounts of biotinyl derivative. Analyses were performed using an automated on-line hydrolyzer followed by HPLC. Compositional analyses determined for known peptides were in excellent agreement with analyses obtained by mass spectrometry. A detailed study of the biotinylated lysine-4 of neuropeptide Y was conducted by automated sequence analysis. Confirmation of the structure of the phenylthiohydantoin derivatives of *N*ε biotinylated lysine was achieved by mass spectrometry. The advantages of the analytical approach described include: 1) the same assay yields quantitative results for both biotin and protein or peptide content; 2) the biotin label containing the spacer arm may be important for reducing the steric hindrance associated with the binding of biotinylated peptides to both avidin and membrane receptors; and 3) the combination of amino acid compositional analysis and automated sequence analysis can allow identification of the specific residues to which biotin is attached. Since several different biotinylated species can be generated during the course of one reaction, such information is valuable for the consistent production of biotinylated probes. **(Support: NIH NS29261, NS07185, and the Robert A. Welch Foundation)**

165.21

DEVELOPMENT OF N- AND C-TERMINAL DIRECTED IMMUNOLOGICAL PROBES TO A 1745-DA PYROGLUTAMYL PEPTIDE DERIVED FROM CHROMOGRANIN B.

M. de Serres*, J. D. McDermed*,T. Flanagan*, O. H. Viveros, and E. J. Diliberto Jr*. Division of Pharmacology and of Organic Chemistry, Burroughs Wellcome Co., 3030 Cornwallis Road, R.T.P., NC 27709.

Specific antisera and a competitive homogeneous RIA were developed to detect the presence of a recently described pyroglutamyl 1745-da peptide (BAM-1745) derived from the endogenous processing of chromogranin B by bovine adrenal medulla chromaffin cells. Antisera directed to either end of BAM-1745 were obtained using analogs of BAM-1745 extended by KCGG or GGCK at the amino- or the carboxy-terminal end, respectively, coupled through the cysteinyl residue to multichain Poly-DL-Alanyl-Poly-L-Lysine by sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate. The resulting directed antisera were found to have markedly disparate epitope requirements when tested for immunological reactivity in a competitive homogeneous RIA against various synthetic analogs of this peptide. Immunoreactivity in micro-pig tissues was examined by RIA using an amino terminal directed antiserum. BAM-1745-like immunoreactivity was found in many of the tissues examined, the highest levels were associated with the pituitary and whole adrenal extracts (12 and 7 nmol/g tissue wet wt, respectively). The highest levels of BAM-1745 like immunoreactivity in brain regions were found to range from 0.4 to 0.2 nmole/g tissue wet wt in cerebellum, hippocampus, striatum, cortex, and septum.

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION III

166.1

CLONING AND REGIONAL EXPRESSION OF A PUTATIVE PROCESSING ENDOPROTEASE FROM RAT BRAIN.

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Recent studies have identified at least three distinct endoproteases in mammals that are related to Kex2, a yeast enzyme involved in the initial proteolytic processing of the precursor of yeast pheromone: furin, PC1/PC3 and PC2. These enzymes all exhibit properties consistent with roles as processing endoproteases: (1) specificity towards paired basic amino acids, typical recognition signals for proteolytic processing found within a variety of protein precursors, and (2) appropriate processing of one or more precursor proteins when co-expressed in transfected cells. Using a cDNA probe for human PC2, we have isolated multiple clones from a rat brain cDNA library that appear to contain a rat homologue of PC2. Partial sequencing shows high homology in the protein coding sequence with those for mouse and human PC2. A major transcript approximately 2.8 kb in size was detected in rat brain RNA using the rat cDNA, consistent with recent reports using mouse and human PC2. Transcripts were much lower or absent in other tissues so far examined using this probe (muscle, liver, kidney, skin, lung). Preliminary Northern blot analysis indicated a widespread regional distribution of rat PC2 mRNA in brain, suggesting this endoprotease may be involved in the processing of multiple neuropeptide precursors within the rat CNS. Data will be presented on the cellular expression and possible regulation of this PC2-like endoprotease in rat brain.

166.2

CLONING AND CHARACTERIZATION OF A cDNA ENCODING A DIPEPTIDYL AMINOPEPTIDASE-LIKE PROTEIN FROM BOVINE, RAT AND HUMAN BRAIN.

N. Yokotani*, C. Hunter, S. Shimasaki*+, K. Doi, R.J. Wenthold and K. Wada. Lab. of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892, and +Lab. of Molecular Endocrinology, The Whittier Institute, La Jolla, CA 92037.

We showed previously the purification of an AMPA subtype of a glutamate receptor from bovine brain (Hunter and Wenthold, *Soc. Neurosci. Abstr.*, 16, 543, 1990). Several peptide sequences were obtained by microsequencing of the protease-digested fragments of the purified protein. Screening of bovine brain cDNA libraries and sequencing of positive phage clones revealed that some of the chemically obtained peptide sequences were encoded by a cDNA unrelated to AMPA receptor cDNAs. The cDNA encods a new dipeptidyl aminopeptidase-like protein which shares 27% and 33% amino acid identity to yeast and rat liver dipeptidyl aminopeptidases (DAPs), respectively. The predicted protein has a membrane topology similar to those of other membrane-bound peptidases. Northern hybridization using a rat homologue cDNA on mRNAs from various rat tissues showed that only brain expressed the transcripts of 3.8 and 4.5 kb. Further analysis using the rat and human homologue cDNAs as well as bovine cDNA showed that all of the three species probably express two different forms of the protein due to an alternative splicing of RNA (803 and 863 aa, respectively, in bovine brain). Expression studies using mammalian cell transfection are currently under way to characterize the enzyme activity of the DAP-like protein.

166.3

PUTATIVE ROLE OF PROHORMONE ENDOPROTEASES (PEPs) *PC1, PC2,* AND *FURIN* IN THE PROCESSING OF PRO-SOMATOSTATIN (PSS).

A. Galanopoulou*, G. Kent*, S. Rabbani*, N. G. Seidah*, and Y.C. Patel. Fraser Laboratories, McGill University, and Clinical Research Institute of Montreal, Montreal, Quebec, Canada.

Mammalian PSS is endogenously processed at dibasic and monobasic cleavage sites to generate respectively SS-14 and SS-28. In the present study we have investigated the putative roles of the recently cloned mammalian PEPs *PC1, PC2,* and *furin* in mediating these cleavages. We have correlated the endogenous expression of these enzymes with cellular SS-14 and SS-28 in rat islet SS-producing tumor cells (1027 B_2) and in 2 non SS-expressing cells (AtT-20, PC12) acutely transfected with PKS5 (prePSS expression vector - gift of K. Sevarino). The relative abundance of *PC1, PC2,* and *furin* mRNA (by Northern analyses with cRNA probes) was compared with percent processed SS-14 and SS-28 (by HPLC analysis followed by RIA) (Table). COS 7 cells transfected with PKS5 constitutively secreted SS-14 and SS-28 (ratio 48%:52%). These data show absence of *PC2* in 1027 B_2, PC12 and AtT-20 cells and suggest a random distribution of *PC1* and *furin* in these neuroendocrine cells. Although processing by unidentified PEPs cannot be excluded, our results provide indirect evidence that (1) *furin*, expressed alone in PC12 cells, can process PSS to SS-14 but not SS-28. (2) *PC1*, abundantly expressed in AtT-20 cells may process at both dibasic (SS-14) and monobasic (SS-28) sites.

	PC1	*PC2*	*Furin*	% SS-14	% SS-28
1027 B_2	++	-	++	60	40
PC12	-	-	+	100	0
AtT-20	+++	-	-	75	25

166.4

IN VIVO CHARACTERIZATION OF THE CLEAVAGE SITE SPECIFICITY OF Neuro 2A, Rin m5F AND AtT20 PROHORMONE PROCESSING ENZYMES.

N. C. Day, H-L. Lin* and H. Akil. Mental Health Res. Inst., University of Michigan, Ann Arbor, MI 48109.

Many peptide hormones and neurotransmitters are synthesized as larger precursor proteins which are endoproteolytically processed at specific cleavage sites to produce the biologically active peptide(s). Endoproteolytic cleavage usually occurs at dibasic amino acid residues in the peptide precursor. An indirect way to characterize the enzymes responsible for endoproteolysis is to examine prohormone processing in heterologous cell lines, known to process endogenous or transfected precursors. In this study, we have assessed the ability of Neuro 2A, Rin m5F and AtT20 cells to process the ßEndorphin/ACTH precursor, proopiomelanocortin (POMC). Wild-type and mutated monkey POMC cDNAs were subcloned into an expression vector employing the cytomegalovirus immediate early gene promoter, and the resulting plasmids transfected into the cell lines. Following extraction and molecular sieving, peptide products were assayed by radioimmunoassay. In AtT20 cells, endogenous mouse POMC was distinguished from transfected monkey POMC by a radioimmunoassay specific for monkey ßMSH. Results showed that wild-type POMC is processed to different extents in the different cell lines: Neuro 2A cells processed the least, whilst Rin m5F cells processed POMC the most extensively. The effect of mutating specific cleavage sites on processing of monkey POMC is being examined in terms of a) sequence specificity and b) cellular milieu. The results will be discussed in relation to expression of PC1 and PC2 (Seidah et al., DNA and Cell Biology 9:415, 1990), two recently cloned putative processing enzymes, in the different cell lines.

166.5

REGULATION OF A NEUROPEPTIDE PROCESSING ENDOPROTEASE IN THE RAT ANTERIOR PITUITARY LACTOTROPHIC CELL LINE, GH_4C_1. L. Greco,* L. Daly,* S. Kim,* L.D. Fricker+ and L. Devi.*. Dept. of Pharmacology,* New York Univ. Med. Center, New York 10016; +Dept. of Mol. Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461.

Several peptide hormones and neurotransmitters are produced by cleavage at the monobasic processing sites. An endoprotease capable of cleaving a dynorphin peptide at the monobasic processing site is secreted from the rat anterior pituitary cell line, GH_4C_1. When characterized by chromatography on FPLC using an ion-exchange column, the majority of the endoprotease activity elutes as a single symmetrical peak around 0.3 M NaCl. The protease inhibitor profile suggests that the activity is due to a thiol protease. These enzymatic properties are similar to a monobasic processing enzyme previously found in bovine pituitary and in the rat brain. The secretory pathway which contains the enzyme activity in GH_4C_1 cells was characterized by stimulation of secretion by thyrotropin releasing hormone, forskolin, phorbol ester, or potassium chloride. The secretion of the enzyme activity was substantially increased by these compounds suggesting that the GH_4C_1 cells secrete the activity via regulated pathway. A hormonal treatment of the GH_4C_1 cells which has been previously shown to produce a substantial increase in the number of secretory granules and ir-prolactin, has been found in this study to elevate the peptide processing enzyme activity 2-fold. This increase is similar to that seen in the carboxypeptidase E activity; another putative peptide hormone processing enzyme activity. These data suggest that the peptide processing activity is regulated to a small but significant extent and is coordinately regulated with carboxypeptidase E activity.

166.6

TRANSFECTION AND PROCESSING OF PRO-ACTH/ENDORPHIN IN GH4C1 CELLS. E.I. Cullen and F. Lin *. Dept. of Pharmacology, New York Medical College, Valhalla, NY 10595

GC and GH3 cells can endoproteolytically process pro-ACTH/endorphin (PAE or POMC) derived from a transfected cDNA (Cullen and Mains, 1989 Endocrinology 125: 1774). Other laboratories have found GH4C1 cells do not process PAE. To facilitate comparison of GC, GH3, and GH4C1 cell processing capabilities, we transfected GH4C1 cells with the same vector and assayed for processing as in earlier work. After incubating transfected cells in medium containing $CdCl_2$, cells were washed three times in serum-free medium, and extracted in 50% acetic acid. Soluble extracts were subjected to Sephadex G-75 chromatography in 10% formic acid, and aliquots of the fractions were dried and assayed by radioimmunoassay using materials generously provided by Dr. R.E. Mains. In contrast to the results obtained in transfected GC cells, the only amidated joining peptide immunoreactivity (JP-IR) detected in transfected GH4C1 cells displayed a K_D of 0.27 $\pm$ 0.02, indicating an apparent molecular weight larger than that of the PAE(1-94)NH_2 found in transfected GC cells. No amidated JP-IR the size of JP(1-18)NH_2 was detected. Thus, processing is less complete in GH4C1 cells than in GC cells, and the ability to cleave at Arg(75)Arg(76) may be absent. Supported by the Pharmaceutical Manufacturer's Association Foundation.

166.7

DIRECT MEASUREMENT OF PROENKEPHALIN PROCESSING IN ADRENAL CHROMAFFIN CELLS. S.P. Wilson, A.P. Rostovtsev* and B.A. Spruce*†. Dept. of Pharmacology, USC Sch. of Med., Columbia, SC 29208 and †Dept. of Biochemistry, Univ. of Dundee, Scotland

The processing of proenkephalin (PE) was examined using pulse-chase radiolabeling of bovine adrenal medullary chromaffin cells. PE and PE-derived peptides of >10 kDa were purified by incubation of cell extracts with an immunoaffinity matrix containing equal portions of the monoclonal antibodies PE-1 and PE-2 directed against PE (*J. Biol. Chem.* **263**, 19788; 1988). The purified peptides were then separated by SDS polyacrylamide gel electrophoresis, blotted to polyvinylidene difluoride membranes, and localized by immunostaining. PE (31/33 kDa) and major PE-derived peptides of 13, 15, 18, 22, and 28 kDa were found on the blots and were analyzed for incorporation of [^{3}H]Leu. After 30 and 60 min of labeling, >75% of the label was found in PE. Radioactivity in PE disappeared with a half-time of about 1 h. Appearance of radiolabel in the PE-derived peptides occurred in parallel with the decrease in PE labeling. No loss in the [^{3}H]PE-derived peptides occurred over times up to 6 h, indicating that these peptides are final products of the PE-processing pathway. Addition of the secretagogue nicotine or the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine during radiolabeling and chase had no effect on PE processing. This work was supported by NSF Grant BNS-8719149.

166.8

STABILITY OF EXOGENOUSLY ADDED PROENKEPHALIN IN TRANSFECTION EXPERIMENTS. J.P. Mathis and I. Lindberg*. Dept. of Biochem. and Mol. Bio., Louisiana State University Med. Ctr., New Orleans, LA 70112.

Tissue culture cell lines transfected with neuropeptide precursor cDNA are widely used for investigating the proteolytic processing of neuropeptide precursor molecules. A potential problem with this method is non-specific proteolytic cleavage of the precursor molecule in culture medium. Therefore, the stability of ^{35}S-radiolabelled proenkephalin added to the medium of transfected and non-transfected cells was measured in order to determine the occurrence and extent of non-specific degradation.

The stability of proenkephalin was measured in the following way. Medium containing 4 - 20 x 10^4 cpm/ml of ^{35}S-radiolabelled, HPLC-purified, exogenous proenkephalin was added to cells. Medium samples were collected at 48h and breakdown of the exogenous proenkephalin was analyzed by gel filtration chromatography or SDS-PAGE. ^{35}S-radiolabelled proenkephalin incubated in the absence of cells did not break down.

The effects of cell density, percent of fetal bovine erum (FBS) in the medium and transient and stable transfection methods on stability of proenkephalin were examined in AtT-20, Cos-1, SK-N-MC and CHO DG44 cell lines. The breakdown of exogenous proenkephalin was increased by 50% at 4 x 10^5 cells/60mm dish when compared with 2.5 x 10^5 cells/60mm dish. Degradation of exogenous proenkephalin was reduced by 50% in 5% FBS medium as compared to serum-free medium. Transfection of cells by the calcium phosphate method *per se* did not affect stability of the exogenous proenkephalin. In SK-N-MC cells, breakdown of proenkephalin was mediated by soluble rather than cell-bound proteases. Therefore, non-specific degradation of precursor molecules does occur in cell culture medium and should be taken into account during studies of neuropeptide precursor processing.

166.9

ENDOPEPTIDASE 22.19 (EC 3.4.22.19), A PUTATIVE ENKEPHALIN-GENERATING ENZYME, IN THE VERTEBRATE RETINA. **E.S. Ferro*, D.E. Hamassaki*, J.C. Bittencourt, A.C.M. Camargo* and L.R.G. Britto. Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP 05508, Brazil.**

In this study we used both biochemical and immunohistochemical procedures to investigate the possibility of endopeptidase 22.19 to convert enkephalins *in vivo*. The specific activity of endopeptidase 22.19 found in pigeon retinae (30.3 ± 7.3 mU/mg) was four times higher as compared to rabbit retinae (7.0 ± 1.1 mU/mg). The enzyme activity was not modified by EDTA, but it was enhanced by dithiothreitol and inhibited by zinc, 5,5'-Dithiobis-(2-nitrobenzoic acid) and dynorphin A_{1-13}. The immunohistochemical experiments revealed labeled neurons in both the inner nuclear layer and the ganglion cell layer of pigeon and rabbit retinae. Double-labeling immunofluorescence experiments demonstrated that about 90% of neurons containing endopeptidase 22.19-like-immunoreactivity contained also leucine-enkephalin-like-immunoreactivity. These results represent an important step towards the demonstration of the possible involvement of endopeptidase 22.19 in enkephalin generation *in vivo*.

166.10

A NOVEL THIOL PROTEASE INVOLVED IN PROCESSING ENKEPHALIN AND TACHYKININ PRECURSORS IS INHIBITED BY α_1-ANTICHYMOTRYPSIN, A COMPONENT OF ALZHEIMER'S AMYLOID DEPOSITS. V.Y.H. Hook, G. Hubbard,* and T.J. Krieger* Dept. of Biochemistry, Uniformed Services University, Bethesda, MD. 20814

The protease inhibitor α_1-antichymotrypsin (α_1-AC) is a normal component of the human nervous system. Identification of the endogenous neuronal protease(s) normally regulated by α_1-AC is important to understanding its role in brain function. In this study, we demonstrate that α_1-AC inhibits a novel thiol protease (purified from bovine chromaffin granules, J.Biol. Chem., in press, 1991), cleaving at Lys-Arg and Lys-Lys sites, that is involved in processing enkephalin and perhaps tachykinin precursors. This protease was inhibited in vitro by human plasma α_1-AC, and α_1-AC immunoreactivity was colocalized with the enzyme in bovine secretory vesicles from adrenal medulla and pituitary. Purification of the endogenous bovine α_1-AC from pituitary by DEAE-Sepharose, chromatofocusing, butyl-Sepharose, and Sephacryl S200 resulted in a 60 kDa bovine α_1-AC protein that was a potent inhibitor of the thiol protease, possessing an IC_{50} value (concentration for 50% inhibition) of 12 nM. It also inhibited chymotrypsin and trypsin with IC_{50} values of 42 and 12 nM, respectively. This is the first demonstration that a neuropeptide precursor processing enzyme is regulated by an endogenous protease inhibitor. Also, these results and recent findings (Abraham et al., Cell;52,487) of α_1-AC localization in amyloid deposits of Alzheimer's Disease suggests a possible role for α_1-AC inhibition of neuropeptide production in neurologic disease.

166.11

A LIGHT AND ELECTRON MICROSCOPIC STUDY OF IMMUNOREACTIVE (IR) CORTICOTROPHIN-RELEASING HORMONE (CRH) CONTAINING NEURONES IN THE RAT NEOCORTEX. P.R.Lowenstein[1], A.F.Shering[1*], J.L.James[1*], P.J.Lowry[2*], L.Linton[2*] and M.G.Castro[1]. [1]Dept.Anat/Physiol., Dundee University, Dundee DD1 4HN, [2]Dept. Biochem./Physiol., Reading University, Reading RG6 2AJ and [3]Dept. Molec. Life Sci., Dundee Inst. Technol., Dundee DD1 1HG, United Kingdom.

CRH is the main secretagogue of anterior pituitary ACTH. Ir CRH, as well as CRH receptors have been identified throughout the CNS. In addition to its neuroendocrine function CRH could act as neurotransmitter in extrahypothalamic areas. To provide the anatomical basis for its role in cortical physiology we have characterised CRH ir neurones in the rat neocortex using light and electron microscopical immunocytochemical techniques. The primary antibodies used were:3B3 and rC70 (Dr.W.Vale), diluted 1:1000. The ABC (Vector) method was used to detect the primary antibodies. Unexpectedly, three different types of ir neurones were encountered: bipolar neurones were found mainly in layers II-III, multipolar sparsely spiny neurones were found mainly in layers V-VI, and smooth dendritic multipolar neurones were located in the white matter. Very few immunoreactive fibres were seen crossing the white matter. Axonal processes originating from bipolar neurones could be followed for long distances and tended to arborize near the cell body. Preliminary examination of the synaptic organization of ir boutons in layers II-III indicates that some CRH boutons establish asymmetric synapses. Our results show that, contrary to previous descriptions, CRH ir neurones in the rat neocortex constitute a heterogenous neuronal group, some of which may establish excitatory synaptic connections.

166.12

BIOSYNTHETIC PATHWAY AND INTRACELLULAR COMPARTAMENTALISATION OF CORTICOTROPHIN-RELEASING HORMONE (CRH). [1,2]M.G. Castro, [3]P.R. Lowenstein, [2]B. Glynn*, [2]A. Perkins*, [2]E. Linton* and [2]P.J. Lowry*. [1] Dept. Molec. Life Sci., Dundee Inst. Technol., Dundee DD1lHG, [2]Dept. Biochem. Physiol., Reading Univ., Reading RG62AJ [3]Dept. Anat. Physiol., Dundee Univ., Dundee, DD14HN, U.K.

CRH is cleaved from a larger precursor (proCRH) by endopeptidases. We have used AtT20 cells transfected with the human (h) proCRH gene (R1 and R4 clones; provided by Drs G. Adler and J. Majzoub) to study post-translational processing and intracellular compartmentalisation of proCRH. The levels of intracellular and secreted ACTH and CRH were determined using immunoradiometric assays (IRMAs) and/or radio-immunoassays (RIAs). Norepinephrine (0.1mM) caused a several-fold stimulation of CRH release in R1 and R4 cells (basal CRH released: 0.05ng/ml/3h for both R1 and R4 cells; stimulated CRH released: 0.44$\pm$ 0.11 ng/ml/3h for R1 cells and 0.19$\pm$0.01 ng/ml/3h for R4 cells). These results indicate that CRH is targetted into the secretory pathway in the transfected cells. Using gel filtration chromatography and HPLC followed by specific IRMAs and RIAs on the eluted fractions, we have demonstrated that the CRH precursor is proteolitically processed in the transfected cells to yield CRH(1-41) and proCRH(125-151), suggesting that these peptides may be the end products of the biosynthetic pathway of the hCRH prohormone.

166.13

DEVELOPMENTAL PATTERN OF THE TYROSYL PROTEIN SULFOTRANSFERASE IN RAT BRAIN

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Unité de Neurobiologie et Pharmacologie, Centre Paul Broca de l'INSERM, 75014 Paris - France.

Cholecystokinin (CCK) is a major hormone and neurotransmitter in the central nervous system. Previous studies revealed the important role of a tyrosine protein sulfotransferase (TPS), in the activation of CCK e.g. tyrosine sulfation (Vargas et al., Biochemistry, 1985, 24: 5938 ; Niehrs et al., J. Biol. Chem., 1990, 265: 8525).
In this report we analyzed the developmental pattern of TPS in rat brain. Ontogenic studies showed that TPS is present in the fetal brain and, also, the PAPS synthetizing system. TPS activity was surprisingly 2.5 fold higher in the newborn rat brain tissue than the mature. The Michaelis affinity constants for PAPS and BocCCK-8(ns) for the TPS activity were similar for newborn and adult membranes, Vmax decreased, however from 0.83 $\pm$ 0.05 to 0.31 $\pm$ 0.02 pmol/mg protein/min in brain membranes from newborn and adult brain respectively.
These results suggest that TPS may play a role in embryogenesis and development as suggested, also, by the presence of CCK-8 in the aerosomal granule of spermatides (Persson et al., Proc. Natl. Acad. Sci., USA, 1989, 86: 6166).

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION IV

167.1

CHARACTERISTICS OF A CCK CLEAVING ENDOPROTEASE PURIFIED FROM RAT BRAIN SYNAPTOSOMES. J.C.Viereck and M.C.Beinfeld. Dept. of Pharmacology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

An endoproteolytic activity which specifically cleaves CCK-33 producing CCK-8 has been purified from a rat brain synaptosome preparation. This activity was assayed by incubating with substrate and separating the product on DEAE Sephadex ion exchanger. The assay product co-eluted with authentic CCK-8 on reverse phase HPLC. Steps including DEAE anion exchange, chromatofocusing, hydroxyapatite chromatography and gel filtration resulted in a several hundred fold increase in specific activity. The endoprotease apparently exists as a 90 kDal molecular weight dimer, which can be dissociated into active 45 kDal subunits. The endoprotease has no activity against several specific substrates for other proteases (trypsin, kallikrein, enteropeptidase) as assayed using flourogenic probes, nor does it display proteolytic activity against analogs of monobasic cleavage sites of several other prohormones. Non sulfated analogs of the cleavage site are also not cleaved by the endoprotease. Thus the amino acid sequence around the cleavage site and sulfation appear to be important in substrate recognition. The specificity and subcellular localization of the endoprotease make it a good candidate for a CCK converting enzyme. Supported by NIH-NS18667

167.2

IN VITRO METABOLISM OF RAT GROWTH HORMONE-RELEASING FACTOR(1-29) AMIDE IN RAT PITUITARY AND HYPOTHALAMUS. L. Boulanger, C. Lazure* and P. Gaudreau. Neuroendocrinology Laboratory, Notre-Dame Hospital Research Center and Montreal Clinical Research Institute, Montreal, Canada, H2L 4M1.

Previous reports showed that in addition to the stimulation of growth hormone (GH) secretion, GH-releasing factor (GRF) may exert a specific negative feedback on its own hypothalamic secretion. The metabolism of GRF in pituitary and hypothalamus has not been documented yet. However, to design peptide analogs exhibiting a longer duration of action than native GRF, vulnerable peptide bonds must be secured from peptidase action, in both tissues. The kinetic of disappearance of rGRF(1-29)NH_2 and the identification of its metabolites were therefore studied in pituitary (237 $\pm$ 51 μg prot./ml) and hypothalamus homogenates (576 $\pm$ 27 μg prot./ml). Analytical HPLC revealed an apparent half-life of 22 $\pm$ 3 min and 25 $\pm$ 4 min respectively. The disappearance of GRF was associated with the successive appearance of 3 major, less hydrophobic, peptides. These degradation products were isolated by preparative HPLC and their identity revealed by amino acids analysis, sequencing and co-chromatography with synthetic fragments. Proteolysis of GRF produced, in both tissues, the fragments 1-21, 1-14 and 1-10. These results show that the main cleavages of rGRF(1-29)NH_2, occur at Lys^{21}-Leu^{22}, Leu^{14}- Gly^{15} and Tyr^{10}-Arg^{11}. Chemical modifications at these critical sites may result in GRF agonists with a greater efficacy.

167.3

PEPTIDASE ACTIVITIES AGAINST N-ACETYLASPARTYLGLUTAMATE ACROSS 50 DISCRETE REGIONS WITHIN THE RAT NERVOUS SYSTEM. S. Fuhrman[1], M. Palkovits[2], and J. H. Neale[1]. [1]Dept. of Biology, Georgetown University, Washington, DC. [2]Lab. of Cell Biol., National Institute of Mental Health, Bethesda, MD.

The dipeptide N-acetylaspartylglutamate (NAAG), which is found in millimolar concentrations in brain tissue, may function in synaptic communication. NAAG is hydrolyzed to N-acetylaspartate and glutamate by membrane-bound peptidase activities, which appear to be extracellular in brain cell culture. These peptidase activities may be involved in the inactivation of NAAG. Alternatively, NAAG could be acting as a sequestered form of glutamate.

The objective of this project is to determine the anatomical relationship between NAAG and peptidase activities which hydrolyze it. Initially, micropunched samples from 50 rat brain nuclei (incl. spinal cord) were assayed to determine the distribution of the NAAG-hydrolyzing peptidase activity. The results indicate a positive correlation between [NAAG] previously identified by immunohistochemistry and NAAG-hydrolyzing enzyme activity; for example, the caudate nucleus and various regions of cerebral cortex are all low in [NAAG] and in the peptidase activity, while the interpeduncular nucleus and locus coeruleus show high levels of the peptidase activity as well as high [NAAG].

Quisqualate is reported to be a non-competetive inhibitor of one peptidase activity against NAAG. The correlations between these enzyme activities and NAAG in control and lesioned rat CNS will be explored.

167.4

PROTEOLYTIC CONVERSION OF OXYTOCIN BY RAT BRAIN SYNAPTIC MEMBRANES: ROLE OF AMINOPEPTIDASES AND ENDOPEPTIDASES. A. Argiolas, M.R. Melis and R. Stancampiano*. B.B. Brodie Dept. of Neurosciences, Univ. of Cagliari, 09124 Cagliari (Italy).

The proteolytic conversion of oxytocin (OXY) and vasopressin (VP) by purified rat brain synaptic membranes was studied at 37°C and pH 7.4. The formed peptide fragments were isolated by HPLC and characterized by amino acid analysis. When OXY was incubated, both C- or N-terminal fragments were found. The most abundant were Cyt^6-OXY 4-9, Cyt^6-OXY 3-9, Cyt^6-OXY 2-9, OXY 1-8 and OXY 1-7. In contrast, only C-terminal fragments, Cyt^6-Arg^8-VP 4-9, Cyt^6-Arg^8-VP 3-9 and Cyt^6-Arg^8-VP 2-9, were found by incubating Arg^8-VP. The formation of C-terminal OXY and VP fragments was prevented by the aminopeptidase inhibitors amastatin and bestatin, while the formation of OXY 1-7 and OXY 1-8 was inhibited by Hg^{2+} and Zn^{2+} ions. The formation of OXY 1-7 was also partially prevented by the endopeptidase inhibitor phosphoramidon. The formation of both C- and N-terminal fragments was inhibited by o-phenantroline. The results suggest that, unlike VP, OXY is metabolized by both membrane-bound aminopeptidases and endopeptidases.

167.5

CORTICOTROPIN RELEASING FACTOR METABOLISM IN THE RAT HYPOTHALAMUS. **J.C. Ritchie, M.J. Owens, C.B. Nemeroff. Depts. Psychiat. and Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.**

The metabolism of neuropeptides may be of great importance in the termination of their actions in the synapse. In order to study the metabolism of corticotropin releasing factor (CRF) we have developed and characterized an HPLC gradient fractionation for r/h CRF_{1-41} and its available synthetic fragments. In an attempt to elucidate CRF_{1-41} metabolism in the rat hypothalamus we prepared 10,000XG homogenate extracts. These extracts were then incubated at 37°C for varying time periods in the presence of excess CRF_{1-41}. Additionally, incubations were carried out in the presence of a wide variety of specific peptidase inhibitors. Fractionation of the enzyme digests showed an attenuation of the CRF_{1-41} peak at all time points and the generation of multiple new peaks. Incubation with specific protease inhibitors altered the pattern of peak generation but did not block completely the degradation of whole CRF. Analysis of the chromatograms reveals that the breakdown of CRF in the rat hypothalamus results in the formation of CRF_{37-41} and several other yet unidentified peptide fragments. (Supported by NIMH MH-42088)

167.6

SUBSTANCE P-METABOLISM *IN VIVO* BY MICRODIALYSIS AND ON-LINE CONTINUOUS FLOW-FAST ATOM BOMBARDMENT MASS SPECTROMETRY. P.E. Andrén and R.M Caprioli*. Department of Psychiatry at Ullerâker, University of Uppsala, S-750 17 Uppsala, Sweden and Analytical Chemistry Center, University of Texas Medical School, P.O. Box 20708, Houston, TX 77225

The putative role of substance P (SP) as a neurotransmitter has focused interest on the mechanisms of its inactivation. Many purified enzymes have been shown to hydrolyse the SP-molecule *in vitro* at a variety of peptide bonds. However, it is not known how these enzymes function *in vivo*. The combination of microdialysis and continuous flow-fast atom bombardment (CF-FAB) mass spectrometry allows endogenous aqueous samples to be introduced directly into the mass spectrometer, so that direct and on-line monitoring capabilities can be effectively used. The purpose of the present study was to assess the feasibility of *in vivo* microdialysis as a technique coupled on-line to a mass spectrometer, which enables continuous monitoring of biochemical events in brain extracellular space, and to study SP-metabolism *in vivo*.

Sprague-Dawley rats, anesthetized with pentobarbital, were fixed in a stereotaxic frame and a microdialysis probe with membrane length of 4 mm was implanted unilaterally in the striatum. The microdialysis probe was used to both introduce a bolus of SP (2.5 nmol/5 uL) as well as collect dialysate to measure reaction products. The perfusate from the microdialysis probe was connected directly onto the CF-FAB-interface on the mass spectrometer. The present results showed that the SP-fragments 8-11, 9-11, 5-9 and 1-6 were the main metabolites formed when SP was infused into the striatum of the living rat. The SP-infusion also yielded the fragment 1-7, but in a smaller amount. It is concluded that the combination of microdialysis and CF-FAB mass spectrometry provides a useful technique for assay of *in vivo* protease activity.

167.7

SUBSTANCE P RELEASE INVESTIGATED IN BOTH THE RAT SUBSTANTIA NIGRA AND SPINAL CORD, USING ANTIBODY MICROPROBES. P. J. Hope*, A.K. Wright*, H.M. Brace*, G. W. Arbuthnott. Department of Preclinical Veterinary Sciences, University of Edinburgh, Edinburgh, EH9 1QH.

Antibody-coated microprobes have been used, for the first time, to study the release of substance P (SP) in two regions of the rat CNS.

Microprobes coated with anti-SP antibodies were inserted sequentially for 10-20 mins either (a) along the mediolateral axis of the substantia nigra (SN) (6 rats) or (b) transversely into the lumbar spinal cord (8 rats). In the region of the SN SP-release was detected following i.p. amphetamine (4 mg/kg). In 2 other rats in which probes had missed the SN by 300μ, no SP release was detected. The origin of this increase in nigral SP may be striatal cells, that contain SP and project to the SN, and which may be excited by amphetamine.

In the lumbar spinal cord SP release was detected in the region of the substantia gelatinosa (SG) following both tibial nerve stimulation (2 min x 50 threshold) and pinch.

These results demonstrate that the antibody microprobe technique can be utilised to detect neuropeptide release in restricted regions of the rat CNS, in both electrophysiological and neuropharmacological studies.

167.8

DIFFERENTIAL EXPRESSION OF NEUTRAL ENDOPEPTIDASE-24.11 (NEP; "ENKEPHALINASE") IN NORMAL, TRANSPLANTED, AND MALIGNANT ASTROCYTES S.A. Back, M. Colon*, C.Y. Endo*, James H. Fallon, F. L. Meyskens, Jr.* and S. Loughlin. Departments of Pediatrics, Medicine, and Anatomy and Neurobiology, University of California, Irvine, CA 92717.

In the CNS of normal animals, NEP is largely expressed by neurons. However, primary human glioma cultures express NEP, suggesting a role for the enzyme in malignant states. We examined the expression of NEP in normal, transplanted and malignant astrocytes using histochemical localization of NEP combined with immunocytochemical localization of the glial marker GFAP.

In normal rat forebrain, little NEP label localized to GFAP-labeled astrocytes, except for occasional cells or processes in layer 1 of neocortex. No astrocytes contained NEP in the striatum. A separate group of animals was examined 5 weeks after receiving transplants of a mixed population of fetal cortical cells (ED 14) into the striatum. At the transplant site, a large encapsulated mixed tumor formed which caused a pronounced mass effect with compression of the lateral ventricle. The tumor contained several distinct populations of cellular elements which co-localized GFAP and NEP. NEP staining in the tumor was markedly greater than in the striatum or cortex on the transplanted or contralateral sides. This suggests that NEP expression is enhanced in glial elements in the tumor relative to normal striatal or cortical neurons. The human astrocytoma cell line U-373 was also used as a glial tumor model to study NEP expression. NEP localized to the cell surface and thin cellular processes suggesting that NEP is membrane-associated. The presence of NEP activity in these cells was confirmed by a colorometric assay using glutaryl-ala-ala-phe-4-methoxy-2-naphthylamide as substrate. The cleavage of this substrate by NEP was confirmed by the potent blockade of substrate cleavage by two selective NEP inhibitors, phosphoramidon and JHF-26, with an ID 50 of 3nM and 1nM, respectively.

These studies support our previous work indicating that in the normal adult rat, NEP is almost exclusively localized to neurons. However, astrocytes show markedly enhanced levels of NEP when they display features of deregulated growth and differentiation. Supported by the Bank of America-Gianinni Foundation NIH-NS26761 and NS15321, ACS-IRG-166Z, The Bud Corbin Neuroscience Award.

168.1

SPECIES DIFFERENCES IN THE PHARMACOLOGY OF 5-HT_2 BINDING SITES. D.L. Nelson, V.L. Lucaites*, and D.B. Wainscott*. Lilly Res. Labs., Lilly Corporate Center, Indianapolis, IN 46285, U.S.A.

Both functional (1) and binding (2) assays have suggested species differences in the pharmacology of the 5-HT_2 receptor. Based on this, the objective of the present study was to examine structural features that allow compounds to discriminate between 5-HT_2 receptors from different species. The 5-HT_2 receptors were measured by [^{3}H]ketanserin binding to cerebral cortical membranes from rat, pig, and monkey. Initial studies were carried out to verify that there was no significant binding of [^{3}H]ketanserin to tetrabenazine-sensitive sites in these tissues and to establish conditions to block [^{3}H]ketanserin binding to α_1-adrenergic receptors. When these conditions were met, it was found that certain ergoline-containing structures could differentially discriminate between 5-HT_2 sites from different species and that this was based, in part, on differences in substituents at the N1 position. Compounds with an N1 isopropyl substituent (LY53857 and LY237733) showed 4-6 fold higher affinity at 5-HT_2 sites in rat membranes than in pig and monkey. Even an N1 substituent as small as methyl produced selectivity for the rat. In contrast, homologs containing an N1 hydrogen (LY86057 and LY193525) had an approximately 10-fold greater affinity for the pig and monkey 5-HT_2 binding sites than for the rat. These data emphasize that species differences in receptor pharmacology must be considered when developing subtype-selective agents and that the rat, though commonly used, is not necessarily the best species on which to base drug design.

1. Killam et al. (1990) J. Pharmacol. Exp. Ther. 252, 1083-1089.
2. Pazos et al. (1985) Eur. J. Pharmacol. 106, 531-538.

168.2

BINDING OF TYPICAL AND ATYPICAL ANTIPSYCHOTIC AGENTS TO 5-HT_{1c} RECEPTORS. B.L. Roth•,**, H.Y. Meltzer** and R.D. Ciaranello•, •Nancy Pritzker Laboratory, Department of Psychiatry, Stanford University and **Department of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Clozapine and similar atypical antipsychotic agents are characterized by their relative preferences for 5-HT_2, as opposed to D_2 receptors (Meltzer et al, JPET, 1989). Because of the close structural, pharmacologic and functional similarities between the 5-HT_2 and 5-HT_{1c} receptors, we determined whether a number of putative atypical antipsychotic agents bind to 5-HT_{1c} receptors as well.

For these experiments 5-HT_{1c} and 5-HT_2 radioligand binding was performed as previously detailed (Roth et al, 1987; 1991) on rat brain membranes under site-specific conditions or in COS-7 cells transiently tranfected with a 5-HT_{1c} cDNA using the vector pSVK3-5HT1c. 12 different atypical agents were studied: setoperone, clozapine, ritanserin, risperidone, tenilapine, rilapine, tiosperone, fluperlapine, SCH23390, amperozide and melperone. Although some of the agents tested showed high affinities for the 5-HT_{1c} receptors (Kd's<50 nM: clozapine, ritanserin, SCH23390) many did not (Kd's>50 nM: setoperone, risperidone, tenilapine, tiosperone, fluperlapine, amperozide, melperone). The atypical nature of these compounds appeared to be better predicted by their binding to 5-HT_2 rather than 5-HT_{1c} receptors.

168.3

CLONING AND NUCLEOTIDE SEQUENCE OF HUMAN(5-HT) TYPE 2 RECEPTOR. Radonna J. Tritch*, Deborah L. Robinson*, Barbara G. Sahagan, Robert A. Horlick*. The DuPont Merck Pharmceutical Co., Biotechnology and CNS Research, Wilmington De.,19880-0400.

Serotonin (5-HT) is a neurotransmitter that interacts with a family of distinct receptors and mediates diverse physiological responses. The sequence of the rat and Chinese hamster 5HT2 receptors have been reported (Julius et al. (1990) PNAS 87, 928-932; Chambard et al. (1990) NAR 18, 5282). Here we report the nucleotide sequence of the human 5HT2 receptor. Using oligonucleotide primers derived from the rat sequence, we have been able to use Polymerase Chain Reaction (PCR) technology to amplify the cDNA for the human 5HT2 receptor. The human amino acid sequence shares an overall 91% identity to both the rat and Chinese hamster sequences.

168.4

DIFFERENTIAL RADIOLIGAND BINDING PROPERTIES OF ^{3}H-5-HYDROXYTRYPTAMINE AND ^{3}H-MESULERGINE IN A CLONAL 5-HYDROXYTRYPTAMINE$_{1C}$ CELL LINE. S. Havlik and S.J. Peroutka. Department of Neurology, Stanford University, Stanford, CA 94305.

^{3}H-5-HT and ^{3}H-mesulergine were used to label 5-HT_{1C} receptors expressed in NIH 3T3 mouse fibroblast cells. Saturation analysis of the radioligand data indicate that ^{3}H-5-HT binding is biphasic. A 2-site model of radioligand binding is significantly more consistent with the data than a 1-site model ($p < 0.01$). The K_D values of ^{3}H-5-HT for 2 populations were 0.5 $\pm$ 0.1 nM and 8.8 $\pm$ 1 nM, while the B_{max} values were 400 $\pm$ 100 pmol/g protein and 3700 $\pm$ 800 pmol/g protein, respectively. Saturation analysis of ^{3}H-mesulergine is monophasic with K_D = 4.2 $\pm$ 0.4 nM and B_{max} = 8800 $\pm$ 600 pmol/g protein. Similar significant differences in apparent B_{max} values were obtained by "cold" saturation analysis. Drug competition studies confirm that both ^{3}H-5-HT and ^{3}H-mesulergine label at least 2 subpopulations of 5-HT_{1C} receptors in NIH 3T3 cells. These data demonstrate that at least 3 distinct populations of 5-HT_{1C} receptors in NIH 3T3 cells can be differentiated by radioligand binding techniques.

168.5

HUMAN 5-HT2 RECEPTOR ENCODED BY A MULTIPLE INTRON-EXON CONTAINING GENE. W. Yang, K. Chen*, J. Grimsby* and J.C. Shih, Dept. of Mol. Pharm. and Tox., Sch. of Pharm., Univ. of South. Calif., L.A. Ca. 90033.

We have recently cloned a human 5-HT2 receptor gene. Since the 5-HT2 receptor gene has been assigned on the human chromosome 13 (Sparks et al., Genomes 9:461, 1991) two human chromosome 13 specific lambda phage libraries (ATCC) were screened. From the library containing EcoRl digested genomic DNA, two positive clones with an insert size of 8.5 and 7.0 kb were isolated. Extensive subcloning and sequencing of these clones showed that the 8.5 kb insert contained exons 1 and 2 of 5-HT2 receptor gene and the 7.0 kb insert contained a part of the third exon. Using the positive fragment in the third exon as a probe, another lambda phage library constructed by HindIII digested genomic DNA was screened. A positive clone which contained a 9 kb insert was isolated and contained the rest of the coding region in exon 3. Sequencing analysis revealed that this human 5-HT2 receptor also consisted of 471 amino acid as in the rat and mouse. The deduced amino acid sequence in these three species shares about 90% identity. The intron and exon organization of human and mouse 5-HT2 receptor gene are identical: they span at least 20Kb and consists of 3 exons. (Supported by NIMH grant R01 MH37020, R37 MH39085, (Merit award), K05 MH00796, and the Welin Professorship).

168.6

CLONED SEROTONIN 5HT_2 RECEPTOR: PHOSPHOINOSITIDE TURNOVER AND GTP INSENSITIVE DOB BINDING. F.G. Szele, J. Zhong, and D.B. Pritchett Children's Seashore House, and Department of Pharmacology, Univ. of PA, Philadelphia, PA, 19104.

Serotonin-2 (5-HT_2) receptors are thought to mediate phosphoinositide turnover by interaction with a GTP binding protein. Addition of non-hydrolysable GTP analogues have been shown to convert the receptor to a low affinity state. We transfected mammalian 293 cells, which normally do not express serotonin receptors, with DNA encoding the 5-HT_2 receptor to assess the binding of ligands and the effect of GTP analogues on that binding. After transfection, Scatchard transformation of saturation binding to cell membranes with ^{3}H-Ketanserin (a 5-HT_2 receptor specific antagonist) revealed a linear, single-site with a K_d of 0.4 $\pm$ 0.01 nM and a B_{max} of 557 $\pm$ 80 fmol/mg protein. ^{3}H-1-(4-Bromo-2,5-dimethoxyphenyl)-2-aminopropane (^{3}H-DOB) (a 5-HT_2 receptor specific agonist) saturation binding generated curvelinear Scatchard plots which were best fit by a two-site model with a high affinity K_d of 0.5 nM (B_{max}=40 fmol/mg protein) and a low affinity K_d of 3.0 nM (B_{max}=122 fM/mg protein). Concentrations of GTPγS or GppNHp as high as 10^{-4} M decreased ^{3}H-DOB by less than 10% and had negligeable effects on ^{3}H-Ketanserin binding. ATPγS had no effect on binding of either compound. However, 5-HT and DOI were effective in stimulating transfected 5-HT_2 receptors to increase phosphoinositide (PI) hydrolysis 5 fold and 8 fold, respectively. These results show that the cloned serotonin receptor is capable of binding agonists as well as antagonists and that it has two affinity states for ^{3}H-DOB. The lack of non-hydrolysable GTP analogue effects together with the robust PI turnover indicates that these receptors couple to a novel G protein possibly G_q which is insensitive to these compounds.

168.7

EFFECT OF ANTIDEPRESSANT TREATMENT ON SEROTONIN2 RECEPTOR-MEDIATED CALCIUM MOBILIZATION IN RAT GLIOMA C6BU-1 CELLS. S.Muraoka*, M.Mikuni, A.Kagaya, K.Saitoh*, M.Ikeda and K.Takahashi. Div. Mental Disorder Res., Natl. Inst. Neurosci. NCNP. Kodaira Tokyo, 187 Japan.

It is known that there exists serotonin2 receptors to couple to phosphoinositide hydrolysis in rat glioma C6BU-1 cells. We have now characterized serotonin2 receptor-stimulated intracellular calcium mobilization and investigated an effect of antidepressants on the calcium movement in C6-BU-1. Serotonin increased intracellular calcium in a dose dependent manner and it was antagonized by ketanserin(KET)with an IC50 of 1nM, indicating the response was mediated by serotonin2 receptors. In the presence of clomipramine(CMI), it inhibited 10uM serotonin-induced response with an IC50 of 800nM, and haloperidol(HPD) had similar effect. When C6BU-1 was pretreated with CMI,HPD or KET for 2hr followed by washing, they inhibited the response to 30,80 or 80%, respectively. Coexposure of KET and CMI showed the similar inhibitory effect as CMI alone. These results indicate that CMI can inhibit serotonin-induced calcium movement by affecting intracellular signaling system, rather than receptor blocking action.

168.8

RESPONSIVENESS OF 5-HT-2 RECEPTOR-MEDIATED CALCIUM MOBILIZATION IS INCREASED IN PLATELETS OF DEPRESSED PATIENTS AND IN C6 GLIOMA CELLS PRETREATED WITH DEXAMETHASONE. M.Mikuni, A.Kagaya S.Muraoka*and K.Takahashi. Div. Mental Disorder Res., Natl. Inst. Neurosci., NCNP, Tokyo, Japan.

The increase in intracellular Ca concentration induced by 10uM 5-HT in platelets from 11 normal controls was 105+6nM, while that from 11 depressed patients was 129+4nM. This result is consistent with our previous observation that 5-HT-stimulated inositol phospholipid hydrolysis in platelets from depressed patients is greater than that of normal controls, suggesting that 5-HT-2 receptor function in platelets is enhanced in patients with affective disorders. On the other hand, we found that 5-HT-2 receptors were responsible for activating Ca mobilization in C6 glioma cells, and that 48-hour treatment with 100nM dexamethasone enhanced 5-HT-induced Ca mobilization in this clonal cells. Co-treatment with 1uM Ru38486 and dexamethasone inhibited this dexamethasone-induced enhancement, whereas Ru alone did not alter 5-HT-induced Ca mobilization in C6 cells. Taken together, these results suggest that the disinhibition of HPA axis may be in part, responsible for enhancing 5-HT-2 receptor function in affective disorders.

168.9

ENHANCEMENT OF SEROTONIN2 RECEPTOR-STIMULATED INTRACELLULAR CALCIUM MOBILIZATION IN HUMAN PLATELETS. A.Kagaya, M.Mikuni, S.Yamawaki*, and K.Takahashi. Div. Mental Disorder Res., Natl. Inst. Neurosci., NCNP. Tokyo, 187 and Dept. Neurol. Psychiat. Hiroshima Univ. Sch. Med. Hiroshima, 734 Japan.

We recently investigated serotonin2 receptor-mediated calcium mobilization in human platelets. To make the mechanism of the receptor function clear, we have studied the modulation of serotonin2 receptor-mediated calcium movement using fura-2. An alpha2-adrenergic agonist norepinephrine, when pretreated 3 min prior, enhanced the response to serotonin up to 120%. This enhancement was not abolished by treatment with 2mM EGTA, indicating that this fascilitation was from internal storage sites. W-7, one of naphthalenesulfonamide derivatives also potentiated the serotonin-induced calcium response up to 145%, and the potentiation was inhibited by chelating extracellular calcium, suggesting that the W-7-induced enhancement was influx from extracellular calcium. Thus, serotonin2 receptor can be enhanced from different pathways to the same effector system.

168.10

INVOLVEMENT OF 5-HT$_2$ RECEPTORS IN PHARMACOLOGICAL EFFECTS OF NAFTIDROFURYL OXALATE

T. Nabeshima[1], K. Niwa*[2], M. Hiramatsu[2], K. Fuji*[2] and T. Kameyama[2]. [1]Dept. Hospital Pharmacy, Nagoya Univ. Sch. Med., Nagoya 466, Japan and [2]Dept. Chem. Pharmacol., Meijo Univ., Nagoya 468, Japan.

Naftidrofuryl oxalate(LS-121) has been used as a therapeutic drug for improving cerebral circulation. In behavioral studies, LS-121 has been reported to have anti-amnesic effects on several animal models. We have suggested the involvement of serotonergic neuronal systems in the anti-amnesic action of LS-121 against the amnesia induced by 5-hydroxytryptophan(5-HTP) and cycloheximide, since LS-121 has affinity only for 5-HT$_2$ receptors among several receptors in the rat synaptic membrane. In the present experiment to confirm the effects of LS-121 on 5-HT$_2$ receptors, the effects of LS-121 on 5-HT$_2$ receptors in the discrete brain areas by using quantitative autoradiography and on (5-HTP + pargyline)-induced head-twitch response were investigated. LS-121 inhibited the [^{3}H] ketanserin binding in the frontal cortex(IC50: 1.53x10^{-7} M), cingulate cortex, nucleus accumbens, caudate-putamen and hippocampus. The potency of LS-121 was less than those of methysergide(9.13x10^{-9} M) and ritanserin(2.69x10^{-9} M). LS-121 inhibited (5-HTP + pargyline)-induced head-twitch response in a dose dependent fashion. These results suggested that LS-121 affects the 5-HT$_2$ receptors as an antagonist and these effects may be responsible for its pharmacological effects.

168.11

EFFECTS OF VARIOUS SEROTONIN RECEPTOR SUBTYPE SELECTIVE ANTAGONISTS ON m-CPP-INDUCED INCREASES IN PLASMA PROLACTIN AND CORTICOSTERONE LEVELS IN RATS. T. Tolliver*, C. S. Aulakh*, J. L. Hill*, J. Tolliver, and D. L. Murphy*. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Administration of m-CPP (a post synaptic 5-HT receptor agonist) to rats increases plasma concentrations of prolactin and corticosterone. In the present study, we investigated the role of various 5-HT receptor subtypes in mediating m-CPP-induced increases in plasma prolactin and corticosterone in male Wistar rats. Saline or various doses of each antagonist were injected intraperitoneally (i.p.) 15 min prior to administrating saline or m-CPP. Animals were sacrificed 30 min after injection of saline or m-CPP. Administration of m-CPP (2.5 mg/kg) significantly increased plasma concentrations of prolactin and corticosterone compared to saline treatment. Pretreatment with nonselective 5-HT antagonist metergoline, and with the partially selective 5-HT$_{1C}$ antagonists mesulergine and mianserin attenuated m-CPP-induced increases in plasma prolactin but not corticosterone. On the other hand, pretreatment with 5-HT$_{1B}$ antagonists iodocyanopindolol and CG361, the 5-HT$_{1A}$ the antagonist spiperone, the 5-HT$_{1A}$ and 5-HT$_{1B}$ antagonist propranolol, the 5-HT$_2$ antagonist ritanserin, and the 5-HT$_3$ antagonists ICS 205930 and MDL-72222 did not affect m-CPP-induced increases in either plasma prolactin or corticosterone levels. These findings suggest that m-CPP-induced increases in plasma prolactin are mediated by stimulation of 5-HT$_{1C}$ receptors while non-serotonergic mechanisms may be involved in m-CPP's effects on plasma corticosterone.

168.12

PROTEIN KINASE C AND 5-HT$_2$ RECEPTORS IN THE SECRETION AND ACTION OF THYROID 5-HT H. Tamir, K. P. Liu*, M. Adlersberg*, S.H. Hsiung*, P.Y. Yu*, and M.D. Gershon. Div. Neurosci., N.Y. State Psych. Inst. and Dept. Anat. and Cell Biol. Columbia University., P&S, N.Y., NY 10032.

Thyroid follicles are comprised of follicular (F) and parafollicular (PF) cells. Thyrotropin (TSH) or ↑ $[Ca^{2+}]_e$ induce PF cells to secrete 5-HT, which activates F cells. 5-HT secretion was studied in MTC cells, a PF-derived cell line. Ca^{2+}-dependent secretion of 5-HT was induced by forskolin, dibutyryl (db)-cAMP, or cholera toxin; however, neither TSH nor ↑ $[Ca^{2+}]_e$ increased cAMP. Instead, TSH and ↑ $[Ca^{2+}]_e$ increased $[Ca^{2+}]_i$. Acute treatment with phorbol esters stimulated 5-HT secretion; staurosporin (0.1 nM) and down-regulation of protein kinase C (PKC) by chronic exposure to phorbol ester antagonized the TSH-induced secretion of 5-HT (but not that due to ↑ $[Ca^{2+}]_e$ or db-cAMP). The actions of 5-HT were investigated on FRTL-5 cells, a F-derived cell line. Membranes from FRTL-5 cells bound the 5-HT$_2$ probe, ^{125}I-DOI, specifically, saturably, and with high affinity; ^{125}I-DOI binding was inhibited by GTP-γ-S. 5-HT increased cAMP, phosphoinositide (PI) turnover, and $[Ca^{2+}]_i$ in FRTL-5 cells; these actions were blocked by ketanserin, spiperone, and (except ↑ cAMP) pertussis toxin (PTx). Immunostaining with an anti-idiotypic antibody confirmed the presence of 5-HT$_2$ receptors on FRTL-5 cells. It is concluded that 5-HT is released from MTC cells by PKC-dependent and PKC-independent mechanisms. 5-HT activates FRTL-5 cell 5-HT$_2$ receptors, which are coupled *via* a PTx-sensitive G protein to PI turnover. The 5-HT$_2$ effect of 5-HT on cAMP is probably indirect. Supported by NIMH grant 37575 and NIH grant NS 12969.

168.13

MEDIATION OF ENHANCED SKF 38393-INDUCED ORAL ACTIVITY BY A SEROTONIN SYSTEM. L. Gong*, E.A. Daigneault and R.M. Kostrzewa. Dept. of Pharmacology, College of Medicine, East Tennessee State Univ., Johnson City, TN 37614.

A neonatal 6-hydroxydopamine (6-OHDA) lesion of nigrostriatal dopamine (DA) neurons is accompanied by serotonin (5-HT) fiber hyperinnervation of the striatum. This study was done, to test whether the 5-HT system could mediate supersensitization of DA D1 agonist-induced oral activity. Rats received 6-OHDA (134 µg, half in each lateral ventricle) at 3d after birth and were studied as adults. An enhanced oral response to the 5-HT1C receptor agonist, m-chlorophenylpiperazine (mCPP) was noted in lesioned vs. control rats. Neither 8-hydroxy-2-(di-n-propyl-amino)tetralin (8-OH-DPAT) nor CGS-12066B, respective 5-HT1A and 1B agonists, induced oral activity. The mCPP-induced oral response was attenuated by mianserin, a 5-HT1C receptor antagonist, but not by ketanserin or MDL 72222, 5-HT2 and 5-HT3 receptor antagonists, respectively. The DA D1 receptor antagonist, SCH 23390, failed to attenuate mCPP-induced oral activity, but mianserin effectively attenuated the oral response to SKF 38393, a DA D1 receptor agonist. These findings demonstrate concurrent supersensitization of a 5-HT system after lesion of a DA system. Also, this 5-HT system is found to mediate the supersensitized response of DA D1 receptors. (Supported by the Scottish Rite Schizophrenia Research Foundation)

168.14

THE PLATELET SHAPE CHANGE REACTION: CHARACTERIZATION AND POTENTIAL USEFULNESS AS A MARKER OF AFFECTIVE DISORDERS. J.R. Magliozzi, D.W. Gietzen, and R.J. Maddock*. Depts. of Psychiatry, University of California, Davis Sch. of Med., Sacramento, CA and University of New Mexico Sch. of Med., Albuquerque, NM.

Blood platelets possess a number of attributes of neuronal cells which may be amenable to investigation in neuropsychiatric disease. Among these is a receptor for serotonin which resembles the brain $5HT_2$ receptor. In normal human subjects, this receptor mediates a shape transition from discoid to spheroid which can be monitored by light scattering, using slight modifications of standard platelet aggregometry methods. The shape change reaction is characterized by inhibition by ketanserin and spiperone at low nanomolar concentrations, but not by the $5HT_3$ inhibitors ICS-205930 and MDL-72222. The reaction was not affected by extracellular EGTA at a concentration range of 0.01-1.0 mM or EDTA at a concentration range of 0.01- 170 mM. In a series of 10 patients with unipolar major depression and 10 normal control subjects the velocity of the phase transition one second after addition of 1 micromolar 5HT was affected by age (b'=-0.62; p=0.004), with a trend noted for the effect of diagnostic group (unipolar major depression vs. control subject; 1.0 ± 0.45 (S.E.M.) mv s^{-1} vs. 3.0 ± 0.71 mv s^{-1}). These preliminary data suggest the platelet shape change reaction may be a useful marker for the $5HT_2$ receptor and its associated effector system function in human affective disorders.

168.15

5-HT-1C MEDIATED EFFECTS ON APOMORPHINE-INDUCED LOCOMOTOR ACTIVITY. P.B. Hicks R.J. Zavodny* and K.A. Young. Department of Psychiatry, Scott & White Hospital/Foundation; Departments of Medical Pharmacology and Medical Anatomy and Neurobiology, Texas A&M College of Medicine, Temple, Texas, 76508.

Serotonin (5-HT) neurotransmission, mediated by various 5-HT receptor subtypes, modifies certain aspects of behavior associated with dopamine (DA) neurotransmission. We determined the effect of the 5-HT-1C/2 agonist DOI and the 5-HT-1C/2 antagonist mesulergine on apomorphine-induced locomotor activity (AILA) in the rat and compared the results to the AILA effects of ketanserine, a 5-HT-2 antagonist. Serotonergic compounds and apomorphine (0.25 mg/kg) were administered SC and locomotor activities (line crossings) were monitored by videotape in 5 min intervals for 30 min. The 5-HT-2 antagonist ketanserine suppressed AILA. The 5-HT-1C/2 antagonist mesulergine potentiated AILA at a low dose (0.1 mg/kg) that had no effect on spontaneous locomotor activity. It is expected that at this dose, mesulergine would be more active at 5-HT-1C receptors. At higher doses of mesulergine (0.5-1.0 mg/kg), a 5-HT-2 antagonist effect to depress locomotor activity predominates (as demonstrated by ketanserine-induced suppression of AILA). The 5-HT-1C/2 agonist DOI (1.0 mg/kg), which has a higher affinity for 5-HT-1C than 5-HT-2 receptors, blocked the AILA stimulation produced by low-doses of the 5-HT-1C/2 antagonist mesulergine. This combination treatment resulted in locomotor activity similar to the control group which received apomorphine only. Therefore, the AILA potentiation of low-dose mesulergine is most likely mediated by 5-HT-1C receptors. These findings suggest a prominent role for 5-HT-1C receptors in the modulation of behaviors associated with DA neurotransmission in the nucleus accumbens.

168.16

REGULATION OF cAMP FORMATION BY $5HT_2$ RECEPTOR ACTIVATION IN THE A1A1 CELL LINE. K.A.Berg[1], B.J. Ebersole[1], W.P. Clarke[2], R. McKay[3], and S. Maayani[1,2]. Depts. of [1]Anesthesiology and [2]Pharmacology, Mt. Sinai School of Medicine, CUNY, NY, NY 10029 and [3]Dept. of Biology and Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

The effects of $5HT_2$ receptor activation on cAMP formation were studied in a cell line derived from embryonic rat cortex (A1A1). 5-HT (EC_{50} =350 nM) enhanced the amount of cAMP (2.5-6 fold) formed in response to1 µM forskolin (FSK) after 15 min of co-incubation in the presence of a PDE inhibitor. The effect of 5-HT was blocked by 10 nM ketanserin and 10 nM spiperone indicating a response mediated by the $5HT_2$ receptor subtype. Similarly, 5-HT potentiated the amount of cAMP accumulated in response to cholera toxin and 5'-N-ethylcarboxamidoadenosine (NECA; an adenosine A_2 receptor agonist). After a 15 min co-incubation, the PKC activator phorbol 12-myristate 13-acetate (PMA) increased cAMP formed in response to FSK and NECA. After a 15 min pre-incubation with PMA, a condition which results in a loss of 5-HT stimulated PI turnover in these cells (Ebersole et al., this volume), 5-HT increased forskolin- and NECA-stimulated cAMP levels. Following exposure to PMA for 24 hrs, a condition which promotes PKC down-regulation, 5-HT still enhanced cAMP formation in response to FSK and NECA while the potentiating effects of PMA were reduced or abolished. These data suggest that $5HT_2$ receptor activation regulates cAMP formation in A1A1 cells by a mechanism(s) in addition to PI turnover and PKC activation.(Support: GM34852 and NS21991)

168.17

$5HT_2$ RECEPTORS ARE LINKED TO PHOSPHATIDYLINOSITOL TURNOVER IN A NOVEL CELL LINE DERIVED FROM RAT CORTEX. B.J. Ebersole[1], K.A. Berg[1], R. McKay[3], and S. Maayani[1,2]. [1]Depts. of Anesthesiology and [2]Pharmacology, Mt. Sinai School of Medicine of CUNY, NY, NY 10029 and [3] Dept. of Biology and Brain & Cognitive Science, MIT, Cambridge, MA 02139.

A novel cell line, A1A1, derived from embryonic rat cortex, was used as a model to study signal transduction systems associated with receptors for 5-hydroxytryptamine (5-HT) in the CNS. Cells were placed in serum-free medium two days before use to minimize effects of the serum present in the growth medium. In cells prelabeled for 48 hrs. with [^{3}H]-*myo*-inositol, exposure to 5-HT (EC_{50}=700 nM) for 10 min. resulted in a 3-fold increase in the amount of [^{3}H]-inositol phosphates ([^{3}H]IPs) accumulated in the presence of 20 mM LiCl. The response to 1 µM 5-HT was blocked by 10 nM spiperone and 10 nM ketanserin, indicating a response mediated by $5HT_2$, rather than $5HT_{1C}$, receptors. Both quipazine and DOI were partial agonists, with intrinsic activities of 0.15 and 0.4, respectively. Pre-treating the cultures for 24 hrs. with 1 µM phorbol 12-myristate 13-acetate (PMA), a condition that promotes down-regulation of protein kinase C (PKC), resulted in a greatly enhanced response to 5-HT (7-fold stimulation), while a 15 min. pre-treatment with PMA, a condition that activates PKC, resulted in a complete loss of 5-HT-stimulated accumulation of [^{3}H]IPs. These results indicate that the $5HT_2$ receptor linked to phosphatidyl-inositol turnover in A1A1 cells can be modulated by PKC. (Supported by GM34852, DA06620, and NS21991).

168.18

ELEVATION OF CATECHOLAMINERGIC METABOLITE LEVELS IN THE RAT HYPOTHALAMUS BY AN AGONIST OF SEROTONIN 1C/2 RECEPTORS, MK212 (6-chloro-2-(1-piperazinyl) pyrazine). D.A. Mayle*, D.W. Robertson, and D.T. Wong. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285

MK212, an agonist at both the $5HT_{1C}$ and the $5HT_2$ serotonin receptor subtypes (1), was injected intraperitoneally into male Harlan Sprague Dawley rats (130-160g), and levels of norepinephrine (NE), serotonin (5HT), dopamine (DA) and their metabolites were measured in the rat hypothalamus. MK212 (0; 0.5; 1.0; 2.0; 4.0; 6.0; 10.0 mg/kg i.p.), administered 30 minutes before sacrifice, caused dose-dependent increases in dopamine (DA), its metabolite, 3-4-dihydroxyphenylacetic acid, and the metabolite of NE, 3-methyoxy-4-hydroxy-phenylglycol sulfate. The time course of MK212 revealed a peak in DA and metabolite levels between 15 and 30 minutes after injection, with a return to control levels two hours after injection. These increases were effectively attenuated by prior administration of serotonergic antagonists, such as metergoline (0.1-0.6 mg/kg i.p.), mianserin (1.0-10.0 mg/kg i.p.), LY53857 (0.3-3.0 mg/kg i.p.), and ketanserin (ineffective at 0.1-0.3 mg/kg, but effective at 1.0 mg/kg i.p.). These indicies implicate either an increase in both NE turnover in noradrenergic neurons and DA turnover in dopaminergic neurons, or simply an increase in NE turnover in noradrenergic neurons (2), due to the activation of serotonin receptors. Serotonergic modulation of NE from rat hypothalamic slices has been demonstrated *in vitro* (3); moreover, the present study supports the possibility of serotonergic modulation of noradrenergic and/or dopaminergic neurons, mediated by the activation of $5HT_{1C}$ / $5HT_2$ receptors, *in vivo*.

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168.19

RADIOIODINATED (R)-DOI AS AN *IN VIVO* LIGAND FOR BRAIN SEROTONIN RECEPTORS. C.A. Mathis and A. Biegon, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

The feasibility of using radioiodinated (R)-2,5-dimethoxy-4-iodo-amphetamine ((R)-DOI) as an *in vivo* SPECT imaging agent for brain serotonin receptors was examined with *in vivo* autoradiography in the rat and *in vitro* autoradiography in postmortem human brain. Following the tail vein injection of (R)-[^{125}I]DOI (specific activity 2175 Ci/mmole), the ligand readily crossed the blood-brain-barrier and accumulated in the rat brain, with cortex-to-blood ratios of ~5:1 at 1 h. The percent of the injected dose found in the brain at 5 min and 1 h were 0.7% and 0.4%, respectively. The distribution of radioactivity at 1 h following injection of 60-250 μCi/kg was similar to the distribution of serotonin 5-HT2 and 5-HT1C receptors. High levels were found in the cortex, known to be enriched in 5-HT2 receptors, and in the choroid plexus which has high concentrations of 5-HT1C receptors. Ketanserin, a selective 5-HT2 antagonist, injected 10 min before (R)-[^{125}I]DOI, produced a dose dependent reduction in brain radioactivity *in vivo*. At doses around 500 μCi/kg, (R)-[^{125}I]DOI appeared to lose its specificity, as a high density of radiolabeling appeared over the thalamus which equaled or exceeded cortical binding. In the postmortem human brain, (R)-[^{125}I]DOI binding was fully displaceable by excess ketanserin. Specific binding was highest in human cortex, particularly occipital cortex. Only moderate to low levels were found in subcortical regions. This pattern is in good agreement with the known autoradiographic distribution of 5-HT2 receptors in the human brain postmortem. The above results indicate that (R)-[^{123}I]DOI may be a useful agent for imaging 5-HT receptors *in vivo*, albeit within a limited dose/concentration range.

168.20

EFFECTS OF AGONISTS, PARTIAL AGONISTS AND ANTAGONISTS ON 5-HT_2 RECEPTORS IN P11 CELLS **R. C. Ferry, C. D. Unsworth* and P. B. Molinoff, Dept. of Pharm., Univ. of Pennsylvania Sch. of Med., Phila., PA 19104.**

Central 5-HT_2 receptors fail to exhibit traditional adaptive/compensatory mechanisms. Although administration of agonists *in vivo* leads to a decrease in 5-HT_2 receptor levels, 5-HT_2 receptors fail to up-regulate following removal of synaptic input or depletion of 5-HT stores. Administration of antagonists leads to a paradoxical decrease in the density of 5-HT_2 receptors. It is not known whether the anomalous behavior of the 5-HT_2 receptor is due to a unique regulatory mechanism or is the result of complexities inherent in the use of *in vivo* models. P11 cells, which express a high density of 5-HT_2 receptors coupled to PI hydrolysis, were used as a model system to study the regulation of 5-HT_2 receptors on cultured cells. Treatment of P11 cells with 1 or 10 μM 5-HT resulted in a 50% reduction in the number of 5-HT_2 receptors as measured by binding of ^{3}H-ketanserin or ^{125}I-LSD. Following removal of 5-HT, the density of receptors returned to control levels within 24 hr. P11 cells were also treated with the partial agonists LSD and DOI. Scatchard analyses of the binding of ^{3}H-ketanserin revealed that exposure to 100 nM LSD or 100 nM DOI led to a reduction in the density of receptors on P11 cells of 30 to 40%, which is comparable to the effect of the full agonist 5-HT. In contrast to studies carried out *in vivo*, the 5-HT_2 receptor antagonist ketanserin failed to elicit a change in the density of 5-HT_2 receptors. No change in the apparent affinity of the receptors for ^{3}H-ketanserin or ^{125}I-LSD was observed following any of the above treatment regimens, indicating that the observed change in B_{max} was not a consequence of residual drug. P11 cells represent a useful model system with which to study the regulation of 5-HT_2 receptors. (USPHS Grant MH 48125)

168.21

AGONIST-INDUCED DESENSITIZATION OF 5-HT_2 RECEPTOR-MEDIATED PHOSPHOINOSITIDE TURNOVER IN CEREBELLAR NEURONS. J. Akiyoshi* and D.-M. Chuang. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

We have previously shown that cerebellar granule cells express 5-HT_2 receptor-mediated phosphoinositide (PI) hydrolysis. The maximal stimulation of 5-HT-induced inositol phosphate accumulation was about 700% of the control and occurred at 10 μM of this agonist. In the study, we investigated agonist-induced desensitization and changes in 5-HT_2 receptor binding sites. Prestimulation of granule cells with 10 μM 5-HT induced a time-dependent decrease of 5-HT-induced PI hydrolysis. This desensitization was evident at 0.5 hr and reached its maximum (about 90% decrease) at 8 hr. Similar time-dependent desensitization of the PI response to 5-HT was observed when DOI (1-(2,5)-dimethoxy-4-iodophenyl-2-aminopropane) was used as the prestimulating receptor agonist; however, DOI appeared to have considerable toxicity for these cerebellar neurons, after more than 8-hr pretreatment. Unexpectedly, 5-HT-induced desensitization was not associated with a decrease in ^{3}H-ketanserin binding. In fact, a small but significant increase in ^{3}H-ketanserin binding occurred after 5-HT pretreatment. This observation is in contrast to that found in carbachol-induced homologous desensitization of the PI response in which marked loss of muscarinic receptor sites occurred after treatment with carbachol for more than 2 hr and progressed with the pretreatment time. Molecular events underlying 5-HT-induced desensitization and changes in receptor binding are under investigation.

168.22

COMPARISON OF THE MOLECULAR REGULATION AND PHARMACOLOGY OF 5-HT_2 RECEPTORS IN BRAIN AND SMOOTH MUSCLE CELLS. L. Rydelek-Fitzgerald[1*], B.D. Wilcox[2], M. Teitler[1*], and J.J. Jeffrey[2]. [1]Dept. of Pharm/Tox and [2]Dept. of Medicine, Albany Medical College Albany, NY 12208

Recently, serotonin was found to be necessary for collagenase production in rat and human uterine smooth muscle cells. Preliminary pharmacological studies suggested that serotonin may be mediating this effect through the 5-HT_2 receptor. In order to definitively establish the presence and functionality of uterine smooth muscle 5-HT_2 receptors, radioligand binding studies were performed in primary cultures of rat uterine smooth muscle cells. Competition of serotonergic agonists and antagonists for the 5-HT_2 receptor agonist radioligand [^{125}I]DOI demonstrated that the uterine receptor has the same pharmacology as the 5-HT_2 receptor in the rat brain. Further, 5-HT_2 receptor mRNA transcripts from rat uterine smooth muscle cell cultures were similar in size to those found in the rat brain. Dose-response relationships with 5-HT_2 agonists in the uterine smooth muscle cell cultures were determined using an ELISA to measure collagenase production. Serotonin and DOI stimulate collagenase production in a dose-dependent manner, indicating these cells should prove valuable in determining 5-HT_2 receptor agonist potency and efficacy. 5-HT_2 receptor agonists (5-HT, DOI, quipazine) induced collagenase mRNA in rat uterine smooth muscle cell cultures. In addition, the agonists increased 5-HT_2 receptor mRNA. The changes in collagenase and 5-HT_2 receptor mRNA were blocked by the antagonists, ketanserin and spiperone. Thus, this in vitro culture system represents a unique model for the study of the molecular biology of 5-HT_2 receptor regulation. Future studies will assess the levels of 5-HT_2 receptors in cell cultures and in vivo to determine whether these levels are regulated to control collagenase production.

168.23

RADIOLABELLING OF MULTIPLE AGONIST AFFINITY STATES OF BRAIN AND CLONED $5HT_{1C}$ SEROTONIN RECEPTORS. S. Leonhardt and M. Teitler. Dept. of Pharmacology & Toxicology, Albany Medical College, 47 New Scotland Avenue, Albany, New York 12208.

$5HT_{1C}$ receptors have been detected with radioligand binding assays using various radioligands (1) and have been cloned and expressed in transfected cells (2). Although this receptor is functionally, pharmacologically and structurally closely related to $5HT_2$ receptors, there are some striking differences in radiolabelling properties. For instance, $5HT_{1C}$ receptors can be labelled with ^{3}H-serotonin, while $5HT_2$ receptors cannot. Detailed studies of the molecular and pharmacological properties of brain and cloned $5HT_{1C}$ receptors using both antagonist and agonist radioligands were conducted in order to investigate the presence, properties, and levels of different agonist affinity states. Saturation studies with ^{3}H-mesulergine and ^{125}I-DOI revealed a lower B_{max} value for specific ^{125}I-DOI binding to $5HT_{1C}$ receptors than for ^{3}H-mesulergine binding in membranes prepared from pig choroid plexus and NIH-3T3 cells expressing $5HT_{1C}$ receptors derived from transfection with a $5HT_{1C}$ cDNA. Binding assays in brain and transfected cell membrane homogenates revealed the presence of two agonist affinity states. These and other studies to be presented reveal molecular similarities and some intriguing differences between radiolabelled $5HT_{1C}$ and $5HT_2$ receptors. The differences may be helpful in designing $5HT_{1C}$ specific agonist and antagonist ligands for studies aimed at determining the functionality of the brain $5HT_{1C}$ receptor.

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169.1

HISTAMINE RELEASES NOREPINEPHRINE FROM THE PARAVENTRICULAR NUCLEUS/ANTERIOR HYPOTHALAMUS REGION IN THE CONSCIOUS RAT. S. L. Bealer, Dept. Physiol., Univ. Tennessee, Memphis, TN 38163

Histamine (HA) increases norepinephrine (NE) release from brain slices and synaptosomes. The present study determined if HA also increases extracellular concentrations of NE in the paraventricular/anterior hypothalamic region (PV/AH) of the conscious rat. A microdialysis probe was positioned adjacent to the PV/AH 24 hr prior to the experiment. During testing, microdialysis probes were perfused with artificial cerebrospinal fluid (ACSF) and dialysate was collected during two 45-minute control periods. ACSF containing HA was then perfused for two 45-min periods followed by one 45-min recovery period. Dialysate NE concentrations were subsequently measured using radioenzymatic assay. Control dialysate NE concentration (23 $\pm$ 5 pg/ml) was significantly increased during dialysis with ACSF containing 1, 3, and 10 ug/ml HA (120$\pm$15 pg NE/ml; 560$\pm$25 pg NE/ml; and 1575$\pm$175 pg NE/ml, respectively). Dialysate NE concentration returned to control levels during the recovery period. These data demonstrate that HA increases extracellular concentrations of NE in the PV/AH region of the brain, possibly through release of neuronal stores. (Supported by USPHS Grant HL-25877 and American Heart Association Grant 88-1103)

169.2

ADRENAL HORMONES AND CIRCADIAN VARIATION ON THE HYPOTHALAMIC HISTAMINE ACTIVITY IN RATS . A. Gillich, & M. J. Meaney. Douglas Hospital Res. Ctr., Depts. of Neurology and Neurosurgery&Psychiatry, McGill Univ., Montreal H4H 1R3, Canada.

Histaminergic cell bodies, found within the posterior hypohalamus, appear to be involved in the regulation of CRF release from neurons within the paraventricular nucleus (PVN). In these studies, we examined the diurnal variation in histamine content in relation to plasma glucocorticoid levels in several brain regions. Long Evans rats were sacrificed at 8PM, 12AM, and 8AM. The PVN, the periventricular nucleus, median eminance, supraoptic nucleus, suprachiasmatic nucleus, mammallary nucleus, and the posterior hypo-thalamus were isolated by the punch technique and assayed for histamine using a single label radioenzymatic technique. We found that the highest histamine levels occured in all areas during the early AM periods except for the posterior hypothalamus. Long Evans rats were then either adrenalectomized (ADX) or sham operated. After 7 days of treatment with various levels of corticosterone replacement, the ADX animals were sacrificed and histamine levels were measured in the same brain regions. The results showed that 1) ADX caused a decreased histamine levels in all ares examined, and 2) these effects could not be totally reversed by corticosterone replacement even with the administeration of high stress levels of corticosterone. These findings suggest that the diurnal variation in hypothalamic histamine activity occurs independantly of glucocorticoid influences. Current studies are underway to examine other substances which may influence histamine activity.

169.3

H_3-HISTAMINE RECEPTORS ACTIVATE K^+ CHANNELS TO HYPERPOLARIZE MAGNOCELLULAR HISTAMINERGIC NEURONS OF RAT POSTERIOR HYPOTHALAMUS. Q.Z. Yang and G.I. Hatton, Neuroscience Program, Michigan State Univ., East Lansing, MI 48824.

Brain neuronal histamine (HA) emanates from the magnocellular neurons of the tuberomammillary (TM) nuclei in the posterior hypothalamus. This relatively small group of neurons projects to almost all brain areas, in some of which evidence exists for HA autoreceptors. We have investigated the possibility that HA receptors play a role in the control of the HAergic neurons themselves. Intracellular recordings were made of 30 magnocellular neurons in hypothalamic slices containing the rostral portion of the ventral TM subnucleus (TMVr), virtually all of which have been shown immunocytochemically to be HAergic. Bath-applied HA (10^{-4}M) depressed both spontaneous and current evoked spikes and hyperpolarized TMVr cells. These effects were selectively mimicked by α-methylHA (H_3-agonist) and selectively antagonized by impromidine (H_3-antagonist). With synaptic transmission blocked by 9.3 mM Mg^{2+}, 0.05 mM Ca^{2+}, HA produced a hyperpolarization (5-40 mV) which was antagonized by the K^+ channel blocker 4-aminopyridine. Na^+ channel blockade with TTX (1 μM) revealed Ca^{2+} spikes which broadened when TEA (5mM) was added to the medium. TEA prevented the hyperpolarizing effects of HA or α-methylHA that were seen in TTX or control medium. Lucifer Yellow injections of some cells confirmed their magnocellular morphology and TMVr location. We conclude that HAergic neurons have H_3-receptors linked to K^+ channels and that these may serve a negative feedback function. Whether activation of these receptors is normally accomplished by recurrent axon collaterals or by inputs from nearby HAergic neurons is not known. Research supported by NIH grant NS16942.

169.4

MODULATION OF TSH SECRETION BY MANIPULATION OF HYPOTHALAMIC HISTAMINE LEVELS. L. Tuomisto[1], R. Tuominen[2] and P. Männistö*[,2]. Dept. of Pharmacol. and Toxicol., [1]Univ. of Kuopio, POB 1627, SF-70211 Kuopio, and [2]Univ. of Helsinki, Helsinki, Finland.

There is some indication of inhibitory but indirect influence of histaminergic systems in the regulation of thyrotropin (TSH) secretion. In this study histamine synthesis inhibitor α-fluoromethylhistidine (FMH, 100 mg/kg 24 h and 50 mg/kg 4 h before, i.p.) and catabolism inhibitor metoprine (20 mg/kg 5 h before, i.p.), were given to male rats. After a cold-stress (60 min at +4°C), hypothalamic histamine was measured by HPLC post-column derivatization method, and hypothalamic TRH and plasma TSH and prolactin by radioimmunoassay. Hypothalamic histamine was significantly decreased by FMH and increased by metoprine. TRH was decreased by cold stimulation, and this decrease was prevented by metoprin. TSH was increased about fivefold by cold-stimulation, and neither FMH nor metoprine significantly influenced the cold response, although there was a facilitatory trend after FMH. Also prolactin increased after cold-stimulation. Both FMH and metoprine inhibited this response. Metoprine also decreased basal prolactin level. The results do not disagree with the previous data that the overall role of histamine in the cold-response of TSH may be inhibitory, but the net effect is not marked. However, the discrepancy between TRH and TSH suggests that factors other than histaminergic inhibition of TRH release are also involved.

169.5

INHIBITION OF HISTAMINE METHYLTRANSFERASE IN BRAIN ELEVATES LEVELS OF HISTAMINE AND PROMOTES ITS OXIDATION TO IMIDAZOLEACETIC ACID. G.D. Prell, A.M. Morrishow* and J.P. Green. Dept. Pharmacology, Mount Sinai Medical Center, CUNY, New York, New York, 10029

In the periphery, endogenous histamine is metabolized by two pathways: (a) oxidation by diamine oxidase, then aldehyde dehydrogenase (AldDH), to form imidazoleacetic acid (IAA), and (b) methylation by histamine methyltransferase (HMT) to form *tele*-methylhistamine (t-MH), which is oxidized by MAO-B, then AldDH, to *tele*-methylimidazoleacetic acid (t-MIAA). Under normal physiological conditions, histamine in brain is mainly, if not solely, methylated by HMT.

Since we discovered IAA present endogenously in brain using GC-MS, we re-investigated the possibility that IAA in brain derives from histamine. In rats injected (icv) with small, physiological amounts of ^{3}H-histamine, radioactivity was recovered mainly as unchanged histamine or as t-MH or t-MIAA, confirming that, in brain, histamine is normally methylated. In rats infused (>4 weeks using osmotic mini-pumps) with α-fluoromethylhistidine (>7.5 mg/kg/day), the irreversible inhibitor of histamine's synthetic enzyme, histidine decarboxylase, brain levels of HA, t-MH and t-MIAA were reduced up to 90%, compared to rats infused with saline. Levels of IAA were unchanged, indicating that histamine is not a physiological precursor for IAA in brain. However, 2h after rats were injected (i.p.) with inhibitors of HMT (e.g. metoprine, or drugs used in Alzheimer's disease: THA, 1-hydroxyTHA or physostigmine), cortical levels of histamine's methylated metabolites declined, while levels of histamine and IAA increased. Also, ^{3}H-histamine (icv), in large, non-physiological quantities, was converted to labelled IAA, t-MH and t-MIAA. It appeared that as quantities of histamine increased, so did the extent of oxidation. These results confirm that under normal conditions, histamine does not form IAA in brain. But when HMT is inhibited, or when histamine is present in large quantities, IAA can derive from histamine through an alternative metabolic pathway. Since IAA is a potent $GABA_A$ agonist, the consequences of inhibition of HMT may not be confined to the histaminergic system. [Research supported by NINDS NS-28012]

169.6

WITHDRAWN

169.7

THE EFFECT OF THIOPERAMIDE ON *EX VIVO* [^{3}H](R)-α-METHYLHISTAMINE BINDING TO BRAIN HISTAMINE H_3 RECEPTORS. SJ Taylor and GJ Kilpatrick. Glaxo Group Research, Park Road, Ware, Herts SG12 0DP, U.K.

The selective histamine H_3 receptor agonist [^{3}H](R)-α-methylhistamine ([^{3}H]RAMH) has been shown to label H_3 receptors in brain tissue homogentates (Arrang et al., 1987; Kilpatrick and Michel, 1991). Using the *ex vivo* binding approach we have examined the effect of prior treatment with the H_3 receptor antagonist thioperamide on this binding.

Rats (male, Lister Hooded, 250-300g) were pretreated with thioperamide (0.02-10 mg/kg i.p.) at various times before being anaesthetised and transcardially perfused with HEPES buffer. Cerebral cortices were dissected and [^{3}H]RAMH binding assessed as previously described (Kilpatrick and Michel, 1991) except that the tissue washing stage was omitted.

Thioperamide pretreatment markedly inhibited specific [^{3}H]RAMH binding, peak inhibition was observed 30min after dosing. Significant inhibition was apparent at doses of 0.2 mg/kg and above. At 30min, the apparent ED_{50} for thioperamide was 1.5 mg/kg. At doses of 2,5 and 10 mg/kg significant inhibition was evident for at least 4 hours. These values were not corrected for the 75 fold tissue dilution that was necessary for the binding assay.

We conclude that *ex vivo* [^{3}H]RAMH binding may be useful in determining the central penetration and time course of agents which interact with the H_3 receptor.

Arrang et al., (1987) Nature 327,117-123
Kilpatrick et al., (1991) Agents & Actions Suppl 33,69-75

169.8

HISTAMINERGIC MODULATION OF HIPPOCAMPAL ACETYLCHOLINE RELEASE. T. Mochizuki*, A. Yamatodani, K. Okakura*, A. Horii** and H. Wada*. Dept. Pharmacol.II & Dept. *Otolaryngol., Faculty of Med., Osaka Univ., Osaka 530, Japan

In the rat, a dense accumulation of histaminergic fibers is found in the diagonal band and medial septal nucleus, where cholinergic neurons with projections to the hippocampus are located. For examination of the modulatory effects of histaminergic inputs on this cholinergic system, hippocampal acetylcholine (ACh) release was determined by *in vivo* microdialysis after electrical stimulation of the tuberomammillary nucleus (TM), where the cell bodies of the histaminergic neurons are situated. Male Wistar rats were anesthetized with urethane, a microdialysis probe was implanted into the CA1-CA3 region. The mean basal ACh release was 0.98±0.04 pmol/20min. Electrical stimulation (200μA) of the TM resulted in a 1.8-fold increase of the release. This enhancement was dependent on the current intensity and was suppressed significantly after administration of α-fluoromethylhistidine (100mg/kg, i.p.), a specific and irreversible inhibitor of histidine decarboxylase. An H_1-receptor blocker, pyliramine (5mg/kg, i.p.) also decreased this enhanced release. On the other hand, hippocampal ACh release increased after administration of thioperamide (5mg/kg, i.p.), a specific H_3-autoreceptor blocker. These results indicate that hippocampal ACh release is modulated by the central histaminergic system, possibly through H_1-receptors.

169.9

INHERITED IMPAIRMENT OF PURINE RECYCLING IN THE MOUSE: CONSEQUENCES FOR BRAIN PURINE AND DOPAMINE SYSTEMS. H.A. Jinnah, T. Page, and T. Friedmann*. Depts of Neurosciences and Pediatrics, Center for Molecular Genetics, UCSD School of Medicine, La Jolla, CA 92093.

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is an enzyme required for recycling the purine bases hypoxanthine and guanine into utilizable purine nucleotides. Strains of mice carrying mutations of the HPRT gene offer a unique tool for studying the biologic consequences of reduced purine recycling in the brain. One of these mouse strains carries a deletion in the HPRT gene and has been maintained congenic with the C57BL/6J strain for 10 generations. A second strain carries a retrovirally-induced disruption of the HPRT gene on an inbred 129/SvEv background. Total purine pools measured by HPLC-UV after microwave irradiation were significantly reduced in brains of both HPRT-deficient mouse strains. For example, ATP and GTP levels were reduced by 36.0% and 32.2% in the HPRT-deficient C57BL/6J mice, and by 49.3% and 51.3% in the HPRT-deficient 129/SvEv mice. HPLC-EC analysis revealed brain dopamine levels were reduced by 30-40%, while the levels of norepinephrine, serotonin, and monoamine metabolites were unaffected in whole brain extracts. Regional analysis revealed dopamine levels were most severely reduced in the caudoputamen. These data show that impairment of purine salvage leads to a reduction in brain purines as well as a potential deficit in dopamine-mediated neurotransmission in the brain. These abnormalities are similar to those which are thought to occur in HPRT-deficient human patients with Lesch-Nyhan syndrome.

169.10

MODULATION OF EXTRACELLULAR ATP^{4-} (P_{2z})-PURINOCEPTOR RESPONSES: ROLE OF PROTEIN KINASE. L. Tenneti and B. R. Talamo, Neuroscience Program; Tufts Univ. School of Medicine, Boston, MA 02111.

ATP is stored and cosecreted with many neurotransmitters and may itself act as a neurotransmitter or modulator. In rat parotid acinar cells, extracellular ATP elevates intracellular calcium (Ca_i) by activating influx of extracellular Ca, probably through a non-selective cation channel. Previous studies have identified this receptor as a P_{2z}-purinoceptor for ATP^{4-}. Evidence for modulation of these P_{2z}-purinoceptor responses was obtained in studies with inhibitors of protein kinases. Preincubation with the catalytic-site inhibitors K252a and staurosporine, as well as the regulatory domain inhibitor sphingosine, specifically potentiated the elevation in Ca_i mediated by extracellular ATP but had no effect on the Ca_i elevation mediated by muscarinic receptors through phospholipase C activation. The lag time for the potentiation, coupled with the observation that K252b, a membrane impermeant analog of K252a, failed to alter the ATP response suggests that intracellular kinases are involved. Neither the peak intracellular Ca^{2+} mobilization nor the sustained Ca^{2+} entry in response to carbachol or to a Ca^{2+} ionophore (BrA23187) were altered by these inhibitors of kinases, supporting the idea that the effect on the ATP^{4-} response was due to enhanced Ca^{2+} influx through a P_{2z}-specific route. Studies showing that ATP^{4-} increases Mn^{2+} influx support this interpretation. Neither H-7, which potently inhibits protein kinase C (PKC), nor phorbol dibutyrate (PDBu), which activates PKC, had any modulatory effect on ATP-mediated Ca^{2+} influx. These observations, coupled with the fact that PDBu did not reverse the effects of sphingosine, argue against the involvement of PKC. Furthermore W-7, a potent inhibitor of Ca/Calmodulin kinase, was ineffective. These results strongly suggest for the first time that P_{2z}-responses (or purinoceptors) can be modulated and that a novel purinoceptor-associated kinase may alter the response to ATP^{4-} in parotid cells.

169.11

CURRENTS EVOKED BY ADENOSINE 5'-TRIPHOSPHATE IN GUINEA PIG CELIAC GANGLION CELLS. V. Gerzanich, S. Matsumoto[1], R.A. North and E.M. Silinsky[2]. Vollum Institute and [1]Department of Anatomy, Dental School, Oregon Health Sciences University, Portland, OR97201 and [2]Department of Pharmacology, Northwestern University, Chicago, IL60611.

Currents activated by ATP (0.2 - 30 μM) in cultured celiac neurons were measured using whole-cell and outside-out patch-clamp technique; pipets contained mostly cesium gluconate, and no ATP or GTP. Whole-cell currents were inward at negative potential and reversed at 0 mV; conductance for inward currents was three times less than for outward. Currents evoked by ATP were reduced by reactive blue 2 or by prior exposure to desensitizing concentrations of α,β-methylene-ATP; they were unaffected by hexamethonium, (+)-tubocurarine or ondansetron at concentrations that blocked similar currents evoked by acetylcholine or 5-hydroxytryptamine (5-HT). The potency of adenosine analogs was 2-methyl-thio-ATP >ATP ≥α,β-methylene-ATP >β,γ-methylene-ATP >ADP >AMP >>adenosine. Channels in excised patches showed very flickery openings and had a conductance of 22 pS at -50 mV; outward currents were not seen. Channel activity was not affected by nicotinic and $5\text{-}HT_3$ selective antagonists. It is concluded that ATP opens ligand-gated cation channels in guinea pig celiac neurons; the receptor is pharmacologically most like the P_{2Y} subtype.

169.12

ACCUMULATION OF EXOGENOUS POLYAMINES IN THE BRAIN AFTER ISCHEMIA. V.H. Gilad, G.M. Gilad and R.J. Wyatt. NPB, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032.

Brain ischemia leads to increased putrescine (Put) synthesis and content while spermidine (Spd) and spermine (Spm) remain unchanged. Nevertheless, extracellular levels of all polyamines (PA) remain unchanged at levels lower than 1 uM. Injections of PA to gerbils after ischemia can protect forebrain neurons from degeneration. Now we sought to determine if PA accumulation from the circulation into the brain and then into brain cells is increased after ischemia. When ^{3}H-Put was injected (i.v.) 5 h after transient global ischemia its accumulation in the hippocampus increased within 1 h to 174% of control. Auto- radiography after ^{3}H-Spd injections also demonstrated increased accumulation in brain parenchyma. In hippocampal slices a temperature- dependent uptake was observed only for Spm and it was transiently increased after ischemia, peaking (150% of control) at 12-13 h and subsiding at 24 h. We conclude that after ischemia, accumulation of exogenous PA is increased both via the (compromised) blood brain barrier and by active cellular uptake mechanisms.

169.13

Brain Family of Aromatic Amine N-Acetyltransferases
S. J. Gaudet and M. A. A. Namboodiri, Molecular Neurobiology Laboratory, Department of Biology, Georgetown University, Washington, DC 20057

The purification of the N-acetyltransferase (NAT) activity from the rat brain has elucidated a family of NATs. A spectrum of NAT activity is observed from arylamine to arylalkylamine specificity. Using an affinity column composed of methotrexate, we have observed different species of NATs based upon their chromatographic behavior. A NAT which is characterized as an arylalkylamine NAT, based upon its specificity towards arylalkylamine substrates, is observed in the effluent of the affinity step, while the arylamine NAT, so called due to its preference towards arylamine substrates, is only observed following soluble methotrexate elution. A third species of NAT is also detected. This NAT also is observed in the effluent and demonstrates affinity for both classes of aromatic amine substrates. These species of NAT can be distinguished by employing other physical parameters as well. The arylalkylamine NAT found in the affinity effluent demonstrates a similar size exclusion HPLC pattern as that seen in the pineal. Two arylalkylamine NAT peaks are observed having apparent molecular weights of 100-KDa and 10-KDa, while a third peak corresponding to 30-KDa demonstrates broad aromatic amine substrate specificity. The other two NATs have an apparent molecular weight around 40-KDa using three size exclusion HPLC columns in series. These two NATs can be further distinguished by their sensitivity to methotrexate inhibition. The NAT which is specifically eluted from the affinity column has a methotrexate IC_{50} value of 62.5 uM, while the other NAT appears insensitive to methotrexate inhibition in the high micromolar range. Further studies will continue to examine the physiological significance of these NAT activities in the brain as well as the possible interrelationship between these NAT proteins.

(supported by NIH grant DK 37024)

169.14

COMPARISON OF CENTRAL AND PERIPHERAL 2-[^{125}I]-IODOMELATONIN BINDING SITES. M. Viswanathan, J.T. Laitinen, A.M. Seltzer, and J.M. Saavedra. Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892

Quantitative autoradiography was used to compare certain biochemical and pharmacological characteristics of 2-[^{125}I]-iodomelatonin binding in the following tissues: chick retina, pituitary of 10 days-old Sprague-Dawley rats, and suprachiasmatic nuclei, area postrema, anterior cerebral artery, and caudal artery of adult rats. Sections of respective tissues were cut in a cryostat at -17 °C, thaw-mounted onto gelatin-coated slides, and dried. Tissue sections were incubated with 2-[^{125}I]-iodomelatonin (100 pM) in the presence or absence of melatonin (1 μM) for 60 min in 50 mM Tris-HCl buffer (pH 7.4) containing 4 mM $CaCl_2$. The effects of dithiothreitol (DTT) which reduces disulfide bridges of proteins or N-ethylmaleimide (NEM) which alkylates sulphydryl groups of the G-protein alpha-subunit were examined. Low concentrations of DTT (0.1 - 2 mM) in the incubation medium had very little effect on 2-[^{125}I]-iodomelatonin binding at all sites except in the anterior pituitary where the amount of binding was significantly increased. Conversely, NEM (0.5 mM) reduced the amount of 2-[^{125}I]-iodomelatonin binding (33-58% of control) in all tissues. The affinities of various serotonin and melatonin analogues for binding sites in all the tissues studied were also similar. We conclude that the biochemical and pharmacological characteristics of the binding sites studied were not distinctly different from one another when compared under the same assay conditions.

169.15

COPPER DISTINGUISHES TWO FORMS OF THE HALOPERIDOL-SENSITIVE SIGMA RECEPTOR. Mark Connor* and Charles Chavkin, Dept of Pharmacology, Univ of Washington, Seattle WA 98195

Two subtypes of the haloperidol-sensitive sigma receptor have recently been characterized in guinea pig and rat brain membranes by radioligand binding assays. We now report that radioligand binding to the sigma-1 site (characterized by a high affinity for haloperidol, (+)pentazocine and (+)3-PPP) is also highly sensitive to Cu^{2+} (as $CuSO_4$), whereas the sigma-2 site is relatively insensitive to Cu^{2+}. Cu^{2+} displaced 3 nM [^{3}H]-DTG in a biphasic manner from both rat and guinea pig brain membranes with an IC_{50} of approximately 20 μM at sigma-1 sites and greater than 10 mM at sigma 2 sites. Zn^{2+} (as SO_4 and Cl_2) has a similar potency to Cu^{2+} at the sigma-1 site but did not discriminate well between the sites. None of the other divalent cations tested (Co^{2+}, Cd^{2+}, Mg^{2+}, Mn^{2+}, Ca^{2+}) were as potent or as selective as Cu^{2+} in displacing [^{3}H]-DTG from haloperidol-sensitive sigma receptors. A fixed concentration of 100 μM Cu^{2+} blocks the ability of haloperidol, (+)3-PPP and (+)pentazocine to compete with [^{3}H]-DTG for binding to the high affinity sigma site in both rat and guinea pig membranes. The IC_{50} values for (+)3-PPP and (+)pentazocine displacing 3 nM [^{3}H]-DTG from the copper sensitive site in guinea pig are about 13 nM and 4 nM respectively, which are in agreement with previously published values for these drugs at the sigma-1 receptor. In rat, DTG has an IC_{50} value vs 3 nM [^{3}H]-DTG at the high affinity site of 12 nM, which also corresponds well with previously published values. These results suggest that the haloperidol-sensitive sigma binding site which is sensitive to the presence of Cu^{2+} corresponds to the sigma-1 site identified previously. Supported by MH 46501.

169.16

DEPOLARIZATION-INDUCED RELEASE OF AN ENDOGENOUS LIGAND FOR THE HALOPERIDOL-SENSITIVE SIGMA RECEPTOR FROM RAT BRAIN. Charles Chavkin, Mark Connor* and John Neumaier[fl], Dept of Pharmacology and Dept of Psychiatry and Behavioral Sciences[fl], University of Washington, Seattle, WA 98195.

The haloperidol-sensitive sigma receptor is a membrane binding site for a group of psychotomimetic and potentially antipsychotic drugs. Endogenous factors that bind to the haloperidol-sensitive sigma receptor have been detected in brain extracts, and radioligand displacement assays have provided indirect evidence of the presence of an endogenous transmitter for the sigma receptor in brain. We now report that depolarization of rat brain slices releases an endogenous sigma factor into the superfusion buffer. Fresh rat brain slices (200 μm) were perfused with oxygenated Krebs-bicarbonate buffer (ACSF) and the perfusate was desalted, concentrated and resolved by C18-HPLC. Sigma activity present was quantified by competition binding assay utilizing rat brain membranes and [^{3}H]-DTG. Depolarization of the tissue slices by perfusion with 50 mM KCl caused a 270 ± 28% increase in the amount of sigma activity recovered (n=4). The sigma activity released co-eluted with a small, nonpeptide factor purified from guinea pig brain extracts. When Mg^{2+} was substituted for Ca^{2+} in the ACSF, depolarization by high potassium failed to induce release of the putative factor (the amount recovered was 89 ± 12% of control, n=4). The depolarization-induced, calcium-dependent release of a sigma factor supports the hypothesis that the molecule purified from brain extracts may be an endogenous neurotransmitter at the haloperidol-sensitive sigma receptor. Supported by MH46501.

NEUROTRANSMITTER MODULATION: EXCITATORY AND INHIBITORY TRANSMITTERS

170.1

MODULATION OF SOMATIC GLYCINE-LIKE IMMUNOREACTIVITY IN PRESUMED GLYCINERGIC NEURONS R. Wickesberg*[1], D. Whitlon*[2] and D. Oertel[1], [1]Department of Neurophysiology, [2]Waisman Center, University of Wisconsin, Madison, WI 53706.

Intracellular recordings have shown that the projection from the dorsal to the ventral cochlear nucleus is inhibitory. Because the inhibition is blocked by 0.5 μM strychnine, it is probably glycinergic. In brain slices from mice, the cell bodies of origin for this projection lose their glycine-like immunoreactivity after being maintained *in vitro* for 7 or 8 hrs without electrical stimulation. The loss of immunoreactivity is prevented by continuous stimulation of the auditory nerve (100 μs shocks, 2/sec), axotomy, or the presence of 0.8 μM tetrodotoxin, 1 μM strychnine, 50 μM colchicine, or 50 μM β-lumicolchicine in the bathing saline. The presence of only 5 μM colchicine does not prevent the loss. After 7 hours *in vitro* without stimulation, the somatic immunoreactivity can be regained by stimulating the auditory nerve for 20 min. The other neurons in the cochlear nuclei, even those that may be glycinergic, respond differently to these manipulations or not at all, and no signs of deterioration were observed. The immunostaining of puncta, which resemble synaptic terminals, appears unaffected by these experiments. These results indicate that in presumably glycinergic neurons, glycine is transferred out of the immunoreactive somatic pool in the absence of synaptic input and that this transfer is blocked by various toxins.

170.2

DEVELOPMENT AND USE OF AN IN VITRO PUSH-PULL PERFUSION TECHNIQUE TO MEASURE NEUROTRANSMITTER RELEASE FROM RESTRICTED PERFUSED AREAS OF THE BRAIN SLICE PREPARATION. B. Anton, M.S. Levine and N. Buchwald. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

The present research describes the use of an in vitro push-pull perfusion technique that allows measurements of neurotransmitter release from the surface of brain slices (500μm). The technique employs a tip-modified concentric push-pull cannula similar to that designed by Philippu et al. (Naunyn Schmiedebergs Arch. Pharm. 276, 1973) in which the inner tube (liquid infusion) is shielded within the outer pull tubing in order to minimize tissue erosion during perfusion. The probe is coupled through sylastic tubes to a peristaltic (manostat) liquid pumping system in which the pull system opens to the atmospheric pressure, thus avoiding tissue resistance changes that induce tissue damage and decrease viability of perfused tissue (Neouillón et al. J. Neurochem. 28, 1977). The slice is placed in a perfused liquid-gas interface chamber. The probe is placed on the surface of the slice to allow administration and sampling of chemicals into a defined area. In an application of the methodology, the resting and K^+-stimulated (50mM) ^{3}H-GABA release profiles during extended perfusion periods (up to 3 hr) were characterized in rat and cat brain slices. Two areas of the brain were assessed, the neostriatum and the frontal cortex. In both structures, the K^+-evoked ^{3}H-GABA release was partially dependent on the presence of external Ca^{2+} (up to 80%), suggesting a neuronal origin of the releasable amino acid. Additionally, in both species ^{3}H-GABA uptake and K^+-evoked release was increased by up to 100% in neostriatum compared to prefrontal cortex. These regional differences in both ^{3}H-GABA release parameters may result from increased GABAergic synaptic density in neostriatum compared to the prefrontal cortex. In summary, this perfusion method may have applications in correlative studies of neurotransmitter release and neuronal firing during specific physiologic and/or drug-altered conditions in the brain slice preparation. Supported by USPHS HD05958.

170.3

N-ACETYLASPARTATE UPTAKE AND METABOLISM BY RAT CEREBELLAR PRIMARY CELL CULTURES. O.H.Saab and J.H.Neale. Department of Biology, Georgetown University, Washington, DC 20057.

The function of N-Acetylaspartate (NAA), which is present in very high concentrations in the central nervous system, has not been elucidated. However, highly elevated levels of NAA have been found in the blood and urine of individuals with certain neurological disorders, such as Canavan disease, the apparent result of a deficiency in aspartoacylase. Immunohistochemical and other methods have indicated a predominantly neuronal localization of NAA in the adult rat brain. The objective of this study was to compare glial and neuronal cells from the rat cerebellum in terms of their uptake and metabolism of NAA. Rat cerebellar cells were maintained in dissociated culture for 9-24 days. The cells were incubated with ^{3}H-NAA under different conditions. While glial cells demonstrated substantial uptake and rapid metabolism of exogenous NAA, granule cells did not show detectible uptake or metabolism. Thus, only the glial uptake of NAA was characterized. These data suggest a differential role between neurons and glia in the metabolism of NAA.

170.4

HPLC-EC ASSAY FOR "FREE" GABA IN HUMAN CEREBROSPINAL FLUID. E. Vontzalidou*, A. Naini, and L.J. Cote. Columbia University, Dept of Neurology, New York, N.Y. 10032

Less than 2% of the total GABA in the CSF is "free" GABA, the remaining source of GABA is in bound form e.g. homocarnosine, homoanserine, and several other unknown conjugates. Most of the chromatographic techniques (isocratic or ion exchange) employed for the measurement of GABA in tissue extract or physiological fluids, use pre or post column derivatization of GABA with o-Phthaldialdehyde (OPA) in the presence of β-mercaptoethanol (β-ME). We found that GABA-OPA-β-ME derivative is unstable and could not be used for the assay of GABA in CSF. In contrast, GABA forms a highly stable derivative with OPA (20mM) in the presence of t- butylthiol (40mM) at pH 9.5. Based on this finding, we have developed an isocratic HPLC-EC assay for GABA in the CSF. The derivative of GABA was separated on reversed phase C18 column with a mobile phase of 0.05M potassium phosphate containing 36% methanol and 5% ethyl acetate adjusted to pH of 6.75. GABA was detected using a Coulochem 5100A electrochemical detector (ESA) operating in an oxidative screening mode (detector 1;+0.25, detector 2; +0.60 volt). Concentrations as low as 0.5 pmoles could be accurately measured in 20 µl injection volume. Recovery studies indicated an accuracy of close to 100% with high precision (between runs CV < 2%).

Supported by the Charles S. Robertson Foundation and the Parkinson Disease Foundation

170.5

A TECHNIQUE FOR CONTINUOUSLY SAMPLING RELEASE OF AMINO ACIDS FROM BRAINSTEM SLICES DURING NEURON RECORDINGS. H. J. Waller[1] and D. A. Godfrey[2], Depts. of Neurological Surgery[1], Otolaryngology[2], Anatomy[2], and Physiology and Biophysics[1,2], Medical College of Ohio, Toledo, OH 43699.

We have developed a simple procedure that permits continuous sampling of perfusate in an interface slice chamber and which does not interfere with concurrent recordings of action potentials from rat dorsal cochlear nucleus. Approximately 1% of the perfusion effluent is diverted by means of a microsiphon, and droplets (e.g., 9.4 µL) can be collected for subsequent measurements. Concentrations of several amino acids have been measured using HPLC. The tip of the microsiphon (polyethylene tubing, 0.61 mm. o.d.) is placed adjacent to but not in contact with the slice, downstream from the region under study. The tubing is supported at intake and collection ends by metal sleeves (20 ga. hypodermic tubing), and can be moved easily without interrupting fluid flow. Measurements were taken at various downstream and lateral positions to provide information about the sampling characteristics of the siphon.

Rates of release of measured amino acids were highest initially and decreased during an experiment. The release of some amino acids (e.g. glycine, serine) was sustained throughout the experiment, whereas that of others (e.g. glutamate, GABA) declined rapidly to low levels. (Supported by NIH grant DC00172.)

170.6

KAINIC ACID CAUSES A DISSOCIATION BETWEEN THE STEADY-STATE CONCENTRATION AND THE KCl-EVOKED RELEASE OF DYNORPHIN B AND GLUTAMATE FROM RAT HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES. J.N. Simpson, R.L. Gannon, J.F. McGinty, and D.M. Terrian. Dept. of Anatomy and Cell Biol., School of Medicine, East Carolina University, Greenville, NC 27858-4354.

In this experiment, the effects of intracerebroventricularly (ICV) administered kainic acid (KA) on the subsequent in vitro release of L-glutamate (GLU) and dynorphin B (dyn B) from isolated rat hippocampal mossy fiber synaptosomes were examined. Hippocampal mossy fiber synaptosomes were immediately prepared at 4.5 hrs, 20 hrs, or 48 hrs after ICV administration of 0.5 ug/ul KA. A 35 mM KCl stimulus was applied to the synaptosomal preparation and the quantity of GLU and dyn B-like immunoreactivity released from the synaptosomes was measured fluorometrically and by radioimmunoassay (RIA), respectively. Hippocampal mossy fiber synaptosomal dyn B concentration was also measured by RIA. At 4.5 hrs, KA caused a 69% decrease in KCl-evoked release of dyn B as compared to control levels when the dyn B concentration in the synaptosomal fraction was decreased by 39%. At 20 hrs, the KCl-evoked release of dyn B remained 34% below control levels when the dyn B concentration in the synaptosomal fraction had increased to 46% above control levels. At 48 hrs, there continued to be a 41% decrease in the KCl-evoked release of dyn B when the dyn B concentration of the synaptosomal fraction remained elevated at 47% above control levels. In contrast, KA caused no change in the KCl-evoked release of GLU at 4.5 hrs as compared to control levels. However, there was a 40% decrease in the KCl-evoked release of GLU from mossy fiber synaptosomes at both the 20 and 48 hr time points. These data indicate a prolonged effect of ICV KA on neurotransmission at mossy fiber synapses in the rat hippocampus, resulting in a depression of the release of GLU as well as the opioid peptide, dyn B. The depression in dyn B release, coupled with increased preprodynorphin synthesis (Lason et al., submitted), may both contribute to the elevated dyn B content in the mossy fiber synaptosomes observed at 20 and 48 hrs after KA injection. Supported by DA 03982 (J.F.M.) and AFOSR 89-0531 (D.M.T.)

170.7

UPTAKE OF EXOGENOUS N-ACETYLASPARTYL-^{3}H-GLUTAMATE AND ^{3}H-GLUTAMATE BY MURINE BRAIN CELLS IN CULTURE. **M. Cassidy and J. H. Neale Department of Biology, Georgetown University, Washington, DC 20057**

N-Acetylaspartylglutamate (NAAG) is an acidic neuropeptide found in high concentration in the mammalian brain. It has been localized to synaptic vesicles and is released by depolarizing stimuli. Peptidase activities which hydrolyze NAAG to glutamate and N-acetylaspartate provide a mechanism of inactivation of the putative neurotransmitter NAAG, or synaptic activation of neurotransmitter glutamate, or both. Intact NAAG, however, is also taken up into cells in both pure glial cultures and mixed neuronal/glial cultures. ^{3}H-NAAG and ^{3}H-glutamate uptake in cultured murine cells were quantitated and partially characterized. Initial results indicate that the NAAG peptidase activity present in both types of cell cultures is not linked to ^{3}H-glutamate uptake. These data support the hypothesis that a substantial portion of the glutamate released from NAAG in the extracellular space is transiently available for activation of acidic amino acid receptors.

170.8

GLUTAMATERGIC TRANSMISSION IN A MODEL OF ABSENCE SEIZURES. M.L. Cordero*, A.E. Negrón*, J.G. Ortiz, G. Santiago*, S. Cardona*, and M.T. García*. Dept. of Pharmacology, UPR School of Medicine, San Juan, Puerto Rico 00936-5067

GABAergic neurotransmission is altered in the C57BL/10Bg sps/sps mouse mutant; a model of generalized absence seizures (Ortiz et al., 1990). In addition, glutamate dehydrogenase (GDH), and glutaminase (GLNase) activities are reduced in these mice. The apparent K_m for the high affinity ^{14}C-GLU uptake (GLU) in the crude synaptosomal (P_2) fraction of adult (2 months old) normal SPS/SPS vs. sps/sps (absence seizure susceptible) mice (38.56 ± 3.59 and 53.66 ± 9.8 µM, respectively) is not altered. In contrast, the U_{max} is increased in sps/sps mice (6.22 ± 0.34 vs 15.98 ± 1.99 nmoles/mg protein, $p < 0.05$). The regional binding of ^{3}H-AP-7, ^{3}H-kainic acid, and ^{3}H proline to crude synaptosomal fraction is decreased in this mouse mutant. The reductions in (1) GDH and GLNase activities; (2) in the binding of the above mentioned ligands; and the increase in U_{max} for GLU uptake are consistent with increased EAA neurotransmission in this model of epilepsy. (Supported by the institutional MBRS/NIH and RCMI programs)

170.9

GLUTAMINE SYNTHETASE HOMEOSTASIS IN CULTURED ASTROCYTES. S.E.Farinelli* and W.J.Nicklas. UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Previously we have demonstrated that in cultured astrocytes, the metabolism of exogenous glutamate through both oxidative and glutamine synthetic pathways is dependent on the functional glutamine synthetase (GS) activity in the cells. In the present study we have determined the turnover rate of GS enzyme in the astrocyte coupled with measurements of GS mRNA levels. By pulsing cultures with methionine sulfoximine (MSO), an irreversible inhibitor of GS, and monitoring the timecourse of recovery of enzymatic activity it is possible to determine the rate of synthesis of new GS enzyme. We examined three conditions previously shown to increase the steady-state level of GS activity in astrocytes: maintaining cells in medium containing no glutamine (Gln), and treatment of the cultures with either hydrocortisone (HC, 1 μM) or dibutyryl cAMP (dbcAMP, 0.5 mM). Elimination of Gln from the medium resulted in a two-fold increase in GS activity compared with those grown in medium containing 5 mM Gln. Both HC and dbcAMP treatment resulted in a 2-3 fold increase in GS activity. Following a pulse of MSO (1 mM for 2 hours) GS enzyme activity rebounded from zero to one-half its steady state level in approximately 2.5 days under all the conditions examined. GS mRNA levels were unaffected by MSO treatment and remained virtually constant throughout the 5 day recovery period. Elimination of Gln from the medium does not result in increased GS mRNA levels whereas in HC and dbcAMP treated cultures GS mRNA levels are elevated when compared with control cultures. This suggests alternative mechanisms of regulating GS activity, ones mediated by hormones and second messengers which act at the transcriptional level and another mediated by Gln, which acts at the translational or post-translational level.

170.10

ALPHA-KETOGLUTARATE TRANSPORT: A MECHANISM FOR REPLENISHING AND REGULATING THE TRANSMITTER POOL OF GLUTAMATE. R. P. Shank and D. J. Bennett*. CNS Research, The R. W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477-0776

Alpha-ketoglutarate (α-KG) is avidly accumulated by synaptosomes, which represents one step in a putative metabolic pathway in which α-KG is supplied by astrocytes to neurons. The uptake of α-KG is mediated by a Na^+-dependent transport process that exhibits complex kinetics. The results of a variety of experiments suggest that uptake is mediated primarily by a single system that exhibits negative cooperativity. This transport process is differentially affected by glutamate, glutamine, malate, membrane depolarization, and a soluble heat-labile substance in rat CNS extracts. Our results suggest that the α-KG transporter possesses two allosteric sites that regulate uptake. One of these is a negative modulatory site that reduces the affinity between α-KG and the transport binding site. Glutamate, malate, and α-KG itself interact with this site. The second site serves to positively modulate uptake by increasing the V_{max}. Glutamine and glutamate interact with this site. Glutamate at concentrations ≤ 3 μM enhances α-KG uptake, but at higher concentrations it inhibits uptake. Hence, glutamate appears to have a dual regulatory effect on the synaptic concentration of α-KG, one of its two primary immediate precursors. Our results indicate that at extracellular concentrations below 3 μM glutamate can promote its own synthesis by increasing the accumulation of α-KG, whereas at higher concentrations glutamate inhibits its own synthesis by reducing the accumulation of α-KG. From a physiological perspective these modulatory effects are equivalent to the functional expression of glutamate autoreceptors.

170.11

DIFFERENTIAL EFFECTS OF TETANUS TOXIN ON INHIBITORY AND EXCITATORY NEUROTRANSMITTER RELEASE IN SPINAL CORD CELL CULTURES. L.C. Williamson, S.C Fitzgerald*, and E. A. Neale. Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Tetanus toxin is a potent neurotoxin which acts in the central nervous system to cause spastic paralysis, likely through disinhibition at the level of the motor neuron. Electrophysiologic analysis of toxin action in murine spinal cord neurons in cell culture demonstrated a short latent period followed by a phase of paroxysmal depolarizing events (PDE) associated with a gradual loss of inhibitory postsynaptic potentials. At greater than 10^{-11} g/ml toxin, the period of convulsant activity is followed by a loss of both spontaneous and evoked excitatory and inhibitory synaptic activity (Bergey et al., J. Neurosci. 3: 2310, 1983). This study examined the effect of tetanus toxin on stimulated and spontaneous release of glycine, GABA and glutamate during the course of toxin action, particularly during PDE. Cell cultures were labeled with ^{3}H-glycine, ^{3}H-GABA or ^{3}H-glutamine; the identity of released radiolabeled molecules was confirmed by TLC. Neurotransmitter release was stimulated by 56 mM K^+ in the presence of 2 mM Ca^{++} at various times after exposure to 10^{-7} g/ml (10^{-10} M) tetanus toxin. Stimulated release of glycine and GABA was blocked completely within 90 min; the stimulated release of glutamate was only partially blocked at this time, with a complete block by 6 hr. Spontaneously released neurotransmitter was measured at 30 min intervals after toxin exposure and examined relative to the radioactivity released from cultures in the presence of 1 μM tetrodotoxin. The spontaneous synaptic release of GABA was blocked totally within 30 min and glycine, within 90 min. In contrast, the synaptic release of glutamate in toxin-exposed cultures was elevated to twice that of controls for 150 min. These studies indicate that toxin-induced convulsant activity can be defined by a reduction in the release of inhibitory neurotransmitters and a concomitant increase in the release of glutamate.

170.12

HETEROGENEITY OF BRAIN L-GLUTAMATE DECARBOXYLASE. J.-Y. Wu, B. Nathan and J. Bao. Department of Physiology & Cell Biology, Univ. of Kansas, Lawrence, KS 66045-2106

Previously we as well as others have reported the presence of multiple forms of soluble L-glutamate decarboxylase (GAD) in mammalian brain, differing in its affinity for pyridoxal phosphate (PLP) [Denner and Wu, J. Neurochem. 44, 957 (1985)], kinetic properties [Spink et al., Brain Res. 421, 235 (1987)] and gene structure [Kobayashi et al.; J. Neurosci. 7, 2768 (1987); Huang et al.; Proc. Natl. Acad. Sci. US 87, 8491 (1990)]. In addition to soluble GAD, we have also isolated, purified and characterized a new form of membrane GAD. The membrane GAD was first isolated from P2 membrane, followed by solubilization with Triton X-100, and purified by a combination of column chromatographies on ion exchange (DE-52), gel filtration (ACA34 and Sephadex G-200), hydroxylapatite and preparative gel electrophoresis. The membrane associated GAD thus purified differs greatly from soluble GAD in molecular size, electrophoretic mobility in non-denaturing PAGE immunochemical properties, and PLP dependency [Wu et al., (Neurochem. Res. in press).] Eventually, we would like to purify membrane GAD to homogeneity and to determine its specific physiological role in GABAergic transmission. [Supported by grants NS 20978 (NIH) and BNS-8820581(NSF)].

170.13

NOREPINEPHRINE AND DOPAMINE DIFFERENTIALLY MODULATE RESPONSES INDUCED BY EXCITATORY AMINO ACIDS IN DEVELOPING HUMAN CORTEX. Z. Radisavljevic, C. Cepeda, M.S. Levine and N.A. Buchwald. MRRC, UCLA-School of Medicine, Los Angeles, California 90024.

The human cerebral cortex is innervated richly by the neuromodulators norepinephrine (NE) and dopamine (DA). These catecholamines alter cortical excitability, at least in part, by modifying responses of cortical neurons to amino acid neurotransmitters. Experiments were performed to study the effects of NE and DA on cortical tissue resected surgically in cases of intractable childhood epilepsy. Tissue was obtained from patients of 7 months to 7 years of age. Brain slices (400 μm) obtained from the cortical samples were maintained in physiological solutions using standard procedures. In these slices the effects of NE, its β-receptor agonist, isoproterenol and DA on responses evoked by iontophoretic application of excitatory amino acids (glutamate (GLU) and N-methyl-D-aspartate (NMDA)) were assessed. In different experiments GLU (0.2M, pH=8), NMDA (0.1M, pH=8), NE (0.2M, pH=5), isoproterenol (0.2M, pH=5) and DA (0.1M, pH=4.5) were applied iontophoretically in the vicinity of the recorded neuron (n=17). Iontophoretic application of GLU (Average current -155±24nA (S.E.),) and NMDA (-127±22nA) evoked membrane depolarizations and action potentials in cortical neurons. Simultaneous iontophoretic application of NE (+142±25 nA) or isoproterenol (+125±25nA) enhanced responses (increased the frequency of action potentials and the duration of the response) evoked by GLU and NMDA. In contrast, application of DA (+197±20nA) enhanced responses to NMDA (increased the frequency of evoked action potentials and the duration of the response), but decreased or eliminated responses to GLU. The differential effects of the two catecholamines may be important in assessing their possible roles in epileptogenic cortex.

171.1

COMPARATIVE ANATOMICAL DISTRIBUTION OF 5HT1A RECEPTOR MRNA AND 5HT1A BINDING IN RAT BRAIN

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The development of selective molecules for the 5HT1A receptor has allowed identification of this site as both a putative inhibitory somato-dendritic autoreceptor on raphe serotonergic cells and as a postsynaptic receptor in selective serotonergic terminal fields. The recent cloning and functional characterisation of the rat 5HT1A receptor gene (Albert et al 1990) indicates features characteristic of the G protein coupled receptor family. Here we describe the anatomical distribution of cells in rat brain expressing 5HT1A mRNA using in situ hybridisation histochemistry and compare this distribution to that of radiolabelled 5HT1A receptor sites.

In general terms, there was a complementary distribution of cells expressing 5HT1A receptor mRNA and 5HT1A receptor sites. High levels of both 5HT1A mRNA and 5HT1A receptors were evident in the hippocampal formation (CA1, CA3,dentate gyrus), entorhinal cortex and raphe nuclei and lower levels in neocortex and thalamus. Although 5HT1A mRNA was not expressed in any regions which did not also exhibit 5HT1A receptors, within both the diagonal band and medial septal nucleus mRNA levels were proportionately higher than 5HT1A receptor levels, possibly reflecting receptor transport or heterogeneity of 5HT1A receptor turnover mechanisms. 5HT1A receptor mRNA and 5HT1A binding sites were undetectable in caudate/putamen and cerebellar regions. The present data indicate the synthesis of 5HT1A receptors both in raphe serotonergic cells and in anatomically specific serotonergic projection areas.

171.2

IN VIVO LABELING OF DOPAMINE AND SEROTONIN UPTAKE SITES IN THE RAT BRAIN WITH [^{123}I]-RTI-55.

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[^{125}I]-RTI-55, a new cocaine analogue, has been found to label central serotonin (5-HT) and dopamine (DA) transporters with high affinity. In this study, the precursor of RTI-55 was labeled with [^{123}I] and the compound evaluated for its in vivo binding in the rat brain. High-specific [^{123}I]-RTI-55 was synthesized from the corresponding stannyl analog and $^{123}I^-$ using chloramine-T as an oxidant (yield = 85%). Rats were injected i.v. with 50 μCi [^{123}I]-RTI-55 Highest accumulation occurred in DA transporter rich regions: Specific to nonspecific (cerebellar) ratios reached 22 in the striatum, 11 in the olfactory tubercles 5 hours p.i. Binding was also high in 5-HT uptake site rich regions: In hypothalamus and pre-frontal cortex, the ratios were 6.5 and 4.1, respectively. Pre-injected paroxetine (5mg/kg) dramatically decreased [^{123}I]-RTI-55 binding in areas rich in 5-HT, but not in those rich in DA uptake sites. Chronic treatment of rats with MDMA significantly reduced [^{123}I]-RTI-55 uptake in regions known to be affected by MDMA neurotoxicity. The results indicate that [^{123}I]-RTI-55 should be useful for SPECT imaging of both, DA and 5-HT uptake sites.

171.3

EFFECT OF IBOTENIC ACID AND AF64A LESIONS OF CAUDATE PUTAMEN ON D_2 RECEPTOR mRNA IN RAT BRAIN.

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Dopamine (DA) containing neurons project from substantia nigra compacta (SNC) to terminate on cholinergic (ACh) interneurons in the caudate-putamen (CPu). D_2 receptors are known to mediate the influence of DA on striatal ACh neurons. D_2 receptors are presynaptic on nigrostriatal terminals and postsynaptic on striatal neurons. We examined the changes in D_2 receptor mRNA after AF64A or ibotenic acid (IA) lesions in CPu. One group of male Long-Evans rats were stereotaxically injected with saline, IA (20 μg/2μl), or AF64A (0.34 μg/2μl) on the left side of the brain. After 15 days, in situ hybridization was performed using a [^{35}S]SαATP radiolabeled dopamine D_2 receptor oligonucleotide (48 base pair) probe. The results show a large decrease in the D_2 message in the CPu with the IA lesion and a smaller decrease with the AF64A lesion as compared to controls. A very small decrease was also seen in the D_2 message in the SNC in both groups. The data indicate that mRNA encoding postsynaptic D_2 receptors in the CPu occurs in ACh containing neurons.

171.4

CHANGES IN DOPAMINE RECEPTOR BINDING AND NEUROPEPTIDE mRNA FOLLOWING A RETROGRADE LESION OF STRIATOPALLIDAL NEURONS.

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We have previously used the retrogradely transported neurotoxin, volkensin, to study changes in D1 and D2 receptors as well as preproenkephalin (PPE) and preprotachykinin (PPT) mRNA after a lesion of striatonigral neurons. In this study, we have used an immunotoxin, OX7/saporin (OX7), to selectively lesion striatopallidal neurons. Three rats were examined 5 days after an injection of 0.5 μg of OX7 in the external segment of the left globus pallidus (GPe). Serial 20 μm striatal sections were examined for receptor binding with ^{3}H-SCH 23390 (D1) and ^{3}H-raclopride (D2) and in situ hybridization with ^{35}S labelled oligonucleotide probes for PPE and PPT mRNA. Specific binding or optical density (OD) was compared contra- and ipsilateral to the lesion using a paired t-test. Specific D1 binding was unchanged (184.1 fmol/mg ± 4.8 (mean ± SE) vs. 181.3 ± 5.4 ns.) while D2 showed a 14% decrease (131.6 ± 7.0 vs 113.3 ± 9.4 p<.01). PPE mRNA expression decreased 27% (OD 0.555 ± 0.043 vs. 0.405 ± 0.037 p<.01); PPT mRNA did not show a significant decrease (OD 0.154 ± 0.01 vs 0.129 ± 0.01 ns). These results suggest that D2 but not D1 receptors are selectively expressed by striatopallidal neurons and are consistant with the previous observation that PPE and D2 receptor mRNA are colocalized in a subpopulation of striatal neurons. The absence of an effect of this lesion on D1 receptor binding or PPT mRNA strongly suggests that the lesion is specific for striatal neurons projecting to GPe. These data are the first demonstration of selective expression of D2 receptor binding sites by striatopallidal neurons.

171.5

RAPID RADIOIODINATION OF SCH-23390, A POTENT D1 DOPAMINE RECEPTOR IMAGING AGENT.

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In recent years, there has been an increasing interest in developing a radiopharmaceutical method of selectively evaluating the concentration of dopamine D1 and D2 receptors in the basal ganglia in order to study the pathology of the central nervous system using SPECT and PET imaging.

One such agent is the iodinated Schering compound, SCH-23390, developed oy Kung's research group. 7-Chloro-8-hydroxy-1-(4'-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (FISCH) is prepared from 7-chloro-8-methoxy-1-[(4'-tri-n-butylstannyl)phenyl]-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine by iodination with $Na^{125}I$ and H_2O_2 followed by demethylation with BBr_3.

The original procedure required 2 hours to effect radioiodination. We have developed a modification which reduces the reaction time to 3 minutes: Aqueous peroxyacetic acid (100 μL, 3.2% w/v) is added to a mixture of the tri-n-butylstannane [50 μL (100 mg/mL)], 200 μL EtOH, 40 mL 1N HCl and carrier-free $Na^{123}I$ in a sealed vial. The reaction is allowed to stand 3 minutes at room temperature and quenched by the addition of 40% $NaHSO_3$ (to reduce excess peracid). The solution is made basic by the addition of sat'd Na_2CO_3 and extracted with EtOAc. The organic layer is dried over Na_2SO_4 and evaporated to dryness under a stream of N_2.

The radiochemical yield after subsequent demethylation is 50%. In vivo autoradiographic studies in the rat demonstrate a binding pattern consistent with the regional distribution and relative density of D1 receptors.

171.6

Differences in Dopamine D2 Receptor Distribution in Hippocampal Formation and Temporal Cortex in Human, Rat and Cat.

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The medial temporal lobe, including the hippocampus and para-hippocampus, is importantly involved in dementia associated with a number of disorders. While there is low density of tyrosine hydroxylase fibers reported in this area (Gaspar et al., JCN., 279: 249, 1989), there is a high densityof D2 receptor binding in cetain regions of the medial temporall lobe in human (Joyce et al., JPET, 1991). Analysis of the cellular and laminar distribution of the D2 receptor was accomplished by autoradiography with [^{125}I]epidepride (Epid). Neurons expressing the receptor were also identified immunocytochemically (IHC) with anti-peptide polyclonal antibodies (McVittie et al., PNAS,1991) in the temporal lobe of the rat, cat and human. Unlike frontal cortex where binding is low with the densest in deep layers, temporal cortex demonstrates a higher density of binding, with highest binding in external (I-III) and internal layers (V, VI). The pattern changes in areas 35 and 36 where binding was densest in layers I-III and low in layers IV and VI. Epid binding and IHC stained cells were conspicuously absent in the entorhinal cortex (area 28) in all three species. Epid binding was high in subregions of hpc in human and cat: CA3, dentate gyrus (DG) and subiculum. Low binding was observed in the rat. IHC staining in human revealed D2 cells in CA3, subiculum, piriform cortex and areas 35-36, but none in DG. In rat, IHC revealed a pattern of specific staining in hpc formation. In dorsal hpc, some pyramidal and basket cells were labeled, as well as in CA1 and CA2 with occassional cell staining in CA3. There were numerous cells stained in the hilus (CA4), but no cells stained in the dorsal DG in the rat. In ventral hpc, large numbers of DG cells were stained, as well as in CA1 and CA3. Piriform cortex showed IHC postitive cells. Staining with preimmune sera was negative. **Funded by MH 43852, MH 43880, AG 09215.**

171.7

DOPAMINE D_1 AND D_2 RECEPTORS COLOCALIZE TO THE SAME CELLS IN RAT MEDIAL PREFRONTAL CORTEX. S.L. Vincent, Y. Khan* and F.M. Benes. Department of Psychiatry and Program in Neuroscience, Harvard Medical School and Mailman Research Center, McLean Hospital, Belmont, MA 02178.

The neuronal localization of dopamine D_1 and D_2 receptor subtypes was investigated in rat medial prefrontal cortex (mPFC) using simultaneous binding of fluorescent derivatives of the antagonists SCH-23390-BODIPY (100 nM) and N-(p-aminophenethyl)spiperone-Texas Red (100 nM), respectively. Fresh-frozen, 10-µm-thick glass-mounted sections were incubated in a solution containing labeled antagonists alone or in combination and then viewed with epifluorescence optics. Specificity of binding for the labeled D_1 and D_2 probes was determined by inhibition with unlabeled SCH-23390 (100nM) or (+)-butaclamol (100 nM), respectively. In addition, because spiperone and its derivitives have a high affinity for serotonin 5-HT_2 sites, mianserin (100 nM) was also used to inhibit binding to this receptor. Low-power, photomicrographic montages through the depth of mPFC were analyzed for the presence of fluorochrome-labeled neurons. The greatest number of neurons labeled with either D_1 or D_2 ligand were found in layers V and VI, and there was nearly complete overlap for the two ligands. Tissue sections incubated with mianserin showed a similar distribution of D_1- and D_2-positive neurons, although fewer cells with intense fluorescence were found in the upper layers of mPFC. These data suggest that D_1 and D_2 dopamine receptor subtypes are colocalized on the same neurons and show a distribution that is similar to that of the mesocortical dopamine projection to mPFC in rat. Supported by MH00423, MH42261 and the Scottish Rite Foundation.

171.8

DOPAMINE RECEPTOR GENE EXPRESSION IN RAT AND HUMAN NIGROSTRIATAL COMPLEX: A NEUROANATOMICAL STUDY. C. Le Moine, E. Normand, V. Bernard, R. Guennoun, F. Tison, I. Aubert, M. Jaber, B. Bloch. URA CNRS 1200, Laboratoire d'Histologie-Embryologie, Université de Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France.

Dopamine receptor mRNAs (D1, D2, D3, D4, D5) were detected by in situ hybridization using radioactive and biotinylated oligonucleotides, in rat and human striatum and substantia nigra. Special attention was devoted to the phenotypical identification of the neurons expressing the dopamine receptor genes. In rat, the D1 gene is expressed in substance P neurons, and in a small number of cholinergic and somatostatinergic neurons. The D2 gene is expressed in enkephalinergic neurons, in 80% of cholinergic neurons and in dopaminergic neurons. All striatal neurons expressing the D2 gene contain the $D2_{444}$ mRNA isoform and express increased levels of D2 mRNA after haloperidol treatment (55% and 148% in enkephalinergic and cholinergic neurons, respectively). During ontogeny, D1 and D2 mRNAs appear, respectively, at gestational days 17 and 14, and DARPP-32 mRNA is present in 66% of the medium-sized neurons and in some cholinergic neurons. Experiments in progress demonstrate D1 and D2 mRNAs in human caudate and putamen neurons as well as D3, D4 and D5 mRNAs in rat striatal neurons. The abundance of neurons labelled with each specific probe suggests co-localization of several receptors within the same neurons. These results demonstrate a neurochemical complexity for dopamine receptor gene expression far more important than previously suspected on the basis of autoradiographic binding studies.

171.9

DISTRIBUTION OF DOPAMINE D1 AND D2 RECEPTOR SUBTYPES IN RAT CENTRAL NERVOUS SYSTEM: AN IMMUNOCYTOCHEMICAL STUDY. C.A. Kitt, A.I. Levey, D.L. Price, R. Maggio*, W. Kao* and M.R. Brann*. Neuropathol. Lab., Dept. Pathol., The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205; NINDS, NIH, Bethesda, MD 20892; University of Vermont, Burlington, Vermont 05405.

Five distinct genes have been identified that encode dopamine receptors (D1-5). To characterize receptor proteins in brain, we made antibodies to fusion proteins (pGEX2T bacterial expression vector) containing sequences unique to D1 and D2 but not in the other subtypes. Antisera reacted specifically with respective fusion proteins on immunoblots; receptor subtypes expressed in transfected cells were specifically immunoprecipitated. Immunocytochemistry revealed intense, complementary patches of D1 and D2 proteins in striatum and olfactory tubercle. In substantia nigra, D1 was located in neuropil and D2 in cells. D1-immunostained cells were seen in layers II/III and V of cortex, hippocampal CA1 pyramidal layer, bed nucleus of the stria terminalis, and portions of amygdala and cerebellum. Thus, antibodies provide cellular and subcellular localization of D1 and D2 gene products with sensitivity and specificity not achievable using autoradiographic techniques. These reagents should be useful for identifying the normal distribution of receptors in brain and alterations in disease.

171.10

IMMUNOCYTOCHEMICAL LOCALIZATION OF MUSCARINIC RECEPTOR PROTEINS IN RAT AND PRIMATE BRAIN AND COLOCALIZATION IN CHOLINERGIC NEURONS. A.I. Levey, C.A. Kitt, C. Heilman*, H. Fedor*, S.M. Edmunds*, D.L. Price and M.R. Brann*. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Balto., MD 21205; NINDS and NIH, Bethesda, MD 20892; and University of Vermont, Burlington, VT 05405.

Native muscarinic receptor proteins (m1-m5) in the brain were studied using subtype-specific antibodies, previously generated to their i3 loops. By immunoprecipitation and immunocytochemistry, three receptor proteins (m1, m2, and m4) accounted for the vast majority of total solubilized muscarinic binding sites and showed marked differences in regional and cellular localization in rat. m1 was present in most neurons in cortex and striatum; m2 receptor was abundant in cortex, basal forebrain, scattered striatal neurons, and was enriched in thalamus and brainstem. m2 and ChAT immunoreactivity colocalized in striatal neurons; however, in basal forebrain, m2 was present in many noncholinergic neurons. m4 receptor was enriched in neostriatum and olfactory tubercle and in patches in neocortex. Similar localization of m1, m2, and m4 was observed in monkeys. These studies indicate that the major receptor subtypes in rat and primate brain have important differences in cellular localization that help clarify their functions.

171.11

LOCALIZATION OF DOPAMINE D2 RECEPTOR PROTEIN IN RAT BRAIN USING POLYCLONAL ANTIBODY. J. Brock, S. Farooqui, K. Ross*, and C. Prasad. Lab. Neurosci., Pennington Biomed. Res. C, Baton Rouge; Dept. Med., LSUMC, New Orleans, LA 70112.

The precise distribution of the dopamine type D_2 receptor (D2) has been mapped for the first time, using an antibody to D2 receptor protein. Polyclonal antisera were collected from rabbits innoculated with an undecapeptide identical to residues 24-34 of the D2 protein sequence. Rat brain slices 40 µm in thickness were incubated with either primary antiserum, the antiserum plus free peptide antigen, or pre-immune serum. Antibody binding was visualized by peroxidase-antiperoxidase (PAP) and light microscopy. PAP complex was seen in discrete areas consistent with the binding of D2 radioligands and known expression of D2 receptor mRNA. Staining was heavy in the striatum, frontal and parietal cortices; staining was more diffuse throughout the medial forebrain bundle and hypothalamus. Unexpectedly, PAP was seen in lateral thalamic nuclei, also in the superior and inferior colliculi. These data suggest that D2 protein was recognizable at all levels of the dopaminergic systems (target tissue, axons, and cell bodies of origin). (Supported by Dept. of Army)

171.12

DOPAMINE D2 RECEPTOR CIRCUITS IN HUMAN CORTEX

J.N. Joyce. Departments of Psychiatry and Pharmacology, University of Pennsylvania School of Medicine, Philadelphia , PA

The primate cortex shows an expanded dopamine (DA) innervation to motor (agranular) and limbic (medial temporal lobe) regions as compared to other species. Comparison with the DA D2 receptor was examined in human postmortem material. The distribution of binding sites for the D2 receptor was visualized by autoradiography with [^{125}I]epidepride (Joyce et al., JPET, 1991). Neurons expressing the receptor were also identified immunocytochemically with anti-peptide polyclonal antibodies. Prefrontal cortex showed low binding in the deep laminae and few D2 positive neurons, but the primary sensory regions examined showed very low binding in all lami-nae. The agranular cortices (motor, cingulate) showed significiantly higher densities of binding sites in the external and internal laminae. Pyramidal neurons in layers II,III, and VI were positively stained for the D2 antibodies. The temporal cortex, except for primary auditory cortex, showed 3-fold higher numbers of binding sites than prefrontal cortex. In the middle and inferior temporal cortex the predominant pattering was of D2 receptors in the deep laminae. At the border of the collateral sulcus this pattern changed such that [^{125}I]epidepride binding sites and D2-positive neurons were located in the upper and lower laminae. The lateral occipito-temporal (LOT) cortex showed dense binding in the external laminae only, whereas the entorhinal cortex (EC) showed very low binding. D2 positive neurons were visible in the LOT but few in the EC. The projection zone of the LOT within the dentate gyrus exhibited D2 binding sites but no D2-positive neurons. In contrast, D2 binding sites and D2-positive neurons were located in the CA3 and subicular fields of the hippocampus and the basolateral nuclei of the amygdala. These data suggest that the D2 receptor is an important mediator of the actions of DA in motor and limbic cortex. **Funded by MH43852, MH 43880, AG 09215.**

171.13

GLUTAMIC ACID DECARBOXYLASE (GAD) IMMUNOREACTIVITY OF MAUTHNER CELLS AND HOMOLOGOUS NEURONS IN GOLDFISH. R. K. K. Lee[1], T. E. Finger[2] and R. C. Eaton[1]. [1]Center for Neuroscience, Univ. Colorado, Boulder, CO 80309-0334, and [2]Dept. Cell and Struct. Biol., Univ. Colorado Med. Ctr., 4200 East 9th Ave., Denver, CO 80262.

The hindbrain is partitioned into a series of 7 segments or rhombomeres. In goldfish, each rhombomere contains distinct clusters of reticulospinal (RS) neurons, some of which are reiterated across adjacent segments. The Mauthner cells and two pairs of segmentally homologous neurons are large and individually identifiable. We have studied the distribution of inhibitory synapses on these neurons using GAD (synthetic enzyme for GABA) immunocytochemistry in conjunction with retrograde labelling (fluorogold or fluorescein dextran) from the spinal cord. Immunoreactive punctate reaction product, presumably synaptic terminals, is present throughout the neuropil of the reticular formation in the vicinity of the Mauthner cells. The lateral dendrites of the Mauthner cells are densely covered by relatively large puncta. There is also a somato-dendritic gradient of GAD-positive terminations on the Mauthner cells. The highest density of immunoreactive puncta appears at the lateral and ventral dendrites; a much lower density is present on the soma. In contrast, GAD immunoreactive terminals are sparser and distributed uniformly across the dendrites and cell bodies of Mauthner cell homologues. These findings suggest that, in spite of developmental and morphological similarities between the Mauthner cell and its homologous counterparts, these neurons may exhibit different functional properties. [Supported by NIH grants NS22621 & DC00147 to R.C.E. & T.E.F. respectively].

171.14

DETECTION OF TWO FORMS OF GAD AND THEIR mRNAs IN RAT BRAIN BY IMMUNOHISTOCHEMISTRY AND NON-RADIOACTIVE IN SITU HYBRIDIZATION. M. Esclapez*, N.J.K. Tillakaratne, A.J. Tobin, and C.R. Houser. Brain Research Institute, UCLA, Los Angeles, CA 90024.

We have studied the localization of mRNAs encoding two forms of glutamic acid decarboxylase (GAD_{65} and GAD_{67}) using digoxigenin-labeled cRNA probes and, in adjacent sections, the immunohistochemical distribution of these forms using specific antibodies. Both GAD mRNAs were present in all subpopulations of GABA neurons examined, but patterns of labeling varied among brain regions. Strong labeling of the two GAD mRNAs was observed in several brain regions including the substantia nigra pars reticulata and reticular nucleus of the thalamus. Lighter but comparable mRNA labeling for both forms was present in many neurons of the superior colliculus and striatum. Other neurons were labeled heavily for only GAD_{67} mRNA, and these included neurons of the globus pallidus, a small population of neurons in the striatum and granule cells of the olfactory bulb. Most neurons that showed heavy labeling for either GAD mRNA also showed immunocytochemical cell body staining for that form of GAD. Due to the cellular resolution and minimal background obtained with the present non-radioactive *in situ* hybridization methods, our findings emphasize the different levels of GAD mRNA labeling among GABA neurons. Supported by Del Duca and Philippe Foundations, VA Medical Research Funds and NS21908.

171.15

LOCALIZATION OF mRNAs ENCODING TWO FORMS OF GLUTAMATE DECARBOXYLASE IN THE HIPPOCAMPAL FORMATION. C.R. Houser and M. Esclapez*. Dept. of Anatomy and Cell Biology and Brain Research Institute, UCLA, and VA Medical Center, Los Angeles, CA 90024.

Neurons containing mRNAs for two forms of glutamic acid decarboxylase (GAD) have been localized in the rat hippocampal formation by *in situ* hybridization histochemistry using digoxigenin labeled cRNA probes. The goals were to determine if the two GAD mRNAs were differentially localized among subpopulations of GABA neurons in the hippocampal formation and to clarify the distribution of GABA neurons in the polymorph or deep hilar regions of the dentate gyrus. The findings indicated that both GAD mRNAs were present in all subpopulations of hippocampal GABA neurons. However, mRNA labeling for the lower molecular weight form of GAD (GAD_{65}) appeared to be slightly greater than that for the higher molecular weight form (GAD_{67}) in many neurons of the polymorph region. This pattern was consistent with preferential labeling of the somata of some deep hilar neurons with an antibody to GAD_{65}. These studies confirm the presence of numerous GABA neurons in the deep hilus of the dentate gyrus that have proved difficult to label consistently with some immunocytochemical methods. Supported by VA Medical Research Funds, NIH grant NS21908, and Del Duca and Philippe Foundations.

171.16

DEMONSTRATION OF GAD IMMUNOREACTIVITY IN RAT HIPPOCAMPAL CELLS IN CULTURE. C.A. Levesque, J.M.R. Harrison*, H.R. Brashear. Depts. of Neurology and Neuroscience, Univ. of Virginia, Charlottesville, VA 22908

The major inhibitory neurotransmitter in the hippocampus is gamma-aminobutyric acid (GABA). GABA-ergic cells and their projections were identified in a well-characterized rat hippocampal pyramidal cell culture (Banker et al. Brain Res. 1977; 126:397) using glutamic acid decarboxylase (GAD) immunohistochemistry. After 16-23 days in culture, living cells on coverslips were fixed with 4% paraformaldehyde, 0.5% zinc salicylate in 0.5 M NaCl, and permeablized with 0.1% Triton. Coverslips were incubated with sheep anti-GAD antibody (kindly provided by Dr. D. Schmeckel) followed by biotinylated antisheep IgG, processed with avidin-biotin-peroxidase complex, and counterstained with ethidium bromide. Strong immunoreactivity without nonspecific staining occurred at an antibody dilution of 1:6000. Approximately 11% of cells showed classical GAD immunoreactivity with marked or moderate cytoplasmic staining. About 9% of cells showed equivocal staining and the remainder were clearly negative. Cell processes exhibited punctate labeling consistent with boutons. Many neurites appeared to make synaptic contacts with GAD-negative cells. Although flat astrocytes are rarely seen in this culture system, those observed were GAD-negative. A subpopulation of cultured hippocampal neurons exhibit a range of GAD immunoreactivity.

171.17

FLUORESCENCE IMAGING OF BENZODIAZEPINE BINDING SITES IN LIVING RAT CORTEX SLICES USING CONFOCAL MICROSCOPY. Q. Gu and M. Cynader. Department of Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

Benzodiazepines are a class of CNS-depressant drugs that have strong anticonvulsant, anxiolytic and hypnotic effects, and function by activating $GABA_A$ receptors to increase permeability of chloride ion channels. In the present study, benzodiazepine binding sites in living rat cortex slices were labelled with the fluorescent benzodiazepine derivate N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-desdiethyl-fluorazepam (NBD-Ro-1986, MOLECULAR PROBES), and visualized with a confocal laser scanning fluorescence microscope. The cortex slices were prepared as for standard electrophysiological slice experiments. Extracellular (field potential) and intracellular recordings were applied to make sure that cortical cells were healthy. After incubation with the NBD-Ro-1986, fluorescent clusters and spots, representing binding sites, were seen and analysed with three-dimensional reconstruction. The binding sites were clearly localized to the surface of cell bodies and processes, and could be displaced by competition with the non-fluorescent benzodiazepine lorazepam, indicating specific labelling. The size of labelled cell bodies varied widely, and cell bodies in the frontal cortex tended to show more intense fluorescence than those in the occipital cortex. These results indicate that various cell populations in the cortex can be differentially affected by benzodiazepines. This technique can also be used to label other membrane receptors or ion channels in living tissues, and the distribution and regulation of these binding sites can be studied at the single cell level.

171.18

PENTOBARBITAL (PB) DECREASES THE DISSOCIATION CONSTANT (K_D) FOR [^{3}H]FLUNITRAZEPAM ([^{3}H]FLU) DIFFERENTIALLY ACROSS BRAIN REGIONS. B.X. Carlson and H.A. Baghdoyan. Department of Anesthesia, Penn State University, College of Medicine, Hershey, PA 17033.

The mechanisms by which hypnotic barbiturates and benzodiazepines (BDZ) induce sedation are not well understood. These pharmacological agents have binding sites on the gamma amino-butyric acid/BDZ (GABA/BDZ) receptor, and it is thought that barbiturate-induced sedation may be produced, in part, by enhancing the actions of BDZ at the GABA/BDZ receptor. Our preliminary data suggested that PB alters the K_D for [^{3}H]FLU differentially across brain regions (Neurosci. Ab.,16: 689, 1990). We now present further evidence supporting the hypothesis that PB alters the K_D for [^{3}H]FLU differentially throughout the brain.

Using standard receptor binding assays, tissue homogenates from six brain regions (cortex, diencephalon, midbrain, pons, medulla, cerebellum) were incubated with [^{3}H]FLU in the absence or presence of PB (0.5mM). Saturation analyses revealed that PB significantly decreased the K_D ($p<0.05$; paired t-test) within each brain region. The decreases in K_D were significantly different between the six regions ($F=7.1;df=5,23;p=0.0004$), and the largest PB-induced decrease in the K_D occurred in the medulla (-42%). There was no significant effect of PB on the B_{max} ($F=3.0;df=1,46;p=0.09$). These data are consistent with the hypothesis that PB may produce sedation, in part, by increasing the affinity of the GABA/BDZ receptor for BDZ differentially within the brain.

Supported by Department of Anesthesia & MH45361 to HAB.

171.19

PERIPHERAL-TYPE BENZODIAZEPINE (τ) RECEPTOR CHARACTERISTICS IN BOVINE PROSTATE: A STEROID DEPENDENT ORGAN. H.L. Grandel*, P.F. Consroe, H.E. Laird II. Univ. of Arizona, College of Pharmacy, Dept. of Pharm/Tox, Tucson, Arizona 85721.

Bovine prostate mitochondria were isolated using subcellular fractionation techniques. Using the isoquiniline carboxamide, PK11195, which is specific for the peripheral-type benzodiazepine receptor (τ), the τ receptor in the bovine prostate mitochondrial and microsomal fractions were characterized. The purified mitochondrial membrane preparation contained the greatest density of τ receptors. Non-linear analyses of data obtained from saturation experiments, revealed that the τ receptor in the bovine prostate mitochondria had a Kd = 0.324 ± 0.033 nM and a Bmax = 1.64 ± 0.018 pmol/mg protein. In comparison to previous work done in this laboratory, (Parola & Laird, **Life Sci.** 48:757, 1991), the prostate contains fewer numbers of τ sites than the adrenal cortex and testes, but is similar to the number of τ receptors found in the kidney and brain. Competition experiments revealed that the rank order of binding potency for τ receptor ligands to the bovine prostate mitochondria was PK11195=Alpidem>>>>>RO5 4864=Clonazepam. The bovine τ receptor has low affinity for benzodiazepine ligands which is consistent with previous reports in this species. To date, the prostate τ receptor has been reported in only one other species, rat. (Katz, et al., **Biochem. Pharmacol.** 40:817,1990). (Supported by Ariz. Dis. Con. Res. Comm. #82-1685, HEL)

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS II

172.1

ALKYLATION OF MELATONIN RECEPTOR UNCOUPLES SIGNAL TRANSDUCTION IN CHICK BRAIN. S.-W. Ying and L.P. Niles. Dept. Biomedical Sciences, Division of Neuroscience, McMaster University, Hamilton, Ontario, Canada, L8S 3Z5.

In order to determine whether the melatonin receptor is sensitive to sulfhydryl (-SH) group alkylation as reported for other G_i coupled receptors, the effects of the irreversible alkylating agent, N-ethylmaleimide (NEM) were investigated. NEM caused a concentration-dependent inhibition of [^{125}I]MEL binding. At least two -SH groups were involved, one of which was highly (50-fold more) sensitive to NEM. Alkylation of the sensitive -SH group by a low concentration of NEM abolished the high-affinity state of the melatonin receptor and led to a complete loss of sensitivity to GTP. In addition, pre-treatment of membranes with a low concentration of NEM uncoupled melatonin receptor-mediated inhibition of adenylate cyclase activity in chick brain. These findings show that the melatonin receptor is coupled to an inhibitory G protein and sulfhydryl groups play an important role in melatonin signal transduction.

Supported by OMHF and NSERC Canada.

172.2

GLYCINE RECEPTOR AND GLYCINE BOUTON CORRESPONDENCE IN THE DEEP CEREBELLAR NUCLEI. S. Chen and D. Hillman, Dept. Physiol. and Biophys., NYU Med. Ctr., New York, NY 10016.

Glycine is recognized as a prominent and powerful inhibitory transmitter in the brainstem and spinal cord while, in the cerebellum, the glycine content is relatively low and GABA is high. The role of glycine in cerebellar signal-transmission has been questioned because of: 1) the coexistence of glycine and GABA in the same neurons and terminals, 2) non-matching of glycine positive terminals to glycine receptors, and 3) the difficultly in distinguishing between the "transmitter" and "metabolic" pools. Our glycine immunohisto-chemistry revealed positive small DCN cells (presumed interneurons) throughout all parts of the DCN. Semithin plastic sectioning showed extensive labeling of glycine fibers and terminals around large DCN cells. Additionally, numerous positive, large neurons (presumed projection cells) were limited to the fastigial nuclei. The GlyR was found along dendrites and around the soma surface of medium and large sized DCN cells in all three nuclear groups. A small dorsal part of the dentate nucleus had an especially dense labeling. The close correspondence between the glycine terminals and glycine postsynaptic sites (GlyR) favors a neurotransmitter role for glycine in these nuclei. Supported by USPHS NS-13742.

172.3

CHARACTERIZATION OF MUSCARINIC RECEPTOR SUBTYPE OF RAT ECCRINE SWEAT GLAND BY AUTORADIOGRAPHY. N. E. Torres*, P. J. Zollman*, P. J. Dyck, and P. A. Low. Neurophysiol. Lab., Dept. Neurology, Mayo Clinic, Rochester, MN 55905.

The muscarinic cholinergic receptor of rat eccrine sweat gland was characterized using quantitative autoradiography and [^{3}H]-QNB as radioligand. The distribution of radioligand was maximal in the secretory coil. Autoradiographic competition binding studies were performed using selective antagonists to M_1 (pirenzepine), M2 (AF-DX 116) and M_3 (4-DAMP) and the classical nonselective antagonist atropine. pK_I for pirenzepine, AF-DX 116, 4-DAMP and atropine were 6.58, 5.47, 8.50 and 8.66 respectively indicating that the eccrine sweat gland muscarinic receptor was predominantly M_3 (Table).

Antagonist	pIC_{50}	Hill Coefficient	Regression Coefficient	pK_I[a]
Atropine	8.08±0.06	0.97±0.07	0.9698	8.66
4-DAMP	7.92±0.04	0.79±0.04	0.9854	8.50
Pirenzepine	6.00±0.04	1.02±0.03	0.9927	6.58
AF-DX 116	4.89±0.04	1.04±0.05	0.9816	5.47

[a] Inhibition constants were converted from the IC_{50} values using the Cheng and Prusoff (1973) equation.

172.4

CHARACTERIZATION OF THE CHOLINERGIC INNERVATION OF THE HIPPOCAMPUS IN THE RAT FOLLOWING FORNIX TRANSECTION. B. W. Fenton*, M. B. Moss, and D. L. Rosene; Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118

Three different markers of cholinergic fibers: hemicholinium (HC), acetylcholinesterase (AChE) and oxotremorine were used to quantify the effect of fornix transection in rat hippocampal subfields. Radioactively labeled HC binds the high affinity choline uptake (HACU) sites on cholinergic axon terminals. Radioactive oxotremorine labels the M2 muscarinic receptor. The Tago AChE method provides quantifiable assessment of cholinergic fibers and diffuse AChE. Unilateral or bilateral lesions of the fornix were made behind the septum with a Scouten wire knife, completely severing the fornix without damaging cingulate cortex. After 35 days the animals' brains were frozen at -70° C, cut on a cryostat and adjacent sections processed with the three methods and analyzed on an imaging system. Unilateral fornix lesions reduced AChE by 78% in all ipsilateral subfields but bilateral lesions reduced it by 85%. Unilateral cases had less depletion in all subfields of the temporal pole, accounting for the greater loss in bilateral lesions. In the unilateral cases HACU density was reduced by 40% overall, but like AChE, the reduction was smaller in the temporal hippocampus. In both bilateral and control cases both AChE and HACU density were uniform from the dorsal to the temporal pole. The gradients of AChE and HACU in the unilateral cases may reflect either sprouting or normal bilateral innervation of the temporal hippocampus through the hippocampal commissure. AChE in the molecular layer of CA1 was largely preserved after either fornix lesion but HACU density was reduced to the same extent as other subfields, indicating that the preserved AChE may be non-cholinergic. Thus AChE is generally useful for localization of cholinergic afferents but may overestimate their loss. In contrast to the reductions of AChE and HACU density, oxotremorine binding showed an 8% loss, suggesting that the majority of M2 receptors are not localized on cholinergic afferents. "Supported by NIH grants AG04321, NS16841, and NS07152, and an Alzheimer's Association grant."

172.5

CELLULAR MAPPING OF m1-m5 MUSCARINIC RECEPTOR mRNA's IN RAT BRAIN. D. M. Weiner, A. I. Levey, and M. R. Brann* HHMI-NIH Res. Schol. Prog; Dept. of Neurology and Neuropathology, JHU; and LMB-NINDS, Bethesda MD., 20814.

We have used a series of subtype specific oligodeoxynucleotide probes to localize, with cellular resolution, the pattern of mRNA expression of the m1-m5 muscarinic acetylcholine receptors. In cortex, the m1 and m4 are found in all layers, in ~90% and ~75% of neurons respectively. The m2 was localized to layers 3-6, the m3 to layers 2,3 and 6, while no cortical expression of m5 was observed. The m1, m3, and m4 are expressed by most neurons in the hippocampal formation. The m2 is seen mainly in the polymorphic layer of the dentate gyrus, as well as in the pyramidal cells in the Ca feilds of Ammon's horn. The m5 is restricted to the pyramidal cells in Ca1 and Ca2. The m2 and m3 are widely expressed in the thalamus, predominately in the anterior, intralaminar, and midline groups, where they display overlapping yet distinct patterns of expression. In the hypothalamus, the m3 is observed in various mammilary nuclei, while m5 is found in the ventromedial nucleus. The m2 and m3 are the predominant receptors expressed throughout various brainstem nuclei. The m2 is widely expressed by cholinergic neurons, however some of these neurons also express other receptor subtypes. These mRNA data, along with data about the distribution of receptor proteins, may help to determine the functional roles these subtypes subserve.

172.6

THE DISTRIBUTION OF CALBINDIN CONTAINING CELL BODIES AND FIBERS IN THE CORTEX OF PRIMATES. S.Iritani, K.Kase*, K.Kobayashi* and T.Kaneko* Tokyo Metropolitan Matsuzawa Hospital, 2-1-1 Kamikitazawa Setagaya Tokyo 156 Japan

Calcium ions play a key role in many aspects of neuronal behavior and certain calcium binding proteins may influence this behavior. We have studied distribution of calbindin 28K immunoreactive cell bodies and fibers in the cerebral cortex of macaque monkey and of post-motem human brain by immunohistochemical technique. Many immunoreactive cell bodies were seen mainly in layer II, III and V, and some cell bodies stained heavily and others stained lighitly. Immunoreactive fibers were also observed mostly in layers II,III and V, and many of fibers run perpendicular to the surface. Immunopositive granular deposits were seen among fibers. These immunoreactive structures were seen as two bands within the cortex. The physiological function of this protein has not been clarified yet, but this characteristic pattern of distribution of calbindin 28K suggests that this protein has significant role in the primate cerebral cortex.

172.7

[^{3}H]-1,25-DIHYDROXYVITAMIN D_3 (SOLTRIOL) BINDING IN THE MIDLINE AND INTRALAMINAR THALAMUS OF THE SIBIRIAN HAMSTER.

I. M. Musiol*, W. E. Stumpf, H. Bidmon*, and Ch. Pilgrim. Dept. of Cell Biology and Anatomy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 and Abteilung für Anatomie und Zellbiologie, Universität Ulm, D-7900 Ulm, Germany.

Autoradiographic studies revealed receptors for Vitamin D in the brain of rats and mice. In the present study, six month old Sibirian hamsters (Phodopus sungorus) were sc injected with tritated 1,25-dihydroxy-vitamin D_3 (1,25-D_3, vitamin D, soltriol) in two pulses of 0,2 ug/100 g bw each in one hour interval, and sacrificed four hours after the first pulse. Thousand-fold excess of unlabeled ligand injected 30 min before and with the first pulse (50% each) prevented nuclear labeling. Autoradiograms were processed according to the thaw-mount method. Neurons with nuclear concentration of labeled hormone are found in most midline and intralaminar nuclei (n.). Strongest nuclear labeling exists in the n. reuniens, n. rhomboideus, n. gelatinosus, and an extented group dorsal to the zona incerta. Nuclear labeling is also present in the n. paraventricularis, n. parataenialis, n. intermediodorsalis, a portion of the n. mediodorsalis, and in the region of n. centromedialis and centrolateralis.

The extensive presence of vitamin D-soltriol receptors in the limbic thalamus of a strongly seasonally adjusted species like the Sibirian hamster, suggests a regulatory role and seasonal modulation of soltriol on thalamic nuclei that receive afferents from the hypothalamus and the reticular formation of the lower brainstem and project to various cortical regions.

172.8

LOCALIZATION OF TRH RECEPTOR mRNA IN THE CAUDAL BRAINSTEM AND SPINAL CORD OF RAT. W. Wu[1], R. Elde[1,2], M.W. Wessendorf[1] and T. Hökfelt[2] Dept. Cell Biol. & Neuroanat., Univ. Minnesota[1], Minneapolis, MN 55455, and Dept. Histology and Neurobiology, Karolinska Institute[2], S-104 01 Stockholm, Sweden.

Thyrotropin-releasing hormone (TRH) is expressed in some serotonergic, bulbospinal neurons and has a facilitatory effect on motor neurons in the spinal cord of rat. Although TRH binding has been demonstrated in spinal cord, the identity of cells which produce TRH receptors has not been determined. Four, 48-mer oligonucleotides complementary to selected regions of the cDNA for mouse pituitary TRH receptor (Straub et al., PNAS 87: 9514, 1990) were individually 3'-end labelled, mixed and applied to cryostat sections of spinal cord and caudal brainstem of rat. Both film and emulsion-dipped autoradiograms were developed.

Hybridization signals were observed over large motor neurons in the facial nuclei in the brainstem. Clusters of grains were also observed over large motor neurons in the ventral horn and over small- to medium-sized neurons in the intermediate gray of the spinal cord. These findings suggest that neurons which are synaptic targets of bulbospinal TRH neurons express TRH receptor mRNA, and that these G-protein linked receptors are post-synaptic structures.

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172.9

TACHYKININ IMMUNOREACTIVE CELLS IN THE RAT VENTRAL PREMAMMILLARY NUCLEUS: SEX DIFFERENCES, CASTRATION EFFECTS, AND GONADAL STEROID BINDING. T.R. Akesson and P.E Micevych, Dept of VCAPP, Wash State Univ, Pullman, WA and Dept of Anat & Cell Biol and Lab of Neuroendocrinol, UCLA Sch Med, LA, CA.

The ventral premammillary nucleus (PMv) in the caudal hypothalamus has reciprocal connections with sexually dimorphic nuclei such as the medial preoptic nucleus, the bed nucleus of the stria terminalis, and the medial nucleus of the amygdala. Lesions of the PMv in female rats block the surge of gonadotropin that normally occurs on proestrus, but other roles of this nucleus have yet to be elucidated. We present several new anatomical observations that suggest the PMv may also participate in neural mechanisms that regulate sexual behavior.

Males have more tachykinin containing cells than do females, and in both sexes, numbers were unaffected by the steroid milieu.

Sex	N	Treatment	# Tachykinin-positive cells
♂	4	Intact	1337 ± 58
♂	4	Castrate	1222 ± 67
♀	4	Ovx - 2 wks	524 ± 77
♀	4	Ovx - E capsule	574 ± 108
♀	3	Ovx - E injection	623 ± 41

We have previously reported a small population of E-concentrating cells along the ventrolateral edge of the PMv and a small proportion of these E-accumulating cells also contain tachykinin. However, using ^{3}H-dihydrotestosterone or R1881, a much higher number of steroid-concentrating cells are seen throughout the PMv. Androgen-concentrating cells that contain immunoreactive tachykinin are common. These results suggest that androgen-receptive tachykinin-containing neurons may form a component of the neural network controlling reproductive function. Supported by HD22869 and NS21220.

172.10

[LEU^{31},PRO^{34}] NEUROPEPTIDE Y IDENTIFIES NEUROPEPTIDE Y RECEPTOR SUBTYPES IN THE RAT BRAIN. D.A. Schober, S.L. Gackenheimer and D.R. Gehlert. CNS Research, Lilly Research Labs, Eli Lilly and Company, Indianapolis, in 46285

Receptor subtypes for neuropeptide Y (NPY) have been previously differentiated into Y_1 and Y_2 subtypes based upon the binding profiles of long C-terminal fragments of NPY, especially the fragment NPY 13-36 which binds Y_2. In this study, our objective was to evaluate the analog [Leu^{31},Pro^{34}]NPY (LP-NPY) for its potential use in the identification of NPY receptor subtypes. LP-NPY has been reported in the literature to be highly selective for the Y_1 receptor subtype. ^{125}I-peptide YY (PYY) binding to rat brain homogenates and tissue slices were performed to determine the binding characteristics of LP-NPY. Results were analyzed using Lundon software for ligand binding and an image analysis system for the autoradiographic studies.

The Y_1 receptor selective analog LP-NPY was found to produce a partial inhibition of ^{125}I-PYY binding in rat whole brain homogenates. Displacement curves using this analog were biphasic with K_i values of 1.01 nM and 13.46 μM. Saturation experiments revealed multiple binding sites for ^{125}I-PYY alone, however, when ^{125}I-PYY was incubated in the presence of 100 nM LP-NPY, the low affinity binding component was eliminated. Two-site analysis of ^{125}I-PYY binding resulted in plots with K_d values of 55.11 pM and 715.6 pM with B_{max} values of 28.64 fmol/mg protein and 131.4 fmol/mg protein, respectively. One-site analysis of ^{125}I-PYY + 100 nM LP-NPY binding yielded a K_d value of 67.8 pM and a B_{max} of 10.12 fmol/mg protein.

Autoradiographic analysis using ^{125}I-PYY in the presence and absence of 100 nM LP-NPY revealed a selective binding pattern in distinct brain regions. Areas that displayed almost complete displacement by 100 nM LP-NPY (Y_1) were the anterior olfactory nucleus, lamina 1-3 of the cerebral cortex , and ventrolateral thalamic nucleus. Those brain regions that were relatively resistant to LP-NPY displacement and appeared to be mainly Y_2, were the lateral septum, globus pallidus, and paraventricular hypothalamic nucleus. Areas where binding to the two sites were evenly distributed include the olfactory tubercle, nucleus accumbens, and gracile nucleus.

^{125}I-PYY was found to label the Y_1 binding site with lower affinity than the high affinity component which appeared to be Y_2. Furthermore, it appears the LP-NPY can be used as a useful tool to reveal the distribution of NPY subtypes in the rat brain.

172.11

IMMUNOREACTIVE-NEUROTENSIN AND -GALANIN IN THE PITUITARY GLAND AND BRAIN OF TWO SPECIES OF Xiphophorus. L. Cepriano and M. P. Schreibman. Department of Biology, Brooklyn College, C.U.N.Y., Brooklyn, New York 11210.

The distribution of neurotensin (NT) and galanin (GAL) in the brain and pituitary gland of Xiphophorus maculatus, the platyfish, and Xiphophorus helleri, the swordtail, was studied by employing immunocytochemistry on Bouin's-fixed, polyfin embedded material. Immunoreactive- (ir-) NT was localized the neurohypophysis and in the three regions of the adenohypophysis. In young animals, NT was not observed in the pars intermedia of the adenohypophysis. Ir-Gal was localized in perikarya and fibers of the nucleus lateralis tuberis of the Xiphophorus brain and in the neurohypophysis and the three region of the adenohypophysis. The localization of ir-NT and -GAL in the NLT, a brain nuclei previously implicated in pituitary function, and pituitary glands of these animals suggests that these peptides are involved in the regulation of pituitary function. [Supported by NASA (NAGW-1704), AID, and PSC-CUNY.]

172.12

THE DISTRIBUTION OF ESTROGEN RECEPTORS IN THE PIG HYPOTHALAMUS. F.J.C.M. van Eerdenburg, S. Chouham*, J.F. Axelson**, D.F. Swaab*** and F.W. van Leeuwen***. Univ. Utrecht, 3508 TD Utrecht, The Netherlands; *Univ. Hassan-II, Casablanca, Morocco; **Holy Cross College, Worcester, MA, USA; ***Neth. Inst. Brain Res., Amsterdam.

The recently described vasopressin and oxytocin containing nucleus (VON) in the pig hypothalamus contains three times as many cells in the adult female as that of the male, which is due to a sexually differential increase in neuron number starting at puberty (Van Eerdenburg et al., J. Comp. Neurol. 301, 138, '90). Since the neuronal number of the VON is influenced by gonadal steroids, a study was initiated to find out whether the VON contains estrogen receptors (ER) and to describe their distribution in the hypothalamus. Using antibodies against the human ER (#H222, Abbott), hypothalami of intact post-pubertal female and male pigs were incubated according to Axelson and Van Leeuwen (J. Neuroend. 2, 209, '90). Nuclear ER staining was found in many areas. The density was more intense in females, e.g. in the medial preoptic area, the area resembling the rat sexually dimorphic nucleus (SDN) of the preoptic area, the bed nucleus of the stria terminalis and the ventromedial hypothalamus. The VON, although surrounded by cells with ER, showed a complete absence of ER. No clear sex differences were observed, except for the SDN which contained more ER cells in females. In conclusion, the distribution of ER in the pig hypothalamus resembles that found in other mammals. Gonadal steroids seem to affect the VON indirectly.

172.13

DIFFERENTIAL EXPRESSION OF ESTROGEN RECEPTOR AND ITS mRNA IN HIPPOCAMPUS AND HYPOTHALAMUS OF MOUSE AND RAT. E. Combatsiaris*[1], M.D. Bergman*[1], W. Schneider*[2], G. Shyamala*[2], E.R. DeSombre*[3], B.A. Brooks[1] and J.F. Nelson[1]. [1]Dept. of Physiology, U. of Texas Health Science Center, San Antonio, TX 78284-7756., [2]Lady Davis Research Inst., Montreal, Canada, [3]Ben May Laboratory of Cancer Research, U. of Chicago.

A recent study of the distribution of estrogen receptor (ER) immunoreactivity in the female rat brain gave evidence of unprecedentedly high levels of ER in the hippocampus (Mol Endocrinol 3:1165). Contrary to results from earlier ligand-binding assays, levels of immunoreactive ER in the hippocampus were similar to those in the hypothalamus. The present study was designed to examine this question further by evaluating the distribution of both ER and its messenger RNA in the brains of mice and rats. Brain regions from ovariectomized C57BL/6J mice and Sprague-Dawley rats were dissected and 1) homogenized immediately for ER measurements either by ligand binding or by immunoprecipitation and western blotting with a monoclonal antibody to ER (H222) or 2) frozen for ER mRNA determinations by solution hybridization/RNase protection assay using a cRNA probe complementary to a transcribed portion of the mouse ER gene. In both mice and rats the amount of radiolabeled estradiol specifically bound (cytosolic + nuclear ER) in hippocampi was 13% of that bound in hypothalami. Western analysis revealed significant ER immunoreactivity in the hypothalamus but negligible ER immunoreactivity in the hippocampus. Furthermore, the amount of ER mRNA in mouse hippocampus was only 24% of that in mouse hypothalamus. These observations do not support the results of the previous study of ER immunoreactivity, but confirm and extend those of earlier ligand binding studies by showing in both mice and rats that concentrations of ER and ER mRNA are markedly lower in the hippocampus than in the hypothalamus.

172.14

ESTROGEN RECEPTORS IN DOPAMINERGIC AND PEPTIDERGIC NEURONS IN THE HYPOTHALAMUS OF THE PRAIRIE VOLE. A.A. Gerall, L. Givon, and D.T. Outlaw*. Dept. of Psych., Tulane Univ., New Orleans, LA 70118.

In the rat, whose ovulation is triggered cyclically by estrogen, autoradiographic assays have demonstrated that ^{3}H-estrogen accumulates in subsets of dopaminergic and beta-endorphin- but not in gonadotropin releasing hormone (GnRH)-immunoreactive (ir) neurons. Using immunocytochemistry, we examined whether the estrogen receptor (ER) is present in similar subsets of neurons in the prairie vole whose ovulation is evoked by copulation. Also, colocalization of ER with oxytocin-ir neurons was determined in the rat.

First, the endogenous neuroactive substances were labelled using a modified Sternberger peroxidase-anti-peroxidase procedure and the chromogen diaminobenzidine. ERs were visualized using the H222sp$_{\Gamma}$ monoclonal antibody (courtesy of Abbott Laboratories), the ABC method (Vector Laboratories) and benzidine dihydrochloride as the second chromogen.

GnRH-ir neurons in the vole's brain did not contain ER-ir. A subset of TH-ir neurons in the periventricular and arcuate nuclei but none in the zona incerta had ER-ir. A small proportion of beta-endorphin-ir neurons in the arcuate nucleus colocalized ER-ir. In the above neurons, ER-ir was confined to their nuclei. ER-ir was most evident in oxytocin-containing neurons in the anterior commissural and paraventricular nuclei of the rat. Although the rat and vole have different ovulatory mechanisms, their ER appear to be colocalized in similar neuronal systems.

172.15

REGIONAL DISTRIBUTION OF MINERALOCORTICOID RECEPTOR IN RAT BRAIN BY WESTERN BLOT ANALYSIS. B.S. McEwen, R.R. Sakai, T.K. Akompong and Z. Krozowski* The Rockefeller Univ., 1230 York Ave., NY., NY 10021 USA and Prince Henry's Hospital, Monash Medical Centre, Melbourne, Australia, 3004.

Regional localization of mineralocorticoid receptor was measured by Western blot analysis in micro-punched rat brain tissue. Cytosolic brain mineralocorticoid receptor content in various brain regions was determined with the polyclonal antibody, MINREC IV, which was raised against a 167 amino acid sequence of the human mineralocorticoid receptor.

Mineralocorticoid receptor protein was present in hippocampus, cortex, preoptic area, bed nucleus of the stria terminalis and amygdala. Cytosolic levels of mineralocorticoid receptor protein were increased in 3 day adrenalectomized rats and correlated with decreased nuclear labelling of both mineralocorticoid (MINREC IV) and glucocorticoid (BuGR) receptors visualized by immunocytochemistry.

These results provide a means by which immunocytochemical labelling of mineralocorticoid receptors may be semi-quantitated with concurrent Western blot analysis. Supported by MH43787 (BSM).

172.16

PLACENTAL LACTOGEN BINDING SITES IN THE PREGNANT RABBIT CNS. L.P.Mangurian and R.J.Walsh. Department of Biological Sciences, Towson State University, Towson, MD and Department of Anatomy, George Washington University, Washington, DC.

The highest concentrations of prolactin receptors in the CNS reside within the hypothalamus and choroid plexus. In an effort to evaluate these areas for potential sites of action of placental lactogens (PL), in vitro autoradiography was used to localize PL binding sites in the brain of pregnant New Zealand White rabbits. Frozen brain and liver sections were incubated in a medium containing ^{125}I human PL (hPL) alone (total binding) or with a 500-fold excess of unlabelled hPL (non-specific binding). Specificity was assessed with excess unlabelled hGH and oLH. An intense autoradiographic reaction occurred over the choroid plexus and liver sections. Excess unlabelled hPL and hGH caused a significant reduction ($p<.001$) in the binding of the ^{125}I hPL while unlabelled oLH had no effect. In contrast, the hypothalamus exhibited a weak autoradiographic reaction that was not affected by any excess unlabelled hormones. The results support a role for the choroid plexus in PL to brain interactions but do not define a hypothalamic site of action.
Supported by TSU Faculty Research Grant (LPM) and BRSG Grant #684-762 (RJW).

172.17

DISTRIBUTION OF CANNABINOID RECEPTOR mRNA IN RAT BRAIN. L. M. Console-Bram[1], J.G. McElligott[1] and S.G. Fitzpatrick-McElligott[2]. Temple University, Pharmacology Dept., Phila., PA 19140[1] and E.I. Du Pont, Medical Products, Glasgow, DE 19702[2].

Histological distribution of cannabinoid receptor mRNA was determined in the brains of both adult and neonatal rats. A 30-base oligonucleotide, complementary to the mRNA, was synthesized from the known cDNA cannabinoid receptor sequence (Matsuda et al., 1990). The oligonucleotide was radiolabelled and used to probe sections of rat brain tissue according to *in situ* hybridization methodology. From these experiments, cannabinoid receptor mRNA in the adult rat brain was found to be localized to the granule cell layer and deep nuclei of the cerebellum, granule and pyramidal cells of the hippocampus, the habenula, diagonal band of Broca, mitral layer of the olfactory bulb, basal ganglia and cerebral cortical layers. Brainstem nuclei expressing receptor mRNA include, the inferior olive, pons and the superior and inferior colliculi. Results from preliminary developmental studies indicate that the pattern of expression of mRNA in the neonatal rat brain (first two postnatal weeks) is similar to that of adult rat brain. The presence of cannabinoid receptor mRNA in the cerebellum, basal ganglia and hippocampus support the involvement of these brain regions in the production of adverse effects such as, difficulty in motor coordination (ataxia) and memory acquisition as a consequence of cannabinoid usage. The localization of cannabinoid receptor mRNA in these brain structures is in agreement with the distribution of receptor protein as reported by Herkenham et al. (1991).

(Supported by NIDA grant T32 DA 07237 and NIH grant DC01094)

172.18

AUTORADIOGRAPHIC DISTRIBUTION OF SIGMA RECEPTORS IN HUMAN NEOCORTEX,HIPPOCAMPUS,BASAL GANGLIA,CEREBELLUM & PINEAL

K.L.R. Jansen[1],R.L.M. Faull[2],M. Dragunow[3],R.A. Leslie[4]
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[2]Anatomy ,[3]Pharmacology, Auckland University,P.B., New Zealand

The distribution of σ receptors in selected regions of human brain was studied using autoradiography.Sections from 8 brains (5 males,3 females),average age 52 (range 26-70) were labelled with 6nM [^{3}H]diorthotolyl guanidine ([^{3}H]DTG) as previously described (Jansen et al.,*Brain Res.*,507, 158 -160).The highest densities among the areas examined were in the substantia nigra pars compacta and over the cerebellar Purkinje cell layer. Pineal levels were modest compared to rat where they are very dense.This may reflect the age of the sample.In cerebral cortex, binding was enhanced in the superficial laminae (II-IVA) and attenuated over a band in the midzone of the gray matter in several areas, especially visual cortex where the band of attenuation corresponded to lamina IVB-IVCα and was followed by a dense band corresponding to IVCβ. There was slight enhancement over the granular layer of the hippocampal dentate gyrus. Binding was modest in caudate and putamen, with less binding in the globus pallidus.The high density over the cerebellar Purkinje and molecular layers was similar to the pattern in rodents. The results of this study support the proposition that σ receptors may be involved in the control of movement and mental status.Supported by NZMRC,NZ Neurological Foundation.

SECOND MESSENGERS V

173.1

G-PROTEIN COUPLING INFIDELITY BY THE MELATONIN RECEPTOR AFTER EXPRESSION IN XENOPUS OOCYTES. P. Barrett[1*], P.J. Morgan[1], S.P. Fraser[2*] and M.B.A. Djamgoz[2*]. [1]Rowett Research Institute, Aberdeen, AB2 9SB and [2]Department of Pure and Applied Biology, Imperial College, London, U.K.

Melatonin receptors inhibit forskolin-stimulated cAMP accumulation via inhibitory G-proteins in *pars tuberalis* (PT) cells. In an attempt to clone the gene for the melatonin receptor this tissue has been used as a source of RNA for microinjection into Xenopus oocytes.

Total RNA was extracted from the PT and either used for selection of poly A$^+$ RNA on an oligo dT column or size fractionated on a 5-20% sucrose gradient. RNA solution (70 nl) was microinjected into oocytes (stage VI) and incubated in modified Barths for 4-10 days. Recordings were made under voltage clamp conditions, whilst oocytes were perfused with frog ringer with or without drugs.

Aluminium fluoride (AlF_4^-) made by mixing 10 mM NaF and 0.1 mM $AlCl_3$ induced a slow inward oscillatory current with a reversal potential of -24 mV. This response resembles the Ca^{2+}-dependent Cl^- current seen in Xenopus oocytes with a variety of stimulants including carbachol which induces these currents through the muscarinic acetylcholine receptor. Using AlF_4^- or carbachol to stimulate oocytes, melatonin (1 mM) blocked the oscillatory current in oocytes which had been injected with poly A$^+$ RNA or size fractionated total RNA extracted from the PT. However, melatonin had no effect on non-injected oocytes. The dose-dependency and pharmacology of the response were consistent for the melatonin receptor. The response to AlF_4^- and subsequent inhibition by melatonin was not altered by the addition of EGTA to the incubation medium, implicating the internal calcium stores in the response to AlF_4^-.

These results demonstrate the expression of a functional melatonin receptor linked to the inhibition of the IP_3/Ca^{2+} signal transduction pathway. Therefore the expressed receptor lacks the G-protein coupling fidelity of its native PT cell.

173.2

DIFFERENCES IN THE FUNCTIONAL RESPONSES OF TWO CELL LINES EACH EXPRESSING PLC-COUPLED MUSCARINIC RECEPTORS. J. Baumgold and R. Paek Dept. of Radiology, The George Washington University Medical Center, Washington, DC 20037

Stimulation of the m1, m3 and m5 muscarinic receptors results in activation of phospholipases C (PLC) and A, increase in intracellular calcium and activation of protein kinase C. Herein we show that these biochemical responses do not always result in the same functional responses.

Fluoresecent oxonol dyes were used to measure changes in the membrane potential of two cell lines each expressing PLC-coupled muscarinic receptors. Both SK-N-SH cells (expressing m3 receptors) and m1-transfected A9 L cells express muscarinic receptors which, when stimulated, elicit a large increase in intracellular calcium and release of inositol phosphates. Despite the similarity in this second messenger response, musarinic stimulation resulted in hyperpolarization of the A9 L cells and depolarization of the SK-N-SH cells. The hyperpolarizing response could be mimicked in the A9 L cells by treatment with the calcium ionophore A23187, suggesting that in these cells, the hyperpolarization is mediated by calcium activation of potassium channels. This ionophore had no effect in the SK-N-SH cells, suggesting that the depolarization was not mediated by intracellular calcium. These studies demonstrate that the functional response of cells may vary considerably, despite being mediated by a similar second messenger response.

173.3

STIMULATION OF TRANSCRIPTIONAL REGULATORY FACTOR ACTIVITY BY SUBSTANCE P (SP). Y. Ohashi*, C. Christian*, M. Gilbert*, and D.G. Payan. University of California, San Francisco and the Howard Hughes Medical Institute, U-426, San Francisco, CA 94143.

We have established stably transfected cell lines expressing functional rat SP receptors (KNRK-SPR). When stimulated by SP, KNRK-SPR cells respond by simultaneously mobilizing intracellular Ca^{2+} and increasing cAMP levels. Our results further demonstrate the independent activation of the PKC and PKA pathways. In order to examine if SP stimulation results in the activation of transcriptional regulatory factors, we have transfected KNRK-SPR cells with plasmids containing the AP-1 and CRE-enhancer elements coupled to the chloramphenicol acetyltransferase (CAT) reporter gene. Stimulation with 10nM SP results in an approximate 2-fold increase in CAT activity both in AP-1/CAT and CRE/CAT transfected cells. These results suggest that SP binding to its receptor stimulates AP-1 and CRE-enhancer activity through PKC and PKA activation respectively, and thus may elucidate the complex cellular responses mediated via the SPR receptor.

173.4

MUTATIONS IN THE THIRD CYTOPLASMIC LOOP OF THE MOUSE M1 MUSCARINIC ACETYL-CHOLINE RECEPTOR WHICH AFFECT G-PROTEIN INTERACTION AND AGONIST AFFINITY. R. A. Shapiro and T. Cislo*. Bristol-Meyers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

Mutations were constructed in the highly conserved carboxy-terminal region of the third cytoplasmic loop of the mouse m1 muscarinic acetylcholine receptor (mAChR) by site-directed mutagenesis. The effects of these mutations on ligand binding and on mAChR coupling to phosphoinositide turnover have been examined following expression in mouse Y1 adrenal carcinoma cells. Antagonist binding affinity was unchanged while agonist binding increased. Those mutations which increased agonist binding also resulted in increased agonist potency. These data suggests that G-protein binding to receptor can significantly alter the potency of agonists to couple to physiological responses.

173.5

TRANSMEMBRANE SIGNALING THROUGH THE PHOSPHOINOSITIDE PATHWAY IN HUMAN CORTICAL MEMBRANES (BRODMANN'S AREA 9). M.A. Wallace and E. Claro,* Dept. of Biochemistry, University of Tennessee, Memphis, TN 38163.

Exogenously supplied substrate was used to measure regulation of phosphoinositide-specific phospholipase C (PLC) in membranes prepared from prefrontal cortex frozen in liquid N_2 at autopsy. Homicide victims, schizophrenic, Parkinson's and Alzheimer's patients were analyzed. Guanosine 3'-o-thiotriphosphate (GTPγS) stimulated PLC activity which was synergistically enhanced by muscarinic cholinergic, serotoninergic or dopaminergic agonists. Muscarinic agonists shifted to lower concentrations the EC_{50} value for GTPγS and this shift was reversed by dopamine for homicide victims, schizophrenics and Alzheimer's patients, but the dopamine effect was not apparent in Parkinson's membranes. Results indicate that: 1) there is a reduction of muscarinic response in Alzheimer's patients which cannot be overcome by supplying agonist to the membranes, 2) there is an altered PLC response to dopamine in Parkinson's patients due to the disease process or l-dopa therapy, and 3) there is a reduced PLC response to serotonin in schizophrenics relative to other neurotransmitters. The usefulness of the PLC assay with exogenous substrate is thus demonstrated for studying transmembrane signaling in human brains.

173.6

NORADRENALINE AND CARBACHOL STIMULATED INOSITOL PHOSPHATE ACCUMULATION IN CEREBRAL ENDOTHELIAL CELLS REGULATED BY PHORBOL ESTER, PERTUSSIS TOXIN, AND C_A^{2+}. J.Xu, Z.X.Qu* and E.L.Hogan, Dept. of Neurology, Med.Univ.of SC, Charleston, SC 29425.

It has been reported that noradrenaline (NE) and carbachol stimulated inositol phosphate (IP) accumulation in confluent murine cerebral endothelial cells (MCEC) maintained in culture. Treatment of MCEC with phorbol 12,13-dibutyrate (PMA) 0.1, 1, and 10μM for 10 min decreased NE-induced IP accumulation to 58%, 68% and 100% and decreased carbachol-induced IP to 85%, 88%, ad 88% respectively. Pretreatment of MCEC with pertussis toxin, IAP 0.01, 0.1, and 1μg/ml for 18 hr inhibited NE-induced IP in a dose-dependent manner (3%, 32% and 72%) but had little inhibitory effect on carbachol stimulation of IP. NE and carbachol-induced IP accumulations were decreased to 67% when calcium was absent in the medium during incubation of MCEC with agonists. IP responses to NE and carbachol were much less in the absence of calcium and the presence of 1mM EGTA. These results suggest that NE and carbachol induced IP accumulations are regulated by protein kinase C. These responses are dependent on physiological calcium concentration. Gi protein is involved in adrenergic receptor mediated PI turnover, but not in muscarinic receptor response. Supported by NS-11066.

173.7

CHARACTERIZATION OF COMPENSATORY CHANGES IN RECEPTOR-STIMULATED PHOSPHOINOSITIDE (PI) HYDROLYSIS FOLLOWING COLCHICINE ADMINISTRATION IN THE RAT HIPPOCAMPUS. P.Tandon[1] S.Barone Jr.[2] and H.A.Tilson[3]. [1]Center for Environ.Med. and Lung Biol. UNC, Chapel Hill and [2]U.S. EPA and [3]METI, RTP, NC 27709.

Compensatory changes in receptor-stimulated PI hydrolysis occur in response to intradentate administration of colchicine (Tandon et al.,1989, J.Neurochem **53**: 1117). To characterize these changes further, bilateral injections of colchicine (2.5 ug/site) were made in the hippocampus of adult male Fischer-344 rats (9 rats/group). The rats were sacrificed 12 weeks later and carbachol-stimulated IP-release measured in the hippocampus. The cholinergic muscarinic receptor is linked to the IP second messenger system via GTP-binding proteins. To observe the involvement of G-proteins in this response, non hydrolyzable analogues of GTP and NaF were used to stimulate PI hydrolysis without stimulating the receptor; a decrease in non-receptor-stimulated IP-release was observed in the hippocampus from colchicine-treated animals. This suggests that G-proteins were not involved in the colchicine-induced increase of carbachol-stimulated IP release. A separate study evaluated membrane function by depolarizing the membrane with increasing concentrations of K^+ and simultaneously stimulating IP release with carbachol. A greater increase in receptor-stimulated IP release was observed in hippocampal slices from colchicine-treated rats, suggesting that colchicine-induced alterations in membrane function and structure affect receptor-mediated IP hydrolysis. Further work is needed to study these membrane changes and their effects on cholinergic-muscarinic receptor function.

173.8

LITHIUM INDEPENDENT MUSCARINIC RECEPTOR ACTIVATED ACCUMULATION OF CDP-DIACYGLYCEROL IN HUMAN NEUROBLASTOMA CELLS. E.B. Stubbs, Jr.*, A.M. Heacock, E.B. Seguin* and B.W. Agranoff. Mental Health Research Institute, Departments of Psychiatry and Biological Chemistry, Neuroscience Laboratory Building, University of Michigan, Ann Arbor, MI 48104-1687.

A lithium-dependent accumulation of CDP-diacylglycerol (CDP-DG), the immediate precursor of phosphatidylinositol (PI), following muscarinic receptor activation in cerebral cortical slices has recently been described. Evidence suggests that lithium perturbs the phosphoinositide signal transduction pathway in the CNS by depleting intracellular levels of *myo*-inositol through the inhibition of inositol monophosphatase, thereby limiting substrate availability for the resynthesis of PI. In the present study, we describe a stimulated accumulation of CDP-DG independent of lithium treatment in cultured human SK-N-SH neuroblastoma cells. Activation of [^{3}H]cytidine prelabeled cells with full muscarinic agonists results in a rapid 4-5 fold accumulation of a single labeled band comigrating on thin layer chromatography with authentic CDP-DG standard. Carbachol-stimulated accumulation of [^{3}H]CDP-DG was dose- (EC_{50}=6 mM) and time-dependent, with maximal accumulation occurring within 10 min, sustained for at least 90 min and completely reversed by the subsequent addition of atropine (10 μM). Oxotremorine-M was as efficacious as carbachol at stimulating [^{3}H]CDP-DG accumulation, whereas oxotremorine, arecoline, pilocarpine and bethanechol were only partially effective. Preincubation of labeled cells with LiCl or inositol had no effect on either the basal or stimulated accumulation of [^{3}H]CDP-DG. The resting intracellular mass of *myo*-inositol was 129 ± 12 nmols/mg protein, approximately 20 x greater than has previously been reported for cultured neurotumor cells or peripheral nerve. Culturing cells in a *myo*-inositol free chemically defined medium substantially reduced the intracellular content of *myo*-inositol. Under these conditions, lithium and inositol sensitivity are observed. These results indicate that in some cells, lithium-induced substrate limitation of PI synthase may not regulate stimulated phosphoinositide turnover. (Supported by grant NIMH PO1 MH42652.)

173.9

INOSITOL PHOSPHATE (IP) ACCUMULATION IN RAT NEOSTRIATAL SLICES UNDER CONDITIONS USED TO MONITOR NEUROTRANSMITTER RELEASE. M. H. Weiler, D. Katz*, H. Lee*, N. V. Cozzi*, and K. DasGupta*[1]. School of Pharmacy and [1]Dept. of Psychiatry, University of Wisconsin, Madison, WI 53706.

To determine whether accumulation of IP is altered by manipulations that significantly alter neurotransmitter release, inositol phospholipid metabolism was examined under experimental conditions used to study acetylcholine (ACh) and dopamine (DA) release from rat neostriatal slices. Slices from Fischer 344 rats (3-mo.) were labelled with myo[2-^{3}H]-inositol, and IP accumulation was determined in the presence of LiCl (10 mM). Effects of nondepolarizing and depolarizing conditions, acetylcholinesterase (AChE) inhibition, and muscarinic agents were tested. Under nondepolarizing conditions, IP accumulation (% of total ^{3}H-inositol incorporation) was 2.4 ± 0.1% (n=3) during a 25 min incubation. If the slices were depolarized an additional 5 min following the nondepolarizing incubation, IP accumulation increased another 78% (4.28 ± 0.28%; n=3, $p<0.05$), indicating that conditions used to stimulate neurotransmitter release also increase IP accumulation. In the presence of physostigmine (PHY), an AChE inhibitor, IP accumulation following a 25 min nondepolarizing/5 min depolarizing incubation was 3.3-fold greater ($p<0.05$) than in its absence. Oxotremorine-M (OXO-M), a muscarinic agonist, enhanced IP accumulation 5-fold (5μM) and 9-fold (100μM) during a 25 min nondepolarizing incubation, and 29% and 14% more during an additional 5 min depolarization. Pirenzepine (50 μM), a muscarinic antagonist, decreased IP accumulation in the presence of OXO-M and PHY by 74% ($p<0.01$) and 68% ($p<0.01$), respectively. These manipulations also significantly affected ACh and DA release, indicating that IP accumulation in neostriatal slices is significantly altered during manipulations used to study neurotransmitter release.

173.10

ANGIOTENSIN II INCREASES PHOSPHOINOSITIDE HYDROLYSIS IN RAT SUPERIOR CERVICAL GANGLION THROUGH AT_1-RECEPTORS. C. Strömberg, K. Tsutsumi, M. Viswanathan and J.M. Saavedra. Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Angiotensin II (AII) interacts with the sympathetic nervous system in maintaining vascular tone. Our preliminary autoradiographic studies have shown, that the sympathetic superior cervical ganglion (SCG) has AII receptors, and that these are mainly of AT_1 type. In this study we wanted to clarify, whether these receptors are coupled to the intracellular phosphoinositide (PI) cycle. Ganglia were taken from 8-week-old male Sprague-Dawley rats, labeled for 2 h with ^{3}H-myo-inositol, and stimulated for 45 min with AII in the presence of 10 mM LiCl. When used, the antagonists were added 10 min before AII. The IP_1-fractions were separated by anion exchange chromatography and the radioactivity was measured in a liquid scintillation counter. The results were corrected for the total incorporation of label. AII elicited a dose-responsive increase in IP_1-accumulation with a maximum effect of 101.3 % over control obtained with the dose 10^{-6} M. The EC_{50} was 2.3 nM. The effect of AII was completely inhibited in a dose-responsive manner by the AT_1-selective antagonist DuP 753, but not by the AT_2-selective displacer PD 123177. The results indicate that SCG contains angiotensin AT_1 receptors and these receptors are coupled to the intracellular PI cycle.

173.11

INOSITOL TRISPHOSPHATE GENERATION IS MEDIATED BY A B2 BRADYKININ RECEPTOR IN NG108-15 NEUROBLASTOMA - GLIOMA HYBRID CELLS. J.E. Rubinstein, M.A. Raio Jr.*, and R.J. Hitzemann. Dept. Psychiatry and Behavioral Sci., Health Sciences Center T-10, SUNY: Stony Brook, NY 11794-8101.

Selective analogs of bradykinin (BK) were used in order to pharmacologically characterize bradykinin-induced phosphoinositide hydrolysis in NG108-15 cells. NG108-15 cells were grown in DMEM with HAT, 10% FBS and 5 μCi [^{3}H]inositol for 2 days in order to prelabel inositol phospholipids. The media was removed and the cells pre-incubated for 15 min in Ringer-HEPES buffer. Lithium was not used in the buffer. BK or analogs were then added and after various times the reaction terminated with perchloric acid. BK receptor antagonists, when used, were added 5 min prior to BK. Inositol phosphates were separated by ion-exchange chromatography.

BK produced a robust concentration- and time-dependent increase in inositol trisphosphate (IP3) as well as IP2 and IP4. The B1 selective agonist [des-Arg^9]BK (BK$_{1\text{-}8}$) was totally inactive. The B1, B2 antagonist $_D$Arg[Hyp^3,Thi^{5,8},$_D$Phe^7]BK (Antag2) inhibited BK action in a dose-responsive fashion. The response of 1 μM BK was totally abolished by 30 μM Antag2. [Thi^{5,8},$_D$Phe^7]BK also antagonized the BK response but was less potent than Antag2. Kallidin (Lys-BK) had agonist properties as expected, and was slightly more potent than BK itself. These results indicate that it is the B2 BK receptor subtype that is linked to Phospholipase C-mediated phosphoinositide hydrolysis in this cell line.

173.12

POLYAMINE - STIMULATED PHOSPHOINOSITIDE TURNOVER IN CORTICAL MEMBRANES OF RAT BRAIN. S. Periyasamy and W. Hoss. Dept. of Medicinal and Biological Chemistry, Univ. of Toledo, Toledo, OH 43606.

The polyamines, spermidine and spermine, and their precursor, putrescine are present in virtually all cells. Although the physiological role of these amines is not well understood, studies have demonstrated that normal cellular growth and differentiation require polyamines. Behavioral studies have shown that polyamines produce sedation, hypothermia and convulsions in the laboratory animals. Recently, it has been demonstrated that there are specific binding sites for spermidine in membranes of rat brain. In the present study, we investigated the effects of polyamines on phosphoinositide (PI) turnover in rat brain cortical membranes. Cortical slices from rat brains were prelabelled for 2 hr at 37°C with [^{3}H]-inositol and a membrane fraction was prepared by homogenization and centrifugation. Release of [^{3}H]-inositol phosphates as a measure of PI turnover was determined using ACCELL QMA anion-exchange SEP-PAK's cartridges. The polyamines, spermine and spermidine produced concentration-dependent increases in PI turnover response in the presence of GTP-γ-S (10 μM) and deoxycholate (1.5 mM) with the EC_{50} values of 40±15 and 60±10 μM, respectively. Spermidine produced maximal stimulation (200% above basal) at 1 mM whereas spermine produced maximal stimulation (130% above basal) at 300 μM. Putrescine also produced an increase in PI turnover, but only at the higher concentrations. Our study clearly demonstrated a stimulatory effect of polyamines on PI turnover. We propose that the increased PI turnover response induced by polyamines is mediated by their specific receptors based on the following observations: 1) the stimulatory effect of the polyamines is dose-dependent and saturable; 2) the effect is GTP-dependent; and 3) specific binding sites for polyamines have been identified. The data suggest that at least some of the cellular effects of the polyamines might be initiated through the phosphoinositide cascade. The work was supported by NIH grants DA06258 and DA04068.

173.13

ETHANOL ENHANCES NEUROTENSIN-INDUCED STIMULATION OF INOSITOL PHOSPHOLIPID METABOLOISM. Radcliffe, R.A.* and Erwin, V.G. Alcohol Research Center, School of Pharmacy, University of Colorado, Boulder, Colorado, USA 80309-0297

Ongoing work in this laboratory suggests that neurotensin (NT), a tridecapeptide, mediates in part, ethanol related behaviors including the differential hypnotic and hypothermic sensitivity to ethanol seen in long-sleep (LS) and short-sleep (SS) mice. NT administered i.c.v. causes SS to become significantly more sensitive to the hypothermic and anesthetic effects of ethanol while virtually no effect is seen in LS. The density of NT receptors in forebrain is greater in SS compared to LS. Endogenous levels of NT in various brain regions are generally higher in LS compared to SS. Both lines of mice show a decrease in NT after acute ethanol administration and an increase in NT after chronic ethanol administration with LS showing greater sensitivity in both cases. It has been shown that NT stimulates the formation of the second messenger inositol-trisphosphate in N1E-115 cells, rat brain slices, and mouse brain slices. Studies have been initiated to investigate the possibility that ethanol alters the NT receptor coupled process of PI metabolism in LS and SS brain slices. Stimulation of PI metabolism by carbachol, a muscarinic agonist which has been shown to be effective in eliciting a PI response, was not affected by the addition of 100-200 mM ethanol in either LS or SS. Ethanol, however, increased the effect of NT on PI metabolism by as much as two fold. Furthermore, enhancement of the NT response was greater in SS than in LS. These observations lend support to the hypothesis that NT receptor coupled processes are modulated by ethanol and that there is a genetic influence on this effect of ethanol. (Supported by NIAAA Grants Number 003527, 00079 and 07330)

173.14

PROGESTERONE STIMULATES PHOSPHATIDYLINOSITOL METABOLISM IN SPECIFIC CNS STRUCTURES OF THE FEMALE RAT S.A. Tischkau,* G.S. Panjwani,* J.W. Wright and V.D. Ramirez, Dept. of Physiology, University of Illinois, Urbana, Illinois, 61801.

The rapidity of onset of many of the actions of progesterone (P) suggests that these effects may not be mediated through the classical genomic mechanism for steroid hormone action. Recently, our laboratory has shown that P binds to specific sites in neuronal tissue plasma membranes. This study was undertaken to determine if the phosphatidylinositol (PI) pathway is a mechanism by which the nongenomic action of P occurs and if this action is specific to areas of the brain which are known target sites for P. The ability of P to activate the PI pathway was determined by incubating various concentrations of P with crude membrane fractions (P_2) prepared from cortex, hypothalamus, corpus striatum, and cerebellum to which ^{3}H-myoinositol had previously been incorporated. Li^+ was included to block reutilization of the myoinositol. P stimulated PI metabolism in the corpus striatum in a dose-dependent manner. 10ng and 100ng P resulted in statistically significant increases ($P<.001$) in the PI response (796±219 CPM, n=8 and 947±154 CPM, n=7, respectively) as compared to basal levels (290±34 CPM, n=16). Similarly, P appears to stimulate the PI response from P_2 fractions prepared from medial basal hypothalamus. 2ng P resulted in a two-fold increase (519±4 CPM, n=2) over basal release (233±38, n=9). The effect of P on the PI response in the cerebellum is more complex. 10ng of P appears to stimulate the PI pathway, (876±53 CPM, n=2) as compared to control values (440±124 CPM, n=5), while, 100ng P (609±231, n=4) is neither stimulatory nor inhibitory. Finally, P has no effect on PI hydrolysis in P_2 fractions derived from cortical tissue. Curiously, basal PI release from the cortex was somewhat higher than other tissues (580±114, n=4). These data suggest that upon binding to a membrane site in the hypothalamus, corpus striatum, and cerebellum, progesterone stimulates the metabolism of phosphatidylinositol. The variation in response between neural tissues may indicate different functions for P in these tissues.

173.15

CAFFEINE AND DOPAMINE MOBILIZE CALCIUM FROM INTRACELLULAR STORES IN ISOLATED CATFISH HORIZONTAL CELLS. C.L.Linn and B.N.Christensen. Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX, 77550.

Catfish cone horizontal depolarize to glutamate (GLU) and the glutamate analogs kainate (KA), quisqualate (QA) and N-methyl-d-aspartate (NMDA). In fura-2 loaded cells and with the cell membrane potential controlled with a voltage clamp, application of these agonists result in significant increases in intracellular Ca^{2+} as a result of influx and Ca^{2+}-induced-Ca^{2+} release.

Caffeine (10 Mm) normally produces a rapid single transient increase in intracellular Ca^{2+}. Dopamine (20 μM) has a similar effect except that the Ca^{2+} transient is only about 25% of the caffeine-induced Ca^{2+} transient. Caffeine mobilizes intracellular Ca^{2+} by its direct effect on the Ca^{2+} store. Dopamine produces no membrane current, presumably does not cross the cell membrane and therefore, unlike caffeine must release Ca^{2+} via a second messenger system.

In some cells, caffeine can trigger Ca^{2+} oscillations. The exact conditions that are necessary for Ca^{2+} oscillations are not clear however, we believe that the cell must undergo a period of cytoplasmic Ca^{2+} load before caffeine is effective in triggering oscillations. Ca^{2+} loading can be readily produced by prior repetitive challenges with one of the GLU analogs. Supported by NRSA grant to C.L.L. and NEI EY-01897 to B.N.C.

173.16

GLUTAMATE AND *TRANS*-ACPD INDUCE FORMATION OF INOSITOL 1,4,5-TRIPHOSPHATE IN RAT HIPPOCAMPAL SLICES: INTERACTION BETWEEN IONOTROPIC AND METABOTROPIC GLUTAMATE RECEPTORS. G. Lonart and K.M. Johnson. *Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550*

We are currently investigating the possible influence of ionotropic glutamate receptors on the glutamate stimulated inositol 1,4,5-triphosphate (1,4,5-InsP_3) formation. Cross-chopped hippocampal slices (350 X 350 μm) were prepared from male Sprague-Dawley rats, washed and incubated for 60 min in Krebs bicarbonate buffer, saturated with O_2/CO_2 (95:5). Gravity packed slices (50 μl) were added to 220 μl Krebs buffer. Additions of agonists/antagonists were made in a volume of 30 μl. Incubation (5 min) was terminated by addition of 700 μl of 1 M TCA. Generation of 1,4,5-InsP_3 was assessed using a radioreceptor assay. Glutamate (1 mM) induced a 58±8% increase in the basal 1,4,5-InsP_3 level (33.1±1.8 pmol/mg of protein). This was not blocked by the AMPA or NMDA receptor antagonists, CNQX (10 μM) or CGS 19755 (10 μM). AP-3 (300 μM), a proposed antagonist of the metabotropic glutamate receptor, produced a 43±5% increase in the 1,4,5-InsP_3 formation alone. This response was not further increased by adding glutamate (1 mM), suggesting a possible agonistic effect of AP-3. However, *trans*-ACPD (100 μM), an agonist of the metabotropic receptor, stimulated the basal 1,4,5-InsP_3 level by 54±8% in a manner that was antagonized by AP-3. Ongoing experiments are being carried out to test the effect of the ionotropic glutamate receptor agonists AMPA and NMDA on the *trans*-ACPD stimulated 1,4,5-InsP_3 formation. Supported by DA-02073.

173.17

INOSITOL PHOSPHATES (IP) BREAKDOWN IN CHICKEN BRAIN: EFFECTS OF MELATONIN (MEL) ANALOGUES, SUBSTANCE P (SP) AND TRH. J.S. Popova* and M.L. Dubocovich. Dept. Pharmacol., Northwestern University Medical School, Chicago, IL 60611.

The direct effect of various agents on phosphatidyl inositol (PI) breakdown (lithium 10 μM) was examined in chick striatum (E) and optic lobes (OL). Chicks (1-2 weeks old) maintained on a 14:10 L:D cycle were sacrificed 4 hours after lights on. Slices were prelabeled with 0.53 μM myo-[2-^{3}H]-inositol. IP were separated (IP_3, IP_2, IP_1) by Dowex ion exchange chromatography. In control OL slices following 30 min incubation with Li, the accumulation of of IP_3, IP_2 and IP_1 was 1.09%, 1.7% and 5.05% of total tissue radioactivity, respectively. Exposure to 1-100 μM melatonin analogues (MEL, 6-Cl-MEL, 2-IMEL, N-Acetyl-serotonin), SP (10 μM) or TRH (10 μM) during 5 min increased IP_1 accumulation by 10-35 %. Exposure to 6-Cl-MEL (10 μM) for 20 sec induced transient increases in IP_3 and IP_2 levels (30 % and 37 %, respectively), while IP_1 levels increased in a time dependent manner from 1 % at 1 min to 30 % at 30 min. The increased in IP breakdown elicited by 6-Cl-MEL was identical in slices from S and OL, and was not modified by sacrificing the chicks 12 h after lights on. Methysergide (10 μM), a non-selective serotonin (5-HT) antagonist, inhibited 5-HT-induced (10 μM) but not 6-Cl-MEL-induced (10 μM) IP_1 accumulation, suggesting that the effects of melatonin analogues on PI breakdown may not be mediated through activation of 5-HT-1c or 5-HT-2 receptor.

173.18

LITHIUM INHIBITION OF NORADRENALIN STIMULATED CYCLIC AMP ACCUMULATION AND AGONIST STIMULATED MASS INOSITOL PHOSPHATE DETERMINATION IN WISTAR RAT CEREBRAL CORTEX SLICES SILLENCE D.J and DOWNES C.P.* Department of Biochemstry University of Dundee UK

One theory for the mechanism of action of lithium in the treatment of affective disorders is that lithium inhibits specific adenylyl cyclases perhaps by inhibiting G-protein function, another is that lithium attenuates the production of inositol phosphates by inducing inositol depletion in hyperactive neurons. NA acting on the alpha-1 receptor potentiates cyclic AMP accumulation and stimulates inositol phosphate production. Data is presented comparing the inositol reversibility of CMPPA accumulation, Inositol phosphate mass and cyclic AMP accumulation . The concentration of lithium required to incur these effects are compared to elucidate which is the therapeutically relevent target for lithium's action.

173.19

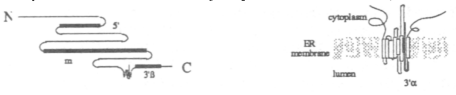
BERYLLIUM IS A POTENT INHIBITOR OF BRAIN *myo*-INOSITOL-1-PHOSPHATASE, BUT UNLIKE LITHIUM DOES NOT POTENTIATE AGONIST INDUCED PI TURNOVER. S.H. Zorn, W.S. Faraci*, A.V. Bakker*, K.G. Pratt*. Department of Neuroscience, Central Research Division, Pfizer Inc., Groton, CT 06340.

Lithium is the drug of choice for the treatment of bipolar depression although it has some limiting side effects. Its action may be due in part to its ability to dampen phosphatidylinositol turnover (PI) by inhibiting *myo*-inositol-1-monophosphatase (IP1ase). The present study was undertaken to examine the properties of $BeCl_2$ as a lithium mimetic agent. $BeCl_2$ is at least three orders of magnitude more potent than LiCl (Ki ≈ 0.5-0.8 mM) as an inhibitor of partially purified IP1ase isolated from rat brain (Ki = 150 nM), bovine brain (K_i = 35 nM), and from the human neuroblastoma cell line SK-N-SH (K_i = 100 nM). Kinetic analysis revealed that $BeCl_2$ is a competitive inhibitor of IP1ase, in contrast to that of lithium (uncompetitive). Inhibition of exogenous [^{3}H]-I-(1)-P breakdown by $BeCl_2$ (IC_{50} ≈ 100-200 nM) was observed to the same maximal extent as that seen with lithium (10mM) in permeabilized SK-N-SH cells reflecting inhibition of cellular IP1ase. In contrast to lithium's potentiation of agonist induced PI turnover in intact and permeabilized SK-N-SH cells and in rat brain slices, $BeCl_2$ was completely inactive in this regard at concentrations up to 0.5 mM. These studies indicate that $BeCl_2$ is a potent competitive inhibitor of brain *myo*-inositol-1-monophosphatase in vitro. The disparity in the actions of $BeCl_2$ and Lithium may suggest that either selective inhibition of IP1ase does not completely explain the action of lithium on the PI cycle, or that uncompetitive inhibition of IP1ase is a necessary requirement to observe functional lithium mimetic activity.

173.20

FUSION PROTEIN ANTIBODIES DIRECTED AGAINST TOPOGRAPHICALLY DISTINCT REGIONS OF THE CEREBELLAR INOSITOL-1,4,5-TRISPHOSPHATE RECEPTOR. D. Lin and W.S. Agnew. Dept. of Cellular and Molecular Physiology and Program in Neuroscience, Yale Univ. Sch. of Med., New Haven, CT 06510

cDNA fragments were prepared by PCR from mouse cerebellar mRNA with primers specific for coding sequences near the N-terminus (5'), middle (M), and carboxyl terminal membrane spanning segments (3'α, 3'ß). These were cloned, sequenced and spliced into pGEX expression vectors to form fusion protein recombinants with glutathione S-transferase. Preparative amounts of antigens were grown in *E.coli*, isolated by affinity purification or by electroelution, and used to raise rabbit polyclonal antisera. Antigen-specific antibodies were then characterized for their utility in western blots, immunoprecipitation, and immunocytochemical labeling.



All four antibodies reacted strongly in western blots performed with a high affinity (Kd = 4 nM) IP_3 receptor purified from rat cerebellum, or with unpurified receptor in cerebellar microsomes. Antibodies M and 3'ß efficiently precipitated the native receptor, while 5' was less effective. Antibodies M and 3'α provided sensitive immunocytochemical markers; strong labeling was observed in the soma, dendrites and dendritic spines of cerebellar Purkinje neurons. Axonal staining of these cells, previously not reported, was also clearly visible. Other novel findings included labeling of relatively rare cerebellar Lugaro cells, hippocampal neurons, as well as pyramidal cells in the cerebral cortex. These antibodies provide useful biochemical and immunocytochemical reagents for studies of receptor structure, expression, and localization.

173.21

PHOTOAFFINITY LABELING OF THE INOSITOL-1,4,5-TRISPHOSPHATE RECEPTOR BINDING SITE. R.J. Mourey, V.A. Estevez*, J.F. Marecek*, G.D. Prestwich* and S.H. Snyder. Depts. of Pharmacology and Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; Dept. of Chemistry, SUNY, Stony Brook, NY 11794.

The inositol-1,4,5-triphosphate (InsP3) binding site is believed to be located in the amino terminal quarter of the InsP3 receptor. To address where in this region InsP3 is binding, we have synthesized an InsP3 photoaffinity ligand, [125I]1-O-[N-(4-azidosalicyloxy)-3-aminopropyl-1-phospho] myo-inositol 4,5-bisphosphate (Prestwich, G.D., et. al. JACS, 113:1822, 1991). This ligand binds to the InsP3 receptor with high affinity and specificity, i.e., InsP4 and InsP6 are weak competitors while other inositol phosphates are inactive.

In crude rat cerebellar membrane fractions, only the InsP3 receptor is specifically labeled, i.e., completely displaced by high concentrations of unlabeled InsP3. Two-dimensional peptide maps prepared from enzymatic (trypsin, thermolysin, St. aureus V8) or chemical (cyanogen bromide) digestion of purified photolabeled InsP3 receptor all yield a single labeled spot which is very acidic. In the published amino acid sequence of the InsP3 receptor, there exists only one very acidic region (a.a. 317-330) in the amino terminal quarter of the receptor. We hypothesize that InsP3 may bind basic residues near this region. Based on the amino acid sequence, we would predict a labeled cyanogen bromide peptide of approximately 14 kDa and a N-chlorosuccinamide peptide of 10.9 kDa. Interestingly, we do resolve a photolabeled peptide of the predicted sizes using a tricine-SDS-PAGE system.

173.22

IP3-SENSITIVE CALCIUM STORES ARE REFILLED BY A PLASMALEMMAL CALCIUM LEAK. T.M. Lo and S.A. Thayer. Dept.of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

The refilling mechanism for IP3-sensitive calcium stores in single NG108-15 hybridoma cells was examined by indo-1 based microfluorimetry. IP3-sensitive calcium stores were depleted by a single 1 minute exposure to bradykinin (30nM). The stores were refilled completely in 20 minutes ($t_{1/2}$ = 2 minutes). Thapsigargin produced a transient release of calcium from the store and inhibited refilling with an EC_{50} of 3nM indicating mediation by the microsomal Ca-ATPase. This effect was partially reversible at submaximal thapsigargin concentrations. The refilling of these stores was also blocked by the removal of extracellular calcium. Verapamil (10uM), diltiazem (10uM), flunarizine (10uM), and nickel (0.2mM) did not effect the refilling of IP3-sensitive calcium stores. However, nitrendipine (10uM) and lanthanum (10uM) blocked refilling of these stores suggesting involvement of a specific channel in replenishing intracellular IP3-sensitive calcium stores. Exposing NG108-15 cells to 10mM Ca^{2+} following Ca^{2+}-free (1mM EGTA) treatment caused a transient increase in calcium. The amplitude of this $[Ca^{2+}]_i$ transient was not altered by depleting the store with bradykinin prior to the calcium challenge suggesting that an additional influx was not recruited during refilling of IP3-sensitive calcium stores. These results suggest that the IP3-sensitive calcium stores in NG108-15 cells are refilled by a plasmalemmal calcium leak channel.

173.23

SUBSECOND KINETICS AND REGULATION OF IP3-INDUCED CA^{2+} RELEASE BY ATP. E.A. Finch[1,3], T.J. Turner[2], and S.M. Goldin[2,3]. [1]Program in Neuroscience and [2]Biol. Chem. Dept., Harvard Med. Sch., Boston, MA 02115; [3]Cambridge NeuroScience Research, Inc., Cambridge, MA 02139.

We have previously reported that extravesicular Ca^{2+} (Ca^{2+}_e) rapidly potentiates and more slowly inactivates IP3-induced $^{45}Ca^{2+}$ release (Finch et al., *Science* 252:443, 1991). The finding that Ca^{2+} is a coagonist for the IP3 receptor suggests functional homology with the structurally homologous ryanodine receptor of sarcoplasmic reticulum. ATP has been reported to allosterically regulate both the IP3 receptor and the ryanodine receptor. We have examined the effects of ATP on the subsecond kinetics of IP3-mediated $^{45}Ca^{2+}$ release from synaptosome-derived microsomes using a rapid superfusion system.

Both Na_2ATP and MgATP rapidly potentiated IP3-induced $^{45}Ca^{2+}$ release (within 100 ms after introduction of ATP). The maximum rate of $^{45}Ca^{2+}$ release was increased at all [Ca^{2+}_e] examined (0.1 - 100 M). The rapid potentiation by Ca^{2+}_e and the biphasic Ca^{2+}_e-dependence of IP3-induced $^{45}Ca^{2+}$ release observed by Finch et al. remained apparent in the presence of ATP, but the amplitude of the $^{45}Ca^{2+}$ release was increased. At 10 uM Ca^{2+}_e 0.1 - 3 mM Na_2ATP produced an≈2-fold increase in the maximum rate of IP3-induced $^{45}Ca^{2+}$ release. The potentiation was also observed with AMP-PNP and ATPγS, indicating that ATP hydrolysis was not required. These results suggest that Ca^{2+}_e and ATP synergistically potentiate IP3-induced $^{45}Ca^{2+}$ release. They further suggest that rapid changes in ATP levels may modulate IP3-induced $^{45}Ca^{2+}$ release. Finally, in the presence of IP3 and ATP changes in cytosolic [Ca^{2+}] are likely to dynamically regulate IP3-induced $^{45}Ca^{2+}$ release.

173.24

LOCALIZATION OF INOSITOL TRISPHOSPHATE RECEPTOR IN NEURONAL CELL BODIES, DENDRITES AND TERMINALS IN THE RAT CENTRAL NERVOUS SYSTEM.

A.H. Sharp, C.A. Ross, T.M. Dawson and S.H. Snyder. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

The inositol trisphosphate receptor (IP3R) releases Ca2+ from intracellular stores in response to stimulation of IP3 production by neurotransmitters and hormones and has been previously localized to the endoplasmic reticulum of cerebellar Purkinje cells. To help elucidate the function of the IP3R, we have now determined the localization of the IP3R throughout the rat brain and spinal cord by an immunoperoxidase method using affinity purified antibodies against the rat cerebellar IP3R and by in situ hybridization. Among the structures labeled, cell bodies and dendrites were intensely stained in the hippocampus, cortex, dorsal cochlear nucleus and olfactory tubercle. A number of the circumventricular organs were strongly stained suggesting a role for IP3 as a second messenger in regulation of water and salt balance. Other labeled areas included the striatum, olfactory bulb and various nuclei in the thalamus, hypothalamus and brainstem. Nerve terminals were prominently labeled in a number of areas including the deep cerebellar and vestibular nuclei and the substantia nigra reticulata suggesting a role for IP3 in modulation of neurotransmitter release.

BEHAVIORAL PHARMACOLOGY III

174.1

THE ANXIOLYTIC EFFECTS OF AHR14042, A NOVEL AZETIDINE, ARE NOT MEDIATED BY BENZODIAZEPINE RECEPTORS. D.J. Bill*, A. Fletcher*, C.A. Boast, H. Morris, R. Tasse, and D.N. Johnson. Wyeth Research (U.K.), Taplow, Maidenhead SL6 0PH,U.K. and Wyeth-Ayerst Research, CN 8000, Princeton, NJ, 08543-8000.

AHR14042 is a novel azetidine molecule [3(4-chlorophenoxy)-N-(2-propenyl) -1-azetidinecarboxamide]. We have examined the activity of this compound in two animal models of anxiety, i.e. the rat Geller-Seifter paradigm and the mouse two-compartment light:dark box model. AHR14042 was also examined in several seizure models in the mouse.

AHR14042 was active in both models of anxiety, inducing effects similar to those of benzodiazepine positive controls. In the mouse light:dark box, AHR14042 significantly increased light compartment rearing and motor activity at doses (0.1-1.5mg/kg sc) which did not modify dark compartment behaviour. AHR14042 significantly increased punished responding in the rat Geller-Seifter paradigm at a dose of 10mg/kg i.p. In both models, the benzodiazepine receptor antagonist, flumazenil (10mg/kg), blocked the effects of diazepam or lorazepam, but not those of AHR14042. When administered up to high dose levels (25mg/kg sc) in mice, AHR14042 had no significant effect on pentylenetetrazol-induced clonic seizures. AHR14042 exerted a weak anticonvulsant effect at high doses in mice challenged with picrotoxin, bicuculline or electroshock. This effect was accompanied by marked sedation/ataxia, suggesting that the compound was inducing a general non-specific effect at these doses. A wide receptor binding profile of AHR14042 did not reveal a significant binding affinity of this compound for any of the major central monoamine, amino-acid, benzodiazepine or opioid receptors. AHR14042 also did not interact with calcium, potassium or chloride channels, or with the adenyl cyclase or protein kinase C second messenger systems. Further work will be focussed on ascertaining the mechanism of action of this novel and interesting anxiolytic.

174.2

EFFECTS OF ALLOSTERIC AGONISTS FOR NMDA RECEPTOR COMPLEX ON METHAMPHETAMINE-INDUCED LOCOMOTOR STIMULATION IN RATS. A.Hashimoto*, T.Nishikawa*, T.Oka*, T.Higuchi and K.Takahashi. Div. of Mental Disorder Res., Natl. Inst. of Neurosci., NCNP, Tokyo, 187, Japan and Tsukuba Res. Lab., Nippon Oil & Fats Co., Ltd. Ibaraki, Japan.

The findings indicating that destruction of cortico-fugal glutamatergic pathways augments amphetamine-induced hyperactivity and stereotyped behaviors have suggested the possible involvement of reduced excitatory amino acidergic transmission in these abnormal behaviors. We have investigated the effects of intracerebroventricular(i.c.v.) injection of allosteric agonists for the NMDA receptor. I.c.v. injection of D-Ser and D-Ala reduced the ability of methamphetamine(MAP) to induce hyperactivity (1.0 mg /kg,s.c.) in a dose-dependent manner without affecting MAP-induced stereotyped behaviors. In contrast, the L-isomer of Ala failed to affect the MAP-induced hyperactivity. This stereospecificity suggests that the antagonism of locomotor stimulatory effects of MAP may be due to stimulation of strychnine-insensitive glycine binding site, since the D-forms are more potent than L-forms as agonists for the allosteric regulation site of NMDA receptor complex.

174.3

MEDIAL HYPOTHALAMIC FACILITATION OF FELINE DEFENSIVE RAGE FROM THE PERIAQUEDUCTAL GRAY IS MEDIATED THROUGH NMDA RECEPTORS. A. Siegel, C.L. Lu* and M.B. Shaikh. Dept. of Neurosciences, N.J. Medical School, Newark, NJ 07103.

The principal excitatory input to the midbrain periaqueductal gray (PAG) with respect to defensive rage behavior (DR) arises from the medial hypothalamus (MH). The present study was designed to test the hypothesis that this pathway utilizes excitatory amino acids (EAA) as a transmitter. Cannula electrodes were implanted into PAG sites for elicitation of DR as well as for infusion of EAA antagonists and NMDA, and stimulating electrodes were placed into sites within the MH from which facilitation of DR could be obtained. Concurrent stimulation of the MH and PAG facilitated DR when compared to single stimulation of the PAG. Then, this dual stimulation paradigm was repeated following microinjections of EAA antagonists into PAG DR sites: kyneurenic acid (KYN, non-specific) [0.1-2.0 nM], DAP-7 (NMDA) [0.1-2.0 nM], CNQX (non-NMDA) [4.0 nM]. The results indicated that both KYN and DAP-7 blocked the facilitatory effects of MH stimulation upon DR in a dose and time dependent manner. In contrast, infusion of CNQX failed to alter the facilitatory effects of MH stimulation. Moreover, infusion of NMDA into PAG DR sites mimicked the facilitatory effects of MH stimulation in a dose and time dependent manner. The results indicate that EAA is a transmitter involved in MH facilitation of PAG elicited DR in which NMDA receptors are utilized. [Supported by NIH Grant NS 07941-21].

174.4

ACQUISITION OF DRUG DISCRIMINATION LEARNING WITH CHOLECYSTOKININ. P.M. Melton & A.L. Riley. The American University, Washington, D.C. 20016.

Recently, the conditioned taste aversion procedure has been demonstrated to be a viable behavioral baseline to examine control by drug states, i.e., drug discrimination learning (Mastropaolo et al., Pharmacol. Biochem. Behav., 32: 1-8, 1989). The sensitivity of the procedure is indexed by the rapid acquisition of drug discriminations using this design and by the variety of compounds for which a discrimination has been established, including the opiate antagonists (Kautz et al., Drug Dev. Res., 16: 317-326, 1989). The present study examined the ability of cholecystokinin (CCK) to serve as a discriminative cue, a compound for which discrimination learning has heretofore been hard to establish. Specifically, every fourth day experimental rats were injected with 13 ug/kg of CCK 5 min before saccharin-lithium chloride pairings and control rats were injected with 13 ug/kg of CCK before saccharin-distilled water pairings. On recovery days, all animals were injected with the CCK vehicle prior to saccharin exposure with no post injection. After 10 CCK-saccharin-lithium trials, 7 of 12 experimental animals acquired the discrimination. In subsequent generalization tests, 10, 18, and 32 ug/kg of CCK resulted in drug-appropriate responding, whereas doses lower than 10 ug/kg did not. Neither the opiate antagonists naloxone (0.56, 1.0, 3.2 mg/kg) nor diprenorphine (1.0, 3.2, 5.6 mg/kg) substituted for the CCK cue.

174.5

EFFECTS OF CHRONIC CAFFEINE EXPOSURE IN MICE ON LOCOMOTOR ACTIVITY AND THE RESPONSE TO ADENOSINE AGONISTS AND ANTAGONISTS. O. Nikodijevic, K. A. Jacobson, and J. W. Daly, NIDDK, NIH, Bethesda, MD 20892.

Chronic caffeine treatment results in an upregulation of central adenosine receptors, but the role of such receptors in tolerance to caffeine is unclear. In mice ingesting caffeine (1 g per liter in drinking water), the locomotor activity relative to vehicle control was greater during the first 24 hours (increasing gradually to 48% stimulation), and then decreased (gradually to 45%) for 5 days, after which time activity stabilized slightly (15-20%) lower than control levels. After stabilization, the A_1-selective adenosine agonist N^6-cyclohexyladenosine (i.p.) was less effective than in controls in decreasing locomotor activity, while caffeine (i.p.) caused a slight stimulation to about the level of locomotor activity in controls. The bell-shaped dose response curve for the locomotor effects of caffeine (i.p.) in mice chronically ingesting caffeine appeared shifted to the left, and the maximum effect was diminished compared to controls. This suggests that multiple processes underlie caffeine habituation, and that adenosine receptor regulation is only in part involved.

174.6

HORMONAL MODULATION OF MUSCIMOL-INDUCED CATATONIA: A NEW MODEL FOR CHANGES IN DRUG SENSITIVITY? L.C. Kaufman*, M.M. McCarthy, D.W. Pfaff and S. Schwartz-Giblin, Rockefeller University, 1230 York Ave, NY. NY. 10021.

The specific GABA-A receptor agonist, muscimol, has been shown to induce catatonia when infused into the lateral hypothalamus or zona incerta of male rats (Wardas et al., Brain Res. 1988). In the present experiments, female rats implanted with 23 g cannulae directed at the ventromedial hypothalamus were tested for catatonia beginning one week post-surgery. Catatonia was determined by placing the animals forepaws on a 9cm high block and timing the latency to descent, with a cut-off time of 5 min. After a pretest, muscimol (25ng) or saline was bilaterally infused (0.25 µl/side) and the animal tested for catatonia at 5, 10, 20, 30, 60 and 120 min post-infusion. Ovariectomized (OVX) rats infused and tested daily for five days showed a significant catatonia after muscimol vs. saline with a peak in catatonia being observed at 60 min post-infusion and increasing catatonia over the 5 day period (two-way ANOVA; $p<.001$). In a separate experiment, hormonally-treated females were infused with muscimol and tested for catatonia. When OVX, estrogen-treated (E-treated; 10 µg for 2 days prior) and progesterone-treated females (P-treated; 1mg 4 hr prior) were infused with muscimol there was significantly higher catatonia in the OVX and E-treated females as compared to P-treated females (ANOVA; $p<.05$). These E- and P-treated females tested at weekly intervals exhibited different patterns of sensitization to muscimol. Catatonia induced by muscimol in E-treated females did not change over the 3 weeks but in P-treated females catatonia was significantly greater at 3 weeks than 1 or 2 weeks ($p<.05$). These results indicate an increasing sensitivity to muscimol when given daily for 5 days and changes in drug sensitivity after weekly administration which are dependent on the hormonal milieu. It is proposed that catatonia testing may serve as a useful index of changes in drug sensitivity after repeated exposure.

174.7

DIFFERENCES AMONG NEUROLEPTICS IN INHIBITING METHYLPHENIDATE-INDUCED STEREOTYPED BEHAVIORS: POSSIBLE RELATIONSHIP WITH VARYING CLINICAL EFFICACY. W. Koek and F.C. Colpaert. FONDAX, Groupe de Recherche SERVIER, Puteaux, FRANCE.

Methylphenidate (MPD) increased in rats the incidence of sniffing, rearing and locomotion, and, at higher doses, exclusively induced stereotyped gnawing. Neuroleptics inhibited gnawing induced by 40 mg/kg of MPD; however, as gnawing was inhibited in a dose-dependent manner, other responses (i.e., sniffing, rearing and locomotion) appeared. Higher doses of neuroleptics also inhibited these latter responses, so that the behavior of the MPD-treated animals became similar to that of normal controls. Only neuroleptics appeared able to normalize the behavior of MPD-treated rats, i.e., to inhibit all of MPD's effects at doses that induced neither complete behavioral suppression nor adverse effects. The neuroleptics differed markedly, however, in terms of the relative doses at which they 1) inhibited gnawing, 2) inhibited the other effects of MPD, and 3) induced complete behavioral suppression or adverse effects. These differences among neuroleptics seem to be compatible with their relative efficacy in suppressing the productive symptoms of schizophrenia.

174.8

IMPLICATION OF ACETYLCHOLINE IN THE INITIATION OF 22 kHz ULTRASONIC VOCALIZATION IN THE RAT. S.M.Brudzynski and D.Ociepa. Dept of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, N6A 5A5 Canada.

It has been recently reported that direct application of carbachol into the rat's basal forebrain can induce 22 kHz ultrasonic vocalization suggesting that the central cholinergic system is involved in initiation of ultrasonic calls. The aim of the present study was to demonstrate that endogenous acetylcholine is implicated in the generation of ultrasonic vocalization in rats. Ultrasonic calls were induced by a mild footshock (max. 1 mA for 1 s) in 17 rats. Bilateral intracerebral injection of physostigmine (10 µg/0.2 µl) into the anterior hypothalamic/preoptic area potentiated and prolonged, by 3.5 times, the duration of ultrasonic vocalization induced by footshock. Local pretreatment with atropine (0.5 µg/0.2 µl) decreased, by 3.7 times, the duration of the footshock-induced vocalization. These results were significant as compared to saline pretreated controls. It is concluded that endogenous acetylcholine is involved in the initiation of 22 kHz ultrasonic calls in rats.
Supported by NSERC of Canada and OMH Foundation.

174.9

MICROIONTOPHORETICALLY APPLIED ACETYLCHOLINE, MORPHINE AND NOREPINEPHRINE MODULATE BED NUCLEUS OF STRIA TERMINALIS NEURONS. N. Dafny and J.H. Casada. Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, Houston, TX 77225.

The bed nucleus of the stria terminalis (BNST) has been implicated in the production of several of the components of the stress response including endocrine, autonomic and behavioral changes. Because of the ability of BNST to regulate several components of the stress response, a study of the effects of neurotransmitters found in BNST may lead to an understanding of physiological coping mechanisms or provide a basis for the pharmacological control of the stress response. The present experiment was designed to determine the effects of three neurotransmitter systems in rats (N=4) anesthetized with 1.25 g/kg urethane. The following drugs were administered via a 6 barrel glass electrode: glutamate, morphine (MS), norepinephrine (NE), acetylcholine (ACh), and a vehicle carrier. Forty-five single BNST neurons were tested with each drug. 52% of BNST neurons responded to MS with increases and decreases in firing rate observed with equal frequency. 72% of BNST neurons responded to NE, predominantly with decreases in firing rate. Finally, 46% of BNST neurons responded to ACh, predominantly with increases in firing rate. The typical BNST neuron responded to more than one drug; 30% of these neurons responded to all three drugs which were tested. Thus NE, ACh, and opiates appear to be important in modulating the firing rate of BNST neurons.

174.10

ENVIRONMENTAL DETERMINANTS OF THE RELATIVE REINFORCING EFFICACY OF NICOTINE. S.I. Dworkin, J. Broadbent, R. Guarino, and J. Robinson. Dept. of Physiology and Pharmacology, Wake Forest University, The Bowman Gray School of Medicine, Winston-Salem, NC 27157.

Male Fisher-344 rats were used to determine conditions under which animals could be trained to self-administer nicotine. For these investigations three behavioral paradigms were utilized to assess the relative reinforcing efficacy of nicotine compared to cocaine. The three paradigms included a fixed-ratio schedule of drug presentation, a schedule-induction paradigm using a fixed-time 1 min schedule of food delivery and a concurrent chained schedule of food, water and nicotine delivery. Lever presses under all three procedures resulted in a 0.2ml infusion of nicotine (3-10 µg/infusion) delivered over 5.6 sec. Under both the fixed-ratio and concurrent chained schedule the initial rates of nicotine self-administration (2-24 infusions) diminished over the first three to five sessions to comparatively low levels (2-6 infusions). Twenty-four hour food extinction probes increased nicotine self-administration under the concurrent chained schedule. Substitutions of cocaine for nicotine resulted in higher rates of self-administration by rats under the fixed-ratio procedure. Single infusions of nicotine to rats that self-administered cocaine but not nicotine under the fixed-ratio procedure, resulted in a decrease in cocaine self-administration. The schedule-induction paradigm produced relatively high rates of responding that resulted in nicotine infusions. However, saline substitutions did not immediately diminish responding by these rats. These data indicate it is more difficult to engender and maintain nicotine self-administration compared to cocaine self-administration, which suggest that cocaine has a greater reinforcing efficacy than nicotine. (Supported by a contract from the R.J. Reynolds Tobacco Co.)

174.11

EVALUATION OF CENTRAL EFFECTS OF NICOTINE IN A DRUG DISCRIMINATION PARADIGM USING MICRODIALYSIS TECHNIQUES IN RATS. H.F.Villanueva, J.H.Johnson*, S.Arezo*, J.R.James*, and J.A.Rosecrans*. Depts. of Pharmacology/Toxicology & Anatomy, Virginia Commonwealth Univ., Richmond VA 23298.

It has previously been shown that rats trained to discriminate peripherally administered nicotine in a two-lever operant drug discrimination procedure will generalize to nicotine injected directly into the dorsal hippocampus and the reticular formation (PBB 15:21-26, 1981). Current research in this laboratory is examining the use of microdialysis techniques to administer nicotine intracerebrally. Nicotine bitartrate or buffered vehicle was delivered via a microdialysis probe inserted through a guide cannula 1-6 hours prior to testing. Brain areas tested include the mesencephalic reticular formation, dorsal hippocampus and nucleus accumbens. Preliminary studies involving the infusion of radioactive nicotine indicate that 2% of the nicotine infused through the probe reached the tissue, resulting in approximately 2.4 µg nicotine reaching the tissue over a 10 minute period of infusion with 25µl of a 30 mmolar nicotine solution. (Supported by the German Research Council on Smoking and Health, Bonn, Germany.)

174.12

THE DISCRIMINATIVE STIMULUS PROPERTIES OF AN ETHANOL-NICOTINE MIXTURE IN RATS. B.D. Youngblood*, D.V. Gauvin, and F.A. Holloway. Dept. of Psychiatry & Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.

Very little is known about the factors or "rules" that govern the perception of drugs in mixtures. This laboratory has previously reported results of drug discriminations (DD) based on a number of legal stimulant mixtures. We now report the results of an **"AND"** drug discrimination (Stolerman & Mariathasan, *Psychopharmacology*, **102**, 557-560, 1990) between an ethanol-nicotine mixture and saline (SAL). Twelve Sprague-Dawley rats were trained to discriminate between SAL and an ethanol-nicotine mixture (1.0 g/kg ethanol - .3 mg/kg nicotine) administered i.p. 15 min prior to a 15 min DD task under a FR10 schedule of food maintained lever-press responding. The elements of the compound stimulus, when tested singly, engendered approx. 50-50 responding on the SAL- and mixture-appropriate levers. A full range of ethanol and nicotine doses were tested in combination with SAL; both drugs failed to engender >90% mixture-appropriate responding up to doses that suppressed rates of responding. However, when the training doses of either ethanol or nicotine were held constant and various doses of the alternate drug element were added in combination, a graded dose-dependent increase in mixture-appropriate responding resulted. These data, in conjunction with Stolerman (*Psychopharmacology*, **101**, S74, 1990), suggest that 1) drug mixtures are not normally perceived as new entities distinct from their component elements; 2) training dose-ratio may influence the characteristics of mixture discriminations; and 3) overshadowing of one drug by another may be an important factor determining the characteristics of cues produced by drug mixtures.

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174.13

FAGERSTROM TOLERANCE QUESTIONNAIRE (FTQ) SCORE, PLASMA COTININE, CAFFEINE AND SMOKING-INDUCED MOOD CHANGES. **C.J. Meliska & D.G. Gilbert*. Psych. Dept., Southern Ill. Univ. at Carbondale, Carbondale, IL 62901**

Plasma nicotine, cotinine (COT), and caffeine (CAF) were assayed before and after 2 and 5 cigarettes, in 8 male and 8 female, overnight smoking-deprived smokers. Before testing, subjects completed the FTQ, an instrument purporting to differentiate smokers based on physical dependence. Mood was assessed before and after smoking. Partial correlation coefficients (controlling for gender and, where appropriate, nicotine boost and nicotine-free baseline values) showed that, prior to smoking: (1) FTQ scores were marginally correlated with plasma COT levels (r = .400, P = .07) and with unpleasant feelings (r = .61, P = .028); (2) plasma COT correlated with negative affect (r = .61, P = .048) and malaise (nausea + sick + unpleasant; r = .70, P = .016); (3) CAF correlated positively with drowsiness (r = .70, P = .016). After 2 cigarettes: (1) FTQ correlated negatively with arousal (Energy + Alert - Drowsy, r = -.75, P = .008) and positively with negative affect (r = .91, P = .001); (2) COT correlated with positive affect (r = .71, P = .050) and reduced anger (r = -.79, P = .020); (3) CAF correlated with reduced anger (r = -.76, P = .040) and malaise (r = -.80, P = .030). After 5 cigarettes COT correlated negatively with arousal (r = -.86, P = .006). Results suggest that plasma COT and CAF levels modulate the mood-influencing effects of smoking.

174.14

EFFECTS OF ANTICHOLINERGICS ON ELEVATED PLUS-MAZE BEHAVIOUR IN THE RAT. C. P. S. Smith*, A. Chape* and G. W. Bennett*. (SPON: Brain Research Association).
Dept. of Phys.& Pharm.,Med. Sch., Q. M. C., Nottingham, NG7 2UH, UK

The Elevated Plus-Maze (EPM) has recently been used as a test paradigm for the evaluation of learning and memory in mice. The present study assessed the effects of two muscarinic cholinergic antagonists, atropine (ATR) and scopolamine (SCOP) on EPM performance in the rat. Male, Hooded Lister rats (250-450 g) received single daily injections of either saline (SAL; 1 ml/kg i.p. n=8 and 10), SCOP (0.15 mg/kg i.p. n=8) or ATR (2.0 mg/kg i.p. n=9) 30 min prior to testing on three consecutive days. On day 1, both ATR and SCOP treated animals exhibited a reduced transfer latency (TL-time taken to reach centre of closed arm from end of open arm) and increased residence latency (RL-time spent within closed arm) compared to SAL controls (Mann-Whitney U test; TL p<0.01-0.05, RL p<0.001-0.05). On days 2 and 3, SAL TL scores were reduced (P<0.001) compared to day 1, whereas ATR and SCOP TL scores were not. The results indicate that ATR and SCOP have both anxiogenic and learning effects in this test.

C. P. S. Smith is a SERC CASE student in conjunction with SmithKline Beecham.

174.15

THE EFFECTS OF SCOPOLAMINE ON VACUOUS CHEWING INDUCED BY SUB-CHRONIC HALOPERIDOL IN THE RAT. R. E. Steinpreis and J. D. Salamone. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.

Long and short term administration of neuroleptic drups produces debilitating extrapyramidal side effects in humans. In rats, neuroleptics produce involuntary movements including vacuous chewing. In the present study, rats were given daily IP injections of either vehicle, 0.4 mg/kg haliperidol (HP) alone, 0.4 mg/kg HP with 0.25 mg/kg scopolamine (SC), or 0.4 mg/kg HP with 0.5 mg/kg SC. Rats were observed in daily 5-min blocks, over a two week period. Observation periods began 50 min after injection. Sub-chronic HP produced a syndrome of vacuous chewing movements in rats that was present in the first few days. Repeated daily co-administration of 0.5 mg/kg SC with HP from the first day resulted in a partial attenuation of the emergence of the syndrome, while co-administation of the lower dose of SC with HP did not. Rats continuing on HP alone after SC was withdrawn demonstrated a rebound increase, showing the highest number of vacuous chewing movements of all groups. In rats previously treated with HP alone, co-administration of 0.5 mg/kg SC with HP after the tenth day did not reduce vacuous chewing responses. These findings have implications for the efficacy and timing of anti-cholinergic reversal of acute-onset extrapyramidal side effects.

174.16

THE DISCRIMINATIVE STIMULUS PROPERTIES OF ACUTE ETHANOL WITHDRAWAL (HANGOVER) IN RATS. D.V. Gauvin and F.A. Holloway, Dept. of Psychiatry & Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190-3000

We have previously reported that acute high dose pretreatments of ethanol produced a rebound "anxiety"-like state in rats trained to discriminate between pentylenetetrazole (PTZ) and chlordiazepoxide in a Drug 1 - Drug 2 discrimination (DD) task (Gauvin *et al.*, *Drug & Alcohol Dep.*, **24**, 103-113, 1989). The ethanol rebound effect was both time and dose dependent. The present study was designed to re-assess this acute ethanol rebound in rats trained in a more typical Drug - No Drug discrimination task. Twenty male Sprague-Dawley rats were trained to discriminate between 15 mg/kg PTZ and saline (SAL) in a two choice DD task under a FR10 schedule of food motivated operant responding. Test sessions were conducted on a randomized subsample of 10 subjects per dose. Ethanol, administered i.p. 15 min. before test sessions, failed to generalize to the PTZ discriminative cue up to doses that totally suppressed responding. However, acute high dose ethanol pretreatments of 2, 3, & 4 g/kg produced a graded dose- and time-dependent shift from SAL- to PTZ-appropriate responding; the peak of PTZ-appropriate responding engendered by these high dose pretreatments were 40%, 76%, and 84% which occurred at 8, 11, and 13 hrs, respectively. This dose- and time-dependent "anxiety"-like rebound produced by high dose ethanol pretreatments is similar to our previously published results and those of Benjamin *et al.*, (*Neuropharm.*, **26**, 1727-1731, 1987) after chronic ethanol exposure. Taken together, these data support D.B. Goldstein's (*Pharmacology of Alcohol*, 1983, pp 106) and McQuarrie & Fingl's (*JPET*, **124**, 264-271, 1958) suggestion that the delayed effects of a single high dose of ethanol (hangover) are qualitatively similar to those elicited by chronic exposure and may differ only in magnitude and time course. (Supported by NIAAA RO1-AA08338)

175.1

LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEURONS MIGRATE ALONG VOMERONASAL AND OLFACTORY AXONS WHICH EXPRESS CC2 GLYCO-CONJUGATES IN RATS. G.A. Schwarting, D.L. Drinkwater*, J.E. Crandall, and S.A. Tobet EK Shriver Ctr, Waltham, MA 02254; and Program in Neuroscience, Harvard Medical School, Boston, MA

LHRH neurons migrate from the epithelium of the medial olfactory placode, across the nasal cavity and cribiform plate to the forebrain, in a wide variety of species. The CC2 monoclonal antibody reacts with the terminal alpha-galactose and alpha-fucose residues of one glycolipid and two glycoproteins (Schwarting and Crandall, Brain Res., in press) which are expressed selectively in the olfactory system of rats. At the ages examined in the present study (E13-15), CC2-immunoreactive glycoconjugates (CC2ir) were demonstrated on vomeronasal cells and axons and also on a dorsomedial subset of main olfactory neurons and axons. At E15, CC2ir was detectable on fibers which run both dorsally and ventrally along the surface of the developing forebrain and also on some fibers which extended directly into the rostral forebrain. In adjacent sections, LHRH-immunoreactive neurons (LR-1, gift of Dr. R. Benoit) were seen at E14 and E15 in the nose adjacent to the vomeronasal organ, across the cribiform plate, and in the rostral and ventral forebrain. Double-label immunocytochemical analysis revealed LHRH neurons in close association with CC2ir vomeronasal and olfactory nerve bundles, and with a complex network of CC2ir fibers across the cribiform plate. LHRH neurons in the nose were associated with CC2ir fibers. By E15, many LHRH neurons had migrated into the rostral forebrain, in some cases maintaining association with CC2ir. These studies raise the possibility that CC2ir glycoconjugates provide a specific chemical guide for a subset of migrating LHRH neurons or their migratory pathways.

175.2

ANTIBODY TO NEURAL CELL ADHESION MOLECULE (NCAM) CAN DISRUPT THE MIGRATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEURONS INTO THE MOUSE BRAIN. M. Schwanzel-Fukuda, S. Abraham, G.R. Reinhard, K.L. Crossin, G.M. Edelman, D.W. Pfaff. The Rockefeller University, New York, New York.

We examined the effects of antiserum to NCAM (A-NCAM) on the migration of LHRH neurons from the epithelium of the olfactory pit into the forebrain, since NCAM appears to be a major component of the migration route (Soc.Neurosci.Abstr.398,'90). Swiss mice, on day 10 of pregnancy, were laporotomized and 1 ul of A-NCAM (10 mg/ml) injected into the area of the olfactory pit of each embryo of one uterine horn. Embryos of the second uterine horn served as uninjected controls. Each embryo of one uterine horn of control animals received 1 ul injection of rabbit IgG (10 mg/ml) into the olfactory pit, or mechanical damage to the olfactory pit. On day 11 or 12, the embryos were collected, fixed in Bouin's solution, and embedded in paraffin. Serial 8 um sections were examined by immunocytochemistry for LHRH, NCAM or IgG. Injection into the olfactory pit was counted as successful only when uptake of A-NCAM or IgG was detected in the epithelium of the olfactory pit. Animals at 12 E that showed such uptake of A-NCAM, had few or no LHRH-immunoreactive cells along the migration route on the nasal septum, or at the entrance to the medial basal forebrain, as is seen in normal 12 E mice. No disruption of the NCAM "scaffolding" resulted from this A-NCAM, but it was sufficient to retard migration of LHRH cells out of the epithelium of the olfactory pit. Untreated control animals, animals injected with IgG or with A-NCAM but that did not show uptake of the antibody, or those which had mechanical damage, showed at 12 E a normal distribution of LHRH cells among the NCAM fascicles on the migration route. These results with A-NCAM provide support for our thesis (Nature,338:161;'89) that LHRH cells migrate from the olfactory placode to the brain. Supported by NIH grant DC00880 (MSF).

175.3

ONTOGENY OF GONADOTROPIN-RELEASING HORMONE (GNRH) NEURONS IN THE CHICK. K.A. Sullivan, A.J. Silverman, Dept. Anat & Cell Biol., Columbia Univ., NY,NY 10032.

GnRH neurons originate in the olfactory placode and migrate into the forebrain (Schwanzel-Fukuda and Pfaff, 1990; Wray,et al., 1990). The current study was undertaken to delineate the time course of the phenomenon, describe the cytology of the developing cells and determine the migratory route. Chicks, ages E3 to E21 (hatching), were fixed by immersion, frozen sections were cut and processed by standard immunocytochemical techniques (Benoit). GnRH+ cells first appeared on E4 in the olfactory epithelium and distributed throughout the midline, along the nasal septum by E5 and E6. By E7, cells had reached the anterior pole of the telencephalon. In these early stages, GnRH+ neurons had large vesicular nuclei and a thin rim of cytoplasm. By E21, they had acquired their characteristic fusiform shape. From E9 to E21, following a dorsal and caudal route over the telencephalon, they turn ventral at the level of the lateral ventricles and distribute to their adult positions. This pathway differs from the ventral route taken by GnRH neurons in the mammal. Studies are currently in progress to examine the substrate for migration. HD 10665 T32HD07093-15.

175.4

PRIMARY CULTURES OF LHRH NEURONS FROM FETAL RAT AND MONKEY OLFACTORY PLACODE. *P. Claude*[†], *C.A. Schulz**, *L.L. Luchansky** *and E.Terasawa*.[†] Wisc. Reg. Primate Res. Ctr. and [†]Neuroscience Training Program, UW-Madison 53715.

Recent reports indicate that hypothalamic LHRH neurons arise in fetal olfactory placode and migrate into the brain before birth, in rodents (Schwanzel-Fukuda and Pfaff, 1989; Wray *et al.*, 1989), and monkey (Ronnekleiv and Resko, 1990). To obtain primary LHRH neurons for further study, we cultured olfactory placode tissue from rhesus monkey (E36) and rat (E12.5) fetuses. The olfactory placode area was cut into small pieces (< 1 mm^3), and plated onto collagen-coated glass coverslips in medium M199 supplemented with 10% charcoal-stripped fetal calf serum. Cultures were maintained for up to 35 days, and medium was assayed by RIA for LHRH. Cultures were immunostained for LHRH, gonadotropin-associated peptide (GAP), neurofilament protein (NF), neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP). *Results:* There were no glial (GFAP-staining) cells in the cultures. Two types of neuron (NF and NSE-positive) migrated out of the explants: LHRH-negative neurons were round and small (5-8μm) had short neurites, and tended to form dense clusters. LHRH-positive neurons were large (up to 25 μm in rat placode cultures) and also GAP-positive. They initially stained lightly for LHRH, but with time in culture, staining intensity increased. They usually associated with each other in chains or networks, and extended long, varicose neurites (up to 800 μm). In monkey cultures there were few LHRH-negative cells, LHRH neurons were larger than in rat cultures, and took longer to mature. Cultured cells released LHRH into the medium, and responded to challenge with high K^+. These cultures contain primary LHRH neurons that migrate and mature in culture and are accessible for study using electrophysiological and microscopic techniques. *(Support: NIH grants HD11355, HD15433 & RR00167.)*

175.5

LUTEINIZING HORMONE - RELEASING HORMONE IMMUNOREACTIVITY IN THE ADULT AND FETAL HUMAN OLFACTORY SYSTEM. LS Patel[1]*,EG Stopa[1],R Chorsky[1]*,B Rubin[2],J King[2] Dept. of Pathology[1] SUNY-HSC, Syracuse, NY 13210; Dept. of Anat. & Cell. Bio.[2] Tufts Hlth Sci. Schls., Boston, MA 02111

Recent studies in fetal brain tissue of rodents, nonhuman primates and birds have demonstrated that cells containing luteinizing hormone-releasing hormone (LHRH) migrate from the olfactory placode across the nasal septum into the forebrain. The purpose of this study was to examine LHRH neurons in components of the adult and fetal human olfactory system. Brain tissues were fixed by immersion in Zamboni's fixative (14 hours), sunk in 30% sucrose, and cut with a cryostat at 40 microns. The immunocytochemical procedures incorporated the use of CR11B73 antibody and the avidin-biotin-peroxidase method on free floating tissues. In the adult human brain (n=3), immunoreactive LHRH was localized in diffusely scattered cell bodies and processes observed in the olfactory bulb, olfactory nerve, olfactory cortex, and nervus terminalis located on the inferior surface of the gyrus rectus. LHRH immunoreactive structures showed a similar distribution in 20 week human fetal brains (n=2), indicating that the migration of LHRH neurons is near completion at this time. These data are consistent with observations made in other species, and suggest that LHRH neurons migrate into the brain after originating in the olfactory placode. Additional studies of human fetal brains obtained earlier in development are presently underway. (AG09301; HD19803)

175.6

REGULATION OF LHRH AND OXYTOCIN GENE EXPRESSION IN ORGANOTYPIC CNS SLICE–EXPLANT CULTURES: EFFECTS OF K^+ AND ESTRADIOL. S. Wray, S. Key*and H. Gainer. Lab. of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

Examination of the regulation of cell-specific neuropeptide gene expression in luteinizing hormone releasing hormone (LHRH) and oxytocin (OT) neurons has been difficult due to the absence of an *in vitro* system which maintains these CNS neuroendocrine cells in primary cultures. We have found that a wide variety of postnatal neuroendocrine cells survive and express specific neuropeptide genes in organotypic slice–explant cultures. Utilizing this model system, we examined the effects of potassium (K^+) and estradiol treatment on peptide mRNA levels in LHRH and OT neurons using semi-quantitative *in situ* hybridization histochemistry. Brain slices from postnatal day 5 rats were cultured in serum-containing media to allow thinning, maintained in defined media (serum-free) for 4-6 days, and exposed to either 40 mM K^+ (4 hrs) or 10 nM estradiol (8 or 24 hrs). To determine if the effect of estradiol on peptide gene expression in LHRH and OT cell subtypes was direct or via interneurons, the cultures were examined under 'normal' synaptic activity and tetrodotoxin activity blockade. Analyses of the OT cultures was performed using X-ray film, providing a density value/culture which corresponds to mRNA levels. Comparisons of these values showed that OT mRNA levels ~doubled after exposure to 40 mM K^+ ($p \leq 0.01$). Densitometric single cell analyses of LHRH and OT neuronal mRNA levels are currently in progress. Single cell analyses will determine if either of these neuroendocrine cell types contain subpopulations which differentially respond to depolarization and estradiol treatment.

175.7

SUBGROUPS OF LHRH NEURONS PROJECT TO DEFINED REGIONS OF THE MEDIAN EMINENCE IN RATS. J.C.King, B.S. Rubin and C.E Lupini*. Dept. of Anatomy and Cellular Biology, Tufts University Health Sciences Campus, Boston, MA 02111.

We have demonstrated that the distribution of LHRH neurons detected by the high threshold PAP technique, i.e. neurons with high abundance of LHRH precursors and intermediates, vary in detection as a function of endocrine state in male and female rats. Three-dimensional analyses of the differences in distribution have revealed a basic microarchitecture of LHRH subgroups. We have postulated that these subgroups receive different afferent inputs and project to distinct selective regions of the median eminence. It was the purpose of this study to test the hypothesis of distinctive projections to the median eminence [ME] using neuro tracing techniques. We delivered PHA-L by iontophoresis with a micropipette tip of 10μ to anesthetized females in a stereotaxic apparatus. Positive current 5 μAmp was pulsed on/off for 7 sec for a total of 20 minutes. Hypothalamic neuronal cell bodies incorporated the PHA-L and fibers could be traced to the ME in animals perfused with either 4% paraformaldehyde in 0.1M Borate Buffer, pH 11 or 3% acrolein in 0.1M Sorensen's phosphate Buffer, pH 7.2. PHA-L was localized using peroxidase-antiperoxidase or avidin-biotin protocols. Neurons located in the rostral preoptic area in the midline just superior to the organum vasculosum of the lamina terminals projected primarily to midline regions of the ME. The restricted ME projection of neurons in this area, which is within the boundaries of one subgroup of LHRH neurons, is consistent with the hypothesis of an organized projection to selected regions of the ME. This study is one in a series of studies directed to elucidating the functional organization of LHRH subgroups. DCB-9004498 (JCK) and HD 19174 (BSR).

175.8

LUTEINIZING HORMONE RELEASING HORMONE (LHRH) NEURONS WHICH UPTAKE FLUORO-GOLD FROM THE VASCULATURE IN THE MALE FERRET. L.A. Berglund and C.L. Sisk, Neuroscience Program & Department of Psychology, Michigan State University, East Lansing, MI 48824.

LHRH-producing neurons are diffusely distributed from rostral forebrain through caudal diencephalon in the ferret. About 70% of the LHRH cell bodies are located in retrochiasmatic hypothalamus in this species. In order to determine whether a distinct subpopulation of LHRH neurons is responsible for LHRH secretion into the vasculature, prepubertal male ferrets received two systemic injections, 5 days apart, of the retrograde tracer Fluoro-Gold (FG). After an additional 5 day post-injection survival period, ferrets were anesthetized and perfused with saline and 4% paraformaldehyde. Immunocytochemistry for LHRH was performed on brain sections using a secondary antibody tagged with tetramethylrhodamine isothiocyanate. LHRH-producing neurons with axon terminals in regions containing fenestrated capillaries were defined as cell bodies which demonstrated both immunofluorescence for LHRH and localization of FG. Double-labelled neurons were scattered throughout the entire population of immunoreactive LHRH (iLHRH) cell bodies. However, less than 30% of iLHRH neurons also contained Fluoro-Gold, and most of these cells contained very faint FG labelling. In contrast, when a matching set of sections from these brains was immunoreacted for neurophysin (NP), a majority of the NP cell bodies in supraoptic and paraventricular nuclei were heavily labelled with FG. The lesser degree of FG uptake in iLHRH cells suggests relative inactivity of the LHRH neurons projecting outside the blood-brain barrier in prepubertal ferrets. Supported by HD26483.

175.9

THE NUMBER OF LHRH-CONTAINING NEURONS DECREASES SELECTIVELY IN THE ARCUATE NUCLEUS DURING PUBERTY IN MALE FERRETS. Y.P. Tang and C.L. Sisk, Neuroscience Program & Dept. of Psychology, Michigan State University, East Lansing, MI 48824.

The neuropeptide luteinizing hormone releasing hormone (LHRH) controls gonadotropin secretion from the pituitary gland. Pubertal maturation is characterized by more frequent release of LHRH and the gonadotropic hormones. In order to determine whether a change in the number of LHRH-producing neurons is correlated with enhanced hormone release at puberty, LHRH-containing neurons were identified immunocytochemically in male ferrets at various ages spanning puberty. Intracerebroventricular administration of colchicine, a blocker of axonal transport, to anesthetized ferrets 24 hr prior to sacrifce did not reveal additional numbers of immunopositive LHRH (iLHRH) cell bodies in any brain area in either pre- or postpubertal ferrets. There were significantly fewer iLHRH somata within the arcuate nucleus in peri- and postpubertal ferrets compared to prepubertal ferrets. A 50% reduction in the number of iLHRH neurons in the arcuate was observed both in ferrets in which puberty onset was induced by a transition from short to long daylengths, and in ferrets which experienced spontaneous puberty in the absence of a change in daylength. The fact that the number of iLHRH neurons is reduced selectively in the arcuate nucleus at a time of more frequent LHRH release suggests that, in the prepubertal ferret, LHRH from arcuate neurons provides an ultrashortloop negative feedback signal that inhibits release of LHRH into the median eminence from non-arcuate LHRH neurons. The decrease in the number of arcuate LHRH-producing neurons at puberty may reduce this negative feedback signal and permit more frequent release of LHRH. Supported by HD26483.

175.10

DETECTABILITY OF LHRH IN SPECIFIC SUBGROUPS IS CORRELATED WITH THE PROESTRUS LH SURGE. B.S. Rubin, J.C. King, S.D. Vath*, and R.A. Strauss*. Department of Anatomy and Cellular Biology, Tufts Health Science Schools, Boston MA 02111.

We have previously reported an increase in the number of LHRH neurons detected by the PAP technique on the evening of proestrus, after the peak of the LH surge, compared to the number detected earlier that afternoon, prior to the surge. We have analyzed the distribution of the detectable LHRH neurons at these two time points by counting LHRH neurons immunoreactive with antiserum 1076 (R.P.Millar) in each section throughout the basal diencephalon. The sections were assigned to one of 4 areas. Area 1 includes the region through the Diagonal Band of Broca, area 2 includes the Organum Vasculosum of the Lamina Terminalis, area 3 includes the Preoptic Area and Anterior Hypothalamus and area 4 encompasses the Medial Basal Hypothalamus. The data suggest that the increase in perikarya number is not randomly localized throughout the entire LHRH neuronal population. Whereas no change in the number of LHRH perikarya was observed in the area of the Diagonal Band of Broca on proestrus, the number of LHRH neurons counted in the Preoptic-Anterior Hypothalamic area on the evening of proestrus was 175% of the number detected in that same region during the early afternoon. Three dimensional mapping of the LHRH neuronal system at these two time points is currently underway to enable a more precise identification of the subgroups in which LHRH detectability varies above/below the threshold for detection with PAP on proestrus. We hypothesize that precise localization of the LHRH subgroup(s) that vary in detectability between early and late proestrus will yield valuable insight regarding the specific LHRH neurons that are involved in the LH surge. HD19174 (BSR) and DCB-9004498 (JCK)

175.11

SEASONAL REPRODUCTION IN THE SYRIAN HAMSTER AND THE INFLUENCE OF EXCITATORY AMINO ACIDS. H.F.Urbanski, M.Pierce* and P.M.Collins*. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Melatonin is known to play a key role in mediating the suppressive effects of short days on the reproductive axis of Syrian hamsters but the link between this indole and the neuronal circuitry controlling LHRH secretion is poorly understood. Recently, we showed that administration of the excitatory amino acid (EAA), *N*-methyl-D-aspartate (NMDA), to male hamsters could overcome the inhibitory effects of short days by stimulating gonadotropin secretion and causing testicular recrudescence. We now sought to test whether activation of EAA receptors could overcome the anti-gonadotropic action of melatonin. Male hamsters were transferred from short to long days (i.e., from 6L:18D to 14L:10D), in order to stimulate testicular recrudescence, and given daily *sc* injections of melatonin (25μg) either in the mornings (AM) or afternoons (PM) for 4 weeks. As expected, only the PM injections blocked the stimulatory action of long days on pituitary gonadotropin content, serum gonadotropin and testosterone levels, and on testicular weight. Interestingly, this inhibitory effect of PM melatonin treatment could be overcome by concomittant *sc* injections of kainate (6.5 mg/kg BW), an EAA that activates non-NMDA receptors. In contrast, concomittant *sc* injections of NMDA (25 mg/kg BW - a dose that can completely suppress the inhibitory effects of short days) was largely ineffective. These results indicate that endogenous EAAs most likely exert their influence on the hamster's reproductive axis by acting through both NMDA and non-NMDA receptors and, furthermore, they suggest that different EAA receptor subtypes are integrated at differnt sites along the photo-neuroendocrine pathway that regulates LHRH secretion.
Supported by NIH grants HD-24312, HD-18185, and RR-00163.

175.12

PHOTOPERIOD REGULATES THE LH RESPONSE TO CENTRAL GLUTAMATERGIC STIMULATION IN THE MALE SYRIAN HAMSTER. Francis J P Ebling, Yui Hui*, Elizabeth S Maywood* and Michael H Hastings. Department of Anatomy, University of Cambridge, Cambridge CB2 3DY, UK.

This study investigated whether a change in glutamatergic function might underlie photoperiod-induced changes in luteinizing hormone (LH) secretion. The experimental approach was to compare the effects of glutamate agonists on LH secretion in reproductively active hamsters kept in long days (LD) with those in photoinhibited hamsters kept in short days (SD) for 6 weeks having regressed testes. Agonists were delivered via a cannula into the III ventricle of freely moving hamsters. In Exp 1, a high dose of NMDA (3 nmole) induced significant ($P<0.01$) increases in serum LH 15 min later in both photoperiods (SD 0.7±0.2 pretreatment increased to 2.8±0.5 ng/ml; LD 1.2±0.1 pretreatment increased to 2.4±0.2 ng/ml). The magnitude of the LH response in SD was twice that in LD. In Exp 2, a lower dose of NMDA (0.3 nmole) revealed a difference in sensitivity. Hamsters in SD responded to this dose (1.6±0.4 ng/ml vs vehicle controls 0.7±0.1, $P<0.05$) whereas those in LD did not (1.6±0.3 ng/ml vs controls 1.4±0.2). In Exp 2, hamsters were also treated with AMPA. This non-NMDA agonist induced a two-fold increase ($P<0.05$ vs controls above) in serum LH in both photoperiods (SD to 1.5±0.3 ng/ml; LD to 2.5±0.6 ng/ml). To investigate the site of action of NMDA, the expression of the *c-fos* gene was used as a marker of neuronal activation. Although NMDA induced widespread *c-fos* expression in many periventricular regions including the medial preoptic area (POA), dual ICC revealed that in neither photoperiod was *c-fos* product induced in GnRH-positive neurons 1 h after 3 nmole NMDA, despite the increases in LH secretion. This observation is consistent with a site of action of NMDA in the median eminence rather than a direct effect on GnRH perikarya in the basal forebrain and POA. In conclusion, these results show that both NMDA and non-NMDA glutamatergic pathways could potentially regulate LH secretion in the Syrian hamster. The increased sensitivity and responsiveness to NMDA in hamsters in SD is consistent with the hypothesis that photoperiod-induced seasonal infertility reflects decreased glutamatergic drive to GnRH neurons. *Supported by MRC grants to MHH (8912713J) and FJPE.*

175.13

CONSPECIFIC MALE ODOR ENHANCES SYNTHESIS OF LUTEINIZING HORMONE IN THE PITUITARY GLAND OF FEMALE RATS: EFFECT MEDIATED THROUGH THE VOMERONASAL ORGAN. G. Rajendren*, R.N. Rosenberg, C.A. Dudley and R.L. Moss. Dept. of Physiology and Neurology, The Univ. Texas Southwestern Med. Ctr. at Dallas, Dallas, TX 75325.

The influence of male rat odor and the effect of removal of the vomeronasal organ (VNO) on the content of luteinizing hormone (LH) in the pituitary gland of regularly cycling female rats was investigated. VNO-removed (VNX), VNO-sham(VNS) or VNO intact (VNI) females were individually housed, and were exposed to clean bedding or male-soiled bedding. Exposure of the females to bedding began on the day of estrus and continued until they were sacrificed on the day of proestrus of the following cycle. On the day of sacrifice, the females were injected with pentobarbital (35 mg/Kg of body weight) to block the preovulatory LH surge. Three to four hours later they were sacrificed by decapitation. The anterior lobe of the pituitary gland was dissected on dry ice, homogenized and the content of LH was determined by radioimmunoassay. The content of LH in the pituitary was not significantly different in the VNX, VNS or VNI females exposed to clean bedding. However, of the females exposed to male-soiled bedding the pituitary content of LH in the VNS and VNI females was significantly higher as compared to the VNX group. The results suggest that male-originating cues can enhance the synthesis of LH in the pituitary and that the effect is mediated through the vomeronasal system. Supported by NIH grant MH41784.

175.14

HYPOTHALAMIC RELEASE OF LHRH IS REDUCED AFTER MATING OR GONADECTOMY IN BREEDING FEMALE, BUT NOT IN MALE, FERRETS. G.M. Lambert, B.S. Rubin, M.J. Baum. Dept. of Biology, Boston Univ., Boston MA 02215 and Dept of Anatomy and Cellular Biology, Tufts Univ., Boston MA 02111.

Mediobasal hypothalami were collected from male and female ferrets which were gonadectomized, or which were in breeding condition and had either achieved or received an intromission with a sexual partner or were left unpaired. LHRH was measured in effluents collected at 10-minute intervals from in vitro perifusion chambers containing the MBH tissues. Estrous female ferrets which had received an intromission 14 minutes prior to sacrifice and ovariectomized females released significantly less LHRH than estrous females which had not been paired; no such effects occurred in females 1 or 2 1/2 hours after mating. The release of LHRH from MBH collected from males was not affected by mating or castration. These data suggest: 1) the post-coital surge of LH in the female ferret is preceded by a release of LHRH that initially depletes neuronal terminals, while LHRH, like LH, is minimally affected by mating in males, and 2) the feedback regulation of LHRH secretion by endogenous gonadal steroids differs between male and female ferrets.
(Supported by RO1 HD21094, RO2 MH00392, and MH09812).

175.15

EVIDENCE THAT NMA INCREASES LH SECRETION BY ACTING INDIRECTLY ON LHRH NEURONS. W.-S. Lee*, R. Abbud*, G.E. Hoffman, and M.S. Smith. Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

NMA stimulates the secretion of LH by increasing the release of LHRH. To determine whether LHRH neurons are activated in association with NMA treatment, we used expression of cFos as a marker of LHRH neuronal activation. During proestrus, LHRH neurons express cFos in association with the LH surge. Treatment with the NMDA receptor antagonist, MK-801, (0.2 mg/kg. s.c.) at 1130 h blocked both the LH surge and cFos induction in LHRH neurons. These data suggest that NMDA receptors are involved in the regulation of LHRH neuronal activation during the LH surge. However, neither systemic (40 mg/kg, 4 injections, 10 min apart) nor intraventricular (2 µg/2 µl, 4 injections, 10 min apart) administration of NMA induced cFos expression in LHRH neurons in female rats, indicating that NMA may act directly on LHRH terminals or on afferent neuronal projections which impinge on LHRH neurons, or both. To differentiate between these possible mechanisms, proestrous rats were treated with pentobarbital (40 mg/kg i.p.,) at 1200 h to block neuronal transmission, followed by either saline or NMA (i.v.) administration between 1500-1600 h. Pentobarbital treatment blocked the proestrous LH surge and cFos induction in LHRH neurons in saline-treated animals. NMA treatment of pentobarbital-blocked rats resulted in no increase in LH secretion, suggesting there were no direct effects of NMA on LHRH neurons. These results demonstrate that although NMDA receptor activation appears to be necessary for LHRH neuronal activation during proestrus, NMA appears to act indirectly on LHRH neurons, perhaps activating afferent neuronal projections to the LHRH neuron. Supported by NIH grants NS 28730 and the Samuel and Emma Winters Foundation.

175.16

GABA-B MEDIATION OF NMDA-STIMULATED LH RELEASE IN FEMALE MICE. G.M. Miller and M.J. Gibson. Div. Endocr. Mount Sinai School of Med., New York, N.Y. 10029.

Our recent findings suggest central inhibitory mediation of GnRH secretion to NMDA challenges, resulting in differential release of LH in male and female hypogonadal mice with preoptic area brain grafts (HPG/POA). As GABA blocks NMDA-induced LH release in pubertal rats, we evaluated the role of the GABA-B receptor in modulating NMDA effects in normal female mice. Five days after ovariectomy, mice were fitted with jugular cannulae and tested the following day. Two NMDA challenges (20mg/kg,iv) were given 2h apart. Ten min before the 2nd NMDA challenge, mice received the GABA-B receptor agonist baclofen (Bac, 5mg/ kg,iv) or saline (Sal). Blood samples (0.1 ml) were taken at 10 min intervals before and after each challenge. Resuspended red blood cells were returned following the next sample withdrawn, and plasma duplicates (25ul) were assayed for LH. Bac-pretreatment attenuated the LH response to the 2nd NMDA challenge: 1: 2.21±0.36 ng/ml, $p<0.01$; 2: 1.28±0.22ng/ ml, ns. In Sal-treated mice, LH increased significantly to both NMDA challenges: 1: 3.04±0.38ng/ml, 2: 3.06±0.38ng/ml, both $p<0.01$. HPG/POA female mice are being tested. These studies implicate GABA-B receptors in GABAergic control over NMDA-induced LH release in female mice. Support: NIH Grants T32DK07645 (GM) & HD19077 (MJG).

175.17

HYPERPROLACTINEMIA SUPPRESSES THE LH RESPONSES TO N-METHYL-D-ASPARTATE AND NEUROPEPTIDE Y IN MALE RATS. M. Selmanoff and S.-K. Park*, Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

This study further characterizes the response of LHRH neurons to N-methyl-D-aspartate (NMDA) and neuropeptide Y (NPY) and determines whether neurons utilizing these compounds are involved in mediating hyperprolactinemic (HP) suppression of LH release. Male Sprague-Dawley rats (250-300g BW) were orchidectomized, adrenalectomized, implanted with a testosterone silastic capsule and a 50% corticosterone pellet and with third cerebroventricular and right atrial cannulae at time 0. Rats received either subcutaneously injected oPRL (2,400 µg/0.25 ml) in a polyvinylpyrrolidone depot or vehicle every 12h. The mean maximal LH increments (ΔLH) to two doses of LHRH (0.4 and 0.8 ng/100g BW) were not altered in HP rats. NMDA (20 mg/kg BW iv)-induced LH release peaked 5 min after injection. The ΔLH (0-5 min) in HP rats (0.16 ± 0.05 ng/ml) was suppressed compared with controls (0.33 ± 0.06 ng/ml). Two doses of NPY (2 and 10 µg/2µl icv) produced dose-dependent LH increments which peaked at 10 min. In HP rats the ΔLH (0-10 min) in response to 10 µg/2µl NPY was suppressed (0.12 ± 0.03 ng/ml) compared with controls (0.25 ± 0.03 ng/ml). Hence, the LH responses to both NMDA and NPY were inhibited in HP rats. This suggests that PRL either acts directly on LHRH neurons to decrease their responsivity to all stimuli, and/or, that PRL acts indirectly to inhibit glutamatergic and NPYergic neurons that are stimulatory to LHRH release. (Supported by NIH grant HD21351).

175.18

IMMUNOCYTOCHEMICAL DETECTION OF A CHICKEN-LHRH-II-LIKE PEPTIDE IN THE RAT HYPOTHALAMO-HYPOPHYSEAL SYSTEM. F. Vandesande, J. van Gils* and F. Bijtebier*. Lab. Neuroendocrinologie, Zoölogisch Instituut, Naamsestraat 59, B-3000, Leuven, Belgium.

Two polyclonal rabbit antisera were prepared against Lys-His-Gly-Trp-Tyr-Pro-Gly-NH_2 conjugated to thyroglobulin. This sequence corresponds to c-LHRH II(5-10). Both antisera recognize c-LHRH II in an immunospotting test and do not cross-react with any other known neuropeptide present in the rat hypothalamo-hypophyseal system. Three groups of adult rats of both sexes were used. (1) six normal rats, (2)six rats sacrificed 48 hours after an intracisternal injection of 50 µg colchicine and (3) two Brattleboro rats. Sections of their hypothalamo-hypophyseal system were stained with the antisera in combination with the PAP technique. In all animals, immunoreactive (ir) perikarya were found in the paraventricular nuclei, the nuclei accessorii, the supraoptic nuclei and the supraoptic retrochiasmatic nuclei. An intense staining was also observed in both the external region of the median eminence and the posterior lobe, where the ir-fibres appeared to accumulate around the blood-capillaries. In the colchicine-treated animals, the staining intensity of the perikarya was much stronger, while the ir in the nerve fibres had decreased. Double staining with anti-vasopressin, anti-oxytocin and anti-somatostatin showed that 100 % of the c-LHRH-II-ir perikarya contained vasopressin, but only 50 to 60 % of the vasopressinergic cells were also ir for c-LHRH-II.

175.19

BRAIN LHRH IMMUNOREACTIVITY IS RETAINED FOLLOWING NEURAL LESIONS THAT ABOLISH THE PHOTOPERIODIC, REPRODUCTIVE RESPONSE IN JAPANESE QUAIL.

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Seasonal reproduction in Japanese Quail is triggered by long days (LD), resulting in secretion of LHRH and consequently LH. Using ablation techniques several regions in the bird brain have been identified that influence this response, but their physiological role remains equivocal. We have tested the possibility that lesions in the posterodorsal area of the infundibular nucleus (p.d.-i.n.c.) in the tuberal hypothalamus prevent photoinduction by causing a deficit in the LHRH system.

Following lesions, male quail were transferred from short days (8L:16D) to LD (18L:16D). After 4-5 weeks, birds whose reproductive response was blocked by the lesions (terminal testicular weight 11±2(8) [Mean±S.E.M.(n)] mg) and those which exhibited normal testicular growth (2687±100(8) mg) were subjected to immunocytochemical analysis for LHRH. The total number of LHRH immunopositive perikarya (blocked birds; 436±80(5), normal birds; 387±50(5), in 90 µm sections) and their regional distribution was similar, with the median eminence staining intensely, in both groups.

It is concluded that the LHRH neuroendocrine system is intact after lesioning and that the p.d.-i.n.c. is probably involved in mechanisms that mediate photoperiodic information.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: STEROIDS

176.1

ESTROGEN RECEPTOR mRNA IN FEMALE RAT BRAIN DURING THE ESTRUS CYCLE: A COMPARISON WITH OVARIECTOMIZED FEMALES AND INTACT MALES.

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Estrogen action in the preoptic area and basal hypothalamus induces female reproductive behavior and facilitates cyclic release of gonadotropins from the pituitary. Autoradiographic, immunocytochemical and *in situ* hybridization studies have detected estrogen receptor (ER) in preoptic/ hypothalamic regions and revealed hormone dependent changes in receptor number and topography. Since dramatic changes in hormonal milieu occur in the cycling female brain, the present study investigated ER mRNA expression during the estrus cycle. Female Wistar rats (200g) were sacrificed during estrus, metestrus, diestrus, proestrus, or 72 hrs after ovariectomy. Additional intact male rats were also sacrificed. Brains were frozen and 20 µm cryostat sections thaw-mounted and hybridized with a ^{35}S-labeled probe for ER mRNA. Section-mounted slides were processed, apposed to x-ray film, and then dipped in liquid emulsion and quantified by computer. After exposure, ER mRNA was detected in brain regions including the medial preoptic area (MPO), arcuate (ARC), and ventromedial nucleus (VMH) of the hypothalamus. At the level of the MPO, ER mRNA levels were highest on metestrus, attenuated at diestrus, and low on proestrus and estrus. In contrast, ER mRNA levels in the VMH increased from metestrus to diestrus and peaked during proestrus, then declined on estrus. However, ER mRNA in the ARC was unchanged during the estrus cycle. When females were ovariectomized message expression was elevated, while levels in the intact male were low. The results indicate that expression of ER mRNA is sexually dimorphic, varies during the estrus cycle, and increased after ovariectomy. These data further suggest that ER mRNA is differentially regulated in nuclei of preoptic and hypothalamic regions of the brain. Supported by NS20311 and NS07332.

176.2

DIFFERENTIAL REGULATION OF ANDROGEN RECEPTORS IN BRAIN AND PROSTATE OF MALE GUINEA PIGS AS SHOWN BY IMMUNOCYTOCHEMISTRY.

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Androgens affect reproductive behaviors and gonadotropin secretion. These effects are mediated through intracellular receptors which affect gene transcription. We compared androgen receptor (AR) regulation in two androgen dependent tissues, the brain and prostate. We developed polyclonal antibodies, in rabbits, to an AR-specific peptide antigen (a.a. 201-222 of the human AR N-terminal region). Using antigen-purified antibodies, we localized AR by immunocytochemistry. Intact animals possessed specific staining for AR in nuclei of prostatic epithelial and stromal cells. Immunostaining was broadly distributed throughout the brain and localized to neuronal nuclei. Brain areas with positive immunoreactivity included piriform and sensory cortex, amygdala, and several nuclei of the hypothalamus. Castration had little or no effect on the distribution or intensity of immunostaining in the brain, however, positive staining was completely abolished in the prostate. These results suggest differential regulation of AR between androgen dependent target tissues. Supported by NIH grants: T32 HD07133 and HD18196.

176.3

ANDROGEN RECEPTOR-LIKE IMMUNOHISTOCHEMICAL LABELING OF NEURONS IN MOUSE AND RAT BRAIN.

A.N. Clancy, R.W. Bonsall and R.P. Michael. Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322 and the Georgia Mental Health Institute, Atlanta, Georgia 30306.

To locate neurons containing androgen receptor-like (AR) proteins, mouse brains were immunohistochemically labeled with a commercially available monoclonal antibody (Affinity BioReagents MA1) raised in rats against protein fragments containing human AR sequences, and rat brains were stained with a polyclonal antibody (Affinity BioReagents PA1-110) raised in rabbits against the same fusion proteins. Frozen sections were incubated with antibodies and further processed by the peroxidase avidin-biotin complex method. Similar patterns of labeling occurred in the brains of both species. Labeled cells were widely distributed throughout the brains and were noted in areas previously identified as containing androgen target neurons by autoradiography including: the medial preoptic area, amygdala, hypothalamus, bed nucleus of the stria terminalis, lateral septum, cortex, hippocampus, several brainstem nuclei, and cerebellar Purkinje cells. However, labeling was also observed in a few brain regions (e.g. the supraoptic nucleus) not labeled autoradiographically. Labeling patterns were generally similar in the brains of intact and orchidectomized animals and in male and female rats. To test the specificity of labeling, some sections were incubated in the absence of anti-AR antibodies while others were incubated in monoclonal anti-AR antibodies that had been preabsorbed with cytosol from rat prostate gland, a tissue rich in androgen receptors: no labeling was observed in either case. Supported by USPHS grant MH 19506, Emory University Research Committee and the Georgia Department of Human Resources.

176.4

LOCALIZATION OF INHIBIN/ACTIVIN SUBUNIT mRNAs IN RAT BRAIN BY IN SITU HYBRIDIZATION.

V. J. Roberts , W. Vale, and P.E. Sawchenko. Dept. Repro. Med., UCSD, La Jolla, CA 92093, and The Salk Institute, La Jolla, CA 92037.

The reproductive hormones inhibin (α/βA or α/βB subunit heterodimers) and activin (β/β dimers) have a broad anatomical distribution both peripherally and centrally. We recently described the central distribution of inhibin/activin subunit proteins in neuronal cell bodies, fibers, and nuclei and reported the presence of mRNAs encoding each of these subunits in all major brain regions (determined by S1 protection analysis). In the present study we examined the detailed *in situ* localization of these mRNAs using ^{35}S-RNA probes and found: 1. βA-mRNA signal was in most regions where βA-immunoreactive (IR) nuclei (inf. olive, medial vest. nu., caudate-putamen) cell bodies (vent. medullary ret. formation) or fibers (amygdala) were present. 2. βB-mRNA signal was present in most regions showing βB-IR neurons (vent. medullary ret. formation, subtantia nigra) and fibers (various thalamic nu., amygdala), and in some areas with exclusively βA nuclear IR (spinal trigeminal nu., cochlear nu.) 3. Areas with βA- or βB-mRNA signal and no corresponding IR were also seen (deep cerebellar nu., hippocampus). 4. α-mRNA signal was usually found in areas along with βA-mRNA signal (piriform cx., hippocampus) but we have not yet succeeded in localizing α-subunit IR in the brain. These results suggest that inhibin/activin proteins are produced in many brain regions where they may be stored, function as intracellular growth factors, or are transported and may act as intercellular communicators.

176.5

NEUROANATOMICAL SPECIFICITY IN THE AUTOREGULATION OF AROMATASE BY ANDROGENS AND ESTROGENS: AN IMMUNOCYTOCHEMICAL STUDY. J. Balthazart, A. Foidart, C. Surlemont*, N. Harada*, and F. Naftolin Lab.Biochemistry, Univ. Liège, B-4020 Liège, Belgium, Molec. Genetics, Fujita Health Univ., Toyoake, Aichi 470-11, Japan, and Dept. Obstetrics/Gynecology, Yale Univ., New Haven, CT 06510, USA.

Testosterone (T) increases brain aromatase activity (AA) in quail and other avian and mammalian species. It was shown both in quail (C.R.Acad.Sci. 1987, 305:569-574) and in rat (Neuroendocrinol. 1991, 53:79-84) that this enzymatic induction results from a synergistic action of androgens and estrogens. These studies provide little information on possible anatomical or cellular specificity of the effect. Using a polyclonal antiserum against human placental aromatase, we have identified aromatase-immunoreactive (ARO-ir) neurons in the quail brain (J. Comp. Neurol. 1990, 301:276-288) and demonstrated that T increases the number of ARO-ir cells in the quail preoptic area (POA)(Brain Res. 1990, 514:327-333) supporting previous evidence that T increases AA in the brain. However, which T metabolites are involved, the actual mechanism of autoregulation and the possibility of anatomical specificity for these effects are not yet clear. EXPERIMENTAL: We disassociated the effects of androgens and estrogens in aromatase induction by comparing ARO-ir in neurons of quail treated with T alone or T in the presence of a potent aromatase inhibitor (R76713), which has been shown to depress AA levels and to suppress T-activated copulatory behavior (Horm.Behav. 1990, 24:510-531). T increased the number of ARO-ir cells in POA, bed nucleus striae terminalis (BNST) and tuberal hypothalamus (TU). The T effect was inhibited by concurrent treatment with aromatase inhibitor in TU, but not in POA and BNST. CONCLUSIONS: T increases AA and ARO-ir. The T effect is blocked by R76713 in areas where ARO-ir and estrogen receptor-ir are generally co-localized (TU) and is not affected in areas with mainly ARO-ir positive, estrogen receptor-ir negative cells (POA, BNST) (J. Neurobiol.1991, 22:143-157). This suggests anatomical differences in the expression or clearance of aromatase which may be differentially sensitive to androgens and estrogens and dependent upon the presence of sex steroid receptors. *Supported by FRFC (2.9003.91, 9.4601.90) and EEC (SC1-0230-C/TT) to JB and NIH HD13587 to FN. R76713 was provided by Dr. R. DeCoster (Janssen Pharmaceutica).*

176.6

A SEXUALLY DIFFERENTIATED, ESTROGEN-RESPONSIVE POPULATION OF NEUROTENSIN NEURONS IN THE ARCUATE NUCLEUS OF THE RAT. M.J. Alexander and S.E. Leeman, University of Massachusetts Medical Center, Worcester, MA 01655.

Previous studies have indicated that a majority of the neurotensin (NT) neurons in the rat arcuate nucleus are also dopaminergic. NT and dopamine have opposite direct effects on prolactin secretion by the anterior pituitary, and evidence suggests a stimulatory role for NT in female-specific regulation of prolactin secretion (*PNAS* 85:9866, 1988). We have used *in situ* hybridization histochemistry to detect mRNA encoding NT and neuromedin N (NT/N) in the arcuate nucleus of male and female rats, and we report a pronounced sex difference in NT/N gene expression restricted to the dorsomedial division of the rostral arcuate. In bilateral sections through this region, proestrous females had nearly three times as many labeled cells in the dorsomedial division as did males (20 ± 2 vs. 7 ± 1). In contrast, there was no sex difference in the number of labeled cells in the ventrolateral division. In the dorsomedial division, cell totals for females on diestrus-1 (9 ± 2) differed significantly from those for proestrous females, suggesting that NT/N mRNA abundance in the arcuate nucleus might be regulated by circulating estrogen levels, which are maximal on proestrus. Indeed, in ovariectomized rats estradiol treatment (2 wks) markedly increased the number of labeled cells in the dorsomedial division, but not the ventrolateral division. These results further implicate NT in female-specific regulation of prolactin secretion and raise the possibility that estrogen, which has complex effects on dopamine synthesis and release, differentially regulates the amounts of releasable NT and dopamine in neurons of the dorsomedial arcuate nucleus in the female rat.

176.7

IMMUNOCYTOCHEMICAL LOCALIZATION OF ESTROGEN RECEPTORS WITHIN NEUROTENSIN NEURONS IN THE FEMALE RAT. J.F. Axelson, F.W. van Leeuwen, W.J. Shannon*, and K.A. O'Shaughnessy*. Psychology Department, Holy Cross College, Worcester, MA 01610 and The Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

In situ hybridization procedures indicate that estrogen selectively increases neurotensin and neuromedin (NT)/N mRNA levels in the anterior medial preoptic nucleus (AMPN) (Alexander et al., 1989). Using the co-localization procedures of Axelson and van Leeuwen (1990), the present study examined whether NT cells in the hypothalamus contained estrogen receptors (ER). Brains from adult ovariectomized, colchicine-treated rats were fixed with 4% paraformaldehyde and 0.5% glutaraldehyde. Vibratome sections were first incubated with estrogen receptor antibody (H222, Abbott) and stained with DAB-Ni+ producing a blue-black nucleus. Methanol-hydrogen peroxide washes were used either before or after ER staining. NT anti-sera (HC-8) was used to provide a brown reaction following DAB. The number of NT cells in the AMPN containing ER was shown to be dependent upon a number of factors including colchicine treatment and duration of post-ovariectomy time period. These data support the hypothesis that NT cells in the AMPN that play a role in LH release are, in part, influenced directly by estrogen feedback via nuclear estrogen receptors.

176.8

COLOCALIZATION OF ATRIAL NATRIURETIC PEPTIDE AND ESTROGEN RECEPTOR IMMUNOREACTIVITY IN SEXUALLY DIMORPHIC NUCLEI IN THE MEDIAL PREOPTIC REGION. R.E. Watson, Jr., R.K. Hutchinson*, J.W. Landis*, and M.C. Langub, Jr. Dept. of Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084.

The medial preoptic region contains a number of distinct sexually dimorphic nuclei, including the anteroventral periventricular nucleus (AVPv), periventricular preoptic nucleus (Pvpo), and medial preoptic nucleus (MPN). These nuclei are characterized by the presence of abundant estrogen receptor- (ER) and atrial natriuretic peptide- (ANP) immunoreactive (ir) neurons. This study was conducted to determine if ER-ir is present in ANP-ir neurons in these nuclei. Young adult female Sprague-Dawley rats were ovariectomized and injected the following day with colchicine (50 µg) intracerebroventricularly. One to two days later, these animals were perfused with Zamboni's fixative. Brain sections (25 µm) were stained for the immunocytochemical localization of ER using monoclonal antibody H222 (kindly provided by Abbott Laboratories) using nickel ammonium sulfate-enhanced DAB as the chromogen, which produced a blue-black reaction product that was confined predominantly to the nuclei of immunopositive cells. ANP-ir was demonstrated using a rabbit polyclonal antiserum against rat ANP (Palm, et al., '89) and DAB which produced the typical reddish-brown reaction product in the cytoplasm of immunopositive cells. Approximately 5-10% of the ANP cells in the AVPv, Pvpo, and MPN were ER-ir. Moreover, single labeled ANP-ir cells frequently appeared to be in direct apposition to ER-ir cells. These results suggest that estrogen is capable of regulating the activity of subsets of ANP-ir neurons in the medial preoptic region, potentially affecting neuroendocrine or cardiovascular function, or the regulation of fluid balance.

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176.9

ULTRASTRUCTURAL EVIDENCE FOR ESTROGEN RECEPTOR IMMUNOREACTIVITY IN GLIA, EPENDYMA, AND ENDOTHELIA. M. C. Langub, Jr. and R.E. Watson, Jr. Dept. Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084.

The presence of estrogen receptors (ERs) in non-neural cells in brain, including glia, ependyma, and endothelia, has not previously been documented with electron microscopy. This study was conducted using ultrastructural immunocytochemistry to examine the possibility that ER-immunoreactivity (ER-ir) is present in non-neural cells in brain. Female guinea pigs (Hartley Strain) were perfused with buffered 4% paraformaldehyde and 0.1% glutaraldehyde and 50µm thick sections were cut on a vibrating microtome. Tissue including the anterior pole of the preoptic region and third ventricle was immunostained for ERs with monoclonal antibody H222 (Abbott Labs) using 3,3',5,5'-tetramethylbenzidine (TMB) as the chromogen (Norgren and Lehman, 1989). Thin sections including the preventricular periventricular nucleus (Pep), anterior compact subnucleus of the medial preoptic nucleus (MPNa), and the organum vasculosum of the lamina terminalis (OVLT) revealed many ER-ir cells which were identified ultrastructurally by the presence of distinct spicule-like TMB crystals in their nuclei. While neurons constituted the clear majority of ER-ir cells, labeled astrocytes, ependyma, and endothelia were also present. Distinct intranuclear TMB labeling was present in approximately 1-2% of the astrocytes at this brain level. No labeled oligodendrocytes were observed. Scattered ependymal cells, including both common ependyma and putative tanycytes, were also labeled. Finally, approximately 5% of the vessels in the OVLT, Pep, and MPNa were lined by ER-ir endothelial and vascular smooth muscle cells. Collectively, these results indicate that in addition to regulating the activity of neurons, estrogen may affect brain function through effects exerted on the genome of a subset of astrocytes, ependymal cells, and endothelial cells.

[Supported by the University of Kentucky Medical Center Research Fund.]

176.10

ESTROGEN RECEPTORS IN AXON TERMINALS IN GUINEA PIG HYPOTHALAMUS: IMMUNOGOLD LOCALIZATION. J.D. Blaustein, J.C. Turcotte, and M.N. Lehman. Neuroscience and Behavior Program and Psychology Dept., Univ. Mass., Amherst, MA 01003 and Dept. Anat. and Cell. Biol., Univ. Cinc. Coll. Med., Cincinnati, OH 45267

While we have confirmed previously that estrogen receptor-immunoreactivity (ER-IR) in hypothalamic neurons is present most abundantly in cell nuclei, we have also detected dense ER-IR in the perikarya and processes of neurons. We have reported with an immunoperoxidase technique, using diaminobenzidine as a chromogen, that ER-IR was present in numerous extranuclear organelles and sites, including axon terminals and distal dendrites. Because results using diaminobenzidine can be confounded by diffusion of reaction product, we used an immunogold-streptavidin procedure. Ovariectomized guinea pigs were perfused, brains vibratome-sectioned, and estrogen receptors immunostained (with H222 antibody) using a biotinylated secondary antibody followed by streptavidin labeled with 1 nanometer particles of colloidal gold. The gold particles were then visualized by silver intensification. Electron microscopic analysis confirmed the distribution of silver deposits in association with cell nuclei, axon terminals containing predominantly round and flattened clear synaptic vesicles, proximal and distal dendrites, and rough endoplasmic reticulum. In addition to suggesting the endoplasmic reticulum as a site of synthesis of the receptor, these results suggest the axon terminal as a potential intracellular site of action for estradiol in the hypothalamus.

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176.11

ESTROGENIC REGULATION OF HEAT SHOCK PROTEINS 90Kd AND 70Kd IN THE RAT VENTROMEDIAL HYPOTHALAMUS AND UTERUS. U.E. Olazábal, D.W. Pfaff and C.V. Mobbs. Rockefeller Univ., New York, NY 10021.

Recent evidence indicates that cellular heat shock proteins 90Kd (hsp90) and constitutive 70Kd (hsp70c) may be involved in the steroid hormonal control of target tissues. Therefore, we carried out this study to determine if estrogen regulates hsp90 and hsp70c protein levels in the ventromedial hypothalamus (VMH), a brain region which controls the estrogen-dependent behavior, lordosis, and in the uterus. Ovariectomized female rats received a single injection of estradiol benzoate (E, 10 μg, s.c.). VMH was removed by microdissection from untreated OVX controls and at 6h, 12h, 18h, or 24h following E, prepared in SDS sample buffer, and normalized with respect to total VMH protein content. Hsp90 and hsp70c were detected by 1-D gel electrophoresis followed by immunoblotting using monoclonal antibodies to these hsps (StressGen). Specific immunoreactive bands were visualized on film using enhanced chemiluminescence (Amersham) and scanned with a laser scanning densitometer. Analysis of hsps levels in VMH showed a time-dependent increase for females, but not males, in both hsp90 and hsp70c which reached maximum levels at 12h (see table). In subsequent experiments, the hsp90 induction in the VMH was replicated at 12h, and estrogen-induced increases for hsp90 and hsp70c were also demonstrated in the uterus.

E t-course		0h	6h	12h	18h	24h
hsp90	VMH	-	+15%	+33% **	0%	0%
	uterus	-	+45%	+46%	+124% *	+41%
hsp70c	VMH	-	+40%	+56% *	+21%	-21%
	uterus	-	+24%	+31% *	+53% **	+84% **

Percentage changes from OVX controls; * $p<0.05$; ** $p<0.01$

176.12

HEAT-SHOCK PROTEINS 90Kd AND 70Kd IN RAT BRAIN AND UTERUS: CELLULAR LOCALIZATION BY IMMUNOCYTOCHEMISTRY AND IN SITU HYBRIDIZATION. S.P. Kleopoulos*, U.E. Olazábal, A.H. Lauber, D.W. Pfaff and C.V. Mobbs. Rockefeller Univ., New York, NY 10021.

We have shown (Olazabal et al., this meeting) that estrogen increases heat shock 90Kd (hsp90) and constitutive 70Kd (hsp70c) protein in the ventromedial hypothalamus (VMH) and uterus. To determine which cell types may be participating in this regulation, we have examined the cellular localization of hsp90 and hsp70c protein and mRNA in brain and uterus. Protein was detected by immunocytochemistry, entailing an avidin-biotin-peroxidase detection procedure, using monoclonal antibodies to hsp90 and hsp70c (StressGen). Hsp90 mRNA was detected using a modified method of in situ hybridization entailing I^{125}-labeled cDNA probes to mouse hsp90. Hsp90 and hsp70c immunoreactivity were readily apparent in neurons throughout the brain, including the VMH, but not in glial cells. Hsp90 and hsp70c immunoreactivity was prominent in secretory cells of the uterus (glandular and luminal epithelial cells) from estrogen-treated rats. In these animals, hsp90 and hsp70c immunostaining was considerably less prominent in stromal and myometrial cells. In ovariectomized rats not given estrogen replacement, hsp90 and hsp70c immunoreactivity was also less prominent. Localization of hsp90 mRNA by in situ hybridization was similar to that obtained for hsp90 protein by immunocytochemistry. Similar findings have been shown for hsp70c (Mobbs et al., Soc. Neurosci. Abs., 15:1989). These data suggest that in VMH and uterus, the cell types most likely involved in estrogenic regulation of heat-shock proteins are neurons and secretory cells, respectively.

176.13

EXPRESSION OF GLUTAMATE-A RECEPTOR mRNA IN GONADOTROPIN-RELEASIMG HORMONE CONTAINING (GnRH) NEURONS OF THE FEMALE RAT. L. Jennes and E. J. DuPré*, Dept. Anatomy, Wright State University, Dayton, OH 45435.

Glutamate is a major excitatory neurotransmitter which, among many other functions, has been implicated to participate in the regulation of GnRH release. The present study was designed to determine the glutamate receptor subtype(s) which mediate the stimulatory signal to the GnRH neurons. Central and peripheral administration of the glutamate agonist AMPA caused a significant increase in circulating LH by actions inside the brain, since exposure of cultured pituitary cells to AMPA had no effect on LH release. Immunohistochemistry for GnRH was combined with "in situ" hybridization for the detection of mRNAs encoding for the glutamate-A receptors. The results show that most GnRH-immunoreactive neurons in the septum-diagonal band complex express the mRNAs for both, "flip" and "flop" sequences of the glutamate-A receptor. It is concluded that glutamate can directly activate the GnRH neurons, at least in part, through binding to and activating postsynaptic glutamate-A type receptors.

Supported by NIH grant HD 24629.

176.14

NEUROENDOCRINE REGULATION: 3D ULTRASTRUCTURE OF A MEDIAN EMINENCE MICROVASCULAR MODULE.

LS Hibbard, TL Arnicar-Sulze*, RA Grothe, Jr.*, BJ Dovey-Hartman*, and RB Page. Washington University School of Medicine, St. Louis, MO; Schering-Plough Research, Lafayette, NJ; and The Pennsylvania State University College of Medicine, Hershey, PA.

To study hypothalamic regulation of the anterior pituitary, we are reconstructing discrete portions of the capillary plexus of the median eminence (ME) of the hypothalamus. These capillary modules, imaged at 1650X, were chosen from a 3D reconstruction of a large volume of a single rabbit ME imaged at lower magnification (100X). TEM digital image mosaics of sections, at 1μ intervals, are aligned by Fourier correlation analyses. The capillary lumens were extracted by region-growing techniques based on the local pixel statistics. The cellular features were extracted by interactive tracing. The reconstruction of one vascular module will be presented, including endothelial cells, pericytes, smooth muscle cells, and the neural-mesenchymal stromal boundary.

(Support: NSF BNS9019153, BNS8506479, and NIH NS15926)

ENDOCRINE REGULATION I

177.1

EFFECTS OF HYPOTHALAMO-PITUITARY DISCONNECTION, THYROID HORMONE STATUS AND DIABETES MELLITUS ON INSULIN-LIKE GROWTH FACTOR II (IGF-2), GROWTH HORMONE-RELEASING HORMONE (GRF) AND SOMATOSTATIN (SRIF) mRNA PREVALENCE IN ADULT RAT BRAIN. A. Levy, M.C.Matovelle* and W.S.Young III. National Institute of Mental Health, Bethesda, MD 20814.

We have used hybridization histochemistry to examine changes in transcript levels encoding choroid plexus IGF-2 mRNA, arcuate GRF- and periventricular SRIF-transcripts induced by pituitary stalk transection, hypophysectomy and drug induced thyrotoxicosis, hypothyroidism and diabetes mellitus. Brains were obtained from male rats after 10 days ingestion of a powdered diet containing either 0.5% propylthiouracil (PTU) or 60μg/kg triiodothyronine (T3), or 10 days after trans-oral surgery or a single intraperitoneal injection of 100mg/kg streptozotocin (STZ). The effects of dietary manipulation were confirmed by demonstrating appropriate changes in pituitary TSH mRNA (231±5.6% and 69±3.6% of control respectively) and the effects of STZ and pituitary surgery by measurement of blood glucose (>22mM by stick testing) and water intake (>200mL/kg/day).

Hypophysectomy lead to increased GRF mRNA and decreased SRIF and IGF2 mRNA levels. Stalk transection induced similar but less pronounced changes. T3 lead to a small increase in SRIF and decreased IGF2, leaving GRF unchanged. PTU only increased GRF mRNA. Finally STZ treatment markedly decreased GRF and IGF2 transcript levels.

Our results support the observations that hypothyroidism and hypoglycemia lead to abnormal somatic growth and confirm the effects of pituitary disconnection and hypophysectomy on GRF and SRIF gene expression. Furthermore, although of unknown significance, endocrine manipulations can also effect levels of IGF2 transcripts in the choroid plexus.

177.2

GROWTH HORMONE RECEPTOR MESSENGER RNA IS COLOCALIZED IN SOMATOSTATIN NEURONS IN THE MALE RAT HYPOTHALAMUS. K.A. Burton*, D.K. Clifton, R.A. Steiner. Departments of Physiology and Biophysics and Obstetrics and Gynecology, University of Washington, Seattle, WA 98195.

Growth hormone (GH), either directly or through insulin-like growth factor-1, has been shown to stimulate the release of somatostatin (SS) peptide from the hypothalamus and to stimulate hypothalamic SS messenger RNA (mRNA) levels. We have previously demonstrated that in the adult male rat, the mRNA for the GH receptor is located in areas of the hypothalamus known to be involved in regulating GH secretion, the periventricular (PeN) and arcuate nuclei. We reasoned that if GH negative feedback involves a direct action on SS neurons in the PeN, then SS neurons in the PeN should express the mRNA for the GH receptor. We tested this hypothesis by performing a double-labeling *in situ* hybridization on basal forebrain tissue sections from the male rat. SS mRNA was hybridized with a RNA probe labeled with digoxigenin-UTP, and the GH receptor mRNA was hybridized with a RNA probe labeled with ^{35}S-UTP. SS mRNA-positive cells were visualized by an alkaline phosphatase color reaction, and the GH receptor mRNA-positive cells appeared as silver grains overlying a cell nucleus. We observed that more than 50% of the SS mRNA-positive neurons in the PeN contained the mRNA for the GH receptor.

Conclusion: The GH receptor gene is co-expressed in SS mRNA-containing cells in the hypothalamus. We infer that GH feeds back directly on those SS neurons in the PeN that participate in orchestrating the pulsatile secretion of GH from the pituitary gland.

177.3

CORRELATION BETWEEN PROLACTIN (PRL) GENE EXPRESSION AND SECRETION AS ASSESSED BY THE COMBINED REVERSE HEMOLYTIC PLAQUE (RHP)/IN SITU HYBRIDIZATION ASSAY: EFFECT OF ESTRADIOL (E2) AND/OR BLOCKADE OF CALCIUM CHANNELS . G.H. Larson and P.M. Wise, Dept. Physiology, Univ. of Maryland Med. Sch., Baltimore, MD 21201.

It has been recently demonstrated that inhibition of calcium entry through voltage-gated channels modulates PRL secretion and mRNA levels in cultured pituitary and GH3 cells. Furthermore, estrogen treatment and/or blockade of these calcium channels enhances the amount or proportion of hormone that is secreted constitutively. The purpose of this study was to assess the effects of nifedipine, an L-type calcium channel blocker, and/or estrogen on PRL secretion and gene expression, and whether either of these treatments influences the coupling of gene expression and hormone secretion at the single cell level. Young rats were ovariectomized (OVX) and after 1 week half of the rats received E2 capsules (180 µg/ml). Three days later pituitary cells were enzymatically dispersed for use in the RHP assay. The cells were incubated with anti-PRL antibody ± nifedipine (10^{-6}M) for 3 hours. Treatment with complement resulted in the lysis of red blood cells in the areas surrounding lactotropes actively secreting hormone. In situ hybridization was performed by incubating slides overnight at 53C with an ^{35}S-labelled antisense riboprobe (2.1 X 10^{8} dpm/µg) transcribed from PRL cDNA. In OVX and E2 rats, 3 hours of exposure to nifedipine suppressed PRL secretion per cell, but did not appear to decrease the level of PRL mRNA per cell. E2 increased PRL secretion and mRNA levels per cell, and resulted in a correlation between the amount of secretory activity and the level of gene expression in individual cells, regardless of treatment with nifedipine. In conclusion, it appears that the ability of E2 to stimulate PRL secretion is coupled to its ability to enhance PRL gene expression within an individual cell. Under these experimental circumstances the blockade of L-type calcium channels does not appear to influence this coupling. (Supported by HD15955 and AG05493)

177.4

LOCALIZATION OF PROLACTIN-RECEPTOR MRNA IN ANTERIOR PITUITARY CELLS. Sufen Chiu* and Phyllis Wise. Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201.

Prolactin (PRL) regulates its own secretion and the secretion of other anterior pituitary hormones. Whether it acts directly at the level of the pituitary is controversial. We have previously reported that Prolactin-Receptor (PRL-R) mRNA is detectable in the anterior pituitary gland (Chiu S et al, 1991, Endocrine Society Abstr. 260). Therefore, we initiated studies to determine which pituitary cell types express PRL-R mRNA. The initial experiments used the newly developed combined reverse hemolytic plaque-*in situ* hybridization (Scarbrough K et al, Mol Endo 5:134-142, 1991) to determine specifically whether cells secreting LH or PRL contain PRL-R mRNA.

Anterior pituitary cells from female rats were dispersed with trypsin and combined with protein A-coupled red blood cells. They were incubated for two hours in the presence of LH antibody or for one hour in the presence of PRL antibody. Treatment with complement resulted in plaques surrounding cells actively secreting LH or PRL, respectively. The cells were then fixed in 4% paraformaldehyde and hybridized to an ^{35}S-labelled riboprobe complementary to PRL-R mRNA (specific activity of 2 x 10^{8} dpm/µg) overnight at 53 C. After a series of washes, the slides were dipped in emulsion and developed within 5 days.

We detected PRL-R mRNA in pituitary cells. However, the mRNA was not localized in cells actively secreting PRL or LH during the experimental period. We speculate that PRL-R mRNA is localized in either non-secreting lactotropes or non-secreting LH gonadotropes or other types of anterior pituitary cells.

177.5

EFFECTS OF HYPO- OR HYPERTHYROID CONDITIONS ON PREPRO-NPY mRNA AND PRECURSOR-VIP/PHI mRNA LEVELS IN THE SUPERIOR CERVICAL GANGLIA (SCG) AND LOCAL THYROID GANGLIA (TG) OF RATS. L. Huffman*, M. Michalkiewicz*, K.E. Vrana*, and G.A. Hedge. Depts. of Physiology and Biochemistry, West Virginia Univ. Hlth. Sci. Ctr., Morgantown, WV 26506.

Both NPY and VIP are present in thyroid nerves and have been shown to alter thyroid blood flow and hormone secretion. The present study was conducted to determine whether hypo- or hyperthyroidism, induced by propylthiouracil (PTU) or thyroxine (T_4), respectively, is associated with changes in prepro-NPY mRNA or precursor-VIP/PHI mRNA levels in the major ganglia supplying nerves to the thyroid gland. Rats (8/group) were treated for six days with vehicle (0.5 ml 0.01N NaOH·day, ip), PTU (1 mg/100gBW·day,ip), or T_4 (5 µg/100gBW·day,ip). SCG and TG were dissected and total RNA was extracted and size fractionated on a denaturing gel for Northern blot analysis. Prepro-NPY mRNA was assessed using a ^{32}P-labeled rat cDNA probe and precursor-VIP/PHI mRNA was determined using a ^{32}P-labeled rat riboprobe. Prepro-NPY mRNA levels detected in the SCG were greater than those in the TG, whereas an opposite pattern was seen for precursor-VIP/PHI mRNA levels. No significant changes in preproNPY mRNA or precursor-VIP/PHI mRNA concentrations were detected in the SCG (8/group) or the TG (4/group) in response to either treatment. We conclude that changes in the thyroid axis in response to these hypo- and hyperthyroid conditions do not include alterations in the steady-state prepro-NPY or precursor-VIP/PHI mRNA concentrations in the major ganglia supplying nerves to the thyroid gland (Supported by NSF DCB-8904470).

177.6

NMDA RAPIDLY INCREASES CELLULAR LEVELS OF LHRH mRNA IN PARALLEL WITH INCREASES IN LH RELEASE. S.L. Petersen, S. McCrone*, M. Keller* and E. Gardner*. Dept.of Anatomy and Neurobiology, Univ. Missouri Sch. Med., Columbia, MO 65212.

We have previously demonstrated that LHRH mRNA levels are low before the estrogen-dependent LH surge, then rise. Similarly, LHRH release into the pituitary portal system increases before the LH surge. Because these changes in LHRH mRNA levels are blocked by antiestrogen implants into the MPOA, we believe that they are cellular correlates of the positive feedback effects of estrogen on LHRH neuronal activity. This interpretation is supported by studies in other neuropeptide systems which show that changes in neuronal activity alter levels of biosynthesis and are reflected in changes in mRNA levels. However, most of these studies have used chronic perturbations of the neuropeptide systems in question. To determine whether changes in LHRH mRNA could be used as an index of acute changes in neuronal activity, we infused intact male rats with either NMDA (13 mg/kg iv), a substance which increases LH release by a hypothalamic action, or with saline. We then used *in situ* hybridization to measure changes in cellular levels of LHRH mRNA at 15 and 60 min after the infusions. LHRH mRNA levels in cells of the rostral preoptic region were significantly elevated by NMDA at both 15 and 60 min, as were serum LH concentrations. Thus, LHRH mRNA levels appear to rapidly reflect changes in LHRH release. Because higher doses of NMDA lesion the arcuate nucleus, changes in levels of mRNAs encoding tyrosine hydroxylase and POMC were also examined.

177.7

GONADOTROPIN-RELEASING HORMONE (GnRH) REGULATES RAT MEDIAN EMINENCE TYROSINE HYDROXYLASE (TH) ACTIVITY *IN VITRO*. D.D. Rasmussen and K.E. Greene. Dept. Reprod. Med., UCSD, La Jolla, CA 92093.

GnRH can stimulate dopamine (DA) turnover in the lateral palisade zone of the rat median eminence (Neurosci. Lett. 45:253), suggesting that GnRH autoregulation could be mediated by DA. However, GnRH can also inhibit rat brain DA synthesis (Nature 296:354), suggesting that previously demonstrated correlations between pulsatile LH and prolactin secretion could be in part mediated by GnRH-induced suppression of hypothalamic DA secretion. Accordingly, we investigated the effect of GnRH on *in vitro* TH activity in adult male rat tuberoinfundibular dopaminergic neurons, as reflected by 3,4 dihydroxyphenylalanine (DOPA) accumulation in the medial portion of the median eminence during in vitro incubation with the DOPA decarboxylase inhibitor 3-hydroxybenzylhydrazine (NSD-1015). DOPA content was determined by HPLC with electrochemical detection. Addition of 10^{-7} M GnRH to the medium inhibited ($p<0.002$, n=13-15/trtmnt) medial median eminence TH activity (73.2±6.3 *vs* 122.0±15.1 pg DOPA/µg protein) whereas 10^{-9} and 10^{-5} M GnRH had no significant effect (97.4±9.6 and 110.0±13.2 pg DOPA/µg protein, respectively). These results demonstrate that GnRH can suppress in vitro TH activity in the medial median eminence and that this response is dose-dependent, suggesting that GnRH may stimulate in vivo prolactin secretion in part by inhibiting tuberoinfundibular DA secretion. In contrast, preliminary results from experiments including lateral portions of the median eminence appear to be consistent with the previously demonstrated stimulation of DA turnover by GnRH, although more studies are required. Further studies will evaluate whether the regulation of median eminence TH activity by GnRH is mediated by specific GnRH receptors. Supported by NIH HD12303, Unit 4 (DDR) and NSF Graduate Fellowship (KEG).

177.8

THE DISTRIBUTION OF PROGESTIN RECEPTOR-IMMUNOREACTIVITY IS SIMILAR TO THAT OF ESTROGEN RECEPTOR-IMMUNOREACTIVITY IN THE MIDBRAIN OF FEMALE GUINEA PIGS. J.C. Turcotte and J.D. Blaustein. Neuroscience and Behavior Program and Psychology Department, University of Massachusetts, Amherst, MA 01003

Estradiol-induced progestin receptor-immunoreactive (PR-IR) neurons in female guinea pigs have been mapped in a number of forebrain areas, including the preoptic area and mediobasal hypothalamus. Using immunocytochemical techniques, we have found that estradiol-induced PR-IR cells are also located in the midbrain central gray (MCG). The distribution of PR-IR cells is similar to that of estrogen receptor-immunoreactive cells in that they are found in the same areas of the MCG, lateral and ventrolateral to the cerebral aqueduct. PR-IR cells, like ER-IR cells, appear to increase in number from rostral to caudal levels before tapering off in the region of the inferior colliculus recess. Midbrain PR-IR cells, however, are far fewer in number than ER-IR cells and seem to be restricted primarily to the MCG, while ER-IR cells are found in other midbrain regions, such as lateral to the MCG. Midbrain PR-IR cells are also more lightly stained than PR-IR cells in other sites, such as the hypothalamus, and have little or no apparent cytoplasmic immunoreactivity. The presence of these cells suggests a role for progestins as well as estrogens in midbrain functions, which may include effects on steroid hormone dependent behaviors such as sexual receptivity.

(Supported by NIH NS 19327 and MH 00885)

177.9

SOME OF THE VENTROLATERAL HYPOTHALAMIC NEURONS CONTAINING SUBSTANCE P AND PROGESTIN RECEPTORS PROJECT TO THE MIDBRAIN CENTRAL GRAY IN GUINEA PIGS. K. H. Nielsen and J. D. Blaustein. Neuroscience and Behavior Program and Psychology Department, University of Massachusetts, Amherst, MA 01003

In estradiol-primed, female guinea pigs, up to 50% of the progestin receptor-immunoreactive (PR-ir) neurons in the ventrolateral hypothalamus are also immunoreactive for substance P (SP-ir). In order to determine the efferent projections of these specific neurons, retrograde tracing was combined with double-label, fluorescent immunocytochemistry. Microinjections of Fluoro-Gold (FG) into the midbrain central gray and adjacent areas labeled neurons in and around the ventrolateral hypothalamus, an area involved in the hormonal control of female sexual behavior. A number of these FG-labeled neurons were SP-ir, and several were PR-ir. Some of the FG-labeled neurons were immunoreactive for *both* substance P and progestin receptors. In contrast, FG injection sites in the red nucleus of the midbrain, which included only a small portion of the central gray, labeled very few neurons in the ventrolateral hypothalamus. Therefore, some of the progesterone target neurons in the ventrolateral hypothalamus express substance P and also project to the midbrain central gray.

(Supported by NIH NS 19327 and MH 00885)

177.10

ABLATION OF THE HYPOTHALAMIC MEDIAN EMINENCE REDUCES THE CONCENTRATION OF VASOACTIVE INTESTINAL PEPTIDE IN THE ANTERIOR PITUITARY GLAND OF MALE RATS. A.J. Carrillo and D.E. Dluzen. Dept. of Anatomy, NEOUCOM, Rootstown, OH 44272.

Recent studies have clearly shown that vasoactive intestinal peptide (VIP) produced in the anterior pituitary gland is regulated by the thyroids, adrenals and perhaps the gonads in a manner similar to that of a pituitary gland-target organ feedback loop. Because the hypothalamus is essential for the functioning of such a feedback loop, this study was designed to determine the influence of this neural center on the anterior pituitary concentration of VIP. To this end, the hypothalamic median eminence (ME) of anesthetized adult male rats was ablated by lowering a triangular shaped knife (2.0 X 1.0 mm) to the base of the hypothalamus and rotating several times. Intact and sham ablated rats served as controls. Fifteen days later all the rats were decapitated (AM) and the anterior pituitary dissected out and frozen for VIP determination using a recently established RIA (Carrillo et al, Endocrinology. 128:131, 1991). In order to evaluate the completeness of the ME ablation, water consumption per 24 hrs. was monitored after surgery and the adrenals and testis were weighed at the time of decapitation. Ablation of the ME resulted in a 211% increase in water consumption and in a reduction of tissue mass of the anterior pituitaries (mg of protein), adrenals and testis (weight) of 59%, 54% and 40% respectively, when compared to controls. Pituitary content of VIP was also significantly ($p < 0.01$) reduced in the ME lesion group (80 ± 13 pg/mg of protein) when compared to sham (518 ± 65) and intact controls (771 ± 162). These data suggest that the hypothalamus is involved in the regulation of pituitary VIP. Supported by Ohio Board of Reagents Research Challenge Program 1989-91.

177.11

VIP/PHI NEURONS IN THE RAT MEDIAN EMINENCE: IMMUNOHISTOCHEMICAL AND IN SITU HYBRIDIZATION STUDIES. S.Ceccatelli, M.J. Villar, T. Hökfelt. Dept. of Histology and Neurobiology, Karolinska Institute, Stockholm, Sweden.

Using immunohistochemistry and in situ hybridization we have analyzed the distribution of vasoactive intestinal polypeptide (VIP) and peptide histidine isoleucine (PHI) in hypothalamus of male and female Sprague-Dawley rats under normal and experimental conditions. Precapillary arterioles along the lateral aspect of the median eminence were surrounded by dense networks of VIP/PHI positive fibers, suggesting that these peptides, via their vasodilatory property, may be involved in control of blood flow through portal vessels. Furthermore, a thick VIP/PHI containing nerve bundle of variable size was observed on the surface of the median eminence in coronal, horizontal and sagittal sections. Also this bundle could be of importance for portal circulation, but VIP/PHI released may reach the anterior pituitary level and play a role in, for example, control of prolactin release. Although different lesions were performed, the origin of the VIP/PHI nerves around lateral blood arterioles and the bundle is still unclear, but is in all probability peripheral. After 48 h hypophysectomy a very large number of PHI immunoreactive fibers could be observed in the median eminence, and with in situ hybridization VIPmRNA could be demonstrated in magno- and parvocellular neurons of the paraventricular nucleus, whereas in control rats it was undetectable. These results demonstrate that VIP/PHI are present in systems of direct neuroendocrine importance i. a. in nerves related to the blood vessels in the median eminence and presumably involved in control of blood flow through the portal system. To what extent the VIP/PHI nerve bundle on the surface of the median eminence might be involved in regulation of pituitary function remains to be established.

177.12

GALANIN NEURONS INNERVATE ANTERIOR PERIVENTRICULAR SOMATOSTATIN CELLS AND MODULATE SOMATOSTATIN SECRETION FROM ARCUATE NUCLEUS-MEDIAN EMINENCE FRAGMENTS. Zs. Liposits, M.M. Valenca*, F.J. López*, I. Merchenthaler and A. Negro-Vilar*. LMIN, NIEHS, NIH, Research Triangle Park, NC 27709.

It has been reported that galanin (GAL) alters growth hormone (GH) secretion via central hypothalamic mechanisms (Ottlecz et al., PNAS 85:9861-9865, 1985; Maiter et al., Endocrinology 126:1212-1222, 1990). Due to the dual inhibitory and stimulatory influence of the diencephalon upon GH secretion, the effect of GAL might be manifested through both growth hormone-releasing hormone (GHRH) and growth hormone-release inhibiting hormone or somatostatin systems. In order to address the modulation of the hypothalamic somatostatin system by GAL, morphological and pharmacological studies were undertaken in male rats. By means of light and electron microscopic immunocytochemical double labeling techniques, we have found that somatostatin-producing neurons in the anterior periventricular area are heavily contacted by GAL-immunoreactive axons. At the ultrastructural level, axo-somatic and axo-dendritic synaptic connections were detected among GAL- and somatostatin-immunoreactive profiles. In vitro incubation of arcuate nucleus-median eminence fragments, containing primarily nerve terminals, in the presence of 0.05-1 μM rat GAL resulted in a significant increase in somatostatin release. These data indicate that the central galanin neuronal system may influence the production and release of somatostatin acting upon both the cell bodies and terminals; thus, the GAL-somatostatin interaction should also be considered as an important step in the mechanisms regulating tonic and pulsatile GH secretion.

177.13

GROWTH HORMONE RELEASING HORMONE (GHRH) IMMUNOREACTIVE NEURONS IN THE HYPOTHALAMUS OF PIGS AND CATTLE. L.S. Leshin[1], C.R. Barb[1], D.Slavin[1]*, E. Taras[2]*, G. B. Rampacek[2]*, T. E. Kiser[2]* and R. R. Kraeling[1]*. USDA-ARS[1], Athens, GA 30613 and Animal and Dairy Science Dept., University of Georgia[2], Athens, 30602.

Hypothalamic GHRH neurons were localized in female immature gilts (n=6) and heifers (n=2) to identify anatomical sites involved in regulation of growth hormone secretion. Coronal and sagittal frozen sections (48-60 μm) of Zamboni's fixed hypothalamic tissue were incubated with GHRH primary antisera (B. D. Schanbacher, USDA-ARS, Clay Center, NB and F. C. Buonomo, Monsanto Co., Chesterfield, MO) for 48 h then visualized by peroxidase-diaminobenzidine or fluorescent immunocytochemistry. Treatment with nonimmune serum lacked specific staining. Incubation of GHRH antisera with GHRH peptides p1-44, b1-44 or h1-29 (2-10 μg/ml, 24 h) prior to immunocytochemistry greatly reduced or blocked specific staining. Rounded or fusiform, bipolar immunoreactive GHRH perikarya were only in ventrolateral portions of the arcuate nucleus with fibers projecting ventrally into the median eminence. In both species, there was very dense innervation of the external lamina of the median eminence. Immunoreactive GHRH was not observed in other hypothalamic areas, suggesting that these GHRH neurons may have only hypophysiotropic functions in gilts and heifers.

177.14

IN SITU HYBRIDIZATION FOR CREATINE KINASE-B MESSENGER RNA IN RAT UTERUS AND BRAIN. H.T. Bergen[1], B.T. Pentecost*[2], H.W. Dickerman*[2], and D.W. Pfaff[1]. [1]Lab. of Neurobiology and Behavior, Rockefeller University, New York, NY 10021, and [2]Wadsworth Center for Laboratories and Research, Albany, NY 12201.

Creatine kinase-B (CKB) is present in both uterus and brain, and in uterus its synthesis (protein and mRNA) is induced by estrogen (E). Malnick et al. (Endocrinology 113: 1907, 1983) proposed that E induction of CKB also occurs in brain. Preliminary immunocytochemical data in our lab showed E induction of CKB in uterus, but not in brain (Schwanzel-Fukuda et al., unpub.). Thus in the present study we used in situ hybridization (ISH), to detect CKB mRNA in uterus and brain, and to examine whether there is site-specific induction of CKB mRNA by E in these tissues. Tissue was taken from ovariectomized (ovx) control rats, and ovx rats that had been injected with E, 2, 8, 24, and 72 hr previously. In the final group (72 hr), the rats also received E at 48 hr. The brains and uteri were removed, frozen, and cryostat-sectioned and processed for ISH by previously published methods employing a ^{3}H-CKB DNA probe complementary to a 3′ fragment of CKB mRNA, and a control sense probe identical to the same 3′ fragment. Quantification of the results consisted of either counting the autoradiographic grains/unit area (uterus) or the number of grains/cell (brain). In uterine smooth muscle, a 2.5 and 3.5 fold induction of CKB mRNA was observed 2 and 24 hr after E, respectively, and levels approached ovx controls at 72 hr. A smaller induction (1.9 fold, 2 hr) was observed in uterine luminal epithelium, with little induction of CKB mRNA in the stroma. In the brain, CKB mRNA was detected in neurons, but not in clearly identified glia, and only occasionally in ependymal cells. Some brain regions had higher neuronal expression than others, but expression was widespread and not limited to neuroendocrine sites. We have not seen evidence of a significant E effect on CKB mRNA levels in brain.

177.15

EFFECTS OF ESTROGEN ON FOS-LIKE IMMUNOREACTIVITY (F-IR) AND ^{3}H-THYMIDINE (^{3}H-THY) INCORPORATION WITHIN UTERINE TISSUES TRANSPLANTED TO THE ADULT RAT CNS. H.J. OKANO, R.B. Gibbs, & D.W. Pfaff. Lab. of Neurobiol. & Behav., Rockefeller University, N.Y., NY 10021

Estradiol (E) stimulates Fos induction and cell division in uterus, but not within E-concentrating neurons in brain (Gibbs et al., Mol. & Cell. Neurosci. 1: 29-40, 1990; Gibbs et al., Mol. & Cell. Neurosci. 1: 250-261, 1990). If uterus were transplanted into brain, would either tissue confer its hormone reactivity upon the other?

Thirty-two adult, ovariectomized, Sprague-Dawley rats were used. Uteri obtained from donor animals were cut into small pieces (~3mm^3) and stereotaxically implanted into the third ventricle adjacent to the arcuate, ventromedial, and anterior hypothalamic nuclei. Two to 5 weeks later, animals received a subcutaneous injection of 10 µg estradiol (E) or vehicle (V). Five, 12, and 24h later, animals were sacrificed and the brains and uteri removed for ICC detection of F-IR using a polyclonal anti-Fos antibody generously provided by Dr. M. Iadarola (NIH). In addition, 5 animals received an IP injection of ^{3}H-Thy (5 µCi/g.b.w.) 22h after receiving either E (n=3) or V (n=2). These animals were sacrificed 2h later. The brains and uteri were removed, processed for ICC, and then for RA detection of ^{3}H-Thy incorporation.

Twenty-nine grafts survived, all of which showed signs of a first-set rejection response including vascular cuffing and infiltration by small mononuclear cells. F-IR epithelial cells (ECs) were detected in all E-treated uteri as well as within all E-treated grafts in which ECs could be identified (12/12). F-IR ECs were not detected within non-E-treated uteri, but were detected within 2 of 7 non-E-treated grafts. ^{3}H-Thy-labeled ECs and stromal cells were detected within 3 of 3 E-treated uteri. Many ^{3}H-Thy-labeled ECs and stromal cells were also detected within E-treated grafts; fewer labeled cells were detected within non-E-treated grafts. The uterine grafts did not confer E-stimulated F-IR or DNA synthesis to surrounding areas of the brain. These data demonstrate that E can stimulate F-IR and DNA synthesis in uterine tissues grafted to an environment where comparable effects on E-concentrating neurons are not observed.

PAIN PATHWAYS: HYPERALGESIA

178.1

MOLECULAR SPINAL RESPONSES TO PERIPHERAL NERVE INJURY. J.L. Culberson, R. Jenkins*, S. Williams* and S.P. Hunt, M.R.C. Molecular Neurobiology Unit, Hills Road, Cambridge CB2 2QH, U.K.

The immediate-early genes c-*fos* and c-*jun* are rapidly and transiently activated in dorsal horn neurons by noxious peripheral stimulation. We have examined the effects of peripheral nerve injury in adult SD rats. The sciatic nerve was exposed surgically (control), and either cut or crushed. In fixed frozen sections of lumbar spinal cord from rats killed two hours after nerve damage, Fos protein-like immunoreactivity appeared (with somatotopic distribution) in many neuronal cell nuclei within the superficial dorsal horn; reactive cells were not seen in motor or dorsal root ganglion neurons. Complimentary localization of central projections was observed in parallel experiments with saphenous nerve lesions. When the survival time was increased from 2 hours to 24 hours, Fos positive neurons were dispersed bilaterally throughout laminae I-VIII and X, but an increase in Jun protein-like immunoreactivity within dorsal root ganglion cells and motor neurons ipsilateral to the injury was seen for the first time. This response was sustained for 7 days, the longest timepoint examined, and was therefore neither rapid nor transient. C-fos induction in second-order sensory neurons may result from injury potentials in the peripheral nerve. C-jun may regulate the long-term response to damage within the injured afferent and efferent neurons themselves.

178.2

EFFECTS OF NEONATAL SCIATIC NERVE LESION ON THE CONTRALATERAL VENTRAL ROOT AFFERENT FIBERS. K. Chung, K. Sheen and J.M. Chung. Sch. Allied Health Sci., Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX, 77550.

In previous studies, we showed that a lesion of the sciatic nerve during the neonatal stage triggers sprouting of afferent fibers in the ipsilateral ventral root. In this study, we examined the possibility that the same type of lesion triggers sprouting of afferent fibers in the contralateral ventral root.

A total of 18 adult (3 month old) Sprague-Dawley rats was used; 5 neonatal sciatic neurectomized (at the age of 3 days) and 13 control rats (10 unoperated normal and 3 sham operated rats). The animals were perfused and the L5 ventral root on the side contralateral to the sciatic nerve lesion was processed for electron microscopic study. The numbers of both myelinated and unmyelinated fibers were counted in electron photomicrographs.

The average numbers of myelinated fibers in the L5 ventral root contralateral to the sciatic nerve lesion were 1697 and 1700 in control and neurectomized rats, respectively. However, the average numbers of unmyelinated fibers in the same root were 19 and 52 in corresponding rats.

These results suggest that a unilateral peripheral nerve lesion during the neonatal period triggers sprouting of unmyelinated fibers in the contralateral ventral root. (Supported by NIH grants NS21266 and NS11255 and a grant from Bristol-Myers Squibb Co.)

178.3

CHANGES IN PCP BINDING SITES IN RAT SPINAL CORD IN AN EXPERIMNENTAL MODEL OF ACUTE, PERIPHERAL INFLAMMATION. S. I. Sloan[1], C.L. Stucky[2], E.M. Jansen[2], M.T. Galeazza[2], V. S. Seybold[2] and L. Aanonsen[1] [1]Biology Dept., Macalester College, St. Paul, MN 55105 and [2]Dept. of Cell Biology & Neuroanat., University of Minnesota, Mpls, MN 55455.

Previous studies reveal that there is modulation of CGRP and SP binding sites in the dorsal horn of the rat spinal cord in a model of peripheral inflammation (Galeazza *et al.*, 1990). The purpose of the present study was to examine changes in PCP receptor binding sites in laminae I/II in this same model. Inflammation was induced by injection of Freund's complete adjuvant (FCA) into the plantar surface of the left hindpaw (75µl, emulsified 1:1 with PBS; s.c.). The thickness of the inflamed paw and the amount of extravasated Evans Blue were significantly increased compared to controls (uninjected rats) at 2 and 4 days after injection (Galeazza *et al.*, 1990). The animal's response to a noxious thermal stimulus was tested 2, 4 and 8 days after FCA injection. A hyperalgesic response was evident ipsilateral to inflammation on days 2 and 4, and contralateral to inflammation on day 4, when compared to controls. Autoradiographic studies were performed on tissue sections of spinal segment L4. These sections were obtained from control rats and from rats 2, 4 and 8 days after FCA injection. The spinal cord sections were incubated in ^{3}H-TCP (5 nM) which was used to label the PCP binding site (Aanonsen and Seybold, 1989). Nonspecific binding was determined by incubation of adjacent sections in ^{3}H-TCP plus 100 µM PCP. Autoradiographic grain densities in the emulsion overlying laminae I/II were quantified using computerized image processing. Specific binding was determined by subtracting nonspecific binding from total binding in the analyzed region. The analysis revealed a significant *decrease* in ^{3}H-TCP binding in laminae I/II ipsilateral to inflammation only on day 2.

The PCP binding site is believed to be associated with the NMDA receptor, an excitatory amino acid receptor subtype. Thus, the apparent modulation of PCP binding sites in this study may reflect changes in release of glutamate from afferents 2 days following inflammation. Supported by USPHS grant NS17702.

178.4

SPONTANEOUS AND EVOKED EXPRESSION OF C-*FOS* IN RAT LUMBAR SPINAL CORD DURING THE DEVELOPMENT OF ADJUVANT-INDUCED ARTHRITIS. C. Abbadie*, F. Morain* and J.-M. Besson. INSERM U 161 and EPHE, 2 rue d'Alésia, 75014 Paris, France.

This study evaluated Fos-like immunoreactivity (FLI) in spinal cord neurons during the development of adjuvant-induced arthritis (AIA), at 1, 2, 3 and 11 weeks (W) postinoculation with Freund's adjuvant. Lumbar spinal cord sections (L2 to L6) from injected and control rats were immunostained using Fos antibody. Quantification of the total number of FLI neurons showed that AIA disease correlated with FLI. FLI was absent in control rats and at 1W, moderate at 2W, maximal at 3W and slightly decreased at 11W. At 3W, which is the acute stage of hyperalgesia, FLI distribution was 6% in superficial laminae (I and II), 6% in the nucleus proprius (laminae III and IV), 55% in the neck (laminae V and VI) and 33% in the ventral horn. Maximal staining was found in L4.

At 3W, FLI was then used to test the responsiveness of spinal neurons to moderate pressure at the ankle joint, under ketamine anesthesia. The number of FLI neurons was significantly higher in arthritic than in healthy rats, particularly in the superficial laminae and in the neck of the ipsilateral dorsal horn with maximal staining in L3 and L4. Similar results were obtained with pressure applied to the dorsal paw. The appearance of spontaneous FLI neurons during AIA disease and their increased number relative to healthy rats following stimulation suggesting these neurons are abnormally active and involved in the hyperalgesia of AIA.

178.5

INFLUENCES OF LOCAL ANESTHETIC UPON EXPERIMENTAL MODELS OF PERIPHERAL NEUROPATHY, C.J. Garrison, P.M. Dougherty, W.D. Willis, S.M. Carlton, Dept. of Anat. & Neurosci., Marine Biomedical Institute, Univ. Texas Medical Branch, Galveston, TX, 77550-2772

Neuropathic pain remains a major complication following various forms of peripheral nerve injury in humans. Two models of peripheral neuropathy in the rat have recently been described which result in hyperalgesia to thermal stimulation. One model is induced by the placement of 4 loose ligatures around the entire sciatic nerve (Bennett & Xie, 1988); the second model is produced by the placement of a tight ligature around one-third to one-half of the sciatic nerve (Seltzer et al., 1990). It was the purpose of this study to compare the effect of the injuries on time course and magnitude of foot withdrawal latency to radiant heat. In addition, to evaluate the hypothesis that hyperalgesia develops as a result of injury discharge, some injuries were induced following local application of lidocaine to the sciatic nerve. Our results from a total of 120 male Sprague-Dawley rats showed that the hyperalgesia in partial-constriction neuropathy (PCN) develops faster than that produced by the partial-transection neuropathy (PTN). In addition, lidocaine reduced the duration and magnitude of the hyperalgesia in the PCN (N=52 vs N=43 animals without lidocaine). The PTN failed to show this sensitivity in 14 animals compared to 11 controls. Based on our results, PCN develops and is maintained, at least in part, by lidocaine- sensitive mechanisms. Lidocaine effected the duration and magnitude, but not the onset, of hyperalgesia. These data suggest that injury-related discharge is one factor which contributes to the generation of hyperalgesia in the PCN model. The factors underlying the PTN model result in a slower onset of hyperalgesia and involve mechanisms which may or may not be shared with the PCN model. (Supported by NS11255, NS27910 and Bristol-Myers Squibb).

178.6

HISTOCHEMICAL CHANGES IN GRACILE NUCLEUS FOLLOWING EXPERIMENTAL PERIPHERAL NEUROPATHY IN THE SCIATIC NERVE OF RATS. K.D. Cliffer, A.A. Cameron, P.M. Dougherty, W.D. Willis and S.M. Carlton. Marine Biomedical Institute and Dept. of Anatomy and Neurosciences, Univ. of Texas Medical Branch, Galveston, TX 77550.

Peripheral nerve damage induces changes in the spinal cord hypothesized to be involved in associated sensory disorders. However, effects in another target for primary afferent fibers, the dorsal column nuclei (DCn), have not been extensively characterized. We stained DCn of rats that were subjected to chronic constriction of the sciatic nerve (Bennett and Xie, 1988) unilaterally for 14 or 28 days. Gracile nuclei from each of the 14-day rats (N=8) and the 28-day rats (N=8) exhibited striking increases in immunohistochemical staining for calcitonin gene-related peptide (CGRP) ipsilateral to the injury. Profiles with increased staining included large fibers or fiber bundles. This contrasted to no obvious change in staining for substance P or galanin in the gracile nucleus, and to a slight decrease in staining for CGRP in spinal cord. Increases in staining for growth-associated protein GAP-43 and with soybean agglutinin, a marker for small primary afferent fibers, also occurred in the ipsilateral gracile nucleus. These changes may be involved in modification of sensory processing in the DCn and contribute to sensory disorders associated with peripheral nerve damage.

Supported by NIH (NS07185, NS08860, NS09743, NS11255, NS27910) and the Bristol-Myers Squibb Co.

178.7

HISTOCHEMICAL CHANGES IN THE DORSAL HORN OF THE RAT FOLLOWING EXPERIMENTAL PERIPHERAL NEUROPATHY. A.A. Cameron, K.D. Cliffer, P.M. Dougherty, W.D. Willis and S.M. Carlton. Dept. of Anatomy and Neurosciences and Marine Biomedical Institute, Galveston, TX 77550.

An experimental rat model of peripheral neuropathy which produces hyperalgesia has recently been described (Bennett and Xie, 1988). We have investigated some of the neurochemical changes occurring in the dorsal horn as a result of the peripheral neuropathy. Four loose ligatures were tied around the sciatic nerve in anaesthetized rats. All animals demonstrated behavioral hyperalgesia. After 28 days the animals were perfused and 25 μm sections of L4 and L5 spinal cord were stained with soybean agglutinin and immunostained for the lectin RL-29, the growth associated protein GAP-43 and the neuropeptides substance P and calcitonin gene-related peptide (CGRP). In the superficial dorsal horn the densities of reactivity to soybean agglutinin, RL-29 and GAP-43 were all increased, while those to substance P and CGRP were slightly decreased. We hypothesize that damage to peripheral axons and the consequent central anatomical changes, possibly including regenerative changes, contribute to the hyperalgesia. (Supported by NS11255, NS09743, NS27910, NS08860 and Bristol Myers-Squibb.

178.8

AN ELECTRON MICROSCOPIC ANALYSIS OF SOYBEAN AGGLUTININ-LABELED PROFILES IN THE RAT DORSAL HORN FOLLOWING EXPERIMENTAL PERIPHERAL NEUROPATHY. E.S. Hayes, P.M. Dougherty, and S.M. Carlton. Dept. of Anatomy & Neurosci., Marine Biomed. Institute, Univ. of Texas Med. Branch, Galveston, TX 77550.

The lectin soybean agglutinin (SBA) labels small diameter dorsal root ganglion cells and primary afferent fibers and terminals in the rat dorsal horn. It has been demonstrated that SBA labeling increases in the dorsal horn in rats with experimental peripheral neuropathy (Cameron et al., Soc. Neurosci. '91). It was the aim of this study to determine, at the ultrastructural level, the profile types which label with SBA in both the normal and the neuropathic dorsal horn.

Following the protocol of Bennett and Xie (1988), 4 loose ligatures were tied around one sciatic nerve (n=3) and a sham-operation was performed on the contralateral side. Fourteen days later, the animals were anesthetized and perfused with an EM fixative. Tissue sections (25μm) from L4 were labeled for SBA and prepared for EM analysis.

Light level analysis revealed an increase in SBA staining on the operated compared to the sham-operated side. On the sham-operated side, EM analysis demonstrated SBA label concentrated in unmyelinated axons and glomerular terminals with dense core vesicles in LI and II. Although colchicine in one animal failed to show any labeled cells at the light level, EM analysis demonstrated SBA-labeled neuronal cell bodies, dendrites and dendrites containing vesicles. In the dorsal horn on the operated side, similar types of SBA-labeled profiles were observed, however, SBA staining was often seen in extracellular spaces, particularly surrounding neuronal somata. It is this extracellular label observed at the EM level which may be responsible for the increase in the density of SBA staining seen at the light level. (Supported by NS11255, NS27910 and Bristol-Myers Squibb).

178.9

NEUROMA FORMATION AND NUMBERS OF AXONS IN AN EXPERIMENTAL PERIPHERAL NEUROPATHY S.M.Carlton, P.M.Dougherty, C.M.Pover and R.E.Coggeshall. Dept. of Anat. and Neurosci., Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

Chronic partial constriction of the sciatic nerve in the rat produces a neuropathy characterized by hyperalgesia to thermal stimuli (Bennett and Xie, 1988). Some important questions to be investigated in this model are the degree of fiber damage, neuroma formation and whether correlations exist between fiber loss and behavioral changes. Two weeks following chronic sciatic constriction in 5 rats, ultrastructural analysis was performed on the injury site and on the nerve proximal and distal to the injury. At the constriction site, the perineurium was disrupted and a neuroma was present in all cases. Axon counts demonstrated 84-99% and 62-84% decreases in myelinated and unmyelinated axons, respectively, in the distal compared to the proximal segment. The majority of the surviving myelinated axons were Aδ fibers. There was considerable disparity in fiber loss from animal to animal, but similar behavioral changes were demonstrated by all animals. We hypothesize that input from the surviving axons will result in increased activity in the neuroma, which in turn will be interpreted as noxious input by the animal. Furthermore, although all fiber populations are decreased, input relayed by unmyelinated axons appears to be crucial in the generation of this model. (Supported by NS11255, NS27910 and Bristol-Myers Squibb).

178.10

ULTRASTRUCTURAL CHANGES IN THE VENTRAL HORN OF THE RAT SPINAL CORD IN EXPERIMENTAL PERIPHERAL NEUROPATHY. H.A. Lekan, P.M. Dougherty, S.M. Carlton, Dept. of Anat. & Neurosci., Marine Biomed. Institute, University of Texas Medical Branch, Galveston, TX 77550.

Animals exhibit hyperalgesia in a rat model of peripheral neuropathy in which the sciatic nerve is unilaterally constricted by ligatures (Bennett and Xie, 1988). The degree of hyperalgesia is determined from paw withdrawal latencies (PWL) from a radiant heat source. All animals exhibit hyperalgesia at post-operative day 14 (14D) with a trend towards recovery by post-operative day 28 (28D). Following testing of PWL, 14D and 28D animals were anesthetized, perfused with an EM fixative and the spinal cords were removed. Tissue sections (40-50μm) from L4-L5 were processed for EM. Light level analysis revealed numerous dark profiles (8-15μm) in the ventral horn on the experimental side in the areas of the motor neuron pool of the sciatic nerve in both 14D and 28D animals. Analysis at the EM level indicated that the dark profiles resembled glial elements. At 14D, >80% of the large neuronal cell bodies (>40μm) were observed to be partially covered by glial processes and had undergone "synaptic stripping". These cells appeared otherwise normal morphologically. No chromatolytic neurons nor degenerating terminals were apparent. At 28D, many of the large cell bodies in this same region were chromatolytic, however glial processes were not observed surrounding the somas. Synaptic contacts were present on these neurons. The ventral horn changes described here parallel those seen following axotomy; however, the changes in the model of peripheral neuropathy are delayed. These morphological changes may lead to alterations in motor function which must be considered when analyzing the results of behavioral testing paradigms.(Supported by NS 11255 and NS 27910 and Bristol-Myers Squibb).

178.11

RESPONSES OF SPINOTHALAMIC TRACT (STT) NEURONS TO MECHANICAL AND THERMAL STIMULATION OF SKIN IN RATS. V. Paleckova, J. Palecek, P.M. Dougherty, S.M. Carlton and W.D. Willis Dept. of Anat. & Neurosci. & Mar. Biomed. Inst., UTMB, Galveston, TX - 77550

The STT neurons are thought to play an important role in transmission of nociceptive information from the periphery to the CNS. The aim of this study was to determine responses of STT cells, antidromically activated from VPL thalamus, to standard mechanical (Brush - brushing the skin with a cotton swab, Press -pressing the skin by a arterial clip with a modest grip, Pinch - noxious pinch with a clip with a strong grip) and thermal stimuli (series of 5s heat pulses, 35s apart grading from 37 - 49°C and 51°C produced by a Peltier device). Activity of STT cells was recorded with carbon filament microelectrodes (3 - 6 MΩ) from 20 rats (Sprague -Dawley) under Urethane anesthesia. Recorded cells were 200 - 1000 μm deep from the spinal cord surface with antidromic latencies from the thalamus ranging from 3.2 - 19 ms. Some of the deeper cells were mostly activated from subcutaneous tissues. 90% of the cells with cutaneous receptive fields had low background activity (< 1Hz). Most of the cells responded with graded activity to standard mechanical stimuli, with about 35% showing afterdischarges. Threshold temperature for evoked activity ranged from 37 - 51°C. Discharge frequencies at 49°C ranged from 4 to 45 Hz with about 60% of the cells showing afterdischarges. Our results indicate that rat STT cells have similar properties as those found in primates. (This work was supported by NIH grants NS 11255, NS 09743, NS 27910 and a Bristol - Myers Squibb grant).

178.12

RESPONSES OF SPINOTHALAMIC TRACT CELLS TO MECHANICAL AND THERMAL STIMULATION OF SKIN IN RATS WITH EXPERIMENTAL PERIPHERAL NEUROPATHY. J.Palecek, V.Paleckova, P.M. Dougherty, W.D. Willis and S.M. Carlton Dept. Anat. & Neurosci. & Mar. Biomed. Inst., UTMB, Galveston,TX - 77550

Placing loose ligatures around the sciatic nerve in rats produces hyperalgesia lasting several weeks (Bennett and Xie 1988). Since the spinothalamic tract (STT) is believed to be an essential pathway for the transmission of nociceptive information, our experiments concentrated on the responses of STT cells in rats in which the sciatic nerve was tied 7, 14 or 28 days previously. All rats used in the experiment showed faster withdrawal of hindpaws with sciatic lesion when exposed to radiant heat. Responses of STT cells from 25 neuropathic rats (Sprague-Dawley) were recorded in response to standard mechanical (BRUSH - brushing the skin with a cotton swab, PRESS - a large arterial clip applied to the skin, subthreshold for pain in humans, PINCH - pinching the skin with a clip with strong grip - perceived as painful in humans) and thermal stimuli (5 s heat pulses 35 s apart ranging from 37 - 49°C and 51°C produced by Peltier device). The most profound changes in STT cells responses were found 14 days after inducing the neuropathy. 50% of the STT cells in these animals showed high background frequencies (5 - 62 Hz) and 87% showed afterdischarges following mechanical stimuli. Even though we did not find a significant shift in the threshold to thermal stimulation, those cells responding to lower temperatures of stimuli usually had high background frequencies and afterdischarges. Furthermore, the afterdischarge rate often surpassed those during the stimuli. These data suggest that changes in STT signalling participates in the generation of neuropathic pain. (This work was supported by NIH grants NS 11255, NS 27910, NS 09743 and a Bristol-Myers Squibb grant).

178.13

INFLAMMATION-INDUCED INCREASE IN PREPROTACHYKININ mRNA IN A SUBPOPULATION OF SPINOMESENCEPHALIC PROJECTION NEURONS K. Noguchi and M.A.Ruda. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

A cascade of molecular, chemical and physiological events have been shown to occur in the spinal cord in a rat model of peripheral inflammation and hyperalgesia. This study investigated the inflammation-induced response of neurons containing preprotachykinin (PPT) mRNA, which codes for substance P and neurokinin A, in dorsal horn projection neurons and local circuit neurons. To identify projection neurons, Fluoro-gold was injected bilaterally into the midbrain parabrachial area. Rats received an injection of complete Freund's adjuvant (CFA) into one hindpaw 10 d later. At 4 d after CFA injection, the animals were killed and the L4,5 spinal cord segments were processed for RNA blot analysis or in situ hybridization using a ^{32}P or ^{35}S-labeled PPT oligonucleotide probe, respectively. RNA blot analysis demonstrated that there was a 60% increase in PPT mRNA ipsilateral to the inflammation. Counts were made of cells labeled with either PPT mRNA, or Fluoro-gold, or both, in laminae I, II and V/VI. The number of neurons exhibiting PPT mRNA was differentially increased in each lamina ipsilateral to inflammation: 80% in lamina I; 30% in lamina II; and 250% in laminae V/VI, although the number of laminae V/VI neurons was less than 10% of the total PPT mRNA neurons. The percentage of lamina I PPT mRNA cells also labeled for Fluoro-gold was 29% ipsilateral and 17% contralateral. The percentage of Fluoro-gold labeled cells also labeled for PPT mRNA in lamina I was 61% ipsilateral and 22% contralateral. These data demonstrate that the induction of PPT mRNA in response to peripheral inflammation and hyperalgesia occurs in both local circuit neurons and spinomesencephalic projection neurons. The identification of spinomesencephalic neurons with inflammation-induced increases in PPT mRNA suggests the participation of tachykinin peptides in nociceptive CNS projection pathways.

178.14

EFFECTS OF LOOSE LIGATION OF THE INFRAORBITAL NERVE ON C-FOS EXPRESSION IN THE MEDULLARY DORSAL HORN OF THE RAT. B.P. Vos, A.M. Strassman and R.J. Maciewicz, Pain Physiology Laboratory, Department of Neurology, Massachusetts General Hospital and the Neuroscience Program, Harvard Medical School, Boston, MA 02114.

Loose constrictive ligation of the rat's infraorbital nerve (IoN) produces behavioral changes which suggest the presence of trigeminal neuropathic pain. In order to identify alterations in patterns of central neuronal activity that may be associated with these behavioral changes, the distribution of fos-like immunoreactivity was determined in the medullary dorsal horn following IoN ligation. Rats received either a unilateral IoN ligation (n=12) or a unilateral sham operation (n=11). The development of changes in facial sensory function was assessed using behavioral testing on post-operative days 3, 6 and 8. Behavioral signs of altered facial somatosensation developed in IoN-ligated rats but not in sham-operated animals. On post-operative day 9, rats were anesthetized with urethane and innocuous or noxious mechanical stimuli (von Frey monofilaments, 2g or 15g; 1.2Hz; 30sec stimulation per min, for 30 min) were applied to the hairy skin of the vibrissal pad (between vibrissae B4-5, C4-5) ipsilateral to the operated side. In order to evaluate c-fos expression related to the IoN ligation alone, 3 rats of each group did not receive stimulation. Animals were perfused 2 hours after stimulation and the caudal medulla was processed for immunocytochemical detection of fos. In the absence of mechanical stimulation, few labelled neurons were found in either the IoN-ligated or the sham-operated animals. In both groups noxious stimulation produced 50% more fos-positive neurons than innocuous stimulation. In IoN-ligated rats, mechanical stimulation produced significantly more fos-positive neurons (for 2g, 180% increase; for 15g, 65% increase), and the labelling was distributed over a larger rostrocaudal distance when compared to sham-operated rats. The increase in labelling in IoN-ligated rats was confined to the superficial dorsal horn, whereas labelling in the deep dorsal horn was similar in IoN-ligated rats and sham-operated rats. These findings indicate that loose constrictive ligation of the IoN that causes behavioral changes consistent with trigeminal neuropathic pain, is accompanied by changes in the pattern of stimulus-induced expression of c-fos in the medullary dorsal horn.

178.15

INFLAMMATION OF THE SKIN AFFECTS FUNCTIONAL NEURONAL CONNECTIVITY IN THE RAT SPINAL DORSAL HORN. A. Eblen-Zajjur and J. Sandkühler. II. Physiologisches Institut, Univ. Heidelberg, Germany.

The functional neuronal network in the spinal dorsal horn is poorly understood. To evaluate the functional connectivity among dorsal horn neurons multiple neuron recordings were made via a single electrode in the lumbar spinal cord of pentobarbital anesthetized rats. Single neuron discharges were identified using a template matching algorithm (Forster et al. J. Neurosci. Method. 31(1990):109) and were analyzed by auto-correlation (AC), cross-correlation (CC) and by joint impulse configuration scatter diagram (JICSD) (bin width 1 ms) after stationarity test. With the skin at the ipsilateral hindpaw intact the CCs at 5/8 (62.5%) dorsal recording sites (depth $\leq$ 600μm) and at 14/53 (26.4%) ventral recording sites (depth > 600μm) had single broad peaks with width of more than 10 ms and zero delays. The CCs at only 2/53 (3.2%) ventral recording sites had bilateral sharp peaks (width < 4 ms and symmetrical delays $\leq$ 7 ms). The strength and the time of the interaction was the same for dorsal and ventral recordings sites. CCs suggestive of monosynaptic connection were not observed. With the glabrous skin at the ipsilateral hindpaw inflamed by radiant heat CCs with bilateral sharp peaks (width < 4 ms and symmetrical delays of 19.8 $\pm$ 9.9 ms $\bar{X}$ $\pm$ SD) were found at 9/18 (50%) of the recording sites. These results suggest that under normal conditions there is a predominant common input via interneurons to neurons recorded at the same site either in the dorsal or in the ventral spinal dorsal horn consistent with a parallel cascade processing model. With the skin inflammated signs of reverberating neuronal circuits were found.

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178.16

THE HYPERALGESIA INDUCED BY A PERIPHERAL MONONEUROPATHY IS ABOLISHED BY NEONATAL CAPSAICIN TREATMENT T.J. Maves, S.T. Meller and G.F. Gebhart, Depts. of Anesthesia and Pharmacology, University of Iowa, Iowa City, IA, 52242.

A recently developed model of a peripheral mononeuropathy in the rat (Bennett and Xie, Pain 1988, 33:87-107), produced by loose ligatures tied around the sciatic nerve, has been characterized by a marked hyperalgesia, a reduction in the number of large myelinated fibers and an increase in the number of small myelinated fibers.

In the present study, we examined the role of C-fiber afferents in this peripheral mononeuropathy model. 4 loose chromic gut ligatures (4-0) were tied around the left sciatic nerve above the point where the sciatic nerve trifurcates. Response latencies for withdrawal of the left and right hind-paws from a ramped heat stimulus were measured in neonatal capsaicin- and vehicle-treated rats on the day prior to surgery, and on days 5, 10 and 20 post-surgery. Capsaicin-treated rats had a significantly greater response latency on all days tested compared to vehicle-treated rats. On the day immediately prior to surgery there was no left paw/right paw difference in latencies in either group. However, on days 5 and 10, vehicle-treated rats showed a markedly lesser response latency on the left compared to the right. This left/right difference was not apparent at day 20. In contrast, the capsaicin-treated rats did not show any left/right difference in response latencies on any day tested.

These results are consistent with a significant role for unmyelinated C-fiber afferents in mediating a peripehral mononeuropathy-induced thermal hyperalgesia.

178.17

PERIPHERAL INJURY ENHANCES EXTRACELLULAR LABELING IN THE SUBSTANTIA GELATINOSA AFTER INJECTION OF WGA-HRP IN THE SCIATIC NERVE, A. Rustioni, J.Valtschanoff, and R. Weinberg, Dept. of Cell Biology & Anatomy and Dept. of Physiology, U of North Carolina, Chapel Hill, NC 27599.

After injection of WGA-HRP into dorsal root ganglia, reaction product is seen in the extracellular space surrounding synaptic boutons in superficial laminae of the spinal dorsal horn (Weinberg et al., 1990). Intraganglionic injection is inherently traumatic; to assess the possible role of injury in this extracellular labeling, WGA-HRP was injected bilaterally into the sciatic nerve of anesthetized rats. After the injection, the sciatic nerve was crushed or transected distal to the injection on one side. After 2-7 days survival, the animal was anesthetized and perfused with aldehyde fixatives. Fifty µm Vibratome sections of spinal cord segment L_5 were cut, reacted for TMB with tungstate stabilizer, and processed for light and electron microscopy.

Labeling in the form of electron-dense crystals and amorphous deposits was inside typical primary afferent terminals. In most cases, labeling could also be seen in postsynaptic elements, including dendrites, somata, and glia. Labeling was usually also present in the extracellular space outside primary afferent terminals. It often nearly surrounded these terminals, but characteristically was excluded from the synaptic cleft itself. The degree of extracellular and transneuronal labeling (as assessed by blind scoring) was significantly greater on the injured side, suggesting that injury leads to release of a variety of proteins into the extracellular space near the synapse. We hypothesize that release of some endogenous peptide may play a role in post-traumatic hyperalgesia. Supported by NINDS award #

178.18

CONTRIBUTION OF PROTEIN KINASE C TO PERSISTENT PAIN FOLLOWING SUBCUTANEOUS FORMALIN INJECTION. T.J. Coderre. Pain Mechanisms Laboratory, Clinical Research Institute of Montreal, 110 Pine Ave. West, Montreal, P.Q., Canada H2W 1R7.

We have recently suggested that noxious stimulus-induced neuronal plasticity occurring in the early period following s.c. formalin injection contributes to tonic pain in the rat formalin pain model. This study assessed the role of various second messengers in the development of this CNS plasticity. Pain behaviour, in response to a 50 µl injection of 2.5% formalin into the hindpaw of rats, was assessed following intrathecal injection of agents affecting various second messengers. Formalin pain scores were unaffected by forskolin and H-9, which affect cAMP and cAMP-dependent protein kinase, respectively. Formalin pain scores were also weakly affected by quincarine, which inhibits the conversion of phosphatidylcholine to arachidonic acid, and W-7 which inhibits Ca^{2+}/calmodulin-dependent protein kinase. By contrast, formalin pain scores were significantly inhibited by neomycin, which inhibits the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol trisphosphate and diacylglycerol (DAG), and by H-7, which inhibits protein kinase C (PKC). Furthermore, formalin pain scores were significantly enhanced by a phorbol ester (PMA) and SC-10 which stimulate PKC. We hypothesize that formalin produces a release of glutamate and aspartate which by acting at NMDA and quisqualate receptors, respectively, leads to an influx of Ca^{2+} and the stimulation of PIP2. The breakdown of PIP2 into DAG leads to the production of PKC, which is activated during high rates of Ca^{2+} influx. By phosphorylating substrate proteins, PKC would produce sustained membrane alterations contributing to tonic pain in the formalin test.

PAIN MODULATION: PERIPHERAL

179.1

POST-SYMPATHECTOMY NEURALGIA: THE RESULT OF CONCURRENT DEAFFERENTATION AND SYMPATHETICALLY-MAINTAINED PAIN MECHANISMS? R.C. Kramis*, R.G. Gillette* and W.J. Roberts. R.S.Dow Neurological Sci. Inst., Good Samaritan Hosp. & Med. Ctr., Portland, OR.

Sympathectomy, when used to relieve chronic pain, often induces a new painful condition proximal to the previously painful region. This post-sympathectomy pain may be moderately intense and of limited duration or extremely painful, persistent and resistant to pharmacological intervention.

Our recent investigations and data from the literature support the hypothesis that post-sympathectomy pain occurs because: 1) wide-dynamic-range spinal neurons are sensitized by nociceptive input associated with the painful condition for which sympathectomy is performed, 2) primary afferent axons (both somatic and visceral) traveling in the sympathetic trunk are unavoidably transected by sympathectomy, and 3) this peripheral afferent axotomy produces degenerative and perhaps regenerative central effects which further heighten the responsiveness of the already sensitized WDR spinal neurons.

We have used electrical stimulation of the lumbar paravertebral sympathetic trunk and extracellular single unit recording from the L4-L5 spinal cord of pentobarbital anesthetized cats to demonstrate convergence of "sympathetic" and somatic afferents onto spinal WDR neurons with receptive fields in the anterolateral and anteromedial thigh, the region which becomes painful in patients following lumbar sympathectomy. Often these same neurons also respond to somatic afferent input elicited by stimulation of sympathetic efferents. This sympathetic efferent/somatic afferent/spinal WDR "loop" may explain the relation of "spontaneous" post-sympathectomy pain to sympathetic hyperactivity in the newly painful (non-sympathectomized) region in patients. The proposed "sensitizing" (deafferentation) effects of transecting afferents in the paravertebral sympathetic trunk remain to be investigated.

179.2

DECREASE IN THE MAGNITUDE OF VAGINOCERVICAL STIMULATION-PRODUCED ANALGESIA IN AGING RATS. S. Chinapen, J.L. Steinman, A. Pierprzny*, and B.R. Komisaruk. Institute of Animal Behavior, Rutgers-The State University, Newark, NJ 07102.

Vaginocervical mechanical stimulation (VS) in sexually mature rats produces analgesia as demonstrated by elevations in tail flick latency (TFL) to radiant heat and in vocalization threshold (VOCT) to electrical shock of the tail. A comparison of VS-produced analgesia in rats tested repeatedly at 4-5 months (mo), 8-9 mo, 12-13 mo, 16-17 mo, 20-21 mo, 24-25 mo and 28-29 mo was made using these two behavioral tests (n's>9 for groups 8-9 mo and older). Rats were ovariectomized at least 1 week before initial testing. VS (100 & 300g) was applied using a force calibrated 1-cc syringe plunger assembly; data were analyzed using correlated t-tests and 1-way ANOVA's.

In each age group, a significant increase above baseline in TFL and VOCT was produced by 100 and 300g VS (t-tests). The baseline levels showed a significant decline from 4 mo to 24 mo of age (.32 to .17 mA for VOCT; 4.21 to 3.06 sec for TFL; p's<.006). There was an overall significant decline in VS-produced analgesia (100g VS) on TFL from 4 to 24 mo ($p<.002$) which was first observed at 16 mo of age. No further significant change was seen at 28 mo on this measure. During both 100 and 300g VS, VOCT was significantly lower at 24 mo than at all previous ages (p's<.003) and also did not show a further significant change at 28 mo. These results demonstrate that the magnitude of VS-produced analgesia declines with increasing age, with decrements in the analgesic effect on TFL appearing at 16 mo and on VOCT at 24 mo of age. These findings raise the possibility that aging induces changes in the female nervous system that reduce analgesic mechanisms by 24 mo of age. [Supported by NIH RR08223 and the Busch Foundation].

179.3

VAGAL ELECTRO-STIMULATION IN AWAKE RATS PRODUCES SIGNS OF PHYSIOLOGICAL DISTRESS BEFORE ANTINOCICEPTION

D. F. Bossut and W. Maixner, Dept. Physiology and Dental Research Center, University of North Carolina at Chapel Hill.

Several observations made in anesthetized or decerebrated animals indicate that electrical vagal afferent stimulation (VAS) produces antinociception. The goal of the following experiments was to determine whether VAS produces a reduction of nociceptive responses before signs of physiological distress in awake, intact animals.

Thoracic or cervical continuous VAS (1 to 100 Hz and 0 to 3 mA) produced a short lived antinociception (increased tail-flick latency) only at levels of stimulation (10 Hz, 1 mA) also producing signs of physiological distress (gasping, cyanosis, immobility, etc). Under anesthesia, inhibition of the digastric reflex started with 2 mA VAS. Blood pressure and heart rate dropped more as the intensity of VAS increased.

It is concluded that electrical VAS does not produce antinociception before obvious signs of physiological distress occur.

Supported by NRSA Fellowship DE05585 to D.F.B. and NIDR grant DE08013 and DE0750901 to W.M.

179.4

EFFECT OF COLONIC INFLAMMATION ON RESPONSES TO NOXIOUS COLORECTAL DISTENSION. M.B. Burton and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, IA 52242

Despite growing knowledge about pain and inflammation, little is known regarding the role of inflammation in visceral pain. However, inflammatory mediators are known to sensitize nociceptors. The following experiments were performed to determine whether colonic inflammation alters responses to noxious colorectal distension (CRD).

Intracolonic administration of 25% turpentine in peanut oil (1 ml) was used to induce inflammation in male Sprague-Dawley rats. Evan's Blue dye extravasation in the colon was measured up to 24 hours after turpentine treatment. Colonic tissue was taken from another group of treated rats for determination of leukocyte infiltration over the same time period. Changes in mean arterial pressure and abdominal electromyographic (EMG) activity were measured in response to a 20 sec phasic CRD before and, in a time-dependent manner, after treatment. The visceromotor threshold, determined by an increasing ramped CRD, was measured over the same time course.

Leukocyte infiltration did not increase at any time measured; however, extravasation was apparent beginning 8 hours after treatment. Changes in EMG activity increased at 2 hours after treatment while, in the other experimental group of animals, the visceromotor threshold decreased at this time.

These results indicate that turpentine induces changes in response to noxious CRD before histological changes are apparent.

179.5

ANALGESIA PRODUCED BY UTEROCERVICAL MECHANOSTIMULATION IN THE RAT: ROLE OF AFFERENT NERVES AND IMPLICATIONS FOR ANALGESIA OF PREGNANCY AND PARTURITION. B.R. Komisaruk[1] and A.R. Gintzler[2]. Inst. Animal Behavior, Rutgers-The State Univ., Newark, NJ 07102[1] and Dept. Biochem., SUNY Health Science Ctr, Brooklyn, NY 11203[2].

Uterocervical mechanostimulation was applied to non-pregnant intact rats via a silastic disc (2mm dia.) implanted in the uterus, abutting against the cervix, and attached to a thread that was externalized through the vaginal orifice. Tail flick latency (TFL) was used as the indicator of pain threshold. Mean pre-stimulus TFL's (4.7-5.4 sec) did not differ among groups. Force of 150g but not 100g increased TFL over pre-stimulation levels (n=7, p<0.03). Using 150g force, TFL increased by 110.4± 40.6% (n=7; pre- vs during-stimulation) in the sham-operated control group. In the hypogastric neurectomy (**HX**) group, TFL increased by 136.0 ± 32.7% (n=8), which did not differ from the control group (p>0.05). By contrast, rats in the pelvic neurectomy (**PX**) group showed a mean increase in TFL of only 1.7 ± 12.5%, which was less than the increase in either the control or the HX groups (p<0.05, n=7). Stimulation of the uterine cervix in rats subjected to **HX + PX** produced a decrease in TFL of 25.0 ± 9.4% relative to both the control and HX groups (p<0.05; n=9). Thus, the analgesia that occurs during pregnancy and/or parturition may result at least in part from mechanical stimulation of the uterine cervix.
Supported by NIH grants NS 19898 (BRK) and HD 18448 (ARG).

179.6

THE CENTRAL PROCESS RESPONSIBLE FOR AßLTM-MEDIATED ALLODYNIA IN SOME PATIENTS WITH RSD IS SENSITIVE TO PERFUSION OF THE MICROENVIRONMENT OF NOCICEPTOR TERMINALS. R.H. Gracely*, S. Lynch and G.J. Bennett. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Mechanical allodynia (touch-evoked pain) is often observed in Reflex Sympathetic Dystrophy (RSD). Increasing evidence indicates that this allodynia can result from perception of normally innocuous tactile input from Aß low threshold mechanoreceptors (AßLTMs) as painful. We recently proposed that this misperception is caused by a central process which is dynamically maintained by ongoing nociceptor input.

AßLTM-mediated allodynia is inferred in part from differential ischemic blocks in which circulatory occlusion simultaneously abolishes Aß function and allodynia while A-delta and C-fiber function is intact. However, in certain patients we have observed an unexpected response in which AßLTM allodynia is abolished rapidly (2-6 min) while all neural function is intact. We propose that this effect is due to ischemic modification of the nociceptor activity maintaining the central process.

We tested this hypothesis in two patients with rapid (2-4 min) ischemic block of AßLTM allodynia on the lower leg. Ischemia produced by a tourniquet applied to mid-calf in a patient with an ankle injury resulted in rapid loss of allodynia both above and below the tourniquet, which returned 3 min after the tourniquet was removed. Elevating the leg without a tourniquet produced a similar loss of allodynia which returned when the leg was lowered, but allodynia did not return if an ischemic block was applied mid-calf before lowering. In the second patient, with a knee injury, leg elevation also abolished allodynia until the leg was lowered. However, an ischemic block at mid-calf after elevation did not prevent return of allodynia above the block.

These results indicate that modification of the peripheral nociceptor input from the injury site is sufficient to abolish allodynia. This modification may be due to decreased capillary perfusion, or change in blood pressure or sympathetic tone that follow limb elevation or circulatory occlusion.

179.7

Aδ AND C FIBER NOCICEPTORS RESPOND DIFFERENTLY TO INJURY. D.L. Tanelian and M.B. MacIver. Pain Research Laboratory, Department of Anesthesia, Stanford University School of Medicine, Stanford, California 94305.

The contribution of Aδ and C fiber discharge activity to acute and chronic pain following injury was studied using the *in vitro* rabbit cornea preparation. Extracellular recording electrodes were used to monitor multiple and single unit discharge from corneal nerves, and receptive fields for mechanosensitive fibers were mapped. A corneal abrasion (2 mm^2) was produced which involved the receptive field of an identified fiber(s). Both Aδ and C fibers responded with high frequency discharge (20 to 50 spikes/s) during injury, but only C fibers continued to discharge tonically (14 +/- 3.1 spikes/s; mean +/- SD, n=28) following injury. In multiple unit recordings, recruitment of previously silent units were often observed during injury. Responses to mechanical and thermal stimuli were increased by 150 to 300 % compared to control ($p < 0.001$; ANOVA), for at least 3 hours after injury. Abrasions which completely destroyed the epithelium, and hence the nerve ending portion of fibers, resulted in abolition of all discharge activity. The results indicate that Aδ fibers respond phasically during injury, while only C fibers continue to discharge following injury. In addition, it appears that nerve endings are essential for sensory transduction and fiber discharge, since truncated fibers do not exhibit tonic activity, and do not discharge in response to stimulation after destruction of the epithelium. Further studies will investigate mechanisms of nerve fiber sensitization following injury.
Supported by NIH grant NS28646-01 A1.

179.8

CROSS-EXCITATION OF DORSAL ROOT GANGLION NEURONS IN NERVE INJURED RATS BY NEIGHBORING AFFERENTS AND BY POST-GANGLIONIC SYMPATHETIC EFFERENTS M. Devor [1], P.D. Wall [2]* and W. Janig [3]*, [1]Life Sciences Inst., Hebrew University Jerusalem, Israel; [2]Dept. Anatomy and Dev. Biol., Univ. College London, UK; and [3]Physiol. Inst., Kiel Univ., FRG.

A small proportion of primary afferent neurons in intact dorsal root ganglia (DRGs) fires spontaneously, and this activity is substantially increased in animals with chronic nerve injury. We now report that such ectopic DRG discharge is augmented by tetanic stimulation of the axons of neighboring afferent neurons that share the same DRG, where the spontaneously active neuron itself has not been stimulated directly. Sensory cross-excitation does not follow stimuli 1:1 like ephaptic coupling, but rather builds up gradually on stimulus onset, decaying gradually after the end of the stimulation. Strong excitations are often followed by afterdepression. Ectopic DRG discharge is also sensitive to sympathetic efferent activity. Repetitive stimulation of postganglionic sympathetic efferents in ventral roots T13 and L1, at frequencies as low as 5 Hz., caused excitation of DRG neurons after a delay of a few seconds. This was sometimes followed by depression. In some DRG neurons, only depression was seen. DRG excitation by sympathetic activity could be mimicked by systemic adrenaline and blocked by phentolamine indicating chemical mediation via α-adreno-receptors. DRG cross-excitation could contribute to ongoing and evoked neuropathic sensory abnormalities.

179.9

UPREGULATION OF VASOACTIVE INTESTINAL PEPTIDE IN SUBSTANCE P EXPRESSING PRIMARY SENSORY NEURONS AFTER INJURY

T. Hökfelt[1], V.M.K. Verge[1], Z. Wiesenfeld-Hallin[3] and M. Eriksson[2]. Dept. of Histology and Neurobiology[1], Pharmacology[2], Karolinska Institutet, Stockholm, Sweden, 104 01 and Sect. Clin. Neurophysiol.[3], 141 86 Huddinge, Sweden.

Peripheral nerve injury leads to downregulation of substance P (SP) and upregulation of vasoactive intestinal peptide (VIP) levels in adult rat primary sensory afferents. The physiological consequences of these changes indicate that the role of SP in mediating C-afferent-induced flexor reflex facilitation is taken over by VIP after axotomy (Neurosci Lett 116(1990)293-298). To examine whether VIP becomes expressed in the same neurons that downregulate SP, the sciatic nerve was sectioned and the co-expression of VIP and SP studied at various time points using *in situ* hybridization with ^{35}S-labelled SP and VIP oligonucleotide probes on adjacent sections of lumbar DRG. At 4 days after axotomy numerous DRG neurons expressed mRNA for both peptides which do not normally coexist in detectable levels in intact neurons.

The above results provide the anatomical substrate in support of the hypothesis that the specific functional consequences associated with axotomy- induced alterations in afferent peptide expression occur within distinct subpopulations of sensory neurons.
Supported in part by the Canadian and Swedish MRCs.

179.10

ALTERATION OF THE SUBSTANCE P CONTENT OF PRIMARY AFFERENT NEURONS BY NOCICEPTIVE STIMULATION. K.E. McCarson and B.D. Goldstein. Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

Substance P (SP) has been proposed as a mediator of nociception in the spinal cord. We have shown that noxious mechanical pinch increases SP release into the superficial dorsal horn. Formalin injection into the hindpaw, a known nociceptive stimulus, has been shown to produce a biphasic increase in dorsal horn SP immunostaining, but decreases SP release in a biphasic manner. This study was undertaken to determine if nociceptive activation of primary afferent neurons affects the SP content of dorsal horn, dorsal root, and dorsal root ganglion tissues. The hindpaws of unanesthetized decerebrate/spinal rats received either an injection of 5% formalin or noxious mechanical stimulation (pinch) as the nociceptive stimulus. Saline injected and naive rats served as controls. After various time intervals the lumbar dorsal horns, the L5 dorsal roots, and the L5 dorsal root ganglia were assayed for SP-like immunoreactivity (SPLI) using radioimmunoassay. Formalin injection produced increases in dorsal horn SPLI content at 20 and 80 minutes. Formalin also increased dorsal root ganglion SPLI content 80 minutes after hindpaw treatment. Noxious pinch slightly decreased the SPLI content of both dorsal horn and dorsal root tissues. Dorsal root SPLI was unchanged by any other treatment. Saline injection also increased dorsal horn SPLI at 60 minutes. SPLI changes in the contralateral dorsal horns were similar to those in the dorsal horn ipsilateral to hindpaw treatment, providing indirect evidence for the collateralization of nociceptive information at the segmental level. The results of this study indicate that both processing of SP precursors and SP release may affect the SPLI content of primary afferent neurons. Supported by a grant from the Medical College of Georgia Research Institute, Inc.

179.11

Norepinephrine (NE) Acts Indirectly to Sensitize C-Fiber Mechano-Heat (C-MH) Nociceptors in Hairy Skin of the Rat.

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Intradermal injection of NE, in the presence of the calcium-ionophore A23187, induces mechanical hyperalgesia in the rat by an action at the α_2-adrenergic receptor (Neurosci. 32: 523, 1990). Since this model of sympathetic dependent hyperalgesia allows characterization of the nociceptor both prior to and after the onset of hyperalgesia, we employed it to study mechanical hyperalgesia induced by NE in C-MH nociceptors in the rat.

45 C-MHs with mechanical thresholds > 2.0 g and thermal thresholds > 42 °C were isolated from the saphenous nerve of pentobarbitol (50 mg/kg) anesthetized 200-300 g male, Sprague-Dawley rats; 30 in controls, and 15 in rats one week after surgical sympathectomy (SYMPx). The mechanical thresholds of C-MHs in control and SYMPx rats were 5.8 ± 0.91 and 5.7 ± 1.38 g (mean ± SEM), respectively. Intradermal injection of neither NE (10 μg) nor A23187 (1 μg), adjacent to the mechanical receptive field of 12 C-MHs, affected mechanical threshold. However, a third injection containing NE + A23187 significantly decreased threshold (33.8 ± 4.34 %, $p < 0.01$) sensitizing 9 of the 12 C-MHs. The three C-MHs not sensitized by NE + A23187, were not sensitized by a fourth injection of prostaglandin E_2 (PGE_2) (100 ng). In a separate group of 9 C-MHs, 6 were sensitized by PGE_2.

SYMPx significantly attenuated the hyperalgesic effect of NE + A23187 (28% decrease, $p < 0.01$). In normal rats, co-injection of indomethacin (10 μg) significantly attenuated the sensitization of C-MHs (n=9) induced by NE + A23187 ($p < 0.01$).

Our data are compatible with the suggestion that a local increase in calcium induces a state in which NE can sensitize C-MH nociceptors. This NE-hyperalgesia is indirectly mediated by sympathetic postganglionic neuron terminal-dependent production of hyperalgesic prostaglandins. *Supported by NIH grant NS21647*

179.12

CHANGES OF RESPONSE PATTERN OF SENSORY AFFERENTS IN RATS EXPOSED TO SUB-BLOCKING CONCENTRATIONS OF LIDOCAINE.

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Traditional understanding of the mechanism of anesthesia due to local nerve blocks is based on the assumption that the absence of sensation in the innervated region results from a complete block of impulse traffic across the nerve site exposed to local anesthetics (LAs). However, somatosensory evoked potentials can be elicited from regions perceived as fully anesthetized indicating that perception of a stimulus can be abolished without fully blocking signal propagation along the neuraxis.

Impulse activity is known to affect the threshold and conduction velocity of subsequently elicited impulses in an axon. So do LAs, especially when impulse activity is present.

We studied changes in axonal excitability and in the response pattern to natural stimulation propagated through a region of nerve exposed to different concentrations of lidocaine insufficient to fully block impulse conduction. In rats anesthetized with Nembutal, the sciatic nerve was exposed to lidocaine (0.1-0.6mM) over a length of 15 mm. Axonal excitability was monitored by tracking latency of conduction in response to electrical stimulation (0.5 Hz - 500 Hz) of the nerve trunk. Changes in response pattern were monitored by recording action potentials from single afferents responding to natural stimulation of the cutaneous receptive field. Lidocaine concentrations as low as 0.1mM induced frequency-dependent changes in response latencies which were accompanied by changes in response pattern without resulting in a complete block of signal conduction. The changes did not correlate with fiber diameter, but did correlate with fiber modality.

Assuming that sensory information is at least in part encoded in the pattern of discharge, then altering of the pattern may blur the information sufficiently to result in changes of perception without abolishing signal transmission .

179.13

EFFECTS OF SYMPATHECTOMY ON A PERIPHERAL NEUROPATHY MODEL IN THE RAT.

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Recently we developed an animal model for neuropathic pain which involves a unilateral tight ligation of the L5 and L6 spinal nerves in rats. The model produces signs of neuropathic pain lasting up to several months, which resembles causalgia in humans. The aim of this study was to determine effects of sympathectomy on this animal model.

The neuropathy was produced in a group of 10 rats according to the previously established model. These animals showed a marked increased in occurrence of paw lifting in response to innocuous mechanical stimuli and a shortened latency of paw withdrawal in response to noxious radiant heat stimuli on the affected hind limb. Three weeks after the surgery when these behavioral signs were at the maximum, surgical sympathectomy was performed under sodium pentobarbital anesthesia by removing the sympathetic chain on the affected side from the L2 to L6 levels.

The sympathectomy produced an almost complete reversal of the responses to innocuous mechanical stimuli, but had variable effects on paw withdrawal latency to noxious heat stimuli.

The data suggest that sympathectomy is effective in alleviating allodynia but not for hyperalgesia in our neuropathic pain animal model. (Supported by NIH grants NS21266 and NS11255 and a grant from Bristol-Myers Squibb Co.)

179.14

ELECTROMYOGRAPHIC (EMG) RESPONSES OF RAT NECK AND JAW MUSCLES TO INFLAMMATORY IRRITANT.

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An EMG study was carried out in 9 anaesthetized rats to determine if the activity of neck and jaw muscles could be influenced by the injection of the inflammatory irritant mustard oil into the deep tissues surrounding the C2 vertebra. EMG baseline activity was recorded bilaterally in the deep neck muscles and ipsilaterally in trapezius, masseter and digastric muscles. Vehicle injection (20 μl, mineral oil) usually induced a transient (1.5 min) increase in EMG activities of all 5 muscles. In contrast, injection of mustard oil (20 μl, 20% allyl isothiocyanate) produced an EMG increase in all 5 muscles that was significantly greater in magnitude (50%, $P<0.05$) and longer in duration (3 min, $P<0.05$). Furthermore, some muscles often showed a second phase of activation which appeared 3.5 - 20 min after the mustard oil injection and lasted 15 - 20 min. These effects suggest that inflammatory irritants injected in deep periarticular tissues of the neck may reflexly enhance the activity of muscles distant from the injection site. Such effects may be related to increased muscle activity associated with trauma to deep tissues. Supported by NIH grants DE04786 and DE09559.

VISUAL CORTEX: EXTRASTRIATE RESPONSE PROPERTIES

180.1

THE RESPONSE OF CELLS IN AREA MT TO STIMULATION OF BLUE-SENSITIVE CONES.

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The contribution of short-wavelength (S) cones to the luminance channel remains controversial within the psychophysical literature. In addition, physiological studies suggest that the S cones have little input to the spectrally non-opponent retinal ganglion cells. Area MT is a region of extrastriate cortex that is believed to rely heavily on the luminance channel and receives the majority of its input from spectrally non-opponent cells. However, we have previously shown that many cells in area MT respond to isoluminant blue/yellow sinusoidal gratings. To determine the contribution of S cones to area MT we recorded from two alert rhesus monkeys using a stimulus configuration designed to isolate S cones.

We first determined optimal tuning for excitatory receptive fields (s.f., velocity, direction) of single units using a range of luminance contrasts and used these parameters for the S cone stimulation. For the tritanopic stimulus, a sinusoidally modulated grating was presented on a CRT using the blue phosphor. An "equiluminizing"veil was placed in front of the screen and flooded with bright yellow light, reducing the luminance contrast of the grating to less than 0.5 percent. We found that the majority of cells respond to S cone stimulation however the signal is markedly lower than the response to a high contrast luminance grating. In addition, we used a series of S cone modulations and adjusted the luminance of the yellow field using neutral density filters. By generating spike density functions we were able to obtain t.v.i. curves and examine the temporal characteristics of the response for many of the cells.

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180.2

SIGNALS ENCODING TARGET ACCELERATION IN VISUAL AREA MT OF MACAQUE MONKEYS.

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Studies of the initiation and maintenance of smooth pursuit eye movements have shown that signals related to both target acceleration and target speed play an important role in the visual guidance of pursuit. We now show that cells in visual area MT discharge in relation to both of these parameters of target motion. We made extracellular recordings from direction selective single units in visual area MT in anesthetized, paralyzed macaque monkeys. Stimuli consisted of random dot textures that moved across an electronic "window" over the cell's receptive field. Motion of the texture provided instantaneous steps or 128-ms ramps of speed in the cell's preferred direction.

Ramps of stimulus speed produced a shaped pulse of firing rate followed by steady firing related to final target speed. Steps of stimulus speed produced a transient that was associated with the onset of motion, followed by a transition to steady-state firing. For each cell we used the steady-state speed tuning curve to calculate the firing rate that could be attributed to stimulus speed, and we subtracted this speed component from the firing recorded during ramps of target speed. In approximately half of the MT cells, this procedure revealed a component of firing rate that could not be attributed to stimulus speed and that appeared as a clear relationship between firing rate and stimulus acceleration. For the remaining 50% of the cells, firing rate could be attributed entirely to stimulus speed. We conclude that the signals carried by neurons in MT can encode all parameters of target motion that are used for the visual guidance of pursuit eye movements. *(Supported by the McDonnell-Pew Program in Cognitive Science and by NIH grants EY03878 and EY02017).*

180.3

NEURONAL SELECTIVITY FOR STIMULUS SPEED AND CONTRAST IN THE PRESTRIATE VISUAL CORTICAL AREAS V4 AND MT OF THE MACAQUE MONKEY.

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V4 and MT are distinctive areas in the prestriate visual cortex of the macaque monkey: V4 projects mainly to the temporal areas, while MT to the parietal areas; color selective cells are present in V4 but absent in MT, whereas directionally selective cells present in MT but absent in V4. Orientation selective cells, however, are found in both areas. In order to further know the difference and similarity of the two areas, we compared neuronal selectivities for stimulus speed and contrast between V4 and MT.

We recorded neurons in V4 (n=85) and MT (n=34) of the same anesthetized, immobilized monkeys (*M. fuscata*), and examined their responses to a slit moving at 10 speeds (0.5-256 deg/s) and to the slit moving at the preferred speed with 6 contrasts (ranging from 0.03 to 1.0 logarithmic values of the lightness difference between the slit and its background). Distributions of peak speeds for cells in V4 and MT overlapped largely, and both had the median at 32 deg/s. Cells tuned to the very slow speeds (0.5 and 1 deg/s) were only found in V4 (7.5%), and cells were more evenly distributed within the range from 8 to 128 deg/s in MT than in V4, although the two distributions were not statistically different ($p>0.05$). Distributions of the cut-off contrast at half maximal response in the two areas also considerably overlapped, but the position of the peak appeared at higher contrast in V4 (0.35) than in MT (0.12). The difference in the two distributions was statistically significant ($p<0.01$). In comparison with the previous data obtained from cells in the lateral geniculate nucleus (LGN), the distributions of cut-off contrast for both V4 and MT cells were in between those for cells in the magnocellular and parvocellular layers of LGN. We suggest that a mostly overlapped range of retinal information is sent to MT and V4 in parallel, but is used differently in these two areas.

180.4

RESPONSES OF MACAQUE V4 NEURONS TO PASSIVELY AND SELF-INDUCED RETINAL IMAGE MOTION. R.G. Erickson and P. Thier, Department of Neurology, University Tubingen, 7400 Tubingen, Germany.

The ability to discriminate self-induced visual motion is necessary for proper spatial orientation, and the brain must possess mechanisms for removing self-induced input from that part of the visual system deciphering motion signals. Recent studies have shown that directionally selective visual neurons within a certain part of the motion pathway, area MSTd, do indeed respond only to retinal image motion not caused by pursuit eye movements (Erickson and Thier, Exp. Brain Res., 1991). The contribution of motion signals to alternate functions such as analysis of form would, however, be expected to require responses invariant with respect to the source of motion. To examine this possibility we have tested area V4, a visual area processing color and form but not direction of motion, by comparing the responses of motion-sensitive cells to retinal image motion caused by stimulus movement as opposed to eye movement.

Recordings from two awake monkeys were obtained while the animals attended to a small target that was either held stationary or moved slowly to induce a low velocity smooth pursuit (5-20 deg/sec). Moving stimuli were swept across a stationary receptive field during stable fixation, and receptive fields were swept across stationary stimuli during pursuit eye movements. Of 25 neurons verified by histological reconstruction to be within V4, all responded well to motion of a bar or slit, none were directionally selective, and all responded equally well to similar retinal image motion regardless of whether the eyes were moving or stationary.

The observed invariance with respect to the source of visual motion is in agreement with the proposed role of area V4 in object recognition and underscores the uniqueness of visual-motion processing occurring in area MST.

180.5

A NEURAL NETWORK FOR FLOW-FIELD PROCESSING IN THE VISUAL MOTION PATHWAY OF HIGHER MAMMALS. M. Lappe* and J.P. Rauschecker. NIH Animal Center, NIMH, Poolesville, MD 20837, U.S.A. and Max Planck Institute for Biological Cybernetics, Tübingen, Germany.

Recent neurophysiological findings from cat and monkey suggest that the visual system may be capable of extracting retinal flow-field information: Neurons in cat area PMLS (Rauschecker et al., 1987) and monkey area MT (Albright, 1988) favor stimuli moving away from the area centralis. This centrifugal direction bias in both species is more pronounced in the visual field periphery and is independent of visual experience (Brenner & Rauschecker, 1990). Other results from monkey areas MT and MST additionally suggest that single neurons themselves may be sensitive to expanding, contracting, or rotating visual patterns. We have designed a neural network model based on these findings, which detects the direction of egomotion with high accuracy.

The network consists of two layers with 4,000 cells. Neurons in layer one (PMLS/MT) prefer movement in different directions, but with an overall centrifugal bias. The second layer (MST) consists of 400 neurons with very large receptive fields responding to global expansions. This is achieved with converging excitatory connections. Each layer-two cell encodes a specific 3-D direction. The direction of egomotion is given by the peak of neuronal activity. The network has been tested with synthetic flow fields and different directions of egomotion within the innermost 10° of the visual field. Varying the centrifugal bias revealed an optimal range in which the mean error (1°) is comparable to humans. Best results were obtained with a strong bias in the periphery and no bias in the center. When there is no bias at all, a large number of peripheral neurons become useless for the task. With a strict overall bias the net is insensitive to differences in flow patterns generated by different movements.

Cells in PMLS and MT show varying degrees of directional selectivity. When we varied the broadness of direction tuning in layer one of our network, we found no effect on the location of the activity peak in layer two. More broadly tuned layer-one cells gave only rise to a higher background activity in layer two. Our neural network provides another example of how precise behavioral performance can be achieved with a large set of imprecisely tuned neurons.

180.6

HOW POSITION-INDEPENDENT DETECTION OF SENSE OF ROTATION OR DILATION IS LEARNED BY A HEBB RULE. M.E. Sereno*, K. Zhang*, and M.I. Sereno, Cognitive Science Department, University of California San Diego, La Jolla, CA 92093.

Some neurons in MSTd detect the sense of rotation or dilation of a pattern independent of the location of the motion singularity (Saito et al, 1986). We constructed a model of how this sensitivity might be learned using a Hebb rule. Our previous work (M.I. Sereno, 1989) showed that a feedforward network with V1-like first-layer units and a Hebb rule, can develop second layer units that solve the aperture problem for pattern translation when trained on a set of rigidly translating velocity fields. The present work added a third layer to the network receiving input from the second layer of MT-like neurons.

The model was trained and tested on rotating, dilating, and translating patterns of various diameters placed at various retinal locations. With large interlayer divergences, some third-layer units learn to detect one or the other senses of rotation or dilation in a position-independent way (Sereno and Sereno, 1991). One explanation for position-independence suggested by Saito et al. and Duffy and Wurtz (1991) is that rotation (curl) or dilation (divergence) is detected locally and then summed across space in MSTd. However, this is difficult to reconcile with the observation that MT units, which provide a major input to MSTd, are *not* sensitive to senses of rotation and dilation. Our model suggests a simpler explanation.

The best direction at each x-y second-layer input location for trained selective units turned out to be surprisingly position-*dependent*. Rotation neurons had centered, circularly-organized local direction selectivity while dilation neurons had radially organized direction selectivity. If we assume each x-y point in the input layer contributes an output equal to the dot product of the local stimulus velocity and the local direction-selectivity, then we show that the output of a second layer unit summing over the input layer is independent of the location of the center of the stimulus. If speed and direction tuning interact multiplicatively, the dot product can be calculated exactly by a group of units with cosine tuning curves.

As soon as the initial random weights (or noise) make a unit sensitive to a particular sense of rotation or dilation, we then show that a Hebb rule and our training set makes that weight vector grow the fastest.

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180.7

MSTd NEURONAL SENSITIVITY TO HEADING OF MOTION IN OPTIC FLOW FIELDS. C. J. Duffy and R. H. Wurtz. Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Neurons in cortical area MSTd of the rhesus monkey respond to planar, circular or radial stimuli which simulate the components of optic flow fields. We have combined planar and radial stimuli to determine how these neurons respond to the unique flow fields produced by such combinations. Stimuli were presented to the central 100° of the visual field and combined the preferred radial motion with one of 8 directions of planar motion. The resulting 8 flow fields differ from radial motion chiefly by having the center of the pattern displaced from the middle of the screen by about 35°. The combined radial and planar stimuli simulate flow fields observed during self-movement with straight ahead gaze when this heading is displaced off to the side.

Single component radial neurons responded best to uncombined radial motion, showing the most heading selectivity. The reduced responses to combined stimuli suggests that these neuron's radial selectivity results from active selection against planar responses. Triple component neurons (responsive to planar, and circular, and radial motion) responded to 4 or 5 combined stimuli, showing the least heading selectivity. The width of directional tuning for heading was proportionate to the width of directional tuning for simple planar motion. However, there was great variability in the relationship between the direction of preferred heading and the direction of the preferred planar motion.

Thus, MSTd neurons respond selectively to a variety of combined flow fields and this selectivity differs between the classes of neurons we have described previously (Duffy and Wurtz, *J. Neurophysiol.*, 1991). Differences between the preferred direction of heading and the preferred direction of simple planar motion might be explained by the way in which combined stimuli distribute the directions of motion across the neuron's receptive field.

180.8

RESPONSES OF DEPTH-MOVEMENT-SENSITIVE NEURONS TO SIZE AND DISPARITY CHANGES IN THE PARIETAL CORTEX OF ALERT MONKEYS. M. KUSUNOKI, Y. TANAKA* AND H. SAKATA*. Dept. of Physiology, Nihon Univ. Sch. of Med., Itabashi, Tokyo 173, Japan

There is a group of neurons that are sensitive to depth movement in the parietal association cortex of the monkey. Previously, we found many of them responded to the size change of visual stimuli (Sakata et al, 1985). Since the change of disparity is another important cue of depth movement, we studied the responses of the parietal depth-movement-sensitive neurons to the disparity change, either separately or combined with the size change, by using a pair of polarized filters with orthogonal planes. Two Japanese monkeys (Macaca fuscata) were used for chronic experiments after training the gaze fixation task. The recording sites of these neurons were localized both in the deepest part of the superior temporal sulcus (MST area) and in the fundus of the intraparietal sulcus (VIP area). Most of the neurons sensitive to depth movement of real objects also responded well to the disparity change of visual stimuli and were suppressed by binocular stimuli moving in parallel on a tangential screen. Some neurons showed vigorous responses when size and disparity changes were presented together. Although most of disparity sensitive neurons preferred the direction straight to or away from the midpoint between two eyes, several neurons were found to prefer oblique direction slanted to the right or left side. In such neurons optimal stimuli to the right and left eyes were often the same in direction but different in velocity. Some of depth-movement-sensitive neurons showed more vigorous response in the light than in the dark to depth movement of real objects. Their responses to changes of size and/or disparity were enhanced by a random dot pattern presented in the background. These characters suggest that these neurons are closely related to the perception of object movement in three dimensional space.

180.9

AREA 21a IN THE CAT AND THE DETECTION OF BINOCULAR ORIENTATION DISPARITY. G.H.Henry*, E.Wieniawa-Narkiewicz*, B.Wimborne*, and W.R.Levick Centre for Visual Sci., John Curtin School of Med. Res. Australian Nat. Univ., Canberra, 2601.

Visual response properties were examined in 115 cells, recorded extra-cellularly in area 21a of the cerebral cortex of 15 anesthetized and paralyzed cats. Every cell had common response properties and their receptive fields consisted of a single uniform discharge region which fired with composite ON/OFF responses to stationary flashing stimuli. All cells were binocular and all were sharply tuned to stimulus orientation (mean 1/2 width at 1/2 height=15.6^0). The optimum orientations favoured the vertical (21%) and horizontal (34%). There was no evidence that area 21a cells were effective detectors of binocular positional disparity. Instead, the sharpness of their orientation tuning together with their binocular drive suggested a possible role in the detection of binocular orientation disparity. Measurements showed that cells in the sample had preferred orientation disparities that ranged over 20^0 and that the tuning to this form of disparity was extremely sharp. It is concluded that while S cells of the striate cortex are suited for the extraction of positional disparity, the detection of orientation disparity is more effectively served by the cells in area 21a.

180.10

BINOCULAR INTERACTIONS IN THE ANTERIOR ECTOSYLVIAN CORTEX OF CATS. H. Jiang, F. Lepore, M. Ptito, A. Ptito, and J.-P. Guillemot. Groupe de Rech. en Neuropsy. Exp., Université de Montréal and Montréal Neurological Institute, Montréal, Qué., H3C 3J7.

AEC is considered a high order cortical association area. AEC neurons have been found to respond to several sensory modalities. The distribution of these modality specific cells along the anterior ectosylvian sulcus (AES) indicates that visual neurons are mainly concentrated in the ventral bank of AES. These neurons have large receptive fields (RF) and are mostly binocular. In order to test for binocular interactions, extracellular microelectrode recordings were carried out along the ventral and dorsal banks of AES in anesthetized-curarized adult cats. The RF for each eye was first determined using light or dark bars. They were then separated using divergent prisms and stimulated simultaneously (both eyes opened). Results demonstrated that: 1) most of the visual units encountered were binocular; 2) some of these cells responded also to other sensory modalities (e.g. auditory, somatosensory); 3) although most of cells showed some kind of binocular interactions, only about 1/4 of the sample showed clear interaction effects. These results taken in conjunction with previous data obtained in our laboratory point to the complexity of this high order area and indicate that it is probably involved in sensory and sensory-motor integration.

180.11

COVERT ORIENTING OF ATTENTION IN MACAQUE: I. BEHAVIORAL PARAMETERS. E. M. Bowman, V. J. Brown, U. Schwarz, and D. L.Robinson. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

It is well established from studies of humans that reaction times to visual targets are modified by antecedent cues (Posner, 1980). The cues are hypothesized to influence the direction of attention. We trained rhesus monkeys to perform a reaction time task with visual cues and altered various parameters which might modulate performance. We used nonparametric statistics to compare the distributions of responses in different conditions.

Reaction times for visual targets which are preceded by cues at the same location (validly cued) are faster than for targets which follow cues at other locations (invalidly cued). This differential responding (validity effect) diminishes with increasing cue-target intervals. When we used different experimental manipulations to speed or slow the animals' performance, the validity effect remained unchanged except when extremely fast responses were generated. The removal of visual landmarks indicating the locus of the target and cue produced a deterioration of the validity effect. When validly cued targets were most frequent, they were consistently responded to faster by both humans and monkeys. When validly cued targets were rare, humans were substantially slowed in responding to them; monkeys were also slowed but not as profoundly. There was a spatial gradient of the effects of cues on reaction times as the cue-target distance increased.

We conclude from these studies that a visual cue leads to a change in performance at its locus. The mechanisms which generate this effect in monkeys are probably involuntary but nonetheless can be modulated.

180.12

COVERT ORIENTING OF ATTENTION IN MACAQUE: II. A SIGNAL IN PARIETAL CORTEX TO DISENGAGE ATTENTION. D. L. Robinson, E. M. Bowman, and C. Kertzman. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Damage to parietal cortex in humans leads to neglect and extinction, and Posner et al (1984) have proposed that these patients cannot disengage their attention. We have recorded from neurons within the intraparietal sulcus in monkeys performing a cued reaction time task. Monkeys fixated a central spot of light and responded with a bar release to the appearance of peripheral targets which followed visual cues.

The discharge of a subset of neurons was best when the peripheral cue had directed attention away from the target within their visual receptive field. The differential activity was transient as was the behavioral effect of the cue. This neuronal activity might contribute to an attentional error signal. For the same neurons there was an enhancement of the visual response generated by active discrimination at the central spot when compared to response with simple direction of gaze at the same point. Here the best response was evoked when attention was actively directed toward the fixation point and not toward the receptive field. Modest response levels were also obtained when the monkeys saccaded repeatedly to a target within the receptive field as well as when the animals repetitiously responded with a bar release to a target in the receptive field. In contrast, infrequent and unexpected targets within the receptive field evoked strong responses.

These data suggest that intense activity from these neurons could be part of an attentional error signal contributing to neuronal events which disengage attention from its current focus. It occurs for targets when attention is directed away from the receptive field. The processes which generate this error signal can be evoked by sensory stimuli as well as various cognitive processes.

180.13

POSTERIOR CINGULATE CORTEX OF RHESUS MONKEY: MECHANISMS OF ORBITAL-POSITION AND POSTSACCADIC SENSITIVITY S. Y. Musil, C.R. Olson, and M. E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, and Department of Psychology, George Mason University, Fairfax, VA 22030.

The posterior cingulate cortex (Area 23) of the rhesus monkey is linked to parietal Area 7a by strong reciprocal pathways and so must contribute to visuospatial and attentional processes that depend on the latter area. We have monitored the activity of posterior cingulate neurons in monkeys performing visually guided eye movements with head fixed and have found that the level of tonic firing depends on two task parameters: the angle of the eye in the orbit and the size and amplitude of the immediately antecedent saccade (S.Y. Musil, C.R. Olson and M.E. Goldberg, "Visual and Oculomotor Properties of Single Neurons in Posterior Cingulate Cortex of Rhesus Monkey," Soc. Neurosci. Abstr. 16:1221, 1990). Thus posterior cingulate neurons carry information that could be used for spatial interpretation of visual signals.

Neurons exhibiting dependence on the orbital angle of the eye and on saccade direction and amplitude as described above, might be influenced by a variety of cues including extraocular efference copy and visual reafferent signals. To dissociate the relative contributions of these factors, we carried out experiments in which eye movements and the angle of the eye in the orbit were held constant while visual context was varied. Manipulations included removing the visible target during eye movements and fixation, imposing darkness on the surroundings and rotating the monkey relative to the surroundings. The results demonstrate that posterior cingulate neurons span a continuum with respect to their dependence on extraocular as opposed to visual cues, ranging from exclusive dependence on saccade metrics and orbital position to strong modulation by visual context.

180.14

SHAPE AND COLOR SELECTIVITY OF INFEROTEMPORAL NEURONS IN THE MACAQUE. H. Komatsu, T. Ideura*, and S. Yamane* Neuroscience Section, Electrotechnical Lab., Tsukuba, Ibaraki, 305, Japan.

Inferotemporal cortex (IT) of the macaque plays an essential role on the visual recognition of objects. Shape and color are both very important parameters for the recognition of objects. Previous studies have shown the existence of neurons that are sensitive to the shape of the visual stimulus and those sensitive to the color. However, no attempt has been made to compare the shape selectivity and the color selectivity of individual neurons systematically. We have tested quantitatively the shape selectivity, and compared it with the color selectivity of the same IT neurons. Monkeys were trained a fixation task, and a visual stimulus was presented at the center of the visual field while the fixation spot was blinked off. Shape selectivity of IT neurons was tested by comparing the responses to seven geometrical shapes. Color selectivity was tested by comparing the responses to 13 or more colors that distributed evenly on the CIE chromaticity diagram. On each test, other parameters were fixed at the optimal values. About 40% of the neurons we have tested were sensitive either to shape or color but not to both. The remaining 60% required a particular combination of shape and color for the maximal activation. These results suggest that color and shape of objects are coded redundantly in IT.

180.15

SELECTIVE NEURONAL RESPONSES TO COMPLEX VISUAL OBJECT-FEATURES ARE ALREADY PRESENT IN POSTERIOR PART OF THE MACAQUE INFEROTEMPORAL CORTEX.

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The macaque inferotemporal cortex (IT) is situated at a higher stage of the pathway responsible for object vision, and contains cells that selectively respond to complex visual features of objects. We previously divided IT into the posterior and anterior parts, which probably correspond to cytoarchitectural areas TEO and TE. To better know their functional differences, stimulus selectivities of cells in the two regions were compared by a method more objective than those in previous studies.

Cells were recorded from IT of anesthetized, immobilized monkeys (*M. fuscata*). The critical feature for the activation of each cell was determined by presenting many 3D objects, simplifying the images of effective objects with an image processor, and determining the simplest feature that evoked the maximal response. If the critical feature was more complex than bars or spots, response to it was then compared with responses to a set of simple stimuli including 32 spots and bars with different size, orientation, color, and contrast polarity and those prepared in relation to the individual critical feature.

There were cells that responded only to the particular complex feature (Elaborate cells), cells that showed some responses (25 to 75% of the response to the critical complex feature) to some of the simple stimuli (Intermediate cells), and cells that responded maximally to some of the simple stimuli (Primary cells). Elaborate cells predominated in the anterior IT, whereas all 3 types of cells were intermingled in the posterior IT. The Elaborate cells in the anterior IT had large receptive fields (10 to 25°) including the fovea. The posterior IT cells, regardless of the type, had small receptive fields (1 to 9°), which occupied various positions within 15° from the fovea. We suggest that integration of visual object information takes place serially: selective responses to complex features appear in the posterior IT and the selectivity becomes more precise and positionally invariant in the anterior IT.

180.16

INFERIOR TEMPORAL NEURONS CONVEY INFORMATION ABOUT STIMULUS PATTERNS AND THEIR BEHAVIORAL RELEVANCE.

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Lesions of area TE in the inferior temporal (IT) cortex of monkeys cause a profound deficit in learning pattern recognition tasks. A pattern recognition system must encode a current stimulus, recall a previous stimulus, compare the two, and decide based on the outcome of the comparison. Therefore, neurons in IT cortex ought to carry signals related to one or more of these processes.

To look for such signals, we recorded 50 single IT neurons from two monkeys performing a sequential match-to-sample task. A complete set of 32, black-and-white, 2-D Walsh patterns were presented under the sample, match and nonmatch conditions. The neuronal response waveforms were decomposed into their principal components (PC's). The weights of the PC's were examined by an analysis of variance (ANOVA), and were used to calculate the information transmitted about the spatial patterns and the behavioral conditions of the stimuli. We found that the neuronal response waveforms were significantly modulated by the pattern independent of the condition in 86% of the neurons, and by the condition independent of the pattern in 66% of the neurons (ANOVA, $p < 0.05$). This modulation conveyed information about both the pattern (0.355 bits) and the condition (0.045 bits). Pairwise comparisons of the various conditions (sample-match, sample-nonmatch, match-nonmatch) revealed that the neurons conveyed significantly more information in each of these than in the control sample-sample pair (t-test, $p < 0.001$). At the 1991 ARVO meeting we reported that IT neurons convey information about recalled stimuli suggesting that some IT neurons perform the comparison step of pattern recognition. The current results suggest that some IT neurons are also involved in the decision step of pattern recognition.

180.17

A CODE SIGNIFYING PATTERN RECOGNITION IN THE RESPONSES OF INFERIOR TEMPORAL NEURONS.

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In the preceding abstract we reported that when IT neurons are stimulated during a sequential match-to-sample task, the responses to a stimulus are frequently different across the sample, match, and nonmatch conditions. Thus, the temporally modulated responses convey information about the behavioral context of the stimulus presentation. This suggests that there might be a consistent coding scheme in IT neurons which signals the behavioral significance of visual stimuli.

To examine the response changes related to the behavioral context of the stimuli we quantified the responses according to the coefficients of the first three principal components. The average response to each pattern in each condition was graphed in a three dimensional space defined by the weights of the principal components. We observed that the responses to all of the stimulus patterns presented in one condition, e.g., the match, were restricted to a narrow region which we abstracted as a plane. In each of 15 neurons conveying large amount of information about stimulus condition, the planes representing the responses to the different conditions occupied different positions in the space. In eight of these neurons, the planes representing the responses to two conditions were shifted in position but remained parallel. These planes were not offset along any single axis in the response space, but rather, the offset represented a change along all of the principal components. In the remaining 7 neurons, the differences in the responses were represented by a combination of differing orientations and displacements of the planes. We hypothesize that in the neurons which show a parallel shift in the response planes, the difference vector between the planes could be a code representing the behavioral relevance of visual stimuli.

180.18

OSCILLATIONS IN THE RESPONSES OF NEURONS IN INFERIOR TEMPORAL CORTEX ARE NOT DRIVEN BY STATIONARY VISUAL STIMULI.

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Several groups have reported that the electrical activity of visual cortex in anesthetized cats shows oscillations in the range of 30 to 70Hz, in response to moving stimuli. This activity is strongest in the local field potential (LFP), but it can also be seen in the timing of action potentials (APs). Is this activity also present in inferior temporal (IT) cortex, an area known to be important for pattern recognition?

We used eight sites in area TE of IT cortex of monkeys performing a sequential match-to-sample task. At each site we recorded both the APs of visually responsive neurons, and the LFP from the same micro-electrode. The stimulus set consisted of 32 B&W stationary Walsh patterns presented 8 to 12 times each. Spike-triggered averaging of the LFP showed no relationship between the LFP and the APs. Both the number and temporal pattern of APs was significantly related to the stimuli at all 8 sites ($P<0.05$, ANOVA). In contrast, spectral analysis of the LFP showed that the frequency of the peak between 20 and 74Hz was significantly related to the pattern of the stimulus at only 2 of the 8 sites ($P<0.05$, ANOVA), and the pattern accounted for only 8.1±2.3% of the variance of frequency. The magnitude of the peak never showed a significant relationship to the stimulus, even when the frequency did. Therefore, any relationship between oscillations in the LFP and visual stimuli is weak at best. Calculating the information transmitted by the temporal pattern of the APs showed that increasing the bandwidth beyond 22Hz added no new stimulus related information. These results suggest that oscillations in the range beyond 20Hz do not play a role in the processing of information about stationary patterns in IT cortex.

180.19

A PREDICTIVE WARNING TONE MODULATES ACTIVITY OF TE UNITS IN MACAQUE.

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This study examined nonvisual and indirect influences on cells recorded in inferotemporal (IT) cortex. In order to see these inputs in isolation a fully split-brain, split-chiasm monkey was used and single units were recorded while the ipsilateral eye was covered. The completeness of the split was verified behaviorally by the failure of inter-ocular transfer. Over 300 units were recorded while the monkey was working on a visual discrimination task. Two phenomena have been observed. First, when the trials were separated by a pseudorandom inter-trial-interval (4-15s) over half of the cells showed a response to a warning tone (67 dB SPL) which indicated that the trial was about to begin. Although the identical tone also sounded to indicate the end of the trial, virtually no cells responded to this latter tone. In a second block of experiments, the majority of sessions were run with a fixed (predictable) inter-trial-interval. In this block, only about 10% of the cells showed a response to the warning tone. The second observation is that following the image presentation and despite the fully split condition some cells show an altered discharge rate locked to the stimulus presentation. No units were found responsive to the onset of the reward (a squirt of fruit juice) nor the panel push necessary to obtain the reward.

181.1

TONOTOPIC MAPPING OF SYNCHRONIZED NEURONAL ACTIVITY IN THE INFERIOR COLLICULUS OF THE RAT.

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Neuronal activity was recorded from the inferior colliculus (IC) of anaesthetized rats using tungsten microelectrodes. Pure tone continuous and burst stimuli were presented from 0.5 to 20 kHz (60-90 dB SPL). Novel stimuli consisted of mechanically induced impulse noises and mixed frequency tone bursts. One-second epochs of neuronal activity were digitized and stored on floppy disk for later analysis. Spikes of individual units were separated and discriminated into leading cell and mass activity categories. Records of neuronal activity were subjected to spectral analysis (FFT). Frequency histograms of interspike intervals were calculated in order to determine significant interactions between neurons. The most striking finding was that localized cell clusters in the central nucleus (ventrolateral division) and the external nucleus of the IC fired in a synchronized manner to reproduce the signatures of both mechanically and electro-magnetically produced sound stimuli. This effect was present only in specified regions of the auditory tectum and followed the general tonotopic organization of the inferior colliculus. The synchronizing effect did not persist after the animal was euthanized and did not appear in other CNS locations.

181.2

IPSI- AND CONTRALATERAL INTERACTIONS BETWEEN NEURONS RECORDED SIMULTANEOUSLY IN THE AUDITORY CORTEX OF BOTH HEMISPHERE. F. de Ribaupierre, G. Simm*, F. Giovannini*, Y. de Ribaupierre* and E. Rouiller. Inst. de Physiologie, Univ. de Lausanne, Bugnon 7, CH 1005 Lausanne, Switzerland.

Functional significance of interhemispheric connections in the auditory system was approached at the neuronal level. Eight single unit spike trains were recorded simultaneously from 7 microelectrodes placed in the primary (AI) and anterior (AAF) auditory cortices on both sides of a cat anesthetized with nitrous oxide and nembutal. Neuronal interactions were studied by cross-correlation techniques for 256 single unit pairs during spontaneous and acoustically driven activity.

For 116 pairs with both members being located on the same hemisphere, interactions observed were typical for the presence of a shared common input (CI), which was present for 18 % of the pairs. These CI occurred between pair members located within the same (AI-AI) or across different areas (AI-AAF), within the same cortical depth or between superficial and deep layers, and for quite a range of best frequency (BF) separations (with a slight preference for pairs having BFs within one octave).

Surprisingly pairs with members on each cortical side also presented CI for 11 % of them. These CI were also observed for all combination of areas, layers and BFs separations, with a preference for short BFs separations and heterotypic positions (17 % of CI for AI-AAF pairs versus 4 % for AAF-AAF or AI-AI pairs). It is quite significant that these interhemispheric neuronal interactions were only about half less frequent than those within one hemisphere.They could be due to the presence of a local branching, in the hemisphere of origin, of the callosal axons.

181.3

INHIBITION SHAPES RESPONSE TO INTERAURAL TIME DIFFERENCES IN THE INFERIOR COLLICULUS OF THE BARN OWL. J.A. Mazer and R. Adolphs, Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

The barn owl, *Tyto alba*, uses interaural time differences (ITD) to encode sound source location in azimuth. Neurons in several nuclei below the level of the inferior colliculus (IC) are sensitive to interaural phase differences at their best frequency. Only at the level of the external nucleus of the inferior colliculus (ICx) does one find neurons capable of responding selectively to a particular ITD without ambiguity of phase, provided that the sound's spectrum contains more than one frequency. ICx neurons are broadly tuned to frequency, suggesting that one way in which phase ambiguity is resolved at this stage is by combining the responses to ITD across several frequency channels. We investigated the role of nucleus ventralis lemnisci lateralis, pars anterior (VLVa) in generating ITD-tuning in the ICx.

VLVa provides polysynaptic input to the contralateral ICx and contains sharply frequency-tuned neurons that are sensitive to interaural phase differences. We microiontophoretically applied agonists (GABA, muscimol) or antagonists (bicuculline methiodide, BMI) of GABA-A receptors to VLVa neurons and found that agonists decreased, and antagonists increased the evoked response rates. In subsequent experiments, we pressure injected these drugs into VLVa while recording evoked responses to ITD from single neurons in the contralateral ICx. We found that: 1. Increasing neural activity in VLVa with BMI led to a decreased response to ITD in the ICx; 2. Decreasing neural activity in VLVa with GABA or muscimol led to an increased response to ITD in the ICx; 3. These effects were greatest when the best frequencies of the VLVa injection site and the ICx neuron recorded from were matched; 4. In some experiments, VLVa injections differentially affected ICx responses at the real ITD (main peak) and at phase-ambiguous ITDs (side peaks) in response to noise stimuli. These results suggest that VLVa provides functional inhibition to ICx in response to ITD, that the projection from VLVa to the inferior colliculus may be topographically organized in the frequency domain, and that VLVa may contribute to side-peak suppression of responses in the ICx.

R.A. is a Howard Hughes Medical Institute Fellow.

181.4

RESPONSE PROPERTIES OF NEURONS IN THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS OF THE MUSTACHED BAT. W.E. O'Neill, J.R. Holt, and M.L. Zettel, Dept. of Physiology, Univ. of Rochester Medical Center, Rochester, NY 14642

The ventral nucleus of the lateral lemniscus (VNLL) is highly developed in echolocating animals, but the response properties of cells within it are almost completely unknown. To determine the response properties of VNLL neurons, we recorded single unit activity in awake mustached bats. Recordings were made passing through the inferior colliculus (IC) on the way to the VNLL, allowing comparison of responses to similar stimuli in both nuclei. Stimuli consisted of 30 ms tone bursts, sinusoidal amplitude modulated tones, linear frequency modulations (12 kHz bandwidth), or wide-band noise bursts (WBN). Cells within the columnar VNLL were isolated over unusually small distances (30-50 μm), and best frequencies varied by as much as an octave from cell to cell. VNLL units were broadly tuned (Q-10dBs from 2 to 11), and often had lower thresholds to WBN. Discharge patterns were of the ON or ONSET type. In contrast to IC cells, latency was invariant with intensity as well as rate of intensity change (rise-time). Thus they resemble "phasic constant latency responders", which are common in FM bats, but rare in the mustached bat IC. Such cells are theoretically important for target range determination and/or fine feature discrimination

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181.5

CORTICO-CORTICAL CONNECTIONS OF RANGE AND VELOCITY PROCESSING AREAS IN THE AUDITORY CORTEX OF THE MUSTACHED BAT. D. C. Fitzpatrick and N. Suga. Department of Biology, Washington Univ., St. Louis, MO 63130.

In the auditory cortex of the mustached bat, two types of biosonar information are initially processed separately; target range in the FM-FM area, and target velocity in the CF/CF and DSCF areas. Since the initial processing stages of these two types of information are entirely separate, we questioned whether range and velocity information comes together elsewhere in the auditory cortex. Consequently, we made injections of anatomical tracers in these different cortical areas, and looked for common targets.

Injections in the FM-FM area labeled two targets in the AC, the dorsal fringe (DF) area and the ventral fringe (VF) area. Both the DF and VF areas are known to contain target ranging neurons. Injections in the CF/CF area also labeled two AC targets. One of these was in the posterior bank of the Sylvian fossa at a relatively dorsal location, which we have called the dorsal intrafossa (DIF) area. The other was in a part of the ventroanterior (VA) area that contains neurons tuned to the second and third harmonic constant frequency components of the biosonar signal. Injections in the DSCF area resulted in labeling in the DIF and VA areas, but the VA area label was in a location posterior to that from CF/CF area injections, in a part of the VA area that contains neurons facilitated by pulse-echo pairs.

To date, we have not labeled any targets in common following injections in range and velocity processing areas, so these processing systems appear to remain separate throughout the auditory cortex.

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181.6

NEURONS IN THE DSCF AREA OF THE AUDITORY CORTEX OF THE MUSTACHED BAT ARE SENSITIVE TO COMBINATIONS OF NON-HARMONIC FREQUENCIES. J.S. Kanwal, D.C. Fitzpatrick and N. Suga, Dept. of Biology, Washington Univ., St. Louis, MO 63130.

The Doppler-shifted constant frequency (DSCF) area includes about 30% of the primary auditory cortex of the mustached bat, *Pteronotus parnellii parnellii*, and overrepresents 60-63 kHz, so that this area is considered to be specialized for processing the second harmonic CF component of a biosonar signal. We unexpectedly found that about 50% of the neurons in this area show facilitative responses to combinations of low (25.05±1.86 kHz; n=43) and high (61.30±1.04 kHz) CF tones. The biosonar signal of the mustached bat consists of four harmonics (CF_1-CF_4 and FM_1-FM_4); 61.30 kHz represents the typical CF_2, but 25.1 kHz is lower than the range of CF_1 frequencies and is contained at the end of a downward FM_1 sweep (30.5-24.5 kHz). These neurons also show a facilitative response to combinations of CF_2 and FM_1. To further characterize the basic response properties of the facilitative neurons, we obtained excitatory, facilitatory and inhibitory tuning curves. The facilitative response was tuned sharply to the high frequency component and broadly to the low frequency component. Moreover, the facilitatory tuning curves were sandwiched between inhibitory tuning curves and the facilitative response exhibited lower thresholds to the high frequency component than the low frequency component. These properties can be useful for processing sounds with a complex acoustic structure, such as communication calls which range from 7-80 kHz and contain both CF and FM sounds. This subpopulation of DSCF neurons may, therefore, process non-biosonar as well as biosonar sounds.

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181.7

EFFECTS OF INHIBITORY AMINO ACID ANTAGONISTS ON AMPLITUDE TUNING IN THE AUDITORY CORTEX OF THE MUSTACHED BAT

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Unlike peripheral neurons, the majority of neurons in the Doppler-shifted constant frequency (DSCF) area of the primary auditory cortex of the mustached bat are tuned to particular amplitudes as well as particular frequencies. This amplitude tuning (non-monotonic impulse-count function) is associated with inhibition at high amplitudes (Suga and Manabe, 1982). Since 79% of collicular neurons are tuned in amplitude (O'Neill, 1985), most of the amplitude tuning of DSCF neurons is created by subcortical nuclei. In the cortex, GABAergic endings are abundant. We therefore studied whether amplitude tuning is sharpened by inhibition within the cortex. A multibarrelled electrode was inserted into the DSCF area, and inhibitory amino acids and their antagonists were iontophoretically applied. All 40 neurons studied showed non-monotonic impulse-count functions. Of these, 35 showed upper thresholds. Bicuculline methiodide (BMI) increased the response magnitude in 15/17 neurons tested, while strychnine increased the response in 6/16. The amplitude tuning curve at a best frequency became broader in 14/17 of neurons by BMI and in 11/16 of neurons by strychnine. Upper thresholds were eliminated in 5/13 neurons by BMI and 2/16 by strychnine. However, BMI and strychnine did not change the non-monotonic impulse-count function into a monotonic impulse-count function. These data indicate that amplitude tuning created in subcortical nuclei is sharpened in the cortex.

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181.8

ACUITY IN RANGING BASED UPON NEURAL RESPONSES IN THE FM-FM AREA OF THE MUSTACHED BAT. M. Suzuki* and N. Suga.
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Bats can detect 60-100 μs differences in echo delay and 0.5 μs echo jitters (J. A. Simmons, J. Acoust. Soc. Amer. 54:157 1973, J. A. Simmons, Science 204:1336 1979). In the auditory cortex of the mustached bat, *Pteronotus parnellii parnellii*, neurons tuned to particular echo delays (target ranges) are clustered in three different areas. The FM-FM area is the largest and represents echo delays from 0.4 to 18 ms. Can the acuity in the behavioral data be explained by the neurophysiological data? That is, what level of acuity in ranging can be theoretically derived from neural activity in the FM-FM area? Using the neurophysiological data (e.g. N. Suga, Neural Networks, 3:3 1990), the theoretical acuity was computed as just-noticeable changes at 75 % correct level in (1) the position of maximally responding neurons along the range axis in the FM-FM area, (2) the weighted sum of responses of all FM-FM neurons, and (3) the center of neural activity over the FM-FM area. The just-noticeable changes depend upon both the stability of neural response and absolute echo delay (target range). Assuming the response of a single neuron fluctuates with 30 % standard deviation of its mean response, the just-noticeable changes in echo delay at the range of 30-120 cm are 110-305 μs, 2.2-34 μs and 2.4-4.0 μs for (1), (2) and (3), respectively. For 10 % standard deviation, the values are 106-240 μs, 0.73-11 μs and 0.80-1.3 μs for (1), (2) and (3), respectively.

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181.9

MNTB PROJECTION TO THE MSO IN RODENTS AND BATS. II: TOPOGRAPHICAL PROJECTIONS AND TERMINAL FIELDS. N. Kuwabara, and J.M. Zook. *Dept. of Zool. and Biomed. Sci. and COM, Ohio University, Athens, OH 45701.*

Previously, we have shown single collateral axons projecting from principal cells of the medial nucleus of the trapezoid body (MNTB) to the medial superior olive (MSO) (Kuwabara et al., *Neurosci. Abstr.* 16:723, 1990). Since MNTB principal cells supply inhibitory input to the lateral superior olive (LSO), their collaterals may provide inhibitory input to the MSO. We are continuing to investigate this projection in the rodent species, *Mus musculus* and *Meriones unguiculatus*, and the bat species, *Eptesicus fuscus* and *Pteronotus parnellii*. MNTB principal cells or their axons were intracellularly labeled with Lucifer Yellow or Lucifer Yellow cadaverine biotin-X in tissue slice preparations of the auditory brainstem.

Here we show that collateral axons of MNTB principal cells project in a topographic pattern to the MSO in all of the species examined. In many cases, the size of the terminal field and the density of axosomatic terminal endings in the MSO were similar to the size and density of the main axon's projection to the LSO. The specific pattern of inputs from the MNTB may play a role in shaping many of the response characteristics of MSO units such as phasic discharge pattern, non-monotonic rate-level function and sharpness of the ITD curve. (Supported by NIH Grants, DC00503, DC00038 and OUCOM)

181.10

TONOTOPIC SHIFTS IN THE AUDITORY PATHWAY OF THE MUSTACHED BAT: COCHLEAR, NEURAL AND BEHAVIORAL CORRELATES. R.F. Huffman and O.W. Henson, Jr.* Curr. in Neurobiology and Dept. Cell Biology & Anatomy, Univ. N. Carolina, Chapel Hill, NC 27599.

Correlates of markedly sharp tuning in the mustached bat *(Pteronotus parnellii)* include: 1) pronounced cochlear resonance, the frequency (CRF) of which is near the 2nd harmonic, constant frequency component (CF2) of the biosonar pulse; 2) numerous sharply tuned neurons throughout the auditory pathway that collectively over-represent the CF2; and 3) Doppler-shift compensation (DSC), whereby the flying bat adjusts the CF2 to maintain the echo CF2 within a narrow band. Previous studies have shown that the CRF changes with flight, noise exposure and body temperature (BT) (Kössl & Vater, 1985, Hear. Res. 19:157; Henson et al., 1990, Hear. Res. 50:259; Huffman et al., 1990, ARO Abst. 13:232). The purpose of this study was to determine whether small temperature-dependent shifts in the CRF produce concomitant changes in the response properties of "CF2 neurons" and CF2 biosonar emissions.

Cochlear microphonic (CM) electrodes were chronically implanted in seven bats, one week prior to experiments. CRF was determined from FFT analysis of CM potentials. Shifts in CRF were induced by controlled adjustments of BT (within normal physiological range for active bats, 36-42°C). DSC was examined by swinging bats on a pendulum toward a target and analyzing the spectral content of emitted pulses and echoes. The preferred echo CF2 and the CF2 emitted from stationary bats both shifted in parallel with changing CRF. In four awake bats, 29 CF2 single and multi-units in the cochlear nucleus and inferior colliculus were characterized. In every case, a CRF shift produced a corresponding change in the best-response frequency (BF). Results were the same for single and multi-units in both nuclei. Although BF's changed reliably with CRF shifts, 72% of the units showed no change in threshold or Q-10dB; this suggests a shift in the position of the place code within the sharply tuned segment of the cochlea. It is concluded that labile cochlear tonotopy in the CF2 region causes shifts in central tonotopy and in the frequency of the emitted CF2.

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181.11

INTRINSIC AND COMMISSURAL CONNECTIONS OF THE RAT INFERIOR COLLICULUS.

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In spite of its importance in the auditory brainstem circuitry, the intrinsic organization of the inferior colliculus (IC) is still poorly understood. Its central nucleus (CN) and dorsal cortex (DC) are tonotopically organized, but the relationships of the CN to the external cortex (EC) and the contralateral IC remain unclear. To analyze the intrinsic and commissural projections of the IC, the anterograde tracer *Phaseolus vulgaris* -leucoagglutinin (PHA-L) was iontophoretically injected at different locations within the CN of adult rats, and visualized immunocytochemically.

Each injection gives rise to four fibrillar, laminar plexuses, two in each IC, that span the IC rostrocaudally. The first ipsilateral plexus, situated in the CN, is parallel to the known isofrequency planes of the IC, and penetrates the DC dorsally. The second plexus is located in the EC, slightly oblique to the pial surface. These two plexuses meet caudally and ventrally. In the contralateral IC, the two plexuses occupy positions symmetrical to those of the ipsilateral side, forming a mirror-like image. The mediolateral and dorsoventral position of the four plexuses changes as the position of the injection site is varied. This systematic variation is especially pronounced when the injection site is displaced along the tonotopic axis of the CN, indicating that the intracollicular projections, both intrinsic and commissural, follow an orderly, tonotopic pattern. The projection from the CN to the EC is tonotopic, thus suggesting the existence of a tonotopic organization for the EC.

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181.12

THE AUDITORY CORTEX INNERVATES THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS

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It has generally been accepted that the descending projection from the auditory cortex to the inferior colliculus (IC) innervates its dorsal (DC) and external (EC) cortices, and that its central nucleus (CN), by contrast, does not receive direct neocortical input (Faye-Lund, 1985, Anat. Embryol., 173:53-70). To study the topography and morphology of the cortico-collicular projection, the anterograde tracer *Phaseolus vulgaris* - leucoagglutinin (PHA-L) was iontophoretically injected at different locations within the primary auditory cortex of adult albino rats.

Each injection gives rise to three fibrillar, laminar plexuses in the IC, two in the ipsilateral and one in the contralateral side, that span the IC rostrocaudally. The plexuses consist of thin fibers with multiple collateral branches, and small *en passant* and terminal boutons. The first ipsilateral plexus, located in the medial half of the IC, has a dorsomedial to ventrolateral orientation, parallel to the isofrequency planes of the IC. This plexus is continuous through the DC and CN, thus proving that the CN receives innervation from the auditory cortex, contrary to the previous tenet. The second plexus is located in the EC, slightly oblique to the pial surface. These two plexuses appear to meet caudally and ventrally. The plexus in the contralateral IC occupies a symmetrical position to that of the first ipsilateral plexus. The mediolateral and dorsoventral position of the three plexuses changes as the injection site is displaced rostrocaudally. These topographic variations conform to a pattern that mimics that of the intracollicular projections (see Saldaña, Soc. Neurosci. Abstr., 1991).

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181.13

FUNCTIONAL ORGANIZATION AND RESPONSE PROPERTIES OF LOW-FREQUENCY, NOISE-SELECTIVE NEURONS IN THE INFERIOR COLLICULUS OF THE PALLID BAT. J. Kennedy and Z.M. Fuzessery. Depts. Psychology and Zoology/Physiology, Univ. of Wyoming, Laramie, WY 82071.

The auditory system of the pallid bat (*Antrozous pallidus*) is specialized for two different spatial tasks. This species emits high-frequency (30-90 kHz) pulses for echolocation, but relies on passive hearing at low frequencies (< 20 kHz) to locate terrestrial prey. This study examines the functional organization and response properties of the low-frequency, noise-selective neurons in the central nucleus of the inferior colliculus (ICC) that may serve passive prey localization .

The tonotopy of the ICC was evaluated through multiunit recording with tungsten electrodes, and recording sites were reconstructed from electrolytic lesions. These studies revealed that the ICC of this bat has a disproportionately large representation (> 50%) of low frequencies (2-25 kHz). In some cases, neurons tuned to frequencies of less than 10 kHz were encountered throughout entire dorsoventral electrode penetrations through the ICC.

Many low-frequency neurons were noise-selective in that their response thresholds for noise were up to 30 dB lower than for tones. They exhibited a high degree of temporal precision in their ability to time-lock to the very short (10 μsec) components of amplitude-modulated noise, and a high degree of response synchrony to amplitude modulation rates of greater than 2000 Hz. The temporal response properties of these cells likely serve in the localization of walking prey through the resolution of ongoing interaural delays in noise.

181.14

AUDITORY AFFERENT INPUT TO THE INFERIOR COLLICULUS OF THE CHICKEN (GALLUS DOMESTICUS). D.W.F. Schwarz, A. Dezso * and I.E. Schwarz*. The Rotary Hearing Ctr., Univ. of British Columbia, Vancouver, B.C., Canada.

If a temporal analysis of the sound waveform occurs after the combination of the binaural input in the laminar nucleus (NL), one would expect faithful phase locking in inferior colliculus (IC) neurons driven with stimuli from both ears (EE-cells). At optimal interaural delays such cells, recorded extracellularly in the anaesthetized chicken, encode the sound period at least as well as cochlear afferents. At the least favourable delays spike counts tend to be lower than during monaural stimulation of either ear, indicating phase locked inhibition. Candidate sources of such phase matched inhibitory contribution are the superior olive (OS) and lateral lemniscal nuclei (VLVa, VLVp and LLv), all of which can be retrogradely labelled from IC with various tracers. OS and LLv contain a similar proportion of neurons projecting to IC of both sides as the NL (ca 0.5%), as they incorporate different dyes (True Blue and Diamidino Yellow) injected into each IC. Thus several pathways exist which might contribute to the conservation, or perhaps improvement, of the temporal code available at the mesencephalic level.

Supported by MRC, Lions' MD19 Hearing Fdn and Rotary Hearing Fdn.

181.15

ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS IN THE CHICK NUCLEI MAGNOCELLULARIS AND LAMINARIS. A. D. Reyes, R. L. Hyson, E. W Rubel Hearing Development Laboratories, Univ. of Washington, Seattle, WA 98195.

Nucleus magnocellularis (NM) and nucleus laminaris (NL) contain, respectively, the second- and third-order neurons of the chick auditory system. NM receives direct input from the VIIIth nerve and projects ipsilaterally and contralaterally to NL. Characterizing the electrophysiological properties of these neurons is important for understanding how sensory information is transmitted through the auditory system. We performed whole cell recordings from these neurons using an *in vitro* slice preparation. The low resistance electrodes used (5-10MΩ) afforded injection of large current and facilitated application of voltage clamp.

Recordings were made from transverse slices (300 - 450μm) of the chick (5-7 days old) brain stem containing the VIIIth nerve, NM, and NL. Patch electrodes were filled with (in mM): KCl (17.5), K-gluconate (65), HEPES (10), NaCl (9), $MgCl_2$ (5), and Na_2ATP (2). The electrode was slowly driven into the slice while applying positive pressure to prevent clogging. Upon contact with a neuron, negative pressure was applied until a tight seal was formed and the underlying membrane was ruptured.

Neurons in both NM and NL exhibited decreased slope resistance at depolarized levels; voltage response to depolarizing current steps were significantly less than to hyperpolarizing steps. Suprathreshold d.c. stimulation caused a single action potential after which the membrane potential remained below threshold for the duration of the step. In NM, EPSPs evoked at rest by stimulation of VIIIth nerve were brief but broadened when the cells were hyperpolarized. Voltage clamp studies revealed that these effects are due to an outward current that: 1) is persistent; 2) is partially activated at rest (-50 to -60mV); 3) increases activation with depolarization, and 4) reaches steady-state values within 2ms. This ionic conductance, by attenuating depolarizing d.c. potentials, may allow neurons of NL and NM to phase-lock to high frequency stimuli. Various pharmacological blocking agents are currently being used to further characterize this conductance. (Supported by DC00395 and DC00858)

181.16

CONNECTIONIST MODELS OF ZEBRA FINCH AUDITORY NEURONS: DYNAMIC NEURONAL PROPERTIES PREDICT RESPONSES TO COMPLEX STIMULI. S. C. Bankes and D. Margoliash. Rand Corp., Santa Monica, CA and Dept. Organismal Biology and Anatomy, Univ. of Chicago, Chicago, IL.

We constructed parametric models of ovoidalis and HVc neuronal responses to natural and artificial auditory stimuli in urethane-anesthetized zebra finches. An exploratory modeling approach was employed where a variety of parametric model architectures were trained and evaluated. Connectionist models of time varying (dynamic) responses were explored although some non-connectionist and static models were also tested. Architectural variations included representing time through delay lines vs. recurrence, variations of the input representation (sonogram, sonogram and RMS amplitude, cochlear model) and variations of the numbers of units and tunable parameters.

Explorations were conducted for each architecture to determine the minimum training set required for good performance. For cells from ovoidalis, models trained with dynamic response to tone bursts (instantaneous firing rate) could predict responses to complex stimuli (bird song). This is a significant result, as a quantitative relationship between central neuronal responses to simple artificial and complex natural stimuli has rarely been achieved. Models trained only with average firing rate information did not accurately predict such responses. This suggests that dynamic models of neuronal responses can capture important information missing in models using only average firing rates, even for cells with "classical" response properties. This modeling paradigm can therefore provide a useful tool for neuroscience. Supported by ONR, NIH and the Searle Scholar Program.

181.17

THALAMIC INPUT AND CYTOARCHITECTURE OF AUDITORY NEOSTRIATUM IN ZEBRA FINCH. E.S.Fortune and D.Margoliash. Dept. of Organismal Biol. and Anat. Univ. of Chicago, Chicago, IL, 60637.

Song learning modifies HVc auditory neurons. Field L (FL) is the recipient of efferents from n. ovoidalis (OV) and is a likely source of auditory input to HVc. We have examined the cytoarchitecture of FL in celloidin embedded material and the organization of thalamic projections to FL and adjacent neostriatum.

The primary thalamorecipient zone is a plate extending dorsocaudally from the dorsal medullary lamina (LMD) coursing across the neostriatum below the hyperstriatal lamina. This plate, designated L2a, is coextant with the internal capsule. Large and small oblong neurons aligned parallel to the major axis of the plate are visible in L2a of Nissl stained parasagittal sections. Dorsocaudal to L2a is L2b of Wild (1990), which is a cloud of densely packed small neurons. A band of small neurons extends in an arch from L2b caudoventrally towards LMD. L3 is the area bounded by this band of neurons caudally, LMD ventrally, and L2a rostrally, and is composed of large clustered fusiform neurons. L1 is a plate of large and small clustered neurons which lies dorsoanterior to L2a. Axons from OV, as demonstrated by HRP and fluorescent tracers, pierce LMD laterally and travel dorsocaudally through L2a, and to a lesser extent through L1 and L3. Axons in L1 and L3 arch into L2a. These axons have at least three types of specializations: large (~8μm) and small (~2μm) varicrosities, and some axons bifurcate before entering L2a. N. ovoidalis medialis, adjacent to OV, appears to send axons to L2b. Neurons in the tractus ovoidalis project to regions caudal to FL and medial to HVc. We continue to investigate parallel auditory pathways to the neostriatum and the sources of auditory input to the song system. Supported by NIH grant NS25677 to DM.

181.18

AUDITORY RESPONSES IN THE NUCLEUS OVOIDALIS ARE NOT SO SIMPLE. B.Diekamp and D.Margoliash. Department of Organismal Biology and Anatomy. Univ. of Chicago, Chicago, IL, 60637.

Neurons in the thalamic nucleus ovoidalis (OV) have been shown to be largely uniform in their responses to acoustic stimuli (Bigalke-Kunz et al., 1987). OV is tonotopically organized, and neurons typically have high spontaneous activities and tonic discharges. Frequency tuning curves of OV neurons display an excitatory area flanked by inhibitory sidebands. Recent anatomical studies, however, indicate that the OV complex may consist at least of three subdivisions. We are studying the response properties of neurons in OV of male zebra finches, looking for physiologically different, functional zones that might relate to the anatomical data.

We are using the technique of analog spike waveform classification, permitting the isolation of several single cells per recording site and the study of a large sample of OV neurons. A stimulus set consisting of simple and complex stimuli is used to thoroughly characterize response properties. All neurons are tested with tone bursts (250 - 7250 kHz) and white noise at intensities between 10 and 80 dB SPL, linear amplitude-modulated (AM) white noise, frequency-modulated tones, harmonics, the bird's own song (BOS) and songs of four conspecifics. To date, the following observations have been made. Neurons vary considerably in their spontaneous activities, latencies, sensitivity and sharpness of frequency tuning, discharge patterns to simple tone bursts, phase locking to AM noise, and response strengths to the five conspecific songs including BOS. Neurons recorded at the same location have either similar, correlated response properties or show different latencies and discharge patterns (tonic or phasic). Characteristic frequencies of neurons at some recording sites differ by more than one octave suggesting substantial scatter in the tonotopic organization of single neurons in OV. These preliminary data indicate that neurons in OV might belong to physiologically diverse populations. (Supported by NIH grant NS25677 to DM.)

181.19

POSSIBLE MECHANISMS OF HIGH FREQUENCY PHASE COMPARISON IN BARN OWLS. W.E. Sullivan, Dept. of E.E. Biology, Princeton Univ., Princeton, N.J. 08544.

Barn owls detect binaural phase differences in high frequency (5 to 9 kHz) sounds for horizontal localization. This involves convergence of phase-locked spikes from the magnocellular cochlear nuclei onto cells in nuc. laminaris. Laminaris neurons fire most strongly when inputs are coherent, suggesting that they act as coincidence detectors.

The present work was designed to understand this process in more detail. It combines an analysis of the synaptic input pattern that would be conveyed by about 100 afferents from each side with a study of spike output mechanisms. Input analysis shows that unitary synaptic conductance changes can be longer than the stimulus period if their shape is asymmetrical. In this case, coherent inputs produce a conductance modulation at the stimulus frequency whereas out-of-phase inputs do not produce this information bearing signal. Both cause modulations at lower frequencies due to random variations in spike arrival.

To explore how laminaris cells could detect rapid conductance changes, a standard spike generation model (Hodgkin and Huxley 1952) was used. Several modifications need to be made to produce maximal selectivity at high frequencies. These include increases in both channel density and gating rate. Increasing the density and gating rate of Na+ channels allows the model to respond to rapid conductance modulations. Increasing K+ channel density and gating rate reduces the response to slower modulations, characteristic of synaptic noise. The resulting spike generator responds poorly to steady or slowly changing excitatory conductances but strongly to rapid modulations of synaptic input.

181.20

COCHLEAR AXON MORPHOLOGY, COCHLEAR NUCLEUS VOLUMETRIC CHANGES, AND THEIR POTENTIAL ROLE IN DEVELOPMENT OF TUNING IN THE DORSAL COCHLEAR NUCLEUS OF THE HAMSTER. L. Schweitzer and T. Cecil*, Department of Anatomical Sciences and Neurobiology, Univ. of Louisville School of Medicine, Louisville, KY 40292

While regressive events (e.g., loss of axonal branches) underlie the development of tuning in some systems, we have determined that axonal morphology in the dorsal cochlear nucleus (DCN) of the hamster is stable after initial ingrowth. WGA-HRP with poly-L-ornithine was iontophoretically injected into the cochlear nerve root of brainstem slices (from hamsters aged PND 2-37) maintained for 4 hours in artificial cerebrospinal fluid (31º C, bubbled with O_2/CO_2). Frozen sections of the slices were reacted with DAB allowing visualization of individual axons in the DCN. On PND 2 the axons are in the deep DCN and reach the top of the fusiform cell layer by PND 10. In addition, topographically-related axons form defined sheets (the anatomical analog of isofrequency planes) by PND 10. After this age, the branching and expanse of the axonal fields changes very little. In contrast to the constancy of the axonal fields, after day 10 the volume of the DCN more than doubles, and the number of large cell bodies within an axon's field decreases by about 30%. Consequently, as development proceeds, each large soma is in the domain of fewer and fewer cochlear axons. These anatomical changes may underlie the successive narrowing of physiological tuning curves reported for auditory cells in developing mammals.

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181.21

LOCALIZATION OF NON-LINEAR TEMPERATURE EFFECTS IN THE FROG AUDITORY SYSTEM. M. B. Carey and R. Zelick. Dept. of Biology, Portland State University, P. O. Box 751, Portland, OR 97207.

Multiunit auditory midbrain recordings in frogs show non-linear temperature effects on threshold (Hubl & Schneider, J. Comp. Physiol. 130:17, 1979), however, recent data show linear changes in VIIIth nerve parameters (van Dijk et al, Hear. Res. 44:231, 1990).

We have examined the effect of temperature on the auditory brainstem response (ABR) in *Rana pipiens*, a technique which provides simultaneous whole-system performance at peripheral and central levels. The ABR was obtained using chronic electrodes, and evoked by 1.0 kHz single-period sinusoids presented at 10/sec. Amplitude and latency of ABR peaks I-IV were measured at a supra-threshold stimulus level (88 dB SPL). Threshold was determined using visual criteria.

Frogs were acclimated to room temperature (19 - 21°C). During recording, temperature was changed from 10 - 32 °C by passing warm or cold water through a copper manifold on which the frog was placed. Cranial temperature was measured by a thermocouple implanted along with ABR electrode.

The temperature Q_{10} for I-II interpeak latency (VIIIth nerve level) was 1.45, while the Q_{10} for the III-IV interpeak latency was 1.36 above 16 °C (similar to the periphery), but 3.62 below 16 °C. Peaks III-IV are generated before the midbrain torus semicircularis level. Threshold was more stable at warm temperatures, with a Q_{10} of 1.00 above 16 °C, but like latency increased markedly (Q_{10} = 1.83) below 16 °C. This represented a change in threshold, from ≈30 dB SPL above 16 °C to 49 dB SPL at 10 °C.

Thus the non-linear temperature dependence in the frog auditory system appears to be localized to the brainstem.

AUDITORY SYSTEM: CENTRAL PATHWAYS IV

182.1

CORTICAL AFFERENTS FROM THE MONKEY MEDIAL GENICULATE COMPLEX: PHA-L TRACING AND CALCIUM BINDING PROTEIN IMMUNOHISTOCHEMISTRY. T. Hashikawa[1], M. Molinali[1,2] and E. G. Jones[1,3]. [1]Neural Systems Lab., F.R.P., RIKEN, Wako 351-01, Japan. [2]Catholic University, Rome, Italy. [3]Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Thalamocortical neurons of monkey medial geniculate complex have been shown to contain either parvalbumin or 28 Kd calbindin. These neurons have layer specific cortical projections (Brain Res., 544: 335-341, 1991). In the present study this differential pattern of the geniculate projections was shown by anterograde transport of Phaseolus vulgaris leucoagglutinin (PHA-L) combined with immunohistochemistry for the proteins.

Electrophoretic injections of PHA-L into small areas about 500 um in diameter, of the ventral and dorsal divisions of the medial geniculate body of *Macaca fuscata* resulted in labeling of geniculocortical fibers, forming plexuses with terminal-like swelling in layers IIIB and IV of primary and neighboring auditory areas. Few fibers projected to other layers. The major plexus in the primary cortex extended several hundred microns in width and continued several millimeters rostrocaudally. Immunohistochemically, most fibers were parvalbumin-positive, and calbindin-positive fibers were rare. These results fit with the previous immunohistochemical findings and also suggest that auditory information from a limited region of the medial geniculate nuclei is distributed along isofrequency bands in primary auditory cortex and in corresponding zones of adjacent areas.

182.2

A METHOD TO COMPUTE THE ORIENTATION AND 3-DIMENSIONAL (3-D) ANATOMY OF LAYERS IN THE INFERIOR COLLICULUS (IC).

S. Ahghari*, D.L. Oliver, and T. Ju*, Dept. Anatomy, Center for Neurological Sciences, UCONN Health Center, Farmington CT 06030; Dept. Electrical and Systems Engineering, UCONN, Storrs, CT 06269-3115.

The fibro-dendritic layers in the central nucleus of the IC may provide an important substrate for integration of monaural and binaural inputs. To graphically reconstruct and quantify the 3-D orientation of these layers, we have studied serial Golgi-Cox impregnated sections in low, mid and high frequency regions of the central nucleus. At each site in each 100 µm-thick section, an argon laser scanning confocal microscope (Bio-Rad) is used to take a series of images at discrete steps along the Z-axis. These images are processed on a Gould IP8500 by an algorithm to preserve the closely spaced layered dendrites and eliminate the non-layered processes. Four different gradient (directional) filters plus median and low pass filters are used to extract the contours of the layers from each image. After processing, the bit-mapped images are converted to vector lists and the objects that represent layered cells are selected interactively. The 2-D orientation of the layered cells in each image is determined by performing a linear regression analysis. To create a 3-D surface model that represents a single layer at that site, the regression lines from each image in the Z-series are connected. The spatial orientation of the resultant surface is calculated with two vector cross products at two opposite corners of each non-planar polygon. The average direction of the layer at that site is represented as the average of the normal vectors for the surface model. All of the 3-D surface models from a single brain are displayed together with the outline of the IC on a IRIS 4D/310GTX workstation (Silicon Graphics). The results show the central part of the central nucleus has a uniform layering pattern, while more medial and lateral parts have more curvature.

(Supported by NIDOCD grant R01-DC00189.)

182.3

COCHLEAR NUCLEUS INNERVATION OF THE CONTRALATERAL LATERAL SUPERIOR OLIVE IN THE NEONATAL GERBIL. L.M. KITZES, J. KIL and G. KAGEYAMA, Dept. of Anatomy and Neurobiology, University of California Irvine, Irvine, CA 92717

A small projection from the cochlear nucleus (CN) to the contralateral lateral superior olive (LSO) has been described in the adult cat and bat. We have observed this projection in the adult gerbil. As part of our study of the development of efferent projections of CN, we have examined the extent of this projection in the neonatal gerbil.

A crystal of Di-I was placed in the ventral CN of gerbil pups ranging in age from one to 14 days. After maintaining the brains in an oven at 37°, the brains were cut in the coronal plane and the projection from CN to the contralateral LSO evaluated using a fluorescence microscope. Some sections were then prepared for electron microscopic analysis by photo-oxidizing labeled fibers in a solution of DAB.

As in the adult gerbil, in the neonate fascicles of fibers course through the ventro-medial limb of the contralateral LSO to the dorsal hilus. On post-natal day 1 there is a dense confluence of labeled fibers within the LSO. The density of these fibers diminishes progressively over the next week, when it resembles the adult pattern. Synaptic profiles of Di-I labeled fibers have been oberved in the contralateral LSO during the first post-natal week. Synaptic profiles were also observed in the contralateral medial nucleus of the trapezoid body (MNTB). No label was observed in cells of MNTB, demonstrating the lack of trans-neuronal transfer of Di-I in this system.

Supported by NS-17596.

182.4

THE EFFECTS OF BARBITURATE AND COCAINE ON THE AMPLITUDE-MODULATION-FOLLOWING RESPONSE (AMFR) OF THE RABBIT: AN INVESTIGATION OF SOURCES. R. Batra, S. Kuwada, N.B. Kluck* and S.J. O'Connor*, Depts. of Anatomy and Psychiatry, Univ. of Conn. Health Center, Farmington, CT 06032.

Since auditory evoked potentials appear to arise from multiple brain sites, they are potentially useful as neurological tools. The AMFR to sinusoidally amplitude modulated tones may be especially advantageous because different generators appear to dominate the response at different modulation frequencies.

In our recordings of the AMFR from the skull of the unanesthethized rabbit, multiple generators are indicated by the way in which the amplitude and latency of the AMFR vary with modulation frequency from ~20-800 Hz. The amplitude shows a sequence of peaks separated by sharp dips, suggesting the summation and cancellation of signals from two or more sources. The latencies, as measured from plots of the phase of the AMFR *vs.* modulation frequency, indicate that different generators dominate the response at different frequencies. The phases lie roughly along three linear segments. The segments at progressively higher frequencies have shallower slopes, indicating progressively shorter latencies.

The responses at different modulation frequencies also differ in their sensitivity to intravenously administered sodium pentobarbital or cocaine. Pentobarbital reduces the amplitude of the AMFR at low frequencies, and the sequence of peaks and dips is abolished. Cocaine enhances the AMFR to low frequencies. In contrast, both pentobarbital and cocaine have much smaller effects on the AMFR at higher frequencies.

Our results suggest that the AMFR may be useful as a neurological tool because different generators might be isolated by varying the modulation frequency. The dominant generators at higher frequencies are consistent with sources in the brainstem, while those at lower frequencies may reside higher in the auditory pathways.

This study was supported by NIH grant NS 18027 to S.K. and NIDA grant DA06060-03 to S.J.O'C.

182.5

PHYSIOLOGICAL IDENTIFICATION OF DORSAL COCHLEAR NUCLEAR NEURONS OF MICE: INTRACELLULAR RECORDING AND DYE-INJECTION IN SLICES. S. Zhang and D. Oertel, Department of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706.

Detailed physiological studies of cells that were labeled intracellularly with biocytin show that different anatomical cell types in the DCN are distinguishable physiologically. All fusiform cells (18) could be unambiguously identified by their frequent, spontaneous, bursts of IPSPs. Most (6/7) cells that are probably cartwheel cells (the spines were generally not labeled by biocytin), and only those cells, had bursts of action potentials superimposed on slow depolarizations. Tuberculoventral cells (TV) (6) and giant cells (2) lack the aforementioned features and differ from one another in three ways. Giant cells have action potentials with more prominent fast undershoots than TV cells. Second, the synaptic responses of TV cells to shocks of the nerve root consist of prominent monosynaptic excitation and weak polysynaptic inhibition, whereas in giant cells an early, strong IPSP and later trains of regular IPSPs almost mask the underlying excitation. Third, only strong (>60V; 2/2 giant cells) shocks to the VCN evoke action potentials directly in giant cells whereas weak stimulation (<30V; 3/3 cells) suffices to stimulate TV cells whose axons in the VCN are not cut.

182.6

THE TWO MIDBRAIN AUDITORY SPACE MAPS IN THE GUINEA PIG EXHIBIT DIFFERENT SUSCEPTIBILITIES TO SENSORY EXPERIENCE. D.J. Withington, K.E. Binns* and M.J. Keating*. Department of Physiology, Leeds University, LS2 9NQ, U.K. &* N.I.M.R., London NW7 1AA, U.K.

Two separate topographically ordered representations of auditory space are found in the guinea pig brain; in the superior colliculus (SC) and the external nucleus of the inferior colliculus (ICX). Both maps have been examined following experiments in which animals were subjected to sensory deprivation, either by dark raising or placement in a continuous noise environment to perturb directional cues on sound location. Terminal mapping experiments were performed on anaesthetized guinea pigs. Electrophysiological data in the form of multi-unit receptive fields were collected in response to free-field presentation of white noise. We have shown previously that the SC space map is highly susceptible to both auditory and visual deprivation during development. Withholding either modality of experience, particularly during a crucial period before the normal emergence of the map, prevents the normal chronological expression of the SC map. In contrast the ICX map, whilst perturbed by auditory deprivation, is unaffected by visual deprivation. The differing susceptibilities of the SC and ICX maps to sensory experience may reflect different functional roles subserved by the maps in the process of sound localization.

182.7

NEURON TYPES IN GERBIL DORSAL COCHLEAR NUCLEUS. T.E. Benson, G.T. Gdowski, and H.F. Voigt, Department of Biomedical Engineering, Boston University, Boston, Massachusetts 02215.

In the course of studies on correlated activity of units in the dorsal cochlear nucleus (DCN) and their correlated anatomy/physiology we have labeled four neuron types with horseradish peroxidase (HRP). Dendrites and axons were characterized by light microscopy from 12 female gerbils.

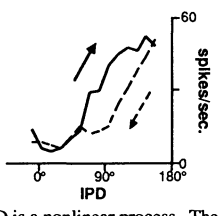
Fusiform cells (n=8) were bitufted with apical dendrites in the molecular layer (MOL) of DCN in cone-like topology. Basal dendritic trees were simpler, less branched and less spiny in deep DCN (DEEP). There was a range in the spininess of apical dendrites among fusiform cells. Apical spines were more "stubby" than basal. Recurrent branches were never observed; in 6 cases axons were traced into the dorsal acoustic stria. **Giant cells** (n=3) had multipolar cell bodies located in DEEP. Spine-sparce dendrites projected extensively in DEEP. A dendrite from each cell terminated in MOL in a complex "tuft" consisting of fine processes. A large diameter, ventrally directed axon emitted two fine branches in DEEP before entering the posteroventral cochlear nucleus (PVCN). **Cartwheel cell** bodies (n=2) were in the fusiform layer or MOL. Spiny dendrites were restricted to MOL, mostly the surface 2/3. Axons ramified locally, restricted to MOL or fusiform layers. A **Golgi cell** in MOL had three sparsely branched dendrites restricted to that layer with fine, short appendages and a curving dendrite (Mugnaini et al., 1980). The fine axon of this cell was directed toward the ependyma and it branched like a T.

Neurons were similar, in most respects, to corresponding ones in other species (e.g. Brawer et al., 1974) and to Golgi-staining results in gerbil (Schwartz et al., 1987). A lack of local branches from rodent fusiform cells suggest differences, as compared with cat, in interactions of fusiform and other DCN neurons. Giant cells in gerbil may influence DCN neurons and project through VCN. Their dendrites may be influenced by MOL parallel fibers through their "tuft" as well as deeper input. [DC00310, DC01099 and 947-ENGR]

182.8

HYSTERESIS IN RESPONSES OF INFERIOR COLLICULUS NEURONS TO DYNAMIC INTERAURAL PHASE STIMULI. M.W. Spitzer, M.N. Semple. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Because an observer's head is normally free to move, binaural difference cues are frequently dynamic. However, little attention has been paid to the influence of dynamic stimulus features on the processing of binaural spatial cues. We studied responses of single inferior colliculus neurons to dynamic interaural phase disparities (IPDs) generated by interaural phase modulation (IPM; Spitzer and Semple, *Soc. Neurosci. Abstr.*, 1990). Digitally-synthesized sounds were presented dichotically to anesthetized gerbils and cats. Responses of single isolated units were recorded extracellularly.



Response profiles for modulation in opposite directions were rarely symmetric. As illustrated by the response of a unit to triangular IPM shown in the Lissajous figure, the increase in firing rate elicited by modulation toward a favorable IPD follows a convex trajectory. In contrast, the decrease in discharge elicited by modulation in the opposite direction follows a concave trajectory (dashed line). Such hysteresis indicates that the encoding of IPD is a nonlinear process. The influence of dynamic stimulus features thus illustrated would impair the ability of neurons to encode absolute, instantaneous IPD. Alternatively, the temporal structure of neuronal discharge may convey information about dynamic features of IPD. Supported by NIDCD grant DC 00364.

182.9

DEVELOPMENT OF EFFERENT CONNECTIONS OF THE COCHLEAR NUCLEUS IN THE RAT. K. Kandler and E. Friauf, Dept. Animal Physiology, Univ. of Tübingen, Fed. Rep. Germany.

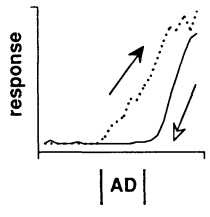
To study the ontogeny of the ascending pathways in the auditory brainstem, small crystals of the carbocyanine dye, DiI were implanted into the cochlear nucleus (CN) of embryonic (embryonic age E18 - E21) and postnatal (P0 - P14) formaldehyde-fixed rat brains. After 2-9 months, brains were cut with a vibratome and examined under epifluorescent illumination. In a second set of experiments, coronal brainstem slices containing both the CN and the superior olivary complex (SOC), were prepared and maintained alive *in vitro* for 8-15 hours. Crystals of biocytin applied to the ventral acoustic stria resulted in retrogradely labeled neurons in the CN and anterogradely labeled fibers in the SOC.

As early as E18, labeled fibers could be observed in the lateral part of the ipsilateral SOC (iSOC), in the medial part of the contralateral SOC (cSOC), bilaterally in the lateral lemnisci and in ventrorostral aspects of the inferior colliculi. However, collaterals were only observed in the iSOC and in the IC. At E20, collaterals were also present in the cSOC and bilaterally in the ventral nuclei of the lateral lemniscus (VNLL). In the contralateral dorsal NLL, collateral sprouting started at E21. Labeled fibers and retrogradely labeled neurons were first observed in the cDCN at P2 and in the cVCN at P3. In the contralateral medial nucleus of the trapezoid body, end bulbs of Held were first found at P3. Since the main afferent pattern to the IC appears adult-like in rat embryos (unpublished), we conclude that the main connections between auditory brainstem nuclei develop in a temporally parallel rather than a serial fashion. Our results also show that there is a time period of about two weeks between the establishment of the main auditory brainstem pathway and the onset of hearing at P12. During this period, spontaneous activity may be necessary for a proper maturation of the auditory system. Supported by DFG Fr 772/1-1

182.10

HYSTERESIS IN RESPONSES OF INFERIOR COLLICULUS NEURONS TO DYNAMIC BINAURAL AMPLITUDE DISPARITY. M.N. Semple. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Most acoustic environments are rich in temporal fluctuations of frequency and amplitude. The two ears are stimulated differently and the stimulus at each ear varies constantly. How do these interaural dynamics contribute to the processing of directional information? Interaural modulation provides a means for examining the neural consequences of dynamic changes in binaural conditions. Dynamic amplitude disparity stimuli (DAD) were generated by modulating the signal amplitude at one or both ears. Stimuli were synthesized digitally and presented dichotically to anesthetized gerbils and cats. Single-unit responses were recorded extracellularly in the central nucleus of the inferior colliculus.



DAD stimuli typically elicit steeper rate/level functions, lower disparity thresholds and greater peak rates than are evoked by static amplitude disparity (SAD) stimuli. Responses to modulation in opposite directions through the same amplitude disparity range exhibit hysteresis (see figure). These properties indicate that the encoding of binaural level is a nonlinear process.

Supported by NIDCD grant DC 00364.

182.11

SPATIAL ATTRIBUTES OF PINNA TRANSFORMATIONS IN THE CAT. J.C.K. CHAN, A.D. MUSICANT* AND J.E. HIND, Department of Neurophysiology, University of Wisconsin, Madison, WI, 53706.

We have described spectral characteristics of free-field to eardrum acoustic pressure transfer functions that occur as a result of sound interacting primarily with the pinna. One consistent finding was a spectral notch that shifts upward in frequency with increasing source azimuth and/or elevation (Musicant et al., Int. Cong. Acoust., 1986; JASA, 1990). We also described a spatial feature, the acoustic-axis. This is the direction from which a particular frequency component is most amplified relative to other directions. Although the acoustic axis is broadly tuned spatially, binaural psychoacoustic experiments have shown that narrow bands of noise tend to bias judgements towards the acoustic axes.

Another spatial attribute is the presence of a distinct region, for each frequency component in the 8-16 kHz range, where the pinna amplification is low, and usually negative. This region of low amplification often appears as a narrow strip in an isoamplitude contour plot. Each strip is oriented at about 45 degrees with respect to the horizontal axis. Strips for the two ears form mirror images of each other and intersect near the median plane. With increasing signal frequency each strip shifts location systematically in a direction orthogonal to its orientation. For each frequency component in the 8 - 16 kHz range, there is a well defined locus near the median plane where pinna amplification is low in both ears. Physiological experiments in our labs have recently demonstrated that these regions of low amplification are detected by the nervous system at the level of the auditory nerve and the primary auditory cortex (Poon et al., Reale et al., Soc. Neurosci. Abstrs., 1991), suggesting that these acoustic features might play a role in sound localization. [Supported by NIH grant DC00116]

182.12

PATTERNS OF RESPONSE OF CAT AUDITORY-NERVE FIBERS TO EARPHONE-REPRODUCED FREE-FIELD CLICKS. P.W.F. Poon*, and L.F. Brugge. Dept. Neurophysiology and Waisman Center, University of Wisconsin, Madison, WI 53706.

Spectral and temporal information about a sound in space is altered by the acoustic properties of the head, pinna and ear canal before being filtered by the transduction mechanisms of the inner ear and relayed to the cochlear nuclei of the brainstem. To assess these tranformations, and therefore the input to the brain, responses of single auditory-nerve fibers of anesthetized cats were obtained to a set of digitally-processed free-field clicks delivered via calibrated earphones sealed into the external auditory meatus. The stimulus set was derived from sound-pressure waveforms generated by a loudspeaker at different locations around a cat's head and recorded at the tympanic membrane as part of a related study (Musicant et al, J.Acoust. Soc. Am. 82:757, 1990). The full complement of these signals represents what we refer to as acoustic virtual space, and the azimuth-vs-elevation map of spike activity derived from responses to this stimulus set is termed a Virtual Space Receptive Field (VSRF). Fibers with CF between 0.4 to 40 kHz were studied. The pattern of activity defining a VSRF depended upon the fiber CF, the stimulus level at which the map was obtained, and on spontaneous discharge rate. The isoamplitude contour map plotted for a single frequency in the stimulus spectrum corresponding to the fiber's CF closely matched the VSRF pattern, although for fibers with CF between 15 and 30 kHz a better correspondence was often obtained with isoamplitude contours for frequencies above CF, especially at high stimulus levels. For fibers of the same CF and at similar stimulus level above threshold, the VSRFs were similar in form, but the spatial contrast between evoked and spontaneous activity was higher for low spontaneous fibers. We conclude that, to the first approximation, VSRFs of single auditory-nerve fibers are governed by the filtering properties of the fiber and the spectral contents of the stimulus. This conclusion is further supported by the agreement between experimental data and results from a two-stage model of the auditory-nerve filter.

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182.13

MONAURAL DIRECTION-DEPENDENT RESPONSES OF NEURONS IN CAT PRIMARY AUDITORY (AI) CORTEX. R.A. Reale*, J.F. Brugge, J.C.K. Chan, A. Musicant*, J.E. Hind, and P.W.F. Poon*. Dept. of Neurophysiology and Waisman Center, University of Wisconsin, Madison, WI 53706.

The dichotic simulation of free-field sounds using insert earphones provides a powerful technique to study the direction-dependent responses of single auditory neurons. Earphone reproduction permits independent variation of acoustical properties including sound pressure level and spectrum both of which play a role in sound localization. Our system accurately simulates the direction-dependent pinna transformation of a broad-spectrum sound (click) presented from a free-field source. We refer to this representation of simulated free-field sounds as acoustic virtual space.

The majority of single neurons in the primary auditory (AI) cortex of the barbiturate-anesthetized cat which respond to tones also respond to clicks. Typically, a cell discharges only when a click stimulus corresponds to certain sound source directions in acoustic virtual space. An aggregation of effective directions forms a Virtual Space Receptive Field (VSRF). VSRFs are strongly influenced by changes in sound pressure level. The shapes of VSRFs can vary from a simple unimodal focus to a complicated pattern. A VSRF resulting from monaural stimulation can exhibit a striking correspondence with the direction-dependent amplitudes of spectral components in the click stimulus. Plotting the amplitudes of single spectral components as a function of azimuth and elevation in acoustic virtual space permits direct comparison with shapes of VSRFs. For a subset of neurons, isoamplitude contours derived for the spectral stimulus component near characteristic frequency (CF) can delineate the VSRF for near-threshold intensity levels. Often, the VSRF is delimited by isoamplitude contours representing both local maxima and local minima. Furthermore, the same contours can exhibit a correspondence with the spatial distribution of first-spike latencies comprising the VSRF. In other cells, VSRFs are better matched by isoamplitude contours for stimulus frequencies different from CF or by contours derived from a range of stimulus frequencies.

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182.14

THE EFFECTS OF NEONATAL AUDITORY CORTICAL LESIONS ON MINIMUM AUDIBLE ANGLES FOR SOUND LOCALIZATION BY THE FERRET. B.J. Rooney and J.B. Kelly. Laboratory of Sensory Neuroscience, Carleton University, Ottawa, Canada, K1S 5B6.

Previous studies have shown that ferrets with unilateral lesions of primary auditory cortex are incapable of localizing sounds within the contralateral field, but experience no difficulty localizing around midline or within the ipsilateral field. The present study was undertaken to determine whether unilateral cortical lesions in neonates would result in the same deficit observed in the adult. The ferret was selected because of the immaturity of its CNS at birth. Unilateral cortical lesions were produced in 4-day old ferrets. As adults, the animals were tested in a two-choice procedure which permitted independent assessment of minimum audible angles around 0, -60 and +60 degrees azimuth. Animals with large cortical lesions showed contralateral deficits similar to those previously reported for adults. Animals with smaller lesions were less impaired than expected whereas those with lesions restricted to the middle ectosylvian gyrus showed no contralateral impairment. Thus, while infant lesions still produced contralateral deficits, there was some indication of functional sparing.

(This research was supported by NSERC.)

182.15

PROCESSING OF PERIODIC SIGNALS IN THE BRAINSTEM OF THE GUINEA FOWL (NUMIDA MELEAGRIS) R.W.Ward Tomlinson and Gerald Langner. Dept. of Zool., Tech. Univ. Darmstadt, Schnittspahnstr. 10, D-6100 Darmstadt, Germany (West)

The Iambus call of the Guinea fowl has a strong sinusoidal amplitude-modulated component. Previous investigations have found neurons in its inferior colliculus analogue MLD nucleus showing marked band-pass tuning to modulation frequency in both their discharge rates and synchronicity. To investigate characteristics of the inputs of MLD, responses of 130 single and multi-units were recorded from auditory brainstem of 12 awake Guinea fowl to pure tones and sinusoidally amplitude modulated tones. Histological confirmation revealed recording sites in or near N. angularis, N. laminaris and N. magnocellularis. The spike counts and synchronization coefficients were measured with respect to pure tone frequency, and modulation frequency. Band-pass modulation frequency characteristics were clearer and occurred more frequently when synchrony was used as a measure of response. With discharge rate as a measure, low-pass characteristics and shallow band-pass characteristics were dominant. Since avian auditory nerve demonstrates only low-pass characteristics to modulation frequency, it can be concluded that additional processing of AM signals is occurring at the level of the cochear nuclei. *(Supported by the DFG, SFB 45. RWWT supported by a fellowship from the Humboldt Foundation).*

182.16

CONDITIONING CHANGES FREQUENCY REPRESENTATION IN GERBIL AUDITORY CORTEX.
H. Scheich and C. Simonis*. Zoological Inst., Technical Univ. Darmstadt, 6100 Darmstadt, Germany

The ^{14}C-2-deoxyglucose method was used to investigate the influence of learning on frequency representation in the auditory cortex of gerbils. During classical conditioning 1 kHz tones as CS were paired with a footshock as US. In the mirror imaged tonotopic fields AI and AAF the two labeled frequency band laminae had a larger distance in comparison to unpaired controls suggesting a shift of representaiton during conditioning. In a second experiment animals were trained in a shuttle-box to avoid the shock before the 2DG session. In trained animals the width of the labeled frequency band laminae was increased compared to controls, suggesting an expansion of the frequency representation in this paradigm. In a third experiment gerbils were trained differentially with 1 and 10 kHz to avoids (CS+) and not avoid the footshock (CS-). In addition to an expansion of CS+ representation the CS- representation was suppressed.
Supported by DFG, SFB 45

182.17

RESPONSES TO COMPLEX SOUNDS IN THE CAT'S ANTERIOR AUDITORY FIELD. B. Tian, J.P. Rauschecker, M. Korte* and J.F. Olsen. NIH Animal Center, NIMH, Poolesville, MD 20837, U.S.A. and Max Planck Institute for Biological Cybernetics, Tübingen, Germany.

More than two dozen different representations have now been described in the visual cortex of cat and monkey. These maps show functional specializations for the processing of different aspects of the visual world, such as form, motion, and depth (Hubel & Livingstone; Ungerleider & Desimone; Van Essen; Zeki). Much less is known about the functional subdivisions of auditory cortex in higher mammals. This is even more surprising since sounds are very important for communication in these species.

We have started to analyze single unit responses in the auditory cortex outside the primary auditory field AI. Our first target was the cat's anterior auditory field (AAF), which is reported to have tuned responses in a tonotopic map (Phillips & Irvine; Reale & Imig). Our results confirm these earlier findings: most cells show narrow frequency tuning when tested with tone bursts. However, virtually every neuron responded much better, and often with sustained firing, when tested with more complex natural sounds, such as finger snaps, key jingle, hissing or kissing. These sounds were digitized on a 486 workstation by means of the SIGNAL program package (Engineering Design). Parts of these standardized complex sounds were then played back during single unit recording. In addition, we used frequency and amplitude modulated synthetic sounds. Semichronic recordings were performed under light gas anesthesia. Almost every neuron in AAF responded briskly to auditory stimulation. More units preferred transient sounds with sharp onsets, rather than signals with slow amplitude modulation. Frequency sweeps often elicited good responses too. Some units responded best when the stimulus was presented at a specific location in space or when it was moved across this location in a certain direction (c.f. Rauschecker & Harris, 1989, for SC neurons).

At present, we cannot say anything about a columnar arrangement of these response properties across the isofrequency domain. It appears puzzling, however, why there should be less order in the organization of auditory cortex than of visual cortex.

182.18

A SECTOR BASED ANALYSIS OF THE RAT AUDITORY CORTEX. E.L. Moore, M.E. Taylor, E.A. Floyd, and H.K. Rucker. Department of Physiology, Meharry Medical College, Nashville TN 37208.

This study was designed to study the organization of the Sprague-Dawley albino rat temporal cortex by employing the combined techniques of auditory electrophysiology, cytoarchitectonic analysis, and retrograde labeling patterns in medial geniculate nuclei (MG). The parietal-temporal cortex of male rats (300-350 gms) was subdivided into twelve sectors employing a 2mm grid design (for identification: columns=w,x,y,z; rows=1,2,3; e.g. sector z3 is caudal-ventral above the tail of the rhinal sulcus). Results have been obtained from 26 animals. Sectors w1 and w2 are characterized by long latency (20-43msec range) responses. All responsive units exhibited a "build-up" type response and were broadly tuned; no tonotopic organization could be discerned. Somatosensory responses were also observed. No acoustically responsive units were located in sector w3. Nissl stained sections of column w did not reveal a distinct layer 4. Medial geniculate (MG) projections to column W1 and w2 were sparse and originated in dorsal MG (MGD) and magnocellular MG (MGM); on the other hand, no MGB projections were evident to sector w3. X1 and Y1 also exhibited retrograde transport to MGD and MGM. Units were broadly tuned with a rough high to low frequency organization (caudal to rostral). The remaining sectors exhibited the same tonotopic organization. However, layer 4 was variable but distinct and the units were more sharply tuned with reliable short (11-13msec) latency responses. Retrograde label was found mainly in MGV and MGD. Our results suggest that the subdivisions of the rat acoustically responsive cortex are more complex than previously reported.[NSF BNS8617837, NSF RII8704121 and NIH SO6GM 08037]

182.19

THE FUNCTIONAL ANATOMY OF MIDDLE LATENCY AUDITORY EVOKED POTENTIALS JOSEPH A. KITHAS*, SHI DI and DANIEL S. BARTH *Department of Psychology, University of Colorado, Boulder, CO 80309-0345 (U.S.A)*

The neural origins of middle latency auditory evoked potentials (MAEP) were studied in rat cortex. MAEP were mapped from the cortical surface with a high spatial resolution electrode array. Spatiotemporal analysis, based on multivariate statistical methods, was then used to relate putative neural generators of the MAEP complex to established cytoarchitectural anatomy. Our data support the hypothesis put forward by others that the MAEP peaks are generated by separate neuronal subpopulations. The topographic distributions of epicortical potentials associated with each peak of the MAEP complex in the present study were different, indicating activation of spatially overlapping but distinct subpopulations of cells within primary and secondary auditory cortex. These data indicate that the MAEP waveform reflects systematic asynchronous activation of both primary and secondary auditory cortex during the processing of simple click stimuli.

182.20

SENSITIVITY TO INTERAURAL TIME DIFFERENCES (ITDs) OF NEURONS IN THE SUPERIOR OLIVARY COMPLEX (SOC) OF THE UNANESTHETIZED RABBIT. S. Kuwada and R. Batra. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

The SOC is considered to be the chief site for processing ITDs in the fine structure and the envelope of sounds. Cells sensitive to ITDs in the SOC are commonly viewed as coincidence detectors. They receive phase-locked inputs from each ear, which can be excitatory or inhibitory. If a cell receives excitatory inputs from both ears, then it should discharge maximally at a particular ITD, independent of frequency. In contrast, if a cell receives excitatory input from one ear, and inhibitory input from the other ear, then it should discharge minimally at a particular ITD, independent of frequency. The ITD that produces maximal or minimal responses will be determined by the relative latencies of the two inputs from each ear. Thus, each combination of inputs leads to a specific prediction about a cell's behavior to ITDs.

We have found SOC cells that have responses consistent with the above predictions. Some cells were excited by monaural tones presented to either ear. They typically responded maximally at the same ITD, independent of frequency and favored ipsilateral delays. Other cells were excited by tones presented to the ipsilateral ear and inhibited by tones presented to the contralateral ear. They typically responded minimally at the same ITD, independent of frequency and favored contralateral delays.

In sum, we find that both mechanisms for processing ITDs that have been postulated may operate in the SOC of the unanesthetized rabbit. Cells of the medial superior olive may code ITDs by discharging maximally, while those of the lateral superior olive may code ITDs by discharging minimally.

This study was supported by NIH grant NS 18027 to S.K.

182.21

DORSAL COCHLEAR NUCLEUS OF UNANESTHETIZED DECEREBRATE CATS: DISCHARGE REGULARITY OF PAUSE-BUILD UNITS. K. Parham and D.O. Kim. Div. Otolaryng., Dep. Surgery, Surgical Res. Ctr., Ctr. Neurological Sciences, Univ. Conn. Hlth. Ctr., Farmington, CT 06030.

We systematically examined the mean and standard deviation of interspike intervals (ISI) and the coefficient of variation (CV) of ISIs versus time in all of our sample of 87 pause-build (PB) units from the dorsal cochlear nucleus (DCN) of decerebrate unanesthetized cats. The units were characterized using both poststimulus time histograms (PSTH) and excitatory-inhibitory area (EI-area) schemes. The PB units in the present sample were distributed across four different EI-area types: III (51%), I/III (25%), II (15%) and IV (9%). A predominant portion of PB units (80% of 44 units with sufficient number of spikes for CV analysis) exhibited mean CVs less than 0.5 in the 20-40 msec time window in response to 50 msec tone bursts at characteristic frequency at 60 dB SPL re 20 μPa; 39% of the 44 units exhibited highly regular discharges (mean CV < 0.35). PB units with mean CVs less than 0.35 were exclusively of types III and I/III. Intracellular depolarizing currents applied to simple-spiking DCN cells in vitro produced highly regular discharges (Hirsch & Oertel, 1988; Manis, 1990). The present in vivo finding is consistent with the in vitro observations in that both indicate regular discharges of certain DCN cells. We suggest that the absence of noticeable chopping in the PSTHs despite the presence of highly regular ISIs of PB units arises from the variability in the absolute latencies of groups of regularly-spaced spikes.

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182.22

PRENATAL AND EARLY POSTNATAL DEVELOPMENT OF AUDITORY THALAMOCORTICAL AXONS. R.K. deVenecia and N.T. McMullen. Department of Anatomy, University of Arizona College of Medicine, Tucson, AZ 85724

The behavioral onset of hearing in rabbits occurs between 6 and 7 days after birth. Simultaneously, supernumerary dendrites appear on layer III/IV nonpyramidal cells in auditory neocortex, presumptive targets of thalamocortical (TC) axons, suggesting that afferent activity may have a stimulatory affect on cortical dendrogenesis. To examine the ingrowth of auditory TC axons, DiI was used for anterograde axonal labeling. A small crystal of DiI was placed into the medial geniculate body of rabbit brains fixed with 4% paraformaldehyde at successive developmental periods from gestational day (GD) 25 to postnatal day (PD) 6. Serial coronal sections of 100-200 um thickness were examined by fluorescence microscopy and TC axons reconstructed from serial photomicrographic slides. At GD-25, axons have entered the cortical mantle and are prominent in the white matter below the presumptive auditory cortex. Growth cones were observed at the tips of the few axons that have entered the grey matter. At birth, axons have penetrated up to layer V where they branch repeatedly. Unbranched axons also extend into the molecular layer but few axons appear to terminate within the cortical plate. However, by PD-3, sparsely branched axons have entered the trilaminar cortical plate. At PD-6, TC axons span the entire extent of the neocortex and branch extensively within the upper layers. From birth until PD-6, the ingrowth of TC axons was most extensive in ventral portions of the auditory cortex where low frequencies are represented tonotopically. These data agree with the appearance of initial behavioral responses to low frequency tones at PD-6.5. Thus, the arrival and ingrowth of TC axons correlates well with previous anatomical, electrophysiological and behavioral data for this sensory neocortex (Arizona Disease Control Research Commission Contract #82-1688).

BASAL GANGLIA AND THALAMUS I

183.1

EXCITOTOXICITY IN THE RAT NUCLEUS TEGMENTI PEDUNCULOPONTINUS, WITH PARTICULAR REFERENCE TO THE LOSS OF CHOLINERGIC NEURONS. P. Winn, E. Rugg*, J. Dunbar*, M. Latimer* and P. Dean. Dept. Psychology, Univ. St Andrews, Fife KY16 9JU, Scotland; Dept. Psychology, Univ. Sheffield, Sheffield S10 2TN, U.K.

The NTPP contains cholinergic neurons which innervate the thalamus, basal ganglia, superior colliculus and other sites. Recent reports suggest that in the basal forebrain certain excitotoxins have selective actions on cholinergic neurons. Various excitotoxins were therefore tested in the NTPP with special reference to loss of cholinergic neurons, identified using ChAT immunohistochemistry. The size of the lesions was computed following examination of cresyl violet stained sections. The largest were made by kainate=AMPA>NMDA=ibotenate>quisqualate=quinolinate. In terms of the number of cholinergic neurons lost the most effective was NMDA>ibotenate=quinolinate=AMPA=quisqualate>kainate. The ratio of cholinergic to general neuronal loss was computed: kainate, both doses of AMPA and the lower dose of ibotenate were relatively non-selective; the higher doses of ibotenate and quinolinate and both doses of quisqualate and NMDA were more selective; and 24nmol quinolinate, with a ratio more than 2X the next best toxin was the most selective. TOH immunohistochemistry showed axons running through the lesioned area indicating that fibers were undamaged. We conclude that in the NTPP different excitotoxins make quantitatively and qualitatively different lesions. Unlike basal forebrain excitotoxic lesions quinolinate not quisqualate made the most selective lesions of cholinergic neurons and unlike septal nuclei lesions ibotenate spared non-myelinated fibres.

183.2

CHOLINERGIC STIMULATION OF RAT SUBSTANTIA NIGRA: COMPARISON OF CARBACHOL, NICOTINE, NEOSTIGMINE, AND EFFECTS OF 6-OHDA LESIONS. W. Inglis*, G. Parker* and P. Winn. (SPON: Brain Research Association) Dept. Psychology, Univ. St Andrews, Fife KY16 9JU, Scotland.

Intranigral carbachol increases feeding, drinking and sexual behavior if there is a pre-existing tendency to respond and low baseline rate. The effects of other cholinergic stimulants have not been compared. Groups of 6-12 non-deprived rats received 0.5ul microinjections into SN of 0.1-5.0ug carbachol, 0.1-5.0ug nicotine, and 1.25-5.0ug or 0.1-1.0ug neostigmine (vehicle, artificial CSF) and their effects on feeding, drinking, locomotion, grooming, rearing and sniffing were examined. Carbachol, nicotine and neostigmine stimulated dose-dependently eating of dry macaroni: amount consumed, latency and duration of feeding were not different; other activities were unaffected. These data show that stimulation of nicotinic or muscarinic receptors or inhibition of AChE in SN potentiates behavior. Nigral ACh is thought to activate DA neurons. A second experiment examined whether unilateral 6-OHDA lesions of nigrostriatal DA neurons affected carbachol-elicited eating. Before lesioning 0.5ug/0.5ul carbachol stimulated significantly more eating than vehicle alone. 6-OHDA lesions were made in 14 rats; 6 rats received sham lesions. After lesioning, carbachol elicited no more eating than vehicle in lesioned rats but elicited eating as before in control rats. HPLC analysis showed lesions had reduced DA concentration in caudate-putamen on the lesioned side by 50%, but had not affected accumbens DA; 5HT was not affected. These data show that DA loss from the caudate-putamen but not accumbens abolishes the effects of intranigral carbachol.

183.3

LONG-TERM CHANGES IN STRIATAL OPIOID BINDING SITES AFTER 6-HYDROXYDOPAMINE (6OHDA) LESION OF SUBSTANTIA NIGRA. J. A. M. Smith*, S. E. Loughlin and F. M. Leslie. Dept. of Pharmacology, Univ. of California, Irvine CA 92717.

Striatal mu and delta opioid receptor densities decrease after lesion of the nigrostriatal dopamine (DA) system, consistent with a localisation of these opioid receptors on DA terminals. Recently, however, it has been suggested that mu opioid receptors are localised on intrinsic striatal neurons and that lesion-induced decreases in density are a result of trans-synaptic changes. In the present study quantitative autoradiography was used to correlate changes in opioid binding site density in rat striatum with the extent of DA denervation at different time intervals after unilateral 6OHDA lesion of the substantia nigra. Mu, delta and kappa sites were labelled using [^{3}H]DAGO, [^{3}H]DADLE (in presence of D-Pro^4, morphiceptin) and [^{3}H]diprenorphine (in presence of D-Pro^4, morphiceptin and DSLET) respectively. [^{3}H]Mazindol binding to DA uptake sites in the ipsilateral striatum was less than 10% of that in the contralateral side at one week post-lesion, and remained at this level at 24 weeks. Delta binding site density was decreased 25% at both 2 and 24 weeks post-lesion. Mu sites, in both patch and matrix compartments, exhibited a similar change at 2 weeks, but were further decreased to approximately 70% of the contralateral side at 24 weeks. Kappa sites were apparently unchanged at 2 weeks, but a 30% decrease was observed at 24 weeks. The short-term changes in mu and delta opioid binding sites may be consistent with a presynaptic localisation for these receptors. In contrast, the delayed loss of a large proportion of mu and kappa sites suggests the presence of a population of postsynaptic striatal opioid receptors which undergo long-term trans-synaptic changes as result of 6OHDA lesion. *Supported by NIH NS19319, NS26761; DC00450; National Parkinson Foundation.*

183.4

GLUTAMATE RECEPTOR BINDING IN THE HISTOCHEMICALLY DEFINED PATCH-MATRIX MOSAIC IN HUMAN STRIATUM. Leon S. Dure IV, John B. Penney, Anne B. Young. Department of Neurology, University of Michigan, Ann Arbor, MI 48109.

The corpus striatum consists of histochemically defined patches and matrix. These regions have so far been defined by variations in histochemical staining, and by differences in mRNA expression as examined by *in situ* hybridization. The patches and matrix differ anatomically with respect to afferent and efferent connections within the brain, but no functional distinctions between these areas have yet been described. Using quantitative *in vitro* autoradiography in frozen sections of human striatum, we performed radioactive ligand binding to receptors specific for the PCP site, the ionotropic quisqualic acid (AMPA) binding site, and the kainic acid binding site. The pattern of receptor binding was correlated with histochemical staining for the patches and matrix defined by acetylcholinesterase activity. There was a correspondence between the acetylcholinesterase-poor patches and areas of increased binding to the kainate receptor. Conversely, the binding to the AMPA site was greater in areas coincident with the matrix. These findings indicate a difference in glutamate receptor subtype distribution within the patch/matrix mosaic of the striatum. Given the potential neurotoxicity of glutamate, a differential distribution of glutamate receptors within the striatum may help to explain the pathophysiology of processes characterized by inhomogeneous striatal pathology, such as Huntington's disease.

This work supported by the Huntington's Disease Society of America (LSD), and USPHS grant NS 15655 (ABY and JBP).

183.5

FIBER ARCHITECTONICS OF THE STRIOSOME-MATRIX ORGANIZATION OF PRIMATE CAUDATE-PUTAMEN. B. Quinn & A.M. Graybiel, Dept. of Pathology, UCLA School of Medicine, Los Angeles, CA 90024, and Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

The striosome and matrix compartments constitute a fundamental organizational system for the neostriatum, and govern heterogeneous distributions of neurotransmitters, receptors, and input-output pathways in this region. This heterogeneity generally remains occult in tissue treated with classical stains such as the Nissl, Luxol Fast Blue, and Weigert, although under certain conditions clusters of striatal neurons can be discerned. We have developed a modification involving hydrogen peroxide catalysis of Schmued's gold chloride fiber stain that reliably demonstrates striosomes as heterogeneous zones clearly delineated by fiber patterns in the caudate-putamen of the squirrel monkey in frozen sections from formalin-perfused brains. Striosomes appeared as zones of reduced fiber impregnation, sometimes surrounded by an annulus of darker-stained fibers. In adjacent tissue sections, the location of striosomes was confirmed by enkephalin immunohistochemistry. These fiber patterns mainly delineated striosomes in the caudal caudoputamen. Under identical conditions, fiber patterns indicating striosomes were not seen in the rat brain. Fiber definition of striosomes may be a valuable adjunct to previously available techniques for the analysis of striosome-matrix architecture in primate brain.

Supported by the United Parkinson Foundation and the American Parkinson's Disease Association, and Javits Award NS25529.

183.6

5'-NUCLEOTIDASE: A NEW ENZYMATIC MARKER FOR STRIOSOMAL ORGANIZATION IN THE RAT CAUDOPUTAMEN. S.W. Schoen and A.M. Graybiel. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

In the mammalian striatum, patchy compartments, the striosomes, can be distinguished from the surrounding neuropil, the extrastriosomal matrix, by differential distributions of a large number of neurotransmitters and related compounds. With a histochemical lead technique, we demonstrate that the adenosine-producing ecto-enzyme 5'-nucleotidase (5'N) follows a similar compartmentalization in the striatum of the adult rat. Serial-section comparisons of the distribution of 5'N enzymatic activity and the distributions of opioid receptor sites (a marker for striosomes visible by ^{3}H-naloxone ligand binding) or calbindin immunoreactivity (a marker for matrix) show that 5'N is differentially concentrated in striosomes, where it assumes a dense neuropil staining. 5'N follows a striosomal distribution in all but the caudal caudoputamen, including the dorsolateral quadrant in which little calbindin staining appears. In practice, 5'N histochemistry proves an advantageous tool for detecting the striosomal architecture in the rat: it can be performed on both unfixed (cryostat) and perfusion-fixed (vibratome or freezing microtome) sections; it reveals a widespread striosomal organization; and it constitutes an inexpensive and fast (results obtained 2 - 3 hr after tissue collection) procedure. 5'N is predominantly associated with glia in the adult nervous system and thus could reflect a specific glial compartmentalization within striatum. This enzyme has, in addition, been localized in modifiable synapses and thus could also mark striosomes as sites of synaptic plasticity.

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183.7

THREE-DIMENSIONAL RECONSTRUCTION AND VOLUME IMAGING OF THE PATCH-MATRIX ORGANIZATION IN NEOSTRIATUM OF RATS USING ^{3}H-NALOXONE RADIOLABELING. D.J. Woodward, J.-Y. Chang, C.R. Gerfen, K.S. Pollan and S.F. Sawyer. *Dept. Cell Biology and Neuroscience, UT Southwestern Med. Center, Dallas, TX 75235 & Lab of Cell Biology, NIMH, Bethesda, MD 20892*

A principle feature of the structural organization of the mammalian neostriatum is the segregation of afferents and efferents into neurochemically-defined compartments, referred to as the patch (striosomes) and the matrix, with the latter occupying approximately 85% of the volume of the neostriatum. As viewed in single sections, patches have an irregular appearance ranging in diameter from 200-800 μm. We have investigated the 3-dimensional structure of patch and matrix across serial sections in the rat neostriatum using an opiate receptor probe to label patch regions. Two adult rats were anesthetized and perfused with 0.3% formalin. Serial sections were cut 20 or 50 μm thick in the coronal plane with a cryostat, mounted on slides and incubated in 5 nM [^{3}H]-naloxone. Sections were apposed to tritium-sensitive film for 2 months to permit visualization of [^{3}H]-naloxone-labeled patches. Autoradiographic images of 130 sections (2.6 mm total thickness) from one animal and 22 sections (1.1 mm total thickness) from the other animal have been digitized, aligned and reconstructed using software from *Biographics*. Volume imaging from different perspectives, aided by rotating views of solid images in video sequences, provided a 3-dimensional view of the organization of the patch system. A "lateral streak" formed a largely continuous shell along the lateral aspect of the neostriatum. Groups of patches observed in multiple serial sections were found to possess a fenestrated and interconnected lamellar organization. This patch-lamellar system was oriented largely parallel to the lateral streak and was most prominent dorsolaterally. The patch-lamellar organization fragments into isolated "islands" in ventromedial regions of the neostriatum. The reconstructed images provide a foundation for integration of data from diverse anatomical and molecular methods. *Supported by MH-44337, AFOSR 90-0416 and Biological Humanics Foundation.*

183.8

NEOSTRIATAL AFFERENTS LABELED WITH 5-HYDROXYDOPAMINE: SYNAPTIC ULTRASTRUCTURE AND RECONSTRUCTIONS FROM SERIAL SECTIONS. J. C. Linder, S.J. Young and P. M. Groves. *Dept. Psychiatry, Univ. Calif. San Diego, La Jolla, CA 92093*

This project analyzed electron micrographs of neostriatum from rats which had been infused intraventricularly with 5-hydroxydopamine (1.5 mg). Serial sections (12-70 / ribbon) were obtained of heavily labeled axons. These axons appeared similar to those presumed to be dopaminergic in previous reports using tyrosine hydroxylase immunocytochemistry. Our sample contained 50 synapses in a total volume 1102.6 μm^{3}. These were analyzed for presynaptic axon diameter, type and area of synaptic specialization, and post-synaptic target. The morphology of some axons and their postsynaptic targets was visualized by computer-assisted three-dimensional reconstruction. We estimate that labeled axons comprise 3% of the neuropil volume and that at least 5% of all synapses within this striatal region were labeled. Labeled axons were relatively straight, unbranched, and of small diameter (0.06-1.5 μm). Variations in diameter occurred along an axon. Enlargements were more often associated with mitochondria than with synapses. Diameter at synapses was 0.1-0.77 μm (mean = 0.26 μm.) Synapses were *en passant*, symmetric, and contacted spine heads (n=15), spine necks (n=13), dendritic shafts (n=21), and an axon (n=1). Axon diameter and synaptic contact area were larger for synapses on dendritic shafts than for those on spines. These tiny synaptic specializations (area: 0.01-0.11; mean = 0.04 μm^{2}) appeared in only 2-3 adjacent serial sections. Synaptic vesicles were almost obscured by the label but appeared sparse and relatively large. Three-dimensional reconstructions facilitated comparison of the locations of these presumed dopaminergic synapses relative to unlabeled synapses along a dendrite. While labeled synapses often contacted spiny dendrites near the base of spines or on spine necks, unlabeled, asymmetric synapses predominated on spine heads. Some aspiny dendrites received labeled synapses at branch points. Reconstructions of individual axons revealed variations in their morphology and the distribution of synapses along their length. A stereo reconstruction of one 10 μm segment of labeled axon demonstrated that it made 6 symmetric synapses with 6 different postsynaptic dendrites, 4 spiny and 2 aspiny. Other reconstructions showed multiple labeled axons contacting a single dendritic segment. These results further characterize dopaminergic influence within the striatum.

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183.9

NIGRAL AFFERENTS IN RELATION TO DOPAMINERGIC NEURONS M. Damlama and J.M. Tepper, Center for Molecular and Behavioral Neuroscience, Rutgers The State University of New Jersey, Newark, NJ 07102.

The normal functioning of substantia nigra dopaminergic neurons is greatly influenced by their afferent inputs as evidenced by significant differences in their physiological characteristics of *in vivo* vs *in vitro* preparations. Although the source and chemical content of many afferents to the nigra have been characterized, their precise postsynaptic targets remain to be determined both at the light and electron microscopic level. PHA-L anterograde transport was used in combination with tyrosine hydroxylase (TH) immunohistochemistry to visualize nigral afferents and their sites of termination in relation to TH-immunoreactive neurons.

PHA-L was iontophoretically injected into one of the following brain loci: the globus pallidus, neostriatum, and subthalamic nucleus in anesthetized male Sprague-Dawley rats. After 10 days, animals were perfused with 4% paraformaldehyde-0.2% glutaraldehyde. Free floating 40 μm parasagittal sections were immunolabeled for PHA-L and reacted with nickel-enhanced DAB. Dopaminergic neurons were identified with TH immunocytochemistry, and the reaction product was visualized with DAB. All tissue was processed with Vector ABC reagents.

Following subthalamic injections, large non-varicose axons were seen traversing pars compacta (PC) rostro-caudally with little sign of terminal arborizations. These same injections resulted in finer and more numerous varicose axon terminals coursing both dorso-ventrally and rostro-caudally along the ventral and caudal extent of pars reticulata. There was no clear preferential association with TH immunoreactive neurons or processes; rather, in several cases, labeled axons appeared to cluster around non-immunoreactive nissl-stained cell bodies in pars reticulata. Pallidal injections resulted in a dense network of varicose terminal labeling throughout both the pars compacta and pars reticulata. There did not appear to be a preferential association of pallido-nigral axons with TH immunoreactive dendrites in pars reticulata. Neostriatal injections resulted in a dense network of fine axons and terminal labeling in the ventral pars reticulata with very little labeling within the pars compacta. Supported by MH45286 and Rutgers University Research Council.

183.10

CONTEXT-DEPENDENT BASAL GANGLIA (BG) ACTIVITY MAPPED WITH ^{14}C DEOXYGLUCOSE AUTORADIOGRAPHY. C.Manetto, T.I.Lidsky, L.L.Brown. Inst. for Basic Research, Staten Island, NY 10314 & Dept. of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

It has been suggested that BG activity is dependent on behavioral context. To evaluate this suggestion, BG activation patterns to perioral stimulation were assessed by glucose utilization autoradiography in rats in situations in which trigeminal feedback either was (context group) or was not (control group) important for guiding movement. Controls showed activation of a specific region of the dorsolateral striatum. In contrast, the context group showed greater glucose utilization in this same dorsolateral area. In addition, ventral striatal regions (including part of the nucleus accumbens), and the globus pallidus were activated only in the context rats. These results indicate that the regional specificity of processing of a given sensory stimulus depends on behavioral context. Moreover, since activation of BG output, via the pallidum, only occurred in the context group, the striatum can act as a gate for behaviorally significant stimuli.

183.11

SOMATOTOPIC MAPS IN RAT STRIATUM: EVIDENCE THAT THE STRIATUM PLAYS A ROLE IN COORDINATION OF THE TWO SIDES OF THE BODY. L.L. Brown, Dept. of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

To determine the organization of somatosensory activity in rat striatum, ^{14}C deoxyglucose mapping studies were carried out in awake rats receiving tactile stimuli of either the hindlimb, trunk, or forelimb, on the left side. The stimuli were 4/sec sweeps of a nylon bristle (1 mm dia.) which traversed 0.2-2.0 cm of the rat's body anteroposteriorly. Patchy areas of activation were identified using image analysis, and their x,y coordinates were measured at 7 different anterior-posterior striatal levels. The results showed a loose somatotopic organization contralaterally, as previously reported from this laboratory, but also ipsilaterally. The activation of both sides was not in symmetric striatal regions, however. In the posterior striatum, the activation ipsilateral to the stimulus was 300 μm dorsal to the activation contralateral. Anteriorly, it was 300-500 μm ventral. The results suggest that each side of the striatum has two separate representations of the body, one from each side, juxtaposed at distances that permit physiological interactions for side-to-side coordination.

183.12

RELATIONSHIPS BETWEEN INTERNEURONS IN THE RAT STRIATUM: PARVALBUMIN NEURONS AND CHOLINERGIC NEURONS. H. T. Chang and H. Kita. Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, 875 Monroe Ave., Memphis, TN 38163

The corpus striatum consists of both projection neurons and interneurons. Several types of striatal interneurons have been identified recently, each containing characteristic neuroactive substances such as acetylcholine, somatostatin, or parvalbumin (a calcium binding protein found in a population of GABAergic striatal interneurons). The present study aims to elucidate the synaptic relationships between cholinergic neurons (ChAT) and the parvalbumin immunoreactive (PV) neurons. A pre-embedding double-labeling immunocytochemical reaction was performed on rat brain sections in which PV neurons were labeled by silver-intensified colloidal gold particles, and the cholinergic neurons by immunoperoxidase reaction products. Our preliminary electron microscopic analysis reveals that both PV and ChAT axon terminals form synapses with unlabeled medium-sized somata with round un-indented nuclei, a typical characteristic of the medium spiny projection neurons. PV and ChAT axon terminals also form synapses with the somata of PV neurons. The somata of ChAT neurons, however, appear to be not innervated by PV terminals. These observations suggest that PV and ChAT neurons have converging influences on both the striatal projection neurons, and the striatal PV interneurons. Whether PV neurons form synapses directly with ChAT neurons, perhaps on the ChAT dendrites, remains to be determined.

(Supported by NIH Grants AG05944, NS25783, NS26473 and a grant from the Alzheimer's Association)

183.13

VENTRAL PALLIDO-STRIATAL PATHWAY IN THE RAT BRAIN. H. Kuo and H.T. Chang. Dept. of Anatomy and Neurobiology, College of Medicine, The Univ. of Tennessee, Memphis, 875 Monroe Ave., Memphis, TN 38163.

The reciprocal connections between the dorsal striatum and dorsal pallidum have been well established in the rat. Comparable connections between the ventral striatum (VS) and the ventral pallidum (VP) have also been reported. In this study we aimed to further characterize this ventral pathway to determine the morphology and chemical properties of ventral pallido-striatal projection neurons, and to determine the morphology of VP efferents in VS. Fluoro Gold (FG) was injected into VS to retrogradely label VP projection neurons. Enkephalin and substance P immunoreactivity were used as pallidal markers to delineate VP from the neighboring substantia innominata (SI). The results showed that most neurons retrogradely labeled by FG were found in VP, but a few were also found in SI. Additional double labeling experiments showed that none of these FG-labeled cells were cholinergic neurons, however, some were immunoreactive for parvalbumin, a calcium binding protein found in many pallidal neurons. The somatic size of these VS-projecting neurons is very similar to those that project to the medial dorsal thalamus. Electron microscopic (EM) analysis revealed that the long dendrites of these VS-projecting neurons formed many synapses. Injection of PHA-L into VP resulted in many anterogradely labeled fibers in VS. EM analysis revealed that some of these labeled axons were myelinated and they formed either symmetrical or asymmetrical synapses with VS neurons.

(Supported by USPHS Grant AG05944 and the Center of Neuroscience, the University of Tennessee, Memphis)

183.14

PALLIDO-NEOSTRIATAL PROJECTIONS OF THE RAT. H. Kita, H. T. Chang and K. Fujimoto. Dept. of Anatomy and Neurobiology, College of Medicine, The Univ. of Tennessee, Memphis, 875 Monroe Ave., Memphis, TN 38163.

Previous studies have shown that the globus pallidus (GP) possesses topographically organized projections to the neostriatum (Str). We have studied these projections in detail using electrophysiological and anatomical techniques. In anesthetized rats, the responses of GP neurons to striatal stimulation were recorded intracellularly. Antidromic responses were evoked from 23 of 68 recorded neurons. The mean antidromic latency was 1.0 ± 0.5 msec. GP neurons projecting to the Str were labeled retrogradely by Fluoro-Gold which was visualized with an immunoperoxidase reaction using anti-Fluoro-Gold serum. Cell count in Nissl stained sections indicated that more than 40% of GP neurons project to the Str. The labeled neurons were seen in all parts of the GP except the caudomedial GP. Most of the GP-Str projection neurons were immunoreactive for parvalbumin, a calcium binding protein which is found in GABAergic GP neurons. PHA-L anterograde labeling revealed that GP-Str fibers formed a moderately dense terminal field within the Str with many boutons en passant. Electron microscopic analysis revealed that the PHA-L labeled boutons form symmetrical synapses mainly with somata and dendritic shafts of spiny neurons. These results suggest that 1) a significant population of GP neurons project to the Str, 2) most of these neurons are GABAergic, and 3) they terminate mainly on striatal spiny projection neurons.

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183.15

RESPONSES OF SUBTHALAMIC NEURONS TO STIMULATION OF THE FRONTAL CORTEX. K. Fujimoto and H. Kita. Dept. of Anatomy and Neurobiology, College of Medicine, Univ. of Tennessee, Memphis, 875 Monroe Ave.,Memphis, TN 38163

The subthalamic nucleus (STH) receives inputs from the frontal cortex (Cx) via both monosynaptic and polysynaptic pathways. In order to investigate the role of each of these pathways, we assessed the responses of STH neurons to stimulation of the Cx in rats which received various brain area lesions. Male Sprague-Dawley rats were used. Some rats were anesthesized with Ketamine and Xylazine, and received quinolinic acid lesion of the neostriatum, and others received ibotenic acid lesion of the globus pallidus 10-14 days before recording. For recording, the rats were anesthetized with urethane, and stimulus electrodes were placed in the Cx. Responses of the STH to Cx stimulation were recorded extracellularly and intracellularly using glass electrodes. STH neurons exhibited excitations with two peaks and a subsequent long lasting inhibition in response to stimulation of the ipsi- or contralateral Cx. The responses of the STH neurons were not significantly affected by the chronic striatal lesion or the acute knife cut of the brainstem immediately caudal to the substantia nigra. However, after the pallidal lesion, the STH responded with a single peak, broad excitation. The reciprocal circuits between the STH and the globus pallidus seem to play some roles in producing two peaks of the excitation. Responses of the STH evoked by contralateral Cx stimulation basically possessed the same patterns as those evoked by ipsilateral Cx stimulation. However, the former had higher thresholds and longer latencies. Responses to the contralateral stimulation completely dissappeared after the corpus callosum was transected. Therefore, contralateral stimulation seems transmitted through the corpus callosum.

Supported by NIH grants NS25783 and NS26473

183.16

TRANSNEURONAL TRANSPORT OF HSV-1 FROM THE PRIMARY MOTOR CORTEX AND THE SMA: EVIDENCE FOR SEPARATE 'MOTOR' CIRCUITS WITHIN THE BASAL GANGLIA. J.E. Hoover and P.L. Strick. VAMC and Depts. of Neurosurg. & Physiol., SUNY-HSC, Syr., NY 13210.

We have used transneuronal transport of herpes simplex virus type 1 (HSV-1) to examine the 'motor' circuits which link the basal ganglia with 2 cortical motor areas: the primary motor cortex and the supplementary motor area (SMA). We injected the arm area of the primary motor cortex (n=2) or the arm area of the SMA (n=2) of monkeys (*Cebus apella*) with a strain of HSV-1 which is transported transneuronally in the retrograde direction. Animals were sacrificed 24-48 hrs. after the development of myoclonic jerks in the arm contralateral to the injected hemisphere. Following tissue processing, numerous neurons infected with virus were found in regions of the ventrolateral thalamus that are known to project to the primary motor cortex or SMA (e.g., VPLo and VLo). In addition, labeled neurons were found in regions of the internal segment of the globus pallidus (GPi) that are known to project to VLo. Surprisingly, transneuronal transport from the 2 arm areas labeled neurons within different regions of GPi. The neurons labeled by injections into the primary motor cortex were located largely within ventral regions of GPi. The neurons labeled by injections into the SMA were located at the same rostro-caudal level, but in more dorsal regions of GPi. These results support the proposal that the primary motor cortex and the SMA are both targets of pallidal output (Holsapple et al., J. Neurosci. '91). Furthermore, they suggest that the 2 motor areas are components of separate basal ganglia circuits. Perhaps, these circuits are differentially involved in the preparatory (SMA) and execution (primary motor cortex) phases of movement. Support: VA Med. Res. Service.

183.17

TOPOGRAPHY AND LAMINAR ORIGIN OF CORTICOCAUDATE PROJECTIONS IN RHESUS MONKEYS. E.H. Yeterian and D.N. Pandya. Dept. of Psychology, Colby College, Waterville, ME 04901, ENRM Veterans Hospital, Bedford, MA 01730, and Boston Univ. Sch. of Med.

Corticocaudate projections were studied by using fluorescent retrograde tracers. The head of the caudate nucleus (HCN) receives projections from the prefrontal cortex, the superior temporal region (STR), the cingulate gyrus, and the ventromedial temporal cortices. The rostral HCN receives projections from medial prefrontal areas 25, 32, 9 and 10, from orbital areas 11, 12, 13 and 14, and from lateral areas 9, 10, 46 and 12. STR projections are derived from the rostral part of the superior temporal gyrus and the upper bank of the superior temporal sulcus as well as the insula. The cingulate projections arise from rostral area 24, caudal area 23, and the retrosplenial cortex. The ventromedial temporal projections originate from the entorhinal cortex, the CA1 sector of the hippocampus, and areas TF, TH and TL of the parahippocampal gyrus. The caudal HCN has similar cortical inputs, but also receives some projections from areas 8 and 6. It receives relatively sparse projections from frontal and temporal proisocortices, the caudal cingulate region, the insula, and the ventral temporal cortices. It appears that cortical areas projecting to a specific region of the HCN are predominantly those that have reciprocal corticocortical connections, and that share similar architectonic features. With regard to laminar origin, the main source of corticocaudate projections is layer V, with relatively few neurons in layers VI and III giving rise to these projections. (Supported by ENRM Veterans Hospital, Bedford, MA, NIH Grant 16481, and Colby Grant 01 2265.)

183.18

PROJECTIONS FROM THE AUDITORY CORTEX TO THE NEOSTRIATUM IN THE ALBINO RAT.
Arévalo, M (1)., Herrera, M.* (3), Garcia-Méndez, J. A.* (2),.and Lanciego, J. L.* (1).
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2.- Cátedra de Histologia. Colegio Universitario de Avila. Univ. de Salamanca. (España).
3.- Dept. de Anatomia. Fac. de Medicina. Univ. de Alicante. (España).

In the present study PHA-L was injected iontophoretically into different zones of the auditory cortex of the rat. Study of the descending ipsilateral projections shows that these course rostrally and curve inwards to penetrate the caudal and lateral regions of the striate body; essentially, the caudate nucleus and putamen. The fibres forming part of the acoustic radiations continue caudally, coursing towards the medial geniculate body and other nuclei of the acoustic pathway. On passing through the striate body the descending fibres give rise to a dense plexus of collaterals forming a right angle and coursing in the dorso-ventral direction, occupying the caudate nucleus and the putamen. Among these fine collaterals with numerous terminals there are thicker fibres also with larger terminals. The terminals are found both in the neuronal fields and among the radiations of cortical fibres.

Iontophoretic injection of HRP into the striate body in areas where the above-mentioned striate plexus is present discloses neurones retrograde labelled within the auditory cortex, at the level of layers IV and V. (Supported by Junta de Castilla y León grant Nº 1115/90, and by FISS grant Nº 88/2040.)

183.19

A GABAERGIC INPUT FROM THE ZONA INCERTA TO PREDORSAL BUNDLE CELLS IN THE RAT. U. Kim*, E. Gregory and W. C. Hall. Department of Neurobiology, Duke University, Durham N.C. 27710

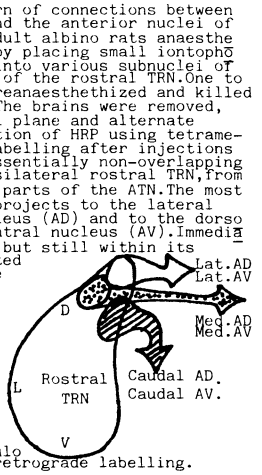
Predorsal bundle cells give rise to the main efferent pathway from the superior colliculus to brainstem centers concerned with initiating orienting movements of the head and eyes. Previous work has shown that a ventral, GABAergic subdivision of the zona incerta projects to the layer of the superior colliculus which contains the predorsal bundle cells. The present experiments demonstrate that this pathway contributes a monosynaptic input to the predorsal bundle cells. Predorsal bundle cells were labeled by the retrograde transport of HRP following injections in either the decussation of the predorsal bundle or in the paramedian pontine reticular formation. In the same animals, the ventral subdivision of the zona incerta was injected with either PHA-L or HRP for an anterograde tracer. The tissue was then processed for HRP histochemistry and/or PHA-L immunocytochemistry and viewed with the light and electron microscopes. Labeled terminals were found in synaptic contact with the proximal dendrites and somas of labeled predorsal bundle cells. The terminals contained pleomorphic synaptic vesicles and formed symmetric contacts. Further experiments using biocytin as both the anterograde and the retrograde tracer extended the main results. That is, labeled incertotectal terminals were found in close apposition to the somas and proximal dendrites of predorsal bundle cells, but some of the terminals were in apposition to more distal dendrites, at sites as far as 100 µm from the cell soma. (Supported by NIH grant EY-08233)

BASAL GANGLIA AND THALAMUS II

184.1

THE ROSTRAL POLE OF THE THALAMIC RETICULAR NUCLEUS (TRN) AND ITS CONNECTIONS WITH THE ANTERIOR THALAMUS OF THE RAT. A.Gonzalo-Ruiz,J.M.Sanz and A.R.Lieberman.Depto.of Anatomy, University College of Soria,Spain and Depto. of Anatomy, University College London, England.

We have investigated the pattern of connections between the rostral portion of the TRN and the anterior nuclei of the thalamus (ATN) in eighteen adult albino rats anaesthetized with Nembutal (45 mg/Kg),by placing small iontophoretic injections of HRP (Sigma) into various subnuclei of the ATN and into different parts of the rostral TRN.One to two days later,the animals were reanaesthethized and killed by cardic perfusion of fixative.The brains were removed, sectioned serially in the frontal plane and alternate sections processed for visualization of HRP using tetramethylbenzidine. Retrograde cell labelling after injections into ATN revealed three major, essentially non-overlapping regions in the dorsal part of ipsilateral rostral TRN,from which cells project to different parts of the ATN.The most dorsomedial part of rostral TRN projects to the lateral half of rostral anterodorsal nucleus (AD) and to the dorsolateral part of rostral anteroventral nucleus (AV).Immediately ventral to this part of TRN,but still within its dorsal portion,are medially located neurons which project to the more caudal levels of both AD and AV. The dorsolateral part of rostral TRN projects to the medial parts of rostral AD and AV and to a second region situated laterally within the posterior subnucleus of AV.No Label was seen in TRN after injections confined to the anteromedial nucleus.Injections of HRP into TRN produced anterograde terminal labelling in the ATN *in a topographic pattern co*rresponding to that of the reticulothalamic projections defined by retrograde labelling.



184.2

AFFERENT PROJECTIONS TO THE NUCLEUS ACCUMBENS CORE AND SHELL IN THE RAT. J.S. Brog*, A.Y. Deutch** and D.S. Zahm. Depts. of Anat. and Neurobiol. and *Pharmacol. and Physiol. Sci., St. Louis Univ. Sch. Med., St. Louis, MO, 63104 and **Dept. of Psychiat., Yale Univ. Sch. Med., New Haven, CT, 06508.

In response to recent reports suggesting that topography provides an incomplete explanation for differences in the connections of the core and shell of the nucleus accumbens, a comprehensive evaluation of the afferents of the compartments was undertaken. The distribution of retrogradely labeled cells following iontophoresis of fluorogold in the core or shell is reported here as a basis for subsequent targeting of confirmatory PHA-L injections. Immunohistochemistry sensitively revealed fluorogold labeled neurons. Involvements of core and shell at injection sites were judged with the aid of substance-P staining. Shell: bilateral subcortical afferents from the basal forebrain, hypothalamus, medial amygdala and brainstem, were predominant. Cortical afferents were rather sparse, ipsilateral, and almost exclusively in the rostroventral medial prefrontal area and the entorhinal cortex. Core: In contrast, bilateral cortical inputs from medial and sulcal prefrontal areas were predominant. Subcortical afferents of the core were less prominent and primarily ipsilateral - in ventral pallidum, VTA, dorsal raphe and the central grey. Shell and core: dense aggregates of labeled neurons were present ipsilaterally in midline and intralaminar thalamic nuclei and hippocampus and bilaterally in the basal amygdala. These results show certain similarities in the connectivity of shell and core, but also reveal considerable specificity of connections unrelated to topography and suggestive of separate systemic affiliations. Supported by USPHS NS-23805 and MH-45124.

184.3

TERMINATION PATTERNS AND OVERLAPPING OF THE STRIATONIGRAL FIBERS IN THE NORTH AMERICAN OPOSSUM. O.B. Ghonegun and J.C. Hazlett. Department of Anatomy and Cell Biology, Wayne State University, Detroit, MI 48201.

The opossum striatonigral projection is topographically organized (Gbonegun & Hazlett, 1989). This study further clarifies this organization by paired injections in the striatum using WGA-HRP and ^{3}H-proline and also determine their termination patterns in the substantia nigra (SN) with striatal PHA-L injections. In the paired injections, eleven animals received twenty-two injections in the rostral, middle, and caudal caudate nucleus, and the putamen. Our results confirm that rostral, middle, and caudal caudate project to medial, middle, and lateral portions of the SN respectively and the putamen projects to lateral portion of the SN. The rostromedial to caudolateral topography is less precise in the caudal one-third of the SN. This feature is related to the SN distribution of tyrosine hydroxylase posi -tive neurons (Hazlett et al., 1991). In the five animals with striatal PHA-L injections, results demonstrate that in SN reticulata (SNr), majority of labeled axon terminals and varicosities appear to contact dendrites, while a smaller component selectively contact cell bodies. This latter contingent involves direct synaptic contact with dopaminergic neurons and the former with GABAergic ones. The cell bodies receiving contacts were interspersed with neuronal somas free from such apositions. Labeled axons were also seen extending medially from SNr onto SN compacta cell somas.

184.4

COMPARTMENTAL DISTRIBUTION OF VENTRAL STRIATAL NEURONS PROJECTING TO THE MESENCEPHALON IN THE RAT. H.W. Berendse*, P. Voorn, and H.J. Groenewegen. Dept. Anatomy and Embryology, Vrije Universiteit, 1081 BT Amsterdam, The Netherlands.

The ventral striatum in the rat is characterized by an intricate neurochemical compartmentation that is reflected in the distribution of most of its afferent fibre systems. The objective of the present study was to establish the compartmental relationships of ventral striatal neurons projecting to the mesencephalon by combining retrograde (cholera toxin B) and anterograde (PHA-L) tracing methods with the histochemical localization of leu-enkephalin. Neurons in the "shell" of the nucleus accumbens, that project to the ventral tegmental area, the retrorubral field, the peribrachial region, and possibly the dorsal part of the substantia nigra pars compacta, are located outside areas of high cell density and weak enkephalin immunoreactivity (ENK-IR). In the rostrolateral part of the nucleus accumbens, neurons that reside inside the large areas of strong ENK-IR surrounding the anterior commissure project to the dorsomedial part of the substantia nigra pars reticulata. Neurons outside these areas innervate the ventral tegmental area and the medial part of the substantia nigra pars compacta. In the border region between the ventromedial part of the caudate-putamen and the dorsal part of the nucleus accumbens, clusters of neurons that project to the substantia nigra pars compacta coincide with patches of strong ENK-IR, whereas neurons in the surrounding matrix issue fibres to the dorsomedial part of the pars reticulata. These results indicate that distinct sets of ventral striatal neurons project either to the substantia nigra pars reticulata or to the ventral tegmental area, the substantia nigra pars compacta, the retrorubral field, and the peribrachial region. Moreover, these sets of neurons respect previously defined compartmental boundaries in each of the various sectors of the ventral striatum. [Supported by NWO Program Grant #900-550-093]

184.5

PATTERNS OF PEPTIDERGIC FIBERS IN THE DORSAL MIDLINE AND INTRALAMINAR THALAMUS OF THE RAT L. J. Freedman[1] and M. D. Cassell[2]. [1]Neuroscience Program and [2]Department of Anatomy, University of Iowa, Iowa City, IA 52242.

The paraventricular thalamus (PV) receives projections from many brainstem and hypothalamic autonomic centers and projects to several structures in the basal forebrain, including nucleus accumbens and central nucleus of the amygdala. Recent studies suggest a dorsoventral topographic arrangement of PV cells projecting to these limbic structures. We have examined the distribution of peptidergic fibers in PV to determine if their is a similar topographic organization of afferents to PV.

Immunocytochemical detection of several peptides was performed using the ABC technique. Three basic patterns were found within PV. Pattern 1, the most common, involves labelling throughout PV, except for a small rostromedial zone, as well as in intermediodorsal nucleus and intralaminar nuclei. Pattern 1 is found using antisera against enkephalin, substance P, neurotensin, cholecystokinin, and somatostatin. Pattern 2 involves labelling in laterodorsal PV and is found using antisera against VIP and αMSH. Pattern 3 involves labelling in ventromedial PV, rostral laterodorsal PV, and intermediodorsal nucleus, and is found using antiserum against vasopressin.

Patterns 2 and 3 resemble the retrograde labelling in PV following injections in amygdala and core of nucleus accumbens, respectively. This suggests that parallel peptidergic inputs may influence outputs to parallel limbic circuits.

184.6

MODULAR ORGANIZATION OF THE STRIATOPALLIDAL SYSTEM. L-N Hazrati and A. Parent. Lab. of Neurobiology, Fac of Med., Laval Univ., Québec, Canada, G1K 7P4.

Injections of the anterograde tracer *biocytine* (Bio) in the sensorimotor (SM) portion of the putamen in the squirrel monkey (*Saimiri sciureus)* produce anterograde fiber labeling in the two segments of the globus pallidus (GP). These fibers are arranged in the form of elongated bands (thin fiber plexuses) lying parallel to the medullary laminae and remaining strictly confined to the SM territory of the GP. Injections of Bio in the associative (AS) portion of the caudate nucleus lead to anterograde labeling in the AS portion of the two GP segments. The labeled fibers form bands orientated parallel to the internal capsule. The functional significance of the band-like pattern displayed by striatopallidal fibers and terminals is unknown. This pattern most likely correspond to the arrangement of the large and discoidal dendritic arborization of the pallidal neurons, which also lie parallel to the external surface of both GP segments. Perhaps the most intriguing aspect of studies is that each injection produces at least two terminal bands separated by zones devoid of anterograde labeling. This suggest that striatal neurons may arborize profusely upon two distant layers of pallidal cells. Indeed, a dual representation of the striatum at pallidal level has been proposed on the basis of autoradiographic tracing study of the striatopallidal projection in the rat. Studies involving intracellular injections of horseradish peroxidase reveal that single striatopallidal neurons arborize twice within the GP in the rat. On the basis of these findings we propose that the striatopallidal system terminates in the form of multiple adjacent modules, each consisting of the entire terminal arborization that covers a single layer of pallidal cells. These modules would originate from groups of striatal cells whose dimensions, shape and regional distribution remain to be determined, particularly in the light of the heterogeneous matrisomal organization of the striatum. These striatal cell groups terminating in the form of modules at pallidal level would represent the functional unit of the striatopallidal system. The fact that each functional unit gives rise to at least two modules suggests the existence of a fair amount of redundancy of information at pallidal level.

184.7

THE STRIATAL COMPARTMENTAL ORGANIZATION IN PRIMATES: LOCALIZATION OF CALBINDIN D-28k AND PARVALBUMIN WITH REFERENCE TO THE STRIOSOMES. P.-Y. Côté and A. Parent, Neurobiology Res. Center, Fac. of Med., Laval Univ., Québec, Canada.

Sections of the striatum of rhesus monkeys (*Macaca mulatta*) were immunohistochemically reacted to reveal the distribution of calbindin D-28k (CaBP) and parvalbumin (PV), two calcium-binding proteins.

Calbindin was heterogeneously distributed in the caudate and the putamen. Small regions where both cell body and neuropil immunostaining was low were encountered mostly in the rostral part of the caudate and putamen. More caudally, these calbindin-poor regions were not found in the putamen and they could not be clearly delineated in the caudate nucleus. Alternate sections stained for the enzyme acetylcholinesterase (AChE) revealed that a striking correspondence exists between the calbindin-poor regions and the striosomes. Most of the striosomes had their counterpart on the alternate sections stained for calbindin. Some calbindin-poor regions, very few in number, did not match the AChE-poor striosomes.

Parvalbumin-positive neurons were distributed in an homogeneous manner in the striatum, but the neuropil showed poorly stained regions that did not match exactly with the striosomal organization. The parvalbumin-positive neurons were disclosed in the striosomes and in the extrastriosomal matrix, whereas the calbindin-positive neurons were confined to the extrastriosomal matrix only. Most calbindin neurons were of medium size, and a few large neurons also displayed calbindin immunoreactivity. The parvalbumin neurons were much less numerous than the calbindin neurons and most of them were aspiny and of medium size although a few larger neurons were noted. These results suggest that calbindin and parvalbumin label different striatal cell populations, namely the medium size projection neurons (CaBP) and a group of aspiny interneurons (PV). We propose that CaBP can be used as a reliable marker of the striosomal organization in primates, as it is the case in rodents, and that PV might be part of a neurochemically distinct subgroup of striatal interneurons in primates. [Supported by FCAR, FRSQ and MRC].

184.8

THE TEGMENTO-NIGRAL PROJECTION IN PRIMATE: CELLULAR ORIGIN AND CHEMOSPECIFICITY. B. Lavoie and A. Parent. Centre de recherche en neurobiologie, Hôpital de L'Enfant-Jésus, Université Laval, Québec, Canada, G1J 1Z4

Recently, the organization of the massive bilateral projection to the substantia nigra (SN) originating from the pedunculopontine nucleus (PPN) has been visualized with the anterograde tracer PHA-L in the squirrel monkey (*Saimiri sciureus*). In the present study the exact cellular origin of this tegmento-nigral projection has been identified following unilateral injections of the fluorescent tracer, fluorogold (FG), in the substantia nigra of squirrel monkey. The results demonstrate that this projection originates mostly from neurons located in the rostral two-thirds of the PPN. At this level retrogradely labeled neurons were bilaterally distributed and were slightly more abundant in the ipsilateral PPN (60%) than in the contralateral PPN (40%). In the caudal third of PPN, only a few neurons were retrogradely labeled and they were mainly localized ipsilaterally. Immunofluorescence (TRICT) studies with antibodies raised against choline acetyltransferase (ChAT) and glutamate (GLU) have revealed that only a few neurons in PPN expressed GLU compared to the large number of neurons expressing ChAT. The study of colocalization of FG and TRICT in PPN neurons showed that a significant proportion of retrogradely labeled neurons were ChAT-positive compared to only a small number that expressed GLU. Further investigations combining WGA-HRP and immunohistochemical procedures should help to determine the distribution and exact proportion of cholinergic and glutamatergic tegmento-nigral neurons in the ipsi- and contralateral PPN. These findings reveal that the PPN exerts a powerful bilateral influence upon the SN and that this influence may, at least in part, be mediated by acetylcholine and, less prominently, by glutamate.
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184.9

SUBSTANTIAL ALTERATIONS IN NEUROCHEMICAL AND METABOLIC INDICES IN SELECT BASAL GANGLIA NEURONS FOLLOW LESIONS OF GLOBUS PALLIDUS NEURONS IN RATS Wm.A.Staines and M.T.C.Hincke*, Department of Anatomy, University of Ottawa,Ottawa,Ontario, Canada K1H 8M5

The globus pallidus (GP) projects massively to the subthalamic nucleus and in an apparently less dense manner to the substantia nigra pars reticulata (SNR) and the matrix compartment of the striatum (CPm). Observations made through visualizing these projections with PHAL transport indicates that select target neurons in all three regions receive equally massive innervation from the GP although the number of fibers/volume tissue is far less in the case of the SNR and the CPm. Experiments were carried out to identify some target neurons of this pathway and to examine the biochemical sequelae to removal of pallidal influence over these cells.

In the striatum, the GP efferents innervated the NPY immunoreactive cell and pallidal deafferentation decreased the number of NPY immunoreactive fibers in the striatum by 99%. A similar decrease was seen in somatostatin positive fibers and NADPH-dependant diaphorase (NADPH-d) staining in striatum but no other CP markers were affected. A mirror image decrease occurred in NADPH-d on the contrateral side. Subsequent to deafferention of nigral neurons of the pars reticulata there was a near total loss in the cytochrome oxidase immunoractivity in the cell body and proximal dendrites. These findings are taken to indicate that pallidal efferents exert a great influence over the steady state of their target neurons.

184.10

ELECTROPHYSIOLOGY AND MORPHOLOGY OF IDENTIFIED THALAMIC NEURONS USING IN VIVO INTRACELLULAR RECORDING AND STAINING. A.Lavin & A.A. Grace. *Depts. Behavioral Neuroscience and Psychiatry, Univ. Pittsburgh, Pittsburgh, PA.* 15260.

Anatomical, electrophysiological and clinical evidence indicate that the thalamus plays a major role in the regulation of motor activity. In these studies, we compared the anatomy and physiology of neurons located in motor vs. non-motor regions of the thalamus and their response to stimulation of basal ganglia afferents. Intracellular recording and staining of thalamic neurons were done *in vivo* in chloral hydrate anesthetized rats. Neurons were identified after recording by intracellular injection of Lucifer yellow (n = 10). The somata of thalamic neurons ranged from 12 to 65 µm and gave rise to three distinct fascicles of dendrites, two from each pole of the neuron and one from central regions of the soma. Injection of hyperpolarizing current pulses usually elicited prominent rebound low threshold spikes upon repolarization of the soma. Neurons recorded in the ventral posterior nuclei (n = 5) had 12-65 µm polygonal somata with dense tufts of dendrites. Spontaneous epsps were not observed in these neurons. In contrast, neurons recorded in ventral anterior and in posterior thalamic nuclei (n = 5) had fusiform shaped somata measuring approximately 20 µm in length. These neurons exhibited spontaneous epsps of 17 ± 5 mV amplitude and cortical stimulation elicited long duration epsps (35 ± 6 msec). In contrast, stimulation of the striatum elicited epsps (6 ± 1 mV) followed by ipsps which often triggered rebound low threshold spikes. In contrast to the hypothesized role of LTS in rhythmic neuron firing, these cells often exhibited irregular LTSs that appear to be elicited during the rebound of spontaneous ipsps.

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184.11

INTRACELLULAR LABELLING OF MEDIUM SPINY NEURONS IN THE PRIMATE CAUDATE NUCLEUS: ANATOMICAL RELATIONSHIP OF DENDRITES TO STRIOSOMAL BORDERS R. H. Walker, [1]G. W. Arbuthnott and A. M. Graybiel. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, and [1]Dept. of Pre-clinical Veterinary Sciences, University of Edinburgh, Scotland.

With the technique of intracellular filling of cells in lightly fixed tissue slices, carried out in combination with staining for striosomes and immunochemistry specific to the intracellular dye, we investigated the orientations of dendrites of medium-sized spiny neurons lying near striosomes in the primate caudate nucleus. Slices 400um thick were cut from lightly fixed squirrel monkey brain. Cells were filled at random using a micropipette filled with an 8% solution of Lucifer Yellow (Sigma). Slices were post-fixed, sectioned at 40um, and stained for butyrylcholinesterase to demonstrate striosomes. Following this they were incubated overnight with an antibody to Lucifer Yellow (courtesy of Drs Kuwada and Knafl) and processed with diaminobenzidine. This sequence permitted the detailed morphology of the filled cells to be clearly distinguished under the light microscope in the presence of staining for the striosomal compartments. Of 206 filled medium-sized spiny neurons, 170 were in the matrix and 36 in striosomes. The dendrites of a number of neurons near striosomal borders, 17 in matrix, 15 in striosomes, avoided crossing distinct compartmental boundaries, whereas dendrites of 5 matrix cells and 4 striosomal cells clearly crossed between compartments. Thus, although many medium-sized projection neurons observe striosome/matrix borders, a subpopulation have the potential for integrating information from both compartments.

Supported by Javits Award R01 25529.

184.12

CO-LOCALIZATION OF EXCITATORY AND INHIBITORY TRANSMITTER MARKERS IN STRIATO-PALLIDAL PROJECTION NEURONS IN THE RAT. K.M. Carnes, J.M. Dubinsky, H. Hodges*, L.E. White, & J.L. Price. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO and Dept. of Physiology, Univ. Texas Health Science Ctr., San Antonio, TX.

The striato-pallidal projection is believed to be inhibitory and to use gamma-aminobutyric acid (GABA) as a neurotransmitter. However, several lines of evidence from our laboratories suggest that a subpopulation of striato-pallidal projection neurons may contain both excitatory and inhibitory neurotransmitters. Recent findings from tissue culture experiments showed that a significant fraction of the synaptic activity recorded from coupled pairs of striatal neurons was excitatory, and could be blocked using the glutamate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3,-dione (CNQX) (Dubinsky, 1989, J. Neurosci. 9:3955-3965). Some of the presynaptic excitatory neurons were labeled with antibodies to glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA. In another study, a portion of the striato-pallidal pathway was labeled *in vivo* with the retrograde tracer, [^{3}H]-D-aspartate, a marker of neurons that possess a high-affinity uptake system for glutamate/aspartate and putatively use these amino acids as neurotransmitters (Carnes et al. 1990, J. Comp. Neurol. *302*:824-852).

In this study, we report that some striato-pallidal projection neurons are double-labeled with antibodies against GAD and with retrogradely transported [^{3}H]-D-aspartate. These *in vivo* anatomical findings, taken together with previous *in vitro* physiological results, demonstrate that a subpopulation of striatal neurons use glutamate/aspartate to mediate excitatory effects on pallidal neurons, and that some of these neurons also contain GAD. Supported by the National Parkinsons Foundation and NIH grants DC00093 and GM07200.

184.13

DYE-COUPLING IS DIFFERENTIALLY AFFECTED BY APOMORPHINE IN ACCUMBENS CORE AND SHELL NEURONS. P. O'Donnell and A. A. Grace. *Depts. of Behavioral Neuroscience and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

Dye coupling has been used as an index of electrical coupling in a number of brain regions. Furthermore, coupling between cells in the retina and in the striatum appears to be regulated by dopamine (DA). In this study, we investigate whether dye coupling (DC) occurs between cells in the accumbens (Acc) and its modulation by the DA agonist apomorphine.

Lucifer yellow was injected intracellularly into 70 accumbens neurons in rat brain slices perfused with either control or apomorphine-containing Krebs-Ringer's solutions. In all cases the stained neurons were of the medium spiny type, with 12-20 µm somata giving rise to 3-6 primary dendrites. The axon emerged either from a major dendrite or from the soma, and projected caudally. In control conditions, 10/22 neurons injected in the Acc core exhibited DC, while 5/20 neurons in the Acc shell showed DC to other neurons. Administration of apomorphine (10-100 µM) decreased the incidence of DC only between neurons in the core region (0/11 cells), and this effect was prevented by adding the specific D1 antagonist SCH 23390 (1-3 µM) to the bath (2/7 cells were coupled). In contrast, apomorphine administration caused an apparent increase in dye coupling between neurons located in the shell region (5/10 cells).

The division of the Acc into core and shell has been been based largely on differences in their connectivity patterns. These results suggest that DA may exert opposite effects on electrical coupling between cells in different regions of the Acc; decreasing the comparatively high level of electrical coupling between neurons in the core while providing an apparent increase of coupling in the shell. Supported by USPHS MH45156 and NS19608.

184.14

ULTRASTRUCTURAL LOCALIZATION OF L-BACLOFEN IMMUNOREACTIVITY IN RAT AND MONKEY SUBSTANTIA NIGRA. G.R. Holstein, P. Pasik, M. Skladany* and G. Martinelli. Depts. Neurology, Cell Bio/Anatomy & Surgery, Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

The goal of this work was the immunocytochemical visualization of L-baclofen in the rat (Sprague-Dawley) and monkey (M. fascicularis) substantia nigra. Experimental animals received an i.m. injection of L-baclofen-HCl (5 mg/kg), controls received an equal volume of saline. Ninety min. post-injection, animals were sacrificed by perfusion. Fifty µm vibratome sections were exposed to a monoclonal antibody raised against L-baclofen (Martinelli et al., 1991; *Neurosci.*). Sections were then processed with the PAP procedure, or the avidin-biotin immunogold method.

Ultrastructural observations of the PAP method revealed large immunoreactive dendritic profiles throughout pars reticulata. Many of these profiles were totally ensheathed by unlabeled axon terminals which formed synapses with the labeled dendrites. Other immunostained dendrites also occupied postsynaptic sites, but were incompletely surrounded by boutons. Small caliber unmyelinated immunoreactive axons were prevalent in the neuropil, and immunostained boutons were occasionally observed. A more restricted immunostain was obtained using pre-embedding immunogold labeling. In general, the silver-enhanced particles were membrane-bound, and formed clusters adherent to outer mitochondrial membranes, cisternae, and the plasma membrane of the neuronal elements, most often visible at synaptic contacts. The deposits were common at points of membrane apposition, both nonsynaptic and synaptic. Identically processed sections from the uninjected animals showed no immunoreactivity.

Baclofen is a specific $GABA_B$ receptor agonist. The present results suggest that the baclofen-sensitive receptors are localized both presynaptically and postsynaptically, and may participate in GABAergic feedback loops in the substantia nigra. Aided by NIH Grants # NS-24656, NS-22953. L-baclofen was generously provided by Ciba-Geigy, Ltd.

184.15

NEUROPEPTIDE Y- AND CHOLINE ACETYLTRANSFERASE-CONTAINING INTERNEURONS IN THE RAT STRIATAL NETWORK.
L. Kerkerian-Le Goff*, J. Vuillet*, R. Dimova* and A. Nieoullon. Neurochemistry Unit, Lab. of Functional Neuroscience, CNRS, 13402 Marseille cedex 9, France.

This study examined the respective position and synaptic relationships of choline acetyltransferase (ChAT) and neuropeptide Y (NPY)-containing interneurons within the rat striatum using dual immunolabelling methods and combination of immunocytochemistry with degeneration of striatal cortical or nigral neuronal inputs. ChAT-immunoreactive (-Ir) neurons (large aspiny) were found to be prevalent in the dorsolateral areas of the striatum whereas NPY-Ir neurons (medium-sized aspiny) were observed to be preferentially localized in the ventromedial part of the structure. Electron microscopic observations indicated that both ChAT and NPY-Ir neurons receive some nigral dopaminergic and cortical afferents and contact striatal spiny presumably projection neurons. In addition, NPY-Ir neurons were found for some of them to also contain the GABA synthetic enzyme, glutamic acid decarboxylase (GAD), and to be frequently contacted by GAD-Ir terminals. Examination of the relationships between the ChAT and the NPY-containing neuronal systems further showed that the two populations of striatal interneurons have reciprocal synaptic interactions, suggesting that their activities may be functionally linked. All in all, these data indicate that the NPY and the cholinergic interneurons have quite similar patterns of connectivity. The reciprocal interactions between these interneurons may serve as a comparative integrator of striatal afferent information allowing coordinate modulation of striatal projection neurons presumably involved in distinct functional loops.

184.16

ENK+ STRIATAL NEURONS AND THEIR INPUT FROM NIGRAL DA+ NEURONS AT THE EM LEVEL.
E.J. Karle, K.D. Anderson and A. Reiner. Dept. of Anatomy & Neurobiology, Univ. of Tennessee, Memphis, TN.

Although information is available on the synaptic inputs to medium spiny striatal neurons in general, little is specifically known regarding inputs to enkephalin-containing (ENK+) striatal neurons. Therefore, we investigated this issue in pigeons, in whom ENK+ striatal neurons and their dendrites and spines can be readily labeled.

Immunohistochemical labeling using an antibody against leucine-enkephalin revealed abundant ENK+ striatal perikarya and dendrites at the LM and EM levels, particularly in the rostral and medial striatum. The ENK+ dendrites were spine-laden. These spines typically received asymmetric synapses at their tips from large terminals containing numerous round vesicles (presumably of cortical or thalamic origin). ENK+ perikarya and dendritic shafts also received synaptic input from ENK+ terminals and from unlabeled terminals whose origin is uncertain. In addition, ENK+ terminals synapsed with various unlabeled cell bodies and dendrites, including "pallidal" type dendrites that were densely coated with ENK+ and other terminals.

EM double-labeling techniques using immunogold in conjunction with PAP/DAB labeling revealed that dopaminergic (DA+) nigral terminals (labeled using anti-tyrosine hydroxylase) made appositions and synapses with ENK+ neurons.

These results show that ENK+ striatal neurons qualitatively receive the same major types of synaptic input as do striatal medium spiny neurons in general. Supported by NS-19620 & NS-28721 (A.R.)

184.17

DEVELOPMENT OF DOPAMINE CELLS IN THE SUBSTANTIA NIGRA STUDIED BY THYROXINE HYDROXYLASE AND BROMODEXYURIDINE DOUBLE LABELING.
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A double immunofluorescence cytochemical technique was used for identification of transmitter phenotype of the cell by tyrosine hydroxylase (TH) and its birth date by bromodeoxyuridine (BrdU). BrdU was injected intraperitoneally (50mg/kg) to pregnant Wistar rats with gestation days of 11 through 20 and to pups at P3 (N=5 for each day). Pups injected with E11 through 20 were sacrificed on postnatal day 0 (date of birth) and 7 and P3 pups on day 4 under ether anaesthesia and perfused transcardially with 20% formalin in 0.1 phosphate buffer (pH 7.4). The brain was removed and coronal sections (30μ thick) were made through SN for immunocytochemical procedures for TH and BrdU antibody.

SN cells were double labeled for TH and BrdU with injections only at E11 through E14. No cells were labeled with injections at E15 and E16. Only small cells were labeled with BrdU with injections at E16 through E19 and large cells at E18 through E20 and P3. In terms of double labeled neurons, maximal labeling (almost 80%) of SN pars compacta (SNc) cells occurred at E12 and E13 injection while maximal labeling (approximately 65-70%) of SN pars reticulata (SNr) cells took place on E13 and E14. By P7, SNr double labeled neurons were reduced almost by 80% but only about 30% of double labeled neurons were lost in SNc. These data indicate that dopamine neurons were born earlier in SNc than SNr and less dopamine cell loss occurs in SNc than SNr post-natally. Supported by USPHS grant NS20702.

184.18

MORPHOLOGICAL DIVERSITY OF PROJECTION NEURONS IN THE NUCLEUS ACCUMBENS OF THE RAT.
G.E. Meredith[1], D.S. Zahm[2], M. Arts[1]*, R. Agolia*[2], and H.J. Groenewegen[1]. [1]Dept. Anatomy and Embryology, Faculty of Medicine, Free University, Amsterdam, The Netherlands; [2]Dept. of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO, USA.

Nucleus accumbens can be subdivided into shell and core regions, each of which is characterized by distinct neurochemical relationships and particular afferent and efferent connections. Within these two accumbal regions, further compartmentation with respect to chemical composition and connectivity, has been described. Inasmuch as striatal outputs are known to come from medium spiny neurons, we have addressed whether the fine morphology of this neuronal subtype varies systematically in the different accumbal compartments. Efferent neurons, identified in nucleus accumbens following injections of Fast Blue in the ventral mesencephalon, were intracellularly injected with Lucifer Yellow (LY) in fixed slices. The tissue was further sectioned and dual-immunostained with antisera against LY and leu-enkephalin (ENK). The ENK immunoreaction was performed to display the accumbal mosaic and the core/shell structure. Computerized (Eutectic) reconstruction and analysis revealed that all neurons were small to medium sized (somal area: 102+/-26 μm) and spiny. Shell cells had fewer dendrites, which were longer, thicker and less spiny than those of core cells. Morphological differences distinguishing cells in and out of the ENK patches in the core were not observed.
Support: Neth. Org. Sci. Res. (NWO) travel grant (GEM), NWO program grant 900-550-093 and USPHS NS-23805.

OCULOMOTOR SYSTEM I

185.1

THE DIRECT CONNECTION BETWEEN THE SUPERIOR COLLICULUS AND OMNIPAUSE NEURONS IN THE CAT.
M. Paré and D. Guitton, Montreal Neurol. Inst. and McGill Univ., Montreal H3A 2B4.

Omnipause neurons (OPNs) are tonically active during fixation but cease firing for saccade duration. Their activity inhibits the saccadic pulse generator and controls it as an on-off gate. Raybourn and Keller (1977) established a direct connection between the superior colliculus (SC) and OPNs. This projection was found to be excitatory and therefore could not inhibits OPNs and initiate saccades. An attempt to explain the function of that connection was proposed by Munoz and Guitton (Rev Neurol 1989). In their model, cells of the SC's fixation area project onto, and excite, OPNs and therefore contribute directly to fixation behavior and participate in saccade initiation throught disfacilitation. Here we report on the nature of the connection between the deeper layers of the SC and OPNs. We recorded OPNs from two alert cats, implanted with SC stimulating electrodes. The characteristics of the gaze shifts elicited at each site were evaluated with parametric studies and indicated the location of the electrodes on the collicular motor map. We found that OPNs could be orthodromically activated from many different sites in the motor map of the SC. 74% of the neurons were mono-synaptically excited from at least one site; other sites elicited either responses having longer latencies (e.g. disynaptic) or no response at all. 23% of the neurons were not driven by the electrical stimulus from any sites. Electrical stimulation of the fixation area of the SC elicited OPN responses, that had the shortest latencies and lowest threshold intensities. Our results suggest a distributed projection from the SC onto the OPNs. They also support the hypothesis that the fixation area of the SC projects preferentially to OPNs.

185.2

ACTIVITY OF OMNIPAUSE NEURONS DURING GAZE SHIFTS ELICITED BY COLLICULAR STIMULATION IN THE ALERT CAT.
D. Guitton, M. Paré and M. Crommelinck, Montreal Neurol. Inst. and McGill Univ., Montreal H3A 2B4.

In the head-fixed cat, electrical stimulation of the superior colliculus (SC) elicits either contraversive fixed-vector or "goal-directed" eye saccades when the stimulating electrode is located in the rostral or caudal parts of the SC, respectively. In the head-free cat, stimulation elicits fixed gaze (eye+head) vectors defined by the site in the motor map being stimulated. Notewhorty, when the caudal portion of the SC is stimulated, the eye movement is still "goal-directed"; the eye trace consists of a saccade followed by a plateau period during which the eye remains immobile at a craniotopic goal location. We have recorded the activity of omnipause neurons (OPNs) during gaze shifts elicited by stimulation of the caudal SC. In the head-free condition, OPNs paused for the gaze duration and not for the duration of the eye saccade. These results concur with our previous report that OPNs exhibit gaze-related activity during visually-guided combined eye-head gaze shifts (Paré and Guitton, Exp Brain Res 1990). In the head-fixed condition, the pause duration corresponded to the eye saccade duration. The pause did not prolong into the plateau period, althought the induced gaze error was not nulled by the small eye movement evoked. In addition, OPNs did not pause when the eye motion was ipsiversive or when the eye, already at the craniotopic goal location, did not move when stimulation was applied. We suggest that two mechanisms gate OPNs: (1) a collicular feedback circuit reactivating the SC's fixation area when head motion is permitted, (2) a brainstem circuit that stops both vestibular quick phases and the "goal-directed saccades" we evoked when the head was fixed.

185.3

EFFECT OF FRONTAL EYE FIELD OR SUPERIOR COLLICULUS LESIONS ON STIMULATION-EVOKED SACCADES ELICITED FROM THE PRIMATE DORSOMEDIAL FRONTAL CORTEX. E. J. Tehovnik, K. M. Lee and P. H. Schiller. Department of Brain & Cognitive Sciences, M.I.T., Cambridge MA 02139

Using electrical stimulation, we have shown that there is an organized topographic map in the dorsomedial frontal cortex (DMFC) for the execution of eye movements and for the maintenance of gaze. The evoked movements are always contraversive and the final eye position, or termination zone, is relatively invariant to initial eye position. As the electrode is stepped caudally in the DMFC, the termination zone shifts from the periphery to the midline of craniotopic space along the azimuth. Furthermore, if electrical stimulation is maintained while the eyes are fixating a target situated in a termination zone, all visually-evoked saccades are inhibited and gaze is maintained. We posit that this region harbours a motor map that specifies eye position in head-space coordinates.

It is known that both the frontal eye fields and the superior colliculus receive direct projections from the DMFC. Both these structures innervate the brainstem which contains the saccadic generator. We lesioned either the frontal eye fields or the superior colliculus to determine if the DMFC utilizes either of these channels to gain access to the brainstem. Our preliminary findings suggest that neither of these lesions abolishes saccades evoked from the DMFC; furthermore, the integrity of the motor map is generally preserved suggesting that other channels are used by the DMFC to have access to the brainstem.

(Supported by McDonnell-Pew and NIH EY00676)

185.4

DIRECTION SELECTIVE SACCADE RELATED MOSSY FIBER ACTIVITY IN THE OCULOMOTOR VERMIS OF MACAQUES. K. Ohtsuka and H. Noda. Dept. of Vis. Sci., Sch of Optometry, Indiana Univ., Bloomington, IN 47405.

The discharge of direction selective mossy fibers was studied during saccades in different directions and amplitudes in the oculomotor vermis of the macaque. Most units did not display background activity, except for a few burst-tonic units, and were located in the white matter. The recording side of units was determined by the direction of horizontal component of evoked saccades during microstimulation. The majority (63%) of units preferred contralateral saccades and they started bursting 23.8 ms (SD 13 ms) prior to the saccade onset. The lead time of the mossy fibers which discharged with ipsilateral saccades was slightly longer; 33.1 ms (SD 16.5 ms). Although the burst duration did not show a high correlation with the saccade amplitude, the burst offset was time locked to the saccades offset. The duration from the peak firing to burst offset was correlated with the saccade duration. Movement field was demonstrated in most units; 60% of these showed the sector type, while 40% showed the vector type. In correlation with the discharge of the previously studied fastigial units, the neural mechanisms subserving the control of saccades by the oculomotor vermis will be discussed (supported by NIH grant EY04063).

185.5

AN UPWARD BIAS OF SACCADES TO REMEMBERED TARGETS IS REFLECTED IN THE MOVEMENT FIELDS OF SACCADE-RELATED BURST NEURONS (SRB's) IN THE SUPERIOR COLLICULUS (SC) OF THE MONKEY. T.R. Stanford, E.G. Freedman*, and D.L. Sparks. Dept. of Psychology, Univ. of Pennsylvania, Phila. PA 19104.

Compared to visually-guided saccades, saccades to the remembered location of a visual target are less accurate, more variable, and display a pronounced upward bias (Gnadt et al., *Vis. Res.* 31:693, 1991). In an effort to examine the neural basis for these errors, we recorded from collicular SRB's and compared movement fields obtained for saccades to visual and remembered goals. Each SRB was recorded while the animal performed an alternating sequence of visual and memory trials. Both trial types required the fixation of a central LED after which an eccentric LED was illuminated. The monkey maintained fixation until the central LED was extinguished, at which time a saccade could be initiated. On visual trials, the target remained on, while on memory trials, the target was extinguished 500 ms before the fixation LED.

Movement fields of SRB's for saccades to remembered targets were shifted relative to those for saccades to visual goals. The shift was consistent with the upward bias observed for saccades to remembered targets. For example, a neuron that discharged maximally before horizontal saccades on visual trials discharged maximally prior to oblique, upward saccades on memory trials.

Other authors have suggested that a bias could result from distortion in the sensorimotor transforms required to make saccades to remembered locations (Gnadt et al., *Vis. Res.* 31:693, 1991). An upward shift in preferred direction for neurons in intraparietal cortex (LIP) has been reported and postulated as a neural correlate of this bias (Bracewell et al., *Soc. Neurosci. Abstr.* 16:622, 1990). The movement field shifts that we've observed for collicular SRB's might reflect a distortion that originates in area LIP. Alternatively, the signal responsible for the upward bias may be added at a site downstream to the SC. Addition of such a signal would alter the relationship between discharge rate and saccade metrics for any antecedent neuron. (Supported by NIH EY01189 and F32-EY06320).

185.6

A Class of Collicular Saccade Related Bursters (SRB) specifies the metrics of impending eye movements after target specification but before movement initiation. Paul W. Glimcher and David L. Sparks. Department of Psychology. University of Pennsylvania, Philadelphia, PA 19104.

SRBs have been described whose discharge pattern includes a low frequency component as prelude to a high frequency burst. Only the burst component has shown a temporal correlation with the impending eye movement (Sparks, 1972). We report here that, in a subset of these units, this prelude is correlated with movement selection. The ongoing activity of the prelude appears appropriate to serve as a motor latch, encoding the impending movement.

The prelude was studied by using a multiple target paradigm which independently cues target presentation, selection and movement initiation. Briefly, animals were trained to fixate a yellow LED. While this fixation light was illuminated, two eccentric yellow *potential target* LEDs were briefly illuminated, one above and one below the horizontal meridian. Several hundred msecs after these targets were extinguished, the fixation light was extinguished and a second yellow fixation light illuminated. After the monkey looked to the new fixation light, its color was changed to either red or green, selecting one *potential target* and deselecting the other. Red signaled that a saccade to the remembered location of the upper target would be rewarded after the fixation light was extinguished. Green similarly specified the lower target. In this manner, the behavior of collicular units can be sampled before, during and after target selection and movement initiation, in both retinal and motor coordinate frameworks. Traditional methods were employed to record the activity of single collicular units.

The class of SRBs reported here become active 300-800msec after the fixation LED turns red/green if and only if the impending movement is encoded by the SRB's burst component. This prelude activity continues as long as the animal fixates the red/green LED (measured up to 4000msec) and is almost perfectly correlated with the impending movement. This signal appears appropriate for encoding the metrics of a selected, impending movement.

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185.7

A MODEL FOR TRANSFORMING AUDITORY SIGNALS FROM HEAD-CENTERED TO OCULOMOTOR ERROR COORDINATES. J. M. Groh and D. L. Sparks. Institute of Neurological Sciences and Department of Psychology, Univ. of Pennsylvania, Phila., PA 19104.

The primate superior colliculus (SC) contains an auditory map of space which appears to be encoded in a motor error rather than head-centered frame of reference (Jay and Sparks, *J. Neurophys.* 57:35-55, 1987). The spatial locations of the receptive fields of the cells in this map shift with changes in eye position, thus encoding the direction and amplitude of the eye movement needed to acquire the target.

Such a map cannot be derived directly from the peripheral sensory receptors, but must be computed neurally instead. The location of an auditory target relative to the head can be determined by interaural timing and intensity differences. Then, subtraction of the position of the eyes in the orbit yields the motor error of the auditory target.

A neural architecture for the implementation of the second portion of this computational process is proposed here. The model's inputs are an anatomical map of auditory target position in head-centered coordinates and frequency coded signals of horizontal and vertical eye position. The anatomical code of auditory target location is first decomposed into frequency coded signals of its horizontal and vertical components using synaptic weights. Then, the appropriate eye position signals are subtracted through inhibitory connections, yielding frequency codes of horizontal and vertical motor error. These frequency codes are transformed back into an anatomical code through a combination of differing thresholds and inhibition. This final stage produces a dynamic map analogous to the auditory map found in the SC.

185.8

ROLE OF HIGH FREQUENCY GROUPED DISCHARGES OF CAT TECTORETICULOSPINAL NEURONS. E.Olivier*, A.Grantyn* and A.Berthoz (SPON: European Brain and Behavior Society).
CNRS, Laboratoire de Physiologie Neurosensorielle, Paris, France.

Tectoreticulospinal neurons (TRSN) of the cat exhibit steady state firing of 700-1100 imp/s when strongly depolarized by transmembrane current (Grantyn et al.,EBR,1983). During orienting movements in alert cats, TRSN bursts contain only short grouped discharges (doublets, triplets, etc.) characteristic of firing in the regenerative mode (Grantyn & Berthoz,EBR,1985). The role of grouped discharges in the control of eye and head movements has been assessed by recording the activity of identified TRSN axons in alert, head-fixed cats during gaze shifts induced by moving visual stimuli.

Grouped discharges (GD) reaching instantaneous rates of 300-800 imp/s are usually present in bursts accompanying orienting in the preferred direction of a given TRSN. GDs generated before saccades often coincide with the onset of a slow eye movement in the direction of the forthcoming gaze shift (pre-saccadic drift). Similarly, GDs appearing during the deceleration phase of the saccade are followed by an increment of saccadic velocity (reacceleration) or by the initiation of a slow eye movement in the same direction (post-saccadic drift). The generation of GDs also correlates with short-lasting EMG increments superimposed on neck muscle activity associated with orienting gaze shift.

The results show that GDs may lead to an augmentation of saccadic eye velocity and induce brief supplementary increments of the ongoing contraction of neck muscles. GDs may also contribute to the triggering of pre- and post-saccadic slow eye movements. These effects could be mediated by direct connections of TRSN with ocular and spinal motoneurons, reinforced by the activity of eye-neck reticulospinal neurons (Grantyn & Berthoz,EBR,1987).

185.9

EFFECT OF BICUCULLINE INJECTED INTO THE FASTIGIAL OCULOMOTOR REGION UPON VISUALLY-GUIDED SACCADES. H. Sato, H. Noda and R. Noda. Vis. Sci. Dept., Sch. of Optometry, Indiana Univ., Bloomington, IN 47405.

To study the effect of impulses from the oculomotor vermis upon visually-guided saccades, the GABA-mediated synapses were blocked by bicuculline injected into the fastigial oculomotor region (FOR) of the monkey. When bicuculline was injected into the FOR, saccades evoked by stimulation of the oculomotor vermis were suppressed for several hours. The changes in the evoked saccades were used as an indicator of the blocking of oculomotor impulses. Saccades from the center to one of 8 targets, evenly spaced LEDs on the 15° perimetric circle, were recorded and their trajectories were reproduced by computer. When the synapses in the FOR were blocked, evidenced by the suppression of evoked saccades, saccades toward the targets presented on the injected side became hypometric. Saccades in the opposite direction became hypermetric. The strongest effect on saccades appeared in the upper-oblique, horizontal, or lower-oblique direction, depending on the experiment and the initial eye position shifted in the direction opposite to that of the strongest effect on saccades. These effects were reversible and the time course of the recovery was closely related to the changes in the evoked saccades (supported by NIH grant EY04063).

185.10

THE EFFECT OF BOTULINUM TOXIN ON SACCADIC EYE MOVEMENTS IN RHESUS MONKEYS. E. J. FitzGibbon, P. Inchingolo, L. M. Optican and M. E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, and Dipartimento di Elettrotecnica, Elettronica ed Informatica, Universita di Trieste, Trieste, Italy.

Botulinum Toxin (BTX) has been used clinically to realign the positions of the eyes in strabismus. We have studied the effect of BTX in two rhesus monkeys after injections in the horizontal rectus muscles of one eye. In each monkey the medial rectus muscle was affected more than the lateral rectus. We recorded eye position of both eyes simultaneously while animals viewed only through the normal eye, in order to study the open-loop response of the weak eye to normal neural commands.

We noted four unexpected characteristics of saccades made by the weak eye to visual targets seen only by the normal eye: 1) There was a directional hysteresis with orbital position dependency: the static position of the weak eye in relation to the normal eye depended on the direction of the previous eye movement. 2) A reduced saccadic step gain of the weak eye that was dependent on orbital-position. 3) There were post-saccadic ocular drifts, whose amplitudes and time constants were continuous functions of orbital position. The ratio of drift amplitude to antecedent saccade amplitude was a nonlinear function of the time constant of the drift, and varied from positive values in temporal positions to negative values in nasal positions. 4) Saccades were slower, and nasally directed saccades began later, in the weak eye.

These effects of BTX can be viewed in terms of a model of the oculomotor plant with a single nonlinear viscoelastic element. BTX not only decreases the innervation-tension gain of the model, but also increases its effective stiffness and viscosity. These changes depended on both orbital-position and saccade-direction.

185.11

SMOOTH-PURSUIT EYE MOVEMENT RELATED ACTIVITY IN MONKEY NUCLEUS RETICULARIS TEGMENTI PONTIS. D.A. Suzuki, T. Yamada*, K.F. Betelak*, & R.D. Yee. Dept. of Ophthalmology, Indiana Univ. Sch. of Medicine, Indianapolis, IN 46202.

The nucl. reticularis tegmenti pontis (NRTP) may be part of a cortico-ponto-cerebellar pathway involved with regulating smooth-pursuit eye movements (SPEM). A major source of cortical inputs to NRTP is the Frontal Eye Field, where lesions cause pursuit deficits. NRTP activity was recorded in alert monkeys trained to fixate a moving 0.25 deg spot. Eye position was monitored with the scleral search coil technique.

In addition to the saccade-related activity reported earlier by Crandall & Keller, a variety of ocular motor related responses were recorded from NRTP. The most common SPEM-related response was direction selective and encoded pursuit eye velocity. A novel finding was the recording of unit activity that appeared to encode smooth-pursuit eye acceleration. When pursuit direction changed ("turnabout"), these cells exhibited peak firing rates that were not related to eye position. Eye position related activity per se was, however, recorded in a different population of NRTP cells.

These results add to the evidence that implicate a role for NRTP in SPEM control. A Frontal Eye Field-NRTP-cerebellum route would parallel the visual motion (MT/MST)-dorsolateral pontine nucleus-cerebellum pathway. This parallel SPEM circuit could account for the recovery of pursuit ability after forming lesions in dorsolateral pontine nucleus.
Supported by a grant and fellowship from RPB Inc., NY, NY.

185.12

INITIAL EYE POSITION DEPENDENCE OF EYE MOVEMENTS EVOKED BY MICROSTIMULATION IN THE MONKEY NUCLEUS RETICULARIS TEGMENTI PONTIS. T. Yamada*, D.A. Suzuki, K.F. Betelak*, and R.D. Yee. Dept. of Ophthalmology, Indiana Univ. Sch. Medicine, Indianapolis, IN 46202.

The characteristics of eye movements elicited by microstimulation in the nucleus reticularis tegmenti pontis (NRTP) were effected by initial eye position. Two monkeys were trained to fixate and track a 0.25° target spot. Pursuit-related neuronal responses were recorded prior to stimulation. The stimulus was delivered through the same recording electrode. 50-400 msec trains of biphasic, 0.3 msec, 10-100 μA pulses were delivered at 100-600 Hz.

Microstimulation elicited "pursuit-like", smooth eye movements. In contrast to stimulation in MT/MST, robust eye movements could be evoked when the animal was in the dark and not fixating, as well as when it fixated a stationary target. The speed of the evoked eye movements depended on initial eye position, even though there was no pre-stimulus neuronal response to eye position per se. The speed of the evoked eye movements did not seem to be related to the speed of the pursuit eye movements. The speed of the evoked, "pursuit-like" eye movements was dependent on (i) initial eye position, (ii) the frequency of the stimulating pulse train, and (iii) the intensity of the stimulating current.

The results support the conclusion that NRTP is involved with the regulation of smooth-pursuit eye movements.
Supported by a grant and fellowship from RPB Inc., NY, NY.

185.13

GABAERGIC INNERVATION OF ABDUCENS MOTONEURONS RETROGRADELY OR INTRACELLULARLY LABELED WITH HRP. AN ULTRASTRUCTURAL STUDY IN THE RAT. H.Bras*, G.Chazal, A.Barbe* and F.Lahjouji*, CNRS UPR 418, 280 Bd Ste Marguerite, 13009 Marseille (France).

GABAergic innervation of abducens motoneurons was studied using a combination of post-embedding gold-immunocytochemistry with retrograde or intracellular labeling of the motoneurons. 1) For retrograde labeling, HRP was revealed in vibratome sections with TMB stabilized with ammonium molybdate. Ultrathin sections were cut from these blocks and were processed for immunocytochemistry. The retrograde labeling of the motoneurons consisted of needle-like deposits distributed in the cytoplasm of their somata and proximal dendrites. GABAergic axon terminals (ATs) were characterized by the presence of colloidal gold particles. The majority of the labeled ATs were densely packed with ovoid and flat vesicles and mitochondria. They were more numerous on the proximal dendrites than on the somata. 2) To visualize and quantify the GABAergic innervation on the whole dendritic arborization, motoneurons were identified and intracellularly stained with HRP revealed on vibratome sections with DAB intensified with cobalt. The neurons were reconstructed in three dimensions using a computer aided microscope. The visualization of the neurons in different views revealed that the dendrites of each neuron appeared to occupy a definite site. The thick sections were cut in 4 μm serial sections, and the exact location of the observed labeled dendritic profiles were determined referencing the 3D reconstruction before ultrathin sectioning and immunocytochemical staining. The quantitative analysis of GABA ATs distribution is currently under study to determine the percentage of synaptic covering and the packing density on the somata and along the dendrites.

185.14

CORTICAL CONNECTIONS OF TWO DIFFERENT EYE FIELDS IN THE DORSOMEDIAL FRONTAL CORTEX OF THE MACAQUE MONKEY. R. Camarda*, G. Luppino*, M. Matelli* and G. Rizzolatti *(SPON: European Brain and Behavior Society) Istituto di Fisiologia Umana Universita' di Parma Via Gramsci 14 I-43100 Parma Italy.

A large sector of the dorsomedial frontal cortex sends projections to the intermediate and deep layers of the superior colliculus. Cytoarchitectonically, this sector corresponds to the dorsal part of area 6aβ and to area 8b of Walker. Physiologically, eye movements can be elicited from the dorsal part of area 6aβ (Supplementary eye field, SEF, Schlag and Schlag-Rey, Exp. Brain Res. 58:208-211, 1985) and from area 8b (Mitz and Godschalk, Neurosci. Lett. 106:157-162, 1989). In this study we examined the possibility that these two cortical sectors may represent two different anatomo-functional areas. Intracortical microstimulation was used to localize the region from which oculomotor responses could be evoked. The caudal and the rostral parts of this region were then separately injected with fluorescent tracers (FB and TB) in the two hemispheres of one Macaca fascicularis. The two injections produced labeling in different prefrontal, limbic, parietal and temporal cortical regions. The caudal injection produced intense labeling in the dorsal and ventral parts of frontal eye fields (FEF), in area 12 and in area 8b. Substantial but less dense labeling was observed in caudal parts of area 9 and area 46. Minor projections originated from area 24, area 7 and from polysensory areas of superior temporal sulcus (STS). The rostral injection produced intense labeling in dorsal and mesial area 10, area 9, dorso-rostral area 46 and in dorsal area 6aβ. Rich projections came also from orbito-frontal cortex as well as from limbic areas 32 and 25. In contrast to the caudal injection, only minor projections were found in the dorsal part of FEF. Finally, few cells were found in the polysensory areas of STS.

In conclusion, architectonic and hodological data suggest that the dorsomedial frontal eye field is composed by two different areas: dorsal area 6aβ, caudally, and area 8b, rostrally. The caudal area is strongly connected with FEF and receives from both visual and auditory-related areas. The rostral one is weakly connected with FEF and receives a robust input from limbic and prefrontal areas.

185.15

SMOOTH PURSUIT EYE MOVEMENTS OBEY LISTING'S LAW IN THE MONKEY. Th Haslwanter*, K Hepp*, D Straumann, MR Dürsteler, BJM Hess. Neurology Dept., Univ. Hospital, CH-8091 Zürich, Switzerland.

Listing's law specifies the amount of ocular torsion as a function of the line of sight by stating that all eye-rotation-vectors lie in a plane. It is known to hold true for fixations and saccades, but its validity for smooth pursuit eye movements has been questioned by different authors.

We have tested Listing's law during smooth pursuit in the monkey, and compared the results with recordings during spontaneous eye movements in the light. Two juvenile rhesus monkeys were chronically prepared with a dual search coil for 3-dimensional eye position measurement. They were trained to follow a light spot. The spot made a smooth, quasi-periodic movement covering the oculomotor range up to ± 20 deg. Eye position recordings were taken over 2 min periods, and saccades were removed. The pursuit movements had a standard deviation from Listing's plane of only 0.66 deg. This value was smaller than that for fixations (0.79 deg).

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185.16

CONJUGACY OF HUMAN SACCADES. R. Bains, W. Cadera*, T. Vilis. Depts. of Physiology and Ophthalmology, The University of Western Ontario, London, Ontario, Canada, N6A 5C1.

The observation that the saccadic system can undergo asymmetric monocular adaptation raises the question as to how the two eyes are yoked. Are they driven by independent saccade generators, or a common saccade generator? The yoking of the two eyes was examined in five normal human subjects using a three-dimensional scleral search coil technique. Subjects made saccades from a central target to 12 radial targets at 30 degree eccentricity. The eyes were 2 meters from the central target, a distance which produced insignificant changes in vergence during saccades. Saccades to the same target showed considerable trial to trial variation in peak velocity, duration, and curvature (deviation from a linear trajectory). However, variations in one eye were mirrored by similar variations in the other eye with a high positive correlation. The mean correlation coefficients for the five subjects were 0.92 for peak velocity, 0.92 for duration, and 0.85 for curvature. The high correlation between the peak velocities suggest that saccades in the two eyes are driven by a common saccade generator. The high correlation between saccade durations and between saccade curvatures suggests that burst activity is guided by a single feedback loop. Thus, it appears that the site of independent adaptive control is distal to the feedback loop, perhaps at the level of the ocular motor nuclei.

185.17

VIOLATIONS OF LISTING'S LAW AND DONDERS' LAW DURING HEAD AND EYE MOVEMENTS. T. Vilis, B. Glenn and D. Tweed. Dept. of Physiology, Univ. of Western Ontario, London, ON CANADA N6A 5Cl.

Previous studies of the three dimensional position of the eye in space, during combined head and eye movements, have suggested that its torsional position is confined to a plane and thus obeys Listing's law as does the eye when the head remains stationary. The present study reexamined this question using five normal human subjects instructed to fixate visual targets over a large range (± 70° or greater). Static eye position in space, at the end of gaze shifts, was constrained to a surface but this surface exhibited a consistent twist similar to that of a Fick gimbal (with Fick torsion=0). During gaze shifts, eye position moved out of and then back into this surface. This dynamic violation of Donders' law was in part due to the VOR acting to stabilize gaze near the end of the head movement.

The observations that the eye in space violates Listing's law statically and Donders' law dynamically suggest that the perceptual rationale for Listing's law, proposed by Hering, (that it maintains self-congruence of the retinal images of oblique lines in the outside world) should be discarded.

185.18

OPTOKINETIC MOVEMENTS AND LISTING'S LAW. D. Tweed, E. Koenig*, D. Fischer*, H. Misslisch*, M. Fetter. Department of Neurology, University of Tuebingen, D7400 Tuebingen, Germany.

Saccades and pursuit follow Listing's law (i.e. they restrict eye position to 2 degrees of freedom by keeping the torsional component = 0). This restriction to 2-D does not hamper these systems, because their job is to control a 2-D variable: gaze direction. But the eye needs all 3 of its degrees of freedom to track en bloc rotation of the visual field: to stabilize the whole field on the retina, eye velocity must be matched to field velocity in all 3 dimensions. Deviations from Listing's law should occur even during horizontal full-field tracking. We used search coils to measure 3-D eye position in 7 human subjects as they tracked a field of light spots which could rotate about any axis. Eye position plots showed ocular torsion restricted to 0 ± 6 deg even during torsional tracking, because each quick phase of nystagmus restores torsion to near 0. To see whether slow phases follow Listing's law, we examined slow phase eye velocities, looking for a particular dependence on eye position characteristic of the law. An analysis program assigned scores to periods of horizontal tracking: 0 when eye velocity was independent of eye position (as required for optimal tracking), .5 for Listing's law. As a control, horizontal sinusoidal pursuit of a single red light spot superimposed on the moving field gave an average score of .50 (range .49 - .52; 3 subjects). For full field tracking, scores averaged .32 (.08 - .53; 7 subjects), i.e. perfect tracking was sacrificed to "partially follow" Listing's law. Scores were < .5 ($p < .05$) in all but 1 subject, even for the first 2 s of motion, suggesting that even the initial seconds of tracking are not accomplished by simple pursuit.

OCULOMOTOR SYSTEM II

186.1

A SURVEY ON THE RESPONSE CHARACTERISTICS OF NEURONS LOCATED IN THE PRETECTAL REGION IN THE SQUIRREL MONKEY. J. Kröller, F. Behrens, O.-J. Grüsser and W. Rüdiger. Physiologisches Institut der Freien Universität, Arnimallee 22, 1000 Berlin 33, Germany

Pretectal nuclei are known to be involved in the pupillary light reflex (OPN, PPN) and in the control of eye movements (NOT). In 2 awake untrained monkeys, recordings of action potentials were obtained from single pretectal neurons of both sides; eye movements were monitored by scleral search coils. The neurons recorded could be tested in 3 paradigms: (a) the monkey was placed inside a rotating drum of vertical black-and-white stripes; (b) a random dot pattern was projected onto a 90x90-degree round screen in front of the monkey which could be moved in any direction or (c) varied in brightness. Within a region of about 1 mm diameter anterior-lateral to the colliculi, we found neurons of the following response characteristics:

(1) Units sensitive to an increase in brightness and (2) units which exhibited an increase in discharge rate to darkening. Sudden steps in intensity elicited a slight overshoot in activity followed by a plateau of high activity. A short phase lead occurred in responses to sinusoidal intensity modulations between 1 and 3 Hz. The response characteristics of neurons sensitive to light or to darkening seem to be similar except for the sign of the response. The units have large uniform receptive fields. (3) Movement-sensitve units appear to respond to the retinal image motion velocity in the ipsiversive direction. There was a wide range of response characteristics to rapid onset of movement. Some transition between groups 1 and 3, and 2 and 3, were observed.

186.2

FIXATION OF A STATIONARY TARGET WITH MOVING PATTERNED BACKGROUNDS BY A MONKEY WITH BILATERAL FOVEAL LESIONS. A.A. Witkofsky, A.A. Skavenski and C.M. Acocella. Psychology Department, Northeastern University, Boston, MA 02115.

Humans and monkeys with macula scotomata readily adapt to the loss by developing a Preferred Retinal Locus (PRL) on intact eccentric retina. Using the PRL, quality of fixation on a small point of light in the dark nearly matches fixation with the fovea. We asked whether this fixation quality would be as robust in the face of prominent moving backgrounds as commonly would be encountered in normal viewing. A macaque monkey was trained to fixate on a small point. After the monkey received bilateral three degree lesions of the fovea, we tested its fixation while a 12 deg arc diameter, checkered background (check sizes of 22, 36 and 48 min arc) moved slowly, horizontally or vertically, in triangle wave motion. The moving background had a small effect on drift direction. But, the directions of the predominate normal drift directions had not changed. The variability of fixation increased only modestly compared to fixation with a dark background. We conclude that the additional stimulation of the eccentric retina had little effect on the effectiveness of slow control during fixation with the PRL.

186.3

SMOOTH PURSUIT EYE MOVEMENTS IN THE PRESENCE OF STRUCTURED BACKGROUND BY A MONKEY WITH BILATERAL FOVEAL LESIONS. C.M. Acocella, A.A. Skavenski, & A.A. Witkofsky. Dept. of Psychology, Northeastern University, Boston, MA 02115.

Loss of central vision due to retinal disease degrades performance of fine visual tasks in humans. Fixation has been shown to adapt rapidly to a new preferred retinal locus (PRL) after elimination of foveae, but saccades adapted incompletely. In the present study, the characteristics of steady-state smooth pursuit performance in the presence of homogeneous or structured backgrounds was examined. A macaque monkey was first trained to smoothly pursue a small target moving with sinusoidal motion. Foveae were then lesioned, leaving a 3 deg bilateral scotoma. Soon after lesions, tracking performance of sinusoidal and pseudorandom motion with the PRL at 2 deg eccentricity was good against dark or homogeneous illuminated backgrounds and dim structured backgrounds. When luminance of the structured background was raised, tracking was marked with frequent saccades and very little smooth component. After practice, however, performance improved dramatically and there was a shift from saccadic tracking back to smooth pursuit. Thus, the smooth pursuit system seems largely unaffected by the loss of foveal vision, and prior reports (e.g. Tamminga, 1983) of the breakdown of peripheral tracking on backgrounds may owe to the subjects' inexperience with the task.

186.4

EFFECTS OF BOTULINUM TOXIN A ON THE PHYSIOLOGY AND MORPHOLOGY OF THE ORBICULARIS OCULI MUSCLE. A.K.E. Horn, J.D. Porter, C. Evinger, Dept. Neurobiol. & Behavior , SUNY Stony Brook, Stony Brook, NY 11794, Dept. Anat. & Neurobiol., Univ. Kentucky, Lexington, KY 40536

Injections of Botulinum Toxin A (BotA) into the orbicularis oculi muscle (OO) is a treatment for lid spasms in humans. Unfortunately, these treatments must be repeated every 2-3 months to prevent spasms. We studied the time course of BotA effect on the blink reflex by monitoring blink parameters prior to and after BotA injection (0.02-0.3 ng) into the OO, and compared the physiological findings with structural changes in the OO. In chronically prepared guinea pigs we measured lid movements with the search coil and the OO-EMG activity during blinks evoked by electrical stimulation of the supraorbital nerve or an air puff. After post injection intervals of 4-42 days, we perfused the animals for histological examination of the OO. Within 2 days of the injection we observed a dramatic decrease of the OO-EMG and blink amplitudes that reached its maximum at 3-4 days (75-80% reduction). Gradual recovery of the blink reflex from BotA treatment started at day 6, with blink amplitudes that recovered fully only after 42 days. The guinea pig OO muscle fiber composition is predominantly type II with some type I, similar to that of other mammals. At day 4 after BotA treatment, the morphology of the OO did not exhibit significant pathology. Profound atrophy of both fiber types was evident at day 21, with significant recovery of fiber size by day 42. The presence of neuronal growth cones by 21 days suggested sprouting as a means of recovery from BotA paralysis. The knowledge obtained from combined physiological and morphological investigations into the recovery from BotA treatment may expose new strategies to eliminate the necessity for reinjection. Supported by NATO-fellowship by the DAAD, NEI grants EY05464 & EY07391.

186.5

INDUCTION OF BLINK REFLEX HYPEREXCITABILITY BY FACIAL NERVE DAMAGE. A CAUSE OF HEMIFACIAL SPASM? C. Evinger, A.K.E. Horn, J.J. Pellegrini, and M.A. Basso. Dept Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11794

Excitability of the blink reflex circuit can be assessed by measuring the EMG of the lid closing muscle, the orbicularis oculi (OOemg), elicited by two identical electrical stimuli delivered to the supraorbital branch of the trigeminal nerve. With interstimulus intervals of less than 1 s., the OOemg activity evoked by the second stimulus is less than that elicited by the first stimulus. In diseases characterized by exaggerated blinking and lid closure, *e.g.* hemifacial spasm, the blink reflex circuit excitability increases. Hemifacial spasm appears to result from compression of the VIIth nerve at the root entry zone. This disease origin suggests that *motor* damage leads to modification of reflex excitability *centrally*.

We investigated whether facial nerve damage or partial paralysis of the orbicularis oculi muscle with botulinum toxin would modify the excitability of the blink reflex circuit in chronically prepared, alert rats. We stimulated the supraorbital branch of the trigeminal nerve while recording the evoked OOemg activity. After establishing the magnitude of the suppression of the second response (2nd response/1st response) as a function of interstimulus interval (25 - 990 ms), we removed 3-4 mm of the zygomatic branch of the facial nerve. This procedure enhanced blink reflex circuit excitability by as much as 60% in the first 10 days. Seventy-five days later, this hyperexcitability was still present. The additional observation that injection of botulinum toxin into the orbicularis oculi muscle of guinea pigs also increased blink reflex circuit excitability demonstrated that muscle weakness induced a central modification in the blink reflex circuit. Supported by NEI EY07391 and a NATO fellowship by the DAAD.

186.6

STUDIES ON THE MECHANISM OF BLINK REFLEX SUPPRESSION. J.J. Pellegrini & C. Evinger. Dept Neurobiology, SUNY Stony Brook, NY 11794

Reflex blinks, voluntary movements and conditioning stimuli such as tones have all been shown in humans to produce suppression of the blink reflex evoked by a subsequent supraorbital (s.o.) stimulus. The mechanism(s) of these effects are unknown. The suppression caused by a reflex blink could result from the stimulus, the movement, or general arousal. In addition, the suppression could occur at a number of different sites within the reflex arc.

We utilized EMG studies and single unit recordings in a chronic guinea pig preparation to investigate the mechanisms by which a supraorbitally evoked reflex blink diminishes the blink to an impending identical stimulus. In guinea pigs, as in humans, this suppression lasts longer than 1 sec., and the later components of the orbicularis oculi (oo) EMG response show stronger suppression than the earlier ones. Our results show that this effect is not due to forewarning: non-blink evoking tones presented to guinea pigs prior to s.o. shock either facilitate the reflex or have no effect. However, both s.o. stimulation and the movement it evokes appear to contribute to subsequent reflex suppression. S.O. shock at intensities subthreshold for the reflex produces reflex facilitation at very short lead times but suppression at longer intervals. Blinks evoked by stimuli other than s.o. shock suppress the s.o. blink, but not as potently as blinks evoked by s.o. shock. The suppressive effects of the reflex blink appear to be exerted at the afferent limb of the reflex arc. Some rostral trigeminal nucleus neurons which respond to s.o. stimulation at latencies shorter than those of the ooEMG response show a time course of double shock suppression that lasts as long as that seen in the ooEMG. Moreover, the observation that large spontaneous blinks sometimes occur following reflex blinks at delays when reflex blinks are suppressed indicates that oo motor units are not fatigued or inhibited. Supported by grant EYO7391.

186.7

NIGROSTRIATAL LESIONS MODULATE THE BLINK REFLEX. M.A. Basso, C. Evinger and R.E. Strecker. Depts. Psychology, Neurobiology & Behavior, and Psychiatry, SUNY Stony Brook, Stony Brook, NY 11794

Humans with extrapyramidal movement disorders exhibit blink reflex abnormalities. For example, blink reflex circuit hyperexcitability, as shown by the reflex's resistance to habituation, is characteristic of Parkinson's disease. To investigate whether an animal model of Parkinson's disease showed blink reflex hyperexcitability, we made unilateral lesions of the nigrostriatal dopaminergic system in rats using 6-hydroxydopamine (6-OHDA) and measured blink reflex excitability. The excitability testing paradigm consisted of the presentation of two identical electrical stimuli (ISI<1 s) to the supraorbital branch of the trigeminal nerve and measuring the evoked EMG of the lid closing muscle, the orbicularis oculi (OOemg) in chronically prepared, alert rats. On the side *contralateral* to the lesion, the OOemg evoked by the second stimulus was less than that elicited by the first stimulus, a result comparable to normal rats. On the side *ipsilateral* to the lesion, however, the OOemg evoked by the second stimulus was frequently larger than the first. This result mimics human Parkinsonism. In addition, the blink reflex ipsilateral to the lesion resisted habituation. Unlike the majority of motor problems which are contralateral to the lesion, unilateral 6-OHDA lesions and hemiparkinsonism most strongly effect the ipsilateral blink reflex. Nevertheless, in one animal, a unilateral 6-OHDA lesion increased blink reflex excitability bilaterally. The presence of vigorous amphetamine induced rotation demonstrated that the lesions were unilateral. Because of its simplicity and reliability, the blink reflex is a valuable counterpart to the more typical measures of motor deficits in models of Parkinson's disease. The paradigm is also amenable for investigating the neural mechanisms by which Parkinson's disease alters reflex excitability. Supported by a Parkinson's Disease Foundation Fellowship and NEI EY07391.

186.8

PUPILLARY AND ACCOMMODATIVE PRE- AND POSTGANGLIONIC NEURONS IN THE MONKEY AND CAT. J.T. Erichsen, C. Evinger and P.J. May, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY, and Depts. of Anatomy and Ophthalmology, U. of Mississippi Med. Ctr., Jackson, MS.

These studies represent a continuation of experiments aimed at anatomically defining the pupil and lens accommodation pathways in the nucleus of Edinger-Westphal (EW) and the ciliary ganglion. In cats and old world monkeys, WGA was injected into the anterior chamber of one eye and the vitreous humor of the other eye. Our expectation was that the anterior chamber injections would preferentially label the iridial pre- and postganglionic motoneurons. By contrast, because of the communicating flow from the vitreous into the aqueous, the vitreous injection was expected to label pathways to the iris as well as to the ciliary muscle. Surprisingly, the number and distribution of the transsynaptically labeled preganglionic neurons that resulted from each injection appeared to be similar. Specifically, in the cat, preganglionic neurons were found in and around the midline EW and among the exiting fascicles of NIII. In the monkey, cells were largely restricted to EW, located dorsal to the oculomotor nucleus, and to the dorsal part of the anteromedian nucleus. The proportion of postganglionic cells in the ciliary ganglion labeled by these two injections was also roughly equivalent. One possible explanation of these data is that the vitreous injection results in little or no uptake by the ciliary muscle, perhaps due to tight junctions present in the ciliary epithelium. In addition, numerous WGA-containing bouton-like endings contacted both labeled and unlabeled postganglionic cells in the ciliary ganglion. An ultrastructural analysis of the ciliary ganglion reveals transsynaptically labeled preganglionic terminals and indicates that there are at least two terminal types. In light of these results, the WGA-labeled boutons on unlabeled, possibly accommodative, ganglion cells may be collaterals from axons of either pre- or postganglionic cells. Such arrangements could yoke the firing of postganglionic neurons or mediate the near response. Supported by grants EY04587 (JE), EY07391 (CE) and EY07166 (PM).

186.9

EYE AND HEAD MOVEMENTS DURING GAZE SHIFTS TO VISUAL TARGETS ARE GOVERNED BY DIFFERENT CONTROL STRATEGIES. L. Ling*, J.O. Phillips, J.J. Plorde*, and A.F. Fuchs. Regional Primate Research Center, Department of Physiology and Biophysics, and Department of Psychology, University of Washington, Seattle, WA 98195

Primates normally use a combination of eye and head movements to acquire visual targets. To determine the contribution of head movements to overall gaze movements, we studied the kinematics of targeted gaze shifts in a monkey free to move his head in the horizontal plane. For the same target step, the variability of gaze and eye trajectories was of a quite different nature. Gaze trajectories, which were relatively stereotyped, differed predominantly in their peak velocities; they saturated for movements of about 25 deg and decreased at larger amplitudes. On the other hand, head trajectories showed a high degree of variability throughout their time course, and the final head contribution at the end of the gaze shift was extremely variable. These data suggest that gaze-associated eye and head movements are controlled differently.

To evaluate this hypothesis, we stimulated the region of the omni-pause neurones (OPNs) during head-free gaze shifts. Stimulation trains of up to 150 msec duration presented randomly during gaze shifts consistently terminated the gaze movement, which then remained fixed in space roughly for the duration of the train; gaze fixation occurred because the eye saccade was terminated and the VOR was turned back on. OPN stimulation, however, did not consistently perturb the associated head movements.

These results suggest that the head and eye movements that comprise a primate gaze shift are controlled separately at the level of the OPNs. We suggest that eye movements may be driven by a gaze signal through a local feedback burst generator, which determines the amplitude of the eye saccade. Head movements, on the other hand, may be controlled by feed forward mechanisms. To ensure the accuracy of the gaze movement, the head velocity must be taken into account by the eye burst generator, which may give rise to the observed decreased peak gaze velocities with large gaze shifts.

This work was supported by NIH grants EY00745, RR00166, EY07991 and a grant from the Virginia Merrill Bloedel Hearing Research Center.

186.10

THE TIMING OF THE RESPONSE OF BRAINSTEM OMNI-PAUSE NEURONES RELATIVE TO SACCADIC EYE MOVEMENTS IN RHESUS MONKEYS. A.F. Fuchs, L. Ling*, C.R.S. Kaneko, W. M.King, and Susan D. Usher *. Regional Primate Research Center and Dept of Physiology and Biophysics, University of Washington, Seattle, WA, 98195.

Brain stem omni-pause neurones (OPNs), which discharge at a steady rate during fixation and cease firing (i.e., pause) during all saccades are thought to play a pivotal role in a local feedback loop that controls the time course of saccades. During fixation the discharge of these inhibitory units keeps the saccadic burst generator inactive, whereas the pause in their activity allows burst neurones to be driven by inputs that specify the metrics of the saccade. Despite their putative central role in the control of saccades, there is little quantitative information about their discharge patterns, especially in monkeys. Therefore, we recorded from OPNs in Rhesus monkeys during spontaneous and targeted saccades.

The pause of every OPN began about 10-15 msec before the saccade. For only a few OPNs were pause and saccade duration well correlated ($r > 0.9$) and the slope of the relationship close to one. For most, the slope was less than one and the correlation coefficients ranged between 0.4 and 0.8. Moreover, almost all OPNs exhibited pauses that ended at the time or after the movement had come to rest. These data suggest that OPNs may chiefly be involved in starting the saccade. All OPNs also cease firing during blinks and often remain silent during a glissade at the end of a saccade.

Occasional OPNs have an excitatory visual response, and a few have pauses that depend on saccade direction. The scarcity of these later types suggests that OPNs may still be regarded as a single, qualitatively homogeneous population.

This study was supported by National Institutes of Health grants EY00745, EY06558, and RR00166.

186.11

ORGANIZATION OF THE OCULOMOTOR AND EDINGER-WESTPHAL NUCLEI IN THE LESSER GALAGO. **Paul J. May and Wensi Sun. Depts. of Anatomy and Ophthalmology, U. of Mississippi Med. Ctr., Jackson, MS, 39216.**

Anthropoid primates have several specializations in the organization of the oculomotor (III) and Edinger-Westphal nuclei (EW). Monkey medial rectus motoneurons are subdivided into three subgroups, the levator palpebrae motoneurons are distributed bilaterally and preganglionic motoneurons are confined within the EW, a pair of cell columns located just dorsal to III. By contrast, the cat III has a unitary medial rectus subdivision, the majority of levator motoneurons are located contralaterally, and the majority of preganglionic motoneurons are located outside the confines of the single midline EW. To determine whether the monkey pattern was general for all primates, we utilized the retrograde tracer, WGA-HRP, to define motoneuron distributions in the nocturnal prosimian, Galago senegalensis. Injections of the medial rectus muscle labeled motoneurons ipsilaterally in a distribution suggestive of the monkey subgroups. Levator motoneurons were distributed contralaterally in the caudal central subdivision as in the cat. Trans-synaptic retrograde labelling of the preganglionic population indicates these cells are located within the EW nucleus as in the monkey. But the EW is a single midline nucleus that runs dorsal to the III and then wraps around its rostral end to extend caudally, between the exiting III'rd nerves, similar to the cat. This analysis was extended to the ciliary ganglion ultrastructure. Postganglionic motoneuron somata are generally devoid of synapses. Most synaptic contacts are in the perisomatic neuropil and the vast majority of presynaptic profiles have pleomorphic vesicles and and make asymmetric contacts. The specialized axosomatic synapses and mutiple synaptic types present in some monkeys were not observed. In sum, these results suggest that the Galago is intermediate in its development, having both monkey and cat features. Supported by NEI grant EY07166.

186.12

RESPONSE FIELDS OF NEURONS IN THE SUPPLEMENTARY EYE FIELD OF THE RHESUS MONKEY ARE RETINOTOPIC. G. S. Russo and C. J. Bruce. Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The trajectory of saccades elicited by electrical stimulation can be affected by the direction of gaze at the time of stimulation. In the supplementary eye field (SEF) this gaze effect has been interpreted as indicating craniotopic coding of saccades (Schlag & Schlag-Rey, J. Neurophysiol., 57: 179, 1987). We recently quantified this gaze effect for saccades elicited from SEF and hypothesized other explanations for this orbital dependence, e.g., summing a population of saccade vectors with orbitally-dependent thresholds (Russo & Bruce, Soc. Neurosci. Absr. 16:899, 1990).

Here, we further studied the coding of saccades in SEF by quantitatively analyzing the effect of gaze direction on response fields of SEF neurons. Of 126 neurons with saccade-related activity, 44 had only visual activity, 24 had only movement activity, and 40 had both. For 38 of these neurons we quantitatively mapped the polar direction of the response field (Ø) at three widely-spaced fixation points. The effect of gaze on Ø was indexed by the regression (K) of gaze eccentricity upon Ø. K=0 indicates a perfectly retinotopic response field whereas K>0 could indicate a craniotopic field. There was little overall effect of gaze on Ø; the mean K (-0.09) did not significantly differ from zero.

At the sites of 21 of these neurons we subsequently elicited saccadic eye movements with electrical stimulation through the recording electrode and measured the average saccade elicited from each of the fixation points that had been used to plot the neuron's response field. Overall, the directions of the elicited saccades matched the direction of the neuron's response field, the mean absolute difference being only 17.5°. As expected, gaze consistently perturbed the direction of elicited saccades; the analogous K having a mean (+0.52) significantly greater than zero, with all but one K being positive. K values of the corresponding neurons were significantly smaller ($t_{20} = 2.76$). Furthermore, elicited and neuronal K values were uncorrelated across sites ($R = +0.03$).

Thus the effects of gaze direction on the trajectory of electrically elicited saccades are not reflected in analogous perturbations of response fields of SEF neurons, indicating that SEF, like the frontal eye fields and superior colliculus, codes saccadic vectors and that the orbital perturbation of saccades electrically elicited from SEF does not derive from a craniotopic coding of saccades there. Supported by PHS grant EY04740.

186.13

ACTIVITY OF MONKEY FRONTAL EYE FIELD NEURONS PROJECTING TO OCULOMOTOR REGIONS OF THE PONS. M. A. Segraves. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

The monkey's frontal eye field projects directly to oculomotor regions of the pons. These projections are an important component of the cortical system involved in the control of saccadic eye movements. I have identified the signals carried from frontal eye field to pons by antidromically exciting 25 corticopontine neurons in 2 behaving rhesus monkeys. Mean threshold and latency were 277μA and 2.0 ms. Most pontine stimulation sites were centered within the nucleus raphe interpositus (RIP), the location of omnipause neurons. In a few instances, the lowest thresholds for antidromic excitation were obtained from sites ventrolateral to the RIP corresponding to the location of the nucleus reticularis tegmenti pontis (NRTP) or basal pontine nuclei, both cerebellar relays.

Twenty-eight percent of the corticopontine neurons were most active during fixation and responded to visual stimulation of the fovea. Forty-eight percent had movement related activity, they were active before and during saccades made within a limited contralateral field. Most of the remaining neurons had activity related to static eye position. Cells with purely visual activity were not found. The anatomical complexity of this brainstem region combined with the spread of stimulation current make it difficult to localize the exact site of termination of these cortical neurons. It is clear, however, that the frontal eye field conveys at least two messages to cells just a few synapses away from extraocular motoneurons. The first message signals when it is appropriate to break fixation and make a saccade, the second message conveys the current position of the eyes and the amplitude and direction of the impending eye movement. For the RIP, these signals may provide the trigger to turn off omnipause cells and initiate a saccade. For the NRTP and pontine nuclei, they may help in the generation of a corollary discharge that is transmitted to the cerebellum.

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186.14

PROPERTIES OF VISUAL STIMULI THAT DRIVE COMPLEX SPIKES IN MONKEY FLOCCULUS. D.C. Gillespie*, R.I. Krauzlis, H.M. Brontë-Stewart and S.G. Lisberger Dept. of Physiology and Neuroscience Graduate Program, UCSF, San Francisco, CA 94143

Complex-spikes (CSs) in the monkey flocculus are known to occur in association with image motion, but their function is unclear. To identify properties of stimuli that elicit CSs, we used small visual perturbations to mimic the image motion that normally precedes CSs. We recorded extracellularly from single Purkinje cells (PCs) in the flocculus of alert monkeys during fixation of a stationary or moving spot. We counted CSs in a 120 ms interval beginning 60 ms after the onset of perturbations that imposed 100 ms of controlled image motion at random times.

We examined the CS activity of PCs in which downward or ipsilateral eye motion caused increases in simple-spike activity. The brief motion of a small spot along a PC's preferred axis was sufficient to evoke CSs with a high probability, but did not cause a measurable eye movement. The probability of evoking a CS was independent of whether image motion was presented during fixation or tracking, and of whether the stimulus was a spot or a large texture. Tests of directionality revealed narrow tuning, with the best direction opposite that for simple spikes. CS responses were direction selective for image speeds ranging from 0.25 to 128 deg/s and exhibited broad velocity tuning between 1 and 32 deg/s. Our data imply that complex-spikes respond to visual motion and that they signal primarily the direction of image motion. *(Supported by NSF Grant BNS 8616509)*

186.15

MIGRATION OF SUPERIOR RECTUS MOTONEURONS TO THE CONTRALATERAL OCULOMOTOR NUCLEUS AS REVEALED WITH DiI IN CHICKEN AND MICE. D. H. Nichols, B. Fritzsch and P. R. Brauer*, Creighton Univ., Div. of Anatomy, Omaha, NE 68178

All vertebrates have oculomotor motoneurons projecting to the superior rectus muscle (SRM) of the contralateral eye. Based on silver stained material it was proposed that these cells selectively migrate over the midline (Heaton, M.B., JCN 198 (1982) 633). Recent experimental analysis in *Xenopus* were not in full agreement with this interpretation (Matesz, C. NSL 116 (1990) 1). We have re-examined this problem in chicken and mice using DiI diffusion in fixed animals. In chicken at E 6¾ retrograde labelling revealed only cells on the ipsilateral side; anterograde tracing showed a distinct innervation of SRM. At E 7 a population of ocular motoneurons was segregated from the ipsilateral population along the midline. At E 7¾ a larger ipsilateral and a smaller contralateral population was found. In mice at E 12 only ipsilateral oculomotor neurons were labelled; anterograde tracing showed a distinct innervation of SRM. At E 13 we found a larger population of ipsilateral cells and a smaller population of cells at or close to the midline; the latter cells all had dendrites to the contralateral side. At E 14 a larger ipsilateral and a smaller contralateral population was found. In both amniotic species examined the developmental pattern is similar and consistent with the suggestion of migration of neuroblasts over the midline. The prior peripheral segregation indicates that contact formation may actually trigger migration.

186.16

FINE STRUCTURE OF THE EYE'S CHOROID AND ITS INNERVATION BY CHOROID NEURONS OF THE CILIARY GANGLION IN THE CHICKEN. M.E. De Stefano and E. Mugnaini. Lab. of Neuromorphology, Univ. of Connecticut, Storrs, CT 06269-4154.

The ciliary and the choroid neurons in the avian ciliary ganglion are both cholinergic but only the choroid neurons express the neuropeptide somatostatin. The two types of neuron innervate different targets in the eye. Recent evidence points to an influence of the target on somatostatin expression by the choroid neurons. Thus, a thorough analysis of the fine structure of the choroidal coat is appropriate. This coat is a complex tissue containing blood vessels, lacunae and trabeculae. The blood vessels are lined by an endothelium of the tight variety (continuous endothelial cells with conspicuous tight junctions and scarce plasmalemmal vesicles). Smooth muscle fibers in the vessels' walls contact the endothelial lining at points where the basal lamina is interrupted. The lacunae are limited by a thin mesothelial lining. The trabecular framework consists of smooth muscle fibers, extracellular collagen matrix, fibroblasts and pigmented cells, many of which are apposed to the mesothelial cells and contact their surface at points. Choroid nerves, ensheathed by Schwann cells, run within the trabeculae and innervate the smooth muscles in the vessels' wall and in the trabeculae themselves with similar boutons containing clear round vesicles and dense core vesicles.The choroid fibers have been immunostained for ChAT and somatostatin and the two markers are presumably colocalized in the same boutons. The only cells in physical contact with the choroidal nerve endings are the Schwann cells and the vascular and trabecular smooth muscle. Supported by a fellowship of the Italian M.P.I. and USA NIH grant NS-09904.

186.17

A NEURAL NETWORK MODEL OF SACCADE PROGRAMMING. David L. Sheinberg*, Gregory J. Zelinsky* & Heinrich H. Bülthoff, Dept Cog & Ling Sci, Brown University, Box 1978, Providence RI 02912.

Human saccadic programming is subject to bottom-up and top-down influences. A neural network model was developed to show how these two processes may interact to produce eye movements. The model consisted of modules loosely corresponding to presaccadic processing in the superior colliculus (SC) and cortex. Both modules received input from a simulated retina. The collicular module was divided into sensory, intermediate, and motor layers and provided a direct interpretation of the visual scene. The cortical module processed inputs based on task dependent variables such as target / non-target differentiation. Topographic connections between units in the cortical and collicular modules allowed target information to selectively enhance locations within the SC intermediate layer. Winner-take-all competition between these integrated neural images was then used to suggest a specific oculomotor target. The system was dynamic in the sense that ongoing cortical enhancement continued to affect the collicular representation while the network settled.

Psychophysical tests were used to assess the predictive validity of the model. The results indicated a qualitative similarity between human and simulated fixations. In particular, saccades to double target stimuli landed at intermediate positions in both cases. A pronounced interaction between saccadic accuracy and latency was also observed. Actual and simulated eye movements became more accurate with longer saccadic latencies. This correspondence suggests that a top-down component may affect saccadic programming by selectively enhancing target locations within a bottom-up computation.

186.18

HUMAN VISUAL-VESTIBULAR INTERACTION DURING PASSIVE AND ACTIVE VERTICAL HEAD MOVEMENTS (HM). J. L. Demer, J. G. Oas*, R. W. Baloh*, and L. A. Hovis*. Jules Stein Eye Institute and Department of Neurology, University of California at Los Angeles, 90024.

To investigate gaze stabilization during vertical HM, we measured gain (eye velocity/head velocity) of eye movements during **passive,** as well as voluntary, **active** pitch HM under several visual conditions.

Eye and head movements of normally sighted subjects were measured using magnetic search coils. Subjects were trained to make **active** sinusoidal HM (0.4 - 6.0 Hz) in synchrony with an audible tone varying in pitch. **Passive,** whole-body rotation (0.4 - 3.2 Hz) was obtained using a servo-driven swing rotating about the interaural axis. The vertical vestibulo-ocular reflex (VOR) was tested in darkness. The visually enhanced VOR was tested in light with **normal vision (1X)** and with **2X binocular telescopic spectacles (2X).**

Robust VOR was observed during both active and passive vertical HM, with gain varying slightly from 1.0. During **active** HM, gain during 1X viewing was 1.0 over the entire spectrum tested. With 2X viewing, gain was ~2.0 at low frequencies, declined with increasing frequency, and remained significantly >1.0 even at maximum frequencies of voluntary HM achieved. During **passive** HM less 2X gain enhancement occurred than during active HM. Significant relative visual enhancement of gain during 2X viewing was observed up to maximum frequencies of 1.5 - 2.5 Hz, but was absent for higher frequencies.

Since visual and vestibular inputs are identical during active and passive vertical HM, these data suggest that **neck proprioception and/or skeletal motor efference copy** produce important additional **gain modulation in visual-vestibular interaction** during active HM at frequencies **above the range of the pursuit system.**

Supported by EY-08656, NS-09828, RPB, and Karl Kirchgessner Foundation.

186.19

CATCH-UP SACCADE AMPLITUDE IS RELATED TO SQUARE WAVE JERK RATE. J.A. Jesberger*, L. Friedman, L.A. Abel, L.F. Dell'Osso, H.Y. Meltzer.

A remarkably strong correlation was noted in normal controls (N=20) between square wave jerk (SWJ) rate during pursuit and mean catch-up saccade (CUS) amplitude (Spearman rho = 0.87, $p < 0.0001$). Eye movements were recorded with IR oculography. Target speed was 5^{o}/s. This correlation was also present in psychiatric patients (N=38)(rho = 0.53, p = 0.0006), although it was significantly weaker than in normals ($p < 0.02$). The relationship between SWJ rate and CUS amplitude was specific: SWJ rate was not correlated with gain (rho = -0.07) or CUS rate (rho = 0.07). Similar strong correlations between SWJ rate during fixation and mean CUS amplitude were also found for normals (rho = 0.73, p = .0002) and patients (rho = 0.52, p = 0.0009). The rates of SWJ during pursuit and fixation were highly correlated (rho = 0.64 for normals, 0.60 for patients). These findings clearly indicate that one factor that predicts CUS amplitude is SWJ rate. The results suggest that saccadic intrusions during tracking keep the saccade correcting system "busy" and delay correction for the position error that accumulates when gain is less than 1.0.

187.1

SEROTONIN DIRECTLY EXCITES NEONATAL HYPOGLOSSAL MOTONEURONS. A.J. Berger, D.A. Bayliss, and F. Viana. Department of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA 98195.

Serotonin (5-HT)-containing nerve fibers and terminals have been demonstrated in the hypoglossal nucleus (*Brain Res. Bull. 23:* 249-256, 1989) and 5-HT-containing boutons have been observed in close apposition to identified hypoglossal motoneurons (HMs) (*Brain Res.538:* 215-225, 1991). These anatomical results suggest that 5-HT can directly modulate the excitability of HMs. To test this hypothesis, we investigated the effects of bath-applied 5-HT to neonatal (P3 to P10) rat HMs in two separate *in vitro* brainstem slice preparations.

One preparation employed conventional intracellular microelectrode recording from thick (400 μm) slices. The other used thin slices (approx. 130 μm) to record whole-cell currents from visualized HMs with patch type electrodes. In all cases (12 HMs to date), bath-applied 5-HT (10 μM) caused either reversible depolarization (current-clamp mode) or generation of an inward current (voltage-clamp mode). 5-HT also caused an increase in spontaneous synaptic activity. Bathing the slice in a nominally Ca^{2+}-free solution containing TTX (1 μM) and 2 mM Mn^{2+} abolished the synaptic effects, but the excitation was maintained.

These results are in contrast to a recent report in which bath-application of 5-HT was found to depress rhythmic hypoglossal nerve activity in the neonatal brainstem-spinal cord preparation (*Neurosci. Letters 111:* 127-132, 1990). Because synaptic effects were not ruled out in that study, we suggest that 5-HT inhibitory effects are probably mediated at a site presynaptic to the HM, and that the direct effects of 5-HT on HMs are excitatory. (Supported by NS 14857).

187.2

THYROTROPIN-RELEASING HORMONE DECREASES A POTASSIUM CONDUCTANCE IN HYPOGLOSSAL MOTONEURONS *IN VITRO*. Douglas A. Bayliss, Felix Viana and Albert J. Berger. Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, WA 98195.

The hypoglossal motor nucleus is innervated by fibers containing the neuropeptide thyrotropin-releasing hormone (TRH) and contains TRH binding sites. We therefore investigated the effects of TRH on hypoglossal motoneurons (HMs) in transverse slices of rat brainstem using conventional single-electrode (3 M KCl) recording techniques (i.e., current- and voltage- clamp). TRH (0.1 to 10 μM), applied either by perfusion or by pressure microinjection, caused a depolarization and/or the development of an inward current in all HMs tested (n=37). These effects were fully reversible, dose- dependent, and showed only modest desensitization with repeated applications. In addition, although TRH increased synaptic activity in most cells, the depolarizing response to TRH was maintained in a tetrodotoxin-containing (0.5 μM) or in a Ca^{2+}-free perfusate containing 2 mM Mn^{2+} in which synaptic effects were abolished. Thus, TRH directly depolarizes HMs. Constant current or voltage steps applied to the neuron during TRH application revealed that the depolarization (inward current) was associated with an increased input resistance (decreased conductance), suggesting involvement of a K^+ conductance. Consistent with this hypothesis, extrapolated instantaneous current-voltage relationships obtained before and at the peak of the TRH response intersected at approximately -100 mV, the expected K^+ equilibrium potential (E_K). Moreover, the TRH-induced depolarization was reversibly diminished when extracellular [K^+] was raised from 3 to 12 mM, and enhanced in amplitude from depolarized potentials (i.e., further from E_K). These data are inconsistent with an effect on a Cl^- conductance, as E_{Cl} would be displaced to depolarized potentials by the KCl-containing electrodes. Thus, we conclude that TRH directly depolarizes hypoglossal motoneurons by decreasing a resting K^+ conductance. (Supported by NS 14857)

187.3

ONTOGENESIS OF MOTONEURON ELECTRICAL PROPERTIES: ROLE OF CALCIUM AND CALCIUM-ACTIVATED CONDUCTANCES. F. Viana, D. A. Bayliss, and A. J. Berger. Department of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA 98195.

We examined the membrane and firing properties of rat hypoglossal motoneurons (HMs) during various stages of development (P2 to P12 and adult). Intracellular recordings were performed in brainstem slices (400-μm) using standard techniques. At P2, HMs were already capable of firing repetitively in response to intracellular current injections. Firing was blocked by TTX.

Firing of young HMs (< P6) differed markedly from that of adult HMs. The action potential of young HMs repolarized less rapidly, was followed by a larger afterdepolarization (ADP) and a longer afterhyperpolarization (AHP). ADP was voltage-dependent, being enhanced in amplitude at negative membrane potentials. The pharmacology of ADP and AHP was similar at all stages. ADP was absent in zero Ca^{2+} solution, enhanced by high Ca^{2+} or Ba^{2+}, and blocked by Mn^{2+}; ω-conotoxin had no effect. AHP was also Ca-dependent: it was absent in zero Ca^{2+}, enhanced by high Ca^{2+}, blocked by apamin, ω-conotoxin, Ba^{2+} and Mn^{2+}. A functional consequence of this prolonged AHP was lower minimal and maximal firing rates for young HMs. Also, young HMs often showed rebound burst firing after hyperpolarizing current pulses. This burst firing was blocked in zero Ca^{2+} and was not present in adult HMs.

Ca^{2+} currents were studied in isolation (intracellular Cs^+, extracellular TEA, TTX and 4-AP) in HMs (P3 to P10) using whole-cell voltage-clamp recordings in a thin-slice preparation. We identified two Ca^{2+} currents: a transient low-voltage-activated (LVA) and a high-voltage-activated (HVA) with inactivating and non-inactivating components. Both LVA and HVA currents were blocked by zero Ca^{2+} or inorganic Ca^{2+} blockers.

Collectively our results suggest a crucial role for Ca^{2+} and Ca^{2+}-activated K^+ currents in the normal firing of HMs at all postnatal ages, with a more prominent role of the LVA Ca^{2+} current at early stages. (Supported by NS 14857)

187.4

FREQUENCY MODULATION OF THE NMDA AND NON-NMDA RECEPTOR-MEDIATED EPSPS IN THE NEONATAL RAT SPINAL CORD. M. Pinco* and A. Lev-Tov. Dept. of Anatomy, The Hebrew Univ. Sch. of Med., Jerusalem, Israel.

Intracellular recordings of EPSPs generated in α-motoneurons by dorsal root afferents in the neonatal rat spinal cord revealed a slow voltage dependent component as the Mg^{++} in the bathing medium was reduced to ¼ of its normal level. The slow component was identified as an NMDA receptor mediated EPSP and could be separated pharmacologically from the predominant non-NMDA component. The present study, characterizes the frequency modulation of the NMDA and non-NMDA EPSP components simultaneously (using time window analysis), and separately (using the specific NMDA and non-NMDA blockers APV and CNQX, respectively). Both components exhibited prolonged EPSP-depression [Lev-Tov et al. Neurosci. Abs. 16, 1015, 1990], with similar kinetics and frequency dependence. As the baseline of transmitter release was lowered by application of 2μM L(-) baclofen to the bath, the prolonged EPSP depression was virtually abolished and could be transformed into frequency facilitation and potentiation. Again, the time course and frequency dependence of facilitation and potentiation were virtually identical for the NMDA and non-NMDA receptor mediated EPSP components. Our findings suggest that the EPSPs generated by dorsal root afferents in α-motoneurons in the neonatal rat spinal cord are mediated by activation of postsynaptically co-localized NMDA and non NMDA receptor sub-types.

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187.5

5-HT_1 RECEPTORS MEDIATE 5-HT-INDUCED DEPOLARIZATIONS IN IMMATURE MOTONEURONS. B.S. Seebach and L. Ziskind-Conhaim. Dept. Physiol., Univ. of Wisconsin, Madison, WI 53706.

The pharmacological profile of serotonin-mediated potentials was studied in the isolated spinal cord of embryonic (Days 18-21) and neonatal (1-to-3-day-old) rats. Serotonin (5-HT) produced long-lasting depolarizations and high-frequency subthreshold potentials. The sub-threshold potentials, but not the long-lasting depolarizations, were blocked by high Mg^{2+} and TTX, indicating that 5-HT affects both moto-neurons and interneurons. 5-CT and α-methyl-5-HT were the only agonists that induced motoneuron depolarizations and subthreshold activity. 8-OH-DPAT depolarized motoneurons without generating subthreshold potentials, while DOI and 2-methyl-5-HT had no effect. Methysergide was the only antagonist that completely blocked 5-HT-induced potentials. Ketanserin, spiperone, and MDL 72222 only partially blocked the long-lasting depolar-izations and subthreshold potentials. These findings suggest that 5-HT_1 receptors, or an unidentified receptor subtype, mediate 5-HT responses in embryonic spinal cord. Supported by NIH grants NS01314 and NS23808.

187.6

DEPLETION SUPERSENSITIVITY TO SEROTONIN IN SPINAL MOTONEURONS OF RAT EMBRYOS. B-X. Gao, M.A. Sweet* and L. Ziskind-Conhaim. Dept. Physiol., Univ. of Wisconsin, Madison, WI 53706.

In embryonic motoneurons, the time course of the changes in serotonin-induced depolarizations was correlated with the growth of serotonergic (5-HT) projections into the ventral horn. To determine the functional role of 5-HT in inducing these changes, 5-HT synthesis was blocked by p-chlorophenylalanine (p-CPA). Starting at Days 7-10 of gestation, daily p-CPA injection (100mg/kg) to pregnant rats resulted in a depletion of 5-HT in the spinal cords of 17 to 21-day-old embryos. Injections of control saline solution did not result in significant changes in the growth pattern of 5-HT projections in the ventral horn. In the absence of 5-HT in the nerve terminals, 5-HT responses increased. In Day 19-21 embryos, the amplitude of 5-HT induced motoneuron depolarizations increased from an average of 7 mV in control motoneurons to 14mV in p-CPA-treated spinal cords. This supersensitivity may be due to an increase in the number or binding affinity of 5-HT receptors, or a change in 5-HT receptor type(s) mediating motoneuron depolarizations. Supported by NIH grants NS01314 and NS23808.

187.7

MULTIPLE EFFECTS OF HYPOXIA ON NEURONS IN DORSAL MOTOR NUCLEUS (X) AND NUCLEUS TRACTUS SOLITARII (NTS). J.B.Dean, E.A.Gallman, W.H.Zhu* and D.E.Millhorn. Dept. of Physiology, Univ. of N. Carolina, Chapel Hill, NC 27599.

Whole-cell recordings were made in NTS and X neurons in 400μm slices prepared from adult rat. Slices were maintained in a medium-gas interface (35-37°C) aerated with (control) 95%O_2-5%CO_2, (hypoxia) 95%N_2-5%CO_2 or 85%N_2-5%CO_2-10%O_2. Four responses were observed during hypoxia (2-15 min) in NTS and X. *1)* 14% of neurons were hyperpolarized, sometimes transiently, accompanied by decreases in synaptic activity, R_{in}, and excitability (spontaneous & evoked spikes) and increased net outward current. *2)* 25% of neurons were depolarized and showed increased excitability, spike broadening, increased or unchanged synaptic activity, and increased net inward current. R_{in} was increased, decreased or unchanged. *3)* 16% of neurons were depolarized as in *2* but with unchanged or reduced excitability. Both depolarization and hyperpolarization were maintained in TTX or high Mg^{++}-low Ca^{++} synaptic blockade. *4)* 45% of neurons were insensitive showing no change in excitability, spike waveform, R_{in}, or synaptic activity. We conclude that some neurons respond to hypoxia with multiple ionic mechanisms whereas other neurons are resistant showing no change in excitability. Maintenance of excitability and synaptic integrity by certain neurons in NTS and X during hypoxia may prevent compromised cardiorespiratory function. (NSF, NIH, ALA)

187.8

AGE-DEPENDENT EFFECTS OF BICUCULLINE AND STRYCHNINE ON DORSAL ROOT-EVOKED POTENTIALS. W-L. Wu* and L. Ziskind-Conhaim. Dept. Physiol., and Ctr. Neurosci., Univ. of Wisconsin, Madison, WI 53706.

Starting at Day 16 of gestation (birth is at Days 21-22), dorsal root stimulation evoked long-latency excitatory and inhibitory depolarizing potentials in spinal motoneurons of rat embryos. The contribution of the inhibitory inputs to dorsal root-evoked potentials was studied by blocking GABA- and glycine-mediated transmission with bicuculline (20 μM) and strychnine (10 μM). These antagonists affected dorsal root-evoked potentials differently at early and late gestational stages. At Days 16-18 of gestation, either antagonists significantly decreased the amplitude of the long-latency potentials, while at Days 19-21 each potentiated it. These findings suggest that some changes in synaptic transmission occur between Days 17 and 19 of gestation. At the same developmental stages, there was a reduction in motoneuron responses to GABA and glycine. The average amplitude of GABA- and glycine-induced depolarizations (in the presence of TTX and high Mg^{2+}) decreased from 9 mV at Day 17 to 5 mV at Day 19. Supported by NIH grants NS 01314 and NS 23808.

187.9

OPIOIDERGIC MODULATION OF REFLEXES EVOKED BY MECHANICAL STIMULATION OF THE HEEL IN THE RABBIT. J. Harris*, T.W. Ford* & R.W. Clarke* (Spon: Brain Research Association). Dept. Physiology & Env. Sci., Univ. Nottingham, Sutton Bonington, LE12 5RD, U.K.

We have examined the effects of the opioid antagonist quadazocine on reflex responses elicited in the motoneurones of gastrocnemius medialis (GM) by controlled mechanical stimulation of the heel. Five rabbits were decerebrated and spinalized at the lower thoracic level under halothane (2-4%)/N_2O anaesthesia. Reflexes were evoked by single pinches of 1s duration applied to the skin of the heel by an electronically-triggered, pressure-driven pinching device. Responses were recorded from the central end of the cut, desheathed GM muscle nerve, and were quantified by integrating the instantaneous firing rate of the whole nerve. Four pinch forces were used: 0.12 ± 0.03, 0.41 ± 0.07, 1.88 ± 0.12 and 4.5 ± 0.12N of which the lowest force was barely perceptible, and only the most intense evoked pain when tested on ourselves. In untreated animals the lowest intensity pinch evoked no response in GM, whereas the strongest pinch elicited a response which had a mean duration of 2.4 ± 0.5s and which consisted of 560 ± 50 spikes during the pinch, and an afterdischarge of 458 ± 165 spikes. (+)-Quadazocine (500μg/kg i.v.) had no effect on reflex responses in GM. (-)-Quadazocine was given i.v. in cumulative doses of 1 to 156μg/kg and caused a dose-dependent increase in reflex responses evoked by all strengths of stimulation. After the top dose of the opioid antagonist, the 0.12N pinch elicited reflexes of mean duration 2.1 ± 0.7s, with 220 ± 73 spikes fired during the pinch and 283 ± 115 afterwards: the corresponding values for the 4.5N pinch were 6.9 ± 0.7s, 690 ± 66 and 1840 ± 441 spikes respectively. These findings show that endogenous opioid peptides tonically and non-selectively inhibit reflex pathways to GM motoneurones from both high- and low-threshold cutaneous afferents.

JH is an SERC scholar. Supported by the AFRC.

187.10

PONTOSPINAL ENKEPHALINERGIC PATHWAYS AND THEIR EFFECTS ON SPINAL REFLEX EXCITABILITY OF THE CAT. H. Zhuo*, S.J. Fung and C.D. Barnes. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Our previous studies have shown that more than 70% of the noradrenergic coerulospinal neurons in the locus coeruleus complex (LC) of the cat co-contained enkaphalin.

The present experiments, performed on decerebrate cats, were done in order to test the effects of the descending enkephalinergic pathways on spinal monosynaptic reflex (MSR) excitability. The MSR of flexor and extensor motoneuron pools were recorded from the cut L7 or S1 ventral root of the spinal cord when stimulating the peripheral nerves ipsilaterally. The effect of Naloxone, an opioid antagonist, on the LC-induced early facilitation phase and the late inhibition phase of the MSR were studied. Both the early facilitation and late inhibition phases of the MSR response were decreased by systematic injection of Naloxone (0.5-2 mg/Kg). The antagonistic action of Naloxone on the descending enkephalinergic influence was dosage-dependent and reversible. Vehicle (saline) control experiments were without effect on LC-induced MSR changes.

The results of the present study indicated the early facilitation and the late inhibition of the MSR conditioned by stimulation of the LC involved the descending enkephalinergic pathways. (Supported by NIH grants NS24388 and 11H-2940-8751).

187.11

LONG-TERM EFFECT OF SPINAL CORD HEMISECTION ON $GABA_B$ BINDING IN THE RAT SUBSTANTIA GELATINOSA. J.S. Kroin, G.D. Bianchi, and R.D. Penn. Dept. of Neurosurgery, Rush Medical College, Chicago, IL 60612.

At present, it is not known why a severe spinal cord lesion in humans often leads to the development of spasticity. To study one of the mechanisms that might accompany spinal cord injury in humans, we examined long-term $GABA_B$ receptor changes following a spinal lesion in rats. Midthoracic hemisections were performed in adult female rats and one year later the lumbar spinal cords were analyzed by quantitative autoradiography (Bowery, et al, Neurosci, 20:365,1987). On the hemisected side, as compared to the normal side, there was a small increase in density (B_{max} increased 12%) of $GABA_B$ receptors in the substantia gelatinosa of the dorsal horn. In addition, there was a moderate change in affinity (K_d increased 26% on the lesioned side). Therefore, in this model of spinal injury, there are small but statistically significant changes in the $GABA_B$ receptor system.

Supported by FDA Grant RFA-FDA-OP-86-1.

187.12

ACETYLCHOLINE RELEASE IN THE ATONIA MEDIATING REGIONS OF THE CAT MEDULLA., T.Kodama, Y.-Y.Lai and J.M.Siegel UCLA. Sch. Med. and VAMC, Sepulveda, CA 91343

The medial medullary reticular formation is an area mediating muscle atonia in REM sleep. We reported in previous studies that a rostral medullary region, N.magnocellularis(NMC), is responsive to glutamate and that a caudal region, N. paramedianus(NPM), is responsive to ACh. The present study was designed to investigate endogeous ACh release during the sleep-wake cycle in these two areas.

Twelve samples were obtained from both NMC and NPM during each stage of wake(W), SWS and REM. A two way ANOVA demonstrated that the amount of ACh release across sleep-wake stages was different in NMC and NPM. In NMC, the mean level of ACh release was significantly higher during REM sleep(55.78±2.87 fmole/min perfusate)($p<0.001$) and W(54.83 ±3.67)($p<0.01$) than that during SWS(49.67±3.13). However, levels of release in W and REM sleep did not differ. In contrast, the mean levels of ACh release in NPM during REM sleep(31.66±1.75) were 31% higher than those during SWS (24.22±1.25) and 35% higher than those during W(23.45±1.64). These differences were significant($p<0.001$). This profile of ACh release in NPM across the sleep-wake cycle resembles that in the dorsal tegmental field, where cholinergic agonists induce muscle atonia. This enhancement of ACh release in NPM during REM sleep supports our hypothesis that cholinoceptive neurons in NPM mediate REM sleep processes.

187.13

GLUTAMATERGIC PROJECTIONS TO PERI-LOCUS COERULEUS α.

J.R. Clements, Y.Y. Lai, S.J. Grant and J.M. Siegel. Dept. of Vet. Anatomy and Public Health., Texas A&M Univ., College Station, TX 77843; Dept. of Psychol, Univ. of Delaware, Newark, DE 19716; and Dept. Psychiatry, UCLA, and Neurobiol. Res. VAMC, Sepulveda, CA 91343.

Microinjection of acetylcholine into the dorsolateral pons has long been known to produce a suppression of muscle tone and REM sleep phenomena. The source of cholinergic projections to this region has been identified as the pedunculopontine (PPN) and laterodorsal tegmental (LDT) nuclei. Recently we demonstrated that glutamate microinjection in the same region also produced muscle tone suppression in the decerebrate cat. To determine the source of the glutamatergic projection to this region, we microinjected WGA-HRP at sites where muscle tone suppression was induced by glutamate injection and then labeled neurons containing the retrogradely-transported WGA-HRP with TMB. In tissue sections containing retrogradely-labeled neurons, NADPH-diaphorase histochemistry was used to identify cholinergic cells and an anti-glutamate antibody was used to immunocytochemically label glutamate-like immunoreactive neurons. Glutamate-like immunoreactive cells projecting to the peri-locus coeruleus α were found in the rostral PPN, FTP and ventral pons, including TRC. The glutamate-like immunoreactive neuronal population projecting to the injection sites was largely distinct from the cholinergic projection, however in caudal PPN some retrogradely labeled cells were both glutamate-like immunoreactive and NADPH positive. The coordinated activation of glutamatergic and cholinergic cells in the PPN may be responsible for the suppression of muscle tone in REM sleep.

187.14

NEURAL MECHANISMS OF ACOUSTIC STARTLE REFLEX: ANATOMICAL ANALYSIS USING C-FOS.

M.-F. Wu, H. M. Fahringer*, T. S. Kilduff, & J. M. Siegel. Neurobiol. Res., VA Med. Ctr., Sepulveda, CA 91343, Dept. Psychiatry, UCLA, Los Angeles, CA 90024, & Dept. Psychiatry, Stanford Univ., Stanford, CA 94305

The neural structures mediating the primarily acoustic startle reflex have been investigated previously using lesioning, electrical stimulation, and anatomical tracing techniques (cf. Davis et al., 1982; Pellet, 1990). Anatomical analysis of the startle circuit at the cellular level, however, has not been done. These kind of analyses are crucial for a better understanding of the elicitation and modulation of startle (Wu et al., 1988).

The fos proto-oncogene (c-fos) has been proposed to function as the third messenger in a stimulus-transcription coupling cascade that converts external stimuli into intracellular long-term genetic action (Curran, 1988). Fos expression in the cell nuclei can be induced by many external stimuli. The present study employed Fos immunohistochemistry to determine the structures activated during startle elicitation.

Cats were given *p*-chlorophenoanaline (PCPA) for 4 days (125 mg/kg/day, IP). This procedure has been shown to enhance startle and reduce habituation (Wu et al., 1990). They were presented with 115 dB, 20 msec noise bursts once every 5 sec for 2-2.5 hr. Cats given no stimulation served as the controls. Cats were sacrificed at the end of the stimulation followed by immunohistochemical procedures for Fos.

Within the primary startle circuit identified previously, Fos-immunoreactive nuclei were found in the cochlear nucleus, nucleus of the lateral lemniscus, and less heavily, ventral pontine reticular formation. In addition, consistent Fos expression was found in structures that have been shown to modulate startle, e.g., inferior colliculus, nucleus cuneiformis, raphe, and central gray. By combining retrograde tracing techniques, this procedure can provide detailed analysis of the neural mechanisms of startle modulation.

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187.15

MEDULLARY CONTROL OF LOCOMOTION AND MYOCLONIC JERKS.

Y.Y. Lai and J.M. Siegel. Dept. Psychiatry, UCLA, and Neurobiol. Res. VAMC, Sepulveda, CA 91343.

Myoclonic jerks are a common clinical problem. We have demonstrated that corticotropin-releasing factor (CRF) and non-NMDA agonists microinjected into nucleus magnocellularis (NMC) of the medulla produce muscle atonia, while NMDA agonists produce increased muscle tone and/or locomotion. The present study was designed to determine the effect of glutamate and CRF on spontaneous or mechanically induced increases in muscle activity and myoclonus. Spontaneous locomotion or myoclonic jerks were present after the second day post decerebration and could also be induced by insertion of a microinjection needle into the medulla. Microinjection of CRF (10 nM), kainic acid (0.1-0.2 mM), and quisqualic acid (1 - 10 mM) into NMC blocked locomotion and myoclonic jerks for only 2.6-5.4 min. However, APV (50 mM), an NMDA antagonist, blocked locomotion or myoclonic jerks for more than 3 h. Thus CRF and non-NMDA agonists not only produce muscle tone suppression but also block spontaneous myoclonic jerks and locomotion. Furthermore, locomotion and myoclonus could be blocked by NMDA antagonists, which do not suppress muscle tone. We hypothesize that locomotion and myoclonic jerks in the decerebrate cat are mediated by NMDA receptors in NMC.

187.16

SPONTANEOUS MOTOR RHYTHM AND GLYCINE EFFECTS ON RHYTHM GENERATION IN MOUSE SPINAL CORD, IN VITRO.

Y. Tao* and M.H. Droge. Dept. of Biol, Texas Woman's Univ., Denton TX 76204.

EMG patterns in the gastrocnemius (G) and tibialis anterior (TA) muscles of spinal cord-hindlimb explants from neonatal mice were investigated. Compared to non-hemisected explants, neither hemisection of the spinal cord nor hemisection plus transection at L1 significantly altered the incidence of spontaneous motor rhythm. Therefore, not only does each half of the neonatal spinal cord contain sufficient circuitry to generate motor rhythm but the more reduced preparations were just as likely to produce such activity. Hemisected preparations, however, exhibited slower spontaneous rhythm. In all explants tested, sequences of spontaneous rhythm occurred in either the G or TA muscle, but not in both muscles simultaneously.

Bath applications of 1-3mM glycine had excitatory as well as inhibitory effects on EMG activity. Glycine as well as its antagonists not only failed to block ongoing rhythmic activity, but could evoke rhythm in previously nonrhythmic explants. The observed excitatory effects from glycine perfusions may reflect: 1)disinhibition; 2) functional immaturity of glycine$_1$ receptors; and/or 3) NMDA-associated depolarizations via glycine$_2$ receptors. Since strychnine (a glycine$_1$ receptor antagonist) and cycloleucine (a glycine$_2$ receptor antagonist)could evoke motor rhythm when given separately or in combination, glycine transmission appears not to be required for motor pattern generation in mice. The fact that synchronized bursting in G and TA muscles occurred only in the presence of either or both glycine antagonists, suggests that glycine does provide some reciprocal inhibition between their motor nuclei.

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187.17

BLOCKADE BY STRYCHNINE OF THE INHIBITION OF MASTICATORY MOTONEURONS INDUCED BY MEDULLARY STIMULATION.

Pablo Castillo,* Cristina Pedroarena,* Francisco R. Morales, and Michael H. Chase. Dept. de Fisiología, Facultad de Medicina, Montevideo (Uruguay), the Brain Research Institute, Dept. of Physiology, University of California, Los Angeles, CA 90024.

Stimulation of the parvocellular reticular formation (PcRF) results in the induction of short latency IPSPs in trigeminal (jaw-closer and jaw-opener) motoneurons (Brain Research 535 [1990] 339-342). The present study was performed to determine whether glycine is the neurotransmitter that mediates these IPSPs. Experiments were conducted on 3 anesthetized cats (Nembutal 40 mg/kg, i.p.). Seventeen motoneurons were recorded before and ten following the intravenous injection of strychnine nitrate (0.2 mg/Kg). IPSPs in trigeminal motoneurons were first evoked by stimulation of the PcRF (80 uA, 0.2 ms; P13-14, L2.0-2.5, H -6.0 to -8.0). Following the application of strychnine, these IPSPs were no longer present. Strychnine also suppressed the short lasting glycinergic component of the inferior alveolar nerve-induced IPSP, whereas the longer lasting GABA-mediated IPSP of inferior alveolar nerve origin was resilient to the effects of this drug. We conclude that glycine, or a structurally-similar inhibitory amino acid, is the neurotransmitter released by the PcRF pathway whose activation results in the non-reciprocal inhibition of trigeminal motoneurons. Supported by NS 09999.

187.18

THE EFFECTS OF EXCITATORY AMINO ACID RECEPTOR ANTAGONISTS, CNQX AND CPP, ON TRANSMISSION OF RHYTHMICAL MASTICATORY DRIVE TO BRAINSTEM INTERNEURONS DURING FICTIVE MASTICATION.

T. Inoue*, N. Hashimoto*, D. Junge and S.H. Chandler. Dept. of Kinesiology and the Brain Research Institute, UCLA, Los Angeles, CA 90024

Previous pharmacological experiments from our lab suggest that transmission of rhythmical masticatory drive from premotoneurons to jaw-opener motoneurons during rhythmical masticatory activity in paralyzed guinea pigs (RMA) is mediated, in part, by excitatory amino acid (EAA) receptor activation. During RMA, many brainstem trigeminal premotoneurons and interneurons are rhythmically active, yet, there is no information regarding the contribution of EAA subtypes in transmission of the masticatory drive signal to these brainstem neurons. Therefore, the present study was performed using classical extracellular single unit recording and microiontophoretic techniques. In ketamine/urethane anesthetized, and paralyzed guinea pigs, repetitive stimulation (30-40Hz) of the masticatory area of the cortex was used to evoke fictive mastication (RMA). Extracellular recordings of rhythmically active brainstem neurons were made through a glass pipette attached to a five-barreled microelectrode. Responses of neurons during RMA were challenged with either kainate, quisqualate, N-methyl-D-aspartate (NMDA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or 3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP). The results are summarized as follows: 1) Application of EAA agonists induced rhythmical spike discharge in previously silent neurons during RMAs, 2) application of the specific non-NMDA antagonist CNQX reduced rhythmical spike activity by greater than 50% whereas CPP, a specific NMDA antagonist, reduced activity less than 50% of control. Simultaneous application of both antagonists at individual doses 1/2 that were shown previously to reduce rhythmical spike discharge produced greater reductions than individual application of either antagonist at 2 times the combined dose. The results suggest that excitatory amino acids may be involved in activation and/or modulation of rhythmical discharge of brainstem interneurons involved in RMA. Supported by NIH grant DE 06193.

187.19

ADRENERGIC AGENTS INFUSED INTO THE MEDIAL PONTINE RETICULAR FORMATION (mPRF) MODULATE REM SLEEP AND UNIT ACTIVITY IN BEHAVING CATS.

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Adrenergic input to the mPRF has been postulated to play a role in the control of REM sleep. In vitro studies from our lab indicate that a majority of mPRF neurons have α_1-mediated depolarizing responses and a minority have α_2-mediated hyperpolarizing responses to norepinephrine (NE), with almost no neurons displaying both responses. We postulated that clonidine (CL, an α_2 agonist) microinjected in vivo would suppress REM sleep and associated unit activity while phenylephrine (PE, an α_1 agonist) might enhance REM sleep, since cholinergic agonists such as carbachol (CARB) which depolarize mPRF neurons induce a REM sleep-like state. A series of microinjections into the mPRF of NE, CL and PE (each 55 nmol, 0.5 µL) were performed at sites effective in producing at least a 3-fold increase in REM sleep in response to CARB (all sites histologically localized to mPRF). REM sleep was significantly decreased in the first hour and over the entire 4 hr recording following both CL (-96% hr 1, -89%, all 4 hr) and NE (-88.0%, -50%). PE significantly increased REM (+395%) in the fourth hour only. Paralleling REM state changes, NE and CL produced a significant reduction in unit activity during REM but not the other states. PE altered unit activity only in the last hour, increasing the firing frequency during REM. These data indicate a REM-suppressive effect of CL, presumably by action at an α_2 receptor. NE had effects on REM which paralleled CL, suggesting α_2 receptors in the mPRF play an important role in REM sleep, and may be preferentially localized to neurons important in regulation of this state.

187.20

EFFECTS OF CLONIDINE, CYPROHEPTADINE AND BACLOFEN ON KNEE SWING IN SPINAL CORD INJURED (SCI) HUMANS. **P.W. NANCE, F. HU*. Dept. of Medicine, U. Manitoba, Winnipeg, Man., CANADA**

In ten SCI subjects, the ability of the lower leg to swing about the knee in response to passive knee extension and flexion was tested before, during and after treatment with putative antispasticity agents: clonidine (an alpha-2 noradrenergic agonist), cyproheptadine (a 5-HT2 antagonist) and baclofen (a GABA-b agonist). Also, the subjects' leg tone was graded using the Ashworth scale (AS). The pendulum score (PS) was recorded using an electrogoniometer attached via velcro straps about the knee (subject sitting and supine), the number of swings greater than 40 degrees over three seconds after the foot was released was summed. In the drug free period, the PS of the subjects sitting correlated with the AS, r=.94. Both scores were reduced similarly by clonidine and cyproheptadine; whereas, the AS was most reduced and the PS was least reduced by oral baclofen. The PS of the subjects in a supine position was minimally changed by these treatments. Intrathecal baclofen in four of these subjects produced the greatest change in all measurements. **Supported by the Rick Hansen Man-in-Motion Legacy Fund.**

SPINAL CORD AND BRAINSTEM: ANATOMY

188.1

ABSENCE OF CGRP mRNA EXPRESSION IN PHRENIC MOTONEURONS. G.C. Sieck, P. Popper, W.Z. Zhan and P.E. Micevych. Mayo Clinic and Foundation, Rochester MN 55905 and UCLA School of Med., Los Angeles, CA 90024.

Calcitonin gene-related peptide (CGRP) mRNA expression in alpha motoneurons is altered by axotomy, spinal cord transection and androgens, all of which influence muscle activity. Because the diaphragm muscle is stereotypically activated with breathing, its activation history is unique compared to limb muscles. Therefore, we examined CGRP mRNA expression in labelled phrenic motoneurons in adult rats. In addition, we examined the influence of 14 days of TTX-induced inactivation of the diaphragm muscle. Phrenic motoneurons were labelled by intramuscular injection of fluorogold and identified in 20 µm transverse sections. Alternate sections were processed for CGRP immunohistochemistry and in situ hybridization with a probe complementary to the 3'-end of αCGRP mRNA. Eight controls and 7 TTX-inactivated spinal cords were studied, with 20-30 phrenic motoneurons per animal identified. The expression of CGRP mRNA was not detected in either control or TTX-inactivated phrenic motoneurons. This contrasted to the clear expression of CGRP mRNA in other cervical cord alpha motoneurons. We conclude that CGRP is either not expressed in phrenic motoneurons or expressed at such a low level that it is not involved in neuromuscular plasticity of the diaphragm muscle.

Research supported by NIH grants HL34817, HL37680, NS23468 and NS21220.

188.2

PSUEDORABIES VIRUS (PRV) DEFINES EFFERENT PROJECTIONS TO PHRENIC MOTONEURONS IN THE RAT. E.G. Finlon & J.L. Feldman Systems Neurobiology Lab. Department of Kinesiology, UCLA, Los Angeles, CA 90024-1527.

Phrenic motoneurons receive diverse projections from brainstem and spinal cord. Conventional labelling studies, which require extracellular deposition of tracers, have serious limitations due to unavoidable contamination by nonspecific transport. Developments in transneuronal tracing techniques (G. Ugolini et al., Science, 243('88): 89) allow exclusive labeling of specific projections to a motoneuron pool. PRV is such a transneuronal tracer (J.P. Card et al., J. Neurosci., 10('90):1974). Following dorsal rhizotomies, 1-2 µL injections of 10^8 PFU of PRV (Becker Strain) were made into the right phrenic nerve of Wistar rats. After processing, PRV positive cells were visualized by a polyclonal antibody and subsequent amplification. Following first order labelling in the phrenic motoneuron pool, numerous cell bodies were labelled in the thoracic spinal cord in inspiratory motoneuron pools, and in the ventral respiratory group (VRG) in the medulla. In addition, cells were labelled in ventrolateral nucleus of the solitary tract, raphe nuclei, parapyramidal region, B3 serotonergic region, and A5 noradrenergic region. Spinal cord segmental interneurons, predominantly ventromedial and ventrolateral to the phrenic motoneurons, appeared with the same time course as the VRG label. No cells were labelled in the upper cervical spinal cord. We are grateful to J.P. Card, Ph.D., E.I. Dupont de Nemours & Co., for his generous gift of PRV and antibody. Supported by NIH Grants NS24742.

188.3

RETROGRADE LABELLING OF RESPIRATORY NEURONS IN LAMPREYS. D.F. Russell. Dept. of Anesthesiology, Washington Univ. Med. School, St. Louis, MO 63110.

The aim was to retrogradely label premotor respiratory interneurons in adult lampreys, either *Petromyzon marinus* or *Ichthyomyzon unicuspus*, from *in vivo* injections. The tracers used were HRP, WGA-HRP, or collidal gold-apoHRP; as reporters, either DAB, TMB, or silver intensification were used. Survival times were 3 weeks to several months. Tracer was injected unilaterally into one of the respiratory motor nuclei, including cranial nuclei IX or X_r, usually sub-ependymally among the somata of respiratory motorneurons. Vibratome sections were cut.

This approach consistently labelled a compact bilateral nucleus rostral to nuc.V, situated dorsally, just under the cerebellum, near the ventricular surface. A unilateral injection into nuc.X gave bilateral labelling of this rostral nucleus. In TMB materal, labelled somata had average diameters ~8µ. This cell group will be called the "subcerebellar respiratory nucleus".

More caudally, there was consistent labelling of a low-density group of medium-size neurons along the rostral and lateral edges of nuc.V. Injection of HRP into nuc.X_r gave solid diffusion labelling of several large crossing fibers in the midline commissure near nuc.V, and also labelled numerous small fibers that decussated more rostrally. Injections of colloidal gold-apoHRP, which appeared to not diffuse, helped define the labelling of neurons near to the injection sites in nuc.IX or X_r; small labelled neurons were observed near the midline in the reticular formation. Caudal to the injection sites, there was consistent labelling of contralateral neurons near obex, situated dorsal to the reticular formation near the ventricular surface.

Supported by NIH grant NS23028.

188.4

ANALYSIS OF THE MOTOR NUCLEUS OF THE EXTENSOR DIGITORUM LONGUS IN THE C57Bl/Ksj-dbm DIABETIC MOUSE. Kathleen M. Klueber and Sue Stansel, Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, KY 40292

Ultrastructural evidence revealed a degeneration of neuromuscular junctions in diabetic muscle resulting in denervation (Feczko and Klueber, Am.J.Anat 182: 224, 1988). However, analysis of the central connections of these denervated myofibers has not been reported. The objective of this study was to determine the size and location of the motor neuron pool to a diabetic muscle. The extensor digitorum longus muscle (EDL) from diabetic (db/db) and control (db/+) mice (n=5/age; 6 and 12 wks) were injected with HRP. After 24 hours, the animals were perfused, the spinal cords removed and processed. HRP reaction products were formed using TMB as the chromogen. Morphometric analysis of the size of the motor neurons revealed a significant change ($p<.05$) in the size of the neurons with the pathogenesis of the disease. The size of the motor neuron pool was not effected. Funded by: NIDDK 1R29DK41553.

188.5

MOTOR INNERVATION OF THE PHARYNX AND LARYNX: AN ELECTRON MICROSCOPIC AND HRP STUDY OF THE NUCLEUS AMBIGUUS IN THE RAT. D.A. Hopkins and D.W. Saxon. Department of Anatomy, Dalhousie University, Halifax, NS B3H 4H7.

Motoneurons innervating the striated musculature of the pharynx and larynx are located in the nucleus ambiguus (NA) but little is known regarding their ultrastructure. Injections (2-6 μl) of WGA-HRP (5%) or CT-HRP (0.25%) into the musculature of the pharynx or larynx resulted in retrogradely labelled neurons in the semicompact (NA_{sc}) and loose (NA_L) formations of the NA, respectively. Pharyngeal and laryngeal motoneurons (25-40 μm diameter) shared similar morphological features: i.e., multipolar, large round nucleus, eccentric nucleolus, large quantities of short stacks of rough endoplasmic reticulum. Neurons in both subdivisions received many synaptic contacts on the somata and dendrites. Axon terminals with round vesicles and asymmetric synaptic contacts (RA) were more numerous on NA_{sc} somata and dendrites while terminals with pleomorphic vesicles and symmetric synapses were more numerous on NA_L somata and dendrites. A few atypical labeled motoneurons (12-20 μm diameter) with an invaginated nucleus, eccentric nucleolus and sparse Nissl substance were also identified in the pharyngeal division of the NA. Pharyngeal and laryngeal motoneurons differ from each other with respect to proportions of types of synapses and from esophageal motoneurons which have very few synapses on their somata and predominantly RA synapses on their dendrites. These differences in afferent inputs may be related to functional differences in each of the subdivisions of the NA. Supported by MRC of Canada Grant MT-7369.

188.6

DIFFERENTIAL LABELING OF ORAL MOTOR SYSTEMS USING PSEUDORABIES VIRUS. R. A. Fay and R. Norgren. Neuroscience Program and the Department of Behavioral Science, Col. of Medicine, Penn. State Univ., Hershey, PA 17033.

Pseudorabies virus was injected into the masticatory and intrinsic tongue muscles of the rat. Injections of 12 ul of viral suspension ($1x10^9$ pfu/ml) resulted in retrograde, transynaptic labeling of neurons in the brainstem and forebrain. Viral injections into the masseter, digastric, and temporalis muscles produced specific myotopic labeling within the trigeminal motor nucleus. Transynaptically labeled neurons occurred in a variety of brainstem structures, but secondary infection from all 3 muscles was restricted to the principal trigeminal nucleus, the Kolliker-Fuse nucleus, and the subcoeruleus area in the pons and the area postrema, the nucleus of the solitary tract, some raphe nuclei, and the paragigantocellular and rostroventrolateral areas in the medulla. Viral injections into the intrinsic tongue muscles resulted in myotopic labeling in the hypoglossal nucleus. In the brainstem, transynaptic labeling overlapped extensively with that following injections into masticatory muscles. In the forebrain, more differences were observed. Infections of the muscles of mastication resulted in weak, but widespread labeling in the cortex and extensive labeling in the hypothalamus. After tongue injections, cortical label was restricted to the frontal and piriform zones, but extensive labeling occurred in both the hypothalamus and the amygdala. Supported by PHS grants DC 00240 and MH 00653.

188.7

CO-LOCALIZATION OF GAMMA-AMINOBUTYRIC ACID (GABA) AND PARVALBUMIN IN CHICK SPINAL MOTONEURONS. E. Philippe, F. Gaulin*, G. Audet* and C. Zhou*. Centre de recherche en neurobiologie, Université Laval et Hôp. de l'Enfant-Jésus, 1401, 18e rue, Québec, Canada, G1J 1Z4.

Chick spinal motoneurons have been shown to express a clear GABA-immunoreactivity (Philippe *et al.*, Neurosci. Lett., 116: 12-16, 1990). However, since GABA is co-localized with acetylcholine, the classical excitatory neurotransmitter of vertebrates neuromuscular system, it seems unlikely that GABA plays the role of an inhibitory neurotransmitter in this system. Moreover, since it has previously been suggested that a dysfunction between amino acids as well as an uncontrolled modulation of intracellular calcium could contribute to neuronal death in a wide variety of neurological diseases, GABA might have a function in the maintenance of cellular interactions in the neuromuscular system. Hence, the aim of this study was to determine whether a co-localization between GABA and calcium binding proteins might be detected within spinal motoneurons.

Immunocytochemistry performed with parvalbumin antiserum, according to the peroxidase anti-peroxidase (PAP) technique, showed a clear and strong immunoreactivity in some chick spinal motoneurons. Such is not the case for calbindin, another calcium binding protein (Philippe and Droz, J. Comp. Neurol., 283: 153-160, 1989). Moreover, combination of pre- and post-embedding immunocytochemical procedures showed that parvalbumin and GABA are co-localized in the majority of motoneurons.

It is interesting to note that the sensory innervation of chick skeletal muscles is assumed, at least in part, by primary sensory neurons of dorsal root ganglia and their related neuromuscular spindles, which are immunoreative to both GABA and calbindin antisera. Hence, it appears that innervation of skeletal muscles is, at least in part, under a GABAergic control. However, the co-localization of GABA with parvalbumin was restricted to the motor innervation, whereas the co-localization of GABA with calbindin was restricted to the sensory innervation of the skeletal muscles.

(Supported by grants of C.R.M. and F.R.S.Q.)

188.8

MOLECULAR SPECIFICATION OF LARGE NEURONS IN THE RAT SPINAL CORD REVEALED BY MONOCLONAL ANTIBODY 8B3. A. R. Tate*, M. F. Barbe, A. F. Pimenta, T. J. Cunningham, P. Levitt. Dept. of Physical Therapy, Temple University, Philadelphia, PA, 19140 and Dept of Anatomy and Neurobiology, Med. Coll. of Pa, Philadelphia, Pa, 19129.

A monoclonal antibody, 8B3, was generated by immunizing Balb/C mice with homogenized motor cortex (Guimaraes, et al., Neuroscience Abst 16: 240, 1990), and was found to crossreact with spinal cord. The distribution of immunohistochemically stained neurons was investigated in the rat throughout the rostrocaudal extent of the spinal cord in both longitudinal and coronal sections. Immunoperoxidase labeling revealed that 8B3 stained a small subpopulation of neurons in the spinal cord. In all instances, immunoreactivity was associated with the surface of somata and dendrites of these neurons. The most prominent group of stained neurons were large diameter cells found in medial and lateral subdivisions of the ventral horn, corresponding primarily to lamina IX. The number of these ventral horn 8B3 immunoreactive cells was the greatest in the lumbar enlargement. In addition, at thoracic and sacral levels, preganglionic motor neurons were stained with 8B3. There were small numbers of the scattered immunoreactive neurons in intermediate laminae V and VI. A third region containing immunoreactive neurons surrounding the central canal, including cells in the dorsally situated Clarke's nucleus. Very few immunoreactive neurons were seen in any of the sections examined in laminae I-III of the dorsal horn. Double-labeling studies are currently being performed to determine whether the phenotype of the 8B3-immunoreactive neurons is motor, ascending projection or interneuronal. The present data suggest that one of these groups may be specified at the molecular level by selective expression of the 8B3 antigen. Supported by NIH grant NS24707.

188.9

C-FOS-LIKE EXPRESSION IN THE BRAINSTEM OF THE CAT DURING CARBACHOL-INDUCED ATONIA. J. Yamuy*, J.R. Mancillas, F.R. Morales and M.H. Chase. Brain Research Institute, Dept. of Physiology and Dept. of Anatomy and Cell Biology, UCLA School of Medicine, Los Angeles, CA, 90024.

The expression of the early proto-oncogene c-fos has been related to neuronal activity. In the present study we were interested in determining whether neurons in areas of the reticular formation that have been implicated in the production of atonia during active sleep became active, as determined by their c-fos expression, when atonia is induced by the pontine injection of carbachol.

Under nembutal anesthesia, 5 chronic cats were prepared for microinjection and the recording of the EEG, EOG, EMG and PGO waves. After recovery, 3 cats were injected with a carbachol solution (4 μg in .25 μl) and 2 cats with saline (.25 μl). Injections were directed to the rostro-medial pontine tegmentum. The cats injected with carbachol entered into a state similar to active sleep, which consisted of atonia, desynchronized EEG and PGO waves, whereas the control cats remained awake. After approximately 2 hours the animals were sacrificed with an overdose of nembutal. Following perfusion and fixation, the brainstem was submerged in Bouins solution. Brainstem frontal sections 7 μm thick were immunostained with a Fos polyclonal antiserum and developed using the alkaline phosphatase and the ABC methods.

Sections at the level of cranial motor nuclei nV, nVI, nVII and nXII were selected for analysis because they sampled the reticular formation at different brainstem levels below the site of injection. Carbachol-injected cats exhibited c-fos-like immunoreactivity in neurons within the paramedian reticular nucleus, magnocellular tegmental field and gigantocellular tegmental field, whereas the control cats displayed low counts of immunostained cells in these areas. C-fos-like expression was also found in other areas which were not the focus of this report.

These results indicate that different patterns of c-fos expression are present during wakefulness and carbachol-induced atonia and that the medial and ventral areas of the reticular formation, which have been implicated in the promotion of atonia during active sleep, exhibit increased neuronal activity during carbachol-induced atonia. Supported by USPHS Grants NS09999 and MH43362.

188.10

GIANT NEURONS IN THE CAUDAL PONTINE RETICULAR FORMATION RELAY THE ACOUSTIC STARTLE RESPONSE IN THE RAT. K. Lingenhöhl, E. Friauf and M. Koch*. Dept. Animal Physiology, Univ. of Tübingen, D-7400 Tübingen, Fed. Rep. Germany.

The caudal pontine reticular formation (PnC) was proposed as a relay station in the acoustic startle pathway (Davis et al., J Neurosci 2:791, 1982). We investigated PnC neurons using electrophysiology and anatomy to test this idea. Intracellular recordings showed that PnC neurons receive excitatory input from both ears with a mean latency of 4.2 ms. The retrograde tracer Fluoro-Gold was injected into the PnC and labeled auditory neurons bilaterally in the dorsal cochlear nucleus, the lateral superior olive and the ventral periolivary nuclei, indicating multiple direct inputs from 1st and 2nd order auditory nuclei to the PnC. Intracellular iontophoresis of HRP into PnC neurons (N=25) identified giant neurons with large dendritic arborization mainly within the PnC. Two types of axonal trajectories were seen: one type (N=23) had a caudal course on the ipsilateral side and could be traced into the upper ventral spinal white matter before fading; the second type (N=2) projected bilaterally with thick axonal branches in caudal and rostral directions. Both axon types showed numerous collateral endings within the reticular formation. PHA-L injections into the PnC substantiated the projections to the spinal cord. Electrical stimulation of the thoracic spinal cord elicited antidromic action potentials in acoustically driven giant PnC neurons with a mean latency of 1.5 ms. Our data show that acoustic information is relayed via giant PnC neurons within 5.7 ms to the thoracic spinal cord, a timespan which matches the latency of the acoustic startle response. Quinolinic acid lesions of PnC neurons greatly impaired or even abolished the acoustic startle response, depending on the number of giant PnC neurons that were eliminated. We conclude therefore that giant PnC neurons are required in the acoustic startle pathway. Supp. by DFG SFB 307.

188.11

WITHDRAWN

188.12

AFFERENT AND EFFERENT CONNECTIONS OF THE MESENCEPHALIC LOCOMOTOR REGION WITH THE PONTOMEDULLARY RETICULAR FORMATION IN THE RAT. C.A. Livingston, S. Pylypas', L.M. Jordan & D.M. Nance. Departments of Physiology and Pathology, University of Manitoba, Winnipeg, MB R3E OW3 Canada

Previous studies indicate that the midbrain locomotor region (MLR) projects monosynaptically onto reticulospinal (RS) neurons located in those regions of the reticular formation (RF) from which the descending locomotor pathways originate. Our goal was to identify the specific population of RS cells in the medial pontomedullary RF that receive direct input from the MLR. We injected 5-20 nl of fluorogold (0.5%, retrograde label), mixed with tetramethylrhodamine dextran (5.0%; anterograde label) into the MLR. In the same animals, we injected 3.0 ul of Fast Blue (2.0%; retrogradely transported) into the thoracolumbar spinal cord. Seven days later the animals were perfused with 4.0% paraformaldehyde. The brains were transversely sectioned (40-100 um, thickness) and the tissue was viewed using either transmitted or epifluorescent optics. The distribution of retrogradely labeled neurons in the pontomedullary RF was compared with that of fibers and terminals anterogradely labeled from the MLR. In the caudal brainstem, numerous cells were retrogradely labeled from the MLR in the gigantocellular, parvocelllular, intermediate and pontine reticular nuclei. Fibers anterogradely labeled from the MLR coursed caudally and ventrally from the MLR and into the lateral pontomedullary RF, or medially and caudally into the medial pontomedullary RF. Fibers and terminals were distributed bilaterally in the pontine reticular nucleus, the dorsomedial tegmental area, the parvocellular, intermediate and gigantocellular reticular nuclei, and the caudal raphe nuclei. Neurons retrogradely labeled from both the spinal cord and the MLR were distributed and closely intermingled within these same nuclei. Despite the coincident distribution of the RS cells and terminals from the MLR, we found few terminal-like structures in close proximity to the somas of labeled RS cells. This suggests that direct input from the MLR onto these cells occurs at synapses located on their dendrites. The close intermingling of RS cells and reticular neurons that project to the MLR suggests that these cells could be synaptically connected. Our results indicate that the MLR and the reticular formation are reciprocally connected.

188.13

CORTICAL AND CEREBELLAR CONVERGENCE UPON SINGLE NEURONS IN THE PRIMATE RED NUCLEUS: AN ELECTRON MICROSCOPIC ANALYSIS. D.D. Ralston. Department of Anatomy, University of California, San Francisco, California, 94143.

The red nucleus of the primate receives major input from the motor cortex and from the deep cerebellar nuclei. Rubral neurons, both in parvicellularis and magnocellularis must then integrate this afferent information. This study in the primate demonstrates convergence of these inputs to the red nucleus from cortical and cerebellar sources. Spatial segregation of cortical and cerebellar inputs exist in each of the divisions of the nucleus: those of cortical origin synapse upon distal dendrites and those of cerebellar origin synapse upon proximal dendrites and somata. Tsukahara, et al, 1975, in the cat, reported sprouting of cortical input to rubral neurons in cerebellar afferent territory after deafferentation from cerebellum, inferring convergence of these two inputs onto the same rubral neuron. In this study convergence of inputs can be demonstrated in parvicellularis in the range of medium sized dendrites of the neuronal dendritic arbor due to the overlap of the two sources of input.. However, the magnocellularis division, where spatial segregation is more marked, requires single neuronal identification for confirmation. Results demonstrate anatomical evidence for the presence of degenerating boutons of cortical origin and WGA-HRP labeled cerebellar terminals onto the same rubral neuron to carry out the task of integration of motor information. The red nucleus therefore is involved in the continuous integration and monitoring of cortical and cerebellar input and, given the possible climbing fiber training of the mossy fibers in cerebellum, may in fact modulate the pontine input to cerebellum in processing motor information. Supported by NS23347 from N.I.H..

188.14

REDEFINING RAT RED NUCLEUS: MULTIPLE LABELLING OF INDIVIDUAL NEURONS FROM SPINAL CORD, INFERIOR OLIVARY NUCLEUS AND CEREBELLAR NUCLEI. D.Y.Yu, S.Na*, J. Wilson* and P.R.Kennedy. Neurosci. Lab, Georgia Tech, Atlanta, GA 30332.

Previous studies (Tucker and Kennedy, Soc. Neurosci. Abstr. 1990, 16(1):729) showed that Red Nucleus (RN) neurons were doubly labelled from the spinal cord and inferior olivary nucleus (ION). About two thirds of RN neurons that projected to the spinal cord also projected to the ION. Huisman et al. (Brain Res., 1983, 264:181-196) showed that RN neurons also projected to both the spinal cord and the cerebellar nuclei (CbN). The possibility that individual RN neurons might project to all three targets was examined using Fluorogold, Diamidino Yellow and WGA-HRP.

Each neuron was examined under brightfield and fluorescent light. The previous findings of double labelling between spinal cord and ION, and spinal cord and CbN were confirmed. A new finding of neurons doubly labelled from ION and CbN was made. More importantly, some RN neurons were triply labelled. We are analyzing labelled neuron classes, their numbers and distributions. In sum, individual RN neurons project to spinal cord, ION and CbN. Grant: NS 24602-03.

188.15

ULTRASTRUCTURAL ANALYSIS OF TH AND 5-HT INPUTS TO THE SNB OF THE MALE RAT. M.G.Leedy, J.C.Bresnahan, and M.S.Beattie, Dept. Cell Biology, Neurobiology and Anatomy, Ohio State Univ., Columbus, OH 43210

While the existence of supraspinal inputs to the spinal nucleus of the bulbocavernosus (SNB) has been proposed, there is presently only limited ultrastructural description of identified inputs to this nucleus. The present study reports quantification of tyrosine-hydroxylase (TH) and 5-HT inputs to somata and dendrites in the SNB. L5/L6 spinal cord segments from adult male rats were sectioned and processed using ABC immunohisto-chemistry for TH and 5-HT. The sections were then flat embedded for sequential light and EM analysis. At the light level, both TH and 5-HT staining were seen in the SNB. 5-HT fibers had an almost exclusive association with the somata, often encircling the perimeter. TH fibers were also seen in proximity to cell bodies, but in addition were scattered throughout the nucleus, often following the motoneuron dendritic arbors. EM analysis supported the light level findings. Sampled 5-HT terminals were frequently in direct contact with somata (45% of terminals were axo-somatic; 27% axo-dendritic). In contrast, the majority of the sampled TH terminals contacted dendrites (17% axo-somatic; 66% axo-dendritic). The quantification of these inputs to the SNB provides the basis for comparisons between spinally intact and transected animals. (NIH grant NS10165)

188.16

Brainstem projections to the upper cervical spinal cord in the macaque. J.O.Phillips, F.R. Robinson, and A.F. Fuchs, Western Regional Primate Research Center, Department of Psychology, and Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195

We identified the areas of primate brainstem that project to the level of the neck motoneuron pools by injecting 3 monkeys with a 1% solution of the tracer WGA-HRP. We injected the upper cervical spinal cord (C1 to C3) in 1 rhesus and 1 cynomolgus monkey and the lower cervical and upper thoracic spinal cord (C6 to T1) in 1 cynomolgus monkey. After 24 hours (upper cervical) or 48 hours (lower cervical), we perfused the monkeys with 3% paraformaldehyde and cut frozen sections of the brainstem and spinal cord. We reacted the sections with TMB using a modified Mesulam technique. We plotted the location of labeled cells with an electronic pantograph under polarized light.

A unilateral 60nl injection into C2 labeled neurons bilaterally in the lateral part of the medial vestibular nucleus and the medullary and pontine reticular formation . The lateral vestibular nucleus also contained labeled cells bilaterally. There were also labeled cells in the contralateral superior colliculus and the contralateral fastigial and interpositus nuclei of the cerebellum. Other areas containing labeled cells included the red nucleus, interstitial nucleus of Cajal, and the mesencephalic area just lateral to the central grey. A 140nl unilateral injection into the lower cervical cord filled cells in many of the same areas but labeled very few cells in the medial vestibular nucleus and more cells in the lateral vestibular nucleus, but only ipsilaterally. There were no labeled cells in the superior colliculus or cerebellum.

A comparison of these results with the data obtained in previous HRP injections of the abducens nucleus suggests that brainstem regions providing pre-abducens and pre-neck motoneuron afferents are largely separate in the monkey. Those regions that do project to both areas (eg., lateral portions of the medial vestibular nucleus) often constitute the overlapping borders of two largely separate populations projecting to each motoneuron pool.

This work was supported by NIH grants EY00745, RR00166, EY07991 and a grant from the Virginia Merrill Bloedel Hearing Research Center.

188.17

PROJECTIONS OF MOTOR CORTEX TO THE PRETECTUM OF THE CAT. B.Hutchins[1], S.L.Liles[2], and B.V.Updyke[3] Department of Anatomy[1], Baylor College of Dentistry, Dallas,Tx. 75246 and Departments of Anatomy[3] and Physiology[2], Louisiana State University Medical Center, New Orleans,La. 70112.

The association of the pretectal complex with the visual system has been well established. There have also been a few studies reporting motor projections to the pretectum, yet there are few studies which have analyzed these projections in detail. Therefore, as part of an integrated study of the cat motor system, the pretectum was analyzed for afferents from specific somatotopic areas of motor cortex. Briefly, the methods were to electrophysiologically stimulate motor cortex in adult animals anesthetized with ketamin and xylazine to define muscular responses and inject ^{3}H-proline into these same cortical areas. Animals were sacrificed 48 hrs. later with an overdose of Nembutol and the tissue was transcardially perfused with 4% para-formaldehyde. After the brains were removed and saturated in a 0.1 M phosphate buffer with 30% sucrose for cryoprotection, 50 μm frozen sections were cut in the coronal plane. Every fifth section was processed for routine autoradiography and counterstained with cresyl violet. Analysis of five cases, reveal afferent projections to only the anterior pretectal nucleus (NPA). Silver grains were typically located over the ventral border of the nucleus without regard for the two subnuclei, compacta or reticularis. These terminal fields often appeared in the shape of a "U" as they closely followed the ventral border. When cases were analyzed with respect to their muscle representations, there appeared to be a crude somatotopy present, with some overlap of terminal fields. Of the three somatotopic regions tested, hindlimb muscles were represented rostrally, the forelimb muscles were represented posteriorly, and the shoulder muscles represented in an intermediate position. Because portions of NPA have been shown to receive both retinal and somatosensory projections, these motor projections may be involved with corollary discharges used in sensorimotor transformations. (Supported by NEI EY06977[1], EY05724[3], and NINDS NS22275[5])

188.18

THE TERMINATION PATTERN AND POSTSYNAPTIC TARGETS OF RUBRO-SPINAL FIBERS IN THE RAT SPINAL CORD M. Antal[1,2], G.N. Sholomenko[1], A.K. Moschovakis[1], J. Storm-Mathisen[3], C.W. Heizmann[4] and W. Hunziker[5] [1]Lab. Neur. Cont., NINDS, NIH, Bethesda, USA; [2]Dept. Anat., Univ. Med. School, Debrecen, Hungary; [3]Anat. Inst., Univ. Oslo, Norway; [4]Dept. Pediat., Univ. Zurich, Switzerland; [5]Cent. Pharm. Res. Dept., F.Hoffmann-La Roche Co., Basle, Switzerland

The spinal projections of the red nucleus were studied in the rat using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L). After injecting PHA-L unilaterally into the red nucleus, labelled fibers and terminals were detected at cervical, thoracic as well as lumbar segments of the spinal cord. Descending fibers were located in the dorso-lateral funiculus, most of them contralateral to the injection site. Terminals were found predominantly in laminae V-VI and in the dorsal part of lamina VII at all levels and on both sides of the spinal cord. The proportion of terminals revealed on the ipsilateral side varied at different segments and represented 10-30% of the total number of boutons. Terminals were also found in the ventro-lateral aspect of Clark's column on both sides.

Synaptic contacts of rubro-spinal terminals and GABA as well as glycine immunoreactivity of their postsynaptic targets were investigated in a correlative electron microscopic study.

To obtain a more global view of the relationship between rubro-spinal fibers and spinal interneurons sections from the cervical and lumbar cord were stained for both PHA-L and calbindin-D28k (CaBP), a calcium-binding protein which has been reported to be a marker of certain subsets of spinal interneurons. CaBP-immunoreactive neurons in laminae V-VI were found to receive contacts from rubro-spinal axons. Labelled boutons impinged mainly upon dendrites, but somatic contacts were also revealed.

188.19

RETROGRADE LABELLING OF SPINAL EFFERENTS IN AN ANIMAL MODEL OF SPASMODIC TORTICOLLIS. P.M. Young*, F. Mullin* & J. Robertson-Rintoul*#. Dept. of Psychology, Lancashire Polytechnic and #Dept. of Biol. & Preclin. Med., University of St. Andrews, U.K. (Spon: European Neuroscience Association.)

The motor efferents to the sternomastoid (SM) and cleidomastoid (CM) were investigated using cholera toxin side chain B conjugated to horseradish peroxidase (CTB-HRP), and a wheatgerm agglutinin-HRP conjugate (WGA-HRP) as retrograde tracers. Under tribomoethanol anaesthesia 10 rats, six guinea pigs and six rabbits received an injection of tracer to the SM or CM. In control animals, injection to the muscles was preceded by section of the spinal accessory nerve (SAN). Twenty four to seventy two hours after injection animals were sacrificed and 80 m frozen sections through the brain stem and cervical cord were processed by the TMB technique. SM and CM receive a motor innervation through both the upper cervical spinal nerves and the SAN. The spinal accessory nucleus and the nucleus of cervical efferents, supplying both the SM and CM, extended from C3 caudally, to a continuity with the caudal component of the hypoglossal nucleus for the SAN efferents. The spinal accessory nucleus supplying SM and CM occupied the medial layer 8 and the lateral layer 9 of Rexed. With CTB-HRP, axons from both subnuclei were seen to join to form the SAN. There was no evidence in this study of retrograde transport of tracer to either the dorsal motor nucleus of the vagus or the nucleus ambiguous. There was however evidence of transneuronal transport of CTB-HRP to the contralateral spinal accessory nucleus and the significance of this finding is under investigation.

LIMBIC SYSTEM II

189.1

GOLGI STUDY OF THE MEDIODORSAL THALAMIC NUCLEUS IN THE RAT. **M.Kuroda and J.L.Price, Dept.Anat. & Neurobiol., Washington Univ.Sch. of Med., St Louis, MO 63110**

The Golgi technique was used to investigate the distribution and domain of neurons in each segment of the mediodorsal thalamic nucleus (MD) in the rat.

All impregnated neurons exhibited small sessile and thorn-like appendages on higher order dendrites, but these appendages were either absent or sparse on the soma and proximal dendrites. The configuration of the dendritic tree varied with the location of neurons. Cells in the central segment of MD had a stellate shape with dendrites radiating in all directions. These dendritic arbors formed a restricted spheroidal dendritic field within the central segment. Within the medial and lateral segments, stellate-shaped neurons were also present, although they were often distorted, such that their major dendrites tended to avoid the boundaries around MD, or between different segments. Cells just lateral to the border between the central and lateral segments were semicircular in shape, with dendrites only in the lateral segment. Impregnated neurons located close to the medial or lateral boundary of MD were fusiform in shape, with their dendrites aligned parallel to the boundary of the nucleus. These findings indicate that major dendrites of neurons do not extend across subdivisional boundaries. Therefore, cells and their dendrites are relatively confined to a single segment of MD.

Supported by NIH research grant DC 00093.

189.2

TOPOGRAPHICAL ORGANIZATION OF THE MEDIAL ORBITOFRONTO-STRIATAL PROJECTION AND ITS RELATIONSHIP TO STRIOSOMES AND ENKEPHALIN PATCHES IN THE MONKEY. M. M. Mizobuchi and S.N. Haber, Dept. of Neurobiology and Anatomy, Univ. of Rochester, Rochester, NY 14642

The ventromedial portion of the striatum receives input from structures related to the limbic lobe. As part of a larger study to understand the organization of limbic striatal input, we injected ^{3}H-AA into discrete areas of the medial orbitofrontal cortex (area 13A). Routine autoradiography was carried out on 50 μm sections throughout the striatum. To compare acetylcholinesterase (AChE)-poor "striosomes" and enkephalin (ENK) immunoreactive patches to the cortical terminal fields, adjacent compartments on either side were processed for AChE and ENK immunocytochemistry (ICC). In addition, sections were processed for both autoradiography and ENK ICC.

A dense patchy distribution of labelled fibers was observed in a narrow strip along the ventromedial aspect of the head of the caudate continuing ventrally into the medial nucleus accumbens. Caudally, label continued in the medial edge in the body of the caudate. No terminal fields were observed in the putamen. All injections produced a similar pattern of terminal labeling, however, medial injections terminated in the medial most edge of the striatum, while more lateral injections terminated just lateral to this. Thus the medial-lateral organization of area 13A was topographically preserved in its projection to the striatum. Comparison between terminal fields and striosomes revealed inconsistent matches. In some areas, striosomes and terminals clearly demarcated each other, and while in other regions, they either avoided each other or were partially overlapped. A similar relationship was observed with the ENK staining. Supported by NIMH MH45573.

189.3

PREFRONTAL CORTICAL PROJECTIONS TO THE AMYGDALOID COMPLEX: A PHA-L STUDY IN THE RAT. F. Mascagni and A.J. McDonald. Dept. of Anatomy, Cell Biology, and Neurosciences. Univ. of South Carolina School of Medicine, Columbia, SC 29208.

The cortico-amygdaloid projections of different areas in the prefrontal cortex (PFC) were investigated using the PHA-L anterograde tract tracing technique. The distribution of fibers in the amygdala seen with injections into different PFC areas appears to be topographically organized. Fibers from the ventromedial frontal cortex (i.e., the infralimbic cortex) terminate mainly in rostral parts of the olfactory amygdala and, to a lesser extent, in discrete portions of the central and basolateral amygdaloid nuclei. In contrast, more dorsal portions of the medial PFC (i.e., the prelimbic area and medial agranular cortex) have strong projections to rostromedial portions of the basolateral amygdala and light projections to the lateral capsular portion of the central nucleus. The lateral PFC (ventral and dorsal agranular insular areas; AIv and AId) sends fibers to the central nucleus and rostral portions of the basolateral amygdala. In addition, AIv projects to rostral portions of the olfactory amygdala. In general, the topographical organization of the projections of different prefrontal fields to the amygdala suggests that these cortical areas are involved in discrete behavioral functions. (Supported by NIH Grant NS19733)

189.4

HIPPOCAMPAL CONECTIONS OF THE AMYGDALA AND CLAUSTRUM IN THE CAT. A. Llamas, A. Román-Guindo* and F. Clascá* Dept. Morfología, Fac. Medicine, Univ. Autónoma, 28029 Madrid, SPAIN.

The connections of the various sectors of the cat's hippocampal formation with the amygdala and endopyriform claustrum (ECl) were studied by using WGA-HRP injected in discrete portions of Fascia Dentata (FD), Cornu Ammonis (CA), Subicular Complex (S) or in the amygdala in 19 adult cats. Hippocampal parcellation was based on the staining patterns present in adjacent serial sections processed for Nissl, Myelin, AChE and Timm's stain.

The rostral two thirds of S receive projections from the ventral part of ECl, but do not project back to it. CA and FD are not connected with the claustrum. The amygdala is only connected with the rostral two-thirds of the temporal stem of the hippocampus, particularly with the rostral third. Projections from amygdala reach the superficial part of the molecular layer of CA1 and prosubiculum. They arise mainly from the superficial division of the accesory basal and the cortical nuclei, and more scarcely from the parvocellular division of the accesory basal and the lateral nuclei. In addition, the lateral nucleus projects to the parasubiculum. Hippocampo-amygdaloid projections arising from the subiculum, prosubiculum and CA1 reach the posterior cortical nucleus, parvocellular and superficial subdivisions of the accesory basal nucleus, and the medial division of the central nucleus.

Present data, together with other reports in the literature show that hippocampo-amygdalar and hippocampo-claustral connections are organized in comparable anatomical patterns in cats and primates. CICYT PB 86-0558 and 88-0170

189.5

COLOCALIZATION OF CALCIUM-BINDING PROTEINS, VC1.1-IR MEMBRANE PROTEIN, AND NEUROFILAMENT PROTEIN (SMI-32) IN NEURONS OF THE BASOLATERAL AMYGDALA. A.J. McDonald. Dept. of Anatomy, Cell Biology, and Neurosciences, Univ. of South Carolina School of Medicine, Columbia, SC 29208.

This study utilized two-color immunoperoxidase techniques to characterize neurons in the rat basal amygdaloid nucleus (ABN) that exhibited immunoreactivity to calbindin (CB+), parvalbumin (PV+), VC1.1-IR membrane protein (VC+), and nonphosphorylated neurofilament protein (SMI32+) antibodies. Previous studies in this laboratory have indicated that CB and PV are extensively colocalized in ABN and constitute a subpopulation of nonpyramidal GABAergic interneurons. The present study demonstrated that virtually all VC+ neurons were PV+, and about 90% of VC+ neurons were CB+. These VC+ neurons, which comprised almost the entire population of large CB+ and PV+ cells, constituted about 1/3 of all PV+ and CB+ neurons. Virtually all somata with moderate to intense SMI32 immunoreactivity were also PV+ or CB+. In addition, there was almost 100% colocalization of VC and SMI32 immunoreactivity. These studies indicate that there is a unique subpopulation of large PV+/CB+ GABAergic interneurons in ABN that constitute virtually the entire population of VC+ and SMI32+ neurons. The functional significance of this colocalization pattern, as well as the role of these neurons in the microcircuitry of ABN, remains to be determined. Supported by NIH Grant NS19733.

189.6

DISTRIBUTION OF NADPH-DIAPHORASE POSITIVE CELLS AND FIBERS IN THE MONKEY AMYGDALA. A. Pitkänen and D.G. Amaral. Salk Institute, La Jolla, CA 92037.

The NADPH-diaphorase (NADPH-d) enzyme histochemical method stains a selective population of neurons in the central nervous system. Although the functional significance of these cells is unknown, they are of interest because they are selectively spared in Huntington's disease and they also seem to be resistant to NMDA mediated neurotoxicity. In the present study we describe the distribution of NADPH-d positive cells and fibers in the monkey amygdaloid complex. Based on the intensity of somal staining, three classes of NADPH-d positive cell types were described. *Type 1 cells*, which were the most intensely stained NADPH-d positive cells in the monkey amygdala, were of different morphological types: pyramidal, modified pyramidal, fusiform or stellate. They were most commonly found in the accessory basal nucleus, basal nucleus, lateral nucleus and in the nucleus of the lateral olfactory tract. The intermediately stained *Type 2 cells*, that were mostly stellate in shape, were the most common. NADPH-d positive cells in the monkey amygdala. They were found in the lateral, basal and accessory basal nuclei. The oval or round, lightly stained *Type 3* cells were mainly found in the medial, anterior cortical and paralaminar nuclei. The highest densities of NADPH-d positive *fibers* were found in the lateral nucleus, parvicellular portion of the accessory basal nucleus and in the anterior amygdaloid area. The lowest densities of NADPH-d positive fibers were found in the amygdalohippocampal area, in the lateral part of the central nucleus and in the intercalated nuclei. In some regions, such as the amygdalohippocampal area, the NADPH-d staining was helpful in establishing nuclear boundaries.

189.7

γ-AMINOBUTYRIC ACID(GABA) SYSTEMS IN THE RAT CENTRAL AMYGDALOID NUCLEUS N. Sun and M. D. Cassell. Department of Anatomy, University of Iowa, Iowa City, IA 52242.

The GABAergic structures of the rat central amygdaloid nucleus(Ce), their co-existence with met-enkephalin, and the possible source of the GABAergic terminals and fibers in Ce have been investigated using immunocytochemical detection of GABA, GAD and enkephalin. Antisera to GABA and GAD revealed a differential distribution pattern of GABAergic immunoreactivity in Ce. Most GABA- and GAD-positive perikarya in Ce were located in the lateral(CeL) subdivision, with some cells in the lateral capsular(CeLC), intermediate(CeI) and caudal part of medial(CeM) subdivisions. Only a few labeled perikarya were observed in rostral CeM. Morphologically these GABAergic neurons resemble the medium-sized neurons of Ce. The distribution pattern of GABAergic neurons in Ce overlaps with enkephalin-positive neurons. Double-labeling indicated that 70-80% of GABA-positive perikarya in Ce are also immunoreactive to met-enkephalin. The staining pattern of GABA- and GAD-positive neuropil did not match the distribution of GABA- and GAD-positive perikarya, as the intensity of GABAergic terminals and fibers was highest in caudal CeM, moderate in rostral CeM, CeLC and CeI, and lowest in CeL. Lesioning the stria terminals resulted in a small decrease in GABAergic immunoreactivity in Ce but a large decrease in the lateral bed nucleus of the stria terminalis(BNST). The result suggests that a complex GABAergic system exists in the Ce, with GABAergic neurons in Ce providing major projections to both the BNST and Ce. The co-existence of GABA with enkephalin in these neurons suggests that both transmitters may be involved in this intrinsic circuit. Supported by NS25139.

189.8

AMYGDALOID CRF NEURONS ARE INNERVATED BY CGRP TERMINALS. E. Harrigan*, D.J. Magnuson and T.S. Gray. Dept. Cell Biology, Neurobiology and Anatomy, Loyola Stritch Sch. of Med, Maywood, Il. 60153

The central amygdala contains a high density and number of CRF neurons and calcitonin gene-related peptide (CGRP) containing terminals. The possibility that CGRP terminals innervate CRF cell bodies in this region was investigated using a double-labeling immunohistochemical technique. The subjects of the study were Long-Evans, black-hooded male rats. Animals were overdosed with sodium pentobarbital and their brains were fixed using 4% paraformaldehyde. The procedure involved application of the nickel intensified DAB staining of CGRP followed by normal brown DAB immunostaining of CRF. This resulted in distinct black punctuate reaction product associated with CGRP terminals and brown reaction product associated with CRF cell bodies.

CRF labeling was primarily localized in the lateral part of the central nucleus. CGRP terminals were densely distributed in the lateral and lateral capsular subdivisions. More than 50% of the CRF cell bodies were innervated by CGRP terminals as judged by the imperceptible distances between CGRP terminals and CRF cell bodies. Thus, there is a significant innervation of CRF neurons in the central amygdala by CGRP terminals. Previous studies have demonstrated the parabrachial nucleus is the source of these terminals and that CRF neurons are projecting to brainstem autonomic neurons. CGRP injected into the central amygdala produces increases in heart rate, blood pressure and plasma catecholamines. Since the effects of central administration of CRF is similar CGRP in the amygdala, the effects of CGRP could be mediated by activation of CRF neurons in the amygdala. The anatomical pathways would likely involve an interaction between the parabrachial nucleus neurons and amygdaloid CRF brainstem projection neurons. Supported by NIH NS 20041.

189.9

AMYGDALA DIRECTLY INNERVATES CRF NEURONS IN THE LATERAL HYPOTHALAMUS. D.J. Magnuson and T.S. Gray, Dept. of Cell Biology, Neurobiology and Anatomy, Loyola University Stritch Sch. of Med, Maywood, Il. 60153

The central amygdala (Ce) projects heavily into the lateral hypothalamus. Numerous CRF immunoreactive neurons are located in this region. The present study investigated the possibility that amygdaloid terminals innervate CRF expressing neurons of the lateral hypothalamus.

Long-Evans, black-hooded male rats were the subjects of the study. The animals were injected with *Phaseolus vulgaris* leucoagglutinin lectin (PHA-L) tracer and allowed to survive for 10 to 16 days. Animals were overdosed with sodium pentobarbital and their brains were fixed using 4% paraformaldehyde. Tissue was processed using brown DAB staining of PHA-L followed by glucose-oxidase blue immunostaining of CRF. This resulted in distinct brown punctuate reaction product associated with amygdaloid terminals and blue reaction product associated with CRF cell bodies.

Numerous amygdaloid terminals were found in the lateral hypothalamus including regions that contained CRF immunoreactive neurons. Approximately 70% of CRF neurons in the lateral hypothalamus were innervated after injections of PHA-L in the medial Ce. Fewer CRF neurons were innervated after PHA-L injections in the lateral Ce. Amygdaloid terminals were especially apparent surrounding the soma of CRF neurons.

Thus, the amygdala directly innervates CRF neurons in the lateral hypothalamus. Previous studies of have demonstrated that CRF lateral hypothalamic neurons innervate the central grey, parabrachial nucleus and dorsal vagal complex. The amygdala could influence autonomic and behavioral adaptations to threat or stressor through this pathway. Supported by NIH NS 20041.

189.10

PATTERNS OF INNERVATION OF THE CENTRAL NUCLEUS OF THE AMYGDALA AND THE BED NUCLEUS OF THE STRIA TERMINALIS IN RAT. E.L. Gustafson, G.M. Zhang* and P. Greengard. Lab. of Molecular and Cellular Neuroscience, The Rockefeller Univ., New York, NY 10021.

First messenger regulation of protein phosphorylation by cAMP-dependent mechanisms is an important component of neuronal function in the mammalian CNS. Dopamine, acting via cAMP, causes an increase in the phosphorylation of the phosphoprotein DARPP-32. While the striatum is the most prominant brain structure containing dopamine and DARPP-32, the bed nucleus of the stria terminalis (BST) and the central nucleus of the amygdala (CeA) also contain both substances. The BST and CeA are closely related structures which share many hodological and neurochemical characteristics. The purpose of the present study was to determine if the BST and CeA are innervated by common dopaminergic axons which bifurcate, or if the cells of origin comprise separate populations. This was accomplished by combining the retrograde transport of two tracers, gold-labeled WGA-apoHRP and fluorogold or rhodamine beads, with tyrosine hydroxylase immunocytochemistry. Preliminary observations indicate that there are three projection patterns of dopaminergic neurons which innervate the BST and CeA. One population innervates only the BST, another only the CeA, and a third, much smaller population innervates both areas. The majority of the retrogradely labeled dopaminergic neurons are found in the lateral substantia nigra, and in the ventral tegmental area. While further studies are clearly needed, these results indicate that the BST and CeA share some common dopaminergic inputs, which may result in common phosphorylation events if dopamine is released simultaneously in both brain areas.

189.11

A COMPARISON OF THE EFFERENTS OF THE BED NUCLEUS OF THE STRIA TERMINALIS AND MEDIAL AMYGDALOID NUCLEUS. H.A. Al-Shamma, S. Numan*, N.A. Bullock*, and G.J. De Vries. Prog. in Neurosci. and Behav., Univ. of Mass., Amherst.

The bed nucleus of the stria terminalis (BST) and medial amygdaloid nucleus (MA) supposedly evolved from one continuous area, which was separated during evolution by the emergence of the internal capsule (Alheid GF, Heimer L, *Neurosci.* 27:1 '88). The BST and MA still share many characteristics such as neurotransmitter content, cell morphology, and steroid sensitivity. Presumably differences in the connections of these nuclei underlie differences in their functions. We compared the efferents of these two nuclei focussing on the areas that contain steroid-sensitive vasopressin neurons, using the anterograde tracer *Phaseolus vulgaris* leucoagglutinin. In general, we confirmed earlier studies showing that the efferents of the MA were concentrated in the preoptic and premammillary areas, while those of the BST were more widely distributed. MA and BST efferents overlapped in some of the areas that contain steroid-sensitive vasopressin innervation such as the perimeter of the diagonal band of Broca. In other areas these efferents did not overlap. For example, the caudal part of the lateral septum receives a dense input from the BST but not from the MA. These differences suggest that the MA and BST contribute differently to the steroid-sensitive vasopressin innervation of the brain.

189.12

INTRINSIC AMYGDALOID PROJECTIONS OF THE LATERAL AMYGDALOID NUCLEUS. C.R. Farb, G. Go*, and J.E. LeDoux. Center for Neural Science, New York University, New York, NY 10003.

The lateral nucleus of the amygdala (AL) receives efferent projections from sensory processing areas in the thalamus and neocortex and is the main channel through which sensory information accesses the emotional and mnemonic functions of the amygdala. While these extrinsic inputs to AL are well-characterized, the intrinsic amygdaloid connections of AL are poorly understood. The purpose of the present study was to examine projections from AL to other amygdaloid areas using modern tracing techniques that allow the production of injection sites restricted to small brain areas. Of particular interest were pathways by which inputs might be relayed from AL to the central amygdaloid nucleus. Pha-L or biocytin was iontophoretically ejected into AL and anterograde transport was examined. In several cases injections were confined within the cytoarchitectural boundaries of AL. Collectively, these produced anterograde terminal labeling in the central (ACE), basolateral, basomedial, intercalated, and cortical nuclei of the amygdala, as well as in the amygdalo-striatal transition area and the piriform cortex. The direct projection to ACE is strongest caudally, where most of ACE is filled with labeled processes. More rostrally, the projection is greatest in the ventral and lateral aspects of ACE. The basolateral and intercalated nuclei are known to project to ACE and thus constitute multisynapic intra-amygdala links between AL and ACE. Supported by MH 38774.

189.13

A SEROTONIN PROJECTION TO THE LATERAL AMYGDALOID NUCLEUS FROM THE DORSAL RAPHE NUCLEUS. N.M.Kheck, C.Farb, and J.E.LeDoux. Dept. of Biology and Center for Neural Science, New York University, New York, NY 10003.

The lateral nucleus of the amygdala is a critical link in the circuitry through which sensory information is endowed with emotional significance. The purpose of this study was to determine the neurochemical identity of brainstem afferent projections to the lateral amygdala. WGA-HRP was injected unilaterally into the lateral amygdala and histochemically visualized with tetramethylbenzidine (TMB) for single label studies; for double label studies the TMB reaction product was stabilized with diaminobenzidine (DAB) and cobalt acetate. Retrograde transport was seen in the thalamus, as previously reported (LeDoux et al, 1990), and in the dorsal raphe nucleus. Other brainstem areas were seldom labeled. An antibody specific to serotonin (Incstar) was used to establish whether the projection neurons in the dorsal raphe contain serotonin. Serotonin was visualized immunocytochemically with DAB, which is distinguishable from the TMB reacted WGA-HRP retrograde label, under light microscopy. Many of the cells containing the retrograde marker were also serotonin-immunoreactive. These findings demonstrate that the dorsal raphe is one of few brainstem areas that projects to the lateral amygdala and that many of the projection neurons contain serotonin. Thus, the serotonin innervation of the lateral amygdaloid nucleus arises at least in part from the dorsal raphe. Through these projections, serotonin may modulate the activity of the amygdala and thereby contribute to its emotional and memory functions. Supported by MH 38774.

189.14

SINGLE MEDIAL AMYGDALA NEURONS ARE MODULATED BY SYNAPTIC CONNECTIONS WITH BOTH THE OLFACTORY BULB AND THE VENTROMEDIAL HYPOTHALAMUS. M. Wong & R.L. Moss, Dept. of Physiology, Univ. Texas Southwestern Medical Center, Dallas, TX 75235

The medial amygdala (m-AMG) has direct reciprocal connections with both the accessory olfactory bulb (AOB) and the ventromedial hypothalamus (VMH). As the accessory olfactory system can modulate reproductive physiology, such as gonadotropin release and lordosis in the rat, the m-AMG may mediate the regulation of hypothalamic function by olfactory inputs. This study investigated the influence of stimulation of the olfactory bulb and VMH on electrical activity in the m-AMG, as well as the effect of iontophoretically-applied drugs. Extracellular single-unit recordings were obtained from urethane-anesthetized ovariectomized female rats (200-300g) with seven-barreled glass microelectrodes. Stimulating electrodes were placed in the VMH and either the AOB or the main olfactory bulb (MOB). AOB stimulation induced orthodromic excitatory (n=61, 21%) or inhibitory (n=194, 67%) responses in 255 (88%) of 288 m-AMG cells. The majority of m-AMG neurons responsive to AOB stimulation also showed orthodromic responses (n=228) to VMH stimulation. Furthermore, 14 other of these AOB-responsive cells were antidromically-activated by the VMH, showing that the AOB can influence m-AMG neurons which project directly to the VMH. While iontophoretic application of GABA inhibited 98% of m-AMG neurons, bicuculline could block some of the orthodromic inhibitory responses from AOB and VMH stimulation. In separate experiments, MOB stimulation also induced orthodromic excitation (39%) and inhibition (50%) in m-AMG neurons, but this orthodromic distribution was significantly different than that due to AOB stimulation. Our results indicate that individual m-AMG neurons can integrate and relay excitatory and inhibitory information directly between the AOB and VMH. Supported by NIH Grant MH47418.

189.15

SINGLE MEDIAL AMYGDALA NEURONS ARE MODULATED BY SYNAPTIC CONNECTIONS WITH THE VENTROMEDIAL HYPOTHALAMUS AND MIDBRAIN, ESTROGEN-PRIMING, AND LOCALLY-APPLIED NEUROTRANSMITTERS. R.L. Moss & M. Wong, Dept. of Physiology, Univ. Texas Southwestern Medical Center, Dallas, TX 75235

The medial amygdala (m-AMG) is an estrogen-receptor-containing region, which can modulate hypothalamic-controlled reproductive function, such as gonadotropin release and lordosis in the rat. This study investigated the influence of stimulation of two structures implicated in sexual behavior, the ventromedial hypothalamus (VMH) and midbrain central gray (MCG), on electrical activity in the m-AMG, as well as the effect of iontophoretically-applied drugs and estrogen-priming. Extracellular single-unit recordings were obtained from urethane-anesthetized ovariectomized female rats (200-300g) with seven-barreled glass microelectrodes. Stimulating electrodes were placed in the VMH and MCG. VMH stimulation elicited excitatory (27%) or inhibitory (45%) orthodromic responses in 72% and antidromic responses in 7% of a total of 221 m-AMG neurons, whereas MCG stimulation induced only orthodromic responses in 43% (19% excitatory, 24% inhibitory). Most cells that were responsive to MCG stimulation also showed orthodromic responses to VMH stimulation in the same direction. Lesioning of the VMH in 4 out of 7 cases eliminated the orthodromic response from the MCG, indicating that the MCG influences the m-AMG at least partially via the VMH. m-AMG neurons were largely excited by iontophoretically-applied glutamate and ACh and inhibited by GABA. Estrogen-priming increased the spontaneous firing rate and the responsiveness to ACh of m-AMG cells. The results of this study suggest that the m-AMG may mediate synaptic and hormonal signals relating to hypothalamic and brainstem control of reproductive behavior. Supported by NIH Grant MH47418.

189.16

ACOUSTIC RESPONSES OF SINGLE NEURONS IN THE LATERAL NUCLEUS OF THE AMYGDALA IN BEHAVING AND ANESTHETIZED RATS. F. Bordi, C. Pavlides, C. Clugnet, and J.E. LeDoux. Center for Neural Sci., New York Univ., New York, NY 10003.

The lateral nucleus of the amygdala (AL) receives inputs from sensory processing areas in the neocortex and thalamus. In order to understand the contribution of these sensory inputs to emotional and memory functions of the amygdala, we have studied the activity of single neurons in AL in freely behaving and anesthetized animals in response to acoustic stimuli. Responses elicited by broad spectrum auditory stimuli (white noise, clicks) were recorded from AL and, for comparison, from the ventral division of the medial geniculate body (MGV) of anesthetized rats. AL units responded with short latencies (15-20 ms) as well as longer latencies (100-150 ms). The threshold for activating these neurons was in the 60-80 dB SPL range. Some of the short-latency response units showed frequency preferences. MGV neurons also responded with short (9-12 ms) and long (100-150 ms) latencies but to much lower intensities and with much stronger frequency preferences than AL neurons. In freely moving animals neurons recorded in AL from implanted microwires responded to broadband acoustic stimuli with short (20-25 ms) and long (100-150 ms) latencies. Spontaneous firing was higher and a greater number of neurons responded to acoustic stimuli in the awake than in the acute AL recordings. In conclusion, acoustic response properties (latencies, breadth of tuning) of AL neurons suggest that they are outputs of auditory processing areas. It remains to be determined exactly how the amygdala converts these auditory signals into emotional and mnemonic representations. Supported by MH38774.

189.17

NEURONAL RESPONSES OF THE MONKEY AMYGDALOID COMPLEX TO DYNAMIC VISUAL STIMULI. F.K.Nahm, T.D.Albright and D.G. Amaral UCSD Group in Neurosciences, La Jolla, CA 92093; The Salk Institute, La Jolla, CA 92037.

The amygdaloid complex has been implicated in the processing of affective information which is an important attribute of complex visual stimuli such as faces. For non-human primates in particular, accurate assessment of facial gestures is critical for normal social interaction. Lesions of the amygdala are known to severely disrupt social behavioral patterns. Developing theories concerning the role of the amygdala necessitates determining which characteristics of complex stimuli are particularly salient for altering amygdaloid neuronal activity. To this end, we have developed a strategy whereby neuronal responses of the monkey amygdala to realistic, dynamic visual stimuli can be studied.

In these studies, single units were recorded in the amygdala of the awake, behaving monkey (*Macaca mulatta*). Two main classes of stimuli were presented: 1) static and dynamic complex images; 2) real-time video footage of conspecific facial expressions. All stimuli were stored on optical laser discs and presented over a computer controlled high-resolution video monitor.

Presentation of the stimuli described above revealed substantial variability in visual responsivity. Baseline firing rate varied considerably across cells, and both excitatory and inhibitory responses were observed. The majority of neurons exhibited differential responses to both static and dynamic stimuli. In several cases, responses to monkey facial expressions were significantly above that of non-monkey images; at times exhibiting selectivity for monkey identity and/or expression. Taken together, these data confirm earlier findings that amygdaloid neurons are highly sensitive to visual stimuli. As more data are accumulated, we hope to determine whether the affective quality of visual stimuli is also relevant to visually responsive neurons in the amygdala. (FKN supported by BNS-9013202 and PHS-5-T32-MH18398-04)

189.18

EFFECTS OF LESIONS IN AND AROUND THE ROSTRAL CENTRAL AMYGDALOID NUCLEUS OF THE RAT ON DRINKING PASSIVE AVOIDANCE. G.D. Coover. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

Electrolytic lesions of the rostral half of the central amygdaloid nucleus (ACE) produce a marked deficit in drinking passive avoidance (dPA) that appears to be due to a reduction in fear and conflict, an "anxiolytic" effect (Coover, Murison and Jellestad, Soc. Neurosci. Abstr., 15:1251, 1989). Small electrolytic lesions were placed in 11 locations in and around ACE to better localize the neurons whose damage produces this deficit.

Lesions of the rostral third of ACE produced a marked deficit in dPA whether they extended dorsally into globus pallidus (rACE+gp) or ventrally into the basomedial nucleus of the amygdala (rACE+bm). These groups required 43 ±2 (±SE) or 45 ±2 footshocks of incrementing intensity to avoid the water spout for 5 min, compared to 20 ±1 for the control group. In contrast, intermediate deficits were produced by lesions placed .7 mm away from rACE+gp rostrally (32 ±4), laterally (33 ±2), caudally (32 ±3) or medially (36 ±5), and also by lesions placed 1.0 mm dorsal to rACE+gp (34 ±3). Lesions did not produce a deficit in dPA when in the caudal third of ACE (24 ± 4), .7 mm dorsal to the middle third of ACE (24 ±3) or .7 mm ventral to either the middle third (26 ±5) or caudal third (23 ±2) of ACE.

The rostral third of ACE continues to stand out as the most dramatic site for lesion-induced anxiolysis.

BRAIN METABOLISM AND BLOOD FLOW II

190.1

RESIDUAL EFFECTS OF TRACER IN SEQUENTIAL DOUBLE LABEL DEOXYGLUCOSE (DG) STUDIES. M.J. Lyon, C.B. Smith, B.W. Agranoff and L. Sokoloff, Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20892.

In DG studies in which there is a high level of variability among experimental animals, it would be advantageous to be able to measure local metabolic rates ($LCMR_{glc}$) twice in the same animal, i.e. during control and stimulated periods or during two periods with different stimuli. Studies employing sequential double label approaches have been qualitative and/or have not adequately accounted for incorporation of the first label during the second experimental period. The purpose of this study was to quantify in rats the degree of incorporation of residual first label during the second experimental period while holding the total duration of the experiment close to the establish optimum of 45 min. In ten barbiturate-anesthetized rats, the proximal portion of the transected right sciatic nerve was placed on a bipolar electrode and stimulated with 2 msec, 15 Hz, 200-400 μA pulses. The stimulus was continuously monitored and maintained for 25 min beginning either at the time of ^{14}C-DG injection or 25 min later. Each rat was killed 50 min after tracer injection. $LCMR_{glc}$ was determined in the medial regions of laminae 2-6 in the dorsal horns of the lumbar spinal cord. When the stimulus is applied during the first or second 25 min after tracer injection, $LCMR_{glc}$ in the dorsal horn ipsilateral to the stimulus was 190% and 150% of the contralateral side, respectively. In an attempt to decrease the body burden of free ^{14}C-DG, an exchange transfusion with fresh blood was carried out at a rate of 4 ml/min starting 10 min after isotope injection and lasting 10 min. In these experiments, the effect of stimulation during the second 25 min was to increase $LCMR_{glc}$ in the ipsilateral dorsal horn to 140% of the contralateral side. These results show that stimulation during the second experimental period from 25-50 min still produces a significant effect on $LCMR_{glc}$ even following transfusion. Sequential double label methods must take into consideration this residual effect which tends to obscure possible differences in $LCMR_{glc}$ between two states.

190.2

ELEVATION OF LOCAL CEREBRAL GLUCOSE UTILIZATION IN SPONTANEOUSLY HYPERTENSIVE RATS BY UNILATERAL SUPERIOR CERVICAL GANGLIONECTOMY. L.Wei*, S-Z.Lin, V.Acuff*, T.Otsuka*, C.Patlak* and J. Fenstermacher. Department of Neurological Surgery, SUNY at Stony Brook, NY 11794

Hyperactivity, cerebral atrophy, and lowered local cerebral glucose utilization (LCGU) have been reported in adult spontaneously hypertensive rats (SHR). The hypertension of SHR has been postulated to rise in part from increased sympathetic nervous system activity. To test this hypothesis, we measured LCGU six months after unilateral removal of the superior cervical ganglion (uGX) from infant SHR and Wistar-Kyoto (WKY) rats (the normotensive control for SHR). Age-matched intact (control) SHR and WKY were also studied. LCGU was measured by the 2-deoxyglucose technique; tissue radioactivity was measured by quantitative autoradiography. Blood pressure was unaffected by uGX. In 7-month-old rats, LCGU in most gray matter areas was lower in control SHR than WKY; the differences were significant ($p < 0.05$) in 16 of 28 gray matter areas. Six months after ganglionectomy, LCGU was slightly higher in some areas of WKY and significantly higher ($p<0.05$) in most areas of SHR relative to their respective controls. Rates of LCGU were similar in uGX-WKY and uGX-SHR. The wide-spread effects of uGX on adult SHR suggest that neural activity was increased throughout the brain of SHR by unilateral ganglionectomy during infancy.

190.3

PARALLEL REDUCED CEREBRAL GLUCOSE UTILIZATION IN GABAERGIC NIGRAL EFFERENT TARGET REGIONS IN 2 RAT MODELS FOR TARDIVE DYSKINESIA. F.I. Tarazi, S.E. Bachus, O. Shirakawa, M. Gottschalk , C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228.

Vacuous chewing movements, proposed as an animal model of tardive dyskinesia, can be induced in rats either by chronic neuroleptic treatment, or by bilateral intranigral microinfusion of isoniazid[1]. Reduced outflow in GABAergic nigral efferents has been hypothesized in dyskinesias[2]. Thus, we have examined here whether an alteration in regional cerebral glucose utilization (rCMRglu) is produced in the nigrotegmental and nigrotectal target regions in rats treated with either 6-12 months of haloperidol (1.5 mg/kg/day po; n=17) or water (n=11); or with bilateral intranigral microinfusion of isoniazid (1.02 micromoles/1 microliter/8 min; n=6) or saline (2.04 osmol n=6) 20 min. prior to [^{14}C]-2-deoxyglucose administration[3]. rCMRglu was significantly reduced ($p<.001$) in both the nigrotegmental and nigrotectal target regions in isoniazid-treated rats relative to saline controls (to 72% and 77%, respectively), but did not differ in control regions. Similarly, in chronic haloperidol-treated rats, local cerebral glucose utilization was decreased in the nigrotegmental target region ($p,.025$), to 81% of control values. These results both strengthen the rationale for using acute intranigral isoniazid treatment as an animal model for tardive dyskinesia, and further implicate alterations in GABAergic nigral efferent projections in vacuous chewing movements in rats, and by analogy, in tardive dyskinesia.

[1]Gunne, Bachus & Gale, Exp. Neurol. 100:459, 1988. [2]Thaker et al., Arch Gen Psychiat 44:522, 1987. [3]Tamminga et al., Synapse 1:497, 1987.

190.4

NEOCORTICAL METABOLIC RESPONSES TO CHOLINERGIC AND NON-CHOLINERGIC DRUGS ARE NOT MODIFIED BY PRIOR UNILATERAL ABLATION OF THE NUCLEUS BASALIS MAGNOCELLULARIS IN RAT. E. De Micheli, D.M. Larson*, H.W. Holloway*, V. Lamour, T.T. Soncrant. Unit on Pharmacology and Pharmacokinetics, Lab. Neurosci., NIA, NIH, Bethesda, MD 20892.

Unilateral ablation of the nucleus basalis magnocellularis (NBM), which reduces cholinergic inputs to the ipsilateral frontoparietal neocortex, transiently depresses regional cerebral metabolic rates for glucose (rCMRglc) in denervated cortical areas, but rCMRglc recovers within 2 wk. To determine the possible effect of cholinergic deafferentation on the responsivity of other transmitter systems, we studied rCMRglc responses to cholinergic, serotonergic and dopaminergic drugs in rats after unilateral NBM ablation. NBM lesions were made in young rats by stereotaxic ibotenate injection. Two wk later, arecoline, physostigmine, nicotine, DOI, m-chlorophenylpiperazine, haloperidol or apomorphine was administered, and rCMRglc was measured with the autoradiographic [14C]deoxyglucose method. No significant left-right rCMRglc asymmetries were found in any drug-treated group as compared to saline controls. rCMRglc effects of all drugs were similar to those found in unlesioned rats. Hence, a reduction in cortical cholinergic inputs (as occurs early in Alzheimer's disease) does not by itself alter the responsivity of cholinergic, dopaminergic or serotonergic neocortical mechanisms.

190.5

REGIONAL CEREBRAL BLOOD FLOW RESPONSES TO ELECTRICAL STIMULATION OF THE DORSAL RAPHE NUCLEUS IN THE RAT.

M.J. Bakalian, M.D. Underwood, V. Arango, and J.J. Mann. Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh 15213.

We have previously demonstrated both an increase and decrease in cortical blood flow (CrtBF) in response to electrical stimulation of the dorsal raphé nucleus (DRN) using laser doppler flowmetry (LDF) (Neurosci. Abstr. *16*:290, 1990). We sought to determine whether the changes in blood flow are restricted to cerebral cortex or widespread throughout the brain.

Rats were anaesthetized (α-chloralose), paralyzed and artificially ventilated. Arterial pressure, heart rate and blood gases were continuously monitored and regulated. The DRN was stimulated electrically (1 s on/1 s off, 200 Hz, 100 μA) and the resulting increase in arterial pressure was controlled by exsanguination. Regional cerebral blood flow (rCBF) was measured in 13 dissected brain regions using ^{14}C-iodoantipyrine (IAP) as tracer. Local CrtBF was monitored using LDF with the probe placed extradurally over the parietal cortex. The stimulation sites were verified histologically.

In unstimulated controls (n = 6), rCBF varied significantly across brain regions ($p < 0.05$) and ranged from 86 ± 11 ml/min/100g in parietal cortex to 51 ± 3 ml/min/100g in corpus callosum. Based on CrtBF responses to DRN stimulation, animals were divided into 2 groups: increased (n = 6) and unchanged or decreased (n = 7) CrtBF. Changes observed in rCBF were in the same direction as the change in CrtBF in 12 of 13 brain regions with the exception of medulla. Increases in rCBF ranged from 49% in parietal cortex to 11% in cerebellum. Decreased rCBF ranged from 25% in hippocampus to 9% in pons. The magnitude of the increase in rCBF did not correlate with that of the decrease in rCBF across brain regions ($r = 0.14$, $p > 0.05$).

We conclude that stimulation of functionally distinct regions of DRN results in either increased or decreased cerebral blood flow across multiple brain regions. The different degree of rCBF changes in the two responses across brain regions suggest the involvement of different effector mechanisms. (Supported by MH46745.)

190.6

THE REGIONAL DISTRIBUTION OF CEREBRAL GLUCOSE UTILIZATION DURING THE "RUSH" PERIOD FOLLOWING COCAINE ADMINISTRATION. A.E. Carnazzola*, M.R. DeJoseph*, D.W. McCandless* and R.A. Hawkins, UHS/The Chicago Medical School, North Chicago, IL, 60064.

The most prominent clinical effect of cocaine on mood is that of euphoria, resulting from the prolonged stimulating action of the neurotransmitter dopamine. The action of dopamine is enhanced by cocaine's ability to block the reuptake of the neurotransmitter. This augmented dopaminergic activity has been seen most prominently in the nucleus accumbens. In the present study we also noted a significant increase in the energy metabolism of the nucleus accumbens. Since energy metabolism of the brain is tightly coupled to its function, we used radiolabeled glucose as a means of identifying neuroanatomical sites that were stimulated or depressed by the administration of cocaine. Comparisons were then made between cerebral glucose utilization (CMR_{Gluc}) in the "rush" phase and controls, and also between the "rush" phase and the steady "high" state. We used three groups (Control, 2.5 and 10 mg/kg cocaine) of male, Long Evans rats which ranged between 270-320 grams. Cocaine was administered by intravenous injection, as this is a common route of administration among cocaine-abusing humans. We observed that the CMR_{Gluc} of the "rush" period differs from that of the steady "high" state. Whole brain glucose utilization in rats given 2.5 and 10 mg/kg cocaine was shown to increase by 53% and 47%, respectively, above control levels. This increase also represented a rise in CMR_{Gluc} above the corresponding steady state levels of 11% and 17%, respectively. All brain regions examined demonstrated an increase in the CMR_{Gluc}, with the greatest increases occurring in certain components of the olfactory, extrapyramidal motor and cholinergic pathways.

190.7

EFFECTS OF SUMATRIPTAN ON AFFERENT AND EFFERENT MECHANISMS OF TRIGEMINAL SENSATION. G.A.Lambert*, J.Michalicek*, D. Tan*, H.Angus-Leppan and P.Boers* Institute of Neurological Sciences, Prince Henry Hospital and University of New South Wales, Little Bay 2036 Australia.

The antimigraine drug sumatriptan was tested for its effects on trigeminal sensation and craniovascular control. In cats anesthetised with chloralose, carotid blood flows were measured with electromagnetic flow probes and cortical microcirculation with laser Doppler flow probes. Each trigeminal ganglion was stimulated electrically. One trigeminal root was sectioned through a craniotomy made through the petrous temporal bone. Cortical circulatory mechanisms were left intact by inserting electrodes under additional halothane anesthesia. Cortical circulatory integrity was tested by subjecting animals to 5% inhaled CO_2. Single unit responses of cervical spinal cord units receiving input from the superior sagittal sinus were studied before and after intravenous injection or iontophoretic application of sumatriptan. Stimulation of the intact trigeminal pathway produced falls in blood pressure and increases in ipsilateral carotid blood flow (+60% at 2 sec^{-1}) as previously reported ("reflex response") but only small and variable effects (-7% at 2 sec^{-1}) on cortical blood flow. Stimulation of the trigeminal ganglion on the lesioned side did not produce changes in blood pressure or carotid flows and only small changes in cortical flows ("antidromic response"). Sumatriptan (100 μg/kg iv) produced a transient biphasic change in blood pressure (+16/-14 mmHg), a prolonged decrease in carotid blood flow (-20%) and a transient increase in cortical flow (+10%). Sumatriptan had no significant effect on responses to trigeminal stimulation except for a slight potentiation of the antidromic dilator response in the cortical circulation. Iontophoretic application of sumatriptan (40 nA) reduced or abolished the response of approximately one third of all units responding to electrical stimulation of the superior sagittal sinus, an effect comparable to the ergot antimigraine drugs. We conclude that pain in the trigeminal system produces changes in cranial blood flow largely through reflex mechanisms and that sumatriptan reduces cranial blood flow without affecting cortical microcirculation.

190.8

WISCONSIN CARD SORT rCBF ACTIVATION IN NORMAL SUBJECTS: DISTRIBUTION IMAGES VS COMPUTED rCBF VALUES. S. Marenco, R. Coppola, D.W. Jones, D.R. Weinberger. Clinical Brain Disorders Branch, NIMH Neuroscience Center at St. Elizabeth's Hospital, Washington DC 20032, USA.

The use of radioactivity distribution images for the analysis of regional cerebral blood flow (rCBF) studies has been advocated for PET studies to avoid arterial sampling. We applied the same concept to Xe-133 inhalation rCBF studies using dynamic single photon emission tomography. 10 normal subjects were studied in two conditions: during execution of the Wisconsin card sort (WCS) and of a sensorimotor control task. 3 slices (2 cm thick) 4, 6 and 8 cm. above and parallel to the CM line were acquired using a 16 mm (FWHM) collimator. rCBF values were computed (Kanno Lassen method) for ROIs defined with the aid of a template adaptable to the individual head size. Count rates for each ROI were obtained for each acquisition interval (first 1.5 minutes of examination [wash in] and one collection for each of the following 3 minutes of examination [wash out]) and for the sum of the four collections (SUM). The ROI count values were normalized to the total count rate in the slice.

Analysis of absolute rCBF values by multiple t-tests showed significant differences between WCS and BAR in two ROIs. 9 ROIs were significant when rCBF values were normalized by the global CBF of the slice. Count rates for the first collection period differed in 10 areas and among them the left dorsolateral prefrontal cortex, where activation was expected to occur, as demonstrated by several previous reports. The analysis of the SUM images yielded 12 areas of statistical significance, including the left dorsolateral prefrontal activation. It is therefore possible that count rate distribution analysis, based on more reliable information than rCBF values computed from only 4 time points, may be more accurate in detecting regional activation. Though the variance due to arterial input is ignored with this method, the assumption that this and other interfering factors (e.g. recirculation) should not differ between two back to back examinations is reasonable.

190.9

REGIONAL CEREBRAL BLOOD FLOW PRIOR TO, DURING, AND FOLLOWING RECOVERY SLEEP. A.R.Braun, T.J.Balkin*, N.J.Wesensten*, K.Berman, P.Baldwin*, S.Stein*, R.E.Carson*, G. Belenky and P. Herscovitch. NIDCD, NIMH and Dept. of Nuclear Medicine, NIH, Bethesda, MD 20892, and Dept. of Behavioral Biology, WRAIR, Washington, D.C. 20307.

Regional cerebral blood flow (rCBF) was measured with $H_2{}^{15}O$ positron emission tomography (PET) in 8 healthy male subjects (ages 21-32) after 24-48 hours of sleep deprivation (pre-sleep), during slow wave sleep (SWS), and following recovery (post-sleep). During each of these states, 30 mCi of $H_2{}^{15}O$ was administered i.v. by bolus infusion and PET scans were performed on a Scanditronix PC2048-15B tomograph (axial and in-plane resolution of 6.5 mm FWHM). Sleep stages were monitored by an experienced polysomnographer. Irregular regions of interest were placed on the reconstructed images, and data were normalized by dividing each pixel value by the mean global flow rate calculated for each scan. Normalized blood flow values were analyzed by repeated measures ANOVA. F-tests for the individual components of variation were used to determine differences between means, when appropriate, and are reported below ($p<.05$, each case).

Normalized rCBF rates were lower in SWS than in both sleep-deprived and post-sleep states in subcortical structures including cerebellum, pons and thalamus. In caudate and putamen, blood flow values did not differ significantly between SWS and sleep-deprived states, but were significantly increased following recovery sleep. On the other hand, in post-rolandic sensory (temporal and occipital) cortices, normalized blood flow rates in SWS exceeded those measured in either pre- or post-sleep states.

Previous PET studies of regional cerebral metabolism in sleep have utilized the fluorodeoxyglucose method which does not afford the temporal resolution possible with $H_2{}^{15}O$ or permit direct comparison with temporally associated pre- and post-sleep states. Although these results may be affected by the normalization process, SWS appears to be associated with differential, regionally specific changes in cerebral blood flow which differentiate it from both sleep-deprived and post-recovery states.

190.10

EFFECT OF NITRIC OXIDE SYNTHETASE INHIBITION ON CEREBRAL BLOOD FLOW (CBF) IN THE CONSCIOUS RAT. I.M. MACRAE, D.A. DAWSON*, J.L. REID*, J. McCULLOCH. Dept. of Medicine, University of Glasgow, G12 8QQ, U.K.

Nitric oxide is the major endothelium-derived relaxing factor of vascular smooth muscle. Inhibitors of the enzyme, nitric oxide synthetase (e.g. N^G-nitro-L-arginine methylester(L-NAME)) give rise to marked hypertension and bradycardia with differential effects on flow in peripheral vascular beds in the conscious rat. We have studied the effect of inhibition of nitric oxide synthesis on CBF.

L-NAME (30 mg/kg i.v.) elicited a significant hypertension (+34±2 mmHg) and bradycardia (-134±6 beats/min) in conscious lightly restrained, male, Sprague Dawley rats. The maximum response was reached within 3 min of administration and hypertension was maintained for the duration of the experiment. Regional CBF 15 mins post-drug was analysed in microdissected areas of brain following a ramped, 30 sec infusion of ^{14}C-iodoantipyrine. CBF was significantly reduced throughout the brain. Control flows in cerebellum, hippocampus, cortex and thalamus were 85±4, 71±3, 102±4 and 91±4 mls/100g/min, resp. This compares with flows following L-NAME of 56±2*, 44±2*, 63±4* and 65±3* mls/100g/min, resp. (*$p<0.001$, Dunnett's test). These results are consistent with a tonic involvement for nitric oxide in the control of CBF.

190.11

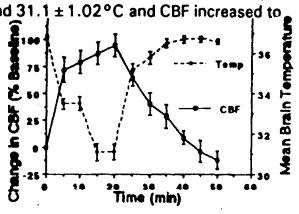
CEREBRAL HEMODYNAMICS IN THE CHRONIC HYDROCEPHALIC RAT. S.C. Kim, P. Tompkins*, P.A. Roberts,* D. Horton,* E. Bates* & M. Pollay. Division of Neurosurgery, University of Oklahoma and Veterans Administration Medical Center, Oklahoma City, OK 73190.

Because of an interest in changes in cerebral hemodynamics with pathologic conditions, a kaolin induced chronic hydrocephalus rat model was used to evaluate cerebral perfusion flow and cerebral blood volume. Hydrocephalus was induced by injection of kaolin into the cisterna magna of rats. Enlargement of the ventricles occured in 8-10 weeks. In one set of experiments hydrocephalic animals were shown to have significantly reduced cerebral perfusion flow (1.77 ml/min/gm control, 1.53 ml/min/gm hydrocephalic, $p<0.05$). In separate experiments, cerebral blood volume tended to be increased in chronic hydrocephalus (11.8 ul/gm) as compared to controls (10.1 ul/gm), although this increase was not significant ($p=0.08$). Since flow is equal to volume divided by transit time, it would appear that transit time through the cerebral circulation is increased in chronic hydrocephalus. Experiments in progress will simultaneously determine brain and choroid plexus blood flow, blood volume, and CSF production rates in a rabbit model of chronic hydrocephalus.

190.12

SELECTIVE BRAIN COOLING INCREASES CBF IN RATS. J Kuluz*, D Smith*, R Busto, MD Ginsberg, WD Dietrich, MYT Globus. Cerebral Vascular Disease Research Ctr, Univ of Miami, Miami, FL 33136.

Previous measurements of CBF during hypothermia have been done with total body cooling. Since body cooling decreases cardiac output and thus CBF, we measured CBF using laser doppler (LD) flowmetry during selective brain cooling while rectal temp was maintained at 37°C. Five adult male Wistar rats underwent eight 20-min periods of brain cooling. For the 1st ten min room temp air was blown across the head followed by liquid N_2 vapor for the next 10 min. CBF was measured by LD in the lateral parietal cortex. CBF was recorded continuously and the mean value for each 5-min period was compared to baseline. Brain temp was measured in the outer parietal cortex contralateral to the LD probe. Halothane was discontinued 45 min prior to brain cooling and rats received 2:1 N_2O:O_2 and pancuronuim for paralysis. $PaCO_2$ was 35-40 torr. Results: Air cooling caused a rapid decrease of brain temp to 33.5±0.26 and 33.5±0.34°C(mean±SEM), and an increase in CBF to 72.1±11.8 and 79.5±10.8% above baseline during the 1st two 5-min periods. With liquid N_2 brain temp fell to 31.1±1.19 and 31.1±1.02°C and CBF increased to 87.3±10.4 and 94.9±10.5% above baseline during the 2nd two 5-min periods. MAP and ICP did not change. Conclusion:Selective brain cooling acutely increases CBF in normal rats as measured by LD. If brain cooling increases CBF after ischemia this may explain its protective effects.



190.13

CARDIOVASCULAR CHANGES AFTER MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) IN RATS. A. D. Perez-Trepichio, J. L. Williams, and S. C. Jones. Department of Brain and Vascular Research, Cleveland Clinic Foundation, Cleveland, OH 44195.

MCAO changes autonomic function by affecting central neural mechanisms. Stroke patients exhibit increases in plasma catecholamines, cardiac arrythmias, and myocardial cell damage (*Stroke* 13: 838, 1982). In animals, MCAO causes autonomic changes acutely, but chronic autonomic changes have not been examined. In this study, we measured mean arterial pressure (MAP) and heart rate (HR) immediately and 24 hr after MCAO.

Sprague-Dawley rats were anesthetized (halothane in 70% N_2O-30% O_2) and ventilated mechanically. The left pterygopalatine artery was cauterized, and saline vehicle (Sham) or a silicone cylinder in saline (MCAO) was injected into the left external carotid artery retrogradely. After initial measurements of MAP and HR, we discontinued anesthesia. 24 hr after MCAO or sham treatment, the rat was reanesthetized. Measurements (means±SE) before (Control) and after treatment were:

	Control	10 min	90 min	24 hr
MAP, Sham	114±4 (8)	106±5 (8)	109±6 (8)	116±6 (8)
MAP, MCAO	112±2 (9)	127±4 (9)*	125±3 (9)*	116±7 (6)
HR, Sham	352±17 (8)	331±11 (8)	353±29 (5)	334±39 (8)
HR, MCAO	343±7 (9)	386±21 (7)*	412±31 (6)	365±11 (6)

* Different from Sham group, $P<0.05$. Number in parenthesis = n.

MCAO produced immediate increases in MAP and HR. 24 hours after MCAO, however, MAP and HR decreased to preocclusion levels and were not different from sham rats. Our findings suggest that by 24 hr after MCAO, changes in MAP and HR are compensated either centrally or peripherally to maintain MAP and HR at normal levels. (Supported by NIH NS24343)

190.14

IR THERMAL IMAGING OF A MONKEY'S HEAD: LOCAL TEMPERATURE CHANGES IN RESPONSE TO SOMATOSENSORY STIMULATION. J.S. George, J.D. Lewine, S.W. Moore* & E.R. Flynn.
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Physiological activation of small populations of cortical neurons is accompanied by changes in regional blood flow and metabolic activity that might produce measurable local temperature changes. Motivated by reports from a Soviet group (Gorbach *et al.* Thermology, 3:108-111,'89), we have begun to investigate the use of thermal imaging for mapping neural activity. An Inframetrics model 600 IR thermal video imager was positioned above the shaved head of an anesthetized macaque monkey. In the first experiment, we attempted to resolve the early timecourse of transient thermal responses to brief electrical depolarization of either the left median or tibial nerve. Stimuli were delivered at 30 second intervals. A sequence of 64 video frames (at 30 Hz) spanning each stimulus (16 pre- and 48 poststimulus) was collected in frame memory of an Imaging Technology video digitizer. Image sequences from 16 trials were summed in main memory. Groups of 16 sequential frames (~.5 sec) were summed, and the prestimulus composite image was subtracted from 3 poststimulus composite images. In a second experiment we attempted to image steady-state changes in temperature distribution associated with repetitive stimulation. A sequence of 64 prestimulus frames was collected to construct a baseline image. Electrical stimuli were then delivered at 5 second intervals for 2 minutes; two sequences of 64 frames were collected and stored. Stimulation was discontinued for 3 minutes, and a poststimulus baseline image was collected. Images in each sequence were averaged, and differences between control and stimulated average images were examined. Difference images for both steady-state and transient conditions demonstrated stimulus-induced warming at locations consistent with *a priori* knowledge of the topographic organization of somatosensory cortex. Although these experiments clearly established the necessity (and difficulty) of adequate thermal control, they also demonstrate the potential utility of thermal imaging techniques for noninvasive characterization of patterns of neural activity.

191.1

AGE AFFECTS EYEBLINK CONDITIONING AND RESPONSE DISCRIMINATION IN HUMANS. J.F. Disterhoft, S.W. Conroy*, L.T. Thompson, B.J. Naughton* & J.D.E. Gabrieli. Departments of CMS Biology, Medicine and Psychology, Northwestern University Medical School, Chicago, IL 60611.

Eyeblink conditioning was originally used by experimental psychologists as an objective paradigm to investigate laws of learning in humans. This simple learning task has been used extensively to investigate the neural substrates of eyeblink conditioning in animal models. We recently showed that trace blink conditioning is impaired in aging rabbits, and that the learning deficit is reversed with i.v. nimodipine (Deyo, et al., 1989). Since trace conditioning is hippocampally-dependent (Moyer et al., 1989), we hypothesize that nimodipine enhances hippocampal function in aging. CA1 pyramidal cell excitability is increased by nimodipine in a dose- and age-dependent fashion both *in vivo* (Thompson et al., 1990) and *in vitro* (Moyer et al., 1991). We are now beginning studies to determine if our behavioral data in the rabbit model can be generalized to the human.

Younger (N=5, mean age=24.6) and older (N=6, mean age=66) humans were trace eyeblink conditioned, with a differential reaction time task to one of two lights as a distractor. All Ss had normal hearing at 3 kHz; were within normal ranges on Benton Visual Retention Test, WMS-R Logical Memory & Paired Associates subtests, WAIS-R Vocabulary subtest, Mini-Mental Status Exam and Geriatric Depression Scale; were in good health on physical exams; and were nonsmokers. Older Ss showed significantly fewer eyeblink CRs in 50 training trials; no difference in UR amplitude was noted. Older Ss blinked more to both lights on the reaction time task. These blinks were apparently generalized CRs as they had similar latency, form, and amplitude as the blinks on tone-puff trials. These data suggest that older humans show reduced acquisition of this hippocampally-dependent learning task. Older Ss also exhibit less response discrimination between stimuli. (Supported by Miles Inc., Pharmaceutical Division).

191.2

EFFECTS OF AGING ON COGNITIVE, VISUOPERCEPTUAL, AND VISUOMOTOR SKILL LEARNING. J. Doyon[1,2], B. Laforce*[1], E. Léger*[1], D. Lacerte*[1], J. Roy*[1], L. Rousseau*[1], P.J. Bédard[2]. [1] École de Psychologie, [2] Centre de recherche en neurobiologie, Univ. Laval, Québec, Canada, G1J 1Z4.

There is now a well-developped body of empirical data in humans showing that normal aging affects secondary memory, leaving sensory, primary, and tertiary types of memory relatively intact (for reviews, see Craik, 1977; Poon, 1985). However, age-related effects on skill learning are still poorly documented. The main goal of the present study was thus to investigate the effects of aging by comparing the performance of young (Mean = 22 yrs.; SD = 2.7) and older healthy adults (Mean = 60 yrs.; SD = 6.3) on cognitive, visuoperceptual, and visuomotor skill learning tasks. A second objective was to explore the possibility of a dissociation between incremental learning capacities and declarative memory for the same stimuli in these two groups. Cognitive, visuoperceptual, and visuomotor skill learning was measured using versions of the Digital Logic Gates Test (Carlson et al., 1989, the Repeated Sequence Test (Nissen & Bullemer, 1987), and the Mirror reading Test (Cohen & Squire, 1980), respectively. These tasks were administered to all subjects on six occasions, once a week over a period of 6 weeks. Measures of declarative knowledge for the material presented during the skill learning tasks were also given either before and after (for the cognitive task) or only after completion of the learning tests. The results for the three tasks showed an equivalent improvement in performance over practice in the two groups, even though the younger subjects were significantly better than the older ones on the measures of declarative memory. These findings suggest that aging does not impair incremental skill learning.

191.3

RESPONSE BIAS IN GERIATRIC DEPRESSION WITH AND WITHOUT COGNITIVE IMPAIRMENT. R.C. Young, S. Mattis*, B. Kalayam*, B.S. Meyers*, and G.S. Alexopoulos*. New York Hospital-Cornell Medical Center, Westchester Division, White Plains, NY 10605.

State-dependent abnormalities in response bias reported in mixed-age depressives (Corwin et al, 1990) have not been investigated in the elderly. Patients (n=35) with major depression by DSM-III criteria treated in a geriatric psychiatry service were given a verbal learning task. Signal detection measures of immediate and delayed recognition memory (d') and response bias (beta and C) were obtained. Testing was performed before, and at two week intervals during, six weeks of pharmacotherapy with nortriptyline. Patients were retrospectively categorized as persistently impaired (n=11) or unimpaired (n=24) based on Dementia Rating Scale scores after optimal treatment. Hamilton Depression Rating Scale scores improved in both groups. Overall there was persistently poorer immediate and delayed recognition memory ($p<.001$ and $p<.03$), more liberal delayed beta ($p<.04$), and more liberal immediate and delayed C ($p<.001$ and $p<.05$) in the impaired group. With treatment, immediate beta and immediate C became more conservative in the impaired group ($p<.07$ and $p<.01$) but not in the unimpaired group. Treatment did not significantly change recognition memory in either group.
MH40726-01A2

191.4

AN AUTOMATED DELAYED NONMATCHING-TO-SAMPLE TASK TO ASSESS VISUAL RECOGNITION MEMORY IN HUMANS. C. Chavoix[1], C. Hagger[2], A. Sirigu*[3], M. Gravelle*[1], and T. Aigner[1]. Laboratory of Neuropsychology[1], NIMH, Bethesda, MD 20892; Clinical Brain Disorder Branch[2], NIMH, St. Elizabeth's Hospital, Washington, DC 20032; Cognitive Neuroscience Section[3], NINDS, Bethesda, MD 20892.

Delayed nonmatching-to-sample (DNMS) with trial-unique 3-dimensional objects is a task of choice to assess visual recognition memory in normal and brain-damaged monkeys. As a step toward validating tasks that measure memory abilities in both humans and monkeys, we developed a computer-automated version (aDNMS) of the classic DNMS (cDNMS) task and compared performance of normal humans on the aDNMS with that of normal rhesus monkeys on the cDNMS. Performance of patients with Alzheimer's disease or schizophrenia was also evaluated on the aDNMS. Two-dimensional nonverbal symbols, and nonverbal visual feedback, projected onto a color computer monitor fitted with a touch-sensitive screen were used in the aDNMS task. Memory performance was assessed in a session of 96 trials of randomized delays (3, 6, 9, 12, 18, and 60 sec) between sample and choice presentations, followed the next day by a session of progressively increasing list lengths (10 lists of 3 stimuli, 5 lists of 6, 3 of 10, and 2 of 15).

Performance of normal humans (n=10) on the aDNMS task did not differ significantly from that of normal monkeys (n=3) on the cDNMS task, in either delay or list length conditions. In addition, patients with Alzheimer's disease (n=3) or schizophrenia (n=5) were significantly poorer than the normal humans. A significant correlation was also found between average performance across all conditions on the aDNMS task and performance on the Wechsler memory test in humans. These results indicate that this automated DNMS is an appropriate test to compare mnemonic abilities in humans and monkeys, and may be useful in the investigation of the neuroanatomical and neurochemical substrates involved in amnesic disorders.

191.5

FUNCTIONAL CORRELATES OF ERP CHANGES OBSERVED DURING RECOGNITION MEMORY TESTING IN HUMANS. Michael E. Smith, Dept. of Psychology, Texas A&M University, College Station, TX 77843.

The scalp-recorded ERP discriminates between items (words) that are, and those that are not, recognized as episodically familiar. This discrimination is correlated with similar changes in hippocampal ERPs (Smith et al, '86, ECN) as well as in changes in the firing of hippocampal neurons (Heit et al, 88, Nature), and it is eliminated following lesions to the temporal lobe (Smith & Halgren, '89, JEP:LMC). However, its' functional interpretation remains unclear. Recognizing that an item has recently occurred can either be based on specific recollection of a study episode, or on some non-specific feeling of familiarity (e.g. Tulving, '89, EJCP). We performed an experiment designed to assess whether the memory-related ERP changes described above are more closely related to one or the other of these forms of recognition. Preliminary results suggest that the ERP discrimination is most closely related to specific memory for the study episode. Supported by NIH and the McDonnell-Pew Program in Cognitive Neuroscience.

191.6

NEURONAL ACTIVITY IN NONDOMINANT HUMAN LATERAL TEMPORAL CORTEX RELATED TO SHORT TERM SPATIAL MEMORY AND VISUOSPATIAL RECOGNITION. M.D. Holmes*, G.A. Ojemann, D.F. Cawthon, and E. Lettich*, Department of Neurological Surgery, University of Washington, Seattle, WA 98195

Extracellular microelectrode recordings from subsequently resected nondominant lateral temporal cortex, in patients undergoing craniotomies under local anesthesia for treatment of medically intractable epilepsy were obtained during performance of visually presented tasks of short term spatial memory and visuospatial recognition. Recordings from 5 populations, each reflecting activity predominantly in 1 neuron, in 2 patients, exhibited statistically significant relation to short term spatial memory or visuospatial recognition: 2 altered activity during spatial memory alone and 3 altered activity during both memory and some measures of recognition. Decreased neuronal activity was observed during the information input phase of spatial memory, and during face, line and complex figure matching tasks. Increased neuronal activity was observed during information retrieval phase of memory and during complex figure matching and recognition of facial expression. Thus, at least some areas of nondominant temporal cortex have a participatory role in spatial memory and visuospatial recognition.
(Supported by NIH Grants NS21724, NS17111, NS20482, and NS07289 and by the Horbach Fellowship).

191.7

IMPAIRED WORKING MEMORY IN UNMEDICATED PARKINSON'S PATIENTS. J. Singh, J.D.E. Gabrieli and C.G. Goetz. Department of Neurological Sciences, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612 and Department of Psychology, Northwestern University, Evanston, IL 60208.

Working memory (WM), defined as the storage of information while simultaneously processing the same or other information, has not been studied in patients with Parkinson's disease (PD). We hypothesized that WM is abnormal in PD because some aspects of WM have been linked to frontal-lobe functions and studies have found PD patients to be selectively impaired on measures of memory sensitive to frontal-lobe lesions. We administered two tests of WM to 5 medication-free, nondemented PD patient and 10 normal control (NC) subjects matched for mean age and education. The WM tests (Salthouse & Babcock,1990) demanded that the subjects answer verbal and arithmetic questions while simultaneously trying to remember the final word or number of a given question. Subjects also took tests of Vocabulary (WAIS-R), verbal Recognition, verbal Recall, Self-ordered Pointing, and Temporal Ordering (the last three are known to be impaired in patients with frontal-lobe lesions). The PD patients obtained significantly lower WM scores by all measures (span, number of trials, and number of items) than the NC group ($p < .01$). The same patients were unimpaired on the Vocabulary and verbal Recognition tests, but performed significantly worse than the NC group on the three tests of frontal-lobe memory capacities (verbal Recall, Self-ordered Pointing and Temporal Ordering tasks). These results suggest that WM involves an integrative frontal-subcortical system and raise the possibility that poor memory performance in PD may relate selectively to deficits in WM capacities. Supported by McDonnell-Pew Grant.

191.8

PRIMARY MEMORY AS STUDIED WITH FDG-PET IN NORMAL CONTROLS AND PATIENTS WITH FRONTAL LOBE EPILEPSY. *B.E.Swartz*[1,2,3], *M.Gee**[1,2], *M. Mandelkern**[2,4], *J.Fuster*[5]. 1.Cal-CEP 2.VAMC Wadsworth 3.UCLA Dept Neurology 4.UCI Dept Physics 5.UCLA Dept Psychiatry. Los Angeles, Cal 90073.

Primary memory has been linked to the prefrontal region in primates. To test whether this was also the case in humans a delayed sample matching task was administered to a group 8 normal adults. A task of immediate sample matching was also administered as a control for attention, and the FDG uptake in each was compared to a resting control scan in the same subject. We then analyzed 6 subjects with frontal lobe epilepsy in the same way to see if the activations induced by the protocol would differ in these two groups. 80 regions of interest were drawn on each scan and each regional value normalized to the mean of the whole brain glucose uptake prior to statistical analyses in an ANOVA framework.

The controls showed task induced increases in 6 frontal, 1 parietal and 2 temporal regions, with decreases seen in two subcortical regions ($p<.05$). The patients showed increases in only 1 frontal, 1 temporal, and 1 visual region. Differences between the controls and patients occurred under all three scan conditions. A difference with respect to orbital frontal cortex was noted between immediate and delayed sample matching tasks. 6 additional subjects are currently being analyzed. The data will be interpreted with respect to know neuronal circuits and theories of frontal lobe function.

191.9

AUTOBIOGRAPHICAL MEMORY IN NORMALS AND BRAIN-DAMAGED SUBJECTS. A. Sirigu,*[1] J. Grafman,[1] I. Litvan,*[2] and J. Toothman*[1] (Spons. J. Grafman). Cognitive Neuroscience Section, [1] Experimental Therapeutics Branch,[2] NINDS, NIH, Bethesda, MD 20892.

We investigated Autobiographical Memory (ABM) in 52 normal subjects (40 to 80 y.o.), 10 patients with dementia of Alzheimer type (DAT, mean age= 74 y.o.) and one patient with amnesia due to cerebral anoxia. In a complementary experiment we studied the effects of scopolamine on ABM in 6 healthy normal subjects. Each decade of the subjects' biography was assessed using a questionnaire that distinguished among four kinds of information: facts ("what"), people ("who"), places ("where") and dates ("when"). Results showed that (1) normal subjects in their eighties obtained lower overall scores than the other age groups. (2) Recall was best for facts and worst for dates. This effect was robust and was present in all age groups. (3) There was no consistent pattern of recall across decades in normals. (4) DAT patients were impaired relative to all normal groups and for all classes of information. Recall of ABMs in DAT patients was most affected for the middle decades, and least affected for the most remote and most recent ones ("primacy-recency" effect). (5) The amnesic patient showed an information-specific temporal gradient in ABM: recall of people's names and of dates was impaired for just the two decades preceding the onset of his illness, while recall of facts and their spatial context was preserved for all decades. (6) Scopolamine at dosage levels that impaired recall of recent information, did not affect ABM in any way. We conclude that: (1) ABM is not organized around a single unitary memory system but may depend on several distincts systems of representation. (2) ABM performances in DAT indicate an increased susceptiblity to interference effects analogous to those found in normal memory (e.g. proactive and retroactive). (3) Cholinergic blockade alone cannot account for the changes in ABM recall found in DAT patients.

191.10

INTACT PRIMING FOR PICTURE NAMING IN GLOBAL AMNESIA. C.I. Kreiser, S.L. Reminger*, M. Verfaellie*, and I.D.E. Gabrieli. Boston University Memory Disorders Research Center, Boston, MA 02130 and Department of Psychology, Northwestern University, Evanston, IL 60208.

Despite recall and recognition deficits, patients with global amnesia (GA) have shown preserved priming (facilitation in processing a repeated stimulus) with a variety of stimuli. To date, however, amnesics have not shown fully intact priming with pictures of common objects and animals. The subjects were 12 patients with GA (7 with Korsakoff's Syndrome and 5 with amnesia of other etiologies, including patient H.M.) and 14 controls. Subjects named 45 pictures. Then they named 90 pictures (45 repeated and 45 new); naming latencies were recorded. Finally, subjects performed a yes/no recognition test for the pictures seen for the first time in the second phase plus 45 foils. Patients with GA were impaired significantly on the recognition test; however, both groups showed equivalent priming by naming previously seen pictures significantly faster than new pictures. In a second study, normal subjects named pictures and then did either a naming or a yes/no recognition test. Within each test, half of the previously seen pictures were rotated 180° about the vertical axis and half appeared exactly as before. Latencies to identify previously seen pictures in the recognition test were slower for rotated pictures than for those not rotated. The effect of rotation upon picture-naming priming was minimal. The dissociation between intact picture-naming priming and impaired recognition memory in GA may reflect separable memory systems with distinct functions. One system records rotation-invariant properties of percepts useful for visual reidentification and another system records variant properties useful for recognizing specific encounters with percepts. Supported by NINDS grant #IP50NS26985.

191.11

EFFECTS OF SEVERE BRAIN INJURY IN CHILDREN ON EXPERIMENTAL COGNITIVE TASKS. H.S. LEVIN, K.Culhane, D. Mendelsohn, H. Harward, J.M. Fletcher, L. Ewing-Cobbs. Division of Neurosurgery, Univ. Texas Medical Branch, Galveston, TX 77550.

To investigate the long-term neurobehavioral effects of closed head injury (CHI) in children, we administered experimental tasks of cognition to 25 children who had sustained severe injuries and to 14 children who had mild/moderate injuries. Severity of CHI was defined according to the lowest post-resuscitation GCS score; severe CHI was defined as a GCS score of less than or equal to 8; mild/moderate injuries had GCS scores of greater than 8. Age at the time of study and age at injury were comparable in the severe (mean age at study=9.8 yrs, mean age at injury=7.7 yrs) and mild/moderate (means=8.7 and 7.3, respectively) groups. A comparison group of 38 uninjured controls was also tested (mean age=9.3 yrs). The tasks involved problem solving, planning, and concept formation. These tasks included the Tower of London, 20 Questions, verbal fluency, and the Go-No go test. General linear model analysis revealed a significant injury severity effect on several of these measures, including the Tower of London (number of trials, p=.01), 20 Questions (% questions which constrained alternatives by referring to semantic categories, p=.02), verbal fluency (total number of words produced, p=.005), and Go-No go (trials to criterion, p=.001). Reclassification of the CHI groups by localization of lesion (via magnetic resonance imaging) revealed differences between children with frontal vs nonfrontal lesions on several measures. We conclude that (1) the late effects of severe injury of the young brain can be elucidated by experimental cognitive tasks; and (2) that prefrontal lesions may result in a distinctive pattern of cognitive deficits.

191.12

MEDIAL TEMPORAL LOBE VS DIENCEPHALIC AMNESIA IN CHILDHOOD. F. Vargha-Khadem, E. Isaacs and K. Watkins , (SPON: European Brain and Behaviour Society), Department of Psychological Medicine, Hospital for Sick Children, Great Ormond Street, London WC1, England.

Chronic anterograde amnesia resulting from either medial temporal lobe or diencephalic damage in childhood is a rare occurrence. Two boys with amnesia of differing aetiology and age of onset showed impairment in the acquisition and retention of declarative knowledge.

Case JF had normal development except for a memory and learning deficit developing from age 4. Case JL became amnesic at age 12 following diagnosis and excision of a craniopharyngioma of the pituitary. MRI scans showed bilateral medial temporal sclerosis and hippocampal atrophy in the case of JF and midline pathology involving the thalamus, mammillary bodies and other diencephalic regions in JL's case. At 13, both patients were impaired in tests of declarative memory whereas performance on tasks of procedural memory was normal.

It is concluded that the evidence from childhood amnesia is consistent with the distinction in adulthood between declarative and procedural memory systems.

191.13

EFFECTS OF THE BENZODIAZEPINE TRIAZOLAM ON MEMORY AND METAMEMORY. R.G. Lister, E.M. Joyce, H.J. Weingartner* and M.J. Eckardt. Lab. of Clin. Studies, NIAAA, DICBR, Bethesda, MD 20892.

To examine the nature of triazolam-induced amnesia, student volunteers received on four separate occasions in a randomized order triazolam (0, 0.25 mg, 0.375 mg, 0.50 mg). On each test day subjects were shown a list of words just prior to drug administration, and a second list 90 min after drug administration. When each word was presented subjects were asked to rate the probability of their remembering the word later. Triazolam caused a marked, dose-related impairment in the free recall of words presented after its administration, but enhanced the recall of words presented before its administration. Although triazolam impaired acquisition processes, at the time of acquisition triazolam-treated subjects rated the likelihood of later remembering words no differently from placebo-treated subjects. In a test of metamemory, triazolam impaired free recall, recognition and feeling of knowing judgments. The cognitive deficits observed with triazolam resemble those seen in some organic amnesias. Triazolam, however, fails to impair retrieval mechanisms.

191.14

INTRA-NEURONAL INFORMATION PROCESSING IN CYTOSKELETAL NETWORKS: THEORETICAL ASPECTS. R. Lahoz-Beltra*[1], A. Samsonovich*[2], S. Rasmussen*[3], A. Scott*[2], S. Hameroff[1]. [1]ABL-Anesthesiology, [2]Applied Math, University Arizona, Tucson, AZ, 85724; [3]CNLS, Los Alamos National Labs, Los Alamos, NM, 87545.

Brain models and artificial neural networks (ANNs) generally consider neurons and synapses as simple switches with weighted connections. However, neurons are complex processing devices supported by cytoskeletal networks including microtubules (MT) and MT associated proteins (MAPs) such as dendrite-specific MAP_2. These intra-neural networks are suitable for molecular level information processing which could subserve neural level functions and provide a substrate for cognition. Cytoskeletal links to neural level events include MAP_2 phosphorylation via protein kinase C, G protein and structural links, Ca^{++} influx and others. We consider theoretical aspects: 1) Energy pumping via phosphorylation and hydrolysis drives coherent nanosecond conformational excitations in cytoskeletal networks. In MT, these coherent "phonons" can localize and give rise to conformational patterns of excited subunits such as MAP attachment sites. 2) Cooperativity among MT subunits and MAPs enables molecular automata behavior suitable for computation and memory. 3) MAP_2 connections among dendritic MT can behave as "molecular synapses" regulating information flow among MT within neurons. 4) Signalling in MT retrograde to membrane depolarization can provide a synaptic regulation function analogous to "back-propagation" in ANNs. We present computer simulation which demonstrate and explore these theoretical aspects.

191.15

SIMULATING HEBB CELL ASSEMBLIES. P.A. Hetherington and M.L. Shapiro. Department of Psychology, McGill University, Montreal, Quebec, Canada H3A 1B1.

Cell assemblies (Hebb, 1949) are self-activating groups of interconnected neurons with connections that grow and persist within a larger neural matrix. Here, computer simulations of Hebb's theoretical cell assemblies produced patterns of activation across a partially interconnected population of simple, simulated neurons. The network was presented with several patterns of input that were stored using variations of Hebb's unsupervised learning rule. During learning, patterns of activation arose which, after learning, persisted in a sparse and distributed fashion. Units within an assembly excited one another and inhibited units in other assemblies. As a result of this learned excitatory interconnectedness, the cell assemblies became active with a given stimulus pattern and persisted in the absence of that stimulus. If the stimulus pattern was degraded, part of the cell assembly was actived, and the rest of assembly became active over successive time steps. In addition, injecting low level random noise diffusely and nonselectively across the entire matrix (of 360 units) caused select cell assemblies to arise one, and only one, at a time.

Various implementations of the Hebb rule, Stent (1973) rule, and correlation rule were evaluated in this network to the extent to which they produced and supported cell assemblies. These simulations showed that: (1) the simple Hebb rule did not allow stable patterns to be stored in the network, but led to sporadic activation; (2) the Stent rule generated stable, but not persistent assemblies; and (3) the correlation rule did not work in this simulated neural matrix. The computational effects of additional neurobiological properties is now being explored.

LEARNING AND MEMORY—ANATOMY III

192.1

DEVELOPMENTAL CHOLINERGIC REGULATION OF ADULT BEHAVIORAL FUNCTIONS. J.E. Sweeney, M. Smith*, E. Bachman*, J.T. Coyle and C.F. Hohmann. INSERM, Unité 161, Paris, France 75014 and Dept. of Child Psychiatry Johns Hopkins University, Baltimore, MD 21205.

We have shown previously that neonatal basal forebrain (BF) lesions in mice produce transient morphological changes in cortex that correlate with the degree of cholinergic deafferentation. We have now examined the behavioral performance of neonatally BF-lesioned mice that were allowed to reach adulthood (when recovery of cortical cholinergic parameters has taken place). Eight Balb/cByJ mice received bilateral electrolytic lesions to the BF. Mice were housed until 6-8 weeks of age and were then tested along with 20 control mice in an activity chamber and then on a passive avoidance task. During the dark cycle, BF-lesioned group exhibited significant increases in motor activity as compared to controls including increased speed (41.1 ± 5.7 m/hr vs 17.6 ± 1.5 m/hr) and increased movement time (38.9 ± 9.1 min/hr vs 26.9 ± 7.4 min/hr). On the 20 min. retention of passive avoidance, BF-lesioned mice exhibited a significantly shorter latency to enter the dark (14.8 ± 8 sec vs 65.6 ± 11 sec). On the 24-hour retention, the BF-lesioned mice exhibited a shorter latency to enter the dark (115 ± 30 sec vs 146.1 ± 15.5 sec). This difference, however, did not reach statistical significance and more mice are being tested. Additionally, all mice are being evaluated on a spatial navigation task. These results suggest that early BF projections to the cerebral cortex play a role in adult behavioral performance. While cortical cholinergic parameters have recovered in adulthood following a neonatal BF lesion, the behavioral deficits remaining are profound.

192.2

EFFECTS OF AGE AND NBM LESION ON MAZE LEARNING AND CORTICAL NEUROCHEMISTRY.
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The effects of age and cortical cholinergic depletion (NBm lesion) were examined using 2 water maze tasks and HPLC analysis. Male F-344 rats (300 gm) were lesioned by bilateral infusion of ibotenic acid (ag/NBm) or underwent sham surgery (ag/con). One year later, a second set of young adults underwent the same procedure (yg/NBm and yg/con). Significant and independent main effects of age and lesion were observed in the impairment of acquisition on the Morris water maze task. None of the groups showed a further decline on this task after a 10-day retention interval. In a water motivated T-maze, only the ag/NBm group showed a consistant impairment in performance. Biochemical analysis revealed a significant decrease in ChAT activity by the NBm lesion but no effect of age. In the anterior cortex, HVA levels were decreased in the aged groups but were not affected by lesion. No other differences in monoamines or their metabolites were noted for cortical or hippocampal areas.

192.3

COMPARATIVE EFFECTS OF NUCLEUS BASALIS OR HIPPOCAMPAL LESIONS AND NORMAL AGING ON RECOGNITION MEMORY IN THE RHESUS MONKEY. L.L. Beason, M.B. Moss and D.L. Rosene. Boston Univ. Sch. Med., Boston, MA 02118.

A decline in memory function has been associated with normal aging in humans and non-human primates. Two structures implicated in both memory function and age-related neuropathology are the hippocampus and nucleus basalis. To assess their role in age-related memory decline, young adult monkeys (5-8 years of age [yoa]) with lesions of either nucleus basalis (sparing the septum and diagonal band, n=4) or the hippocampus (n=3) or control operations (n=3) were compared with normal aged monkeys (n=5; 26-27 yoa) on the Delayed Recognition Span Task (DRST). The DRST requires the animal to identify the novel stimulus added to an increasing array of stimuli. The number of correct responses made before an error constitutes a "recognition span score". The DRST was administered to all groups in spatial and color conditions. Consistent with earlier findings in our laboratory, the results showed that the recognition span of animals with nucleus basalis lesions did not differ from controls on either the spatial or color conditions. In contrast, animals with hippocampal lesions as well aged monkeys had significantly lower recognition spans on both conditions of the task (spatial 1.9 and 1.9, color 2.2 and 2.5, respectively) relative to controls (4.0 and 3.5) but there was no significant difference in degree of impairment between the aged and hippocampal groups. These findings raise the possibility that age-related changes in the hippocampal formation and not the nucleus basalis may underlie the decline in memory that occurs in normal aging. The findings also suggest that the anterograde memory deficits in Alzheimer's disease may pbe due to pathology in the hippocampal formation or associated structures of the temporal lobe limbic system and not a consequence of damage to the nucleus basalis. "Supported by NIH grants AG-04321 and AG-00001".

192.4

PERFORMANCE ON DELAYED NON-MATCHING TO SAMPLE IS NOT IMPAIRED IN RATS BY AGING UP TO 30 MONTHS. Susan M. Koger, Robert G. Mair, & Russell L. Knoth. Psychol. Dept., Univ. of New Hampshire, Durham, NH 03824.

In a longitudinal study, rats were trained on spatial delayed non-matching to sample (DNMTS) prior to 6 months of age and then re-tested at 12 (n=7), 24 (n=5), and 30 (n=3) months of age. At each age, animals were first retrained at a 3 s interval and then tested for 150 trials at each of 4 retention intervals (0.1, 3, 9, 15 s) presented in a counter-balanced order (600 trials total). Accuracy decayed as a function of both retention interval and response latency at all ages. There was no evidence that DNMTS performance decreased for individual animals as they aged. These results suggest that with extensive training at an early age, there are no consistent age-related effects on this measure of working memory in rats within the life-span tested.

192.5

IMPAIRMENT OF VISUAL ATTENTIONAL PERFORMANCE FOLLOWING CHOLINERGIC NUCLEUS BASALIS LESIONS IN RATS. J. L. Muir, T.W. Robbins and B. J. Everitt+ Departments of Experimental Psychology and Anatomy+ University of Cambridge, CB2 3EB, U.K.

Recent studies suggest that cognitive functions other than learning and memory may be significantly affected by loss of cortical acetylcholine. In order to investigate further the role of forebrain cholinergic projections in attentional function and to clarify cholinergic specificity, the effects of infusions of the excitotoxin AMPA, which effectively and with considerable selectivity destroys cholinergic cells of the nucleus basalis magnocellularis (nbM) (70% reduction in cortical ChAT activity), while producing little damage to pallidal and other basal forebrain neurons, were investigated.

These lesions impaired the rats' accuracy in localising brief visual targets in a 5-choice serial reaction time task and lengthened the latency to respond correctly several months following surgery. The behavioral deficits observed using this excitotoxin were in many cases even more profound than those following quisqualic acid-induced lesions of the basal forebrain (50% reduction in cortical ChAT activity). Furthermore, the AMPA-induced deficits could be exacerbated or ameliorated by certain behavioral challenges: thus, reducing the stimulus duration significantly impaired, while increasing this duration significantly improved performance of the task. The results provide further support for a role for the basal forebrain-neocortical cholinergic projection in attentional function.

192.6

NUCLEUS BASALIS STIMULATION ALTERS SPONTANEOUS AND STIMULUS-EVOKED RESPONSES OF SOMATOSENSORY CORTICAL NEURONS. M.A.Howard and D.J.Simons. Dept. Neurosurg., U.Wash., Seattle, WA 98195 and Dept. Physiol., U.Pitt., Pittsburgh, PA, 15261

Responses of SmI barrel cortex neurons to controlled whisker stimulation were examined in urethane anesthetized rats before and after electrical stimulation of the Nucleus Basalis Magnocellularis (NBM). Twenty-six of 60 cells showed statistically significant alterations in spontaneous activity (SA). Short-term effects of <5 min consisted of abrupt decreases (n=6) or increases (n=2) in SA. Eighteen cells showed long-term effects that could last > 60 min. Most (n=14) showed abrupt or gradually developing decreases in SA. In cells with diminished SA, stimulus-evoked activity either decreased slightly, remained unchanged or increased, sometimes substantially; on average, responses to whisker movement increased 50% despite an average decrease in SA of >50%. Both acute and long-term effects of NBM stimulation could be prevented by systemic injection of atropine. NBM activation can thus alter cortical excitability for long periods and in some cases selectively enhance the net response to a tactile stimulus. Supported by NS08438 and NS19950.

192.7

EVIDENCE FOR SPECIFIC RELATIONSHIP BETWEEN DISCRIMINATION PERFORMANCE AND CHOLINE ACETYLTRANSFERASE IN THE POSTERIOR CINGULATE CORTEX OF THE RAT. H.M.Marston, B.J.Everitt and T.W.Robbins. Dept. of Expt. Psychology, Univ. of Cambridge, Cambridge, CB2 3EB, UK.

Differential reductions in the level of posterior cingulate (PCg) and hippocampal (Hip) choline acetyltransferase activity (ChAT) were achieved by a series of quisqualate lesions to the septum and the diagonal band. The behavioural consequence of these, and ibotenate hippocampal lesions were compared in the acquisition and performance of a conditional visual discrimination. The task required the rat to respond either to the left or the right dependent upon a preceding visual cue that either flashed slow or fast.

A strong correlation (r=0.82) was found between *post mortem* PCg ChAT activity and errors made in performance. There was little correlation with Hip ChAT (r=0.40), nor were hippocampal lesioned animals severely debilitated. These animals were however more susceptible to the interpolation of a response delay. Finally it was found that animals with large PCg ChAT reductions were less susceptible to the deleterious effects of physostigmine and scopolamine compared with Sham controls on the task.

The results suggest a significant role for PCg cholinergic function in the mediation of conditional behaviour independent of the mnemonic role of the hippocampus.

192.8

IMPAIRED REVERSAL LEARNING BUT PRESERVED LATENT INHIBITION FOLLOWING EXCITOTOXIC LESIONS OF THE BASAL FOREBRAIN IN THE RAT. T.W. Robbins, A.C. Roberts, J.L. Muir, A.S. Killcross* and B.J. Everitt+(SPON: European Brain and Behaviour Society). Departments of Experimental Psychology and Anatomy+, University of Cambridge, CB2 3EB, U.K.

Injection of the excitotoxin ibotenic acid into the region of the cholinergic cell bodies of the nucleus basalis magnocellularis (nbM) produces a wide range of behavioral deficits. However, interpretation of these data is confounded, both by the non-cholinergic damage produced by this neurotoxin and the lack of behavioral specificity. The present study therefore uses the more selective excitotoxins quisqualic acid and AMPA to destroy cholinergic cells in the nbM and investigates the effect of this lesion on tests specific for associative learning and selective attention.

In Experiment 1 lesioned rats were impaired on reversal of a visual discrimination task which required them to switch their responding either towards or away from the lever paired with the cue light. Experiment 2 showed that the performance of lesioned rats during extinction of a conditioned response was insensitive to the presence of stimuli previously associated with reward. In contrast, there was no effect of the lesion on latent inhibition, a test of selective attention. The results will be discussed in relation to the intimate direct connections that exist between the orbitofrontal cortex, the amygdala and the nbM.

192.9

DIFFERENT PASSIVE AVOIDANCE AND MILK MAZE DEFICITS WITH DIFFERENT SUBSTANTIA INNOMINATA LESIONS. R.C. Meyer and G.D. Coover. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

Basal forebrain lesions affecting passive avoidance (PA) and milk maze (MM) performance have involved damage to portions of the substantia innominata (SI). We examined the effects on drinking PA (dPA), step-through PA (sPA) and MM performance of three electrolytic lesions in the rat all at V -8.2 mm relative to bregma: caudolateral SI (cSI, L ±4.2, A -1.9), middle SI (mSI, L ±3.5, A -1.2) and rostromedial SI (rSI, L ±2.8, A -0.5). Our cSI lesions damaged the rostral third of the central amygdaloid nucleus, where lesions produce a deficit in dPA that appears to be "anxiolytic" in character.

Large deficits in dPA were produced by cSI and also mSI lesions, but not rSI lesions. The control group (CON) took a mean ±SE of only 21 ±2 footshocks of incrementing intensity to avoid drinking for 5 min, while cSI and mSI took 42 ±4 and 37 ±3, and rSI took 27 ±3. In sPA, only cSI showed a significant deficit, mSI was intermediate, and rSI was indistinguishable from CON. In the MM, rSI was very slow over test sessions to reach the hidden platform (60 ±16 sec) compared to mSI (26 ±12), cSI (14 ±4) and CON (11 ±2).

Lesions in SI produce different deficits in PA and spatial tasks depending on lesion location. PA deficits from cSI and mSI lesions appear to stem from an anxiolytic effect rather than a learning deficit.

192.10

MAP-2 EXPRESSION IN CHOLINOCEPTIVE PYRAMIDAL CELLS OF RODENT CORTEX AND HIPPOCAMPUS IS ALTERED BY PAVLOVIAN CONDITIONING. Nancy J. Woolf, Stacey L. Young, Michael S. Fanselow, and Larry L. Butcher. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

Immunohistochemical studies have revealed that only a subpopulation of pyramidal cells in the cerebral cortex and hippocampus are cholinoceptive. These cells additionally contain high levels of certain cytoskeletal proteins, including MAP-2. Recently, we found that cholinergic deafferentation decreases MAP-2 in cholinoceptive pyramidal cells, indicating that levels of this protein are subject to modulation. The present study was done in order to determine whether or not a learning paradigm would have any modulatory effect on the expression of MAP-2 in these cholinoceptive pyramidal cells. Hooded rats were randomly assigned to one of three groups: tone paired with shock, tone unpaired with shock, and tone only. After 5 days of training the paired group evidenced conditioning to the tone relative to controls. The rats were immediately sacrificed after the last session. MAP-2 staining density was assessed in the pyramidal cell layer of the cortex and hippocampus. The density of MAP-2 staining in various cortical regions varied as a function of experimental group ($p<.05$, ANOVA, repeated measures). In contrast to the other groups, tone-conditioned animals exhibited significantly higher MAP-2 staining in auditory regions of the cerebral cortex compared with the hippocampus ($p<.005$) and visual and limbic cortices ($p<.05$). [Support: USPHS grant NS 10928 to L.L.B.]

192.11

EFFECTS OF MEDIAL SEPTAL LESIONS ON AN OPERANT DELAYED GO/NO-GO DISCRIMINATION IN RATS. R. Numan. Psychology Department, Santa Clara University, Santa Clara, CA 95053.

Male hooded rats received either a medial septal lesion (N=11) or a control operation (N=10). The rats were then tested on an operant go/no-go discrimination task (30 min sessions, 5 days/wk, 45 mg food reward) first without a delay contingency (20 days), followed by 25 days under an 8-sec delay. A discrete trial procedure with symmetrical reinforcement was used. When the rat was placed in the operant chamber it was dark, except for back-illumination of a centrally located press panel. Depression of this panel extinguished the back-light and initiated a random presentation of either the "go" stimulus (2800 Hz tone) or the "no-go" stimulus (10 Hz pulsing light) for 3-sec. When this stimulus terminated the chamber was darkened for 0.1 sec (0-sec delay) or 8.0 sec (8-sec delay). The delay was followed by the availability of a lever for 2-sec. If the rat pressed the lever on "go" trials, or refrained from pressing the lever on "no-go" trials a food pellet was delivered. At the end of the 2-sec lever availability period, or following a lever press, the central press panel was again back-illuminated to begin the next trial. The data (% correct responses) were averaged over 5-day blocks. Overall, the septal lesioned rats performed significantly *better* than the control rats under both 0-sec delay ($p < 0.025$) and 8-sec delay ($p < 0.001$) contingencies. While there was not a significant difference between the groups during the last block of the 0-sec delay (septals 93% correct, controls 89% correct, $p > 0.05$) or the first block of the 8-sec delay (septals 54% correct, controls 53% correct, $p > 0.05$), the septal lesioned animals performed significantly better than the controls during all the remaining phases of the experiment. For example, during the final 5-day block of the 8-sec delay, the septal rats averaged 71% correct and the control rats averaged 61% correct ($p < 0.001$). The results will be discussed in terms of various theories of septo-hippocampal function.

192.12

INTERACTION OF OPERANT HISTORY AND SEPTAL LESIONS IN THE RAT ON VARIABLE-RATIO RESPONDING. Alex Poplawsky. Dept. of Psychology, Bloomsburg Univ. of Penna. Bloomsburg, PA 17815.

The over responding of rats with septal lesions on a variety of schedules of reinforcement can be altered by training procedures. Since few studies have investigated the effects of septal damage on Variable Ratio (VR) behavior, rats with septal lesions or control operations were trained to leverpress for food on a VR 20 schedule of reinforcement for five sessions. This was followed by 44 sessions of VR schedules that ranged between VR 60 to 100 before the rats were returned to the VR 20. Rats with septal lesions responded at higher rates than control rats during the early VR-20 sessions; however, both groups had equivalent response rates after the higher VR-requirement sessions . These findings suggest that the operant history of the rat is an important factor when interpreting the effects of brain damage on food-reinforced lever-pressing.

192.13

ATTENTION DEFICITS PRODUCED BY UNILATERAL OR BILATERAL MEDIODORSAL THALAMIC LESIONS IN THE RAT. J.M. Vargo and P.J. Best. Department of Psychology, Miami Univ., Oxford, OH 45056.

Bilateral lesions of the mediodorsal thalamic nucleus (MD) produce deficits in spatial context recognition (K.A. Stokes & P.J. Best, Neurosci. Abs., 13: 1067, 1987) similar to those seen following bilateral medial precentral prefrontal cortex (Agm) lesions (J.M. Vargo et al., Neurosci. Abs., 16: 1095, 1990). These animals fail to react to a familiar environmental cue when it is out of place. Unilateral but not bilateral AGm lesions produce neglect (deficits in orientation to unilaterally presented stimuli) (J.M. Vargo et al., Neurosci. Abs., 16: 1095, 1990).

Long-Evans hooded rats with unilateral or bilateral MD lesions were tested for neglect (degree of head turning toward unilaterally presented stimuli was recorded). Unilaterally lesioned animals demonstrated unilateral orientation deficits. Bilaterally lesioned animals did not demonstrate bilateral neglect. In the same animals, bilateral but not unilateral lesions led to deficits in spatial context recognition.

192.14

IN THE RAT, BOTH ANTERIOR AND POSTERIOR PORTIONS OF MEDIAL THALAMUS MUST BE LESIONED TO IMPAIR DNMTS PERFORMANCE. R.G. Mair, G.D. Fox*, Y.-P. Zhang*, J.K. Robinson. Dept. Psychol. University of New Hampshire, Durham, NH 03824.

Impairments in DNMTS performance have been associated with lesions involving lateral portions of the internal medullary lamina (L-IML) produced by either: pyrithiamine-induced thiamine deficiency (Robinson & Mair, this meeting); or radiofrequency (RF) methods. We pretrained 32 rats on DNMTS and assigned 8 to each of four treatments: RF lesions of the complete L-IML site, anterior portions of the L-IML site, posterior portions of the L-IML site, and sham surgery. Animals with complete IML lesions showed DNMTS deficits comparable to previous studies, performing significantly worse than either sham operated controls or animals with partial IML lesions. The animals with partial IML lesions did not differ significantly from sham operated controls.

192.15

MK-801 PROTECTS RATS FROM BRAIN LESIONS AND BEHAVIORAL IMPAIRMENTS FOLLOWING PYRITHIAMINE-INDUCED THIAMINE DEFICIENCY(PTD). John K. Robinson & Robert G. Mair. Psychol. Dept., Univ. of New Hampshire, Durham, NH 03824.

Rats were pretrained on spatial delayed non-match to sample (DNMTS) and given one of four treatments: PTD, PTD + MK-801 (3 mg/ kg, followed by 1.5 mg/kg t.i.d. for the last three days of PTD treatment), pair-fed control, and pair-fed + MK-801 control. Following recovery, the PTD group was impaired compared to all other groups on several measures of DNMTS performance; including: reacquisition at a 3s delay; performance at delays from 0 to 15s; and delay producing 75% accurate performance with a staircase procedure. The PTD group retained the capacity to perform DNMTS accurately (mean = 92.8% correct) following extensive training at short delays. The PTD group made more errors learning a radial arm maze task, although they reached criteria for both discrimination and working memory problems. These impairments are consistent with the effects of RF lesions centered on the internal medullary lamina (IML) and involving the mediodorsal nucleus (MDn) of thalamus.

Histological analyses showed: thalamic lesions involving IML & MDn in 17/17 PTD rats; 1/12 PTD + MK-801 rats; and none of the two control groups. These results demonstrate that MK-801 treatment prior to the onset of seizures protects from PTD-induced lesions.

192.16

Mamillary bodies or medio-dorsal thalamic lesions differentially modify anxiety in mice.

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Several studies have shown that damage to the mamillary bodies (MB) and the medio-dorsal thalamus produced emotional disturbances. Thus, the MB are an important site of antianxiety action of benzodiazepines, and lesions of this area and/or the mammillothalamic tract induced deficits in various tasks involving negative reinforcers (Kataoka et al., Brain Res., 1982, 241, 374-377). The MD receives afferents coming from the amygdala which has anxiogenic functions. In this study, we have decided to determine whether emotional disturbances might be found following lesions of these structures in mice. Anxiety was measured in an elevated plus maze , a motivation artifact free test measuring strength of antagonism between exploratory tendency and avoidance of open novel spaces . Results showed that MB-lesioned subjects spent significantly more times in the open alleys than both control and MD subjects, whereas the opposite effect was observed for MD animals. Thus, MB-lesioned subjects were "hypo-anxious"whereas MD-lesioned ones were " hyper-anxious" .The relationships between the emotional and memory disorders resulting from diencephalic lesions will be discussed.

192.17

CEREBRAL DEOXYGLUCOSE METABOLISM FOLLOWING CHRONIC ETHANOL CONSUMPTION IN MICE: A SELECTIVE EFFECT ON DIENCEPHALIC STRUCTURES. B. Bontempi*, D.J. Beracochea, C. Destrade, R. Jaffard. Lab. Neurosciences Comportementales & Cognitives, Univ. Bordeaux 1, URA CNRS 339, 33405 Talence-cedex, France.

Ten week-old male mice were submitted to a 12% v/v alcohol consumption during 17 months and then withdrawn at least 8 months before the experiment began. Controls were either pair-fed or had received ad. lib. water and dry food. The (^{14}C)-deoxyglucose (2-DG) was injected in the jugular vein 10 min before the animals were placed in a T-maze. They were allowed to freely visit the apparatus during 20 min. Then, the animals were removed from the maze and sacrificed 5 min later; the brains were processed for autoradiography. The regional uptake of 2-DG was analysed using the relative 2-DG method. A significant decrease of labelling was found in diencephalic areas, mainly in the mamillary bodies and thalamic nuclei. A slight but not significant decrease was found in the hippocampal formation, whereas no decrease was observed in other cortical and subcortical structures. These results are consistant with the hypothesis that the memory disorders observed following chronic alcohol administration in mice may be related to diencephalic rather than hippocampal or cortical damages.

192.18

PERFORMANCE ON A NON-SPATIAL MEMORY TASK FOLLOWING LESIONS TO THE REGION OF THE MAMMILLARY BODIES. V. Sziklas and M. Petrides. Dept. of Psychology, McGill University, Montreal, Quebec, Canada H3A 1B1.

In the present study, rats with extensive lesions to the mammillary region (MB-R), the amygdala (A), or a control operation (OC) were trained postoperatively on a high interference non-spatial delayed nonmatching-to-sample task (DNMS) and on a spatial DNMS task. On the non-spatial task, only two stimuli, a black and a white arm, were used repeatedly throughout testing. A greater proportion (90%) of animals with MB-R lesions were able to acquire the non-spatial task, within the limits of testing, as compared with the OC (38%) and A (50%) groups. In sharp contrast, the performance of the MB-R group was significantly impaired on the spatial task in comparison with both the OC and the A groups. These findings suggest that lesions to the MB-R may not result in a global memory impairment and that this region may be selectively involved in spatial learning and memory.

192.19

INCREASE IN IPSILATERAL LH SELF-STIMULATION AND BEHAVIORAL ASYMMETRIES INDUCED BY UNILATERAL LESION OF THE TUBEROMAMMILLARY NUCLEUS. U.Wagner*, H.-T.Weiler* and J.P. Huston, Institute of Physiological Psychology, University of Düsseldorf, D-4000 Düsseldorf, Germany

The subnuclei of the tuberomammillary nucleus (TM) are located in the posterior part of the hypothalamus. The neurons of this nucleus innervate extensive parts of the brain with several transmitters; they represent the only source of histaminergic projections in the brain. This is the first study dealing with the effects of a lesion in this region on sensorimotor behavior and self-stimulation. Unilateral electrolytic lesions of the TM had the following effects: 1. An asymmetry in thigmotactic scanning behavior; at 11 days but not 1 day post-lesion, the rats scanned the walls of an open field more with the vibrissae contralateral to the lesion side. 2. More ipsiversive than contraversive turning. 3. Application of the histamine precursor histidine led to a compensation of these asymmetries. 4. Beginning with the first post-lesion day an increase in lateral hypothalamic self-stimulation rate ipsilateral to the lesion was found. Response rates continued to rise for two weeks post-lesion. Recently we found evidence for an involvement of the TM in asymmetries, and recovery therefrom, induced by unilateral removal of the vibrissae (Weiler et al., Neurosci., 1990,37,463-469). Thus, the TM may function in reinforcement, sensorimotor asymmetry and behavioral neuroplasticity.

192.20

GENETIC INFLUENCE ON RADIAL MAZE PERFORMANCE IN MICE. Dudley Peeler. Department of Neurosurgery, University of Mississippi Medical Center, Jackson, MS 39211.

Recombinant inbred (RI) mouse strains have been used to investigate possible major gene determinants of behaviors reflecting learning, memory and emotion. Major single gene influences have been described for various measures of activity and shuttle box performance of shock avoidance. In this study, the CXB set of 7 RI strains and their BALB/cBY and C57BL/6By progenitors were tested for working memory (number of re-entries) on an 8-arm radial maze. A 2-hr deprivation schedule and food incentive provided motivation to explore the maze. Male and Female adult mice were allowed two, 5-min familiarization sessions followed by 5 test sessions, one session per day. All strains showed a decrease over the 5 test sessions in the number of re-entries before 8 arms were entered. However, there was a significant strain effect and significant interaction between strain and session. There were differences in terms of mean number of re-entries for all test sessions (BALB/c = CXBE > all other strains), and in terms of mean number of re-entries in the final session (BALB/c > CXBI = CXBK). The pattern of decline of number of re-entries over test sessions also differed among strains. Failure of some mice to enter all 8 arms during the familiarization sessions indicated a different strain distribution pattern which was essentially like that found in one study of shuttle box performance. Genetic effects upon working memory (number of re-entries) differ from those reported for various measures of activity and for shock avoidance learning. There is apparent similarity between genetic effects upon initial exploration of the maze (emotionality) and shuttle box performance.

193.1

ENHANCED LEARNING OF A SEXUALLY DIMORPHIC SPATIAL TASK IN MEADOW VOLES FOLLOWING BRIEF EXPOSURE TO 60HZ MAGNETIC FIELDS. M. Kavaliers, L. Eckel, K.-P. Ossenkopp and N. Yu.*, Univ. Western Ontario, London, Ontario, Canada.

Meadow voles, Microtus pennsylvanicus, are polygamous microtine rodents that display marked sex differences in activity and spatial performance. Adult male voles have been shown to range more widely in the wild and to perform better on laboratory measures (radial and symmetrical mazes) of spatial ability than adult female voles. Meadow voles are also excellent swimmers and have been shown to make extensive aquatic excursions in the wild. In this study we examined the spatial abilities of adult male and female meadow voles in the Morris water maze task. The males showed significantly faster acquisition and better retention of this spatial task than did females.

A variety of factors have been shown to affect spatial learning in rodents. In the present study we also examined the effects of exposure to weak (1 gauss) 60 Hz magnetic fields on performance of the spatial task. Exposure of the voles to the magnetic fields during the daily testing procedures (maximum daily exposure 6 minutes) significantly enhanced the acquisition of the learning task in both males and females. The magnetic stimuli may be providing directional information and/or affecting a number of modulatory systems (endogenous opioid systems, protein kinase C activity and calcium ion levels) which have been previously implicated in learning.

193.2

EFFECTS OF ISCHEMIA ON HIPPOCAMPAL SINGLE-UNIT ACTIVITY IN FREELY BEHAVING RATS. P.A. Garcia, S.J.Y. Mizumori, and B.T. Volpe, Dept. Psychol., Univ. Utah, S.L.C., UT 84112 and Dept. Neuro. & Neurosci., Burke Inst. Med. Res., Cornell Univ. Med. Ctr., White Plains, NY 10605.

In hippocampus, transient forebrain ischemia primarily causes a severe loss of CA1 pyramidal cells. Using the stereotrode recording method, the electrophysiological properties of hippocampal cells were recorded from 4 ischemic (4 vessel occlusion method) and 4 sham operated Wistar rats while they performed a radial maze task. Consistent with previous results, ischemic animals showed impaired acquisition, but asymptote performance was equal to that of controls. A total of 161 units were recorded following acquisition of the task. Preliminary unit analysis revealed the following pattern of results. Place specificity of CA1 complex spike (CS) cells was not different for ischemic (n=2) and control animals (n=14). This was also the case for CA3 CS cells. However, place cells recorded in the hilar region were significantly less specific for ischemic animals (n=11) compared to controls (n=5). For all populations tested, theta cells showed no difference between groups in terms of place specificity. Other analyses revealed that CA1 CS cells had a significantly higher mean firing rate in ischemic animals, and ischemic CA3 CS cells showed a significantly higher spike amplitude.

CA1 (n=6 ischemic,4 sham), CA3 (n=5,4), hilar (n=8,3), subiculum (n=11 ischemic only), and stratum granulosum (n=10,4) theta cells showed statistically significant movement sensitivity. Also, ischemic theta cells were rhythmically modulated in a manner similar to that observed for controls. Thus, CA1 retains functionally active theta cells despite a significant reduction in pyramidal cells. Also, these data provide evidence for the hypothesis that CA3 place fields are maintained by direct entorhinal input.
[Supported by URC Grant 15155 and MH40090]

193.3

MNEMONIC PROPERTIES OF VISUAL-SENSITIVE HEAD DIRECTION CELLS IN LATERAL DORSAL THALAMUS. S. J. Y. Mizumori and J. D. Williams*, Department of Psychology, Univ. Utah, Salt Lake City, UT 84112

While more is becoming known about the behavioral correlates of cells recorded in different subfields of the hippocampal formation, the functional contribution of its many afferents is not clear. The present study addresses this issue by investigating the nature of information provided by afferents arising from the lateral dorsal nucleus (LDN) of the thalamus.

Single unit activity of LDN neurons was recorded while rats (n=4) performed 15 working memory trials daily on an 8-arm radial maze. Of 26 LDN units, 8 exhibited head direction firing patterns that were similar to those observed for postsubicular cells (Taube et al. *J. Neurosci.*, 1990). These head direction cells were found in the dorsolateral aspect of the LDN, which projects directly to postsubiculum. The directional preference (DP) of the LDN cells was disrupted if rats were placed on the maze in darkness. If the room light was then turned on, the reliability of the DP was restored. When rats began maze trials in darkness 30 sec after being allowed to view the room, DPs were maintained. Delays of more than 1 min resulted in altered DPs. Thus, directional specificity was initialized by visual input, and maintained briefly when visual cues were absent. Many cells had DPs which varied depending on the arrangement of visual cues in a lit room. When tested in subsequent dark trials, the DPs of these cells corresponded to the *most recent* visual input. DPs during passive movement through space were not different from DPs during active translational movements by the rat, suggesting a limited contribution by motor systems. These data suggest that the LDN may provide the hippocampal formation with a visual reference framework for use in computations concerning visuospatial learning.
[Supported by BRSG Grant S07 RR07092]

193.4

DIFFERENTIAL FUNCTIONS OF SUBCLASSES OF HIPPOCAMPAL THETA CELLS. A. M. Lavoie, S. J. Y. Mizumori and K. E. Ward*, Department of Psychology, University of Utah, Salt Lake City, UT 84112

Hippocampal theta cells (presumed interneurons) may be classified according to immunohistochemical characteristics. The present study was conducted to compare the functional properties of oriens, pyramidale, lacunosum-moleculare/ radiatum and stratum granulosum (SG) interneurons recorded in dorsal and ventral hippocampus. Stereotrode recording was used to monitor single unit activity of hippocampal theta cells (N=271) while rats (N=16) completed 15 trials daily on an 8 arm radial maze. Upon initial analysis it was clear that theta cells recorded in dorsal hippocampus exhibited different firing patterns than those in ventral hippocampus. There were no significant regional differences between CA1, CA3 and SG interneurons, so the cell classes were collapsed for subsequent analysis.

The predominant finding to emerge thus far involves differential firing rates as a function of various behavioral conditions. Dorsal pyramidale theta cells (n=29; which may correspond to parvalbumin interneurons) showed a significant increase in firing as a function of translational movement. This result is consistent with the finding that place cells, usually recorded in the dorsal hippocampus pyramidal cell layer, require movement input (Foster et al.,1989). Dorsal oriens theta cells (n=25; possibly corresponding to somatostatin or parvalbumin interneurons), on the other hand, demonstrated no significant movement sensitivity. As found previously, dorsal SG interneurons (n=34) showed significant movement sensitivity. Examination of different classes of ventral hippocampal theta cells revealed no significant effect of movement (N=106). The differential sensitivity of dorsal and ventral hippocampus to movement may reflect distinct patterns of innervation.
[Supported by BRSG Grant S07 RR07092]

193.5

LIMBIC SEIZURES, BUT NOT KINDLING, IMPAIR PLACE LEARNING IN THE MORRIS WATER MAZE: RETROGRADE AND ANTEROGRADE DEFICITS. R.D. Kirkby, R.K. McNamara, R.W. Skelton & M.E. Corcoran, Dept. Psychology, Univ. Victoria, P.O. BOX 3050, Victoria, British Columbia, Canada, V8W 3P5.

Kindling or seizure induction in the hippocampus produces retrograde, but not anterograde, amnesia on the radial arm maze (e.g., Olton & Wolf, Behav. Neural Biol., 33: 437, 1981). The present study examined the effects of kindling and pretraining stimulation-induced seizures (SIS) in the medial perforant path (MPP), septum or amygdala, and posttraining SIS in the MPP on place learning in the Morris water maze.

Bipolar electrodes were implanted in the MPP, septum or amygdala and rats were kindled to three stage 5 seizures. Control rats were handled in a similar manner without receiving stimulation. Rats were trained (8 days, 4 trials/d) to locate a submerged platform maintained in a constant position in a pool of cool (22±1°C), opaque water. SIS were triggered in half the rats 25-45 min. prior to training, and a group of MPP rats received SIS immediately posttraining. Kindling *per se* did not produce place learning deficits, regardless of the site of stimulation. Pretraining SIS in the MPP or septum produced severe place learning deficits, with rats taking longer to locate the escape platform and failing to show a quadrant preference during the probe trial. Pretraining SIS in the amygdala produced a small but significant impairment of place learning. Pretraining SIS in all locations failed to impair the rats' ability to swim to a single visible platform. Posttraining SIS of the MPP also impaired place learning. These results demonstrate that both pre- and posttraining SIS in the limbic system, but not kindling, impair place learning in the Morris water maze.

193.6

STRENGTH AND DIRECTION OF LATERALITY: EFFECTS ON SPATIAL LEARNING N.S. Waters, L.M. Schrott, G.W. Boehm*, G.D. Rosen, G.F. Sherman, A.M. Galaburda, and V.H. Denenberg Biobeh. Sci. Grad. Deg. Prog., U. Conn., Storrs, CT 06269, and Dept. Neurol., Harvard Med. School and Beth Israel Hosp., Boston, MA 02215.

177 mice of 15 NXSM recombinant inbred lines (Eicher & Lee, *Gen.* **125**:431-446) and the two parental lines were tested on a battery of behavioral tests, including the lateral paw preference (LPP) test (Waters & Denenberg, *Phys. Beh.* submitted), the Collins paw preference test (Collins, *J. Hered.* **59**:9-12), and the Morris water escape test. The latter consisted of 5 days of standard testing followed by one day in which the escape platform was moved to a new location. The animals were sacrificed and examined for the presence of cortical ectopias (Sherman et al., *Acta Neuropathol.* **74**:239-242). Performance on the Morris maze was analyzed in terms of strength or direction of paw laterality, and the presence of cortical anomalies. The Collins paw measure was not associated with spatial learning, but LPP was. Right pawed ectopics were significantly impaired in acquisition of the task. This finding coincides with the finding of Denenberg et al. (*Neurosc. Abs.* **16**:450; *Br. Res.* submitted) that ectopic animals who are left pawed on the Collins paw preference test performed better on a simple water escape task. Strength of LPP lateralization did not affect Morris maze acquisition, but strongly lateralized animals showed significant improvement in learning the new location over initial learning, while weakly lateralized subjects did not. This improvement indicates acquisition of a learning set (Whishaw, *Phys. Beh.* **35**:139-143), and that highly lateralized individuals acquire this set more easily than less lateralized subjects. Supported, in part, by NIH grant HD20806.We wish to thank Dr. E. Eicher, who supplied the RI mice.

193.7

CAUDATE NUCLEUS AND HIPPOCAMPAL UNIT RECORDINGS IN RATS PERFORMING A SPATIAL NAVIGATION TASK. S.I. Wiener, B. Delord* and A. Berthoz. CNRS Laboratoire de Physiologie Neurosensorielle URP2, Paris, FRANCE.

The caudate nucleus and the hippocampus have been implicated in cognitive processing of spatial information in egocentric and allocentric reference frames, respectively. To better understand how these complementary processes may be performed in parallel, simultaneous recordings were made from fine wire multiple electrodes in these two structures in freely behaving rats.

In an uncovered 50 cm cubic box with water reservoirs in the corners and in the center of the floor, Long-Evans rats (under 23.5 hr/day water deprivation) were rewarded at the corners on a win/shift basis with interleaved rewards at the center reservoir through automated control. Head position, heading angle and visits to the reservoirs were monitored with a video system detecting two light bulbs on the headstage as well as with photodetectors positioned over the reservoirs.

Neurons in the rostrodorsal portion of the caudate nucleus (AP 4.2, ML 2.2 relative to bregma) had reductions in their tonic level of activity synchronous with the departure from the water cups. The caudate neuron activity was spatially selective. In contrast, the hippocampal neurons (AP 2.0, ML 2.0), as previously reported, had spatially selective increases in activity during goal approach movements.

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193.8

THE RELATION OF ROTATION TO EGOCENTRIC AND ALLOCENTRIC SPATIAL LEARNING IN THE RAT. D.P. Crowne, P.A. Tokrud* and P. Brown*, Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, CANADA N2L 3G1.

There is a well-grounded relation between amphetamine-induced rotation and the learning and retention of left-right discrimination. In this experiment, we asked whether that relation is a more general one, extending to allocentric spatial learning or is limited to tasks requiring egocentric spatial orientation. Rats were sucessively trained on 2 spatial tasks: navigation in the Morris water maze, requiring place localization by means of distal (allocentric) cues, and delayed spatial alternation in a water T maze, an egocentric task considerably more difficult than left-right discrimination. Rotational behavior was established following injections of *d*-Amphetamine sulfate, and rats were classified in 3 groups: nonrotators, mid-rotators, and strong rotators. Rotational behavior was not related to latency, quadrant entries, or heading error in water maze learning but was significantly related to delayed spatial alternation learning but not retention. Nonrotators made nearly 40% more errors in reaching criterion than rotators. Our results extend the generality of the earlier left-right discrimination findings in showing that rotation is also related to difficult egocentric spatial learning. But they emphasize that if a rose is a rose is a rose, spatial is not spatial is not spatial (necessarily).

193.9

SPATIAL NAVIGATION IN THE SPLIT-BRAIN RAT. M.F. Novotny and D.P. Crowne. Dept. of Psychology, Univ. of Waterloo, Waterloo, Ont. N2L 3G1, CANADA.

Hemispheric specialization and interocular transfer of spatial learning in the Morris water maze was examined. Rats underwent split-brain (optic chiasm and corpus callosum), chiasm (optic chiasm), callosum or chiasm-sham surgeries. They were tested monocularly on the distal cue version of the water maze. Performance of the hemispheres did not differ significantly. The lack of hemispheric differences may have been due to the subcortical involvement in spatial navigation. Overall, the split-brain rats were significantly slower and entered more non-target quadrants than the other 3 groups, ($p<.01$). All groups displayed a decrement in performance when the platform location was reversed, which demonstrated the animals had learned the task. The results suggested the isolation of the hemispheres produced a visuospatial deficit. A lack of transfer for latency, but not quadrant entries was found when the 2nd eye was tested, and did not differ as a function of group.

193.10

DOES LEARNING PRODUCE LONG-LASTING CHANGES IN PERFORANT PATH-DENTATE EVOKED POTENTIALS? J.M. Hoesing, R.W. Skelton, J. Evanson & R.J. Sutherland. Depts. of Psychol., Univ. of Lethbridge, Lethbridge, AB, T1K 3M4 and Univ. of Victoria, Victoria, BC, V8W 3P5, Canada.

The hypothesis that long-lasting changes in synapse strength (LTE) in the hippocampal formation represent the neural bases of place learning was examined. Perforant path-evoked potentials in the dentate gyrus of rats were examined during exposure to a novel, complex environment. A robust, long-lasting increase in the size of the population spike and a less consistent increase in the field EPSP was observed. Further investigation demonstrated that the increases in evoked potentials were not due to changes in the behaviour of the rat at the time the stimulus pulses were delivered. In a second experiment rats with electrolytic lesions in the perforant path were unable to acquire place navigation in the Morris water task. Rats with ibotenic acid lesions in the vicinity of the perforant path were not impaired in the Morris water task. In a third experiment, perforant path-dentate evoked potentials were examined during place learning in the Morris water task. A small, but statistically reliable, increase in the field EPSP was observed, but the size of the population spike was unchanged.

These results provide some support for the notion that LTE-like changes in the perforant path-dentate synapses underlie certain forms of learning in the rat.

193.11

IS LTE IN THE HIPPOCAMPUS NECESSARY FOR PLACE LEARNING? R. J. Sutherland, H. C. Dringenberg, J. M. Hoesing and R. W. Skelton. Depts. of Psychol., Univ. of Lethbridge, Lethbridge, AB, T1K 3M4 and Univ. of Victoria, Victoria, BC, V8W 3P5, Canada.

Recent evidence suggests that activity in hippocampal circuitry provides animals with a representation of information that is essential for recent place learning. Long-lasting changes at synapses (LTE) in the hippocampus may be the basis for the initial storage of place memories. To examine this idea we interfered with these processes by means of bilateral LTE "saturation" of the perforant path-dentate connection with repeated, tetanic perforant path stimulation and by means of injections of the NMDA receptor antagonist MK-801.

At least 2 wk prior to tetanic stimulation or drug treatment rats were trained to swim to a hidden platform in the Morris water task. Each day the goal was placed in a new location. Neither saturation (as measured by induction to maximal population spike area and field EPSP slope) nor MK-801 treatment (0.05, 0.08, or 0.1 mg/kg, ip) affected learning to locate the platform position each day. The rats were then tested in a pool in a new room. Despite saturation or MK-801 treatment, they showed normal acquisition of navigation to the hidden platform in one trial. The rats displayed normal retention of this information for 24 hr. In rats that are naive to the task, LTE saturation or MK-801 treatment impairs initial performance.

These results suggest that place learning can proceed normally despite interference with perforant path-dentate LTE provided the animals have acquired the procedural requirements or behavioural supports for the task.

193.12

PLASTICITY OF RAT HIPPOCAMPAL PLACE CELL RESPONSIVENESS RELATIVE TO LOCATION OF REWARDING ICSS DELIVERY. T. Kobayashi*, M. Fukuda, T. Ono and S. Eifuku*. Department of Physiology, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama 930-01, Japan.

Electrophysiological studies show that hippocampal pyramidal cells fire when the animal is in a particular region of the environment. It has been demonstrated that spatial firing patterns of hippocampal cells are not fixed, but can be changed readily. The plasticity of the hippocampal cells might be a factor in spatial learning. Using a spatial navigation task which can be easily acquired within several trials, we tested several types of plastic responsiveness of place cells.

In a circular open field, rats were trained in a spatial navigation task that required them to visit two invisible reward areas, alternately, to get electrical stimulation of the lateral hypothalamus as reward. During the task, unit activity from the dorsal hippocampus was recorded. Some hippocampal cells of naive rats gradually developed location specific firing as the task was acquired. After learning the task, the location of a reward area was moved into a place field, after which the hippocampal cells tended to fire within and immediately before entering the new reward area. The results suggest plasticity of the place cells in the hippocampus, and an important role in learning and memory of place, and that some of the plastic neurons might not be place neurons per se, but their activity might reflect integrated inputs.

193.13

PLACE FIELDS OF SINGLE HIPPOCAMPAL CELLS ARE SMALLER AND MORE SPATIALLY LOCALISED THAN YOU THOUGHT. M. Recce*, A. Speakman and J. O'Keefe*. Department of Anatomy and Developmental Biology, University College, London, WC1E 6BT U.K.

Hippocampal complex spike cells in freely moving rats have spatially specific firing properties or place fields which represent the animals spatial memory of an environment. Many methods have been used to analyse the correlates and plasticity of place fields. Previously published descriptions of place fields have reported a range of sizes, activity in more than one contiguous region (double fields), and the absence of topography. It is clear that all of these descriptions rely on the assumption that single cells have been successfully isolated.

Using the newly developed, high resolution, tetrode recording technique (Recce and O'Keefe, 1989, Soc. Neurosci. Abstr. p1250) we find several differences from previous reports. In particular fields are smaller, restricted to one contiguous region of the environment and in some cases show a degree of topography. Our data include 48 place cells recorded from 11 rats in one of three different environments.

By resampling our tetrode data under the lower resolution conditions corresponding to earlier methods, the previously reported field properties emerged. For example, there are several cases in which neuronal waveforms separable by the tetrode method were confounded when examining only one or two electrodes (stereotrode). In these cases the confounded waveform was associated either with a double field or with a larger place field. In light of these results, we strongly advise the use of the tetrode method in all experiments where isolation of hippocampal single units is necessary.

(Supported by the Medical Research Council of the U.K.)

193.14

A NEURAL NETWORK MODEL OF HIPPOCAMPAL PLACE CELLS THAT DEMONSTRATES PLASTICITY OF PLACE FIELDS. Robert E. Hampson, Don W. Drawbaugh*, and Sam A. Deadwyler, Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1083.

Hippocampal place cells demonstrate place-specific firing in a variety of experimental tasks. Whether these "place fields" are static maps of spatial locations or representations which can be shifted to new positions by varying behavioral stimuli has been discussed previously (Deadwyler *et al.*, Psychobiology **17**:221-227, 1989). We have developed a neural network based on hippocampal neuroanatomy to simulate place fields and study theories of place cell functions.

The neural network model is organized in a layered design with input, output and hidden layers (Drawbaugh, '91 Intl. Joint Conf. on Neural Networks **II**:717, 1990). Inputs consist of encoded positions in a simulated environment, and outputs that correspond to hippocampal place cells. Once the model is "taught" all of the possible navigational paths through the environment, the output demonstrates emergence of place-correlated firing as the model encounters all possible positions. Once the initial set of "place fields" are expressed in this manner, they remain unchanged until the model is reset (*i.e.* to a new environment). An optional input consists of a bias which denotes significance of a location or the "satisfaction value" associated with occupying specific locations (such as obtaining food or water). When the significance cue is used, only the locations labelled with the highest significance values will demonstrate place fields. Furthermore, when one location is significantly higher in significance, than all others, only that location produces place fields.

Thus, the model demonstrates both the static and plastic features of place fields and suggests a mechanism whereby the hippocampal spatial map can be adjusted to encode location relative to its significance for the subject. Comparison with animal derived experimental results will be presented.

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193.15

LAYERED NETWORK MODELING OF CROSS-MODAL TRANSFER EFFECTS ON RELEARNING AFTER VISUAL CORTEX LESIONS IN RATS. E.R. Delay, N.J. Matsushima*, and D.Q. Nguyen*. Dept. Psychology., Regis College, Denver, CO 80221.

Kehoe's layered network model of associative learning (Psychol. Rev., 95:411-433, 1988) combines a threshold concept for adaptive units with the Rescorla-Wagner delta rule. This model was applied to data from visual decorticate rats given postoperative training to relearn a brightness discrimination using procedures previously described (Delay & Rudolph, Neurosci. Abs., 15:762, 1988). The raw data indicated that postoperative training with an auditory intensity S+ reduced the visual relearning deficit more than additional training with the preoperative brightness S+. Model simulations suggested that visual cortex lesions did not alter thresholds of adaptive units nor total connective weight of the US. However, the rate of change parameters, alpha(j), were reduced by the cortical lesions. Post-operative training with auditory intensity cues before retraining on the brightness discrimination increased the rate parameter to a greater extent than comparable training with the original visual cue. The model suggests that cross-modal transfer effects on recovery may reduce lesion deficits through processes using convergent input to common adaptive units.

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193.16

NEURAL NETWORK ERROR SURFACES: LIMITATION OF NETWORK SIZE, INPUT SIGNAL DYNAMICS, AND METAKNOWLEDGE IN MEMORY STORAGE. G. J. Mpitsos. Hatfield Marine Science Center, Newport, OR 97365.

Simple neural networks may provide access to questions that would be impossible to address presently in biological systems. For example, using error backpropagation, we found that: (1) The ability of networks to learn increases as the size of the network increases, but only with marginally larger networks; network size eventually prevents learning, even for simple tasks. (2) These networks learn nothing about the dynamics of their input signals, but the dynamics nonetheless has a decisive role in the learning process. (3) Learning in the presence of noise enhances later learning when no noise is present.

The present work analyzed error surfaces, arising from the responses of the network to systematic changes in the strength of its synapses, to account for the above findings: (1) As network size increases, high error volumes in multidimensional space increase exponentially by comparison to low error regions. Error gradients become increasingly flatter, the length of the synaptic weight-error trajectory increases, and low-error regions become increasingly difficult to find. (2) Chaotic point processes (as might occur in spike trains) and random noise are statistically identical, but the short-term determinism of chaos provides for efficient searches of error space. Limit cycles and continuous signals pose certain problems. (3) Noise-exposed and noise-unexposed networks reach equally low errors during learning. But networks exposed to noise learn more of the teacher error-surface than do networks not exposed to noise. Such noise-exposed networks are able to learn future tasks rapidly when the tasks involve error surfaces having some similarity to the surface in the first task. Thus, synaptic weights not only encode information about a given task, but also a form of metaknowledge relating to how they reached their settings. Biological analogies are presented. Supported by AFOSR-89-0262

193.17

A MODEL OF CONDITIONING BASED ON CS FACILITATION OF US-ELICITED NEURONAL ACTIVITY. D.J. Weisz. Departments of Neurological Surgery and Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

A major goal of the model systems approach to learning and memory is the identification of critical site(s) at which repeated pairings of conditioned (CS) and unconditioned stimuli (US) result in an alteration of neuronal activity such that the CS eventually comes to elicit overt conditioned responses (CRs). The most likely candidates for these critical sites are those that are necessary for normal conditioning and, in addition, receive information about both the CS and US. This research has focused on CR development as the primary goal, and much progress has been made in many model systems in identifying structures that are critical for CR development. Furthermore, electrophysiological studies have identified neuronal populations that exhibit activity that correlates with the appearance of CRs.

In these studies, however, little attention has been given to the effects that the CS can exert on behavioral and neuronal events elicited by US presentation at the very beginning of training and long before the development of overt behavioral CRs. In contrast to the research that has focused on the appearance of overt CRs, the present hypothetical model is based on the effects the CS exerts on US-elicited activity from the very first pairing. Specifically, this model argues that the neuronal plasticity that underlies behavioral conditioning develops out of CS alterations of US-elicited neuronal responses. Not only must a CS and US converge on a neuron, but they must do so in a way such that the CS can alter the neuron's response to the US. Furthermore, the magnitude of the influence predicts the potential for plasticity at that site. For example, improper timing or weak stimulus intensity could reduce the CS's influence on US-elicited activity and therefore slow or prevent neuronal plasticity. Although the model has been formally developed using data from studies of the conditioned nictitating membrane response, it is easily applied to other model systems. (Supported by MH 42800)

193.18

HEBB RULE IS NECESSARY BUT NOT SUFFICIENT FOR PLASTICITY OF FUNCTIONAL CONNECTIONS IN THE ADULT MONKEY - BEHAVIORAL RELEVANCE IS REQUIRED. E. Ahissar, M. Ahissar, E. Vaadia and M. Abeles Dep. of physiology, The Hebrew University - Hadassah Medical school, Jerusalem 91010, Israel.

Multiple single units were extracellularly recorded in the auditory cortex of adult monkeys. The aim of the study was to find the crucial conditions for plasticity of functional connections (F.CN.) as revealed by cross-correlation histograms. The monkey was trained to touch a switch for the trial to start. Then a train of short duration (30ms) auditory stimuli (US) were delivered. After a few seconds (0.8 to 2.2) the characteristic of the US was changed and the monkey had to remove his hand quickly to gain a drop of juice. 77 pairs of neurons were studied under three conditioning paradigms: **Full-, Hebbian- and Pseudo-conditioning.** In each pair, one of the neurons was defined as the "CS" neuron (CSN) and the other as the "CR" neuron (CRN). The F.CN. between the CSN to the CRN was studied. An auditory stimulus which was most effective in activating the CRN was used as the "US". **Contiguity** between the firings of the two neurons was caused by delivering the US every time and immediately after the CSN fired a spike, causing the CRN to fire a spike at high probability. **Behavioral relevance** of the US was achieved during the performance of the task. During Hebbian-cond no task was performed and during Pseudo-cond no contiguity was present (table). Strengthening of F.CN.s, as expressed by **Gain** (= strength after / strength before), was evident only in **Full-conditioning** (table). We believe that changes in F.CN.s represent changes in the relevant neural network configuration. These results, thus, suggest that both contiguity and behavioral relevance are necessary for learning at the network level.

	Full	Hebbian	Pseudo
Contiguity	+	+	-
Behav. rel.	+	-	+
Gain (S.D)	**2.8**(1.5)	**1.3**(.5)	**0.9**(.2)

193.19

A NEURAL NETWORK MODEL OF ADAPTIVELY TIMED REINFORCEMENT LEARNING AND HIPPOCAMPAL DYNAMICS. Grossberg, S. and Merrill, J. W. L.* Center for Adaptive Systems, Boston University, Boston, MA 02215.

A neural model is described of how reinforcement learning is adaptively timed and modulates the course of recognition learning, attention switching, memory search, selective forgetting, and the timing of goal-oriented actions. The model suggests how NMDA receptors in the dentate-CA3 region of the hippocampus may participate in adaptively timed reinforcement learning, and helps to explain the changes in trace conditioning, delay conditioning, and reversal conditioning that are caused by hippocampal ablations. The model distinguishes between cerebellar influences on motor learning and hippocampal influences on adaptive timing of reinforcement learning. The model clarifies how hippocampal damage eliminates attentional blocking and causes symptoms of medial temporal amnesia. It suggests how normal acquisition of subcortical emotional conditioning can occur after cortical ablation, even though extinction of emotional conditioning is retarded. The model also distinguishes emotional conditioning from conditioning of adaptive timing. The model suggests how an increase in US duration increases amplitude but not timing of emotional conditioning, and how an increase in CS intensity but not US intensity "speeds up the clock". Computer simulations fit parametric conditioning data from the rabbit NMR paradigm, including primary and secondary adaptively timed conditioning, and conditioning using multiple ISIs, gradually or abruptly changing ISIs, and partial reinforcement.

193.20

CONJECTURE UNIFYING THREE "CONSTANTS": SHORT TERM MEMORY CAPACITY, NUMBER OF PYRAMIDAL CELL INPUT SOURCES, AND ALPHA RHYTHM PERIOD.
R. B. Glassman. Dept. of Psychology, Lake Forest Col., Lake Forest, IL 60045.

The fact that immense long term memory evolved suggests that short term memory holds only 7±2 chunks for some important reason. If every combination of n chunks in STM is tested for meaning, this requires

$$T = \sum_{r=0}^{n} C(n,r) = 2^n \text{ tests.}$$

If n=7, then T=128. Pyramidal neurons have about 7±2 segregated input sources in hippocampus and cortex (i.e. cortical layers). If each meaning-test involved briefly activating a combination of inputs (differently in each member of a momentary complement of many neurons), sufficient recovery from graded potentials might allow about one test every 0.6-1.0 msec, thus 128 tests in 1/13 - 1/8 sec, the period of the alpha rhythm. This entails a conception in which underlying alpha paces desynchronized cortical areas lit by attention. During every cycle, a complement of pyramidal neurons awaits a match between exogenous inputs, due to a set of 7±2 chunks, and myriad memory-fragments in endogenous inputs to the same cells.

LEARNING AND MEMORY—PHARMACOLOGY: EXCITATORY AMINO ACIDS

194.1

NMDA RECEPTOR ANTAGONIST MK-801 INHIBITS ACQUISITION OF CLASSICAL FEAR CONDITIONING IN GOLDFISH. R.E. Davis, X. Xu,* P.D. Klinger.* Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan, 48104-1687.

Investigations in mammals indicate that selective antagonists of NMDA (N-methyl-D-aspartate) receptors inhibit acquisition and/or retention of learning but not the expression of learning. The results are consistent with the thesis that NMDA receptor functions mediate activity-driven synaptic plasticity which may be the neuronal basis of some forms of learning. The present experiments examined the effects of the NMDA antagonist MK-801 on learning and memory in the goldfish. Classical conditioning of fear evoked by an electrical bodyshock US to a neutral light CS was employed as it is a rapidly acquired form of associative learning. The branchial suppression response (BSR), a defensive fixed action pattern, was measured as the response index of conditioning. Intracranial injection of .1 µg (ca. .01 mg/kg) or higher of MK-801 30 min prior to the training session impaired retention in a subsequent test session increasingly with the dose. Fish given 2 µg MK-801 immediately following the training session showed normal retention. Thus, the deficit produced by pretrial MK-801 was not a result of lasting behavioral toxicity - nor of a disruption memory consolidation. Giving .5 µg MK-801 during training and during testing did not improve retention, revealing no evidence of state-dependent learning. Giving .5 µg MK-801 only during testing did not impair expression of the conditioned BSR in the retention trials. The results suggest that pretrial MK-801 impaired retention by inhibiting acquisition and, moreover, that it inhibited acquisition by impairing learning processes as opposed to perception of the CS or US or performance of the BSR.

194.2

NMDA ANTAGONIST MK-801 BLOCKS ASSOCIATIVE FEAR CONDITIONING BUT NOT NONASSOCIATIVE SENSITIZATION OF CONDITIONAL FEAR.
J. P. DeCola, J. J. Kim & M. S. Fanselow. Psychology Dept., UCLA, Los Angeles, CA 90024.

Previously we demonstrated that administration of the NMDA antagonist APV, blocks associative fear conditioning. The present experiments assess the role of NMDA dependent processes in the sensitization of conditional fear. Sensitization can be demonstrated behaviorally as an enhanced response to a Conditional Stimulus (CS) when conditioning is proceeded by a series of Unconditional stimuli (US) alone preexposure trials. In our paradigm the sensitization pretreatment is done by exposing rats to a series of 15 (1s, 1 mA) footshocks in a distinct context the day prior to a single pairing of a novel CS, a unique context, and a shock US (1s, 1mA). The sensitized animals exhibit enhanced fear in the novel context as compared to a nonsensitized group. Control groups show that this effect is not due to generalization of fear between the two contexts. Administration of the NMDA antagonist MK-801 (.2 mg/kg, i.p.) during the sensitization treatment in the first context did not block the sensitization of conditional fear to the second novel context. However, it did block the acquisition of conditional fear to the context where the pretreatment shocks were delivered. These data indicate that similar to the NMDA antagonist APV, MK-801 blocks associative fear conditioning. Furthermore, this associative failure is not caused by an inability to process the US. Also, a nonassociative sensitization of fear exists that is mediated by an NMDA-independent process.

194.3

INFUSION OF AN N-METHYL-D-ASPARTATE ANTAGONIST INTO THE AMYGDALA BLOCKS EXTINCTION OF FEAR-POTENTIATED STARTLE.
W.A. Falls, M.J.D. Miserendino & M. Davis. Depts. of Psychology & Psychiatry, Yale Univ. Med Sch., 34 Park St., New Haven, Ct. 06508.

Paired presentation of a neutral stimulus, such as a light, with an aversive stimulus, such as shock, leads to the acquisition of a variety of behavioral effects that are used to define a state of conditioned fear. If the same light is then presented in the absence of shock, it loses its ability to produce these behavioral effects. The reduction in conditioned fear is called experimental extinction and is thought to involve the formation of a new memory rather than a decay or erasure of the original memory. We have previously shown that intra-amygdala infusion of N-methyl-D-aspartate (NMDA) antagonists blocks the acquisition of conditioned fear-potentiated startle (Miserendino et al., *Nature*, 345, 716, 1990). Moreover, the amygdala is also important for extinction because intra-amygdala infusion of the non-selective excitatory amino acid (EAA) antagonist γ-D-glutamyl-glycine blocked extinction of fear-potentiated startle (Falls et al. *Neurosci. Abs.*, 1990, 316.6). The present study employed more selective EAA antagonists to further examine the role of amygdaloid EAA receptors in extinction of fear-potentiated startle.

Rats were implanted with bilateral cannulae aimed at the basolateral nucleus of the amygdala and one week later underwent training in which a light was paired with footshock. Five days after training, fear-potentiated startle was assessed by measuring the amplitude of noise-elicited startle in the presence or absence of the light. On the next two days, the rats were infused with either DL-2-amino-5-phosphonovaleric acid (AP5; 1.5, 6.25, 12.5, 25 nmol), 6-cyano-7-nitroquinoxoline-2-3-dione (CNQX; 12.5 nmol) or vehicle immediately before receiving 30 light-alone extinction trials. One day later, fear potentiated startle was again assessed without drug infusion.

AP5 dose dependently blocked extinction of fear-potentiated startle whereas CNQX did not. This effect showed anatomical specificity because AP5 infused into the interpositus nucleus of the cerebellum did not block extinction. Moreover, these results could not be explained by reduced salience of the light or by cytotoxic effects of the compounds tested. Therefore, these data implicate and NMDA-dependent process within the amygdala in the extinction of conditioned fear and may provide the basis with which to begin studying the mechanisms of extinction.

194.4

INFUSION OF AN N-METHYL-D-ASPARTATE ANTAGONIST INTO THE AMYGDALA BLOCKS ACQUISITION OF FEAR-POTENTIATED STARTLE TO AN AUDITORY CONDITIONED STIMULUS.
M.J.D. Miserendino, S. Campeau & M. Davis, Psychiatry Dept., Yale University, New Haven, CT. 06508

The amygdala appears to be critically involved in a variety of aversive conditioned responses. NMDA receptors, which are implicated in neural plasticity underlying learning, occur densely in the lateral and basolateral nuclei of the amygdala. We previously showed that intra-amygdalar infusion of NMDA-selective antagonists blocked acquisition of fear conditioning to a visual stimulus as measured by fear-potentiation of the acoustic startle reflex (Miserendino et al. Nature, 345 716-718, 1990). We now examine the generality of this finding by testing whether an NMDA receptor-mediated process in the amygdala might similarly be involved in the acquisition of fear-conditioning to an auditory conditioned stimulus. Rats were implanted with bilateral cannulae aimed at the basolateral nucleus of the amygdala. Following recovery, rats received two training sessions on consecutive days, each session consisting of 5 noise-footshock pairings (noise=2 KHz bandwidth noise, 70 dB; footshock=0.6 mA; ISI=2 min). Immediately prior to training, rats were infused with the competitive NMDA antagonist DL-2-amino-5-phosphonovaleric acid (AP5; 3.125, 12.5, 50.0 nmoles) or ACSF vehicle. One day later, rats were tested for conditioned fear by comparing the magnitude of the startle reflex to a noise burst presented either alone or in the presence of the previously fear-conditioned 2 KHz noise. Local infusion of AP5 blocked acquisition of conditioned fear to this auditory stimulus, an effect that was linearly related to dose ($F(1,20)=11.03$, $p<0.005$), without altering reactivity to the footshock unconditioned stimulus or baseline startle amplitude in the absence of the conditioned stimulus. These data extend our previous findings and support the idea that across different sensory modalities, the amygdala may be a common locus of neural plasticity for associative fear conditioning.

194.5

TIME-DEPENDENT IMPAIRMENT AND ENHANCEMENT OF INHIBITORY AVOIDANCE LEARNING BY INTRA-AMYGDALA 2-AMINO-5-PHOSPHONOPENTANOIC ACID (AP5) INFUSION. D.L. WALKER and P.E. GOLD. Neuroscience Graduate Program & Department of Psychology, University of Virginia, Charlottesville, VA 22903.

Peripheral and central injections of *N*-methyl-D-aspartate (NMDA) receptor antagonists disrupt performance on a variety of learning and memory tasks. The present experiment examined the effects on memory of direct intra-amygdala injections of AP5. Rats received bilateral intra-amygdala injections of AP5 (1, 10, 25, or 50 nmol) 15 min before or immediately after inhibitory avoidance training, or 15 min prior to test (48 h later). Pre-training (25 and 50 nmol) but not pre-testing (50 nmol) injections of AP5 significantly impaired retention. The amnesia was not secondary to seizures (though AP5 increased the occurrence of low-frequency, high-amplitude EEG) or to drug-induced analgesia. Injections dorsal to the amygdala were ineffective. Surprisingly, post-training injections of AP5 enhanced performance on the retention test -- i.e., AP5 impaired (pre-training) or enhanced (post-training) retention depending on a 15-min difference in time of drug administration. One interpretation is that the impairment reflects a requirement for NMDA receptor activation during acquisition, while the enhancement reflects post-training regulation by NMDA receptors of other neurotransmitters (e.g., noradrenaline) which retroactively modulate memory storage. Or, post-training AP5 may prevent subsequent learning that would normally produce retrograde interference for the learned avoidance response. These results indicate that antagonism of NMDA receptors in the amygdala influences acquisition and storage of a learned avoidance response. [Supported by ONR (N0001489-J-1216), NIA (AG 07648), and NSF (BNS-9012239); DLW is an NIMH predoctoral trainee (5-T32-MH18411)].

194.6

PRETEST INTRA-AMYGDALA INJECTION OF LIDOCAINE OR GLUTAMATE ANTAGONISTS IMPAIRS RETENTION PERFORMANCE IN AN INHIBITORY AVOIDANCE TASK. K.C. Liang, Dept. Psychology, Natl' Taiwan Univ., Taipei, Taiwan 10764, R.O.C

The amygdala is implicated in memory processing of emotional experience. However, whether the amygdala may be a neural substrate underlying long-term storage of affective memory remains controversial. Such a view implies that memory retrieval after different retention intervals would be equally affected by altering amygdala functioning prior to retention tests. In view of the evidence that local anesthetic infusion produces reversible neural inactivation and that memory processing in the amygdala involves glutamate transmission, the present study examined the effects of pretest intra-amygdala injections of lidocaine or various glutamate antagonists on retention of an inhibitory avoidance response tested at different times after training.

Male Sprague-Dawley rats were implanted with cannulae aiming at the basolateral amygdala. They were trained on a one-trial step-through inhibitory avoidance task with a 1.25 mA/1 s footshock and tested for retention 1, 2 or 21 days after training. Drugs (1 µl) were infused into the amygdala 5 min prior to the retention test. In Exp. I, rats received vehicle (Veh) or 2% lidocaine. Results indicated that lidocaine significantly impaired retention when given before a 2-day retention test, but had no significant effect when given before a 21-day retention test. In Exp. II, rats received Veh, an N-methyl-D-aspartate antagonist--dl-2-amino-5-phosphonovaleric acid (APV, 1.25 µg), or a kainate/quisqualate antagonist--6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 1.0 µg dissolved in DMSO). Results indicated that in a 1-day retention test, pretest CNQX impaired retention performance significantly, but pretest APV failed to produce a significant deficit. In contrast, neither CNQX nor APV affected retention performance when given prior to a 21-day retention test. These findings suggest that successful retrieval of an emotion-related memory requires the integrity of amygdala functioning for a limited period of time after its formation and eventually becomes independent of the amygdala. Such findings are inconsistent with a notion that the amygdala is the permanent storage site of memory. Supported by a grant NSC 80-0412-B-002-148 from the National Science Council of the Republic of China.

194.7

POSTTRAINING GLUTAMATE RECEPTOR BLOCKADE IMPAIRS OLFACTORY LEARNING IN NEONATAL RAT PUPS. D. A. Weldon and C. M. Lorusso*. Dept. of Psychology, Hamilton College, Clinton, NY 13323.

Norepinephrine, dopamine, and glutamate receptors appear to play an important role in early olfactory conditioning in neonatal rats. Administration of receptor antagonists for these neurotransmitters before training can prevent learning. The critical time for the effect dopamine receptor antagonists, however, is during a posttraining period (Weldon, et al., Behav. Neurosci. 1991). The present experiment was designed to investigate whether posttraining administration of an N-methyl-D-aspartate (NMDA) receptor antagonist would impair olfactory conditioning.

Six day old Sprague-Dawley rat pups were exposed to anise odor paired with tactile stimulation (stroking the skin with a paint brush) for a 10 minute conditioning session. Pups received either vehicle (saline) or the noncompetitive NMDA antagonist MK-801 (0.3 mg/kg, i.p.) either 30 minutes before or immediately after the conditioning period. The next day, the pups were placed in a testing chamber for a total of 6 minutes, and the amount of time that they spent over the conditioned odor was recorded. Treatment with MK-801 produced a statistically significant reduction in the conditioned preference for the anise odor, regardless of whether the drug was administered before or after the training period. The data indicate that posttraining activation of NMDA receptors is required for normal olfactory learning to occur in neonatal rats.

194.8

THE NMDA RECEPTOR/CHANNEL COMPLEX AND ASSOCIATIVE LEARNING: OPPOSITE EFFECTS OF PCP AND D-CYCLOSERINE. L.T. Thompson & J.F. Disterhoft. Department of CMS Biology, Northwestern University Medical School, Chicago, IL 60611.

Activation of the neuronal NMDA receptor may play a critical role in many forms of associative learning, via receptor-mediated changes in intracellular calcium levels. We evaluate here the effects both of non-competitive blockade of the receptor-associated cation channel and of allosteric agonism of the glycine coagonist binding site on the receptor on long-interval trace eyeblink conditioning in rabbits. Previous work from our laboratory has shown that this task is hippocampally-dependent (Moyer *et al., Behav. Neurosci.*, 1990, *104*: 241-250). Recently, we demonstrated that a monoclonal antibody (B6B21) binding to the NMDA receptor's glycine coagonist site in hippocampus facilitates acquisition of this task (Disterhoft *et al., Soc. Neurosci. Abtr.*, 1990, *16*, 264) and that acquisition alters the number of binding sites for the NMDA antagonist MK-801 in rabbit hippocampus (Thompson *et al., Soc. Neurosci. Abtr.*, 1990, *16*, 263).

Groups of 6 rabbits per dose and treatment were trained to a behavioral criterion of 80% conditioned responses (CRs) using 500 mec trace eyeblink conditioning, with pseudoconditioned animals as controls. Phencyclidine (PCP, a common drug of abuse), which non-competitively blocks the NMDA receptor's cation channel, blocked aquisition of the task in a dose-dependent fashion. Doses as low as 1 mg/kg completely blocked conditioning, while unconditioned eyeblink responses were unaffected. D-cycloserine, a partial agonist at the strychnine-insensitive glycine binding site, facilitated acquisition of the task without altering unconditioned responses. Doses of 6 mg/kg of D-cycloserine improved acquisition rates by more than 50%, similar to the behavioral improvement observed after intraventricular treatment with B6B21. Both PCP and D-cycloserine cross the blood-brain barrier readily after systemic administration.

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194.9

EFFECTS OF A GLUTAMATE AGONIST AND ANTAGONIST ON WEAK MEMORY FORMATION IN CHICKS. D.W. Lee, A. Hittelman*, S.Shrawder*, E.L. Bennett, & M.R. Rosenzweig. Department of Psychology, University of California, Berkeley, CA, 94720.

In rodents, glutamate (GLUT) enhances memory, whereas GLUT antagonists (such as AP5) impair memory. In 2-day-old chicks, memory for a 1-trial peck avoidance task using 100% methyl anthranilate (MeA, a liquid aversant) is impaired by both GLUT and AP5. The lack of evidence for enhancing effects of GLUT in chicks may be due to a ceiling effect obtained with very strong training. To allow for memory enhancement as well as impairment, we investigated the effects of GLUT and AP5 using weak training (10% MeA).

Bilateral i.c. injections (10 µl/hemisphere) were made into the region of the IMHV 5 min pretraining using either saline (SAL); .001, .01, .1, 1, 10, 25, or 50 mM GLUT; or 250 pg or 250 ng AP5 per chick. When tested at 24 hr, memory for weak training is impaired by both GLUT (50 mM) and AP5 (250 pg and 250 ng) at the same doses that impair memory for strong training. Results with the lowest doses of GLUT indicated a trend towards memory enhancement. This was further tested by injecting either SAL or .001, .005, .01, or .05 mM GLUT. From these two experiments we conclude that, in the chick, GLUT does not produce significant memory enhancement.

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194.10

MEMORY FACILITATION BY NMDA RECEPTOR BLOCKADE

C. Mondadori, Ciba-Geigy Pharma Research Division, Basle Switzerland and L. Weiskrantz, Dept. of Experimental Psychology, University of Oxford, Oxford, England. (SPON: European Brain Behaviour Society)

A single administration of MK 801 (0.003 - 0.1 mg/kg i.p.) or CGP 37849 (0.01 - 3 mg/kg i.p.) 30 or 60 minutes before the training trial of a passive avoidance task reliably improved subsequent retention performance of mice after a retention interval of 3 days. Since similar memory facilitating effects were observed when the compounds were administered after the learning trial ("posttrial"), the effects on retention were not likely to have been due to indirect influences via modulation of perception, motivation, emotion etc. The posttrial facilitation effects were found to be time-dependent: they were still detectable even when an interval of 8, but not 16 hours was allowed between the learning trial and the treatment. The similarity of the results after competitive (CGP 37849) or noncompetitive (MK 801) NMDA-receptor blockade indicated that the observed memory effects were most likely mediated via NMDA-mechanisms.

194.11

WHAT ASPECTS OF A PASSIVE AVOIDANCE TASK ARE RESPONSIBLE FOR MEMORY FACILITATION BY NMDA BLOCKADE? L. Weiskrantz*, Dept. of Experimental Psychology, University of Oxford, Oxford, England, and C. Mondadori*, Ciba-Geigy Pharma Division, Basle, Switzerland. (SPON: European Brain and Behaviour Society)

When NMDA blockers are administered post-trial to mice, thereby avoiding possible interfering effects of the drugs at the acquisition stage, memory in a one-trial mild passive avoidance task is reliably enhanced (Mondadori et al., Exp. Br. Res., 1989, 75: 449). The present study analyses which features of the task govern the animal's behaviour, and which are important for the facilitating effect of MK-801 on retention. The results of behavioral manipulation of task components demonstrate that the memory of undrugged control animals is strongly based on perception of the floor of the apparatus, and is only weakly controlled by an instrumental response contingency, or by the spatial locus of the reinforcer. But the drug does not enhance this perceptual control. Instead, it facilitates memory for the spatial locus, and raises the influence of this factor from a sub-threshold to supra-threshold level. It follows from the results that whatever is important for control animals may be different from the factor on which this drug treatment operates.

194.12

DIFFICULTY DETERMINES THE REFERENCE OR WORKING MEMORY IMPAIRING EFFECTS OF EXCITATORY AMINO ACID ANTAGONISTS IN GERBILS. Maurer, S.A.*, Storch, F.E.*, Morris, H. and Boast, C.A. Wyeth-Ayerst Research, Princeton, NJ 08543.

Working memory (WM) and reference memory (RM) impairments have been reported following treatment with excitatory amino acid antagonists (EAAA) (Shapiro & Caramanos, Psychobiol., 1990, 18:231). We assessed the effects of a noncompetitive EAAA, MK801, a competitive EAAA, CPP, and the muscarinic antagonist, scopolamine, in two cognitive tasks with WM and RM components that vary in the relative degree of difficulty of the two components. Specifically, gerbils were trained on a split stem T-maze (Olton, Neurobehav. Tox. & Teratol., 1983, 5:635) in which WM is more difficult than RM and other gerbils were trained on a radial maze (Olton & Samuelson, J. Exp. Psychol., 1976, 2:97)) in which RM is more difficult than WM. In the T-maze, MK801 (0.1 mg/kg i.p., 30 min prior) selectively impaired WM. CPP (30 mg/kg i.p., 2 hr prior) and scopolamine (0.3 mg/kg i.p., 30 min prior) impaired both components of the T-maze, but the magnitude of the effect was greater on WM than on RM. In the radial maze all three drugs impaired both components, but the magnitude of the RM impairment was greater than that of the WM impairment. These data indicate 1) that both RM and WM can be impaired by EAAA and 2) that apparent selective effects on either WM or RM can be due to the degree of difficulty of each component of a given task.

194.13

NMDA ANTAGONIST AP5 IMPAIRS SPATIAL WORKING MEMORY PERFORMANCE IN TRAINED RATS. Z. Caramanos and M.L. Shapiro. Dept. of Psychology, Mcgill University, Montreal, Quebec, H3A 1B1.

NMDA receptor activity is necessary for inducing glutamate-dependent LTP. Non-competitive NMDA antagonist MK801 (0.06 mg/kg i.p.) blocks acquisition of spatial working memory (WM) in a radial arm maze (RAM) task. However, MK-801 did not impair WM performance in rats pre-trained for 34 days (Shapiro & Caramanos, 1990). Thus, NMDA-dependent synaptic plasticity may be required for establishing the neural representation of a spatial environment, but not for WM operations upon such a representation. We now report that the competitive NMDA antagonist AP5 does impair spatial WM performance in trained rats. Rats were trained on a standard, 8 arm RAM task for 20 days. WM errors (returning to an arm visited during the first 8 choices of a trial) decreased from a mean of 1.9 during the first four days of training to 0.4 during the last 12 days. After training, rats were implanted with osmotic minipumps that infused either artificial CSF (ACSF) or a 33 Mm AP5 solution (0.5 ul/hr) into the left lateral ventricle, a regimen known to block LTP induction and spatial reference memory acquisition (Morris, 1989). Two days after surgery, the rats were tested for another 12 days. Rats given ACSF (n=8) continued performing the WM task well. However, the rats given AP5 (n=7) were impaired for the full 12 days of post-op testing, making a mean of 1.7 WM errors--performance similar to that shown at the start of training. The AP5 group also had mild motor deficits, but these disappeared after the first few days of testing. Thus, spatial WM can be impaired by competitive NMDA antagonists in trained rats. These results suggest that: (1) >20 days of training is necessary to establish spatial representations of the environment sufficient for WM performance given NMDA receptor blockade; or (2), spatial WM memory depends upon NMDA receptor mechanisms that are unaffected by the doses of MK801 that block spatial learning.

194.14

NMDA ANTAGONIST MK-801 IMPAIRS SPATIAL WORKING MEMORY PERFORMANCE IN A NEW, BUT NOT IN A FAMILIAR, PLACE. M.L. Shapiro & C. O'Connor* McGill Univ., Montreal, Quebec H3A 1B1.

NMDA receptor blockade impairs glutamate-dependent LTP induction and spatial learning in rats. MK-801 (MK), a non-competitive NMDA antagonist, impairs acquisition, but not performance, of spatial working memory (WM) in the radial maze (Shapiro & Caramanos 1990, Psychobio., 18(2):231). Does MK produce spatial learning deficits by impairing the rule learning needed for spatial tasks, or by blocking the acquisition of spatial representations? If impaired rule learning underlies WM acquisition deficits, then rats pre-trained and then given MK should have good WM in both familiar and new places. If, however, impaired *representational* learning underlies WM acquisition deficits, then pre-trained rats given MK should have impaired WM only in new places. To distinguish between these possibilities, rats were trained in a spatial WM task in a familiar room, tested with MK after criterion performance was achieved, and tested in an unfamiliar spatial environment with MK. Thirty two female SD rats were trained to perform a spatial WM task in an 8 arm radial maze. After reaching criterion performance, the rats were injected with either MK-801 (0.06 mg/kg) or saline (i.p., 30 min. before testing), and tested in the same maze for 5 days. WM performance was unimpaired by MK, as shown previously. The rats were then tested in the same task, but in a different room. Rats given MK performed the WM task poorly when tested in the new room, and did not improve in 15 days of testing. In contrast, control rats performed the WM task well, and consistently outperformed rats given MK ($F(1,29) = 22$, $p < .05$). Thus, MK-801 blocks storage of spatial representations, which are required for spatial WM performance, and the learning deficit produced by the drug is not an impairment in rule learning.

194.15

MK-801, AN NMDA RECEPTOR ANTAGONIST, IMPAIRS LEARNING OF A SPATIAL AND A NONSPATIAL DELAYED-NONMATCHING-TO-SAMPLE TASK. S. Das and R.M. Douglas, Departments of Psychology and Opthalmology, University of British Columbia, Vancouver, B.C. V6T 1Y7

The effects of a noncompetitive NMDA receptor antagonist, MK-801, were assessed on a spatial and a nonspatial delayed-nonmatching-to-sample task. Saline controls reached criterion on both tasks after 400 trials, whereas rats treated with .1 mg/kg MK-801 (i.p.) 30 min. prior to testing were unable to reach criterion on either task at a minimal delay (testing was discontinued after 900 trials). A lower dose (.06 mg/kg) did not impair acquisition of either task as compared to controls. In contrast to rats which received MK-801 prior to training, those rats given the higher dose of MK-801 (.1 mg/kg) after reaching criterion were not impaired and could remember the sample following a 30 sec. delay. These results suggest that NMDA receptor dependent processes might be necessary for learning the nonmatching-to-sample rule but are not involved in working memory.

194.16

EFFECTS OF THE NON-COMPETITIVE NMDA-RECEPTOR ANTAGONIST MK-801 ON SPATIAL MEMORY, MOTIVATION, AND MOTOR PERFORMANCE OF RATS IN A T-MAZE. P. Bialobok*, J.M. Ordy, T.M. Wengenack, W.P. Dunlap* (SPON: G.J. Thomas). Fisons Pharmaceuticals, Rochester, NY 14623, Univ. of Rochester, Rochester, NY 14642, and Tulane University, New Orleans, LA 70118.

The NMDA receptor complex for excitatory amino acid neurotransmitters may play a critical role in "long term potentiation" (LTP) and hippocampal neural mechanisms of learning and memory. The non-competitive NMDA receptor antagonist MK-801 has been reported to block the induction of NMDA-dependent hippocampal LTP and also inhibit learning and memory without effects on performance. One controversy in the NMDA receptor blockade strategy of correlating LTP and memory is the equivocal evidence for the selective effects of MK-801 on LTP, learning, and/or memory independent of general or non-associative effects on performance. The aims of this study were to evaluate the effects of MK-801 at low doses on spatial memory of rats in a T-maze in which drug effects on spatial memory can be dissociated from effects on motivation and motor performance. In a within-subject design, male, Sprague-Dawley rats were injected with either saline, 0.025, 0.05, 0.1, or 0.2 mg/kg, i.p. of MK-801 and tested 30 min post-injection in the T-maze for effects on spatial memory, at 10, 90, and 180 sec delays, and on start, choice, and goal speeds. According to analyses of variance followed by Newman-Keuls tests of significance, there was a significant and dose-dependent impairment of spatial memory, with increased start speed, or motivation, choice speed, and goal speed, or motor performance. In view of the significant dose-dependent effects on spatial memory impairment and the significant increases in hyperactivity and motor speed, it may be concluded that MK-801 effects on memory may not be selective or be dissociated from non-associative effects on other sources of performance even at very low dose levels.

194.17

DRUG EFFECTS ON REPEATED ACQUISITIONS IN THE MORRIS SWIM TASK. Julian Keith, Carmen McLamb, Erin Yard, Carl Schmidt, and Mark Galizio Dept. of Psychology, UNC-Wilmington, Wilmington, NC 28403.

The Morris swim task (MST) is widely used to evaluate drug effects on the navigation abilities of rats. Performance on the place version (where the platform is hidden) of the MST is impaired by some drugs at doses that do not impair performance on the cued task (where the platform is visible). Taken at face value this pattern of results suggests that these drugs can selectively disrupt spatial learning while not affecting other (i.e. sensorimotor, motivational, and procedural) factors critical for successful performance. We describe a repeated acquisitions procedure that permits evaluation of acquisition of a new place navigation task and performance of a previously learned task within a session. Subjects were tested (5 days/week for approximately 3 mths) in two pools, alternating pools on each trial (6 trials/pool/day). In one pool (the performance pool) the platform was in the same location each day. In the other pool (the acquisition pool) the platform location changed each day. Results of studies evaluating alcohol, Chlordiazepoxide (CDZ) and MK-801 will be presented. Inconsistencies between our results and those of several previous studies will be discussed. Supported by NC ARA # 9101.

194.18

MK-801 REDUCES BEHAVIORAL DEFICITS ASSOCIATED WITH X-RAY-INDUCED HIPPOCAMPAL GRANULE CELL HYPOPLASIA. G.A. Mickley[+], J.L. Ferguson and T.A. Nemeth[+*]. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145 and [+] USAF School of Aerospace Medicine, Brooks AFB, TX 78235-5301

MK-801 (NMDA antagonist) has been shown to protect newborns from hypoxia-induced brain damage. Here, we tested if MK-801 could attenuate behavioral deficits associated with radiation-induced hypoplasia of fascia dentata granule cells. We pretreated neonatal rats (N=20) with MK-801 (0, 0.1 or 0.2 mg/kg, i.p.) before each of 6 fractionated, head-only doses of X-rays (12 Gy total) administered during the first 16 days *post partum*. Other rats (N=18) received the same drug treatments but were sham irradiated. At age 16 months, water-escape latencies to a submerged platform were measured in a water maze. Irradiated rats with hippocampal damage exhibited impaired learning (longer latencies to find the platform) than did sham-irradiated subjects. Moderate doses of MK-801 (0.1 mg/kg) facilitated the learning of the water maze by irradiated subjects, whereas higher doses (0.2 mg/kg) provided no such benefits. In fact, this high dose significantly impaired the learning of the water maze by sham-irradiated rats. Thus, early MK-801 treatment produces dose-dependant behavioral protection for adult rats exposed (as neonates) to a procedure that causes selective hypoplasia of hippocampal granule cells.

194.19

A COMBINATION OF 0.0125 mg/kg DIZOCILPINE (MK801) AND 0.125 mg/kg SCOPOLAMINE (SCOP) IMPAIRS DETOUR MAZE PERFORMANCE IN ADULT AND AGED RATS. *E. Bresnahan, N. Muth*, E. Spangler, and D. Ingram.* Essex Community College, Baltimore, MD 21237; Gerontology Research Center, NIA, Baltimore, MD 21224

Male Fischer-344 rats (N=15) received extensive training with all possible problems (P) and 3-P sequences in a detour (D) maze. Rats had to run to a goal through a path containing pairs of U-shaped D's deviating bilaterally at 3 locations. Each P required: 2 forced runs with the entrance to 1 side of a D-pair blocked at 1 or more locations (sample); and 2 errorless choice runs with both entrances of a D-pair opened (delayed choice trials). To avoid 0.4 mA footshock, the rat must procede to the goal without stopping. Errors during choice runs were punished by a shock pulse. At age 11 mo, 7 of the 15 rats were tested on a new 4-P sequence (Exp 1). On wk-1, more errors occurred on a 0.05 mg/kg MK801 session than on 0.025 mg/kg or saline (SAL) sessions (Spangler, et al., Soc. Neurosci. Abst., 16:316.1, 1990). On wk-2, compared to SAL and non-SAL sessions, a combined (COMB) drug session (MK801-0.025 mg/kg s.c. & SCOP-0.3 mg/kg i.p.) increased errors ($p < 0.001$). For the next 11 mo all 15 rats were tested extensively on this 4-P sequence. At 23 mo, 10 surviving aged rats were tested on SAL and drug sessions (Exp 2). On wk-1, both 0.025 mg/kg and 0.05 mg/kg MK801 doses increased errors ($p's < 0.05$). On wk-2, the 3 drug sessions were: (a) COMB - MK801-0.0125 mg/kg s.c. and SCOP-0.125 mg/kg i.p.; (b) MK801 only; and (c) SCOP only. More errors occurred on COMB than on all remaining sessions except that of MK801 ($p's < 0.05$); errors during MK801 were no greater than those on other sessions; and no differences existed among control sessions. Thus, with this well-learned 4-P sequence, MK801-0.05 mg/kg continued to disrupt choice performance at 23 mos; this small dose of 0.025 mg/kg was disruptive at 23 mos; whereas it was not at 11 mo; and at 23 mo, COMB in smaller doses increased errors; whereas neither MK801 nor SCOP alone at these smaller doses was disruptive. Within subjects evaluation of DMTS performance in this D-maze is both efficient and meaningful for drug evaluation studies.

194.20

REPEATED ACQUISITION OF FOUR-LEVER SEQUENCES: DIFFERENTIAL EFFECTS OF SCOPOLAMINE, MK-801, AND NMDA. J. Cohn and D.A. Cory-Slechta. Environmental Health Sciences Center, Univ. Rochester School of Medicine & Dentistry, Rochester, NY 14642.

The cholinergic and glutaminergic (NMDA) neurotransmitter systems are known to serve as biological substrates for learning. Analyses of the behavioral mechanisms by which drugs influence learning processes may yield a more complete understanding of the relative role different neurotransmitter systems play. Prior studies from this laboratory have shown a differential role for the cholinergic antagonist scopolamine and the NMDA antagonist MK-801 in learning, as indicated by different patterns of errors in a 3-member response sequence paradigm (3M; Soc. Neurosc. Abstr. 16:766, 1991). In this study, the effects of those same compounds as well as NMDA itself, were examined on the acquisition of 4-lever (4M) response sequences (e.g. Left (L), Right (R), Left (L), Center (C), excluding perseverative sequences such as LLLR) to determine the extent to which task complexity modified drug effects. Baseline accuracy was approximately 55%, substantially lower than that obtained on the 3M. Each drug produced different patterns of errors across the sequences, some of which differed from previously observed 3M effects. As on the 3M, MK-801 produced strong decreases in overall accuracy primarily due to an incease in perseverative errors early in the sequence. Skipping errors were more prevalent on the 4M than on the 3M paradigm with MK-801, particularly later in the sequence. Early and mid-sequence errors were predominantly perseverative with scopolamine, followed by a rise in skipping errors later in the sequence, in contrast to a predominance of skipping errors on the 3M. NMDA increased the likelihood of a correct response (returning to the start of the sequence) following an error. These studies indicated that different neurotransmitter systems can impact learning through different underlying behavioral processes. The pattern of skipping and perseverative errors induced in the 4M relative to the 3M sequences by MK-801 and scopolamine administration suggests that these drugs may have multiple behavioral mechanisms of action. NIH grants ES05017 and ES05501.

194.21

COMPARISONS OF THE ANTI-ISCHEMIC DRUGS REMACEMIDE AND MK-801 ON CNS SAFETY PROFILES IN NON-ISCHEMIC RATS. T.M. Wengenack, J.M. Ordy, and P. Bialobok*. Fisons Pharmaceuticals, Rochester, NY 14623.

Remacemide, (±) 2-amino-N-(1-methyl-1,2-diphenylethyl)-acetamide, is an anticonvulsant currently in Phase II clinical trials. The desglycinated metabolite of remacemide is a non-competitive NMDA antagonist. Studies with remacemide in the rat four-vessel occlusion (4-VO) model of global ischemia have indicated significant improvement of 4-VO memory impairment and reduction of hippocampal CA1 neuronal damage. Studies with the non-competitive NMDA receptor antagonist, MK-801, in global and focal models of ischemia have also reported neuroprotective efficacy in some rodent models. Because there is no currently approved neuroprotective reference drug for cerebral ischemia, comparisons were made of remacemide and MK-801 on CNS safety profiles in non-ischemic rats. Efficacy-safety studies of neuroprotective candidates are considered critical for establishing therapeutic safety margins in drug treatment of cerebral ischemia. Specifically, adverse drug treatment effects on memory, body temperature, glucose levels, and glucocorticoid concentrations may interact with ischemia effects on these variables and thus exacerbate neuronal damage. Remacemide, at 10 and 20 mg/kg, i.p., had no adverse effects on memory, psychotomimetic signs, body temperature, blood glucose, and corticosterone levels. MK-801, at 0.1, 0.2, and 1.0 mg/kg, i.p., produced significant, dose-dependent impairment of memory, psychotomimetic signs, biphasic hypo-hyperthermia, increased blood glucose, and corticosterone concentrations. Compared to the adverse CNS profile of MK-801, remacemide had no demonstrable effects on CNS and physiological variables that could exacerbate the effects of cerebral ischemia.

195.1

MICROSTRUCTURAL ANALYSIS OF LICKING BEHAVIORS IN ZUCKER FATTIES AND LEANS, AND IN RATS GIVEN NEUROPEPTIDE-Y ICV. K.E. Asin and L. Bednarz*, Neuroscience Research Div., Pharmaceutical Discovery, Abbott Labs, D-47H, Bldg-AP10, Abbott Park, IL 60064.

Recent reports have provided considerable information regarding the licking behaviors of rats. These studies have indicated that these behaviors can be assessed quantitatively and that particular feeding-related variables change in a predictable manner depending on the palatability of the fluid being consumed.

The analysis of licking behaviors has also been useful for characterizing how various pharmacologic treatments, particularly anorectics, act to suppress feeding. In the present study, we examined how different patterns of licking might promote increased food intake and obesity. We therefore chose to study the licking behaviors of obese (Ob) and lean (Ln) Zucker rats, and we also examined feeding in albino rats following ICV administration of NPY.

Female Zucker rats were trained to consume various concentrations of sucrose (0 - 0.8 M) and Ensure™ (0-100%). Overall, there were considerable differences between groups. Obs failed to increase their burst size (BS) as much as Lns in response to increasing palatability, showed shorter interlick intervals (ILI) and made fewer licks/ml (L/ml), suggesting greater licking efficacy. Although sucrose intakes were similar between groups, there were concentration-dependent differences in their drinking patterns. Ensure™ intakes were higher in Obs; this was accompanied by a **reduction** in burst number (BNo) and the ILI.

In another study, NPY (10 μg/rat) was infused into the lateral ventricle of rats trained to drink 0.05M sucrose. NPY increased intakes 25%, primarily by producing a 2-fold **increase** in burst number, which was accompanied by a shift of the ILI distribution from longer (>0.50 sec) to shorter (<0.23 sec) intervals. BS and lick rate were **reduced** by NPY; L/ml was unchanged. Thus, the enhanced intakes of Ob and NPY-treated rats reflect different alterations in licking patterns.

195.2

NEUROPEPTIDE Y PRODUCES CONDITIONED TASTE AVERSIONS (CTAs) TO LIQUID DIETS IN LEAN BUT NOT OBESE ZUCKER RATS. A. J. Sipols, F. Koegler*, M. Shimoji*, and S. C. Woods Department of Psychology, University of Washington NI-25, Seattle, WA 98195

Neuropeptide Y (NPY) elicits hyperphagia when administered intraventricularly (IVT). This phenomenon is more robust in lean (Fa/Fa) and heterozygous (Fa/fa) Zucker rats than in their obese (fa/fa) siblings, and this genotype-dependent effect has been seen with solid food, wet mash, calorically dense liquids, and non-caloric liquids. Lean and heterozygous Zucker rats also develop more robust CTA's in response to IVT NPY than do obese animals. We now report that Zucker genotype is also associated with susceptibility to NPY-induced CTA's to calorically-dense liquids. Male Zucker rats (N=20) with I3VT cannulas were adapted to solid food (1 8 hr/day), with vanilla Ensure available for 30 min after 4-hr deprivation. After adaptation to the schedule, subjects in each genotype received IVT saline (1 ul) or NPY (9.5 ug) before access to 5 ml of novel flavored Ensure (chocolate or strawberry). On the following day, subjects were presented with both chocolate and strawberry Ensure. Homozygous (Fa/Fa) (48 ± 31 vs. 85 ± 20%, $p<0.05$) and heterozygous lean (Fa/fa) (52 ± 17 vs. 113 ± 31%, $p<0.05$) rats developed significant CTAs following NPY whereas obese (fa/fa) (65 ± 22 vs. 77 ± 19%) subjects did not. The lack of CTA formation in obese Zucker rats is independent of the caloric density of the novel liquids.

195.3

FEEDING RESPONSES TO PERIFORNICAL HYPOTHALAMIC INJECTION OF NEUROPEPTIDE Y IN RELATION TO DAILY RHYTHMS OF EATING BEHAVIOR. B.V. Aramakis, W.J. Thomas* & B.G. Stanley. Dept. of Psychology, Univ. of California, Riverside, CA 92521.

To determine whether the perifornical hypothalamus (PFH) might exhibit a daily rhythm of sensitivity to the eating stimulatory effect of neuropeptide Y (NPY), rats were given PFH injections of NPY (78 pM/10 nl) or artificial CSF vehicle at 6 different times of the 12/12 hr light/dark cycle. The injections were given in the first, middle, and last hour of the light phase, as well as in the first, middle, and last hour of the dark phase (n = 10 rats/time). Food intake was measured 1, 2 & 4 hours postinjection. Analysis of the results revealed that there was a marked effect of injection time on food intake following injection of either NPY or CSF. Food intake measures following both types of injection showed that the largest intakes occurred at the end of the light phase and the early to middle portion of the dark phase. Subtracting the CSF scores from the NPY scores at each time to reveal the increases produced by just the peptide, established that NPY elicited significant eating throughout the light/dark cycle. There was a tendency for the NPY to be most effective at the end of the light and throughout the dark phase, as compared to the early to mid-light phase. These findings suggest that in the PFH there is, at best, a modest daily variation in the sensitivity to the eating stimulatory effects of NPY.

195.4

NEUROPEPTIDE Y (NPY) INCREASES RESPONDING OF RATS UNDER PROGRESSIVE RATIO SCHEDULES OF FOOD REINFORCEMENT. D.C. Jewett, D.W. Schaal, J. Cleary, T. Thompson & A.S. Levine. University of Minnesota and VA Medical Center Minneapolis, MN 55455 and 55417.

In the present study, progressive ratio (PR) schedules (i.e., schedules in which the number of responses required for food increases with each reinforcer) were used to assess the effects of NPY on the reinforcing efficacy, or value, of food. The number of responses rats emitted in the last completed ratio before stopping (i.e., the break point) was taken as the index of reinforcer value. The effects of NPY were also compared to those of food deprivation. Five rats were initially maintained at 80% of their free feeding weights and trained to press for food under a PR 1 schedule. When responding showed no increasing or decreasing trends, rats were given free access to food which decreased break points. After responding stabilized again, rats were injected icv with 0.3, 1.0, 3.0, 5.0 and 10.0 μg of NPY 30 minutes before the session. The effects of 12, 24, 36 and 48 hours acute food deprivation were also tested. Effects of NPY and food deprivation were expressed as a percentage of the break points obtained when rats were maintained at 80% of their free-feeding weights. NPY increased mean break points in a dose-dependent manner (0μg= 7.3%; 0.3=30.3; 1.0=41.1; 3.0=39.82; 5.0=51.0; 10.0=50.1). All levels of acute food deprivation also increased break points. Break points obtained after each dose of NPY were higher than those obtained after 12 (22.2%) and 24 hours (25.4%) food deprivation; break points after 5.0 and 10.0 μg of NPY were comparable to those obtained after 48 hours (70.4%) food deprivation.

195.5

CHARACTERISTICS OF AND MECHANISM UNDERLYING CHOLECYSTOKININ (CCK) CLASSICAL CONDITIONING IN NEONATAL RATS. A. Weller[1], E.M. Blass[2], G.P. Smith[3], & J. Gibbs[3]. [1]Dept. of Psychology, Bar Ilan Univ., Ramat Gan, Israel; [2]Depts. of Psychology & Nutrition, Cornell Univ., Ithaca, NY 14853; [3]Bourne Behavioral Lab., New York Hospital-Cornell Medical Center, White Plains, NY 10605.

When an odor that had previously predicted and been paired with i.p. injections of CCK (0.25-2.0 ug/kg) was presented to 11-day-old rats, the following ensued: 1. Behaviors affected by injections of CCK were similarly affected by the conditioned odor. Specifically, ultrasonic vocalizations (UV) in isolated rats were reduced (230 vs 100 UV) as was independent feeding (4.7 vs 3.5 ml milk) during exposure to the conditioned odor. Yet more time (45 vs 27%) was spent over the normally aversive orange odor by rats conditioned with CCK. 2. Behaviors not affected by CCK injections such as activity and heat escape were likewise not affected by the conditioned stimulus. 3. Pretreatment with the Type A CCK receptor antagonist MK329 prevented conditioning formation. 4. Treatment with MK329 prior to testing prevented the expression of conditioning.

These data imply, in neonatal rats, that a stimulus associated with administration of exogenous CCK gains control over behavior by sensitizing Type A afferents. A model of a specific mechanism will be proposed and alternative models considered.

195.6

DEVAZEPIDE COMPLETELY REVERSES CCK-INDUCED SATIETY IN SHAM FEEDING RATS. L. D. Melville*, G. P. Smith, J. Gibbs & J. Sechzer. Bourne Laboratory, NY Hospital-Cornell Medical Center, White Plains, NY 10605.

The selective, potent CCK_A antagonist devazepide (DV) blocks the satiating effect of exogenous, peripherally administered CCK-8 in a variety of test conditions in which rats eat normally and all of the endogenous postingestive satiating mechanisms are stimulated. We wished to investigate the effect of DV when CCK-8 was given (ip) prior to sham feeding, which minimizes or eliminates those postingestive satiety mechanisms. Male Sprague-Dawley rats (n=8) were prepared with chronic gastric cannulas for sham feeding. Rats were deprived of food, but not water, for 17 hours. DV (0.025-1.0 mg/kg) or vehicle was given ip at -30 minutes; CCK-8 (8 or 16 ug/kg) or vehicle was given at -5 minutes. Sham fed intakes of a sweet milk diet were measured for 30 minutes. All doses of DV significantly blocked the satiating effect of CCK-8; complete reversal occurred with 0.05, 0.1, and 1.0 mg/kg DV.

	SHAM INTAKES (ml/30 min)		
DV (mg/kg)	Vehicle + Vehicle	Vehicle + CCK-8	DV + CCK-8
0.025	61.0 ± 2.8	30.1 ± 4.3	54.3 ± 3.6**
0.05	58.7 ± 2.7	34.5 ± 4.9	59.6 ± 3.5***
0.1	50.9 ± 4.1	33.4 ± 4.2	52.0 ± 3.8***
1.0	53.3 ± 2.6	34.6 ± 3.8	59.3 ± 4.1**

Note: Data are mean ± SE. **p<.01, ***p<.001, DV + CCK-8 significantly larger than vehicle + CCK-8.

These results demonstrate for the first time that DV is a very potent antagonist of the satiating effect of exogenous CCK-8 during sham feeding. Since sham feeding minimizes or eliminates all other endogenous satiating mechanisms and thus removes potential interactions between CCK-8 and other mechanisms, the sham feeding rat provides a sensitive and simple assay system for studying the interaction between CCK-8 and CCK antagonists.

Supported by MH40010 and MH00149 (GPS).

195.7

A-71623, A NOVEL CCK-A TETRAPEPTIDE AGONIST: COMPARATIVE ANORECTIC EFFECTS IN RAT, MOUSE, DOG AND MONKEY. A. M. Nadzan, K. E. Asin, L. Bednarz*, P. Gore*, A. Nikkel, W. Montana*, K. Shiosaki* and R. Craig*, Neuroscience Research Division, Pharmaceutical Discovery, D-47H, AP-10-3, Abbott Laboratories, Abbott Park, IL 60064.

We have reported recently on a nonsulfated CCK-A tetrapeptide agonist A-71623, Boc-Trp-Lys(ε-N-2-methylphenylaminocarbonyl)-Asp-(NMe)Phe-NH_2, which exhibits high affinity (IC_{50} = 4 nM) and selectivity (1200-fold) for CCK-A receptors (Shiosaki, et. al., J. Med. Chem., 33, 2950, 1990) and potently suppresses food intake in several species. In the present study, the anorectic efficacy of A-71623 is compared in rat, mouse, dog and monkey.

Male mice and rats (deprived) were maintained exclusively on a liquid diet of ENSURE™ presented twice daily. A group of sated rats, maintained on rat chow, was allowed daily access (60 min.) to the liquid diet. Male cynomolgus monkeys (N=5) were trained to drink a liquid diet of ENRICH™ available twice daily. Female beagles were maintained on dog chow, with normal chow being removed in the AM prior to access to a gravy + chow mixture for 3 hours. Dogs, monkeys and sated rats were injected i.m. with either vehicle or A-71623 at 15-30 min. prior to the feeding period. Deprived rats and mice were injected i.p. approximately 10 min. prior to the AM feeding session.

A-71623 suppressed food intake in all species studied. Monkeys were the most sensitive to A-71623 (ED_{50} = 3 nm/kg @60 min., i.m.). In dogs and rats, A-71623 was equipotent at the 60 min. time point, but dog intakes were reduced less than rats at later times. A-71623 was more potent in rats than in mice when administered via the i.p. route. These results demonstrate that A-71623 is a highly selective CCK-A agonist with potent anorectic actions in several mammalian species. The relatively small size of this peptide offers the potential for development of orally-effective CCK-based anorectic agents.

195.8

ENDOGENOUSLY RELEASED CCK INHIBITS GASTRIC EMPTYING THROUGH A-TYPE NOT B-TYPE RECEPTORS. A.J. Strohmayer, D. Greenberg D.R. Lewis, G.P. Smith and J. Gibbs. Dept. of Psychiatry, Cornell University Medical College: North Shore Univ. Hospital, Manhasset, NY 11030 and The New York Hospital, White Plains, NY 10605.

Intragastric infusions of Soybean Trypsin Inhibitor (STI) slow gastric emptying (GE) and release endogenous CCK (Strohmayer et al. 1990, Liddle et al. 1984). The present study was designed to identify the CCK receptor subtype(s) (A and/or B) which mediate the GE effect of STI.

Male Sprague-Dawley rats (n=9 per group; body weight = 250g) were maintained on a liquid diet (BioServ) for at least 10 days prior to testing to insure that stomachs were free of solids. For testing, rats were 17h food deprived. Fifty minutes prior to a gastric gavage, rats were injected (sc) with either Devazepide (A-type CCK antagonist; 0.5 mg/kg), L-365,260 (B-type CCK antagonist; 1 mg/kg) or vehicle. Rats were then gavaged with STI (230 mg/rat) in 5ml 0.15M NaCl, or 5 ml 0.15M NaCl alone. Ten minutes later, rats were sacrificed, their stomachs were removed and weighed to determine GE.

As in previous studies, intragastric STI significantly slowed GE to 43% compared to 61% by saline alone. Pretreatment with the A-antagonist completely reversed the STI effect (GE=62%). The A-antagonist alone had no effect on GE (GE=64%). Pretreatment with the B-antagonist did not reverse the slowing of GE by STI, in fact it augmented this effect (GE=10%). The B-antagonist alone had an agonistic effect on GE (GE=22%).

The slowing of GE by STI was totally reversed only by Devazepide. We therefore conclude that the effect on GE by endogenous CCK acts through A-type CCK receptors.

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195.9

EXOGENOUS ADMINISTRATION OF CCK-8 INHIBITS GASTRIC EMPTYING THROUGH A-TYPE NOT B-TYPE RECEPTORS. D. Greenberg, A.J. Strohmayer, D.R. Lewis, G.P. Smith and J. Gibbs. Dept. of Psychiatry, Cornell University Medical College: The New York Hospital, White Plains, NY 10605 and North Shore Univ. Hospital, Manhasset, NY 11030.

Injections of CCK are known to slow gastric emptying (GE) in rats, dogs, monkeys, mice and humans. Green et al. (Am. J. Physiol., 1988) showed this effect was partially mediated by CCK A-type receptors in rats. In the present study the specific CCK receptor subtype (A or B) involved in GE was identified.

Male Sprague-Dawley rats (n=9 per group; body weight = 250g) were maintained on a liquid diet (Research Diets Inc.) for at least 10 days prior to testing to insure that stomachs were free of solids. For testing, rats were 17h food deprived. Fifty minutes prior to a gastric gavage, rats were injected (sc) with either Devazepide (A-type CCK antagonist; 0.01, 0.1, 0.5, or 1 mg/kg), L-365,260 (B-type CCK antagonist; 1 mg/kg) or vehicle. Rats were then gavaged with 5ml 0.15M NaCl, and were injected (ip) with 8 μg/kg CCK-8, 8 μg/kg desulfated CCK-8, or 0.15M NaCl. Ten minutes later, rats were sacrificed, their stomachs were removed and weighed to determine GE.

With CCK alone GE was 16%. When desulfated CCK was given at the same dose 60% of the load emptied. When saline was given, 64% of the load emptied. Pretreatment with the B-antagonist plus CCK resulted in only 13% GE. Pretreatment with the A-antagonist resulted in a dose related attenuation of the slowing of GE by CCK, with 1 mg/kg being the most effective.

Since desulfated CCK was ineffective at slowing GE and L365-260 was not able to reverse the effects of sulfated CCK, we conclude that the B-type receptor is not involved in the CCK effect on GE. The A-type receptor is involved in this effect but may not completely account for it.

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195.10

ATTENUATION OF CCK SATIETY BY JMV-180, AN ANTAGONIST OF LOW AFFINITY PANCREATIC CCK A RECEPTORS. L.A. Netterville*, S. Weatherford, G.J. Schwartz, W. Danho*, J.W. Tilley* and T.H. Moran. Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD. 21205, Roche Research Center, Hoffmann-LaRoche Inc., Nutley, N.J. 07110.

The synthetic cholecystokinin (CCK) analog, Boc-Tyr(SO_3)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester (JMV-180) has been demonstrated to differentially interact with high and low affinity pancreatic CCK A receptors in that it is an agonist at the pancreatic high affinity site but an antagonist at the low affinity site. The present study utilized this compound to address whether the receptor site mediating CCK's effects on food intake was pharmacologically similar to either the high or low affinity pancreatic CCK receptor. Male Sprague-Dawley rats were adapted to 1 hr daily access to a 0.5 kcal/ml glucose solution following a 6 hr daytime deprivation. Seven min prior to glucose access, rats received IP injections of various dosages (vehicle or 0.1, 0.32 or 1.0 μmol/kg) JMV-180, and, 5 min prior to glucose access, received 3.2 nmol/kg CCK-8 IP. Intake was monitored at 15 min intervals for the 60 min test. CCK alone resulted in a 70 % suppression of intake at the 15 min time point and maintained a 55 % suppression through the other time points. JMV-180 significantly attenuated this suppression at all dosages such that by the 1.0 μmol/kg dose, intake was no longer significantly suppressed by CCK. These results suggest that exogenous CCK inhibits intake through an interaction with CCK A receptors which are functionally similar to the pancreatic low affinity binding site.

195.11

CCK-A RECEPTOR ANTAGONIST IS MORE EFFECTIVE PERIPHERALLY THAN CENTRALLY FOR ATTENUATION OF SUPPRESSION OF SHAM FEEDING BY INTESTINAL OLEATE. L.A. Brenner and R.C. Ritter, Dept. of VCAPP, Washington State University, Pullman, WA 99164.

Previously, we reported that peripheral administration of a CCK type A (CCK-A) receptor antagonist, MK-329, but not a CCK type B (CCK-B) receptor antagonist, L-365,260, attenuates the suppression of sham feeding by systemic injection of exogenous CCK-8 or by intraintestinal oleate. These results suggest that suppression by oleate is mediated by endogenous CCK acting at CCK-A receptors. Though generally considered a "peripheral" type, the CCK-A receptor is also present in the brain. To determine whether CCK mediating the suppression of sham feeding by intraintestinal oleate acts at a central nervous or peripheral site we injected MK-329 into the lateral ventricles of intraintestinally infused, sham feeding rats. MK-329 at doses of 0.18, 0.37 or 0.74 umol/rat icv failed to attenuate suppression of sham feeding by oleate. These doses were sufficient, however, to attenuate or abolish suppression of sham feeding by oleate when administered peripherally. Suppression of sham feeding by intraperitoneal CCK-8 (2ug/kg), previously shown to be more sensitive than oleate to attenuation by ip MK-329, was abolished by icv injection of 0.18 umol/rat. This icv dose of MK329, however, was higher than ip doses that abolished CCK-induced suppression of sham feeding. Assuming that icv injection produces brain antagonist concentrations at least as high as those achieved by peripheral injection, then these results argue against participation of central CCK type A receptors in suppression of food intake by intraintestinal oleate and suggest mediation by endogenous CCK acting at peripherally located CCK-A receptors. Supported by NINDS Grant NS20561.

195.12

INDIVIDUAL, BUT NOT COMBINED, INFUSIONS OF PANCREATIC GLUCAGON AND CHOLECYSTOKININ REDUCE MEAL SIZE IN MEN. N. Geary, V. Hinton†, H.R. Kissileff & F.X. Pi-Sunyer†, Obesity Research Core Center, St. Luke's/Roosevelt Hospital, NY NY 10025.

Exogenous pancreatic glucagon (PG) and cholecystokinin octapeptide (CCK-8) produce functionally synergistic satiety signals in rats. To determine whether these peptides interact similarly in men, 8 normal weight men received 3 ng/kg-min PG, 2 ng/kg-min CCK-8, PG plus CCK-8, or saline. IV infusions began 15 min after a 500 ml soup preload and 5 min before a lunch of macaroni and beef with tomato sauce. Meal size (MS) was less after either PG (708 g, $p<.05$) or CCK-8 (587 g, $p<.01$) than after saline (856 g, SED=43 g). Simultaneous infusion of PG and CCK-8 did not reliably reduce MS (742 g, $p>.05$). Indeed, the decrease in MS after simultaneous infusion (113 g) was less than predicted by the sum of the two individual effects (417 g, $p<.05$). Psychophysical ratings failed to detect non-specific side effects after any of the infusions. In further tests, 1.5 ng/kg-min PG and 1 ng/kg-min CCK-8 had neither individual nor interactive effects on MS.

That PG and CCK-8 each reduced MS in a subjectively specific way suggests they participate in the physiological control of human appetite. Because their simultaneous infusion resulted in an infra-additive MS effect, PG and CCK-8 may interact antagonistically in humans.

195.13

POTENT ANTAGONISM OF THE FEEDING INHIBITORY ACTIONS OF EXOGENOUS CCK-8 BY A SELECTIVE PYRAZOLIDINONE TYPE A CCK RECEPTOR ANTAGONIST (LY219057). J. J. Howbert[1], N. R. Mason[1], R. F. Bruns[1], L. A. Netterville*[2], G. J. Schwartz[2], and T. H. Moran[2]. [1]Lilly Research Labs., Eli Lilly & Co., Indianapolis, IN 46285 and [2]Dept. of Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Recent studies have demonstrated that feeding inhibitory actions of exogenous and endogenous cholecystokinin (CCK) are mediated by type A CCK (CCK-A) receptors. The present experiments evaluated the ability of a novel pyrazolidinone CCK antagonist (LY219057) to block the feeding inhibitory actions of exogenous CCK. In *in vitro* binding, LY219057 showed good affinity for the pancreatic CCK-A receptor (IC50 = 50 nM in rat pancreatic membranes) and moderate selectivity relative to the brain CCK (CCK-B) receptor (IC50 = 1300 nM in guinea pig brain membranes). For the feeding experiments, male Sprague Dawley rats were adapted to 1 hr access to a 0.5 kcal/mL glucose solution following a 6 hr daytime deprivation. Thirty minutes prior to glucose access, rats received IP injections of various doses (vehicle or 0.1 to 100 µg/kg in half log steps) of LY219057 and, 5 min prior to glucose access, received 4 µg/kg CCK-8. Intake was recorded at 15 min intervals for the 60 min test period. CCK alone resulted in a 60% suppression of intake at 15 min and a 50% suppression of intake at the other time points. Beginning at a dose of 0.32 µg/kg, LY219057 attenuated CCK's inhibition of glucose intake in a dose-dependent fashion. This attenuation was maximal at a dose of 10 µg/kg. The potency of LY219057 in blocking CCK-induced suppression of intake appears significantly greater than would be predicted from its relative affinity for pancreatic CCK-A receptors.

195.14

MICROINJECTION OF NALOXONE INTO THE SUBSTANTIA NIGRA OF RATS BLOCKS EATING INDUCED BY STRESS. M.F. Hawkins, B. Cubic*, A.A. Baumeister, and J.C. Barton*. Dept. of Psychology, Louisiana State Univ., Baton Rouge, LA 70803

Stress produced by pinching the tail elicits eating in rats which is reversible by naloxone. Little is known about what sites in the CNS may mediate these effects. Male Sprague-Dawley rats were maintained on free access to food and water. Five minutes after naloxone (3, 10, 20, or 30 nmol) or water was injected into the substantia nigra (0.5 µl/side) food intake was recorded for four minutes during continuous tail pinch. Immediately afterward, nociceptive threshold was assessed with tail-flick and hotplate tests. Naloxone produced a significant [$F(4,34)=11.41$; $p<.0000$] and dose related [$r(37)=-.71$, $p<.0000$] reduction in the feeding elicited by tail pinch. Naloxone had no effect on gnawing (spillage) or nociceptive threshold. These results suggest that the SN may be an important site for the regulation of stress induced feeding. Additional studies in our lab are attempting to determine the degree of neuroanatomical specificity of this effect.

195.15

METHADONE AND FEEDING: DIFFERENT EFFECTS IN THE HOME CAGE AND THE OPERANT CHAMBER. J.M. Rudski, T. Thompson*, D.W. Schaal*, J.P. Cleary*, C.J. Billington*, and A.S. Levine. Univ of MN, Mpls, MN 55455 and VA Medical Center, Mpls, MN 55417.

Levine et al. have shown that mu-opiate agonists increase food intake in free-feeding rats at times when feeding is a low probability behavior. Thompson et al. have shown that methadone, a predominantly mu-opiate agonist, decreases food reinforced operant behavior in food-deprived animals. To examine this apparent paradox of opiates' effects on feeding, food-reinforced operant behavior at a time when feeding is usually not observed was measured for 4 hours in satiated rats living in an operant chamber. One group of rats received methadone 5.0 mg/kg and the other received vehicle for 10 days. Pellets were delivered under an FR 1 reinforcement schedule. Differences for each rat between mean intake of the last 5 baseline days and last 5 drug days were compared. Results were not statistically significant; the methadone group increased their food intake a mean of 0.68 g and the saline group increased their intake a mean of 0.13 g. Thus, methadone does not result in decreases in food-reinforced operant behavior observed in food-deprived animals. Furthermore, increases similar to those reported in free-feeding following mu agonists are not seen when food was contingent on an operant response. This suggests that the increases observed in free-feeding do not reflect a significant increase in food motivation similar to that induced by food deprivation, since an additional response requirement to procure food resulted in methadone failing to increase food-maintained behavior.

195.16

EVALUATION OF AGING AND GENDER EFFECTS UPON NALOXONE HYPOPHAGIA FOLLOWING REGULATORY CHALLENGE OR PALATABLE SITUATIONS IN RATS. A.K. Islam, I.W. Beczkowska, R. Basile*, M.L. Cooper* and R.J. Bodnar. Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367.

Aging reduces the sensitivity of rats to hypophagia induced by the opiate receptor antagonist naloxone during spontaneous feeding. Naloxone reduces food intake under other circumstances including the regulatory challenge of food deprivation for 24 h and the palatable situation of intake of a high-fat diet. Different opiate receptor subtypes are responsible for different forms of ingestive behavior. To determine whether aging and gender differentially influence different forms of naloxone hypophagia, intake of male and female rats at different ages (4, 8, 14, 20 and 26 mo) was examined following systemic naloxone (0, 0.25, 1, 2.5 and 5 mg/kg) in two paradigms: a) reintroduction of food after 24 h of deprivation and b) intake of a high-fat diet. Both aging and gender affected basal intake following deprivation with reduced intake observed in older and female rats respectively. Neither variable consistently altered high-fat intake. Aging but not gender increased the sensitivity to naloxone hypophagia following deprivation. Neither gender nor aging altered the dose-response function of naloxone hypophagia following intake of a high-fat diet. Overall, rats were more sensitive to naloxone hypophagia in the palatable relative to the deprivation situation.

195.17

INVOLVEMENT OF KAPPA AND MU OPIOID RECEPTORS IN SUCROSE INTAKE IN RATS. I.W. Beczkowska and R.J. Bodnar. Dept. of Psych., Queens College, CUNY, Flushing, NY 11367

General opioid antagonists like naloxone and naltrexone (NTX) reduce intake of palatable liquid diets, including sucrose and saccharin solutions. Studies using selective mu and kappa agonists and antagonists have implicated these receptor subtypes in general palatable effects. The present study evaluated whether ingestion of a palatable (10%) sucrose solution was differentially altered in 5-min blocks over a 1 h period by central pretreatment with a general (NTX), mu-selective (beta-funaltrexamine; BFNA) or kappa-selective (nor-binaltorphamine; nor-BNI) antagonist in rats. Central NTX reduced sucrose intake over the 1 h time course with inhibition ranging from 30% (5 ug) to 47% (50 ug). Central norBNI significantly reduced sucrose intake over the 1 h time course with 5 and 20 ug doses producing inhibition of 47%. In contrast, the reduction in sucrose intake by central BFNA (20 ug) did not begin until 10 min of ingestion and the magnitude of inhibition did not exceed 30%. These data support the previously-stated role for kappa receptors as integral modulators of palatability, but indicate that the mu receptor also participates to a lesser degree in these effects (Supported by NIDA Grant 04194).

195.18

WITHDRAWAL FROM DAILY NALTREXONE STIMULATES EXCESS SUCROSE INGESTION IN NAIVE RATS. W.C. Lynch and L. Moe,* Dept. of Psychology, Montana State Univ., Bozeman, MT 59717.

In previous work we reported that daily naloxone treatment blocks ingestion of preferred sucrose solutions and stimulates sucrose intake following NAL withdrawal (Lynch, W.C. and Burns, G., Appet. 15: 23-32, 1990). The withdrawal effect may be due to modification of opioid receptor function resulting in supersensitivity to sucrose taste. In order to rule out possible effects due to taste experience, the present experiment measured intake following withdrawal from repeated naltrexone (NALT) in animals with no prior sucrose experience. Twenty Sprague-Dawley albino rats (200gm male) were randomly assigned to two groups. One group was injected with NALT (2mg/kg, sc) and the other with saline (1ml/kg, sc) for 13 days (10am daily). Animals had free access to lab chow and water and body weights were recorded daily throughout testing. At 24, 48 and 72h following the last injection, all animals were given 2h access to a 20% (w/v) sucrose solution and intake was recorded hourly. NALT had no effect on normal body weight gain. Following withdrawal, however, sucrose intake in the NALT group was significantly elevated at 24h and 48h, returning to control levels by 72h. Thus NALT withdrawal transiently increases sucrose ingestion in naive animals and this effect has a time course similar to the upregulation of opioid receptors following withdrawal from chronic NALT.

195.19

EFFECTS OF CENTRALLY ADMINISTERED NALOXONE ON THE INTAKE OF CARBOHYDRATE AND FAT. B.A. Gosnell and D.D. Krahn. Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109-0656.

We have reported recently that baseline preference for carbohydrate vs. fat is an important determinant of the effect of morphine on diet selection (Gosnell et al., *Pharmacol. Biochem. Behav.* 37:207, 1990). In this experiment, we examined the effects of centrally administered naloxone on diet selection in rats selected for different baseline preferences for fat and carbohydrate. Male Sprague-Dawley rats (n=32) were given *ad lib* access to 2 diets: a fat/protein diet and a carbohydrate/protein diet. Both diets contained equal amounts of protein (20% of total energy). After adaptation to these diets, groups of fat-preferring and carbohydrate-preferring rats were formed (n=7). All rats were then placed on a food restriction schedule (diets available 5 hrs/day). Cannulas for icv injections were implanted after adaptation to this schedule. Fat-preferring rats consumed 86 ± 1% of total daily calories from the fat diet prior to food restriction; the restriction schedule did not change this preference (89 ± 3% of daily calories). Carbohydrate-preferring rats consumed 38 ± 2% of total calories from the fat diet. After adaptation to food restriction, preference for the fat diet increased significantly (55 ± 5% of daily calories). Rats were given icv injections of naloxone (0, 20 or 50 ug) 10 min before diets were presented; intake was measured at 1, 2, and 5 hrs. Each rat was tested with all doses. In fat-preferring rats, both doses of naloxone reduced 1 hr intake of the fat diet; intake of the carbohydrate diet was low and was unaffected by naloxone. In carbohydrate-preferring rats, naloxone (50 ug) reduced the intake of both diets by an approximately equal amount (in calories). These results, together with our previous findings, indicate that the effects of opioid manipulations on diet selection must be interpreted in light of baseline diet preferences. (Supported by NIDA Grant DA05471)

195.20

DIFFERENTIAL EFFECTS ON FEEDING BEHAVIOR AND LOCOMOTOR ACTIVITY OF MU, DELTA AND KAPPA AGONISTS INFUSED INTO THE NUCLEUS ACCUMBENS AND VENTROLATERAL STRIATUM. V.P. Bakshi* and A.E. Kelley. Dept. of Psychology, Northeastern University, Boston, MA. 02115.

Previous evidence has indicated that systemic administration of opiates can affect both locomotor activity and feeding behavior. In this study, we investigated the role of specific receptor subtypes as well as different striatal regions in the regulation of these behaviors in sated animals. In 6 separate experiments, 8 rats were implanted with cannulae aimed at either the nucleus accumbens (N.Acc.) or the ventrolateral striatum (VLS). Equimolar doses of either a mu (DAGO), delta (DPEN) or kappa (U50,488H) agonist were bilaterally infused into these sites. The doses were: DAGO (0, .025, .25 and 2.5 µg/.5 µl); DPEN (0, .031, .31 and 3.1 µg/.5µl); U50,488H (0, .019, .19 and 1.9 µg/.5µl). Behavior was rated every 10 min. over a 4 hr. session. Total food intake and spillage were calculated at the end of the testing period. When injected into the N.Acc., DAGO elicited delayed yet robust locomotor and feeding responses. The .25-µg dose produced a biphasic effect on general activity; an initial suppression was followed by a significant enhancement over the saline baseline. Feeding was markedly potentiated by the highest dose. In contrast, administration of the highest dose of DPEN produced an immediate and significant increase in locomotor activity, but only a mild enhancement of feeding. U50,488H treatment did not significantly alter either behavior. Administration of DAGO into the VLS augmented both feeding and locomotor activity, whereas infusion of DPEN into this region only elicited locomotor activity. An experiment to determine the effects of U50,488H infused into the VLS is in progress. The results in general indicate that different opioid receptor subtypes may play different roles in the regulation of ingestive and locomotor behaviors. In addition, the relative involvement of different striatal subregions is discussed.

195.21

MU OPIOID ACTIVITY IN ANTERIOR CINGULATE CORTEX MAY MEDIATE STIMULATION-INDUCED FEEDING. T. Wolinsky, D. Gilady* and K.D. Carr. Millhauser Labs, NYU Medical Center, NY, NY 10016.

In a recent in vivo autoradiographic study, we demonstrated that feeding elicited by lateral hypothalamic electrical stimulation reduces ^{3}H-diprenorphine binding in supragenual cingulate cortex (Stein, Carr & Simon, 1990). This suggests competitive displacement by an endogenous opioid peptide that is released during feeding. In an effort to verify the involvement of supragenual cingulate opioid activity in stimulation-induced feeding, a follow-up microinjection experiment was conducted. Since supragenual cingulate contains mu and delta receptors but seems devoid of kappa receptors (Mansour et al., 1988), effects of mu- and delta-selective compounds on stimulation frequency threshold for eliciting feeding were determined. Bilateral microinjection of the mu-preferring antagonist, naloxone (20.0 ug), produced a significant (34% ± 5.8) increase in feeding threshold ($F_{4,16}$=14.4, $p<.001$; Dunnett t, $p<.01$) while the delta-selective antagonist, naltrindole (5.0 ug) did not (Dunnett t, $p>.05$). Bilateral microinjection of the mu-selective agonist, DAMGO (1.0 ug) produced a significant reduction (25.8% ± 2.6) in feeding threshold (Dunnett t, $p<.01$) while the delta-selective agonist, DPDPE (10.0 ug) did not. These findings support our interpretation of the autoradiographic results and suggest that mu opioid activity in supragenual cingulate cortex plays a significant role in stimulation-induced feeding.

Supported by DA 03956.

195.22

EFFECTS OF CHRONIC NALTREXONE INFUSIONS ON THE ACQUISITION AND MAINTENANCE OF DIET PREFERENCES. D.D. Krahn, B.A. Gosnell and D.H. Averbach*. Dept. of Psychiatry, Univ. of Michigan, Ann Arbor, MI 48109.

Opioids may play a role in the acquisition of taste preferences. Opioid agonists increase preference for sweet tastes while opioid antagonists or opioid receptor deficiency decrease preference for sweet tastes. We hypothesized that opioids might be important in diet preferences also and performed an experiment to determine whether chronic infusion of the opioid antagonist naltrexone causes changes in the acquisition of diet preferences or changes already established diet preferences. Two groups of 10 male, Sprague-Dawley rats were implanted with minipumps which delivered naltrexone (200 ug/kg/hr) or saline for the first 7 days of self-selection from high CHO (80% CHO, 20% protein) and high fat (80% fat, 20% protein) diets. After 7 days, the minipumps of the naltrexone-treated group (Group I) were replaced with saline-containing minipumps while the minipumps of the saline-treated group (Group II) were replaced with naltrexone-containing pumps and 7 more days of food intake were measured. Intakes of each diet were measured daily. Group I displayed an unexpected response to naltrexone as 6 of 10 rats ate more than 80% of calories from the high CHO diet while 3 of 10 ate more than 90% of calories from the high fat diet. After the switch to saline, Group I showed a gradual moderation of these extreme preferences such that, under saline treatment, most rats ate a higher percentage of calories from the diet which was least preferred with naltrexone. Under initial saline treatment in Group II, no rats ate more than 80% of either diet. After switching to naltrexone, only one rat (which ate 95% high CHO) in Group II developed the extreme preference for one diet seen during naltrexone treatment in Group I. Overall, naltrexone-treated rats in each group ate significantly less total calories than under saline conditions. Thus, the effects of opioid antagonists on diet preferences are modified dramatically by previous experience with the diets available for selection. (Supported by NIDA grant 05471).

195.23

SPINAL VISCERAL AFFERENTS CAUDAL TO T_{13} ARE SUFFICIENT TO MEDIATE THE SATIETY ACTION OF BOMBESIN. C. Walsh*, S. Mindell*, T.C. Kirkham, D. Greenberg, J. Gibbs & G.P. Smith. Bourne Laboratory, The NY Hospital-Cornell Medical Center, White Plains, NY 10605.

The combination of total abdominal vagotomy (vgx) and disconnection of spinal visceral afferents caudal to T_2 abolishes the inhibitory action of peripherally-administered bombesin on food intake at a test meal in rats (Stuckey et al, 1985). Since vgx alone does not alter this satiety effect of bombesin, spinal visceral afferents entering the cord caudal to T_2 are sufficient to mediate the effect. To delineate further the extent of the visceral afferents involved, we tested the satiety effect of bombesin in groups of rats with the following surgical lesions in addition to vgx: bilateral greater splanchnic nerve section (n=5); bilateral central dorsal rhizotomy (dr) T_3-T_{13} (n=4); dr T_3-T_{10} (n=18); dr T_3-T_{10} + cord section at T_{10} (n=6); and appropriate sham lesions. After overnight (17h) food deprivation, one dose of tetradecapeptide bombesin (Bachem; 2,4, or 8 $\mu g/kg^{-1}$) or vehicle control was injected intraperitoneally immediately prior to 30 min access to liquid food (BioServ, 0.37 $kcal\text{-}ml^{-1}$). When vgx was combined with dr T_3-T_{10} + cord section at T_{10}, the satiating effect of the largest dose of bombesin was abolished. No other lesion significantly changed the satiating potency of bombesin. The differential results between rats with vgx + dr T_3-T_{13} (full satiety effect of bombesin) and with vgx + dr T_3-T_{10} + cord section at T_{10} (no satiety effect of bombesin) demonstrate that spinal visceral afferents entering the cord caudal to T_{13} are sufficient to mediate the satiety action of bombesin.

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195.24

POTENTIATION OF BOMBESIN-INDUCED SUPPRESSION OF FOOD INTAKE AFTER COMBINED VAGOTOMY AND CELIAC GANGLIONECTOMY. **S.C. Weatherford, J. McQuade, L.D. Melville, G.P. Smith and J. Gibbs. Department of Psychology, University of Washington, Seattle, WA 98195 and Department of Psychiatry, New York Hospital-Cornell Medical Center, White Plains, NY 10605.**

Gibbs and Smith (1988) have shown that combined high thoracic spinal-visceral disconnection and total subdiaphragmatic vagotomy (TSV) block the satiety effect of bombesin (BBS) in rats, while neither lesion alone is effective. Similarly, we have observed (unpublished observations) that celiac ganglionectomy (CGX) alone does not affect BBS-induced suppression of food intake. In this study we examined the effect of BBS on 18-min intake of 20% sucrose in rats that had received both TSV and CGX and in sham-operated controls.

RESULTS: Values are mean±SEM % suppression

	Dose ($\mu g \cdot kg^{-1}$)			
	2	4	8	16
Sham	9±5	9±13	38±12*	45±6*
TSV/CGX	36±8*#	37±13*#	59±4*	56±12*

Tests were conducted at 2 p.m. after a 6 hr fast. *# $p < 0.05$, * BBS vs. vehicle, # sham vs. TSV/CGX.

CONCLUSIONS: Inasmuch as the minimal effective dose and the magnitude of the response to BBS were enhanced in the TSV/CGX rats, these results suggest that not only do these neural substrates not mediate the satiety effect of BBS, but that their integrity may function to attenuate BBS-induced satiety. [supported by NIH DK33248 (JG).]

195.25

GRF EFFECTS ON MACRONUTRIENT SELECTION. P.R. Dickson and F. J. Vaccarino, Dept Psychology, University of Toronto, Toronto, Canada, M5S 1A1.

Growth hormone releasing factor (GRF) is a peptide which releases growth hormone from the anterior pituitary and stimulates food intake centrally. Microinjection of GRF into the suprachiasmatic nucleus/medial preoptic area (SCN/MPOA) stimulates intake of chow by non-deprived male rats. The present study extends these findings to examine how GRF affects intake of macronutrients (fat, protein and carbohydrates). Male Wistar rats with SCN/MPOA cannulae were habituated to macronutrient-specific diets for 2 weeks and then tested with GRF (1 pmol) and vehicle (.01% ascorbic acid). Intake (controlled for spillage) was recorded at 1, 2, and 4 hours post-injection. Diets were isocaloric, visually and texturally similar, and nutritionally complete. Results suggest that GRF specifically stimulates protein intake, and that there is a reliable time course to this effect. These results are in keeping with evidence implicating opiates in the expresion of GRF-induced feeding, and further suport the hypothesis of a central-peripheral integration of function for GRF.

195.26

CHRONIC INTRAHYPOTHALAMIC INSULIN INFUSIONS DIFFERENTIALLY AFFECT DIURNAL RHYTHMS OF FOOD INTAKE AND BODY TEMPERATURE IN THE RAT. K.M. Andrews*, M.K.McGowan†, & S.P. Grossman. Committee on Biopsychology & †Department of Anesthesia & Critical Care, University of Chicago, Chicago, IL 60637.

Insulin infused intrahypothalamically significantly decreases body weight and alters daily food intake patterns; day-time intake increases while night-time intake falls. To test whether insulin non-specifically influences other diurnal rhythms, rats were implanted with a thermosensor i.p. in order to measure daily pattern of body temperature. Body temperature was recorded hourly. Following baseline temperature readings, rats received chronic infusions of 1.5μU/hr of insulin into the ventromedial nucleus of the hypothalamus. In this experiment, insulin infusion shifted food intake patterns in a manner consistent with previous reports but differentially altered the diurnal pattern of body temperature. Insulin shifted the peak of the temperature cycle away from the light period; body temperature reached its zenith at a later point of the dark period, reaching significance only after the infusion terminated. Also, unlike the dampening effect of insulin on the food intake cycle, insulin significantly increased the core temperature range over a 24h period. Mean daily core temperature rose significantly under insulin infusion relative to both saline infusion and pre-op levels and remained significantly elevated during the post-infusion period. This mirrors insulin's effects on body weight, which was significantly depressed during infusion and post-infusion. These data suggest that insulin's effects on food intake patterns are not the result of a non-specific alteration of circadian rhythms. However, they imply that intrahypothalamic insulin may exert its effects on body weight by altering both food intake and metabolic processes.

INGESTIVE BEHAVIOR: NEURAL, HORMONAL AND GI

196.1

AN EXAMINATION OF POSTINGESTIVE MECHANISMS THAT SUPPRESS MEALS INITIATED IN RESPONSE TO CONDITIONED CUES. L.Whitten, S. Callaghan* and H.P. Weingarten, Dept. of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

Cues conditioned to food reliably elicit meal initiation and large meals. We examined postingestive factors that suppress intake in response to the presentation of food-associated conditioned cues.

We trained male Long Evans hooded rats (approx 280 gm) to associate a tone-light conditioned stimulus (CS) with food by presenting the CS for 4 min prior to each of six daily meals for 12 days. The meal consisted of 7 ml of a nutritionally-adequate liquid diet. On test days, maintenance food was removed and rats were provided with a 7 mls liquid diet premeal. One hour later, the CS was presented followed by a test meal of 15 mls liquid diet. Meal sizes induced by presentation of the CS were determined by measuring the amount remaining in the food cup 15 minutes after meal delivery.

First, we compared the ability of intraperitoneal (ip) and subcutaneous (sc) glucose to suppress eating. Glucose, at doses of .5, 1.0 and 1.5 g/kg, was injected 30 min prior to CS onset. Equivolumes of ip or sc .15M saline was the control. With ip injections, intake suppression was directly related to dose; overall, ip injections were more effective than sc in suppressing meals. Second, we tested the synergy between ip glucose and gastric distension. Treatments were .5 g/kg ip glucose and 10 mls intragastric .15M saline administered either alone or in combination 30 min prior to CS onset. Control condition was .15M ip saline and sham gavage. Neither glucose nor distension alone suppressed meal size. However, the combined treatment resulted in a 28% suppression of eating. These studies provide results encouraging future searches for postingestive mechanisms capable of suppressing conditioned eating.

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196.2

MEAL SIZE IS DETERMINED BY STOMACH VOLUME. V.E. MENDEL AND M. PALIESCHESKY. DEPT. AN. PHYSIOLOGY, UNIV OF CALIF., DAVIS, CA 95616

Voluntary consumption of food in mammals in all species is an episodic process. This suggests that either the brain monitors energy levels in tissues, including blood, or that the amount of food eaten in any feeding episode may be determined by volume and rate limiters in the gastrointestinal tract. Daily food intake can be determined by changing either meal size or meal frequency or both. This study was designed to determine whether size of the first meal following periods of fast is limited. Twelve Sprague-Dawley, male rats, averaging 389gm were placed in individual Meal Pattern Analysis modules. Six of the animals received a 15% casein basal diet (BD), the remaining six rats were offered a BD+30% corn oil diet (BD+30). Baseline food intake data were collected for 10 days and the first meal consumed following lights out during the last 3 days of baseline period was averaged and used as the zero meal size. Rats were randomly fasted 12, 24, 36, 48 hr. and size of the first meal was measured. Food was introduced 10 min. before lights out. First meal size (1st MS) at zero hr. averaged 2.7±0.30gm BD and 2.6±0.30gm BD+30. The 1st MS increased to 4.9±0.46 ($P<0.02$) gm on average after 12 hr. fast and did not significantly increase with fasts of greater duration. These results suggest that meal size is limited by stomach volume.

196.3

THE ROLE OF DELAYED GASTRIC EMPTYING IN ANOREXIA INDUCED BY INTESTINAL INFLAMMATION IN THE RAT. K.J. McHugh, H.P. Weingarten and S.M. Collins*, Intestinal Disease Research Unit and Department of Psychology, McMaster Univ. Hamilton Ont. Canada L8S4K1.

We have recently characterized a dramatic suppression of feeding that occurs in a rat model of intestinal inflammation induced by intra-rectal (i.r.) administration of trinitrobenzene sulfonic acid (TNB) and ethanol. We investigated the role of gastric emptying in the reduction of food intake in this model. Intestinal inflammation was induced by infusing 30mg TNB in 0.25ml of 50% ethanol in male Sprague Dawley rats. Control rats were infused with the 50% ethanol vehicle only. The rate of gastric emptying was measured by infusing a test meal of 15ml liquid diet (evaporated milk, sucrose, water and vitamins) by gavage directly into the stomach. Either 60 min or 90 min post- gavage rats were sacrificed, their stomachs removed and stomach contents emptied and dried. The percent of the original test load remaining in the stomach was determined by comparing the dry weight of remaining stomach contents with the dry weight of 15ml of liquid diet. Results were as follows:

Group	% remaining at 60 min	% remaining at 90 min
TNB+EtOH	55.9 ± 4.2	55.6 ± 6.9
EtOH	44.1 ± 5	37.4 ± 3.7
	$p<.05$	$p<.001$

Thus inflammation of the colon leads to a delay of gastric emptying. Although the mechanism mediating this effect is unclear, cytokines (eg. IL-1, TNF) released by the inflamed bowel segment may be relevant. Our results leave open the possibility that delayed gastric emptying may contribute to the decreased food intake that accompanies intestinal inflammation.

Supported by Medical Research Council of Canada.

196.4

A ROLE FOR HIPPOCAMPUS IN THE CONTROL OF INGESTIVE BEHAVIOR. **T.L. Davidson* and L.E. Jarrard. Dept. Psychol. Sci., Purdue Univ., West Lafayette, IN 47907, and Dept. Psychol., Washington & Lee Univ., Lexington, VA 24450.**

Rats with ibotenate (IBO) lesions of the hippocampus were compared to controls with respect to (1) feeding behavior; (2) the ability to use food deprivation and exteroceptive stimuli as discriminative cues. For 12 days postsurgery, feeding, drinking, and activity of the rats was monitored by an automated system. The rats were then given Pavlovian discrimination training. For half of the rats in each group, 24-hr food deprivation plus tone signaled shock and 0-hr food deprivation plus clicker signaled no shock. The remaining rats had the reversed contingency. Both groups were then tested in extinction (i.e., without shock) with deprivation cues alone, then with auditory cues alone. Discrimination was indicated to the extent that more freezing (i.e., skeletal muscle immobility) was elicited by the previously shocked cue (deprivation or auditory) than by the nonshocked cue.

Relative to controls, IBO lesioned rats were markedly impaired in their ability to use food deprivation cues, but not auditory cues, as predictors of shock. In addition, the feeding behavior of the IBO lesioned rats indicated that they were also unable to use their deprivation cues to predict when feeding was followed by rewarding consequences. The results suggest that the hippocampus may be involved in the utilization of information provided by food deprivation states.

196.5

HORMONAL AND SOMATIC CHANGES IN RATS PAIR FED BY COMPUTER OPERATED FEEDERS TO GROWTH RETARDED DORSOMEDIAL HYPOTHALAMIC NUCLEI LESIONED (DMNL) ANIMALS. L.L. Bellinger, T.W. Castonguay and L.L. Bernardis. Dept. Physiol., Baylor Coll. Dent., Dallas, TX 75246; Univ. Maryland, College Park, MD 20742; V.A. Med. Ctr., Buffalo, NY 14215.

Rats with DMNL are hypophagic and show reduced linear and ponderal growth, but have normal body composition and anabolic hormone concentrations. In the present study weanling Sprague Dawley rats were either bilaterally DMNL and ad lib fed (body weight, [BW], 93±3.5g, n=7); sham operated (BW, 90±3.4g, n=7) and pair fed to the DMNL group (SPF); or sham operated (BW, 92±2.7g, n=17) and ad lib fed (SAD). Pair feeding (Bioserve, 45 mg Rodent Grain Pellet) was accomplished using 14 photobeam-computer-activated pellet feeders in which the food availability of an individual SPF rat was "yoked" to the ad lib intake of a DMNL rat. Food intake, BW, nasoanal length (NAL), Lee Obesity Index, body composition using Model SA-2 EM-SCAN were calculated over the 3 week experiment. At experiment's end, blood was collected in the middle of the light phase after a 4 hr fast and assayed for insulin, corticosterone and T_3; correct lesion placement was determined histologically. DMNL rats were hypophagic ($P<0.01$) and gained less BW ($P<0.01$) than SAD. The ΔBW gains were similar in DMNL and SPF. The SPF showed the most ($P<0.01$) NAL growth retardation. Fat percentages calculated by EM-SCAN Model SA-2 were unreliable and Lee Obesity Indices were all in normal range at experiment's end. Plasma T_3 were similar in DMNL and SPF, but slightly lower than SAD. Corticosterone concentrations were similar in the three groups, whereas insulin was reduced ($P<0.05$) in SPF compared to SAD, while DMNL was not. The data indicate that the hypophagia of the DMNL rats may be producing some, but not all, of the physiological manifestations following lesioning. Supported in part by Baylor College of Dentistry, NIH DEO9725-01 and V.A. Research Funds.

196.6

Decreased Intestinal Transit following Area Postrema Ablation (APX). M. Kathleen Gruver*, Tom H. Burchard*, Nancy J. Kenney, Dept. Psychology, University of Washington, Seattle, WA 98195.

Gastrointestinal (GI) transit is reduced following APX. These studies examine the longevity of this effect and the possibility that APX-induced hypophagia may underlie the change of GI transit.

In the first study, conditioned taste aversion (CTA) development and GI transit of APX and control (CONT) rats were monitored 4 months after surgery. After an 8-day exposure to a novel AIN diet, APX rats demonstrated an aversion to AIN compared to CONT rats. GI transit was measured 2 hr after injection of 200 ul of a 20% charcoal suspension into the stomachs of urethane anesthetized (1.25 g/kg) rats. The charcoal traversed 9.1% of the total intestinal length of APX rats as compared with 23% of controls. Thus, decreased GI transit is a chronic effect of APX and may underlie the CTA induced by APX.

In the second study, GI transit of APX, ad-lib-fed CONT rats and intact rats whose daily food intake was restricted to the amount eaten by the APX rats (DEP rats) was studied. GI transit of APX rats (7.0%) was suppressed compared to that of CONT (30.0%) or DEP rats (27.5%). GI transit of CONT and DEP rats did not differ. Thus, hypophagia alone does not account for decreased GI transit of APX rats.

196.7

EFFECTS OF AREA POSTREMA LESIONS ON TASTE REACTIVITY IN RATS. L.A. Eckel and K.-P. Ossenkopp. Dept. Psychology, Univ. of Western Ontario, London, Ontario, CANADA N6A 5C2.

The present study examined the effects of intra-oral infusions of varying concentrations of sucrose solutions on taste reactivity in rats with area postrema lesions (APX) or sham lesions (APS). The taste reactivity test involves videotaping the activity of lingual, masticatory, and facial muscles during direct oral contact with specific taste stimuli. In the first phase of the experiment, rats were habituated to the test chamber while distilled water was infused via a chronically implanted intra-oral cannula. The second phase of the experiment involved the infusion of four concentrations of glucose (.01 M, .03 M, .30 M, and 1.0 M), each for a period of 3 minutes at a rate of .78 ml/min. During all infusions, the resulting oral and somatic responses to the taste stimuli (taste reactivity test), were videotaped and analysed at one-fifth the normal tape speed. The results revealed comparable enhanced ingestive responses by both groups to the sucrose solutions in comparison to the distilled water. Furthermore, these responses were concentration dependent such that both groups showed sucessively stronger ingestive sequences in response to the increasing concentrations of sucrose ($p<.05$). This finding indicates that lesioning the area postrema is not disrupting the rat's ability to discriminate between the varying concentrations of sucrose, relative to the APS group.

(Supported by a grant from NSERC to KPO).

196.8

INTRAVENTRICULAR INJECTIONS OF GLUCOCORTICOIDS IN ADRENALECTOMIZED AND SHAM OPERATED SPRAGUE-DAWLEY RATS: EFFECTS ON DIETARY SELF-SELECTION. T.W. Castonguay, M.E. Bligh*, and K. Kamara* Department of Human Nutrition and Food Systems, University of Maryland, College Park, MD 20742.

Adrenalectomy (ADX) promotes a change in the dietary selection patterns of adult male rats. ADX rats typically reduce protein and fat intake when compared to sham operated controls. Systemic corticosterone therapy restores dietary selection patterns so that the proportion of calories taken from protein, fat and carbohydrate in the diet of ADX rats does not differ from that of sham operated controls. The present study was conducted to test the relationship between glucocorticoid replacement of ADX rats that was limited to the brain and dietary self-selection patterns. Sixty-four male Sprague-Dawley rats were surgically fitted with intraventricular (ICV) cannulae and either bilaterally adrenalectomized or given sham operations. Patency of the ICV cannula was verified by ICV injection of 150 ng Angiotensin II followed by at least 5 ml of water consumed within 1 h of injection. Adx rats were then assigned to one of four dose groups (0, 5, 50 or 500 ug glucocorticoid [either corticosterone or corticosterone acetate] in volumes ranging from .2 - 10 ul.)

Sham operated rats were given vehicular injections only. Vehicles used included ETOH, 50% ETOH in isotonic saline, or Tween 80 + carboxymethylcellulose + ETOH. Each rat was injected and weighed daily, and its daily intake of separate protein, fat and carbohydrate determined. Results from these procedures failed to demonstrate any specific effect of ICV glucocorticoid replacement. Of particular note was that all rats with the exception of those given very low volume vehicle injections (0.2 ul/d) failed to gain weight during the injection period. (Sponsored by a grant from the Maryland Agricultural Experiment Station Competitive Grants Program.)

196.9

CENTRAL BLOCKADE OF GLUCOCORTICOID RECEPTORS IN THE ZUCKER RAT. V.R.Burden*, B.D. White and R.J. Martin. Dept. of Foods and Nutrition, University of Georgia, Athens, GA 30602.

In the Zucker obese rat, it appears that glucocorticoids are necessary for hyperphagia and the development of obesity. While peripheral delivery of the glucocorticoid antagonist, RU 486, has resulted in food intake and body weight gain parallel to lean Zuckers, it is not known whether this occurs through a peripheral or central site of action. This study was designed to investigate the effect of centrally administered glucocorticoid antagonists. Lean (fa/?) and obese (fa/fa) male Zucker rats (8-9 weeks) were implanted with cannula directed toward the lateral ventrical of the brain. Rats received glucocorticoid antagonists (RU 318, RU 486) or vehicle at a rate of 20 ng/hr through mini-osmotic pumps attached to the cannula. After 18 days of infusion there were no statistical differences in body weight or food intake among rats receiving the different compounds within each phenotype. At this time, the osmotic pumps were replaced with new pumps containing the glucocorticoid antagonists that delivered at a higher rate of 1 ug/hr. After 13 days body weight gain was significantly decreased ($p<.05$) in obese rats receiving RU 486 compared to rats receiving vehicle. Total food intake was also reduced in the RU 486 treated obese rats ($p<.05$). The antagonists had no significant effects on body weight or food intake of lean rats. These results show that obese Zuckers are more susceptible to blockade of central glucocorticoid action than lean Zuckers.

196.10

EFFECTS OF CENTRAL TYPE I AND TYPE II GLUCOCORTICOID RECEPTOR STIMULATION ON FOOD INTAKE, BODY WEIGHT GAIN, AND FOOD EFFICIENCY. B.D. White, R.G. Dean* and R.J. Martin. Dept. of Foods and Nutrition, University of Georgia, Athens, GA 30602.

In this study, we further examined the role of Type I and Type II glucocorticoid receptor stimulation on food intake and body weight gain, by the central administration of specific agonists and antagonists. Forty-eight male Sprague-Dawley rats (approx. 175 g) were divided into five groups. Each was implanted with an ICV cannula connected subcutaneously to an osmotic pump containing vehicle. After one week, four of the groups were ADX and the pumps were replaced with pumps containing either ALDO and RU486, RU362 and RU318, RU362 and ALDO, or vehicle (propylene glycol). Each was delivered at a rate of 20 ng/hr. The remaining group was sham-operated and received vehicle. After two weeks, food intake or body weights of the treatment groups were not different from the ADX-vehicle treated group. Replacement pumps, delivering at a rate of 1 ug/hr, were then administered. From the point of the second pump replacement, Type II receptor stimulation, alone or in combination with Type I receptor stimulation, resulted in lower weight gains as compared to the other groups. Food intake was not different between the groups. Differences in food efficiency mirrored differences in body weight gain. Type I receptor stimulation resulted in a restoration of body weight in ADX rats.

196.11

GLUCO- AND MINERALOCORTICOID RECEPTOR MODULATION OF FOOD INTAKE. D.L. Tempel, T. Kim*, S.F. Leibowitz. Rockefeller Univ., N.Y. N.Y. 10021.

Glucocorticoids, which bind to both type I and type II steroid receptors, are involved in the control of food intake. Mineralocorticoids, which only bind to type I receptors, may also play a role in feeding behavior. These experiments studied the impact of adrenalectomy (ADX) and subsequent corticosterone (CORT) and aldosterone (ALDO) administration on food intake in male Sprague-Dawley rats maintained on pure diets of protein (P), carbohydrate (C) and fat (F). Feeding was monitored over the 24h period, and during the first 2h of the dark feeding cycle. Results indicate that, in animals eating ≥20% of each diet over 24hr, ADX decreases total intake by 30-40%, due to a decline in both C (-30%) and F intake (-40%). At dark onset, a strong suppression of C is observed (-93%), accounting for most of the decrease in 24h C intake, whereas, only a small decline (-15%) in F intake, accounting for ≤15% of the decrease in daily F intake is evident. C feeding (2h and 24hr) is restored by CORT at doses of 0.25-≥2.0 mg/kg (s.c.), while F intake (2h and 24h) is stimulated only by higher doses (≥2.0mg/kg s.c.). ALDO (0.1mg/kg s.c.), strongly enhances F intake, both in the early dark and also throughout the 24 hr feeding period. ALDO also potentiates C intake at dark onset, but this effect is small relative to ALDO's effects on fat intake. These results are consistent with those found after hypothalamic (PVN) implant of CORT and ALDO, and may indicate a central site of action for these hormones. These data indicate that there may be diurnal variations in CORT's effects on food intake and further suggest that F may be modulated by the type I mineralocorticoid receptor, whereas, C intake, specifically at dark onset, may be dependent upon the type II glucocorticoid receptor or a combination of both type I and type II activity.

196.12

EFFECTS OF TYPE I AND II CORTICOSTERONE RECEPTOR AGONISTS ON VENTROMEDIAL HYPOTHALAMIC LESION-INDUCED OBESITY. T.L. Thomas and L.D. Devenport, Dept. of Psychology, University of Oklahoma, Norman, OK 73019.

Ventromedial hypothalamic (VMH) lesions result in rapid weight gain and obesity. While this syndrome is known to be dependent on corticosterone (Cort) for its full expression, it is not known whether Cort acts via stimulation of type 1 or II Cort receptors. To test this, female S-D rats received bilateral VMH lesions, followed 2 weeks later by adrenalectomy (ADX), and 1 week later by mini-pump implantation for delivery of various doses of Aldosterone (type I agonist), RU 28362 (type II agonist), or combinations. Weight gains and food intakes were measured throughout. Results replicated previous findings that VMH lesion causes animals to eat more and gain more weight, and that ADX reverses this effect. VMH lesion was found to invert the normal response to both receptor agonists. Therefore, it is type II receptor stimulation that supports the enhanced weight gain following VMH lesion. It does this by a combination of altered metabolic efficiency and increased food intakes.

196.13

ACTIVATION OF CENTRAL TYPE II ADRENAL STEROID RECEPTORS ENHANCES RATE OF WEIGHT GAIN IN UNDERWEIGHT ADRENALECTOMIZED RATS. P.K. Green, C.W. Wilkinson, S.C. Woods. Depts. Psych. and Psychiat. and Behav. Sci., Univ. of Washington, Seattle, WA 98195 and VA Med. Ctr., Tacoma, WA 98493

We have previously reported that corticosterone (CORT) acts directly on the central nervous system to modify central regulators of adiposity. Intraventricular (IVT) administration of CORT enhances rate of weight gain in underweight adrenalectomized (ADX) lean rats, and slightly decreases rate of weight gain in sham-ADX rats, and in ADX rats at control body weight. Since it has been reported that there are two types of corticoid receptors with differing affinities for CORT located in the rat CNS, we investigated the receptor type through which CORT acts to increase weight gain. Male Long-Evans rats weighing 280-320g were restricted to half baseline food intake (pelleted chow, 13g/day) for 10 days, and then given ADX or sham-ADX. The next day, animals received a single IVT injection of 100 ng aldosterone (ALDO), dexamethasone (DEX), RU-28362, or synthetic CSF vehicle. Animals were then restored to ad lib chow, and body weight was monitored for six days. IVT-ALDO, a specific type I receptor agonist, slightly suppressed weight gain in underweight ADX rats over the 6 days following treatment (VEH: 28.6 g; ALDO: 23.1 g, n.s.). IVT-DEX, a mixed type I and II receptor agonist, and RU-28362, a specific type II receptor agonist, significantly increased weight gain (VEH: 27.2 g; DEX: 43.7 g; RU: 46.6 g, $p < .05$). We therefore conclude that CORT acts centrally via type II corticoid receptors to enhance weight gain in underweight lean rats.

196.14

"SELF-IMPOSED" INGESTIVE BEHAVIOR STRATEGIES IN RATS: EFFECTS OF CHRONIC CALORIC RESTRICTION ON HORMONES, GROWTH, AND BODY TEMPERATURE. J.K. Nishita, C.E. Serface*, and P.D. Martorano*. Dept. of Psychology, San Jose State Univ., San Jose, CA 95192-0120.

Our previous research has attempted to describe and analyze the "self-imposed" ingestive behavior strategies of female rats during long-term caloric restriction in our novel apparatus. In these studies, we have described changes in ingestion, body weight, hoarding behavior, and stress-related hormones (Nishita et al., 1989, 1990a,b). When housed in our novel apparatus, rats must crawl through a Plexiglass cylinder to obtain daily food and water. We hypothesized that these rats will manifest neuroendocrine changes similar to those produced by other stress-related paradigms. In the present study, the stress effects of the physical restriction produced by two different cylinder sizes (Small (SM) = 3.3 cm, Large (LG) = 4.2 cm, i.d.) were compared in male and female rats.

Both SM and LG rats showed weight loss and decreased linear growth compared to controls housed with No Cylinder (NC). SM male rats showed significantly lower rectal temperatures (36.0°C) than LG (36.4°C) and NC (36.7°C) rats following six weeks of housing in our apparatus. SM female rats (n = 11) showed elevated plasma vasopressin (21.5 pg/ml) compared to LG (7.1 pg/ml) and NC (6.1 pg/ml) littermates. However, plasma osmolality, sodium, and potassium were not significantly different among these groups. Furthermore, adrenal weights and plasma corticosterone levels did not differ among the SM, LG, and NC female rats following 100-120 days of continuous housing.

196.15

BLOOD GLUCOSE LEVEL AFFECTS INGESTION IN THE GASTROPOD, LIMAX FLAVUS. C.B. Phifer and F.H. Perkins. Louisiana Scholars' College, Northwestern State University, Natchitoches, LA 71497.

Blood nutrient levels affect feeding behavior in mammals and some other vertebrates but the relationship between fluctuations in nutrient levels, particularly glucose, and feeding has proven to be quite complex and resistant to interpretation. Results from a few previous studies have indicated that invertebrate animals, particularly molluscs, might regulate blood glucose levels, but the possible role of blood glucose in ingestive-behavior control has not been investigated. In this study on the terrestrial slug Limax flavus, food deprivation was shown to be associated with decreased blood glucose levels (32.4 ± 2.8 mg/100ml for fed, 7.3 ± 1.3 for 14-day fasted). A probable role for glucose level in ingestion was demonstrated when glucose injections that raised the blood glucose level of fasted slugs to that of free-feeding slugs also inhibited food intake. Control injections of mannitol, a non-metabolizable alcohol sugar, had no effect on intake. Continuing studies may reveal the physiological significance of these results. The accessibility of the gastropod CNS and relative ease of neurophysiological study may make this simple invertebrate preparation a valuable model for experiments on blood glucose regulation and nutritive control of feeding.

196.16

SUMMATION OF REWARDING EFFECTS PRODUCED BY HYPOTHALAMIC STIMULATION AND INTRAORAL SUCROSE INFUSION. K.L. Conover & P. Shizgal. Concordia Univ., Montreal, Quebec H3G 1M8.

Competition and summation between the rewarding effects produced by tastants and by electrical stimulation of the lateral hypothalamus (LH) were measured in a preference paradigm. In the competition test, rats with open gastric cannulae were offered a forced, two-alternative choice between a 0.055 ml infusion of 1M sucrose and a train of LH stimulation; the parameters of the sucrose infusion (the "standard") were fixed whereas the frequency of the LH stimulation (the "alternative") was varied across trials. In the summation test, LH stimulation again served as the alternative but the standard was the combination of a sucrose infusion and the equi-preferred frequency of LH stimulation as determined in the competition test. The combination of the LH stimulation and the sucrose infusion acted as a more potent reward than either of its individual components; the frequency of the alternative had to be increased by 26-58% in order to match its value to that of the compound standard. These findings indicate that the rewarding effects of hypothalamic stimulation and sucrose can be evaluated in a common system of units and combined.

196.17

COMPARISONS AMONG THE CALMING AND ANTINOCICEPTIVE EFFECTS OF SUCROSE, GLUCOSE, FRUCTOSE, AND LACTOSE IN INFANT RATS. D.J. Shide[1] and E. M. Blass[2]. [1]Dept. of Psychiatry, Johns Hopkins Univ., Baltimore, MD 21205; [2]Depts. of Psychology & Nutrition, Cornell Univ., Ithaca, NY 14853.

These experiments compared the quieting and antinociceptive effects of different sugars. In Exp. 1, individually housed 10-day-old Sprague-Dawley rat pups received 1% BW intraoral infusions of either 0.22, 0.44, or 0.66 M concentrations of sucrose, glucose, fructose, lactose, distilled water, or no infusion. Ultrasonic vocalizations were recorded. In Exp. 2, Day 10 pups received an infusion of 0.22 M sucrose or glucose solution, or 0.44 M fructose or lactose solution, while separated from the mother but group-housed as a litter; paw-lift latencies from a 48° C hot-plate were determined immediately after infusion termination. In both experiments pups were injected with either naltrexone (1.0 mg/kg) or saline 30 min prior to testing to evaluate the contribution of endogenous opioids.

Sucrose and glucose were equally effective calming agents, at all concentrations tested, fructose at the higher concentrations (0.44 & 0.66 M); naltrexone reversed taste-mediated calming at the lowest (0.22M), but not the higher, sugar concentrations. Sucrose, glucose, and fructose elevated pain threshold in a naltrexone-reversible fashion. Lactose, the milk sugar, was ineffective at calming or elevating pain threshold.

196.18

DUODENAL LIPID INFUSIONS SUPPRESS SHAM INTAKE OF SUCROSE, POLYCOSE, AND A LIQUID DIET. L.A. Foster, K. Nakamura, R. Norgren, D. Greenberg[1], and G.P. Smith[1]. Dept. Beh. Sci., Col. Med., Penn State Univ., Hershey, PA 17033 and Dept. Psychiatry[1], New York Hosp.-Cornell Med. Center, White Plains, NY 10065.

A lipid infusion into the duodenum will suppress sham-fed intake of liquid food in rats before significant absorption of the lipid occurs. In this study, a duodenal infusion of lipid decreased intake of a liquid diet, a solution of glucose polymers (Polycose), and a sucrose solution. Rats were fitted with a chronic gastric fistula and a duodenal catheter (n=4 for each diet). After an overnight fast, the rats sham fed either a nutritionally complete liquid diet, a 10% Polycose solution, or a 0.3M sucrose solution for 90 min. Twelve min after the start of the sham feeding session, a duodenal infusion of Intralipid (from a concentration series) or saline was given over a 20 min period. Fluid intake was measured every 5 min throughout the 90 min test period. Although the efficacy varied depending upon the oral stimulus, a duodenal infusion of lipid did suppress sham intake of all 3 solutions during at least 30 min of the test period ($p<0.02$). These results support earlier observations that food deprived rats treat a sucrose solution in a manner similar to more complex liquid foods. In addition, the data demonstrate that intraduodenal lipids will suppress sham intake of diets that do not contain any fat. Supported by PHS DC 00067, DC 00240, DK 38757, MH 00653, MH 15455[1], and the International Life Sciences Institute[1].

196.19

STIMULUS EFFECTS OF OREXIGENIC OPERATIONS: INSULIN, 2-DEOXY-GLUCOSE (2-DG), AND FOOD DEPRIVATION. K.J. Schuh*, D.W. Schaal*, J. Cleary*, T. Thompson* & A.S. Levine. Univ. of MN and VA Med. Ctr., Minneapolis, MN 55455 and 55417.

Three groups of rats (4 rats per group) were trained to discriminate the stimulus effects of insulin (1.0 U/kg) or 2-DG (125.0 mg/kg) from saline, or 2 hrs since feeding from 23 hrs since feeding (i.e., relative food deprivation and food satiation). 15 presses on one lever were reinforced with sweet water following administration of insulin or 2-DG or when relatively food deprived, and 15 presses on the other lever were reinforced after saline or when relatively food satiated. 15 presses on the condition-inappropriate lever resulted in an 8-s period of darkness (time-out). After training, other doses of 2-DG (25.0, 75.0, 175.0 mg/kg) were tested under conditions in which the lever first chosen produced a reinforcer. Rats chose the saline-appropriate lever after saline or 25.0 mg/kg, the 2-DG-appropriate lever after 125.0 and 175.0 mg/kg, and both levers equally after 75.0 mg/kg. Rats trained to discriminate food deprivation were tested after being fed 6 and 14 hrs prior to testing. Rats chose the lever associated with food satiation after 2 and 6 hrs, but chose the opposite lever after 14 and 23 hrs since being fed. Insulin (0.25, 0.5, 1.0 and 1.5 U/kg) was tested in rats trained to discriminate 2-DG. 1.0 and 1.5 U/kg resulted in 2-DG-appropriate responding, although in some rats response rates were greatly suppressed by these doses, making firm conclusions difficult. Lower doses of insulin resulted in saline-appropriate responding. These results indicate that 2-DG, insulin, and food deprivation are discriminable and that their stimulus effects may substitute for one another.

196.20

HEPATIC ENERGY METABOLISM CORRELATES WITH FEEDING IN RABBITS. L. O'Farrell and D. Novin. Neuroscience Program and Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Slow hepatic portal glucose or fructose infusion (1 ml/min) has previously been shown to decrease subsequent chow intake in rabbits, while fast infusion (3 ml/min) increases it. A possible mechanism for these effects was investigated in the present research by testing whether liver metabolism correlated with feeding.

Rabbits were infused with either fast glucose, slow glucose, fast fructose or slow fructose, which included tracer amounts of U-^{14}C-glucose or U-^{14}C fructose, and tritiated water.

Hunger-stimulating infusions caused more uptake of tritium into hepatic lipid than satiety-inducing infusions. Satiety-inducing infusions caused more uptake of monosaccharide into hepatic glycogen and hepatic mitochondria than hunger-stimulating infusions.

The results suggest hunger may be induced by diversion of incoming carbon skeletons away from oxidation and toward lipid synthesis in the liver of rabbits. Satiety may be related to increased hepatic glycogen synthesis.

196.21

ATTENUATED BODY WEIGHT RECOVERY IN AGED MALE BUT NOT FEMALE LONG-EVANS RATS Collin R. Park*, Sabine M. von Preyss-Friedman*, Robert S. Schwartz & Stephen C. Woods Program in Physiology/Psychology, and Departments of Medicine and of Psychology, University of Washington, Seattle WA 98195

We have previously found that aged male Long-Evans rats have a decreased rate of weight loss and attenuated weight regain compared to young rats. We now extend this model to aged female rats. Food intake and body weight were determined daily on 18 mo. old female Long-Evans rats. Other measures taken include plasma insulin and glucose, body temperature and general activity.

After a 2-week Baseline period, food was restricted to 70 % of baseline intake given as two meals separated by 6 h. until animals had lost 25 % of their baseline weight. Animals were then allowed ad lib access to food. Control animals had 100% of their baseline food intake available throughout.

Restricted animals averaged 55 days to reach criterion, and 2 weeks to recover, although hyperphagia continued for over 7 weeks (18.9 g vs 25.7 g, $p=0.02$, within-subjects).

In contrast, aged male Long-Evans rats require over 5 weeks to regain body weight. Our results suggest that abnormalities of body weight regulation are more pronounced in aged male then female Long-Evans rats.

197.1

GALANIN IMMUNOREACTIVE (GAL-I) CELLS WITHIN THE MEDIAL PREOPTIC AREA(MPOA) OF THE RAT: SEX DIFFERENCES AND RESPONSE TO GONADAL STEROIDS. C.B. Eckersell*, R. Mills*,B. Padgett* and G.J. Bloch Dep't of Psych.,Brigham Young U.,Provo,UT, 84602

GAL-I cell bodies and fibers are found in the rat brain, including the MPOA (Melander et.al, Eur.J.Pharm.124,'86; Levin et.al, J.C.N. 261, '87). Estrogen(E) concentrating cells co-contain GAL, E affects GAL-immunoreactivity, and increases GAL mRNA levels (Bloch et.al., Neurosci.Abst.15,'90; Kaplan et. al, P.Nat.Acad.Sci. 85,'88; Gabriel et.al Neuro-end.51,'90). The number and density of GAL-I cells was determined within the sexually dimorphic anteroventral periventricular nucleus and central part of the medial preoptic nucleus (AVPv, MPNc; Simerly et.al; Bloch et.al; J.C.N. 225,275,'84,'88) in 18-day gonadectomized (Gxd) rats. There were significantly greater numbers of GAL-I cells within the MPNc, and fewer within the AVPv, of the Gxd male than the Gxd female. Estrogen increased GAl-I cell numbers and densities within the AVPv of the male, and the MPNc of rats of both sexes. These findings support data suggesting that GAL within the MPN affects gondal steroid-sensitive functions (Neurosci. Abst. 16, 17,'90,'91).

197.2

GALANIN (GAL) MICROINJECTED INTO THE MEDIAL PREOPTIC AREA (MPOA) FACILITATES MALE-TYPICAL COPULATORY BEHAVIOR IN THE FEMALE. J.G. Kohlert, P. Butler*, C. Lowry*, R. Hammond*, D. Schwitters*, J. Peterson*, S. Kurth, S. Shillingford* and G.J. Bloch. Dep't of Psychology, Brigham Young Univ., Provo, UT, 84602.

Since steroid concentrating cells that contain GAL-immunoreactive material have been found in sexually dimorphic regions of the male and female rat MPOA (Bloch et al., Neurosci.15,'89), and microinjections of GAL into the MPOA of male rats significantly increased measures of male sexual behavior (Butler et al.,Neurosci.16,'90), female and male sexual behaviors were assessed in gonadectomized adult females. There were no significant differences in lordosis and proceptive behaviors in females given 0.3ul microinjections of 0(vehicle), 10, 50, 100, or 500 ng GAL into the MPOA. However, when given testosterone at a level comparable to that of intact males, 50 and 500 ng GAL microinjections caused an increase in mount frequencies and a significant reduction in mount latencies. General arousal was unaffected by GAL. Coupled with previous findings, these data suggest that GAL affects male-typical copulatory behavior in both males and females. Supported by BYU research funds.

197.3

ONSET OF THE HORMONE-SENSITIVE PERIOD FOR SEXUAL DIFFERENTIATION IN FEMALE RATS. R.W. Rhees, S. Sephton*, G.J. Bloch, and D.E. Fleming. Depts. of Zoology and Psychology, Brigham Young University, Provo, UT 84602.

Sexual differentiation of many brain structures and functions is dependent on levels of testosterone during the last few days of gestation and the first few days postpartum. If testosterone is present during this perinatal period, masculinization and defeminazation of sexual behavior, reproductive physiology, and central nervous system morphology occur. The purpose of the present study was to determine the precise onset of the prenatal component of the period. To avoid complications induced by endogenous testicular activity, only females were used. Free testosterone was used because of its short half-life, so that the effects induced by its administration on a specific day of gestation could be evaluated. Pregnant rats were injected SC with sesame oil or 5 mg testosterone (T) on day 16, 17, 18, 19, 20, 21, or 22 of gestation. Female offspring exposed to T displayed an increase in anogenital distance (16-18), delayed vaginal opening (16-19), abnormal vaginal morphology (16-20), and lower levels of lordotic behavior (18-22). Exposure to T, on any of the prenatal days, however, did not alter their ability to exhibit an LH surge. We conclude that the onset for altered reproductive morphology occurs at least as early as day 16 whereas the onset of sexual behavior sensitivity occurs precisely on day 18 and that the normal pattern of adult LH release is not altered by prenatal androgen treatment using this specific paradigm.

197.4

CHRONIC TESTOSTERONE (T) IN FEMINIZED MALE RATS DURING 15-30 DAYS OF AGE: SEX REVERSAL OF BEHAVIORAL FUNCTION IN ADULTHOOD. S.D. GALE, G.J. BLOCH, R.W. RHEES, AND D.E. FLEMING, Psychology and Zoology, Brigham Young U., Provo, UT, 84602.

Lordosis behavior in male rats can be markedly stimulated in adulthood under special circumstances such as after microinjection of CCK into the medial preoptic area [Bloch et al, Physiol. Behav. 46,'89], or after pulse administration of estrogen [Sodersten et al, Endoc. 112,'83;Olster et al, Horm.Behav. 6,'88]. Last year we reported that chronic free T during 15-30 days of age can permanently defeminize endocrine and behavioral function in females [Gale et al, Neurosci.16,'90]. To extend this finding to the male, 7 mm T capsules or nothing (blank) were implanted into 15-day-old males that had been castrated neonatally, and the capsules were removed 15 days later (30 days of age). T-implanted males showed significantly lower levels of lordosis behavior and proceptive behaviors when compared to blank-implanted males (neonatally castrated controls). Thus, as in the female, chronic androgen during a period well beyond the perinatal time can also permanently defeminize reproductive function in the male.

197.5

INTRACEREBRAL ADMINISTRATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) ANTISENSE OLIGODEOXYNUCLEOTIDE REDUCES LORDOSIS BEHAVIOR IN THE RAT. M.M. McCarthy, S. Schwartz-Giblin and D.W. Pfaff. Rockefeller University, NY., NY. 10021

Antisense oligodeoxynucleotides to mRNA hybridize and specifically block protein translation in a variety of cell culture systems. GABA in the ventromedial hypothalamus (VMH) and midbrain central gray (MCG) facilitates lordosis, but in the preoptic area (POA) GABA is inhibitory (McCarthy et al., 1990, 1991). We administered a 15-mer antisense oligonucleotide spanning the start codon for the GABA synthetic enzyme, GAD, into the brain of hormonally-induced sexually receptive females. The antisense oligo was either unmodified (ODN) or contained a methyl-phosphonate group (MP-ODN). Controls consisted of oligos containing the same bases in a scrambled order. Female rats were implanted with 23g guide cannulae directed at the POA, VMH or MCG. Injection cannulae (28g) were loaded with crystalline ODN or MP-ODN mixed with cholesterol (1:1 for ODN; 1:10 for MP-ODN) and placed in the brain for 3 days after which they were removed and examined, indicating some inconsistencies in rates of oligo delivery. Nonetheless, either ODN or MP-ODN in the VMH or MCG caused a significant decrease in lordosis at 1, 2 and 3 days post implantation compared to pretest levels. In the VMH, there was also a smaller nonsignificant reduction in lordosis in animals receiving scrambled ODN and MP-ODN. There was no effect on lordosis of ODN or MP-ODN in the POA. In a separate experiment, females were bilaterally infused into the VMH with ODN (500ng) dissolved in oil (0.5µl/side; n=11). There was a significant 50% reduction of lordosis compared to scrambled controls at 1, 2 and 3 days post-infusion with full recovery by 6 days. The precise mechanism of GAD antisense ODN and MP-ODN reduction of lordosis remains unclear but may involve hybridization arrest of GAD mRNA as well as some non-specific disruption of protein synthesis, and is likely to depend on the hormonal milieu.

197.6

EFFECTS OF ALPHA-ADRENERGIC AGONISTS AND ANTAGONISTS INFUSED INTO THE MEDIAL PREOPTIC AREA (MPOA) AND MEDIAL BASAL HYPOTHALAMUS (MBH) ON LORDOSIS IN THE GUINEA PIG. K.F. MALIK, J.I. MORRELL, and H.H. FEDER. INST. OF ANIM. BEHAV., RUTGERS UNIVERSITY, NEWARK, NJ 07102.

We sought to characterize central targets mediating alpha-adrenergic modulation of lordosis.

Exp. 1. Ovariectomized (OVX) females were given 10ug estradiol benzoate (EB) subcutaneously and 40hrs later infused via bilateral cannulae with 2ug/0.5ul of the alpha-adrenergic agonist clonidine (CLON) or saline (SAL). CLON infused in the MBH (N=10) transiently (15min to 2.0hr) increased, up to 92%, the time (secs) animals held lordosis ($p<.01$) compared to SAL (N=8). CLON infused in the MPOA had no effect on lordosis.

Exp. 2. OVX females received 10ug EB, then 40hrs later 500ug progesterone (subcutaneously); 4hrs later the alpha-adrenergic antagonist phentolamine (PHEN; 5ug/0.5ul) or SAL was infused. PHEN in the MBH (N=8) transiently reduced the time that animals held lordosis posture by as much as 64% (1.0 hr) after infusion ($p<.01$) compared to SAL (N=8). Conversely, PHEN (N=8) in the MPOA increased time in lordotic posture by 74% (1.0hr) after infusion (N=9 SAL; $p<.01$).

These results suggest that alpha-adrenergic neurotransmission accounts in part for opposing actions of the MPOA and MBH in the regulation of lordosis. (Supported by HD 04467 to HHF and JIM)

197.7

α_1-ADRENERGIC AGONISTS ACT ON VENTROMEDIAL HYPOTHALAMUS TO CAUSE NEURONAL EXCITATION AND LORDOSIS FACILITATION. L.-M. Kow, G.D. Weesner*, D.W. Pfaff. The Rockefeller University, New York, NY. 10021

To investigate the suggestion that norepinephrine (NE) acts through α_1-adrenergic receptors (α_1-) in the brain to facilitate lordosis, we first infused α_1-agonists, phenylephrine (PhE) or methoxamine (MA) into the lateral ventricle of estrogen-primed female rats. Both PhE (0.2 and 1.0 µmol/rat) and MA (0.2, 0.5, and 1.0 µmol/rat) facilitated lordosis in a dose-dependent fashion, while MA at 0.1 µmol/rat had no effect. Infusion of a ß-agonist, isoproterenol (Isop, 0.2 µmol/rat), was not effective, either. Neural mechanisms potentially underlying the facilitatory effect were then studied. Since the ventromedial hypothalamic nucleus (VMN) is a likely site of action, the effects of PhE and MA, both at 10 µM, on the electrical activity of single VMN neurons *in vitro* were recorded. MA excited 78% of 65 neurons tested, and never caused inhibition. PhE excited 61% of 64 neurons, but also caused inhibition (9%) and biphasic responses (3%). Although excitations by PhE and MA differed in time courses, both were blocked by α_1-antagonist, prazosine (PZ, 0.5 µM), and also by chloroethylclonidine (CEC, 100 µM), an α_{1b}-antagonist.

To see if the excitatory action on VMN neurons was responsible for the facilitation of lordosis, PhE or MA was infused bilaterally into the VMN of ovariectomized rats. At 2 or 4 nmol/site, both PhE and MA facilitated lordosis in estrogen-treated, but not in untreated rats. Infusion of MA outside the VMN, or infusion of Isop or an α_2-AR agonist, clonidine, at doses similar to or higher than those of MA had no effect. The facilitatory effect of MA was abolished or attenuated by a preceding infusion of PZ or CEC (both 2 nmol/site), respectively. Thus, our combined electrophysiological and behavioral investigations indicate that, NE can facilitate lordosis by increasing the activity of VMN neurons, and that the effect is mediated largely through α_{1b}-receptors, known to be coupled to the hydrolysis of phosphoinositides.

197.8

GABAERGIC-SEROTONERGIC INTERACTIONS IN REGULATING LORDOSIS. Victoria Luine and Maya Frankfurt. Hunter College and Rockefeller University, New York, N.Y. 10021.

We investigated interactions between hypothalamic GABA and 5HT in regulating lordosis. OVX rats, primed with 5 ug of EB and 500 ug of progesterone (P), were given Baclofen, a GABA B agonist, and lordosis was tested 3 h. later. Baclofen inhibited lordosis quotients (LQ) and quality scores in a dose-dependent manner (2.5 to 10 mg/kg). To assess possible GABA-5HT interactions, intra-hypothalamic 5,7-DHT was given to deplete 5HT. In these rats, Baclofen, 7.5 or 10 mg/kg, significantly reduced LQs to approximately 60. In unlesioned rats, Baclofen reduced LQs to 26 (7.5 mg/kg) and 0 (10 mg/kg), suggesting that part of Baclofen's inhibitory effect may be mediated through interactions with 5HT terminals in the preoptic-hypothalamic area. Baclofen effects on activity of 5HT and NE containing terminals in the medial preoptic nucleus (mPOA), ventromedial nucleus (VMN) and midbrain central gray (MCG) were evaluated with Pargyline using HPLC with EC detection for measurements. 3 h. post Baclofen administration to EB + P primed rats, 5HT levels increased 54% in the mPOA; 5HT turnover decreased 3-fold in the mPOA, was totally inhibited in the VMN, and increased sixfold in the MCG. NE turnover was unaffected in the VMN but increased tenfold in the mPOA. These results indicate that effects of GABA on lordotic behavior may involve an interaction of this transmitter with 5HT. (Grant HD12011).

197.9

SERUM 1,25 DIHYDROXYVITAMIN D_3 (SOLTRIOL) LEVELS INFLUENCE SEROTONIN LEVELS IN THE HYPOTHALAMUS OF THE RAT T.H. Privette, W.E. Stumpf[1], R.A. Mueller[2]* and B.W. Hollis[3]* Dept Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC, [1]Dept of Cell Biology and Anatomy, [2]Dept of Anesthesiology, UNC School of Medicine, Chapel Hill, NC, [3]Dept of Pediatrics, USC School of Medicine, Charleston, SC.

Vitamin D precursor is synthesized in the skin, dependent on ultraviolet wavelengths of electromagnetic radiation from the sun. Due to the dependence of vitamin D synthesis on sunlight and to the strategic localization of its receptors, vitamin D has been suggested as a tranducer of the seasons Receptors for the steroid hormone 1,25 dihydroxyvitamin D_3 have been demonstrated in various brain nuclei including the midbrain raphe nucleus suggesting a potential influence on synthesis of serotonin. Serotonin levels in post mortem human hypothalami are higher in the fall and lower in the spring (Acta Psychiat Scand 61:[supp 280] 75-85, 1980) which is also reflective of the serum levels of vitamin D during these seasons We studied rat hypothalami following treatment with high, normal or low calcium diet which is known to produce low, normal or high serum levels of vitamin D, respectively (Endocrinology 121:278-283, 1987). Neurochemical analysis revealed enhanced levels of serotonin in the group with high serum vitamin D levels and attenuated levels of serotonin in the group with low serum vitamin D levels compared to the control group. A metabolic by-product of serotonin (5-HIAA) did not vary among groups suggesting an influence by vitamin D on synthesis but not release of serotonin. These findings suggest Vitamin D may influence serotonin synthesis in neurons of the raphe nucleus, which results in an enhanced storage of the transmitter in raphe nucleus terminal fields in the hypothalamus, and provides further support for vitamin D as a seasonal neuroendocrine regulator.

197.10

EFFECTS OF NONCOMPETITIVE NMDA RECEPTOR ANTAGONISTS ON LORDOSIS AND MOTOR BEHAVIORS IN FEMALE RATS. A.Fleischmann, P.A.Vincent and A.M.Etgen. Depts. Psychiatry & Neuroscience, Albert Einstein Coll. Med., Bronx, NY 10461.

MK-801 and dextrorphan, selective noncompetitive antagonists at NMDA receptors, were used to evaluate the effect of NMDA receptor blockade on sexual and motor behaviors in female rats. Ovariectomized rats were treated with estradiol benzoate (EB) plus progesterone (P) and were tested for sexual and motor behaviors 4 hr after P. Sexual behavior was inhibited in animals that received 0.5 mg/kg MK-801 30 min before EB, but not if the MK-801 injection was delayed until 24 hr after EB. Moderate doses of MK-801 (≥0.05 mg/kg) and 30 mg/kg dextrorphan also inhibited lordosis when injected 30 min before behavior testing. However, when 30 mg/kg dextrorphan was administered 30 min before P, sexual behavior was not affected. Thus NMDA receptor blockade can interfere both with the estrogen priming process and with the performance of lordosis behavior.

The effect of MK-801 administered after EB+P on motor behaviors was also assessed. Drug doses that inhibited sexual behavior also antagonized "vertical" motor behaviors such as rearing and jumping. In contrast, low doses of the drug increased general locomotion and circling behaviors. Only very high doses (0.4 mg/kg) inhibited lateral movement and righting reflexes. These data indicate that different features of movement are affected differentially by NMDA receptor antagonism. Supported by MH41414 and RSDA MH00636 to AME.

197.11

EFFECTS OF ANTERIOR ROOF DEAFFERENTATION ON ULTRASOUND PRODUCTION AND LORDOSIS IN FEMALE HAMSTERS. Q. R. Floody. Dept. of Psychology, Bucknell Univ., Lewisburg, PA 17837.

Studies of the mechanisms controlling female-typical mating behavior have focused on the ventromedial hypothalamus (VMN), reflecting, in part, the extent and reliability of the decrements in lordosis caused by VMN lesions (Pfaff & Sakuma, J. Physiol., 288, 203, 1979). However, opposed changes of comparable size are produced by horizontal cuts projecting forward from the anterior commissure (anterior roof deafferentation, or ARD; Yamanouchi, Physiol. Behav., 25, 721, 1980). These data suggest the existence, at least in the female rat, of a lordosis-inhibiting system of forebrain structures, possibly including the preoptic area and lateral septum.

To assess the generality of this system, each of 17 ovariectomized hamsters was subjected to either control surgery or ARD. Both before and after this treatment, females were primed with estradiol benzoate (1.0-10.0µg/100g) and progesterone (500µg) prior to tests of ultrasound production and lordosis. Specifically, ultrasound rates were observed before and after exposure to stimulus males. Tests of lordosis also included measures of responsiveness to males, but focused on the extent to which females already emerging from lordosis could be returned to this posture (lordosis reinstatement) by light manual stimulation along the dorsal midline.

Consistent with previous observations (Kirn & Floody, Behav. Neurosci., 99, 1142, 1985), the results documented a clear facilitation of ultrasound rates by ARD. In contrast, the same treatment had no consistent impact on the duration of male-elicited lordosis. In part, this probably reflects contamination by variability across males, since ARD did facilitate lordosis reinstatement responses to manual stimuli. Nevertheless, these changes in lordosis seem less dramatic than those produced by ARD in female rats. Accordingly, these data suggest species differences in the dependence of lordosis on forebrain lordosis-inhibitory mechanisms.

197.12

SELECTIVE MU OPIOID RECEPTOR AGONISTS INHIBIT WHEREAS DELTA AND KAPPA RECEPTOR AGONISTS FACILITATE LORDOSIS BEHAVIOR IN THE FEMALE RAT: DIFFERENTIAL MODULATION BY PROGESTERONE. J.G. Pfaus and D.W. Pfaff. The Rockefeller University, New York, NY, 10021.

Previous studies indicate that selective opioid receptor agonists infused into the lateral ventricles can inhibit (mu) or facilitate (delta) lordosis behavior in ovariectomized (OVX) rats treated with estrogen and a low dose of progesterone. Both effects are reversible with naloxone. The present study investigated whether these effects depend upon the presence of progesterone. Sexually experienced OVX rats were implanted stereotaxically with guide cannulae aimed at the right lateral ventricle. One group of rats was treated with estradiol benzoate (EB; 10 ug) 48 h and progesterone (P; 250 ug) 4 h before testing, whereas the other was treated with EB alone. Rats were infused with different doses of the selective mu receptor agonist D-Ala^2,MePhe^4,Gly-ol^5-enkephalin (DAMGO), the selective delta agonist D-Pen^2,D-Pen^5-enkephalin (DPDPE), or the selective kappa receptor agonist U50-488H. The females were placed with a sexually vigorous male in a bilevel chamber for three tests of sexual behavior, beginning 15, 30, and 60 min after infusion. DAMGO reduced lordosis quotients and magnitudes significantly in rats treated with EB and P, but not in rats treated with EB alone. In contrast, DPDPE and U50-488H increased lordosis quotients and magnitudes significantly in both steroid-treatment groups. Measures of proceptivity, rejection responses, and locomotor activity were not affected significantly by any drug treatment. These results suggest that the facilitation of lordosis behavior by delta and kappa receptor agonists is independent of progesterone treatment, whereas the inhibitory effect of the mu receptor agonist requires the presence of progesterone. The site(s) of action of these drugs are currently being investigated.

197.13

BEHAVIORAL RESPONSES AS A FUNCTION OF ESTRUS CYCLE IN WKY RATS. W.P. Paré and E. Redei, VA Medical Center, Perry Point, MD 21902 and U. Pennsylvania, Philadelphia, PA 19104

Previous animal studies attempting to demonstrate gender differences in response to stress have been equivocal. The inability to demonstrate an effect may be attributable to the fact that these studies did not control for genetic and estrus cycle differences. The Wistar Kyoto (WKY) normotensive rat strain is hyperresponsive to stressor stimulation (Physiol. Behav., 46, 993, 1989). WKY, as well as Wistar and Fischer-344 female rats were exposed to the ulcerogenic procedure of water plus restraint. WKY rats revealed significantly more stomach ulcers as compared to Wistar and Fischer-344 rats. In addition stress ulcer incidence was significantly greater in WKY rats during proestrus as compared to anestrus and diestrus. Thus ulcer severity was a function of the strain and estrus cycle factors. In a subsequent study, proestrus female WKY rats were judged as more emotional in the open field test of emotionality as compared to anestrus and diestrus rats. The same animals were also observed in the Porsolt forced-swimming test of depression where WKY proestrus females were judged as more behaviorally depressed. These studies suggest that the steroid hormone milieu may be responsible for these behavioral changes and that these effects can be demonstrated in a stress-susceptible strain.

197.14

EFFECTS OF SYSTEMIC AND CENTRAL PROLACTIN INJECTION ON FOOD INTAKE, WEIGHT GAIN AND ESTROUS CYCLICITY IN FEMALE RATS. Miriam Beth Noel and Barbara Woodside*, Department of Psychology, Concordia University, Montreal, Quebec, Canada.

Previous research has suggested that prolactin may contribute to the hyperphagia of lactation (Gerardo-Gettens,Moore, Stern and Horwitz, 1989). In the current study the effects of peripheral and central prolactin administration on food intake, weight gain and estrous cyclicity in female rats were compared. Prolactin was administered twice daily at 0800hr and at 1900hr either subcutaneously at 3mg/kg or 1mg/kg body weight or by intracerebral ventricular (icv) infusion (2µg/0.5µl) for 10 days to female wistar rats. Control animals received similar injections of vehicle. Food intake, body weight and vaginal smears were taken daily. Results showed that peripheral administration of prolactin increased food intake and weight gain and disrupted vaginal cyclicity. In contrast, icv administration of prolactin increased food intake but had no effect on weight gain or cyclicity. These data suggest that prolactin acts both peripherally and centrally to regulate energy balance in the female rat.

197.15

EFFECTS OF SCOPOLAMINE ON SEXUAL BEHAVIORS OF FEMALE RATS. S. M. Ross*, C. S. Menard, M. M. Brazier*, and G. P. Dohanich. Dept. of Psychology and Neuroscience Program, Tulane University, and Dept. of Psychology, Loyola University, New Orleans, LA 70118.

The muscarinic antagonist, scopolamine, reliably inhibits lordosis in female rats under a variety of conditions. However, the effects of scopolamine, as well as other cholinergic agents, on other components of sexual behavior exhibited by female rats have not been reported. In the present experiments, the effects of scopolamine on lordosis, solicitation, pacing, attractivity, and locomotion were evaluated in female rats. To activate sexual receptivity, ovariectomized Long-Evans rats were injected with estradiol benzoate (EB, 1 µg/kg, i. m.) at 72, 48, and 24 hours before behavioral testing. Progesterone (500 µg, i. m.) was injected at 5 hours before testing. Behaviors were tested in a partitioned arena that allowed females to escape the stimulus male by exiting into a neutral compartment. Under this condition, females were able to determine or pace their contacts with the male. Females injected with scopolamine (1 mg) displayed a reduced incidence of lordosis (LQ = 28) and solicitation (SQ = 6) compared to females treated with saline vehicle (LQ = 94, SQ = 43, $p < .01$). Although females treated with saline spent significantly more time in the neutral compartment than in the male compartment ($p < .01$), females treated with scopolamine divided time equally between the compartments. While females treated with scopolamine did not display typical pacing behavior they did not avoid contact with the male. In addition, locomotor activity generally was reduced following treatment with scopolamine. Females treated with saline or scopolamine received equal numbers of sexual contacts, although females treated with scopolamine received significantly fewer intromissions and ejaculations and significantly more mounts without intromissions ($p < .01$). On almost all measures, the behaviors of females treated with scopolamine were similar to behaviors exhibited by nonreceptive ovariectomized rats that had received no hormone treatment. The results indicate that the muscarinic antagonist, scopolamine, disrupted various components of sexual receptivity including lordosis, solicitation, and pacing. However, scopolamine did not appear to reduce female attractivity nor cause females to avoid a stimulus male.

197.16

SIGNALS GENERATED IN CNS GLUCOSE METABOLISM AFFECT ESTROUS CYCLES IN SYRIAN HAMSTERS. J. E. Schneider', A. J. Hall', D. G. Friedenson', M. H. Brown and G. N. Wade. Neuroscience and Behavior Program, Department of Psychology, University of Massachusetts, Amherst, MA 01003.

Estrous cycles are inhibited by signals generated when there is a decrease in oxidation of metabolic fuels. We examined the relative importance of peripheral versus central metabolic signals. Food deprivation-induced anestrus was prevented by feeding hamsters either of the peripherally oxidized substrates, shortening or fructose, or by prefattening hamsters prior to food deprivation. These results might suggest a role for peripheral metabolic signals. However, food deprivation-induced anestrus was not prevented by any of these manipulations when the hamsters were treated with 2-deoxy-d-glucose (2DG), a general inhibitor of glucose utilization. Thus, fuels oxidized peripherally may prevent anestrus by sparing glucose for the central nervous system. Estrous cycles were interrupted in *ad libitum*-fed hamsters treated with inhibitors of glucose metabolism (2DG) and fatty acid oxidation (methyl palmoxirate) when the drugs were given concurrently, but not separately. The results of the food and drug manipulations were the same whether the hamsters had received bilateral subdiaphragmatic vagotomies or sham surgeries. Thus, vagally-mediated information about peripheral fuel availability is not critical for the effects of decreased metabolic fuels on estrous cycles. (Supported by NS10873, AM32976 from NIH, and BNS8719361 from NSF.)

197.17

EFFECTS OF DIABETES MELLITUS ON ESTROUS CYCLICITY AND COPULATORY BEHAVIOR IN FEMALE SYRIAN HAMSTERS H. Y. Li and G. N. Wade. Neuroscience and Behavior Program and Psychology Department, University of Massachusetts, Amherst, MA. 01003

Food deprivation inhibits ovulation and estrous behavior in Syrian hamsters (*Mesocricetus auratus*). In diabetetic hamsters, utilization of metabolic fuels, particularly carbohydrates, is greatly impaired. Diabetes mellitus was induced in female hamsters by intraperitoneal injections of 60 mg/kg streptozotocin for 3 successive days which led to decreases in body weight, glycosuria, and irregular estrous cycles. Furthermore, diabetic hamsters which stopped estrous cycles showed decreased response of lordosis behavior to exogenous estradiol benzoate (5 µg) and progesterone (200 µg) when tested with male hamsters. It was also demonstrated that daily injections of 10 U of U-100 Lente insulin were sufficient to restore estrous cycles, increase body weight, and prevent glycosuria. Withdrawal insulin treatment on specific days of the estrous cycle reduced the number of corpora lutea compared with hamsters that were given continuous insulin treatment.

Supported by NS 10873, DK 32976 and MH 00321.

197.18

CENTRAL AND PERIPHERAL EFFECTS OF RU486 ON AGONISTIC RESPONDING AND PLASMA HORMONE LEVELS IN HAMSTERS. D.M. Hayden-Hixson[1] and C.F. Ferris[2]. [1]Dept. of Psychiat., Duke Univ. Med. Ctr., Durham, NC 27710; [2]Dept. of Physiol., Univ. Mass. Med. Ctr., Worcester, MA 01655.

The agonistic behaviors of male golden hamsters (N=48 dyads) treated with the glucocorticoid antagonist, RU486, were examined 5min after acute microinjection (100nl) in the anterior hypothalamic area (AHA) and paraventricular nucleus (PVN), or 15, 30, and 45min after subcutaneous (sc) injections (210µg). RU486-treated animals were tested for aggressive/submissive responding in a neutral arena pairings with vehicle-treated (AHA or PVN) or cholesterol-treated (sc) opponents. Two hours after treatment, trunk bloods were collected and assayed for cortisol, ACTH, and testosterone.

Agonistic responding was not affected by any of the RU486 treatments. Males receiving PVN injections of RU486 had significantly elevated circulating ACTH levels compared to sc or AHA injections. ACTH levels were also significantly higher in males receiving 10^{-6}M RU486 injections than 10^{-4}M or 10^{-2}M injections in AHA. These results suggest RU486 has pronounced, differential effects on adenohypophyseal function when administered in AHA, PVN or sc, but no effect on aggressive/submissive behavior. (Work supported by NIH grant #NS23557).

197.19

SCOPOLAMINE INHIBITION OF LORDOSIS: EFFECTS OF VARIABLE DOSES AND REPEATED ADMINISTRATIONS OF ESTROGEN. C. S. Menard, T. J. Hebert, and G. P. Dohanich, Dept. of Psychology and Neuroscience Program, Tulane University, New Orleans, LA 70118.

Previous evidence indicates that the muscarinic antagonist, scopolamine, inhibits lordosis in female rats. In the first experiment, the effects of various doses and repeated administrations of estrogen on the scopolamine inhibition of lordosis were examined. Ovariectomized rats were injected for 3 days with estradiol benzoate (EB, i.m., .25, .5, or 25 μg) followed by progesterone (500 μg, i.m.) 5 hrs before behavior testing on day 4. Lordosis was inhibited 15 min after scopolamine injection (1 mg, i.p.) in females primed with all doses of EB ($p<.01$). However, scopolamine inhibition after priming with .25 or .5 μg EB was greater than inhibition after priming with 25 μg EB ($p<.01$). When hormone priming was repeated on a second week, scopolamine continued to inhibit lordosis in females that received .25 μg EB but was less effective in females primed with .5 μg EB. Scopolamine failed to inhibit lordosis in females treated with 25 μg EB ($p<.01$). Consequently, the inhibitory effect of scopolamine on lordosis was dependent both on the dose and number of EB treatments. To examine if this estrogen-dependent reduction in scopolamine inhibition could be counteracted, various doses of scopolamine (1, 2, 4 mg, i.p.) were administered to females primed with the highest dose of EB (25 μg) and progesterone (500 μg). Lordosis was inhibited equally 15 min after all scopolamine doses during the first week. As in the first experiment, scopolamine failed to inhibit lordosis at all doses during the second week of testing in females primed with 25 μg EB. In addition, lordosis was not inhibited in females that received saline on the first week and the highest dose of scopolamine (4 mg) on the second week. Therefore, the failure to inhibit lordosis on the second week was not due to repeated exposure to scopolamine, but rather to repeated exposure to a high dose of EB. Data indicate that the ability of scopolamine to inhibit lordosis can be reduced by increasing the dose or the number of estrogen exposures. Because higher doses of scopolamine failed to restore its inhibitory effect on lordosis it is unlikely that repeated exposure to estrogen induces an upregulation of muscarinic receptors.

197.20

P-3-BSA IMPLANTS IN THE VTA RAPIDLY FACILITATE RECEPTIVITY IN HAMSTERS AFTER PROGESTERONE PRIMING TO THE VMH. C.A. Frye and J.F. DeBold, Psychology Dept., Tufts Univ., Medford, MA 02155.

Recent evidence suggests progesterone (P) may have behaviorally relevant actions on neuronal membranes in the ventral tegmental area (VTA). When progesterone conjugated to BSA (P-3-BSA), which cannot permeate the cell membrane, is applied to the VTA concurrent with normal P to the VMH, estrogen-primed hamsters are maximally receptive after 2 1/2 hours. As the reverse treatment is ineffective, this suggests non-genomic effects in the VTA may require concurrent genomic activation by P in the hypothalamus.

In the present experiment we exploited the different time courses necessary for genomic and non-genomic actions of steroid hormones. We assessed whether estrogen-primed hamsters would be immediately receptive after P-3-BSA applied to the VTA, when P had been aimed at the VMH 2 hours earlier. Ovariectomized hamsters were implanted with chronic cannulae, one aimed above the VMH and the other over the contralateral VTA. These animals were later estrogen-primed and tested for sexual receptivity two hours after P or P-3-BSA containing tubes were applied above the MBH. Immediately after this pretest, the opposite hormone containing insert was applied over the VTA. The following week the contents of the tubes were reversed. Facilitation of receptivity only occurred when P was applied to the VMH and 2 hours later P-3-BSA was inserted into the VTA. The reverse treatment was not effective. These data demonstrate that the action of progesterone in the VMH takes 1-2 hours but in the VTA the effect of a progestin occur in 5 minutes. These time courses are consistent with a genomic action in the VMH and a non-genomic action in the VTA.

197.21

FOOD DEPRIVATION SUPPRESSES STEROID-INDUCED ESTROUS BEHAVIOR IN OVARIECTOMIZED SYRIAN HAMSTERS. R. W. Dickerman, H. Y. Li, and G. N. Wade, Neuroscience and Behavior Prog. and Dept. of Psychology, University of Massachusetts, Amherst, MA 01003.

Hamsters fail to ovulate or show estrous behavior when access to metabolic fuels is limited by food deprivation (FD). Plasma estradiol (E_2) concentrations are depressed by FD, but lack of estrous behavior may also be due to decreased neural responsiveness to E_2. The hypothesis that FD decreases neural responsiveness was tested by measuring lordosis duration in steroid-primed ovariectomized hamsters following FD. FD or fed hamsters were injected with E_2-benzoate (5μg) at -48 hours and progesterone (200μg) at -4 hours. At time=0, time spent in lordosis out of 5 min. in the presence of a male was recorded. There were two FD regimens with food withdrawn for 48 hours starting at either -96 hours or -48 hours. Both treatments reduced lordosis time. When FD and E_2 were coincidental lordosis was shown for 70±24sec. vs. 202±30sec. in fed controls. When FD preceded E_2 treatment lordosis was shown for 159±21sec. vs. 212±14sec. in fed controls. Reduction in lordosis time was greater when FD and E_2 treatment were coincidental compared to when FD preceded E_2 treatment. FD animals lost 19.6±1.0 grams which was 18.6% of pretreatment body weight. Previous work has suggested that FD affects metabolism of exogenous E_2. However, plasma E_2 concentrations were not different between FD and fed animals in either treatment regimen at time=0. These data show that FD decreases induction of estrous behavior by exogenous steroids in hamsters. Supported by NS10873, DK32976 and MH00321.

197.22

ACTIVATION OF THE ACCESSORY OLFACTORY SYSTEM IN THE FEMALE RAT BY EXPOSURE TO MALE-SOILED BEDDING AND REPEATED MATINGS. C.A. Dudley, G. Rajendren*, and R.L. Moss, Dept. of Physiology, Univ. of Texas Southwestern Med. Ctr., Dallas, TX 752355

The activity of the accessory olfactory system in ovariectomized female rats was examined by c-fos immunohistochemistry after estrogen administration (EB; 5μg), EB administration followed 48 h later by exposure to male-soiled bedding, and EB administration followed 48 h later by several copulatory episodes with male rats. Animals were sacrificed and perfused immediately after treatment was complete. Vibratome sections (40μ) through the accessory olfactory bulb (AOB), medial amygdala (mAMYG) and ventromedial hypothalamus (VMH) were processed immunohistochemically using the ABC technique with DAB as the chromagen. EB administration alone was not sufficient to induce appreciable amounts of c-fos activity in any area examined. EB administration combined with exposure for 1 or 2 hr, but not 10 or 30 min, to bedding soaked with male urine was effective in inducing intense c-fos activation in the mitral and granular cell layers of the AOB. Staining in the mAMYG was less intense and was scattered in the baso and medial portions of the nucleus. A circular pattern of c-fos activity was noted in the VMH. When EB-primed females were mated four times for 15 min each with 15 min intervals separating the tests, very intense c-fos activity was observed in the granular cell layer of the AOB, in the mAMYG close to the optic tract, and in a circular area in the lateral portion of the VMH. The results indicate discrete activation of the accessory olfactory system by sexually relevant stimuli. Supported by MH41784.

MONOAMINES AND BEHAVIOR: DOPAMINE

198.1

EFFECTS OF 6-OHDA LESIONS AND DRUG CHALLENGE ON EXTRAPYRAMIDAL- VERSUS TARDIVE DYSKINESIA-LIKE EFFECTS IN RATS. S.K. Johnson, H. Fisher* and G.C. Wagner, Depts. of Psychology and Nutritional Sciences, Rutgers University, New Brunswick, N.J. 08903

Age is a strong predictor of both extrapyramidal side-effects and tardive dyskinesia associated with neuroleptic administration. This susceptibility in the aged may be due to the loss of dopaminergic cells. The present study used 6-OHDA to create 35% lesions of striatal dopamine and then assessed rats for haloperidol-induced catalepsy and chewing behavior. Lesioned animals were significantly more sensitive to haloperidol-induced catalepsy than sham-lesioned animals, but chewing behavior was not significantly different between the two groups. Rats were also administered drug challenges of D1 and D2 agonists, serotonergic and cholinergic agonists and antagonists at the beginning and end of chronic haloperidol (2 mg/kg) treatment as well as after withdrawal from haloperidol. It was observed that serotonergic agents had opposing effects on catalepsy versus chewing, while cholinergic agents acted in the same manner on both catalepsy and chewing bouts.

198.2

URIDINE AND STIMULANT-INDUCED ACTIVITY. C.S. Myers*, M. Napolitano*, H. Fisher*, and G.C. Wagner, Depts. of Psychology and Nutritional Sciences, Rutgers University New Brunswick, NJ 08903

Chronic administration of uridine has been shown to alter both dopaminergic activity and dopaminergic-mediated behavior. The present studies further investigated this effect using amphetamine (AMPH) and cocaine (COC)-induced activity and rotation in rats with unilateral dopaminergic lesions.

10 adult, female, individually housed Sprague-Dawley rats with free access to food and water received daily IP uridine (16 mg/kg) and 10 received an equal volume of saline. Activity was assessed for 10 min in a photocell chamber 30-min after IP AMPH or COC (0.5 - 4.0 mg/kg). 20 otherwise comparably treated rats had unilateral dopaminergic lesions and were assessed for stimulant-induced rotation.

Uridine exerted no effect on body weight, activity, or rotation under baseline conditions. AMPH and COC increased activity at low doses and decreased activity at higher doses and caused a dose-dependent increase in the number of rotations. For both tests, uridine-treated rats exhibited a significant increase in their sensitivity to AMPH but not COC indicating that uridine may differentially affect cytoplasmic versus vesicular release of dopamine.

198.3

QUANTIFYING OPERANT BEHAVIOR DEFICITS IN RATS WITH MODEST BILATERAL 6-HYDROXYDOPAMINE LESIONS OF THE VENTROLATERAL STRIATUM. R.-M.Liao, S.C.Fowler, & M.J.Kallman; Dept. of Psychology, Ntal. Cheng-Chi Univ., Taiwan, ROC., & Dept. of Psychology and Pharmacology, Univ. of Mississippi, MS., USA.

Rats (N=6) with bilateral 6-OHDA lesions (6 ug/1 ul/site) to the ventrolateral striatum (AP=0.9mm, L=±3.7mm, D=-5.7mm; referred to bregma) were compared to a vehicle-treated group (N=6) using specially modified operant chambers, which allowed measurement of successive behaviors occurring within a cycle of the operant/licking complex. Determined by HPLC analyses, the lesioned group exhibited dopamine levels that were 35 % of the control group. During the first postoperative week, the lesioned rats exhibited increased forelimb response duration ($p=0.06$). The frequencies of head-entry into the reward well and licking of the reward significantly dropped in the lesioned rats ($p<0.05$). Furthermore, the lesioned rats significantly increased the latencies required to switch from one motor response to another ($p<0.05$). These results suggest the important role of the ventrolateral striatum in the control of behavioral sequences.

198.4

SHOCK-ELICITED BEHAVIORAL DIFFERENCES IN RAT MODELS OF RISK FACTORS CONTRIBUTING TO CHRONIC ABERRANT BEHAVIOR. C.J. Stodgell, S.R. Schroeder* and R.E. Tessel Dept. of Pharmacol. and Toxicol., Univ. of Kansas, Lawrence, KS 66045.

Spontaneously Hypertensive rat pups (SHR), and 3 groups of Sprague-Dawley (SD) rat pups [controls, microcephalic rat offspring generated by IP injection of methylazoxymethanol (MAM) to pregnant dams, and rat pups neonatally depleted of brain dopamine by intracisternal injection of 6-hydroxydopamine (6HD)] remained with their mothers until weaning. Animals of each group were then housed 3/cage (Group Housed; GH) or 1/cage (Socially Isolated; SI) for at least 30 days until exposed to a scrambled electric foot shock (six 1.6 mA, 150 msec shocks/2 sec every 10 sec) for 1 hr, and the behaviors occurring during the hr period were recorded on video tape. The frequencies with which various behaviors occurred during selected 1-min observation periods were then scored. The behaviors and group differences observed were as follows (">" = $p<0.05$; "≥" and "=" = $p>0.05$; lack of housing designation indicates absence of difference based on housing condition): Locomotor activity (MAM>SD>SHR=6HD); Maintained Rearing (a stereotypy; MAM>SD≥6HD>SHR); Cage Biting (aggression; SHR-SI>SHR-GH-SD-SI>SD-GH>MAM=6HD). No self-injurious behavior was observed. Surprisingly, the 6HD groups but no other groups rapidly learned to avoid the scrambled shocks. These data suggest that genetic, organic and environmental risk factors alone and in combination can markedly and predictably determine the types of behavior elicited by stress in rats, and suggest that respondent mechanisms may importantly contribute to the chronic incidence of some aberrant behaviors in man. (Supported by NICHHD grant 1 PO1 HD26927)

198.5

THE EFFECTS OF SELECTIVE PREFRONTAL DOPAMINE (DA) LESIONS ON COGNITIVE TESTS OF FRONTAL FUNCTION IN PRIMATES. A.C. Roberts, M.A. de Salvia*, J.L. Muir, L.S. Wilkinson*, B.J. Everitt+ and T.W. Robbins, Departments of Experimental Psychology and Anatomy+, University of Cambridge, Cambridge, CB2 3EB, U.K.

Degeneration of the mesocortical DA pathway which occurs in Parkinson's disease (PD) may contribute to the frontal-like cognitive impairments associated with this disorder. The present study therefore examined in marmosets the effects of selective prefrontal DA lesions on a series of behavioral tests sensitive to frontal lobe damage. These included attentional set-shifting, serial discrimination reversal learning and object retrieval (a detour problem using a transparent barrier), tests all requiring behavioral inhibition but at different levels of psychological control of responding. Injection of 6-hydroxydopamine into the prefrontal cortex of monkeys, pretreated with specific noradrenaline (NA) and serotonin (5-HT) uptake blockers, resulted in selective DA depletion (90% loss of DA, < 50% loss of NA, < 10% loss of 5-HT). The lesion impaired both serial reversal learning and object retrieval but improved performance of individual animals on the attentional set-shifting task. These results will be compared to the behavioral pattern of changes on the same series of tests seen following lesions of the frontal cholinergic system and in patients suffering from PD.

198.6

BEHAVIORAL DEFICITS CAUSED BY STRESSORS AND LATERALIZED CHANGES IN THE MESOCORTICAL DOPAMINE SYSTEM. *J.N. Carlson, K.L. Rossman*, R.W. Keller, and S.D. Glick Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208*

Rats exposed to stressors that cannot be controlled exhibit profound behavioral and physiological disturbances that have been used as an animal model of human depression. We have previously shown that uncontrollable stress has differential effects on shock escape learning in rats having left- and right-sided turning preferences and causes lateralized changes in the mesocortical (PFC) dopamine (DA) neurochemistry. In the present study we have attempted to relate the behavioral effects of such treatment to specific alterations in PFC DA function by measuring both indicies in the same subjects. Left and right sided male Long-Evans rats were exposed to controllable (CS) or identical uncontrollable (UCS) footshock and were tested for footshock escape behavior on the next day. Following testing, bilateral samples of the PFC were removed and analyzed for DA and metabolites. As seen in previous studies, right sided rats were more susceptible to the behavioral effects of UCS as evidenced by more escape failures and longer escape latency. A lower concentration of DA and its metabolite homovanillic acid (HVA) was measured in the right side than in the left side of the PFC in these animals. Across groups a significant inverse correlation was seen between mean shock escape latency and the DA concentration in the right side of the PFC. This relationship was strongest in animals with the most successful escapes (CS). These findings suggest that the behavioral disruption observed following UCS may depend upon an alteration of DA function in the right PFC.

(Supported by ES 04032)

198.7

ELECTRICAL SELF-STIMULATION OF RAT MEDIAL FOREBRAIN BUNDLE INCREASES BOTH ENDOGENOUS DOPAMINE AND PULSE LABELED [^{3}H]L-DOPA STRIATAL METABOLISM

W.P. Melega, J.R. Barrio*, M.E. Phelps, R.F. Ackermann Division of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, CA 90024

Changes in homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) / dopamine (DA) concentration ratios subsequent to electrical stimulation are generally accepted as evidence for increases in dopamine turnover. Our rat self-stimulation/reinforcement model effected parallel changes in [^{3}H]-DA and endogenous DA metabolism, indicative of increased turnover of both amines. The combination of procedures comprised: a) an awake, freely moving animal b) active driving of an operant/reward pathway via electrical stimulation of the substantia nigra and ventral tegmental area and c) labeled and endogenous DA metabolite analyses Increases in DA and [^{3}H]DA turnover were observed (n=6), primarily in either caudate-putamen (CP) or nucleus accumbens (NA)/olfactory tubercle (OT). Ipsilateral increases (40-60%) in both labeled and endogenous DOPAC/DA and HVA/DA ratios and stimulated/non-stimulated regional DOPAC and HVA ratios were detected.

These experiments demonstrate for the first time that, in vivo, [^{3}H]L-DOPA probes the functional state of an activated dopaminergic system. Such changes in DA metabolism suggest fundamental alterations in DA metabolism regulation consequent to increased availability of amino acid precursors, and/or to activation of aromatic amino acid decarboxylase, rather than mere increases in steady state DA function.

198.8

EFFICACY OF ANTIPARKINSONIAN DRUGS ON THE SPONTANEOUS PAW REACHING AND SENSORIMOTOR DEFICITS CAUSED BY COMPLETE UNILATERAL DOPAMINE DEPLETION OF THE RAT FOREBRAIN. B.C. Gray*, C.S. McNeish*, R.J. Mandel[1], and R.E. Strecker, Dept of Psychiatry, SUNY @ Stony Brook, NY, 11794, [1] Dept of Psychol., Univ. IL, Champaign, IL.

Rats bearing unilateral dopamine (DA) depletions of the striatum and subsequent DA-rich neural grafts show a complete recovery of drug-induced asymmetric behavior, but exhibit no improvement on some behaviors such as spontaneous paw reaching. One hypothesis holds that the grafts do not contain enough DA neurons and/or their outgrowth has not reached enough different target sites to produce complete recovery. This hypothesis would be supported if peripherally injected antiparkinsonian drugs reversed the deficits previously shown to be resistant to graft-induced recovery. Rats received complete unilateral DA denervation via the injection of 6-OHDA into two midbrain sites (N=7). Rotation was measured following several doses of amphetamine, apomorphine, and a carbidopa/levodopa combination to determine sub-rotational doses. Also, these rats exhibited marked contralateral sensorimotor neglect, and had an impaired ability to use their left forelimb to grasp and consume 45mg food pellets in the staircase test (Montoya et al.1990). Suprisingly, pretreatment with various sub-rotational doses of carbidopa/levodopa (i.p.), or apomorphine (s.c.) did not produce a performance improvement in either behavioral tests. Thus, merely providing the denervated receptors with DA agonists via drug treatments, or by analogy, with multiple graft placements appears inadequate to reverse all behavioral deficits. Instead, the complete normalization of the spontaneous behavioral deficits may require a more complex reconstruction of the damaged circuitry than is provided by either drug injection or the standard DA graft paradigm (e.g., greater re-afferentation of the graft by the host may be required). Alternately, the severe (>95%) DA depletion in this rat model may be similar to end stage Parkinson's patients who also may no longer respond well to medication. Hence, some surviving DA terminals may be needed to provide effective functional regulation of the DA produced from levodopa. Supported by P. D. Fdn fellowships to CSM & BCG.

198.9

CELLULAR EFFECTS OF L-DOPA AND SELECTIVE DOPAMINE COMPOUNDS IN RAT NEOSTRIATUM. Nantwi, K.D. and Schoener, E. P., Dept. of Pharmacology, Wayne State Univ., Detroit, MI 48201.

The effects of specific dopamine receptor agonists and antagonists on spontaneously active neostriatal neurons were examined in urethane-anesthetized rats. Adult male Sprague-Dawley rats (200-250gm) were mounted in a cranial stereotaxic apparatus after surgical preparation for intravenous drug administration, recording systemic arterial blood pressure, and providing ventilatory assistance as needed. Extracellular single-unit activity was detected with glass-coated, platinum-iridium microelectrodes, stereotaxically guided into the striatum, and recorded continuously throughout the experiment.

L-DOPA was administered following pretreatment with the peripheral aromatic amino acid decarboxylase inhibitor carbidopa (1.0mg/kg). Neuronal depression and excitation were observed after a low dose of L-DOPA was given (0.25mg/kg), while depression predominated at higher doses (0.50 and 1.0mg/kg). These changes in activity were reflected in the average firing rate and duration of action, and were antagonized with haloperidol. The D-1 agonist SKF38393 also induced depression (62%) and excitation (38%), both of which were sensitive to SCH23390. Quinpirole, the D-2 agonist, primarily depressed activity (86%) in a dose-dependent, eticlopride reversible manner.

These experiments reveal that selective activation of dopamine receptors in the striatum can evoke changes in spontaneous neuronal behavior. Responsiveness of D-1 and D-2 receptors appears to be more balanced in the striatum than reported for the nucleus accumbens. This distinction may have potential therapeutic value in developing selective dopamine compounds for psycho/motor deficits.

198.10

CONDITIONING-RELATED SINGLE-UNIT ACTIVITY IN THE NEOSTRIATUM DURING CLASSICAL EYELID CONDITIONING IN RABBITS: EFFECTS OF HALOPERIDOL. I.M. White, G.V. Rebec & J.E. Steinmetz, Prog. in Neural Sci., Dept. of Psych., Indiana Univ., Bloomington, IN, 47405.

Multi-unit recording from the cerebellar interpositus nucleus (INP) during classical eyelid conditioning indicates that haloperidol disrupts conditioning-related activity in the INP and reduces the percent conditioned response (CR) (Sears & Steinmetz, Pharm. Biochem. Beh., 1990). To determine if haloperidol disrupts CRs by altering modulatory forebrain regions, the present study examined neuronal activity in the neostriatum during eyelid conditioning and assessed the effects of haloperidol in this task. During the acquisition period (0-50% CR) animals received a 90 dB auditory CS. During the training period (51-100% CR) they received an 80 dB CS. A total of 192 single-units were recorded over the course of conditioning. During the acquisition phase, 55 units were conditioning related (18 CR-, 14 CS-, 13 US-, 10 CS+US-units) and 20 units were non-specific. During the training phase, 90 units were conditioning related (37 US-, 36 CR-, 8 CS-, 9 CS+US-units) and 27 units were non-specific. Units that were responsive to CRs increased activity as percent CRs increased. Haloperidol (0.5 and 1.0 mg/kg, s.c.) was administered when percent CR at 80 dB CS was 51% or greater for at least two consecutive sessions. At both doses of haloperidol, a variety of unit patterns was observed. Some CR-related units showed either an increase or a slight decrease in activity after the low dose, whereas some CR units decreased activity after the high dose. Most US units and CS+US units were inhibited at a both doses. In most cases, however, such inhibition by haloperidol was greater to the lower CS-intensity (80- dB). Disruption of percent CRs was greater with the higher dose and to the lower intensity CS. These findings indicate that haloperidol disrupts CRs and also affects neostriatal activity. Although the neostriatum is not essential for eyelid conditioning, such disruption may produce a failure to modulate processing of sensory information and alter information to areas that project to the cerebellum. [Supported by NIMH Grant #44052 to JES and Scottish Rite Schizophrenia Research Grant to GVR].

198.11

THE EFFECTS OF INTRASTRIATAL DOPAMINE DEPLETIONS ON INSTRUMENTAL RESPONSES IN A NOVEL COST/BENEFIT PARADIGM. C. V. Alma and J. D. Salamone. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.

A novel food-choice paradigm was utilized to determine if rats would lever press on an FR5 schedule to obtain a more-preferred food (Bioserve Pellets) or consume less-preferred food (Lab Chow) that was freely available in the operant chamber. Prior to intrastriatal dopamine depletion, rats exhibited high frequencies of bar pressing per 30-min experimental period, and concomitant low consumption of the freely available, less-preferred lab chow. Bilateral 6-hydroxydopamine (6-OHDA) injections into either anteroventromedial (AVMS) or dorsolateral (DLS) striatum shifted instrumental response choice, such that level pressing frequency decreased and lab chow consumption increased. These changes in lever pressing frequency and chow consumption occurred for both 6-OHDA placement groups. Rats with AVMS and DLS dopamine depletions showed recovery of function in the post-surgical period and complete recovery was evident by day 21 after surgery. These results indicate that intrastriatal dopamine depletion suppressed a complex instrumental response for food. Nevertheless, the behavior of the rat remained directed toward the acquisition and consumption of food.

198.12

HALOPERIDOL EXAGGERATES PROPRIOCEPTIVE-TACTILE SUPPORT REFLEXES AND DIMINISHES VESTIBULAR DOMINANCE OVER THEM. A. J. Cordover,* S. M. Pellis and P. Teitelbaum. Dept. Psychol., Univ. Florida, Gainesville, FL 32611.

Rats made immobile and cataleptic by the dopamine receptor blocker, haloperidol, maintain their stable static equilibrium by employing a variety of allied postural support reflexes. Under some test conditions competition between such reflexes occurs, and in haloperidol-treated rats, unlike undrugged controls, proprioceptive-tactile stimuli appear to be dominant over vestibular stimuli. We investigated this relationship in rats by testing their air-righting with and without simultaneous contact of the tail on a wooden platform. The rats were inverted and held upside down by the shoulders and pelvis 40 cm above the ground, with either none, or varying degrees of tail contact on a small wooden platform. Undrugged rats showed the normal pattern of righting, involving cephalocaudal recruitment of axial rotation, whether contacting the platform or not. The haloperidol-treated rats (2.5 mg/kg) gripped the platform with their tail upon release, which interferred with the righting reflex. Furthermore, some undrugged labyrinthectomized rats also gripped with their tail when released, and this appeared to be exaggerated under haloperidol. This suggested that a tactile signal from the tail is present in all rats, but in normal rats receiving simultaneous tactile and vestibular stimulation this is ignored, and the vestibular signal is dominant which triggers air-righting. Therefore, it appears that proprioceptive-tactile triggered postural support reflexes are dominant over vestibular ones in haloperidol-treated rats, reversing the normal relationship.

198.13

EFFECTS OF REPEATED STRESS ON MESOLIMBIC, MESOCORTICAL AND NIGROSTRIATAL DOPAMINE RELEASE: AN ELECTROCHEMICAL STUDY IN FREELY-BEHAVING RATS. M.D. Doherty & A. Gratton, Douglas Hosp. Res. Ctr., McGill Univ., Montréal, CANADA.

The present study was designed to characterize the effects of repeated, once daily exposure to either tail pinch (TP) or restraint (RES) stress on dopamine (DA) release in nucleus accumbens (NAcc), prefrontal cortex (PFC) and striatum (CPu). Male rats each implanted with a voltammetric probe in one of these three DA terminal fields were subjected to either 10 min of TP or 15 min of RES every day for 5 consecutive days. Acutely, TP produced reliable increases in DA levels in all three regions. While there was some indication, especially in NAcc and PFC, of enhanced DA release with repeated exposure to TP, this effect was highly variable both between animals and between test days. In contrast, the magnitude and the rate of DA release in NAcc and to a lesser extent in CPu increased with each daily exposure to RES. This effect was particularly noticeable in NAcc. Compared to the first exposure which caused a weak and transient (5-6 min) increase in NAcc DA levels, the fifth exposure to RES produced a sustained, 5-6 fold greater increase in DA levels. Such day to day changes in RES effects were not observed in PFC. In fact, compared to TP the effects of RES on PFC DA release were highly variable. Of the PFC 9 animals tested, RES either had no effect on any test day (n=3), increased DA levels on only the first two test days (n=3) or increased DA levels on only some of the 5 test days. Surprisingly, subsequent tests revealed that TP could stimulate PFC DA release in three animals that were unresponsive to RES. The present data indicate that a relatively mild stressor such as TP can stimulate DA release in limbic, cortical and striatal terminal fields. Furthermore the data show that mesolimbic but not mesocortical DA release is potently enhanced, or sensitized when the animal is repeatedly exposed to a more intense stressor such as RES. *Supported by MRC.*

198.14

PARTIAL 6-OHDA LESIONS POTENTIATE THE EFFECTS OF RESTRAINT STRESS ON AMPHETAMINE-ELICITED LOCOMOTION AND DOPAMINE RELEASE. K.E. Banks, J. B. Mitchell & A. Gratton, Douglas Hosp. Res. Ctr., McGill Univ., & Ctr. Studies Behav. Neurobiol., Concordia Univ., Montréal, CANADA.

Recently, we have shown that partial (60-80%) depletion of DA levels potentiates the reward attenuating effects of neuroleptics (Doherty & Gratton, 1990). In the present study, we examined how partial DA depletion modifies the sensitizing effects of repeated exposure to stress on locomotion and on mesolimbic DA release elicited by d-amphetamine (d-A). The locomotor activity of 4 groups of male rats--lesion-stress, intact-stress, lesion-control, intact-control--was monitored during 150 min sessions in computer-monitored activity chambers. Following 5 days of habituation to the test chambers, the activity elicited by d-A was monitored in all animals. On each of the following 5 days animals in the stress groups were restrained for 30 mins in the chambers 30 mins after the beginning of session; control animals were left undisturbed in the test chambers. Five and 13 days following the last stress session, the activity of all animals in response to a d-A challenge was examined. The data show that, compared to the control groups, locomotor activity elicited by d-A on the two challenge days was significantly higher in the two stress groups. Furthermore, d-A-elicited locomotion following repeated restraint was significantly higher in the lesioned animals than in the intact animals. High-speed chronoamperometry was then used to monitor DA release in nucleus accumbens of intact and lesioned rats subjected to the same treatment. The preliminary results of this study indicate that, following repeated stress, mesolimbic DA release induced by d-A is enhanced in lesioned animals when compared to intact animals. Taken together, these data indicate that repeated exposure to a relatively painless stress potentiates the facilitatory effects of d-A on DA neurotransmission. The enhanced effects of restraint stress in partially depleted animals suggest that the surviving A10 DA neurons are hyperresponsive to the sensitizing effects of stress or that the lesion eliminated an opponent (possibly mesocortical) DA system that inhibits the development of sensitization (see Mitchell & Gratton, this meeting). *Supported by FCAR.*

198.15

ELECTROCHEMICAL MONITORING OF MESOLIMBIC DOPAMINE RELEASE ASSOCIATED WITH DAILY COCAINE ADMINISTRATION IN FREELY-BEHAVING RATS. E.A. Kiyatkin, R.A. Wise and A. Gratton*, Ctr for Studies in Behav. Neurobiol., Concordia Univ. and *Douglas Hosp. Res. Ctr, McGill Univ., Montréal, CANADA.

Following repeated daily cocaine (COC) administration rats exhibit an enhanced locomotor response to this and other psychostimulant drugs. Enhanced mesolimbic and nigrostriatal dopamine (DA) release has been reported to be associated with the sensitized behavioral response to COC. However, the daily changes in the pattern of DA release during the development of behavioral sensitization to COC have yet to be characterized. The present study used high-speed chronoamperometry to obtain this information in freely-behaving rats. Male rats each implanted with a voltammetric electrode in the nuc. accumbens (NAcc) received COC (15 mg/kg/day i.p.) on 5 consecutive days and also 3-4 days later (challenge). Changes in extracellular DA concentration were monitored for 2 hours prior to and 4 hours following COC. On day 1, COC caused a transient, 30-40 min decrease in DA levels followed by a longer lasting (approx. 100 min) increase. However, the initial decrease in DA after the first COC injection was no longer evident on subsequent days (2-5). Instead, DA levels began to rise 5-10 mins after injection, peaking in the 150-200 nM range 50-60 mins later and returning to baseline levels approximately 2 hours after injection. Changes in the amplitude and duration of DA increases in response to COC were highly variable both between animals and between days. However the greatest daily change in peak DA levels occured between days 1 and 2; more progressive increases were recorded on subsequent days. The amplitude and latency measures of DA release in response to the COC challenge were similar to those obtained on days 2-5. The present data suggest that mesolimbic DA release is significantly enhanced after only 1 injection of COC. A suppression of the transient inhibition of DA release following the first injection of COC was found to be the most reliable and enduring change associated with subsequent COC administration. This effect may reflect a decreased effectiveness of negative feed-back mechanisms that regulate A10 DA cell activity. *Supported by NIDA and FCAR.*

198.16

ACCESSORY OLFACTORY SYSTEM STIMULATION ELICITS DOPAMINE RELEASE WITHIN THE NUCLEUS ACCUMBENS. A. Gratton and J.B. Mitchell, Douglas Hosp. Res. Ctr., Dept. of Psychiatry, McGill Univ., Montreal, CANADA H4H 1R3.

Previous studies from our laboratory have found that the presentation of sex-related olfactory cues increase extracellular dopamine (DA) concentrations within the nucleus Accumbens (nAcc) of freely-behaving male rats. In the present study, male rats were anesthetized with chloral hydrate, and extracellular DA concentrations in the nAcc were measured using high speed chronoamperometry. The electrochemical signal was obtained by applying a +0.55 V pulse to a carbon fiber electrode, relative to a Ag/AgCl reference electrode, at a rate of 5 Hz. Air was passed through bedding obtained from cages that housed estrus female rats, and directed into the nasal cavity of anesthetized male rats. Application of olfactory stimuli resulted in an increase in the electrochemical signal recorded within the nAcc. The peak amplitude and the duration of bedding-elicited increases in the electrochemical signal were increased by pretreatment with the specific DA reuptake inhibitor GBR-12909 (10 mg/kg, s.c., 30-60 min prior to testing). Using pulled-glass capillary micropipettes, depolarizing concentrations of potassium (120 mM KCl, 2.5 mM CaCl2) were applied, using pneumatic pressure, directly to the accessory olfactory system. The application of potassium to the vomeronasal nerve layer of the anterior accessory olfactory bulb, and to the accessory olfactory bulb, itself, elicited reproducible increases in the electrochemical signal recorded within the nAcc. The reversibility of the oxidation reaction and the amplifying effects of GBR-12909 suggest that DA was the primary contributor to the electrochemical signal. The results of these experiments suggest that the accessory olfactory system, which is thought to be activated by molecules of low volatility, such as pheromones, is able to activate mesolimbic DA system.

Supported by a grant from NSERC of Canada.

198.17

INCREASED SENSITIZATION OF MESOLIMBIC DOPAMINE RESPONSES TO PRIMARY INCENTIVE STIMULI IN RATS WITH 6-OHDA LESIONS OF THE PREFRONTAL CORTEX. J.B. Mitchell and A. Gratton, Douglas Hosp. Res. Ctr., Dept. of Psychiatry, McGill Univ., Montreal, CANADA H4H 1R3.

Repeated, intermittent presentation of stimuli, such as stress or psychomotor stimulants, that activate the mesolimbic dopamine (DA) system leads to an enhanced, or sensitized, response upon subsequent activation of this system (see Banks et al., this meeting). Several recent reports suggest that the mesocortical DA system may function, in part, to inhibit mesolimbic DA release. Thus, animals were given 6-OHDA lesions of the medial prefrontal cortex or left intact, and were then given repeated daily presentation of one of two primary incentive stimuli, a highly palatable food or sex-related olfactory cues, bedding from cages that housed estrus female rats. During presentation of these stimuli, extracellular DA concentrations were measured within the nucleus Accumbens using high speed chronoamperometry. The electrochemical signal was obtained by applying a +0.55 V pulse, relative to a Ag/AgCl reference electrode, to a carbon fiber electrode, at a rate of 5 Hz. Among intact animals, presentation of both food and bedding reliably elicited an increase in the electrochemical signal. The response to bedding, but not food, increased across days. Depletion of prefrontal cortex DA, resulted in an enhanced response to both food and bedding, and the response to both stimuli increased across days. Furthermore, the basal oxidation current, obtained during the first 20 min in the testing apparatus, was relatively constant across days for the intact animals receiving repeated presentation of food, but increased across days for the intact animals exposed to the bedding, and especially for the lesioned animals receiving either stimulus. These results indicate that naturally rewarding stimuli increase activity within the mesolimbic DA system, and that the response to sex-related cues, but not food, sensitizes with repeated, intermittent presentation. Furthermore, the augmented response and facilitated sensitization found in animals with DA depletion of the medial prefrontal cortex suggests that the mesocortical DA system functions, in part, to dampen the reactivity of the mesolimbic DA system.

Supported by a grant from NSERC of Canada to AG.

198.18

IN-VIVO MICRODIALYSIS MEASURES OF DOPAMINE AND HOMOVANILLIC ACID OVERFLOW IN THE NUCLEUS ACCUMBENS AFTER REPEATED MORPHINE INJECTIONS: TESTS WITH REPEATED PROBE IMPLANTATIONS. J.P. Druhan, D. Funk, H. Rajabi and J. Stewart. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

This study examined whether enhanced release of nucleus accumbens (NAS) dopamine (DA) after repeated morphine injections could be detected using in-vivo microdialysis with repeated probe implantations. Levels of DA and homovanillic acid (HVA) were measured in rats on two occasions (6 days apart) 18-24 h after implantation of removable microdialysis probes into the NAS. After collecting at least 6 baseline samples (20 min/sample), the rats were injected with morphine (5 mg/kg IP or 8 nmol/0.5 ul into the VTA) and samples collected for 3 h. The same doses of morphine were injected twice in the period between each test at two day intervals. On the first test, both systemic and intra-VTA morphine injections increased DA levels by about 50% of the baseline values and they increased HVA levels by 100-150%. Behavioral sensitization was observed on the second test after both IP and intra-VTA morphine injections, however DA levels were increased only to the same extent as on the first test by each of these treatments. Similarly, HVA levels were increased to the same extent on the each test with intra-VTA injections, although there was a slightly greater increase in HVA levels during the second test with systemic morphine. This lack of evidence for neurochemical sensitization with repeated microdialysis tests contrasts with previous ex-vivo results, and further raises questions about the feasibility of using repeated probe implantations to assess the development of sensitization within animals.

198.19

EFFECTS OF NOCICEPTIVE AND "PSYCHOSOCIAL" STRESSORS ON REGIONAL DOPAMINE UTILIZATION. S. Uysal, K.D. Carr, J.W. Schweitzer* and A.J. Friedhoff. Millhauser Labs, NYU Med. Ctr., NY, 10016.

It is well established that mild inescapable footshock selectively activates mesoprefrontal dopamine (DA) neurons (e.g. Thierry et al., 1976; Reinhard et al., 1982). The anatomical specificity of the DA response to stressful stimulation may, however, depend upon the nature and intensity of the stressor employed (e.g. Dunn, 1988). In the present study, rats were exposed to one of three stressors on an acute basis and effects on DA utilization in caudate, accumbens, and medial prefrontal cortex (mpfc) were determined. Rats in the first group received a nociceptive stressor consisting of 0.3 sec tailshocks delivered every 30 sec for 20 min. Rats in the second group "witnessed" the tailshock treatment through a wire mesh partition. Rats in the third group were placed in the treatment chamber following removal of each tailshock/witness pair to be stimulated by residual olfactory cues. A control group remained in home cages. Following treatment, rats were sacrificed by decapitation and DA terminal regions were micropunched from frozen brain sections. Tissues were assayed for DA, HVA, and DOPAC by HPLC. Ratios of DOPAC: and HVA: to DA were calculated as indices of DA utilization. None of the treatments altered DA utilization in the caudate. Tailshock increased HVA:DA ($F_{3,24}$=7.03, $p<.01$; Dunnett t, $P<.01$) and DOPAC:DA ($F_{3,24}$=9.71, $p<.01$; Dunnett t, $p<.01$) in mpfc and HVA:DA in accumbens ($F_{3,22}$=3.58, $p<.03$; Dunnett t, $p<.05$). "Witness" stress increased HVA:DA in the mpfc only (Dunnett t, $p<.05$). "Olfactory" stress increased both ratios in the mpfc, but not significantly. These results are compatible with proposals that mesocortical DA neurons are activated by diverse stressors and also support proposals that anatomical specificity of the DA stress response may vary according to nature of the stressor.

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198.20

GLUTAMATE-DOPAMINE FUNCTIONAL INTERACTION WITHIN THE STRIATUM AND THE NUCLEUS ACCUMBENS.

M. Amalric, A. Ouagazzal*, and A. Nieoullon. Neurochemistry unit, Functional Neurosc. Lab., CNRS, 13402 Marseille cx 9 (France).

Antagonists of gutamatergic transmission at the N-methyl-D-aspartate (NMDA) receptor, like MK-801, have been shown to induce a locomotor hyperactivity in rats. This study was conducted to see whether this enhanced motor activity could be mediated by the D1 or D2 dopamine (DA) receptor subtypes and if this was dependent on DA neuronal activity in the two main DAergic pathways. MK 801 (0.05 - 0.3 mg/kg s.c.) induced a robust and dose-dependent increase in locomotor activity. MK 801 (0.3 mg/kg) was then challenged to different doses of a D1 receptor antagonist (SCH 23390, 10, 20 and 40 µg/kg s.c.) or a D2 receptor antagonist (raclopride, 100, 200 and 300 µg/kg s.c.). At doses known to potently antagonize amphetamine induced locomotor activity, SCH 23390 only slightly antagonized MK 801 effect. This effect was not affected by raclopride, except at the highest dose known to produce a catatonic effect. Furthermore, a 6-OHDA induced destruction of DA nerve terminals within the nucleus accumbens (N. Acc.) would not prevent MK 801 stimulant effect. In contrast, the 6-OHDA lesion of nigrostriatal nerve terminals resulted in an enhanced activation following MK 801 injection. In another experiment, microinjection of the NMDA receptor antagonist AP5 (5, 10 µg) in the N. Acc. induced a dose-dependent increase in locomotor activity and in the striatum induced a stereotyped behavior. These results suggest that the modulatory action of NMDA receptors on locomotor activity is not closely linked to the dopaminergic transmission in the mesolimbic system, while striatal NMDA receptor blockade antagonizes behavioral impairements due to DA nigrostriatal dysfunction.

199.1

DECREASED PROSTAGLANDIN D2 SYNTHESIS IN THE CNS OF TRISOMY 16 EMBRYOS- A MODEL OF DOWN'S SYNDROME D. Minc - Golomb* and S.I. Rapoport. Lab. of Neurosciences, NIA, NIH, Bethesda,MD 20892.

Fibroblasts of Down's Syndrome (DS) embryos have been found to exhibit a reduced ability to release prostaglandins (PGs) (Minc-Golomb et. al. Mouse Molecular Genetics Meeting - CSH, N.Y. Abstr 143, 1990). In order to examine whether similar alterations in PG synthesis occur in CNS, the release of PGD2 from different brain areas was compared between normal and trisomy 16 mice (Ts16), an animal model of DS based on the homology between human chromosome 21 and mouse chromosome 16. Hemispheres and cerebelli of 8 Ts16 and 8 normal mice were removed and placed in DMEM. The amount of PGD2 released to the medium in 60min was determined by RIA. PGD2 release from the cerebelli of Ts16 fetuses was significantly less by 22% than that in littermate control embryos (3.84±0.34 and 4.94±0.27pg/mg respectively, mean±SEM n=8, $p<0.05$). A similar difference was found in the hippocampus. However, no difference was found in the release of PGD2 from the whole hemisphere (Ts16: 4.18±0.67; control: 4.14±0.45pg/mg tissue). These findings suggest that there are regional disturbances in arachidonic acid metabolism in the CNS in Ts16 fetuses. As PGD2 is an important neuromodulator in the CNS, the alteration described may contribute to CNS disturbances which characterize DS.

199.2

SOMATOSTATIN EXPRESSION IN TS16 CULTURES INFLUENCED BY THE PRESENCE OF NON NEURONAL CELLS.

P.Corsi,C.DiBenedetta,M.L.Oster-Granite,J.T.Coyle.
Istituto di Fisiologia Umana, Univ. di Bari, Italy and Depts. of Neuroscience and Pharmacology, Johns Hopkins Univ., Baltimore, MD 21205.

Since the gene encoding pre pro-somatostatin (ppSS) is located on mouse chromosome 16 (MMU16) we have studied the effects of Trisomy (Ts) of MMU16 on SS immunoreactivity (IR) in primary disaggregated cultures of neocortex prepared from gestational day 15 Trisomy 16 mice and their littermate euploid controls. Prymary cultures of papain dissociated cells were plated at different plating density ranging from $2.5x10^5$ to $1x10^6$ in poly-D-lysine coated dishes to examine the effect of different plating density on Somatostatin expression in both type of cultures. Two culture models were used: 1) neuron enriched cultures obtained by exposing to 5uM cytosine arabinoside (AraC) for 48h between day 3 and 5 after plating and whole cell cultures obtained by using the same protocol but untreated with AraC to investigate the effect of non neuronal cells on Somatostatin content in both group of cultured cortical cells. Immunocytochemical analysis indicated an increase in the relative density of SS-IR neurons ranging from 1-3 fold above euploids in Ts16 whole cultures at 2 and 3 weeks in vitro, although differences in SS expression disappear in cultures treated with AraC. The morphology of SS-IR neurons studied in Ts16 cultures and euploid sister cultures revealed a more complex pattern of staining for the Ts16 SS-IR neurons compared to euploids. Our data suggest that even though an extra copy of ppSS gene in Ts16 results in SS expression in an increased number of cortical neurons in whole cell cultures also a functional interaction between glial cells and neuron secreting Somatostatin occurs.

199.3

SPROUTING OF SEROTONERGIC FIBERS IN THE FOREBRAIN AND CORTEX OF NEONATAL MALE BRINDLED MICE. P.M. Martin, M. Ohno, and K. Suzuki. Brain and Development Research Center, and Dept. of Pathology, Univ. of North Carolina, Chapel Hill, N.C. 27599.

Mottled brindled (MO^{br}) is an X-linked mutation in the mouse, maintained in hybrids of the C3H and C57BL strains. Hemizygous males (MO^{br}/y), who usually do not live beyond postnatal (P) day 15, have low copper concentrations in the brain, an almost total lack of fur pigmentation, and neuronal degeneration of the cerebrum. Since copper is a cofactor for dopamine-ß-hydroxylase, hemizygous males have decreased concentrations of norepinepherine in the cortex and other regions of the cerebrum, along with high concentrations of 5-HT in the brainstem, and high concentrations of 5-hydroxyindoleacetic acid, the metabolite of 5-HT, in the hindbrain and all areas of the cortex and forebrain (Satoh et al., J Neuropathol Exp Neurol, in press). In the present experiment, 5-HT immumoreactive cell bodies in the dorsal raphe nucleus, and 5-HT immunoreactive fibers in corex and forebrain have been compared in hemizygous males and control littermates at P10 and P12 (n = 2 pairs at each time point). Immunohistochemical staining was carried out with free-floating, 40 micrometer, Vibratome sections, using 3,3'-diaminobenzidine-HCl (DAB) or DAB-nickle staining. No differences were observed between the 5-HT immunoreactive cell bodies in brainstem nuclei of the hemizygous males and their littermate controls. However, 5-HT immunoreactive fibers were more dense throughout the cortex and forebrain areas of the hemizygous males in comparison to the same areas of normal male littermates. This increase in density of 5-HT immunoreactive fibers was apparent at both ages. Additional pairs of animals at ages P7 and P14 currently are being tested. This increase in density possibly reflects sprouting of collateral serotonergic fibers, and may be similar to the sprouting which has been observed following neonatal 6-OHDA lesioning of dopaminergic or noradrenergic pathways in rat pups. (Supported by Grants HD-03110, ES70126, NS24453 and ES01104).

199.4

INCREASED EXPRESSION OF NEUROPEPTIDE Y-IMMUNOREACTIVE NEURONS IN MURINE TRISOMY 16 CORTICAL CULTURES. Maria T. Caserta Depts. of Psychiatry and Neurobiology and Physiology. Northwestern University Medical School and Evanston Hospital, Evanston, IL 60201.

Murine trisomy 16 has been used as a model of human trisomy 21 and may be useful in elucidating the neurobiological mechanisms that are involved in mental retardation and the predisposition to Alzheimer's disease in individuals with trisomy 21. Trisomy 16 fetuses, however, die in vitro and therefore, studies of neuronal development and differentiation must be done in vitro. We have previously shown that somatostatin (SMST) - immunoreactive neurons were overexpressed in cortical cultures derived from 15 day in uterotrisomy 16 mouse fetuses when compared to their euploid littermate controls (Caserta et al., 1990, Mol.Br.Res.7:269-272). The gene for pre-pro-SMST has been mapped to chromosome 16 in the mouse. Since SMST and neuropeptide Y (NPY) are frequently co-localized in mammalian brain and both of these neuropeptides are depleted in Alzheimer's disease, the expression of NPY-immunoreactive neurons in cortical cultures derived from trisomy 16 mouse fetuses and their euplid littermates was investigated. Neuronal plating density and dissociation protocols were varied to maximize neuronal survival in trisomic cultures and identical conditions were employed for the euploid control cultures. The NPY peptide was visualized by immunocytochemistry and the cultures were examined at varying ages in vitro. All cultures were counterstained for the expression of neuron-specific enolase to identify neurons. There was an increased expression of NPY-immunoreactive neurons in trisomy 16 cortical cultures when compared to control cultures (4% vs. 7%) These studies suggest that NPY and SMST expression may be regulated by similar genetic and/or developmental mechanisms in trisomy 16 cortex.

199.5

HIGH DOSE ASPARTAME HAS NO EFFECT ON EEG SPECTRAL PARAMETERS IN PHENYLKETONURIC HETEROZYGOTES (PKUH). Ch. Benninger, P. Matthis*, L.M.J. de Sonneville, B. Lanz-Englert*, F.K. Trefz*, and H. Bickel*. University of Heidelberg, Heidelberg, Germany.

The effects of aspartame (APM) on EEG, cognition, and biochemical parameters were evaluated in 48 adult (21 male, 27 female) PKUH whose carrier status had been proven by DNA analysis. Subjects received APM (either 15 or 45 mg/kg/day) and placebo in capsules in a randomized, double-blind, placebo-controlled, crossover study for twelve weeks on each treatment. EEG's were recorded at baseline and at the end of each treatment (weeks 12 & 24). The relative powers (%) in the delta, theta, alpha, and beta bands between 1.0 - 25 Hz were computed from six leads using the fast Fourier transform. The mean power frequencies across all bands, across the major alpha-theta bands, and the half-power frequency were also computed. There were no significant differences in the clinical EEG evaluation, or in any of the EEG spectral parameters, when APM was compared to placebo. There were no significant effects of treatment on urinary organic acids, or adverse experiences. In addition, the results of a computerized battery of neuropsychological tests (the de Sonneville visual attention tasks) demonstrate that APM has no effect on cognitive function. These results reaffirm the safety of APM in PKUH and refute the speculation that APM causes changes in neurological function.

199.6

AGE-RELATED MOLECULAR AND MORPHOLOGICAL CHANGES IN THE CEREBELLUM AND HIPPOCAMPUS OF SPASTIC HAN-WISTAR RATS. R.W. Cohen, T.P. Duong, R.S. Fisher, J.B. Watson, A.T. Campagnoni, N.A. Buchwald, and M.S. Levine. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

A genetic mutation of a strain of Han-Wistar rat is characterized by the postnatal development of ataxia, tremor and limb rigidity. Our previous findings suggested that a dysfunction in glutamate neurotransmission occurred in the cerebellum of these rats which led to losses in Purkinje cells and a reorganization of the granule cell layer (Cohen, et al., Mole. Brain Res. in press, 1991). To investigate the role of glutamate receptors in this disorder, we obtained clones of GluR1, GluR2 and GluR3 mRNA (Boulter, et al., Science 249:1033-1037, 1990; gift from S. Heinemann and M. Hollmann). Northern blot analysis revealed decreased expression of each glutamate receptor mRNA in mutant cerebellum (ages 45-50 days). The results suggest that cerebellar cell death may reduce overall receptor mRNA levels and changes in glutamate conductance may be due to elevated expression of a different receptor or preferential assembly of a multimeric, cerebellar glutamate receptor with potent kainate binding affinity. Parallel morphological studies assessed the time course of the defect. Mutant and normal rats were examined histologically at 30, 40, 56 and 60 days postnatally. Coronal sections from cerebellum and hippocampus were stained with Cresyl violet or processed immunochemically for neurofilament proteins. Mutant cerebella showed losses of Purkinje cells starting at 40 days. Labeling mutant cerebella with a neurofilament antibody also revealed cell loss and Purkinje cell axonal swellings that extended into the granule cell layer. Loss of pyramidal cells in the CA3 region was observed after 50 days in the mutants, corroborating the results of Wagemann et al. (Neurosci. Letts. 121:102-106,1991). Axonal swellings were not observed in the hippocampal neurons. Based on the evidence, we propose that the spastic Han-Wistar rat disorder represents a disturbance in cerebellar glutamate transmission. The hippocampus may also be involved. Supported by NICHD HD05958 AND HD25831.

199.7

Manganese and Epilepsy: Mn^{2+}/Mg^{2+} Effects on Rat Brain Glutamine Synthetase Activity. G.F. Carl, J. Williams*, V. Livingston* and B.B. Gallagher. Med. Coll. Ga. and VAMC, Augusta, GA 30912

It has been repeatedly demonstrated that blood Mn concentrations are lower in epileptics than in a control population. Lower blood and brain Mn concentrations have also been demonstrated in genetically epilepsy prone rats (GEPR) when compared to their non-epileptic progenitors. GEPRs also exhibit lower brain glutamine synthetase activity. Since both the Mn concentration and the glutamine synthetase activity in GEPR brain were lower, we examined the effect of variations in Mn^{2+} concentration on the activity of the enzyme purified from both GEPRs and their progenitor strain, genetically epilepsy resistant rats (GERRs), to determine if the decreased brain Mn concentration might account for the decreased brain glutamine synthetase activity observed in the epileptic animals.

By varying the Mg^{2+} concentrations from 0 to 25 mM and the Mn^{2+} concentration from 0 to 5 mM, we observed that Mn^{2+} inhibits the Mg^{2+}-stimulated activity of brain glutamine synthetase in a concentration dependant manner at physiological pH, 7.2. We conclude that the decreased Mn^{2+} concentration observed in GEPR brain is not contributing to the decreased activity of glutamine synthetase in the brains of these animals. The relationship between decreased glutamine synthetase activity, decreased Mn^{2+} concentration and seizure susceptibility remains undefined.

199.8

REDUCED ACTIVITY OF A NAAG-HYDROLYZING ENZYME IN BRAIN REGIONS OF 21 DAY-OLD SEIZURE-SUSCEPTIBLE DBA/2 MICE. J.L. Meyerhoff[1], R.E. Carter*[2], D.L. Yourick[1] and J.T. Coyle[2]. [1]Dept. Med. Neurosci., Walter Reed Army Inst. Res., Wash., DC 20307. [2]Div. Child Psychiatry, Johns Hopkins Univ. School of Med., Balt., MD 21205.

N-Acetyl-aspartyl-glutamate (NAAG) is a dipeptide in brain, which is localized to neurons, releasable by depolarization from brain tissue, increased by kindling, and excitatory when applied to neurons. NAAG is hydrolyzed into glutamate (GLU) and N-acetyl-aspartate (NAA) by N-acetylated-α-linked acidic dipeptidase (NAALADase). Thus, NAALADase could affect the availability of GLU at certain synapses. NAAG might be an excitatory neurotransmitter, but it could also function as a precursor/storage form of GLU. We recently found increased NAALADase activity in adult genetically epilepsy-prone rats (GEPRs). In contrast, DBA/2 mice, at the time of maximal seizure susceptibility (21 days), had NAALADase activity about 50% lower in several brain regions as compared with C57BL/6 seizure resistant mice. Separate genes reportedly regulate seizure spread vs susceptibility in DBA/2s, and different genes control juvenile vs adult-onset audiogenic seizures. Therefore, it is important to determine whether NAALADase activity is related to seizure susceptibility or seizure spread, and to compare the ontogeny of regional brain NAALADase in GEPRs, DBA/2s, and mouse strains susceptible to seizures as adults.

199.9

ENHANCED STIMULATION OF DOPAMINE RELEASE BY SEROTONIN IN WEAVER MICE. J.A. Richter, H. Yu, B. Ghetti and J.R. Simon. Indiana Univ. Sch. Med., Indianapolis, IN 46202.

The weaver mutant mouse has about a 70% loss of nigro-striatal dopamine (DA) neurons. Previously we found that there was a compensatory increase in the basal release of DA (expressed as a fraction of the tissue content) from weaver striatal slices, and the amphetamine-evoked fractional DA release was also higher. In the present study we examined the effect of serotonin (5-HT) on weaver striatal DA release since 5-HT is known to increase DA release from normal striatum.

Striatal slices were superfused in vitro and release of endogenous DA was assayed by HPLC-EC. A concentration-related increase in fractional DA release was induced by 5-HT perfusion of +/+ striatal slices and the effect increased over 40 min of 5-HT exposure. When a 20 min perfusion with 10 uM 5-HT was followed by perfusion with normal medium, the evoked DA release peaked in the fraction following the end of the 5-HT exposure. In contrast to these results in +/+, the evoked DA release from weaver striatal slices occurred with less delay. Basal fractional release was once again higher for weaver than +/+, and the 5-HT-evoked fractional DA release was greater in the weaver than in the +/+. These results suggest that weaver striatum is more sensitive to the stimulatory effect of 5-HT on DA release. The mechanism for the greater and more rapid effect is under investigation. Supported by R01 NS14426 & P01 NS27613.

199.10

DOPAMINE UPTAKE IN THE SUBSTANTIA NIGRA: EFFECT OF THE WEAVER GENE. J.R. Simon, A.D. Miller* and B. Ghetti. Indiana Univ. Sch. of Medicine, Indianapolis, IN 46202.

The aim of the present study was to establish conditions for assessing dopamine (DA) uptake into DAergic elements in the substantia nigra (SN) of the mouse and then determine the effect of the weaver gene (wv) on this transport process. The SN, including the pars compacta and pars reticulata, was dissected from a 0.7 mm coronal section and chopped into 0.1 mm x 0.1 mm slices. Uptake of [3H]-DA was determined by incubating the slices at 37°C for 15 min. Approximately 60-70 % of the estimated DA uptake was inhibited by 1 uM fluoxetine, indicating a significant contribution from serotonergic terminals. DA uptake measured in the presence of 1 uM fluoxetine was temperature sensitive and inhibted by GBR12909 and nomifensine, suggesting the presence of a classical DA uptake carrier in the SN. Heterozygotes (wv/+) have a severe reduction in the number of TH-immunopositive dendrites in the pars reticulata of the SN (Triarhou and Ghetti, 1989). However, as measured by the method described above, DA uptake into SN slices from wv/+ mice did not differ significantly from that observed in +/+ mice. It is possible that the uptake is occurring into soma as opposed to dendrites or that homozygosity is required for an effect on the transport system in the SN. Uptake studies in the SN from wv/wv mice are currently in progress. (Supported by P01 NS27613)

199.11

CHANGES IN STRIATAL GLUTAMIC ACID DECARBOXYLASE ACTIVITY IN WEAVER MUTANT MICE. H. Yu., B. Ghetti and J.R. Simon. Indiana Univ. Sch. of Medicine, Indianapolis, IN. 46202

The weaver mutant mouse (wv/wv) has a defect in the nigrostriatal dopaminergic pathway. Morphological and neurochemical evidence indicates that these dopaminergic neurons play a role in regulating the activity of GABAergic neurons in the striatum. The objective of the present study was to determine if the loss of tonic DA inhibition in weaver mice results in alterations in striatal GABA neurons. We assessed GABA uptake and glutamic acid decarboxylase (GAD) activity in striatal homogenates from age matched +/+ and wv/wv mice. In 9 and 12 month old mice, GABA uptake was unaffected in wv/wv mice compared to +/+ mice (N=6). Striatal GAD activity, determined at a sub-saturating substrate concentration of 25 mM Glu, was significantly increased in 10 month old wv/wv mice (75±6.6 vs 100±5 nmol/mg prot. N=4), and in 45 day old wv/wv mice (73±4.5 vs 102±8.5 nmol/mg prot. N=4). When saturating concentrations of Glu were employed, no differences in GAD activity were observed between the 2 genotypes at either 45 days or 10 months of age. GAD activity in the substantia nigra determined using 25 mM Glu, did not differ between +/+ and wv/wv mice. The alterations in GAD suggest that a population of striatal GABA neurons is affected by the dopamine depletion in the weaver mutant mouse, and that GAD from wv/wv striatum may itself be altered. (Supported by R01 NS 14426)

200.1

HYPEREXCITABLE GRANULE CELL RESPONSES EVOKED *IN VITRO* AFTER PROLONGED *IN VIVO* STIMULATION. B.W. Strowbridge[1], P.S. Buckmaster[1] and P.A. Schwartzkroin[1,2]. Depts. of [1]Physiology & Biophysics, and [2]Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Mossy cells and somatostatin-immunoreactive neurons appear to be especially vulnerable in the dentate gyri in temporal lobe epileptic patients with presumed hippocampal foci (de Lanerolle, N., *et al.*, 1989, *Brain Res.* 495: 387-395). A similar pattern of hilar cell loss can be produced in rats with prolonged perforant pathway stimulation (Sloviter, R., 1987, *Science* 235: 73-76). In order to study further the role of hilar neurons in regulating dentate gyrus excitability, we have examined synaptic responses of granule cells of stimulated and control animals *in vitro*. The normal synaptic response of granule cells to perforant path stimulation (an EPSP which may elicit a single action potential, followed by an IPSP) was observed in all granule cells in control animals (n=21) and some cells in stimulated animals (n=11 of 22). In those slices in which granule cells responded normally, we also obtained normal intracellular recordings from hilar mossy cells (n=8), suggesting that hilar cell loss was not pervasive. Other granule cells (n=11 of 22) from stimulated animals responded to stimulation with a prolonged depolarization that could trigger a burst of action potentials. In these cells, the short latency (Cl^--mediated) IPSP was diminished or absent, but the late, K^+-mediated IPSP component was present. In the presence of glutamate-receptor antagonists, CNQX (10-40 μM) and APV (20-30 μM), stimulation near the granule cell recording electrode elicited inhibitory synaptic responses with both early and late components (n=2). These results support the hypothesis that prolonged *in vivo* stimulation affects the local circuitry that activates the inhibitory neurons that mediate the early IPSP recorded in granule cells.

Supported by NIH grants NS20482 and NS07097.

200.2

IN VITRO VISUALIZATION OF HIPPOCAMPAL MOSSY FIBER SPROUTING AFTER STATUS EPILEPTICUS IN RATS. M.M. Okazaki and J.V. Nadler, Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

We have developed a method for visualizing dentate granule cells and their mossy fibers in the rat hippocampal slice after extracellular ionophoresis of biocytin (Okazaki and Nadler, Soc. Neurosci. Abstr. 16:123, 1990). The present study applied this methodology to visualize mossy fiber sprouting into the inner portion of the dentate molecular layer after status epilepticus (SE). SE was induced in adult male Sprague-Dawley rats by one of two methods: 325 mg/kg (i.p.) of pilocarpine preceded by 1 mg/kg (i.p.) of methylscopolamine or 18 mg/kg (s.c.) of kainic acid. Pilocarpine-induced SE was terminated after 3 h with 50 mg/kg (i.p.) of phenobarbital. At least 4 weeks later, horizontal 400 μm-thick slices of the caudal half of the forebrain were prepared and incubated in a static interface chamber. Alternate slices were set aside for Timm's staining. Biocytin was ionophoretically applied to stratum lucidum of area CA3b. This procedure labeled the dentate granule cells and their mossy fibers by retrograde transport of the tracer. Biocytin was visualized with the NBT/BCIP chromogen reaction in sections prepared from these slices.

Whenever Timm's staining indicated the growth of mossy fibers into the dentate molecular layer, many small diameter biocytin-labeled fibers were observed to penetrate the dentate granule cell layer from the hilus and then to course parallel to the layer. These fibers were studded with relatively large varicosities. Their abundance correlated with the intensity of the Timm's reaction in the same region; they were rarely observed in sections from control animals. This is the first demonstration of mossy fiber sprouting that does not depend on a histochemical marker. (Supported by NIH grant NS 17771.)

200.3

EVIDENCE FOR HYPEREXCITABILITY IN THE ISOLATED HIPPOCAMPAL CA1 AREA OF LONG-TERM KAINATE-LESIONED RATS C. L. Meier, A. Obenaus and F. E. Dudek. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024

Loss of Schaffer collateral input to CA1 pyramidal cells due to kainate-induced lesions may lead to synaptic reorganization in the CA1.

Simultaneous intra- and extracellular recordings were obtained in CA1 minislices prepared from 5 control rats and 5 animals 41 to 363 days after subcutaneous injection of kainate (18 mg/kg). Stimulation of the stratum radiatum close (less than 500 μm) to the recording site evoked an EPSP-IPSP sequence in all 9 CA1 neurons in control slices but only in 4 out of 9 neurons recorded in slices from kainate-treated animals. In the other 5 neurons, stimulation of the stratum radiatum evoked an EPSP only. The mean duration (measured at baseline) of the EPSP in slices from kainate-treated animals was significantly longer than the stimulation-evoked EPSP in slices from controls (44 ± 7 ms vs 14 ± 3 ms; mean ± SEM, t-test, $p<0.02$). In 8 out of 10 slices from kainate-injected rats, the extracellular response consisted of 2-3 population spikes. Two slices showed only a single population spike similar to the response seen in all 7 control slices. In slices from both groups, bath application of 30 μM bicuculline for 20-100 min led to stimulation-evoked bursts of 4-8 population spikes and prolonged EPSPs with 2-6 action potentials. Therefore, in bicuculline, there were no apparent differences in stimulation-evoked responses between control and kainate-treated rats. Spontaneous bursts were never observed in the isolated CA1.

Our preliminary results confirm the results of Nakajima et al. (1991) (Hippocampus, 1, 67-78) and are consistent with the hypothesis that kainate treatment leads to loss of inhibition within the CA1 area, which can persist for more than a month.

200.4

EVIDENCE FOR G PROTEIN MODULATION OF GENERALIZED ABSENCE SEIZURES. O. Carter Snead III. Dept Neurology, Univ. Southern Calif; Div Neurology, Childrens Hospital Los Angeles, Los Angeles, CA 90027

γ-Hydroxybutyric acid (GHB) is a naturally occurring compound which has the ability to produce absence-like seizures in rats (Snead, Epilepsia 1988;29:361). It is known thant $GABA_a$ agonists exacerbate experimental absence, but we have recently demonstrated that the $GABA_b$ agonist baclofen also prolongs spike wave discharge (SWD) duration in the GHB model of absence. Since the $GABA_b$ receptor is coupled to a G protein, we sought to determine G protein involvement in the GHB model of absence. G protein inactivation (Andrade et al, Science 1986;234:1261) by pertussis toxin administered icv 3 days prior to GHB induction of absence seizures in rats resulted in a significant ($p<0.05$, n=6 , nonparametric ANOVA) decrease in SWD duration in this model of absence. In view of these results, the effect of GTP on both [^{3}H]GHB and [^{3}H]GABA ($GABA_b$) binding in synaptosomal membranes was determined. GTP resulted in a 40-60% inhibition of binding of both ligands to their respective receptor sites. These data raise the possibility that GHB-induced absence seizures are mediated through a G-protein which is coupled to both the $GABA_b$ and GHB receptor.

200.5

USE-DEPENDENT CHANGES IN DENTATE GRANULE CELL FIELD RESPONSES RECORDED FROM NAIVE AND KINDLED RATS. L.J. Burdette, G.J. Hart*, & L.M. Masukawa. Depts of Neurology & Research, Graduate Hospital, Philadelphia, PA

Low frequency (1 Hz) stimulation of the perforant path results in a decrease in the population spike (PS) amplitude of dentate granule cell field responses. To determine if this decrease reflects the long-lasting time course of late inhibition, we compared waveform changes in naive and kindled rats during trains of single pulses (30 pulses, 0.1 ms, 60% maximum PS amplitude) to the strength of paired pulse inhibition (interstimulus intervals: 20, 50, 100, 200 ms) delivered at 0.05, 1 and 5 Hz. No significant waveform changes were evident in either group during trains of 0.05 Hz stimulation. Both groups exhibited a similar decrease in PS amplitude to single pulse trains of 1 Hz stimulation, despite the presence of significantly enhanced paired pulse inhibition recorded at 0.05 Hz from kindled rats. A similar, but more pronounced, decrease in PS amplitude preceded afterdischarge initiation elicited by 5 Hz stimulation (single or paired pulses) in both groups. When the amplitude of the first PS was matched during 1 Hz paired pulse stimulation to that recorded during 0.05 Hz, kindled rats exhibited a greater decrease in late inhibition to 1 Hz paired pulses than that observed in naive rats. These results suggest that the decrease in PS amplitude observed with repetitive low frequency stimulation is not due to late inhibition, but instead may reflect some mechanism involved in seizure initiation.

200.6

THE NMDA-ANTAGONIST, MK-801, DIFFERENTIALLY AFFECTS AFTERDISCHARGE DURATION AND SEIZURE DEVELOPMENT IN PERFORANT PATH KINDLING. ME Gilbert. ManTech Environmental Technology Inc., RTP, NC, 27709.

In amygdala kindling, NMDA antagonists have been reported to possess strong antiepileptogenic, but only mild anticonvulsant properties. MK-801 retards the development of amygdala and perforant path (PP) kindling and reduces afterdischarge (AD) durations induced by amygdala stimulation. However, AD's evoked by PP stimulation appear to be prolonged by MK-801 (Gilbert and Mack, 1990). The following study was designed to evaluate further the effects of MK-801 on AD and seizure development in response to PP stimulation. A crossover design was utilized in which male Long Evans rats were stimulated once daily for 10 sessions in the PP (60 Hz, 2 s, 800μ a), 30 m following MK-801 (1mg/kg, ip) or saline. Following a 1 wk rest period, stimulation resumed with the drug conditions reversed. MK-801 suppressed kindling development, but 3/12 animals had displayed generalized seizures (GS, stages 3-5) by the 10th session. When stimulation was resumed under saline conditions following the rest period, 8/10 animals displayed GS by the 4th stimulation session. Naive, saline-treated control animals required a mean of 7.8 sessions to evoke GS. MK-801 also resulted in longer ADs. Mean AD durations dropped after the rest period when animals were stimulated under saline conditions. These data suggest that the conditions that modulate focal AD duration in the PP may be distinct from those in the amygdala. Savings in kindling development following high dosages of MK-801 indicates that a significant progression of kindling has occurred under this drug regimen.

200.7

HIPPOCAMPAL MOSSY FIBER ZINC AND KINDLING SUSCEPTIBILITY IN INBRED MICE. HD Rees, RC Green and DD Savage. Dept. of Neurol., Emory Univ. School of Med., Atlanta, GA 30322; Dept. of Pharmacol., Univ. of New Mexico School of Med., Albuquerque, NM 87131.

The relationship between mossy fiber zinc and genetic differences in olfactory bulb kindling rates was studied in 5 strains of mice. Six to ten male mice of each strain were killed at age 5-7 weeks, before onset of movement-induced seizures in EI mice, and without elicitation of audiogenic seizures in DBA mice. The brains were frozen and a quantitative histofluorescence procedure utilizing the chelation of zinc and TS-Q was employed to measure zinc density in stratum lucidum of CA3 in sections of dorsal and ventral hippocampus. Results (mean ± SD) are as follows:

Mossy Fiber Zinc Density in Dorsal/Ventral Hippocampus (femtograms/100 μm²)
Kindling Rate (Number of Stimulations to First Stage 5 Seizure)

	DBA/2	C57Bl/6J	EI	ddY	C3H/He
Ventral Hipp	3.29 ± 0.43	3.12 ± 0.19	2.37 ± 0.13	2.27 ± 0.21	2.06 ± 0.16
Dorsal Hipp	3.44 ± 0.61	3.63 ± 0.45	2.59 ± 0.13	2.82 ± 0.13	2.70 ± 0.08
Kindling Rate	4.0 ± 1.4	9.6 ± 2.2	14.8 ± 3.0	18.5 ± 2.5	22.6 ± 3.8

At both dorsal and ventral levels of the hippocampal formation, one-way ANOVA followed by Scheffé post-hoc test indicated that DBA/2 and C57Bl/6J strains had significantly higher mossy fiber zinc levels than the other 3 strains. Comparison by two-tailed *t*-test of mossy fiber zinc levels in the epileptic EI mice to those in the genetically similar but non-epileptic ddY mice revealed no significant differences in either the ventral (p=0.73) or dorsal (p=0.24) hippocampus. Mossy fiber zinc density values were correlated by linear regression with olfactory bulb kindling rates for these strains derived from earlier experiments in animals of similar age (Green & Seyfried, *Epilepsia* 32:22, 1991). The coefficients of determination (r^2) were 0.93 in the ventral hippocampus and 0.63 in the dorsal hippocampus. These findings suggest that strains with higher levels of hippocampal mossy fiber zinc kindle more rapidly.

200.8

AMYGDALA KINDLING, ANXIETY AND CORTICOTROPHIN RELEASING FACTOR (CRF). R.E. Adamec. Depts. of Psychology/Basic Medical Science, Memorial University, St. John's, NF, Canada

Male Wistar rats were kindled in the right medial amygdala until four stage 5 seizures were evoked. One week later anxiety was assessed in the elevated plus maze. One kindled group was simply tested. Additional kindled groups were tested in the plus maze one hour after an icv injection of either saline, CRF or the CRF antagonist, α-helical CRF. Parallel groups of implanted control animals were tested in the plus maze in the same manner as kindled rats. Controls were handled like kindled rats, but not kindled. Kindling alone increased anxiety in the plus maze relative to controls. CRF did not alter anxiety in handled control animals. In contrast, icv injections of saline or CRF were equally anxiolytic in kindled animals. Kindled rats injected icv were less anxious than uninjected kindled rats, and did not differ from controls. α-helical CRF reversed the anxiolytic effect of icv injection in kindled rats, but had no effect on anxiety in handled rats. Kindled rats given α-helical CRF were as anxious as uninjected kindled rats, and more anxious than controls given α-helical CRF. These findings suggest that amygdala kindling increases anxiety for some time after kindling has ceased. Moreover, kindling seems to alter the behavioral effects of injection stress mediated by endogenous CRF. Normally, stress induced increases in endogenous CRF are anxiogenic. Kindling seems to change this effect of CRF to an anxiolytic action.

200.9

RECOVERY FROM UNILATERAL CORTEX DAMAGE IN KINDLED RATS: EFFECT OF SEIZURE STAGE ON SOMATOSENSORY DEFICITS. T.D. Hernandez, J.C. Green*, L.C. Russell* and J.M. Droogan*. Department of Psychology, University of Colorado, Boulder, CO 80309

It has been reported that generalized seizures induced by chemoconvulsants or electroconvulsive shock facilitate recovery from behavioral deficits (i.e. sensorimotor asymmetries) produced by cortical damage. In contrast, anti-convulsant administration following cortex damage interferes with behavioral recovery and can reinstate behavioral deficits in fully recovered animals. The present studies were undertaken to assess the effect of focal epileptogenesis (using amygdala kindling) on behavioral recovery following unilateral frontal cortex damage in rats.

Animals were chronically implanted with a stimulating electrode in the amygdala during the same surgery in which they sustained a unilateral electrolytic lesion of the frontal cortex. Daily electrical stimulation of the amygdala began 48 hours after surgery. Recovery from somatosensory asymmetry (as measured by the bilateral tactile stimulation test) was facilitated during the early stages of kindling when compared to implanted/unstimulated controls. In addition, when multiple Stage 5 seizures were administered to fully kindled animals that had completely recovered from somatosensory asymmetry, the asymmetry was reinstated. Thus, seizures administered during the *development of kindling* had very different effects on behavioral recovery than did seizures administered within the *kindled state* itself. The degree to which these results may be a function of post-lesion plasticity and/or seizure severity will be discussed.

200.10

EFFECTS OF GLUCOCORTICOIDS ON COCAINE-INDUCED KINDLING AND BEHAVIORAL SENSITIZATION. M.A. Kling, D. Pluznik*, M.D. DeBellis*, J. Demas*, J.R. Glowa, and M.A. Smith. Clinical Neuroendocrinology Branch, NIMH/DIRP, Bethesda MD 20892.

Repeated administration of cocaine to rats produces both kindled seizures and behavioral sensitization (e.g., stereotypic behavior). Previous studies have shown that glucocorticoids and their antagonists can influence the development of local anesthetic-induced kindling. We report here a study of the effects of the glucocorticoids dexamethasone (DEX) and corticosterone (B) on cocaine-induced kindling and sensitization. Male Sprague-Dawley rats, 250-300g, were pretreated with DEX (250 μg/rat/d), B (10 mg/rat/d), or vehicle i.p. at 1600 h X 3 d prior to initiating daily i.p. injections of cocaine (40mg/kg/d) at 0900 h. Rats were observed for 30 min. in an open field for seizure activity and stereotypy, rated on a 0-7 scale. DEX-treated animals kindled significantly more rapidly than B-treated rats or controls, with 90% of DEX-treated rats developing seizures by day 5, compared with 40% of B-treated rats; 50% of controls kindled, all between days 8 and 17 (X^2=11.8, p<0.02). A second experiment showed that DEX given at 25, but not 2.5 μg/rat/d, significantly increased kindling frequency compared with controls (X^2=9.2, p<0.01). Neither steroid significantly affected the development of cocaine-induced sensitization of stereotypy. These results suggest that glucocorticoids (DEX>B) enhance the development of cocaine kindling but not sensitization, compatible with the idea that these phenomena are mediated by different neural substrates.

200.11

ANALYSIS OF EXCITATORY AMINO ACID RECEPTORS IN THE DISCRETE BRAIN AREAS OF KINDLED RATS. K.Akiyama[1], Y.Yoneda[2*], K.Ogita[2*], T.Itoh[1*], A.Daigen[1*], I.Sora[1], I.Kohira[1*], H.Ujike[1*] and S.Otsuki[1*]. [1]Department of Neuropsychiatry, Okayama University Medical School, Okayama 700, JAPAN, and [2]Department of Pharmacology, Setsunan University, Hirakata 573-01, JAPAN.

The present study examined the binding of excitatory amino acid receptor subtypes in the five brain areas (amygdala, hippocampus, striatum, frontal cortex and cerebellum) of the kindled rats, by using extensively washed and Triton X-100-treated membranes. 7 days after the last amygdala kindled seizure, specific [^{3}H]MK-801 binding decreased significantly only in the amygdala of the kindled rats as compared to the control in the equilibrium assay conditions. There was no significant change in the [^{3}H]MK-801 binding in the amygdala or hippocampus 7 days after the last hippocampal kindled seizure, or 28 days after the last amygdala kindled seizure. Nor was there significant change in NMDA-sensitive [^{3}H]glutamate, [^{3}H]glycine, [^{3}H]spermidine, [^{3}H]kainate or [^{3}H]AMPA binding in any brain area 7 days after the last amygdala kindled seizure, or in the hippocampus 28 days after the last amygdala kindled seizure. These results indicate that NMDA receptor channel in the amygdala undergoes down-regulation only transiently, but that none of the excitatory amino acid receptor subtypes exhibit long-lasting change at steady state following the completion of amygdala kindling.

200.12

DIFFERENTIAL EFFECTS OF CONDITIONING STIMULATION IN THE STRIATUM ON KINDLED SEIZURE DEVELOPMENT. M.J. Berg*, C.D. Applegate, J.L. Burchfiel. Comprehensive Epilepsy Program, University of Rochester Medical Center, Rochester, NY 14642.

Activation of the striatum has been reported to have an anti-convulsant effect in a variety of rodent seizure models. In this study we evaluated the influence of striatal activation on kindled seizure development. Male, Sprague-Dawley rats were implanted with bipolar stimulating electrodes into either the ventral (N=8) or dorsal (N=4) striatum and the ipsilateral basolateral nucleus of the amygdala. Conditioning stimulation (5s train of 1ms pulses, 3Hz, 1.5mA) was delivered to the striatum 1 minute prior to each amygdala kindling stimulation trial. Results demonstrated an effect of striatal stimulation on kindling rates which differed based on the site of the conditioning stimulation. Activation of the ventral striatum significantly increased kindling rates, while activation of the dorsal striatum was ineffective in altering the rate of amygdala kindling in comparison with controls. The mean number of trials to the first stage 5 seizure was 15.3+/-0.8 (p<.01) for the ventral striatal group, 6.5+/-0.3 (p>.1) for the dorsal striatal group and 8.5+/-1.0 for control animals (N=6). The increased number of trials in the ventral striatal group was totally accounted for by an increased number of stage 1-2 trials. No differences in afterdischarge durations or thresholds were observed among groups. These observations support a role of the striatum in the development of amygdala kindling and suggest a striatal influence on afterdischarge propagation. This effect may be mediated by topographically organized striatal output to the substantia nigra.

200.13

THE PIRIFORM CORTEX AND KINDLING: BEHAVIORAL AND PHYSIOLOGICAL EVIDENCE FOR A COMMON SUBSTRATE. J.L. Burchfiel and C.D. Applegate. Dept. of Neurology, University of Rochester School of Medicine, Rochester, NY 14642.

A growing body of evidence suggests that the piriform cortex (PC) is uniquely sensitive to the effects of limbic kindling. Male, Sprague-Dawley were implanted with bipolar electrodes into either the olfactory bulb (OB) or septal nucleus (S) and the ipsilateral PC, and kindled using staandard protocols from either the OB or S. Following primary kindling, transfer kindling of the PC was assessed. Afterdischarge (AD) characteristics were recorded on each trial during primary and transfer kindling.

Each OB AD during primary kindling elicted a PC AD with identical characteristics to that elicited by direct stimulation of the PC. During primary S kindling, the emergence of an "endogenous" PC AD was delayed and was directly related to the development and expression of the initial stage 3 seizure. The PC was virtually fully kindled following primary kindling of either OB or S, requiring only 1-2 stimulations to exhibit a stage 5 seizure. Data indicate that the PC is a highly involved structure in both OB and S kindling. Experiments to test whether the PC also is involved in the kindling of dorsal hippocampus and other structures are currently being conducted.

200.14

FAST AND SLOW KINDLING RAT STRAINS: *IN VITRO* RESPONSE OF PYRIFORM AND PERIRHINAL CORTEX TO MAGNESIUM-FREE PERFUSATE. Heather J. Lennox* and Dan C. McIntyre. Dept. of Psychology, Carleton Univ., Ottawa, ONT Canada K1S5B6.

Prolonged exposure of a coronal slice including the amygdala, perirhinal cortex and pyriform cortex to magnesium-free (0-Mg) perfusate produces spontaneous epileptiform activity, and continued hyperexcitability in a reduced form after re-exposure to normal-Mg perfusate. Thus, this treatment might be an *in vitro* analog of *in vivo* kindling (Hoffman & Haberly (1989) J. Neurosci. 9:206-215). In the present study, electrophysiological properties of such slices from two rat strains previously bred as prone (fast) and resistant (slow) to amygdala kindling were observed under normal and 0-Mg perfusates. First, the identifying interstrain difference *in vivo*, the kindling rates, emerged *in vitro* when slices from slow rats, compared to fast rats, were slower to exhibit regular epileptiform burst discharge activity. Second, frequent independent pyriform discharges in slow slices seem to be an *in vitro* analog of the *in vivo* interictal spikes prevalent in the slow strain. Third, the pyriform cortex of slices from the slow strain were surprisingly excitable in 0-Mg. And finally, kindling-like effects were produced in both strains by prolonged 0-Mg-induced hyperexcitability: increased excitability was seen in measures of both evoked and spontaneous population discharges during normal reperfusion after 0-Mg.

200.15

C-FOS EXPRESSION IN PILOCARPINE-INDUCED STATUS EPILEPTICUS. L.E.A.M. Mello, A.M. Tan* and D.M. Finch, Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Injection of a single dose of pilocarpine (320 mg/kg, i.p.) was used to induce status epilepticus (SE) in Sprague-Dawley rats (male, 200-300 g). At various times after the induction of SE, animals were sacrificed and processed immunocytochemically for c-fos expression. Analysis with light microscopy was performed to determine which areas showed c-fos expression. After 15 min of SE, c-fos expression could already be seen in neocortex (layer IV) as well as in the subicular complex, entorhinal cortex and striatum, but not in the hippocampus or any thalamic nuclei. After 1 or 2 h of SE, c-fos could be seen in the olfactory tubercle, diagonal band, supraoptic nucleus, amygdala, hippocampus, subicular complex, entorhinal cortex, piriform cortex, neocortex, striatum, medial geniculate and thalamus (paraventricularis, intermediodorsal, reticular, laterodorsal and rhomboid nuclei). Several areas showed remarkably preferential staining: layer II of entorhinal and piriform cortices; dentate gyrus and CA1; amygdaloid laterodorsal, central and basolateral anterior nuclei; and the marginal zone of medial geniculate. This selective pattern of c-fos expression in SE presumably indicates which brain areas are involved in seizure genesis and propagation, but c-fos expression may also reflect an adaptive process. Further studies can contribute to a more complete understanding of the mechanisms leading to a state of chronic epilepsy after pilocarpine-induced SE. Supported by NIH Grants NS 23074 and NS 16721, and FAPESP (Brazil).

200.16

CONSTANT HIGH DOSE PYRIDOXINE TREATMENT STARTED PRENATALLY INCREASES REGIONAL SEROTONIN LEVELS IN EPILEPSY-PRONE BALB/C MICE.
J. Vriend, A. H. Greenberg,*, N. A. Alexiuk* and S. Dolina*, Dept. of Anatomy, Univ. of Manitoba and Manitoba Inst. of Cell Biology, Winnipeg, MB, Canada R3E 0W3

Regional concentrations of serotonin (5HT) and 5HIAA were determined in extracts of brain tissue of selectively bred epilepsy-prone (EP) and epilepsy-resistant (ER) substrains of Balb/c mice. Significantly decreased levels of 5HT were found in cortex, but slightly increased levels of 5HT and 5HIAA were observed in brainstem of EP mice compared to ER, at 6 weeks of age. Constant pyridoxine treatment started prenatally (100 mg/L in the drinking water) increased significantly 5HT concentrations in hypothalamus and hippocampus compared to non-treated EP animals. The level of 5HT in their cortex was restored to 92% of the level observed in normal ER mice. In contrast similarly treated ER mice had decreased 5HT concentration in their cortex, without significant changes in 5HT levels in other brain structures. These data show that ER and EP mice are differentially sensitive to pyridoxine treatment. A disorder of pyridoxine dependent L-amino acid decarboxylase, susceptible to early correction by pyridoxine is considered as a possible mechanism for the genetically enhanced convulsibility in EP Balb/c mice.

200.17

EFFECT OF PRECOLLICULAR TRANSECTION ON AUDIOGENIC SEIZURES IN GENETICALLY EPILEPSY- PRONE RATS (GEPRs). R. A. Browning, C. Wang, and P.C. Jobe. Southern Illinois Univ. Sch. of Med., Carbondale, IL, 62901 and Univ. of Illinois Coll. of Med., Peoria, IL, 61656.

Previous studies have demonstrated that electroshock or pentylenetetrazol (PTZ)-induced seizures characterized by running-bouncing (R/B) clonus or tonic extensor convulsions occur as a result of seizure discharge in the brainstem (Browning & Nelson, Exp. Neurol. 93: 546, 1986). In order to test the hypothesis that audiogenic seizures (AGS) also emanate from the brainstem and do not require forebrain circuitry, we have examined the response of two strains of GEPR (GEPR-3s and GEPR-9s) to seizure-evoking auditory stimuli 3 hours after a precollicular transection or sham surgery performed under ether anesthesia. Following the transection, 50% of the GEPR-9s (N=8) displayed their typical tonic seizure in response to the usual auditory stimulus, and 50% showed the tonic seizure in response to sound after receiving a subconvulsant dose of PTZ (25 mg/kg,i.p.). In contrast only 20% of the GEPR-3s (N=5) displayed their usual R/B clonic seizure in response to auditory stimulation after the transection, but the remaining 80% became audiogenic seizure-sensitive after pretreatment with PTZ. All sham-operated rats exhibited their usual seizure in response to sound stimulation 3 hours after sham surgery. The effect of precollicular transection was also assessed in normal rats rendered sensitive to AGS by the infusion of NMDA into the inferior colliculus (IC, 10 nMoles, bilaterally). Three hours after transection all (N=5) the normal rats displayed audiogenic or audiogenic-like seizures after receiving an IC infusion of NMDA. These findings support the hypothesis that the anatomical substrate necessary for AGS resides within the brainstem. However, the present study also suggests that the threshold for sound-induced seizures is elevated 3 hours after a precollicular transection. Whether the latter is due to surgical trauma or the removal of a seizure-facilitating system in the forebrain is not known. The greater resistance of GEPR-3s to AGS after transection is consistent with known threshold differences between GEPR-3s and GEPR-9s (Browning et al., Epilepsy Res. 6: 1,1990).

200.18

Genetical analysis of neocortical-spike-and-wave patterns in inbred rat strains. R. Urioste, Z. Horváth, X-G. Li*, E. Pierre*, D. Vadi*, M. Hsu and C. Vadász[1], G. Buzsáki, Center for Neuroscience, Rutgers University, Newark, NJ 07102 and Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962[1]

Spontaneously occurring high voltage spike-and-wave spindle patterns (HVS) were examined in 6-month-old rats of the Fischer 344 (F344) and Brown Norway (BN) strains and their F1 hybrids. Neocortical activity and movement were monitored for 12 night hours in each rat and the analog data were digitized (100 Hz) and recorded on laser disks. HVS episodes, occurrence of movement and slow-wave periods (sleep, drowsiness) were detected by a software program. The intra-HVS frequency was significantly slower in F344 rats (7.4 Hz) than in the BN strain (8.1 Hz) and the F1 cross. HVS-associated tremor was very rare in F344 but frequent in BN and F1 rats. A significant sexual dimorphism in BN and F1 rats but not in F344 animals and marked strain differences in males were observed in the total duration of HVS during awake immobility. The incidence, average duration and total duration of HVS were significantly higher in F1 hybrids than in the parental strains. The results suggest that control of HVS involves genotype-dependent sex effects and genetic additive and dominance effects. Heterosis in F1 hybrids can be attributed to overdominance or dipersion of genes.

200.19

AUDIOGENIC SEIZURE SUSCEPTIBILITY IN WSP AND WSR MICE. D.J. Feller and C.A. Le Fevre*, VA Medical Center and Oregon Health Sciences University, Portland, OR 97201.

Mice have been genetically selected for susceptibility (WSP) and resistance (WSR) to handling-induced convulsions during withdrawal from chronic ethanol treatment. The neurochemical basis for this difference is unknown. We have shown that hippocampal mossy fiber zinc is decreased in WSP compared to WSR mice. Reduced hippocampal mossy fiber zinc has also been found in rats predisposed to audiogenic seizures. These observations led us to test naive WSP and WSR mice for audiogenic seizures. Mice were tested using a 121 dB sound source at ages 17, 22, 28 and >70 days. Mice were exposed to the bell for 60 sec or until a response was observed. WSR mice did not respond at any age to audiogenic stimulation, while WSP mice displayed peak susceptibility at age 22 (58%). Percentages for each seizure category were: wild running, 18%; spasms, 8%; clonic, 3%; tonic, 3%; lethal, 26%. WSP mice did not have audiogenic seizures if tested at >70 days. However, exposure to 121 dB stimulation increased handling-induced convulsion scores above baseline levels (4.2 compared to 1.0). Differences between WSR and WSP mice in susceptibility to audiogenic seizures and increased handling-induced convulsions after a 121dB stimulus are correlated responses to selection. Therefore, these traits may share some common genetic basis with ethanol withdrawal seizures, and may thus be regulated to some extent by a similar neurochemical mechanism which may involve hippocampal mossy fiber zinc.

These studies were supported by a grant from the VA Medical Center.

EPILEPSY: BASIC MECHANISMS II

201.1

LIMBIC STATUS EPILEPTICUS IN THE RAT REQUIRES ACTIVATION OF THE BASOLATERAL AMYGDALA. L.E. White and J.L. Price. Dept. Anat. & Neurobiol., Washington Univ., St. Louis, MO 63110.

Limbic status epilepticus was induced in adult albino rats by 40 min of continuous electrical stimulation of either the basolateral amygdala, the anterior olfactory nucleus, or the anterior piriform cortex (White and Price, 1990, Proc. Soc. Neurosci. *16*:281). In each experiment, one of four types of limbic status was induced; of these, Types II and III were encountered most frequently (25% and 60%, respectively). Type II status is characterized by discontinuous locomotor activity and low frequency (1-2 Hz) sharp waves observed in depth electroencephalographic recordings. Type III status includes all of these features, with recurrent episodes of facial and forelimb clonus during discrete segments of high frequency (10-20 Hz) activity. [^{14}C]2-deoxyglucose autoradiography and Fos immunocytochemistry revealed that the anatomical substrates of both types of status include the basolateral amygdala, and its efferent targets. Type II status also involved restricted parts of the olfactory cortex, but not the piriform cortex. Type III status involved the entire amygdala and olfactory cortex, the insular cortex, the ventral striatum, the ventral hippocampus, and the substantia nigra.

The possibility that activation of the basolateral amygdala is required for the expression of limbic status was investigated by acutely injecting muscimol or lidocaine into this region during Type II or Type III status. Deactivation of the basolateral amygdala resulted in the complete cessation of Type II or Type III status. For each type of status, [^{14}C]2-deoxyglucose autoradiography revealed that the entire anatomical substrate was deactivated, except for the amygdalohippocampal area. These findings implicate the basolateral amygdala as a primary generator of limbic status epilepticus activity. Supported by NIH DC00093.

201.2

ANATOMICAL CONNECTIONS OF THE AREA TEMPESTAS IN THE RAT. N. Sahibzada[1], W. Chen[1], T. Halonen[2] and K. Gale[2]. [1]Dept. of Psychology, Univ. District of Columbia and [2]Dept. of Pharmacology, Georgetown Univ. Medical Center, Washington, DC.

The area tempestas (AT), a deep prepiriform cortical site, has been demonstrated to be involved in the generation of limbic seizures in the rat. Microinjection of GABA antagonists or glutamate agonists into the AT elicit seizures that are characterized by facial and forelimb clonus accompanied by rearing and falling. The AT, as functionally defined, is highly discrete, with a radius of approximately 1mm. In order to characterize afferent and efferent projections of the region containing the AT, we applied fluorescent tracers Fluoro-Gold and Fluoro-Ruby (a few animals received WGA-HRP) into this site. Bicuculline methiodide (60ng) was included in the tracer infusions in order to functionally verify the accuracy of the infusion site. Animals exhibiting convulsive seizures within 3 min of the the infusion were considered to have correctly placed cannulae.

After a period of 2-14 days the animals were perfused, their brains removed and sectioned. Brains injected with WGA-HRP were processed according to the TMB protocol of Mesulam (1978). Our results show that the AT receives afferent projections from a number of structures which include: dorsal tegmental nucleus, piriform cortex, entorhinal cortex, olfactory bulb, midline thalamic nuclei, pedunculopontine tegmental nucleus, locus coeruleus and amagydaloid nuclear complex. Several of these structures also appear to be reciprocally innervated by AT. Based on these initial findings, we are verifying efferent targets of AT by placing retrograde tracers in those targets in order to identify cells of origin in AT.

Supported by HHS grant #NS28130.

201.3

GLUTAMATE RECEPTORS IN PIRIFORM CORTEX REGULATE SUSCEPTIBILITY TO SEIZURES EVOKED FROM PREPIRIFORM CORTEX. T. Halonen, H. Zrebeet, K. Gale. Department of Pharmacology, Georgetown University Medical Center, Washington, D.C., 20007.

The area tempestas (AT) is an epileptogenic site within the prepiriform cortex from which bilateral limbic motor seizures can be evoked with unilateral application of GABA antagonists or glutamate agonists. In the present study, we evaluated the functional relationship between the AT and a region of the posterior piriform cortex (PC) (6-7mm caudal to the level of AT) in which strong increases in immediate early gene expression and glucose metabolism occur in response to seizures evoked from AT.

Seizures were evoked by the unilateral application of bicuculline methiodide (BIC) (118 pmol in 120 nl) into AT. Glutamate antagonists were applied unilaterally to the caudal PC (lateral to amygdala) of the same hemisphere as the AT injection, 5 min prior to BIC in AT. Antagonists selective for NMDA-sensitive receptors only partially attenuated the AT-evoked seizures, with relatively high doses of CPP (10 nmol or more) or AP7 (20 nmol or more) required for obtaining a significant protective effect. Kynurenic acid, a relatively low potency antagonist of both NDMA and non-NMDA receptor subtypes, was effective in completely blocking AT-evoked seizures when applied into PC at a dose of 100 nmol. These observations suggest that the homolateral posterior PC may be a critical initial target for the relay of propagated seizures triggered from AT and that non-NMDA receptors, in addition to NMDA receptors, are important mediators of this activity. Studies with focal application of selective antagonists of non-NMDA receptors will allow us to determine the relative contribution of the various glutamate receptor subtypes in PC for propagation of the AT-evoked seizures.

Supported by HHS grant #NS28130 and fellowship #NS08750.

201.4

EFFECT OF LIDOCAINE HYDROCHLORIDE MICROINFUSED INTO THE PONTINE RETICULAR FORMATION ON MAXIMAL ELECTROSHOCK SEIZURES IN THE RAT. M.L. Maring, D.C. Smith[1], and R.A. Browning. Depts. of Physiology and [1]Psychology, School of Medicine, Southern Illinois University, Carbondale, IL 62901.

The pontine reticular formation (PRF) has been implicated in the expression of generalized tonic seizures. Specifically, bilateral lesions of the pontine tegmentum involving the superior cerebellar peduncles and the nucleus reticularis pontis oralis (RPO) have been shown to attenuate the tonic components of generalized seizures induced by maximal electroshock (MES), pentylenetetrazol and sound stimulation (audiogenic) (Browning et al., *Epilepsia* 22:583, 1981). While attenuation of tonic seizures following lesions of the RPO indicates a critical role of this structure in the expression of such seizures, it is possible that neurophysiological changes may have occurred during the post-surgical recuperative period, prior to seizure testing. In order to confirm the observed effects of RPO lesions on tonic seizures, we have investigated the effects of reversible inactivation of the RPO on MES. Reversible inactivation of the RPO was accomplished through the use of microinfusions of lidocaine HCl, a local anesthetic which produces reversible neuronal inactivation via a voltage-dependent blockade of sodium channels. Sprague-Dawley rats were pretested to ensure the presence of full hindlimb extension (HLE) in response to MES. The rats were bilaterally implanted with epidural 23 gauge guide cannulae directed at the RPO. One week after implantation the rats were infused with lidocaine HCl (1 µl of 2%, 0.5 µl of 5% or 1 µl of 5%) or saline bilaterally, and ten minutes later subjected to transcorneal MES (0.2 sec, 150 mA). While lidocaine HCl, in the doses employed, failed to alter the latency to or duration of HLE (in those displaying HLE) compared to vehicle-infused controls, a reduction in the incidence of HLE was observed. Bilateral infusions of 1 µl of 2%, 0.5 µl of 5%, or 1 µl of 5% lidocaine HCl reduced the incidence of full HLE in 22% (N=9, p= .13), 38% (N=8, p= .05) and 75% (N=4, p= .03) of the rats tested, respectively. These findings support the proposed critical role of the RPO nucleus in the expression of tonic seizures.

201.5

AUDIOGENIC SEIZURE SENSITIZATION BY UNILATERAL SUBSTANTIA NIGRA LESIONS IN RESISTANT WISTAR RATS **N. Garcia-Cairasco and M.C. Doretto. Department of Physiology, School of Medicine of Ribeirao Preto, University of Sao Paulo, 14049, Ribeirao Preto - SP - Brazil.**

Lesions or neurochemical manipulations of substantia nigra reticulata (SNR) have shown that this structure is differentially involved in control of threshold and seizure spreading in several epilepsy models. Muscimol and excitatory amino acid antagonists block audiogenic seizures (AS) in innately susceptible (S) rats and unilateral SNR lesions can sensitize resistant (R) rats to AS. In order to evaluate the participation of different nigral sites in these effects, we made a series of small (5 mCoulombs; n=28), medium (10mC; n=57) and large (15mC; 3 points of 5 mC each; n=19) unilateral electrolytic SNR lesions in R rats. Animals were evaluated at 5, 10 and 15 days post-surgery. Bilateral SNR lesions (15 mC type) were made in S animals and evaluated at 1, 3, 10 and 15 days post-surgery. The behavior was evaluated using a neuroethological method (Garcia Cairasco and Sabbatini,Braz.Med. Biol. Res. 16:171-181, 1983). Small unilateral lesions induced AS susceptibility in 17% of R animals with 5% of them displaying tonic-clonic AS. Medium size lesions induced AS susceptibility in 50% of the animals with 18% of these exhibiting tonic-clonic seizures similar to those displayed by naturally S animals but with predominance of wild running (gyri) contralateral to the lesioned SNR. The AS severity was higher at day 5 post-surgery, decreasing at days 10 and 15. Large SNR unilateral lesions destroying the whole SNR failed to cause tonic/clonic seizures, although procursive behaviors (giry, jumping and atonic falling) occur in 10% of the animals. Bilateral large SNR lesions did not modify AS in S animals, only inducing a statistically significant increase in the AS latency. The present data suggest: 1) The AS severity after SNR lesions is not a linear function of the lesion size; 2) Functionally different and antagonistic AS related substrates may exist in the SNR; 3) Neurochemical and hodological characterization of these areas should be important for a better understanding of their role in AS.Financial support: Capes (PICD), Brazil.

201.6

DESTRUCTION OF DENTATE GRANULE CELLS PROLONGS HIPPOCAMPAL ELECTRICAL AFTER-DISCHARGE BUT NOT THE RATE OF KINDLING ELICITED BY STIMULATION OF THE PERFORANT PATH. C. L. Mitchell and M. I. Barnes*. LMIN/NIEHS/NIH, Research Triangle Park, NC 27709.

Colchicine (C) induced destruction of dentate granule cells (DGC) lowers the threshold for behavioral seizures elicited by stimulation of the perforant path (PPS) (Barnes and Mitchell, FASEB J. 5:A211, 1991). In contrast, kindling induced by stimulation of the entorhinal cortex is delayed by destruction of DGC (Frush et al. Exp. Neurol. 92:92, 1986). We were interested, therefore, in determining the effect of destruction of DGC on kindling induced by PPS. Rats were implanted with stimulating electrodes in the perforant path and recording electrodes in the dorsal hippocampal formation (HF). Kindling commenced 8 weeks after bilateral destruction of DGC in the dorsal and ventral HF. Destruction of DGC had no effect on the threshold for after-discharge (AD) before or after kindling. Neither did it affect the number of stimulations required to attain kindling. However, the duration of AD was significantly longer (2-3x) in C lesioned animals compared to those receiving artificial cerebrospinal fluid. This was true for the threshold for AD, the AD at the first kindling trial and the last kindling trial. These data suggest that DGC may inhibit the duration of AD induced by PPS or that other post lesion changes occur which result in a prolongation of the AD.

201.7

ANALYSIS OF NEUROPEPTIDE mRNA EXPRESSION AT DIFFERENT STAGES OF AMYGDALA KINDLING. J.B. Rosen, C.J. Cain*, S.R.B. Weiss, R.M. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20982.

In situ hybridization was used to assess the spatiotemporal changes in neuropeptide mRNA during the evolution of amygdala kindling. Twenty-four hrs following a stage 1 or stage 5 amygdala kindled seizure or 2 weeks following a stage 5 seizure, autoradiograms with 35-S labeled oligonucleotide probes to mRNA of somatostatin (SOM), enkephalin (ENK), dynorphin (DYN) and thyrotropin releasing hormone (TRH) were analyzed by computer-assisted image analysis.

Consistent increases in SOM mRNA in the retrosplenial and fronto-parietal cortices were seen only after stage 1 seizures. ENK mRNA was increased in the pyriform cortex ipsilateral to the stimulation and bilaterally in the entorhinal cortex 24 hr after both stage 1 and 5 seizures. There was no change in DYN mRNA at stage 1. However, DYN mRNA in the dentate gyrus showed a decrease 24 hr (3 of 4 rats) and two weeks after a stage 5 seizure (3 of 5 rats). TRH mRNA was expressed ipsilateral to the stimulation in the pyriform and entorhinal cortices after stage 1, and ipsilateral or bilateral after 24 hr after stage 5. Dentate gyrus TRH mRNA levels were inconsistently increased after stage 1 but were increased bilaterally 24 hr after stage 5. Our results demonstrate both transient and more persistent alterations in regional expression of neuropeptide mRNAs which progressively evolve during the process of kindling.

201.8

DECREASED INHIBITORY NEUROTRANSMISSION IN THE AMYGDALA-KINDLED RAT: A POTENTIAL MECHANISM OF EPILEPTOGENESIS. K.M. Ryder*, W.F. Collins Jr., D.D. Spencer, and M.J. During. Dept. of Surgery, Yale Sch. of Med., New Haven, CT 06510.

An imbalance of excitatory and inhibitory influences, favoring excitation, may underly the initiation and spread of epileptiform discharge. Potassium stimulated release of GABA from slices in animal models of epilepsy has not conclusively demonstrated a depressed GABA response, and GABA release in response to glutamate (GLU) stimulation has not been studied. In normal brain tissue, GLU-induced GABA release is not calcium-dependent and is thought to involve reversal of the GABA transporter. We used microdialysis to investigate GABA release in response to potassium and GLU perfusion in both hippocampi of freely-moving amygdala-kindled rats. One week after the last seizure, dialysis probes were implanted; the probes were perfused with artificial extracellular fluid the following day. The perfusate was analyzed for GABA concentration by HPLC. After baseline collections, either 56mM potassium or 5mM GLU (pH 7.4) was perfused through the probe. GABA release in response to potassium perfusion was not attenuated in kindled rats, while GLU-induced release was significantly lower. GLU-induced GABA release is not blocked by the absence of calcium, while it is attenuated after 1 hour preincubation with nipecotic acid, a GABA transporter inhibitor. These *in vivo* studies demonstrate that GLU-induced GABA release is not calcium-dependent in the rat hippocampus, and that there is a depressed GABA response to GLU perfusion in kindled rats.

201.9

INDUCTION OF VIMENTIN IMMUNOREACTIVITY IN THE BRAINS OF AMYGDALA KINDLED RATS. G.O. Ivy, M. Khurgel and R.J. Racine. Division of Life Sciences., Univ. of Toronto at Scarborough, Ont., M1C 1A4 and Department of Psychology, McMaster Univ., Hamilton, Ont. L8S 1B9.

Increased GFA content in astrocytes has been shown to accompany the establishment of the kindled state. We now report that vimentin (VIM), a functionally different cytoskeletal protein, accumulates in certain astrocytes following the development of kindling. The brains of amygdala kindled rats and electrode implanted control rats were processed for immunoreactivity (IR) to anti-VIM, anti-GFA and anti-BrdU (a marker of cell proliferation). While no VIM-IR was present in astrocytes of control animals, anomalous VIM-IR was detected in astrocytes in the amygdala-pyriform area as well as in hippocampus of kindled animals. Also, the extent of VIM-IR appeared to correlate well with both levels of afterdischarges and behavioral measures of seizures. There was a parallel, though much more extensive, increase in GFA-IR in these brain regions. No astrocyte proliferation was seen one week following the development of kindling. These results demonstrate that astrocytes differentially modify the composition of their cytoskeleton in response to abnormal levels of neuronal activity.

This research was supported by NSERC.

201.10

MODULATION OF POSTSEIZURE REFRACTORY PERIOD BY THE ADENOSINE SYSTEM IN AMYGDALA KINDLED RATS. **R. J. Kleinsorge*[1], M. F. Jarvis[2], and R. F. Berman[1]. [1]Dept. of Psychology, Wayne State Univ., Detroit, MI. 48202, [2]Rhone-Poulenc Rorer Central Research, Horsham, PA 19044.**

"Kindled" seizures can be produced in rats by giving low-intensity electrical stimulation to the amygdala. Kindled animals are typically refractory to further seizures for a short time following the initial seizure. The hypothesis that the refractory period is due, in part, to the activation of the brain adenosine system following a seizure was tested. Rats were electrically stimulated for 1 sec, once per day, through an electrode stereotaxically implanted into the amygdala until fully generalized kindled seizures were produced. Trials of paired stimulations, separated by increasing time intervals out to 25 min, were then given to determine the refractory period. Adenosine receptors were then up-regulated by injecting animals for14 days with theophylline (75 mg/kg/day). Seizure severity at the end of the previously determined refractory period was then tested. Chronic theophylline treatment significantly increased [^{3}H]-cyclohexyladenosine binding in thalamus, hippocampus and cerebellum. In addition, the seizure refractory period was significantly shortened, although the response to the initial stimulation was unchanged. The effect of receptor modification lasted about nine days after the last theophylline injection. These results support a role for adenosine in the kindled-seizure refractory period. (Supported by NIH Grant GM 08167).

201.11

RELATIONSHIP BETWEEN MAXIMAL DENTATE ACTIVATION AND AFTERDISCHARGE PRODUCTION IN THE ANESTHETIZED RAT. J.L. Stringer and E.W. Lothman. Dept. Neurology, Univ. Va. Med. Ctr., Charlottesville, Va. 22908.

Recently, a phenomenon has been described in the dentate gyrus, termed maximal dentate activation, which is defined by the appearance of bursts of large amplitude population spikes associated with a negative shift of the DC potential and a secondary rise of the extracellular potassium level. Previous work has linked maximal dentate activation to kindling of afterdischarges, either when they are elicited in the hippocampus or outside of the hippocampus in the amygdala. Recording bilaterally in the dentate gyrus, it was found that maximal dentate activation occurred on both sides, with the side ipsilateral to the stimulus (either CA3 or angular bundle) being activated first. An afterdischarge did not appear unless bilateral maximal dentate activation had occurred. With repeated stimulation, the time to onset of maximal dentate activation on the two sides of the brain became nearly equal. This was associated with the appearance of afterdischarges. However, complete synchronization of the onset of maximal dentate activation was not necessary for afterdischarge production. Maximal dentate activation and afterdischarges could be readily elicited in rats in whom the hippocampal commissures had been cut. It appears that, in the intact brain, the lack of maximal dentate activation on one side of the brain can function as a "brake" for epileptic activity, preventing afterdischarges. Once this brake is removed, by cutting the hippocampal commissures or by initiating maximal dentate activation, the dentate gyrus readily expresses afterdischarges.

201.12

EPILEPTIFORM POTENTIALS IN SLICES OF PIRIFORM CORTEX FROM KINDLED RATS ORIGINATE IN DEEP STRUCTURES. W.H.Hoffman* and L.B.Haberly. Neuroscience Training Program and Dept. of Anatomy, Univ. of Wisconsin, Madison WI 53706

In previous studies from this laboratory it has been shown that bursting activity evoked by a variety of manipulations in slices of piriform cortex induces persistent epileptiform EPSPs by an NMDA dependent process. These epileptiform responses have recently been shown to be driven by deep cells concentrated in the endopiriform nucleus and adjacent structures that underlie the piriform cortex (Hoffman & Haberly, J. Neurosci., in press). Since similar epileptiform responses occur in the piriform cortex of kindled rats *in vivo* (Racine et al, Br. Res. 322:101; Russell & Stripling, Br. Res. 361:61), the present experiments were undertaken to determine if kindled epileptiform activity in slices of piriform cortex also originates in deep cells. Rats were kindled by standard methods through bipolar electrodes chronically implanted in the olfactory bulb. Experiments were performed on 7 rats that had undergone 30 to 82 class 5 seizures and 3 littermate controls that had received low rate stimulation. Slices were prepared 1 to 50 days after the last seizure. Long latency epileptiform (all-or-none) components were observed in intracellular and extracellular responses to stimulation of excitatory fiber systems in standard bathing medium in 6/7 kindled rats. These epileptiform components closely resembled those induced in slices from normal rats by bursting activity. No epileptiform activity was observed in implanted or normal controls. Three observations suggest that these responses originated in deep cells: (a) they were typically high in amplitude and suprathreshold in cells in the endopiriform nucleus vs small and subthreshold in superficial pyramidal cells, (b) local injection of glutamate and KCl through micropipettes evoked epileptiform potentials only in the endopiriform nucleus and adjacent claustrum, and (c) local injection of Co^{2+} blocked epileptiform EPSPs only from the endopiriform nucleus and adjacent portion of the claustrum. These results provide further evidence that cells at the deep boundary of piriform cortex play an important role in epileptogenesis and suggest that studies of epileptiform activity induced by bursting in vitro may have implications for kindled epileptogenesis. Supported by grant NS19865 from NINDS.

201.13

ELECTROPHYSIOLOGY OF PAIR-PULSE RESPONSES IN HIPPOCAMPAL CA1 IN VITRO. Xiao-Wen Fu, L. Stan Leung and D. Zhao*. Depts. Clin. Neurol. Sci. and Physiology, Univ. Western Ontario, London, Ontario N6A 5A5 Canada.

Previous studies in our laboratory indicate that the pair-pulse response in the hippocampal CA1 is facilitated by kindling and modulated by behavior. The electrophysiology of the pair-pulse response evoked by str. radiatum stimulation in hippocampal CA1 in vitro was studied here. Both field and intracellular data indicated that the excitatory postsynaptic potentials (EPSPs) in pyramidal cells were facilitated by about 20-30% at an interpulse interval (IPI) of 30-100 ms. Pair-pulse facilitation of the population spike varied from >200% at low stimulus intensity to about 20% at high intensity, the maximal facilitation also occurred at 30-100 ms IPI. Bath perfusion of bicuculline methiodide (25-50 μM) significantly increased the pair-pulse index of the intracellular EPSPs (EPI= slope of 2nd EPSP/ slope of 1st EPSP) to >1.5 at 40-90 ms IPI, as did perfusion of 8mM $[K^+]_o$. Perfusion of 10mM $[Mg^{2+}]_o$ reduced the EPI to near unity at all IPIs, with or without bicuculline. High Mg^{2+} may suppress pair-pulse facilitation by suppressing a presynaptic Ca^{2+} current; thus, the effect of bicuculline on the EPI may be presynaptic, though postsynaptic effects have not been totally excluded. The contribution of the spike afterhyperpolarization (AHP) was studied by stimulation at intensities that sometimes evoked a first spike and sometimes not. The effect of the AHP on the EPSP and the latency and amplitude of the spike evoked by the 2nd pulse at 30-200 ms IPI was small. Putative glial cell recordings indicated that even at submaximal stimulus intensities, a single afferent pulse evoked depolarization of 2-5 mV suggesting that $[K^+]_o$ increased by 0.5-1 mM, which may facilitate the spike response to the 2nd pulse. In summary, bicuculline or high $[K^+]_o$ (or kindling) enhances the pair-pulse facilitation of the EPSP, possibly through suppression of presynaptic GABA-A inhibition. (Supported by NSERC and NS25383).

201.14

EFFECTS OF ANTICONVULSANTS AND NMDA ANTAGONISTS ON HIPPOCAMPAL LTP AND KINDLING-INDUCED PLASTICITY. L. Stan Leung and B. Shen* Depts. Clin. Neurol. Sci. and Physiology, Univ. Western Ontario, London, Ontario N6A 5A5 Canada.

Recently, we found a robust long-term potentiation (LTP) of the commissurally activated basal-dendritic synapse on CA1 pyramidal cells in freely moving rats (Leung, Neurosci Abstr 16:652). Here, we studied the effects of 2 NMDA antagonists APV and MK-801 and 2 anticonvulsants (phenytoin, U54494A) on the LTP induced by 2 types of tetanic stimulations in chronically implanted rats: (1)Primed bursts (PB: 8 bursts, each of 1 pulse followed 190ms later by 10 pulses at 100 Hz): no afterdischarges(ADs) were evoked. (2) 1-s, 200 Hz high-frequency tetanus followed by an AD (HF). Counter-balanced, repeated tetani were given. In undrugged rats, LTP was evoked by the PB; a smaller LTP was evoked after an AD (of >15s duration) by HF, preceded by 5-30 min depression. Intraventricular (icv) 20μg D,L-APV blocked the LTP induced by PB or HF ($P<0.0001$;ANOVA), but had no effect on the AD duration or the early depression following an AD. At 30 min post-PB, LTP after APV was 1.26 $\pm$ 0.22 times the baseline population EPSP slope values, vs 2.09 $\pm$ 0.36 after icv saline injections. MK-801 (0.5mg/kg ip) had a weaker (but significant) effect on blocking LTP. U54494A (50mg/kg ip) and phenytoin (40mg/kg ip) had no effect on the PB-induced LTP. However, U54494A and phenytoin attenuated the AD duration and depression induced by HF, thus apparently enhancing the HF-induced LTP. In conclusion, (1) LTP following a PB or HF (with AD) in behaving rats was blocked by NMDA antagonists. (2) A long-term depression and an even more persistent LTP followed an AD (evoked by HF). (3) U54494A and phenytoin had no effect on the LTP induced by PB, but they enhanced the LTP following a HF perhaps by partially blocking the AD and the AD-induced depression. Supported by UpJohn, NS-25383 and NSERC.

201.15

KINDLING IN THE PERIRHINAL CORTEX. I. KINDLING RATE AND SEIZURE CHARACTERISTICS. M. E. Kelly, J. N. Armstrong and D. C. McIntyre. Department of Psychology, Carleton Univeristy, Ottawa, Ontario K1S 5B6.

Evidence suggests that the pyriform cortex (PC) plays an important role in the genesis and/or maintainence of secondarily generalized limbic-kindled seizures. In *in vitro* experiments, we have shown that the PC and overlying perirhinal cortex possess a strong disposition for developing spontaneous rhythmic burst discharges, and that the relationship between these two areas changes as a result of previous *in vivo* amygdala kindling.

Since the PC manifests rates of kindling faster than any other structure in the limbic system, we compared the PC, amygdala and perirhinal cortex for their respective rates of kindling. The perirhinal cortex kindles faster than the PC and amygdala. Further, the latency to onset of forelimb clonus was much shorter in the perirhinal subjects. These findings suggest that the perirhinal cortex may be more intimate with the mechanisms of secondary generalization than the PC or amygdala.

201.16

Regulation of α-CaM Kinase II Gene Expression in Kindling. J.B. Perlin, E.R. Jakol*, C.M. Gerwin*, and R.J. DeLorenzo. Depts of Neurology and Pharmacology, Medical College of Virginia, Richmond VA 23298-0599.

The Rapidly Recurring Hippocampal Seizure (RRHS) or 'rapid kindling' model was used to explore regulation of the activity of type II calcium/calmodulin-dependent protein kinase (CKII). In crude synaptic plasma membrane, CKII activity was found to be 56% inhibited 6 weeks following administration of the kindling paradigm. Limited inhibition of CKII was also found in this same fraction immediately after kindling. Diminished activity could not be accounted for by loss of the enzyme itself as CKII was present on polyacrylamide gels in equal proportion to kinase from naive and surgical control rats. In addition, biotinylated calmodulin binding to CKII was not reduced compared to either control group suggesting that neither proteolysis nor loss of calmodulin-binding capacity had occurred.

To assess pretranslational regulation of CKII expression, steady state levels of mRNA encoding CKII were measured by slot hybridization assays. Blots were probed with an oligonucleotide complementary to the 3' noncoding tail of the α-subunit of CKII (c.f. Burgin et al, J. Neurosci. 10:6(1990):1788-98). An increase in mRNA for the α-subunit of CKII was observed following electrically-induced hippocampal seizures in both non-kindled and kindled animals, suggesting that a transient increase in CKII transcription occurs following limbic seizure. However, long-term expression of α-CKII message (6 weeks) after kindling was not different from either control group. Thus, sustained alterations in CKII activity following kindling arise at the level of post-translational modification.

201.17

TIME COURSE OF SELECTIVE NEURONAL DAMAGE AFTER STATUS EPILEPTICUS (SE) TRIGGERED FROM A KINDLED AMYGDALA FOCUS: A SILVER IMPREGNATION STUDY. L.E. Markert, D. C. McIntyre, M. E. Kelly and D. C. S. Roberts. Department of Psychology, Carleton Univeristy, Ottawa, Ontario K1S 5B6.

After development of 6 generalized kindled seizures, rats were exposed to amygdala stimulation triggering either partial or generalized SE. The time course of brain damage was studied using Nadler and Evenson's silver staining method after 2, 6, 24, or 72 hrs of partial SE, or 2 hrs of generalized SE.

Six hrs of partial SE produced argyrophilic cells in dorsomedial thalamus, endopyriform n., pyriform and perirhinal cortex ipsilateral, but not contralateral, to the kindled amygdala. Bilateral damage appeared in reuniens n. and, unexpectedly, bed n. of stria terminalis. No hippocampal damage was observed after 24 hrs, when ipsilateral CA1 showed darkened nucleoli and, within 3 days, argyrophilia. Novel fibers were seen in thalamus and cerebral peduncles, but not substantia nigra reticulata (SNR). On the other hand, generalized SE caused extensive argryophilia bilaterally in neocortex, and massive silver granules in SNR.

201.18

EFFECT OF CHRONIC NALOXONE TREATMENT ON BENZODIAZEPINE RECEPTOR LEVELS IN NORMAL AND KINDLED RATS. L. Rocha, J. Engel Jr., H.T. Chugani, K. Tatsukawa * and R.F. Ackermann. UCLA School of Medicine, Los Angeles, CA 90024.

We found that chronic naloxone pretreatment facilitates amygdaloid kindling and enhances postictal depression. Benzodiazepine (BZ) receptors were studied in naloxone pretreated rats (NA) (n=3), kindled rats with naloxone (N+K) (n=3) or saline (S+K) (n=3) pretreatment and control saline pretreated rats (S) (n=3). Stimulation electrodes were implanted in anterior amygdala. Animals received chronic treatment (14 d) with osmotic minipumps s.c. containing either saline or naloxone solution (the delivery rate was 75 µg/h). S and NA rats were sacrificed 48 h after completion of treatment. After the minipumps were withdrawn, S+K and N+K received daily amygdaloid kindling trials until they experienced 5 stage V seizures or 30 kindling trials. They were sacrificed 24 h after the last stimulation. BZ binding sites were labeled with 3H-flunitrazepam (2 nM) and their levels were determined by quantitative autoradiography. Compared to S group, NA group presented 90% increase of BZ receptors in substantia nigra; S+K animals showed an enhancement of 78% in dentate gyrus (78%), whereas N+K rats presented 81% and 130% increase in dentate gyrus and substantia nigra respectively. These data show that chronic naloxone treatment alters BZ receptor binding in substantia nigra, an effect that persists during the kindling process possibly contributing to the seizure suppressive effect.

201.19

PENTYLENETETRAZOLE (PTZ) KINDLING IN DIAZEPAM-SENSITIVE AND -RESISTANT MICE, M.C. Anderson and E.J. Gallaher. Depts. of Medical Psychology & Pharmacology, Oregon Health Sciences University and Portland Veteran's Administration Medical Center, Portland, OR. 97201

In this study we induced chemical kindling in diazepam-sensitive and -resistant (DS & DR) lines of mice by daily administration of pentylenetetrazole (PTZ). These mice were derived from heterogeneous stock (HS/Ibg) mice in our laboratory, and were initially selected for differential sensitivity to sedative effects of diazepam. They have subsequently been tested for a number of behavioral and neuro- chemical parameters. DS mice are 15 fold more sensitive to motor impairment produced by diazepam as compared to DR mice, but show no differences to anti-convulsant properties of diazepam or in acute response to chemical convulsants acting at the GABA-benzodiazepine binding site (Gionet & Gallaher, 1989, Soc. Neurosci. Abst. 15:668). In the current study, DS and DR mice were treated daily with 35 mg/kg PTZ to induce sensitivity to the convulsant activity of PTZ (PTZ-kindling). Mice were monitored daily following PTZ treatment for the presence of myoclonic jerks, mild clonic seizures characterized by facial clonus and rearing, tonic-clonic hindlimb extensor seizures and death due to *status epilepticus*. At the end of 1 week, DR mice were fully kindled and showed a decreased threshold to tonic-clonic hindlimb extensor seizures induced by intravenous PTZ, in addition, 64% died in *status epilepticus*. DS mice failed to show a kindled response. Pilot EEG studies showed that kindled DR mice displayed intermittent spiking 24 hours following treatment with PTZ. These data suggest differences in the underlying functional mechanisms, possibly GABAergic, governing convulsive behavior between DS and DR lines of mice.
Research supported by NIAAA Grant # 5T32 AA07468-04, NINCDS Grant # 23927 and VA Medical Research Service.

TEACHING OF NEUROSCIENCE: COURSES AND PROGRAMS

202.1

ORGANIZATION OF AN INTERDISCIPLINARY INSTITUTE FOR NEUROSCIENCE AT NORTHWESTERN UNIVERSITY. P.E. Meyers. Institute for Neuroscience and Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

During the planning phases of a graduate program for a large interdisciplinary institute for neuroscience at Northwestern University we sought to satisfy a number of objectives; 1) identify and present a common core of information to all students, 2) provide the benefits of both interdisciplinary and departmental programs, 3) provide a broad exposure to research in neuroscience, and 4) promote a sense of community among the members of the institute and rapidly incorporate new students into the community. The institute is dispersed over two totally separate campuses and includes 88 faculty in several schools and departments.

The salient features of this program include: all students take a series of common core courses, students become affiliated with individual departments and satisfy modest course requirements for that department, students are exposed to the research of institute members through seminars and special events, students conduct at least brief research projects on both campuses of the University, and the institute promotes a number of special events that combine social and scientific opportunities for all of its members.

After two years of experience with this program, we are reviewing our success, and invite discussion.

202.2

THE NEUROBIOLOGY OF DISEASE: A GRADUATE COURSE FOCUSSING ON HUMAN NERVOUS SYSTEM DISEASES AND DISORDERS. *E.A. Kravitz and W.J. Koroshetz, Depts. of Neurobiology and Neurology, Harvard Medical School and MGH, Boston, MA 02115.* Aimed at graduate students, MD/PhD, MD students and advanced undergraduates, this course is designed to introduce students to the diseases and disorders of the nervous system. The course involves patient presentations, clinical and basic science core lectures, literature readings, student seminar presentations and general discussion of each topic. The course meets two evenings a week and a major disease or disorder is covered each week. Topics covered in a typical year include Muscular Dystrophy, Myasthenia Gravis and Myasthenic Syndrome, Multiple Sclerosis, Huntington's, Parkinson's and Alzheimer's Diseases, Amyotrophic Lateral Sclerosis, Epilepsy, Chronic Pain, Viral Attack on the Nervous System, Stroke and Affective Disorders. Approximately 20 students register for the course and between 20 and 30 regularly attend. Each week the first session begins with a patient presentation by a clinician, who is told to address his/her remarks to non-specialists. Clinical and basic science core lectures finish these sessions. The second session each week is reserved for student presentations of assigned readings and general discussion. The goal of the course is to educate students about the diseases and disorders of man, to show them in a clear and direct way that the fundamental research they are performing is relevant to the problems of mankind, and to leave them prepared to make extrapolations between what they are doing and the important unsolved diseases of man.

202.3

USE OF CLINICAL CORRELATIONS IN THE TEACHING OF NEUROSCIENCE. M.E.Melnick. Dept of Physical Therapy. Wayne State Univ., Detroit, MI 48202.

Students in a professional program consistently desire clinical information while learning basic science courses. Case histories can be used in many ways to enhance learning of neuroanatomy. Traditionally, lesion questions have been used in which the student must detail the specific signs and symptoms expected with a variety of lesions or a detailed evaluation of a patient is presented and the student must then decide upon the probable localization of the lesion. Use of CT and PET scans as well as MRIs are often more effective than diagrammatic pictures. These applications can be taken a step further and incorporate clinical decision making and evaluation techniques with the basic science. The student is given a detailed description of the patient as they are sitting in the waiting room and as they move to the treatment room. From this description the student must begin a problem solving approach that requires a listing of the problem(s), the possible tracts or area(s) of the nervous system that could be involved and the methods they would use to determine which of their possibilities is most correct. Students have found this last approach challenging, helpful in integrating the material.

202.4

•WHY EXCELLENT STUDENTS MISS EASY QUESTIONS: THE PROBLEM OF SYNONYMS AND MULTIPLE MEANINGS IN FUNCTIONAL NEUROANATOMY. • T. R. Anthoney. Behav. & Soc. Sci., Sch. of Med., South. Ill. Univ., Carbondale, IL 62901.

•A student knows a term but not its synonyms. S/he learns an accepted meaning of a term but is unaware of alternative accepted meanings. An instructor asks questions using a synonym or an alternative meaning. Result?—wrong answers and confusion.

A rare occurrence? Probably not. Review of 26 recent textbooks of functional neuroanatomy, including those used most at schools of medicine or osteopathy in the USA and Canada in 1985, revealed 2-12 synonyms for each of 474 terms and multiple meanings for 368 terms. Many terms with multiple meanings are basic—e.g., aphasia, ataxia, basal ganglia, bradykinesia, brainstem, bulbar, cerebral and cerebrum, cerebral peduncle, hemiplegia, interneuron, involuntary movement, medial lemniscus, nerve and nerve root, neuroglia, nystagmus, peripheral nerve, recent memory, rigidity, scotoma, somatosensory, spinothalamic tract, tegmentum, tic, tremor, and visual field(s).

Though continued work toward a standardized set of terms and meanings is desirable, the Nomina Anatomica experience suggests that the problem will remain substantial. A reference text, indexed by term for rapid access to synonyms and multiple meanings, seemed desirable and was written. Details of its preparation and contents are described. Also, sample questions with multiple incompatible correct answers are given, as are questions involving synonyms.

202.5

USE OF EASY-SHAPING MATERIAL TO FOSTER THE TEACHING-LEARNING PROCESS OF NEUROEMBRIOLOGY. M.C. Márquez-Orozco, A. Márquez-Orozco and A. Lastiri*. Embriol. Dept. Sch. of Med. and Center Inv. Educ. Serv. UNAM. P.O.B. 70-553 México 04510 D.F. MEXICO.

Psychometric tests were applied to 180 freshman students of the School of Medicine (UNAM) to analize their IQ. their abstraction capacity and spatial perception, as well as their analysis and synthesis of thought. We found that 85% of the students had difficulties to establish space relations and make abstractions. Half of this population randomly selected, was submitted to a teaching-learning strategy which included the use of easy-shaping material made of wheat flour, aimed at testing whether this didactic resource fosters acquisition of the knowledge of CNS development. The other half of the students underwent traditional teaching, lacking this resourse. Models that change shape according to the different stages of the CNS development were made, using colored material for each of the different structures of the CNS developing sequence. Results were evaluated through the institutional test of achievements and a year after applying this strategy, the same psycometric tests were given to the same students. Achievement, measured in terms of their capacity for spatial abstraction and perception, and abstraction and synthesis of thought, were significatively higher ($p < 0.01$).

202.6

A CHANGE IN THE TEACHING LABORATORY OF HUMAN PHYSIOLOGY. M. Najar-Joa*, B. Barrera-Mera, and P. Vergara*, Depto de Fisiologia Fac. Medicina UNAM Mexico 04510 D.F.

When in 1985 a curricular change in the medicine studies at our faculty was announced, it looked as a nice opportunity to make also a change in the practicing activities at the laboratory, thus acheiving a more scientific approach in the teaching of Human Physiology. The academic activities were divided in two phases. In the first one the old model was used, with the teachers directing the activities and the students learning the techniques and being progressively prepared to handle the concepts of hypothesis, variables and analysis. The second phase was named "Free Projects Phase" and was aimed to prepare students to develop a research project based on their own ideas. The change of model was not easy one. Teachers' response was divided among positive and negative opinions: fortunately there were more positive responses. The students were more receptives although some of them were afraid of this new approach. The most frequent complaint of the students was the lack of freedom to develop their original ideas. A very difficult aspect was on the teachers' side, their different approaches. Due to the lack of resources to handle all the projects, it was necessary to direct the work to those projects for which the required material was available. The students response, however was amaizing. Some students bought their own material or equipment, or went to other institutions to ask for information and support. More than 60 per cent of the projects were on Neroscience Research.

202.7

EXPERIENCES IN TEACHING HUMAN PHYSIOLOGY TO MEDICAL STUDENTS. E. Gijón and X. García. Dept. of Physiol. Sch. of Med. Universidad Nacional Autónoma de México. Ap. P. 70-250, México, D.F. 04510. MEXICO.

Due to changes in the medical curriculum and a decrease in the number of students for each group, our groups instead of being formed by 80 or more students are actually formed by an average of 30 students. The change in the curriculum started in 1985, so it is known as Plan 1985. The time of one semester with 200 hours was changed to one year with 320 hours for Human Physiology. The time for theory and the reduction in the number of students led to a change in the traditional lecture for student oral presentation tutored by their professor. The traditional exercises of laboratory have been extended to an innovative program called Directed Projects. The first change allowed us to know what the student understood of every topic with his own words, and the second change to introduce the student to the scientific method. The effectiveness of both changes have positive results, as it can be judged by the improved grades obtained by the students, and the increase in the % of accreditation of the course.

202.8

NEUROSCIENCE IN AN EXPANDING OPTOMETRIC CURRICULUM. P. Simmons and W.H. Ridder, III, Southern California College of Optometry, 2575 Yorba Linda Blvd., Fullerton, CA 92631.

In a growing national trend, optometrists are becoming increasingly responsible for the diagnosis and/or treatment of both ocular and systemic disease. Thus neuroscience, along with other biomedical sciences, is becoming a more important component of optometric training. The challenge in the optometric curriculum is to provide additional material in biomedical sciences without sacrificing traditional optometric training in optics and visual science. At the Southern California College of Optometry, neuroscience is taught in a lecture/laboratory combination in which lectures focus on clinically relevant neurophysiology, and laboratories provide intensive training in the clinical correlates of neuroanatomy, with emphasis throughout on the sensory and motor aspects of the visual system as well as the cranial nerves. In later portions of the course, students are asked to determine the anatomical correlates of clinical symptoms or signs, including those functions evaluated in a complete optometric examination, such as visual fields and oculomotor functions. The aim of this course design is to provide a broad background in basic neuroscience while keeping the number of class hours relatively low, currently approximately 30 hours each for lecture and laboratory. Thus, students are able to integrate an increased amount of neuroanatomical knowledge into their 4-year professional program.

202.9

SHOULD A SCIENCE ORIENTED TEACHING BE DIRECTED TOWARDS MEDICAL STUDENTS? EXPERIENCES IN THE TEACHING LABORATORY THROUGH DIRECTED PROJECTS. L.P. Solano-Flores, R. Guevara-Guzmán, G. Reyes-Guerrero*, and M.P. Rosas-Arellano*. Fisiología, Medicina-U.N.A.M. A.P. 70250, 04510 México, DF.

WHAT?, HOW?, and WHY? These are the questions to be kept in mind whenever trying to understand nature by means of a scientific pathway. A laboratory is a place where nature variability can be restricted in order to OBSERVE -to answer the "WHAT"-, to DESCRIBE -to answer the "HOW"-, and to INTERPRET -to answer the "WHY"-. The Teaching Laboratory is the optimal tool to awake, to develop, and to mature the inquiring, critical, analytical and integrative attitudes needed to understand nature. One question: Should a science oriented teaching be directed towards medical students? The answer: Since a great similitude essentially exists among the physiological, pathological and the therapeutical phenomena, then "every physician should be an experimenter" (Claude Bernard). Thus, since the utmost way to understand science is precisely making science following the complete path, the above ideas have been limited in the Directed Projects Phase of the Teaching Laboratory in the Human Physiology Course for medical students. In order to achieve this, a deep and huge effort is required from both students and academic staff to change the established conceptions and methods to acquire knowledge. This experience, however, is worthwhile to live. The purpose of this work will be to present our experiences on this teaching modality.

202.10

TEACHING NEUROSCIENCE IN AN UNDERGRADUATE INSTITUTION: A GRADUATE MODEL IN AN UNDERGRADUATE INSTITUTION. M. J. SAARI. Neuroscience Research Unit, Nipissing University College, North Bay, Ontario, Canada P1B 8L7.

An undergraduate neuroscience teaching model based on undergraduate research participation is described. The model involves extensive "hands-on" laboratory experience with an emphasis on research productivity and dissemination of results through conference papers and manuscripts. The model attempts to create a typical graduate neuroscience laboratory experience using the student/advisor apprenticeship relationship commonly found in graduate schools. The students involved are undergraduates. The model was developed in order to allow undergraduates the opportunity to gain research skills and to demonstrate their potential for research productivity thereby allowing an early start to their careers as research neuroscientists. Student case studies are presented to demonstrate the effectiveness of the model.

202.11

A NEUROSCIENCE PROGRAM AT A LIBERAL ARTS COLLEGE. J. J. Ramirez. Dept. of Psychology, Davidson College, Davidson, NC 28036.

The objective of our educational program is to enhance the training and enrich the experience of students interested in CNS. To this end, we are developing a curriculum in Neuroscience. This program consists of three levels of training: 1) an introductory and advanced course in neuroscience, 2) advanced seminars, labs, and independent studies, and 3) a research project in which original research is conducted by students. An integral component of each level involves training in laboratory methods, with the aim to guide students from the structured lab exercises of the introductory course to a level of competence at which the students may productively pursue independent research. Behavioral, neuroanatomical, and neurophysiological components of the program are presently in place. The students emerge from our program with an understanding of the theoretical and empirical literature in neuroscience, a sophisticated appreciation of scientific methods and analysis, and extensive laboratory skills. As a result, these students are well prepared to pursue careers in science, medicine, or public policy. Supported by The Pew Charitable Trusts.

202.12

TEACHING NEUROSCIENCE: PUT PRINCIPLES BEFORE DETAIL. F. Delcomyn. Department of Entomology and Neuroscience Program, University of Illinois, Urbana, IL 61801.

Beginning students studying neuroscience are often so overwhelmed by the presentation of the minute facts and detailed experimental results of the subject that they loose any sense of their meaning. When teaching beginning students it is imperative to provide a context within which specific facts or experimental results can be understood. The principle will be illustrated with examples from two different neuroscience areas. The first example, from the area of membrane channel diversity, will describe a sample of the enormous variety of channel types present in nerve membranes. The second, from the area of sensory-motor integration, will describe results of several experiments in which sensory feedback has been manipulated during a motor performance. Each set of material will be described as it might be in typical textbooks. However, further explanatory, context-setting material will be provided and highlighted, so that in each case viewers of the poster will be able to examine the factual material with or without specific consideration of the context of the information. In this way, viewers will be able to judge the added effectiveness of an attention to context and underlying scientific principles.

202.13

TEAM-TAUGHT *INTRODUCTION TO NEUROSCIENCE* COURSE. L. J. Achor, L. M. Barker*, J. R. Flynn, J. H. Patton, and C. A. Weaver III*. Dept. of Psychology, Baylor Univ., Waco, TX 76798-97334.

We offer an entry-level team-taught course on introductory neuroscience, which is used by 250 students per year to partially satisfy the university requirement for three laboratory science courses for the bachelor's degree. It is taken by both science majors and non-science majors.

The goals for this course include (1) the presentation of a balance of classic and current information on theory, methods, and findings in neuroscience; (2) the enhancement of scientific literacy; (3) increased understanding of scientific methodology; (4) encouragement of intellectual skepticism and critical thinking; and (5) the development of greater understanding of those individuals whose thinking and behavior are different from the norm.

Our primary purpose in team teaching *Introduction to Neuroscience* has been to enable a group of professors to bring to the course their individual expertise in major areas of the rapidly expanding field of neuroscience. Each of the five members of our team teaches a three-week unit. To enhance the flow of information and smooth the transition from one professor to another, lecture outlines and abbreviated notes are provided to the students at the beginning of the semester.

Computer exercises simulating scientific procedures and phenomena in neuroscience are used to facilitate learning of the neuroscience curriculum.

202.14

AN UNDERGRADUATE COURSE IN BEHAVIORAL NEUROSCIENCE. C.H. Vanderwolf. Dept. of Psychology, Univ. Western Ontario, London, Ontario, Canada, N6A 5C2.

Undergraduates who have completed introductory biology, psychology, and a brain-behavior lecture course may enroll in a laboratory course (13 weeks) comprising 3 hrs laboratory and 1 hr lecture/week. Laboratory exercises include: (A) neuroanatomy and neurohistology, 7 weeks; (B) brain lesions and behavior, 1 week; (C) recording brain electrical activity and behavior, 1 week; (D) drugs and behavior, 1 week; and (E) electrical stimulation of the brain, 1 week. Demonstrations are also given. Evaluation is based on written and practical examinations on A plus written reports on B-E. A laboratory manual for topic A (Vanderwolf, C.H., & Cooley, R.K. The sheep brain: a photographic series, London, Ontario: A.J. Kirby, 1990, 105 pp.) and instructions for topics B-E have been prepared. The course has been refined over a period of over 20 years.

202.15

AN INTEGRATED APPROACH TO TEACHING NEUROSCIENCE. C.A. Paul* and B.S. Beltz Dept Biol.Sci, Wellesley College, Wellesley, MA 02181

A multi-disciplinary approach is used to examine aspects of structure, function and biochemistry in the adult leech nervous system. Students are expected to relate their findings to developmental mechanisms illustrated in the lecture.

1. Immunocytochemical localization of transmitters: Amine and peptide antibodies are utilized to ask: "Are labelling patterns for particular transmitters distinctive or similar?" These studies illustrate how determinate developmental mechanisms result in stereotyped patterns of organization.

2. Anatomical characterization of identified neurons: Neurons identified immunocytochemically are injected with lucifer yellow by iontophoresis to answer: "What is the anatomical profile of a particular neuron?"; "Is this profile the same between segmental ganglia and between animals?" Students learn that there is tight developmental control over primary branching patterns but less regulation of the secondary pattern.

3. Physiological characterization of the identified cells: Thresholds, action potential shapes and response properties of the identified neurons are determined. Extracellular stimulation of peripheral nerve roots and connectives, while recording intracellularly, can verify whether projections identified by dye injection can be demonstrated physiologically.

4. Independent projects: Students formulate new questions, utilize the above methods to generate and analyze novel data, and compose a research report.

This sequence fulfills four goals in addition to teaching the scientific method: (1) to "demystify" science by using state of the art technologies; (2) to provide an opportunity for discovery by exploring current problems; (3) to illustrate the interdisciplinary nature of science by using several methods to address related questions; and (4) to learn to communicate ideas and think critically.

(Supported by NSF - ILI grants #USE- 8851888 and #USE-9152022)

202.16

UNDERGRADUATE PARTICIPATION IN THE DEVELOPMENT OF A SCIENCE COMPETITION: AN INTEGRATIVE METHOD TO PROMOTE SCIENTIFIC AWARENESS. A. Bettica*, M.A. Behr*, and A.C. Santucci, Depts. of Biology and Psychology, Manhattanville College, Purchase, NY 10577.

Recent trends of undergraduate majors indicate a decreasing interest in the sciences and allied fields. These trends parallel discouraging reports demonstrating a decline of scientific and technological aptitudes within the general population of the United States. Several recent programs developed to enhance scientific literacy have been suggested such as large-scaled endeavors aimed at changing curricula at all educational levels (e.g., Project 2061). Moreover, individual faculty have implemented innovative teaching techniques as a way to enhance scientific literacy and student participation. With these goals in mind, a collaborative effort of the science departments of a small liberal arts college has been initiated. This effort focused on the development of an integrative annual science competition drawing on available community and educational resources. High school students from 200 area schools were invited to submit proposals of their current research projects. The undergraduate science majors played a significant role in determining the applied categories for entrants, judging the scientific merit of the presentations for a special honor society award, and coordinating the integrative aspects of the program. In addition, the students were exposed to various presentations of faculty research and attended an awards luncheon highlighted by a keynote lecture on recombinant DNA applications. Although the focus of this year's events was not specifically neuroscience in nature, this integrative program is a potential mechanism to promote education in neuroscience and other contemporary fields.

202.17

A SUCCESSFUL FORMAT FOR COVERING TWELVE NEUROPHYSIOLOGY LABORATORIES IN FIVE LABORATORY SESSIONS. J. Hore, J. Van Dijk-Smith* and M. Robinson. Dept. of Physiology, University of Western Ontario, London, Ont. Canada N6A 5Cl.

Objective: To expose 48 third year science students to a large number of neurophysiological concepts and phenomena in an active learning laboratory situation. Constraints: 5 three hour sessions, minimum budget, but 12 teaching assistants. Method: Students sign up for one of 12 experiments that illustrate important concepts presented in concurrent lectures. They include: Rat motor cortex, Rat evoked potentials, Human eye movements, Frog and human vestibular function, Rat Parkinson's disease, Frog spinal reflexes, Rat VMH lesion, Human EEG, Rat dorsal column recording, Rat limbic lesion, Human evoked potentials. Over the first 3 laboratory periods students develop expertise in their chosen experiment. On week 4, half of the students present their experiment in a 10 min demonstration to their classmates in a "bell ringer" format, i.e. 2 members of the group present 11 times to rotating groups of two. On week 5 roles are reversed. Emphasis is placed on the 2 visitors having a hands-on experience and being able to ask questions. Objectives and answers to objectives are provided for all experiments. This format in which any one student sees the 11 other experiments in a student-teaching-student situation has proven to be highly successful.

202.18

"THE BRAIN IS WIDER THAN THE SKY": AN HISTORICAL SURVEY OF BRAIN IMAGERY IN THE POETRY OF EMILY DICKINSON. R.A. Johnson and L.L. Butcher. Behavioral Neuroscience Program, Department of Psychology, UCLA, Los Angeles, CA 90024-1563.

The word *brain* appears 26 times in the poems of Emily Dickinson (1830-1886) of Amherst, Massachusetts. That is the same word frequency as *cold*, *secret*, *sunset*, and *winter*. Though far less than *I* (1682 times) or *you* (378 times), it is more than *moon* (25), *june* (21), *music* (22), *circumference* (17), *notwithstanding* (7), *amen* (1), and *scientist* (1). One piece, written in 1862 (published 1892), begins:

> The Brain is wider than the sky—
> For— put them side by side—
> The one the other will contain—
> With ease— and You— beside—

For over 15 years, undergraduate Psychobiology majors in L.L. Butcher's classes at UCLA have been asked to consider the above stanza and respond to the question, "In 25 words or less, what did she mean?" Excerpts and an informal cluster analysis of hundreds of responses will be presented, alongside a collection of commentary on the poems. While raised in a latter-day New England Trinitarian (Puritan) community, Emily Dickinson developed a profound self-consciousness and facility with both humanistic and scientific knowledge. Of particular interest is a consideration of her exposure to the science of the day, started while studying under Edward Hitchcock, president of Amherst College and professor of both geology and moral theology, and continued at Mount Holyoke Seminary. Attendees of the meeting will be invited to comment on the efficacy and merits of citing historical and literary sources in a neuroscience survey course.

202.19

AN OPTIMAL SEQUENCE IN NEUROSCIENCE FOR PHYSICAL AND OCCUPATIONAL THERAPY STUDENTS. S.S. Palmer. Dept. of Physical Therapy, Texas Tech Univ. Sch. of Allied Health, Lubbock, TX 79430.

The optimal time for learning neuroscience in therapy programs is between gross anatomy and clinical courses. An optimal sequence of topics was hypothesized to start from spinal cord and work rostrally. Labs would start with meninges, blood vessels and peripheral nerves, and work inward to CNS at each caudal-to-rostral level. Two other sequences would be imposed, one moving from structure to normal and pathological function, the other moving from disease to research methodologies and potential clinical therapies. This plan resulted in the following sequence: neurons/glia & meninges/ventricles; arteries/veins; spinal cord development, investments, vessels & dermatomes; spinal cord reflexes, disorders & nerve plexuses; brain stem & cerebellum; cranial nerves & brain stem sections; development of brain, diencephalon & pituitary; autonomic & limbic systems, hemisected brain, basal ganglia, cortical areas & lobes; motor & sensory systems; aphasias, EEG, epilepsy, evoked potentials & white fasciculi; aging/dementias, motor disorders, alcoholism, parasagittal, coronal & horizontal sections; regeneration/plasticity, immunocytochemistry & imaging; EMG, neurologic exam & cases. The course that sequentially dismantles the human brain and builds upon a structural foundation with clinical and research directions was judged superior to other sequences by students, and it produced a better knowledge of neuroscience as determined by testing.

202.20

USE OF MINITHEMES TO ENCOURAGE CONCEPTUAL UNDERSTANDING OF NEUROPHYSIOLOGY. J.A. McMillan. Biology Dept. and WAMI Regional Medical Education Program, Montana State Univ., Bozeman, MT 59717

The greatest challenge in teaching neurophysiology is facilitating development of a strong conceptual framework of basic principles as early as possible. One strategy is the use of take-home minithemes, limited to one side of an 8.5 X 11" paper.

Two formats for the minithemes, in which students assume different roles, are especially successful. In the first (expert medical witness), they must determine if a given set of data do or do not support a stated hypothesis. Often one piece of information is designed to make the data inconclusive; this helps students become comfortable with inconsistencies. In the second (basic scientist), a set of data consistent with two hypotheses are given and students must design an experiment which would enable them to determine which hypothesis is correct.

Minithemes are evaluated for accuracy, originality, logic and grammar. A satisfactory performance (S) gets full credit and a non-satisfactory performance (NS) gets no credit. An "NS" can be changed to an "S" by submitting a satisfactory revision of the minitheme. This enables faculty to be very critical, both of content and style, without jeopardizing the student's grade.

Students consistently consider the minithemes to be a valuable learning experience. They typically report that the exercise helps them understand the material better and that they enjoy the opportunity apply their knowledge to a practical situation. Furthermore, most of them appreciate the fact that all aspects of their efforts, including writing skills, are evaluated critically but constructively by faculty in the course.

202.21

YAWNING AS CLASS DEMONSTRATION OF FIXED ACTION PATTERN, RELEASING STIMULUS, CONTAGION, AND FACE PERCEPTION. R. R. Provine. Dept. of Psychology, Univ. of Maryland Baltimore County, Baltimore, MD 21228.

Yawns are easy to produce for study in the classroom because most stimuli associated with yawning trigger yawns - even reading or talking about yawns. The students are part of this demonstration! Yawning is an ethological "fixed action pattern" (FAP) that is the likely product of a central motor pattern generator. Yawns meet the classical criteria of FAP's: they are more complex and long lasting than reflexes, they occur at "typical intensity," and once initiated, they go to completion (try to stifle a yawn). Research by the author shows yawns to be most frequent in subjects who are sleepy, bored, or stretching. Yawning is not influenced by blood levels of CO_2, O_2 or by exercise.

Observed yawns are ethological "releasing stimuli" for the FAP of yawning. The resulting "contagious" yawning response is a primitive, neurologically programmed social coupling process that synchronizes the behavioral and physiological state of a group. The search for the elements of the yawning face that trigger yawns is a non-invasive approach to the neuropsychological problem of face recognition, a problem traditionally approached using brain damaged humans (prosopagnosics) and face-specific neurons in the brains of non-human species. Research shows our neurological yawn detector to be independent of axial orientation and seems tuned to the overall configuration of the yawning face - specific features such as the yawning mouth are not necessary to evoke yawns.

202.22

SENSORY ADAPTATION: EXTRACELLULAR RECORDING FROM THE LOCUST FOREWING HINGE STRETCH RECEPTOR. R.M. Robertson. Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

Elevation of each of a locust's wings is monitored by a wing hinge stretch receptor. The stretch receptor comprises a single multipolar sensory neuron innervating a connective tissue strand spanning skeletal elements at the base of the wing. The adequate stimulus for the sensory neuron is stretch of the strand caused by elevation of the wing. This provides a simple preparation for demonstrating and investigating the process of sensory adaptation. Experiments using it can be performed by students who have limited manual dexterity and/or little experience with recording techniques.

The legs and head of a locust are cut off and the gut is removed by pulling it through the neck. The animal is mounted on a support which allows free movement of the forewings. One of the forewings is extended laterally and fixed to a simple device which will hold the forewing at desired wing elevations. A monopolar silver wire hook electrode is inserted through the neck into the thorax and is positioned near the base of the forewing under investigation. The electrode must be moved in search of activity of the stretch receptor which is easily recognized as it fires at around 10 Hz when the wing is extended laterally from the thorax. Once the electrode is in position the opening at the neck can be sealed with a mixture of petroleum jelly and mineral oil to prevent desiccation. Preparations like this will remain viable for more than 24 hours. Measurements are made of the frequency of discharge of the sensory neuron at different times after different elevation/depression movements of the wing.

202.23

CURRENT EVENTS: A STUDENT LABORATORY EXERCISE FOR EXAMINING IONIC CURRENTS UNDER VOLTAGE CLAMP IN SNAIL NEURONS . B.R. Johnson, M.L. May* and P.D. Brodfuehrer. Neurobiology and Behavior, Cornell University, Ithaca, NY 14853 and Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

We use the circumesophageal ganglia preparation from the land snail, *Helix aspera*, to teach electrical properties of neurons in the undergraduate laboratory. Snails are inexpensive, easy to maintain, the neurons are large (up to 200 μM in diameter), and remain viable for at least several hours in chilled saline. Here we describe an exercise that is designed to teach intracellular voltage clamp techniques and examine the ionic basis of neuronal activity. First, students simulate the procedures for the voltage clamp experiments using commercially available software for the Macintosh. This ensures their familiarity with the experimental paradigm before using the voltage clamp amplifiers. Second, they describe the time and voltage dependency of the total ionic current in identified neurons using different voltage steps from a holding potential. Third, each student group describes the same parameters of an isolated ionic current using pharmacological and stimulation techniques to separate it from the total membrane current. For example, a group may describe the early potassium current by pharmacologically blocking other currents and then comparing the currents obtained with depolarizing steps to 0 mV from holding potentials of -50 mV and -80 mV. Results from all student groups are pooled during a general discussion of the contribution of each individual current to neuronal activity. Supported by grants from the NSF (DIR9051880), the Howard Hughes Medical Institute and Apple Computer, Inc.

202.24

AN INTEGRATED LABORATORY PROGRAM FOR PHYSIOLOGICAL AND MORPHOLOGICAL EXAMINATION OF MOTOR CONTROL. R. R. Hoy, B.R. Johnson, and S.J. Zottoli. Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853 and Dept. of Biology, Williams College, Williamstown, MA 01267.

Crayfish are widely used for undergraduate laboratory exercises because they are inexpensive, easy to maintain, and there is a large background of research literature for reference. In addition, they are cultured, thus their use causes little environmental impact, and they can easily substitute for vertebrate preparations in the student neurobiology laboratory. We have organized an integrated series of laboratory modules for the neurosciences using this animal. These emphasize scientific methodology, principles of nervous system function, and physiological and morphological techniques of experimental neurobiology. Here we present two integrated modules and provide handouts. The first is an exercise using extracellular recordings to monitor spontaneous activity in a purely motor nerve (6 axons) that innervates only the superficial flexor muscle (contributes to posture) in the crayfish abdomen. Students describe patterns of neural activity, and from the amplitude distribution of the action potentials, they predict the number and relative sizes of motor neurons innervating the muscle. In the second module, students are taught cobalt backfilling techniques to visualize the motor neurons within the central nervous system, and thus directly test their predictions. Their results lead to discussions of motor control and functional morphology of neurons. Subsequent modules will focus on basic synaptic mechanisms and their plasticity. Supported by NSF grants BNS8809445 and DIR9051880, the Howard Hughes Medical Institute, the Grass Foundation and Apple Computer, Inc.

202.25

A LABORATORY EXERCISE IN SOMATOSENSORY PSYCHOPHYSICS THAT IS EXPEDITIOUS, INEXPENSIVE, AND SUITABLE FOR LARGE CLASSES J.D. Greenspan, Depts. of Neurosurgery and Physiology, SUNY Health Science Center, Syracuse, NY 13210

A procedure is described which allows a large class (>100) to determine tactile acuity in the form of two-point discrimination. The key to this laboratory is having a large number of stimulating apparati that are reasonably accurate. Such a device can be made with a six-inch plastic ruler and paper clips in less than five minutes for less than $1.00 each.

This laboratory was conducted by a first year medical school class (N=160). One stimulating device was given to every three students. Each of the three students in a group took turns as the subject, experimenter, and record keeper. They were instructed to use a method of limits to determine two-point thresholds on four locations of their right hand: the fingertip of digit 2, the proximal part of the distal phalanx of digit 2, the middle of the proximal phalanx of digit 2, and the thenar eminence. The students could see for themselves the dramatic regional differences in tactile acuity, as well as the practical problems associated with an "objective" assessment of perception. Furthermore, class means could be determined and presented to the students at a later date. These results can be compared to published data on mechanoreceptor density in the human hand (Johansson and Vallbo, 1979). Also, these results can be discussed with respect to the size of cortical representation of different body regions (Kandel and Schwartz, 1985, Chap. 25).

202.26

TO STUDY THE EFFECT OF SENSORY DEFICITS ON FOOD SEARCHING BEHAVIORS AND RECOVERY OF FUNCTIONS WITH PLANARIANS. S.HSIAO AND J.MASSENGALE*. Dept. of Psychology, Univ. of Arizona, Tucson, AZ 85721

Students can easily measure various behaviors of planarians. This inexpensive animal regenerates missing parts readily and is unique as the most primitive species to possess organ systems, with bilateral symmetry, and cephalo-caudal/dorso-ventral axes. Planarian food searching behavior was studied as a function of unilateral lesioning and regeneration of chemosensory auricles. 20 planarians, 1.5 cm long, housed in petri dishes (7.5 cm dia.) with 200 ml tap water and food-deprived for 11 days, were randomly divided into 4 groups of n=5: Intact Control, Left Auricle Excised, Right Auricle Excised, Surgery Control (tail tissue excised). In PRE-SURGERY test, a live tubifex was placed into the dish 4.2 cm away from the head. The head location was recorded every 10 sec for a 10 min period. Surgery consisted of removal of an auricle with about 1 mm of indentation toward the eye, or of about the same amount of tail tissue. The test was repeated the next day (POST-SURGERY), and upon complete auricle regeneration in about a week (POST-RECOVERY). We measured the time to capture the prey (600 sec assigned to that failed to capture), the distance to prey when a straight move to the prey was commenced, and the number of times animals circled within 24 mm of prey. Results: The auricle-lesioned animals (a) approached very near to the prey before commencing a direct move toward it (3.5 vs 13 mm), (b) circled around the prey with the lesioned side toward it, but (c) the time to capture prey was not altered. No deficit was detected in the tail-excised and recovered groups. (UofA SBS Small Research Grant.)

202.27

OIL RED O: A SIMPLE PATHWAY STAIN FOR USE IN THE NEUROSCIENCE TEACHING LABORATORY. M. LeGare and B. Winegar. Dept. of Psychology, California State Univ., Sacramento, 95819-6007; Dept. of Pharmacology, Sch. of Med., U.C. California, San Francisco, 94143-0450.

One solution to the time-money problem in the undergraduate neuroscience teaching lab is our development of Oil Red O (ORO), a lipid stain (Humason, 1967), for staining mounted rat brain sections. Rats are sacrificed with nembutal and perfused through the heart with 10% formalin. Extracted brains are placed in 10% formalin for >24 hours. Frozen sections (25-35 microns) are made on a clinical freezing microtome, mounted in a water bath on slides which have a thin coating of 50% glycerol-50% fresh egg albumin, and placed on a slide warmer at 40° C until dry (30-40 min.). The ORO (Sigma) stock solution is 250-500 mg ORO in 100 ml of 99% ETOH (saturated). The staining solution is 120 ml stock solution and 80 ml water which is allowed to stand for 10-15 minutes and then filtered. The slides are rinsed twice in water, stained for 20 minutes followed by a final rinse. Slides are removed one at a time, enclosed with glycerol gelatin (Sigma), covered with a #1 cover slip and ringed with Preserva-slide. Pathways are red-orange to dark red. Cellular areas are pink. Our students do all work except for the animal sacrifice and brain extraction. The slides are examined and pathways drawn using low power (x25) light microscopy and projecting microscopy. Students use Paxinos and Watson (1986) for pathway identification.

References: Humason, G. Animal Tissue Techniques. Freeman & Co.. 1967; Paxinos, Q. & Watson, C. The Rat Brain in Stereotaxic Coordinates. Academic Press, 1986.

202.28

UNIVERSITY RESEARCH EXPERIENCE FOR HIGH SCHOOL SCIENCE TEACHERS AS A MEANS TO INTRODUCE NEUROSCIENCE INTO THE SECONDARY CURRICULUM. Joseph, G.[1]◆& DeRiemer, S.A.[2] [1]W. Taft H.S., Bronx, NY 10457, & [2] Dept. of Biological Sciences, Columbia Univ. NY NY 10027.

Columbia University has introduced a program to expose New York high school science teachers to the experience of conducting basic research. The program currently involves 20 teachers who spend 8 weeks each summer for two years in Columbia laboratories. They are given weekly seminars by prominent scientists on topics of contemporary interest and have year round use of Columbia facilities including libraries, stockrooms, and computers. At the end of each summer, teachers present their scientific work and develop a plan for the incorporation of this experience into their teaching. Following is one example of how such programs can lead to innovative teaching of neuroscience at the secondary level.

W. Taft H.S. in the Bronx serves a low-income, minority population of students coming from primarily single parent homes. Eighty-five of the 2500 students are currently enrolled in Regent's (Upper level) Biology. The changes introduced into the teaching of these students following a summer of laboratory research include the following: 1) Substituting an emphasis on the use of scientific reasoning (generation and evaluation of hypotheses) for answering questions instead of a reliance on "right" answers from the teacher or text; 2) The relating of first hand experiences and information collected during the summer to capture the interest and excite students; 3) Reconstruction in the classroom of the preparation and experiments done in the Columbia laboratory. For example, peristalsis in an isolated intestinal section was used to demonstrate the presence of nerves outside the brain, the autonomy of isolated organs, the complex, coordinted control of contractions produced by the myenteric system, and the effects of neurotransmitters. Program supported by a grant from the Howard Hughes Foundation.

202.29

THE STAR PROGRAM: A COOPERATIVE VENTURE IN SCIENCE EDUCATION BETWEEN THE UNIVERSITY OF SOUTHERN CALIFORNIA HEALTH SCIENCES CAMPUS AND FRANCISCO BRAVO MEDICAL MAGNET HIGHSCHOOL IN EAST LOS ANGELES. R.E. Brinton◆, D.C Moot* and M. Mayo* ◆ Depart of Molecular Pharmacology and Toxicology, School of Pharmacy University of Southern California, Los Angeles, CA 90033 and * Francisco Bravo Medical Magnet High School , Los Angeles, CA 90033.

The STAR Program for Science, Technology And Research provides junior and senior high school students interested in learning about scientific exploration the opportunity to participate in research projects. The goals of the STAR Program are to: 1. Expose Students to The World of Science 2. Teach Students the Discipline of The Discovery Process. 3. Create an Environment That Nurtures Curiosity. 4. Provide Role Models of The Investigative Explorer. STAR students participate in research projects as an integral part of their curriculum and work in the laboratory 3 afternoons or more a week. A total of 30 research laboratories at the USC Health Science Campus participate in the STAR Program. Bravo Medical Magnet High School, a public high school located in East Los Angeles, has an enrollment of 60% Latino-Am, 18% African-Am, 16 %Asian-Am, 5 % Anglo-Am and 1% Native Am. Of the 12 STAR students of 1990, **92%** went onto college, **25%** of STAR Students were Latino-Am and **100%** of the Latino-Am students went on to colleges such as Penn State and Berkeley. Two of the 12 were early acceptance students, one to Cal Tech and the other to Penn State. Of the 30 participating laboratories 50% are conducting research related to neuroscience. Of the 1990 STAR students **30%** are continuing to do research as part of their undergraduate curricula. The class of 1991 has tripled in size relative to the 1990 STAR class with **30%** of the current students presenting their research at the Los Angeles County Science Fair. Last year's winner was a STAR student whose research project was "The Effect of Stress Hormones on Hippocampal Nerve Cell Growth in Culture."

202.30

PRINCIPLES OF NEUROSCIENCE AS AN ELECTIVE COURSE IN HIGH SCHOOL. J. Hart Romeo*, C. Poole*, I.G. McQuarrie. Department of Science, Lakewood High School, Lakewood, Ohio 44107.

An educational initiative designed to increase awareness and understanding of neuroscience has been developed at Lakewood High School in Ohio. Principles of Neuroscience is offered as an elective course, and, to capture the widest possible audience at the school, requires only a general biology course as a prerequisite. The course also functions as a feeder for the advanced physiology and anatomy course offered to junior and senior students.

Written to provide intellectual stimulation and challenge, the course utilizes input from neuroscientists, neurosurgeons, and educators. The format includes lecture, discussion, presentation by members of the scientific community, development of a familiarity with the neuroscience literature, essay examination, and an individual investigative or research project. Students are encouraged to develop links with the neuroscience community, visit laboratories, and assist in on-going research where possible. Course topics include neural physiology, cell biology of neurons, the chemical basis of synaptic transmission, reactions of neurons to injury, and axon regeneration. Special integrated topics include the physiology of sleep and dreaming, neurochemistry of seizure activity, sexual differentiation of the nervous system, the development and aging of the brain, biochemistry of mental disorders, biochemical approaches to learning and memory.

202.31

THE PITTSBURGH NEUROSCIENCE EDUCATION PROGRAM FOR JUNIOR HIGH SCHOOL STUDENTS. Weisz, D.J.*+, Bradler, J.E.+, Hurrianko, A.E.+, Pandalai, S.P.+, and Smith, I.D.+ Departments of Neurological Surgery* and Behavioral Neuroscience+, University of Pittsburgh, Pittsburgh, PA 15260.

Over the past two years faculty members from the Departments of Behavioral Neuroscience and Neurological Surgery and several graduate students from the Department of Behavioral Neuroscience at the University of Pittsburgh have gone into the junior high school classrooms in the Pittsburgh City School System to talk to approximately 1500 seventh grade students about neuroscience. The goals of this expanding project are to introduce students to research in neuroscience, to illustrate how neuroscientific research can help people with nervous system disorders, and to generate a high level of awareness concerning the necessity of both animal and human research. Individual presentations are given to approximately 20-75 students and are focused on two or three demonstrations depending on the length of classroom sessions. Demonstrations include recording of EEG, EMG, and EKG, comparison of diseased and normal human brains, activation of motor responses by electrical stimulation, and recording of sensory evoked potentials. Benefits of neuroscientific research to paralyzed individuals, Parkinson's patients, and people with sensory deficits are demonstrated with the use of slides or videotape. Student participation in the discussion and demonstrations is actively encouraged.

202.32

LABORATORY EXERCISE IN COMPARATIVE NEUROANATOMY: BREAKING DOWN BARRIERS TO ANIMAL DISSECTION IN THE CLASSROOM. M.C. Fields* and R.D. Fields. Sidwell Friends School, Washington D.C. 20016.

Many classroom teachers face the dilemma of whether or not to use organisms, yet feel a need to expose precollege students to animal dissection. In an effort to overcome student objections, minimize the number of organisms used and avoid wasting any part of an animal, I use common "seafood items" to illustrate features of the nervous system. Squid, octopus, lobster, fish and shark can give students hands-on knowledge about giant axons, nerve cords, brain structure, visual, auditory and electrosensory systems. Knowledge of comparative anatomy can be gained from "cleaning" these organisms and ending the session with a seafood feast. In the context of food preparation, students lose the inhibition of cutting into animals, and the use of lower organisms is acceptable to more students, including some vegetarians. Through this practical exercise, individuals address the important "animal rights" issues involving the justification of using animals for food, clothing, and scientific research. Student response is outstanding: they enjoy the experience, learn the material better, and relate the lab exercise positively to other students and parents. This activity tends to break down many biases that students (and teachers) have about classroom dissections, and it can be used to lead into the standard dissection of the earthworm, frog, and pig.

202.33

PROJECT SEED: A COLLABORATIVE UNIVERSITY / PUBLIC SCHOOL DISTRICT EFFORT TO REVITALIZE EARLY SCIENCE EDUCATION. L. Gonzalez, J.M. Bower and J. Pine. Divs. of Physics and Biology, California Institute of Technology, Pasadena, CA. 91125

Caltech in association with the local Pasadena Unified School District has been involved for the last six years in a program to develop an exemplary K-5 public science education program. The program is strongly based on "hands-on" science activities used by regular classroom teachers. The activities themselves are in the form of experimental "kits" used in the classroom for five to six weeks. The kits are derived from existing materials invented in the 1960's and concern a broad range of scientific subjects including biological and, even, neurobiologically related topics. These include, for example, a fourth grade kit called "Action Reaction" in which students perform reaction time experiments on themselves, and a fifth grade kit that involves children exploring the physiology and behavior of crayfish.

Project SEED also has a strong teacher training component based on interactions between Caltech and community science professionals and classroom teachers. The training program is somewhat unusual in that it is organized around the use of the science materials themselves. The principle training emphasis is, accordingly, on the process of doing science rather than the factual base for the experiments being performed. With this approach discussions about science content arise naturally from actually doing science.

Following a successful three year pilot in one test school, the local school board has recently adopted Project SEED as the science curriculum for the entire district (22 schools). As a result, Caltech is now in the midst of training 430 classroom teachers in the use of hands-on science materials. Within two years, experimental science will constitute a significant component of the elementary educational experience of every one of the 10,000 young children in the Pasadena public schools. Planning is currently underway to extend this approach through all grade levels.

Project SEED is supported by the NSFgrant TPE-9050276, the Pasadena Community Bank, Rockwell International, Apple Computer Inc., and the Caltech Presidents Fund.

202.34

TEACHING NEUROSCIENCE TO ELEMENTARY STUDENTS. V.J. Sanders*, C.Mathes, M. Derosa, P.Dassonville, and A.B. Scheibel. Brain Res. Inst., UCLA, Los Angeles, CA. 90024

Students in the Interdisciplinary Neuroscience Ph.D. Program at UCLA have started a community outreach program teaching the fundamentals of neuroscience to elementary school children (ages 7-12). **The program is aimed at exposing students to elements of the sciences that would otherwise not be accessible and at promoting scientific research as a career.**

Sessions consist of one hour long presentations given on consecutive weeks; usually two sessions are given per visit (for example: 2nd grade: 10:00-11:00, 3rd grade: 11:00-12:00.) The first visit teaches the anatomy and function of the human brain. Students are asked about the location and the appearance of the brain, connections with the rest of the body, and activities and substances that are "good" or "bad" for the brain. Visuals include one whole human brain, two hemispheres, coronal sections, and a spinal cord. Sheep, cat, and mice brains are used for comparison. The second visit teaches electrical and chemical communication between cells. Analogies are made to multi-party telephones (action potentials traveling along axons and dendrites) and bursting water balloons (synaptic release). Visuals include a patellar reflex hammer as an example of speed and efficiency of communication between cells and a tuning fork as an example of transduction of sound waves into electrical signals within the auditory cortex. The students are asked questions as often as possible in order to maintain attention; similarly, questions from the students are answered immediately to promote interaction.

Since April, 1990, 25 classes (8 schools) have been visited, including a school for the blind. The program has received great enthusiasm from both students and teachers. [Sponsored by the Brain Research Institute at UCLA, the LA chapter of ARCS (Achievement Rewards for College Scientists), and the W.F. Muller Foundation.]

202.35

TEACHING NEUROSCIENCE THROUGH A LABORATORY EXPERIENCE: YOU CAN'T START TOO YOUNG. L.L. Stark and T.J. Carew. Interdepartmental Program in Neuroscience and Depts. of Psychology & Biology, Yale University, New Haven, CT 06520.

In an effort to expose children to science at an early age, our laboratory has annually opened our doors to a second-grade class (7-8 yrs. old). For three hours, we attempt to provide students a perspective on science, emphasizing neuroscience, which they can't get in the classroom. In our laboratory we investigate behavioral plasticity and its underlying cellular mechanisms in the marine mollusc *Aplysia*. Because our research spans many levels, the children can participate in behavioral, anatomical, developmental, and physiological experiments and demonstrations. In groups of 4-5 the children rotate through 5 stations. STATION 1 emphasizes OBSERVATION. The children examine the body plan of a dissected *Aplysia* and learn to look through a stereomicroscope to observe and draw neurons in an *Aplysia* central ganglion. STATIONS 2 & 3 emphasize EXPERIMENTATION, one examining feeding behavior and the other learning (habituation of siphon withdrawal). We pose questions, ask the children to generate and discuss hypotheses, and conduct experiments to test ideas. Finally, we introduce them to statistical analysis, using a computer to analyze and graph results. STATION 4 allows an INTEGRATION of science with the children's experience of growing: they examine stages of the *Aplysia* life cycle from egg, through metamorphosis, to adulthood. STATION 5, the "Electricity Room," is a DEMONSTRATION. Emphasizing the role neurons play in the children's own brains, we explain how neurons communicate through action potentials and show them different patterns of neuronal firing.

Our goal is to demystify the process of science and the people who do it, encouraging the children to view science as an exciting process of discovery. Then we all get ice cream.

TEACHING OF NEUROSCIENCE: COMPUTER-BASED EDUCATION

203.1

Computer-assisted education (CAE): a new approach and a module for teaching neuroanatomy. Louis Cornacchia*[1], Ellen Mower*[2], and James Hatton[1], 1 Div. of Neurosurgery and 2 Office of Learning Resources, UCSD, La Jolla CA.

Education in the neurosciences maximally taxes the teaching process because it involves the communication and synthesis of a voluminous, diverse and conceptually challenging data set. We have developed software to simplify the construction of educational modules and we have used this system to develop CAE software for teaching neuroanatomy.

To harness more of the potential benefits of CAE beyond "automatic page turning," the one-on-one tutoring session can be referenced as the archetype of the information transfer scenario. The tutor provides more than a source of information. For instance, the tutor provides a history of the interaction which is used to plan future studies and to guide the student on how to best learn the material considering his or her learning style and cognitive assets. Secondly, the tutor is capable of "natural language" and free-form querying of the database which allows the student to test formulations and hypotheses. Also, the tutor provides a mechanism by which a student can assess his progress.

We have implemented a system that provides a close facsimile of the one-on-one session and a platform for further research in this area. This system provides for both hierarchical and direct-access organization of neuroanatomic information. It provides for detailed record-keeping regarding the interaction of the user with the subject matter. Furthermore, the journaled information contains sufficient detail that, when evaluated by the author, can be used to optimize the lesson for more efficient learning. We provide facilities for instructor-, author-, or student-directed questioning to help the system assess progress. The system provides a flexible interface to the database, allowing the student to adjust the system to his personal learning style. Finally, we have implemented a lesson construction system that provides for rapid, simple construction of CAE software without prior programming experience. Future implementation of natural language and artificial intelligence algorithms will provide for context-sensitive remediation and automatic adaptation of the environment to the user's learning style. A neurophysiology module is currently under development.

203.2

NEURODATABASE: A MULTIMEDIA NEUROSCIENCE DATABASE FOR RESEARCH AND TEACHING S.L. Wertheim and R.L.Sidman
New England Regional Primate Research Center,
Harvard Medical School, Southborough, MA 01772-9102

NeuroDatabase is an information tool for neuroscience that is being developed to aid research and teaching. It focuses on the acquisition, analysis and presentation of two and three-dimensional data sets. It is distinguished from most other computer-based neuroscience tools by the presence of a flexible underlying database. This design enables a neuroscientist to handle the data of current and future experiments and teaching exercises as well as to engage in collaborative efforts. Each laboratory can have its own, private copy of the database to which local data can be added. The vocabulary, data storage structures and tools for creation of teaching materials are shared.

NeuroDatabase has four major functional modules: Microscopy, Laboratory Notebook, Database and Education. The Education module allows the student to browse freely through the visual database, or to obtain guided instruction by using the Guided Tours, glossary, programmed instruction or testing functions.

The software requires a Macintosh II with 8 MB RAM, hard disk and 19" color display. Sony rewritable optical disks (650 MB) are used for archival storage and data exchange. The software was created using SuperCard (Silicon Beach) and Oracle (Oracle Corp.). 3D reconstruction will be done using the Silicon Graphics Iris.

203.3

THE GRAPHIC BRAIN. T.J.Voneida, T.J.Teyler & P.J.Vertucci, *Neurobiology Dept., NEOUCOM, Rootstown, OH 44272.*

Two areas of neuroscience that are difficult to teach using traditional means (textbooks, lectures and slides) are the three-dimensional anatomy of the brain and the physiological changes that occur in neurons as a function of time. Conventional neuroanatomy teaching involves extensive reiteration of two-dimensional material to (hopefully) develop a three-dimensional representation of brain structures in the student's mind. We are using a graphics computer to develop neuroanatomical teaching materials to better illustrate the three-dimensional anatomy of the brain.

The second area is cellular neurophysiology, including the binding of receptors by ligands, opening of channels, movement of ions across membranes, and generation of electrical potentials. These dynamic events vary in time and space, and are difficult to convey via books or slides. We believe that the computer animation of physiological events at the cellular, membrane, and molecular levels will make the teaching of neurophysiology not only more effective, but more interesting. We have created computer models of neural membranes, receptors, ion channels, ions, and neurotransmitters, and show these elements interacting in normal and abnormal states.

These computer-generated instructional segments will be initially videotaped and used in the classroom to illustrate certain key points. Ultimately, we hope to create an interactive electronic textbook of neuroscience. We forsee that individual student interaction with graphic images on a computer will provide an opportune medium for neuroscience instruction. In addition, the flexibility of the computer will allow us to tailor the content from professional levels (medical & graduate) to high school.

203.4

SuperBrain: A MULTIMEDIA COMPUTER APPLICATION FOR INTERDISCIPLINARY NEUROSCIENCE EDUCATION. R. P. Hammer, Jr. and S.A. Jacobson, Dept. Anatomy & Reprod. Biol., Univ. Hawaii Sch. Med., Honolulu, HI 96822, and Dept. Psychiatry & Biobehav. Sci., UCLA Sch. Med., Los Angeles, CA 90024.

The field of neuroscience lends itself well to an interdisciplinary educational approach in both medical and graduate education. The introduction of problem-based medical curricula provides the opportunity to construct a learning tool which presents current information in both basic and clinical neuroscience. ***SuperBrain*** is a Macintosh-based computer application which incorporates graphics, videodisc displays, on-screen animation and sound together with textual descriptions. The application is oriented toward clinical problems, whereby one utilizes navigational menus containing diagnostic categories and differential diagnoses to explore the structural, functional, chemical, pathological, diagnostic and therapeutic bases of brain disease. The user may change topics as desired using "buttons" and/or textual "links." An additional menu allows direct access to basic neurobiological information on brain structure and chemistry. User evaluations reveal that the most valuable features of the application are graphic animations of processes such as synaptic function, NBME-type questions with direct user feedback, graphical interfaces and linkage of clinical disorders with underlying basic mechanisms. This application facilitates exploration of the biological substrates of brain function and dysfunction, illustrating the relevance of current neuroscience research findings. This concept should be useful for neuroscience instruction in graduate, medical, postgraduate and continuing medical education.

203.5

NEUROQUIZ: A MODULAR PROGRAM FOR REVIEWING NEUROANATOMY ON COLOR MACINTOSH COMPUTERS. T. Gibbs, G. Convers* and J.L. Kubie, Deptartment of Anatomy and Cell Biology, S.U.N.Y. Health Science Center at Brooklyn , Brooklyn, NY 11203 and Deptartment of Pharmacology, Boston University School of Medicine , Boston , MA 02118

The Neuroquiz programs for Macintosh computers provide an interactive review of neuroanatomy. Each module is a series of 2 to 25 photographic images of brain specimens arranged around a theme, such as coronal sections or external views of the human brain. The image quality is dramatic with high resolution and photographic quality color. Student reaction has been highly favorable.

Each module can be run in one of three modes. In Review mode a list of identified structures accompanies each picture. The student can click on an item in the list and the appropriate structure will highlight, or click on a structure in the photo and its name will highlight. Drill mode is a self test in which a series of brain images is presented. The student is asked to click at the location of a named structure on the current image. The program responds with immediate visual and audible feedback, and the correct structure is highlighted. A running score is maintained. All structures on a photo are presented in random sequence; incorrectly identified structures repeat. When all structures are correctly identified, the next photo is presented. In Quiz mode, the student is asked to identify a series of randomly selected structures and the student's name and score may be saved to disk.

Neuroquiz is programmed in Supercard and runs off a file server that currently serves 6 computers. The programs are intuitive -- students learn to use them in under 5 minutes. The programs are also robust and need little supervision. The skeleton of each module is identical. It is an easy job, requiring no programming, to add new modules or to edit old ones. Conversely, the skeleton of an existing module can be modified or replaced while retaining existing photos and structural information. We currently have modules that cover external morphology, coronal sections, horizontal sections, floor of the fourth ventricle, dissection of the lateral ventricle, gyri and sulci, and a Pal-Weigert atlas. Several enhancements, such as the addition of voice, are in late stages of development and will be presented.

203.6

A COMPUTER IMAGE ACCESS DATABASE FOR TEACHING NEUROSCIENCE. D.E. Hillman, M.S. Nachbar*, X. Hom*, and A. W. Hunger*. Depts. of Physiol. & Biophys. and Microbiol., NYU Med. Ctr., New York, NY 10016.

Three neuroscience teaching modules have been generated for image and graphic illustration of the brain surface and the brain in sections. A program was written on a Macintosh II™ system with a high resolution color display (SuperMac Technology, Sunnyvale, CA 94086) using a software authoring environment, SuperCard™ (Silicon Beach Software, San Diego, CA 92126), to control image access, graphics and text manipulation. Two modules define various surface views of the human telencephalon, diencephalon (midsagittal), cerebellum and brainstem while a third displays sections through the brainstem and diencephalon. The images are displayed as cursor selectable frames in SuperCard windows on a high resolution color monitor. Cursor interrogation of objects in images highlights a graphic overlay of the specific region while a scrollable list highlights the object name. Alternatively, the the object name is interrogated while the corresponding image region is highlighted. Object descriptions are requested by another information button. Access of overlapping objects is afforded through a hierarchic modality scheme which switches modes for gross, detailed or functional regions. Self-testing of objects by name or location is afforded by a routine of the software. The acquisition of digitized color images was through a 35 mm slide scanner (Barneyscan Corp., Alemeda, CA 94501) for sections and a bed scanner (Truvel Corp., Chatsworth, CA 91311) for surface structures. Images were manipulated for size, orientation, and color using Photoshop™ (Adobe System Inc., Mountain View, CA 94039). The approach provides student's 24 hour access to a tutorial of self-help teaching-aids.

203.7

INTEGRATING INTERACTIVE COMPUTER MODULES INTO THE NEUROSCIENCE CURRICULUM. **Kerry D. Walton, Dept. of Physiology & Biophysics, NYU Medical Center, 550 First Ave., New York, NY 10016.**

While the content of science courses given in colleges and medical schools has increased tremendously in recent years, many new graphic techniques are now available that make the teaching of difficult concepts more achievable. Such an approach is significant in a wider sense as today's student must be able to integrate the material within each course and among related curricula. This issue is especially critical in Neuroscience courses as they are rich in concepts which are supported by a multitude of facts that must be known and understood. With this in mind and to return laboratory exercises to the classroom, over the last three years the Neuroscience Course faculty at NYU School of Medicine has developed, with student help, a set of interactive computer programs that allow self-paced, random access to information and the blending of conceptual and quantitative elements. These modules have been incorporated into four main areas of the course: (1) providing computer modeling capable of displaying real-time numerical solutions to sets of equations (synaptic transmission) or animation during lectures (sensory processing); (2) allowing students to perform computer based experiments (single cell and single channel properties); (3) acting as interactive neuroanatomical atlases (brain surface structures, brain sections, histology, MRI, spinal cord); (4) student self tests and review. Ours is a Macintosh-based system with digitized images and 19" color monitors. Some modules were developed as part of the Hippocrates Project.

203.8

BRAINSTORM, AN INTERACTIVE COMPUTER ATLAS FOR STUDYING HUMAN NEUROANATOMY. G. P. Coppa, *E. J. Tancred and P. Dev. SUMMIT, Stanford Medical School, Stanford, CA. 94305 and *School of Anatomy, UNSW, Sydney, Australia.

Using Macintosh computers and SuperCard software, we have developed a highly interactive program to enhance the study of human neuroanatomy. It includes a series of digitised color images (cards) of myelin-stained cross-sections and gross sections of the human brain and spinal cord, as well as diagrams and information (text) cards. When a structure or system is selected by clicking on it in the image or structure list, the corresponding shape is outlined and information on its connectivity and function can be presented from an underlying database. The database also provides extensive cross-references, allowing the user to follow a structure through consecutive cross-sections or to move to other representations such as diagrams, text or gross dissections. A Quiz button on each card presents randomly selected structures from the card and uses the database to generate questions and multiple-choice answers on identification and theory. The program also features several animations, including a clinical examination of cranial nerves, which illustrates the effect of lesions and tests the user's ability to recognize randomly selected lesions.

The effectiveness of BrainStorm as a teaching tool will be assessed in a controlled study at UNSW in August-September 1991, but preliminary results obtained at Stanford in early 1991 showed that 78% of students who used BrainStorm found it more effective for studying the internal anatomy of the brainstem than either practical classes or textbooks. The cross-sections and the quiz mode were the most popular aspects of the program.

203.9

***Neuroimage*: A CBL Program To Study Correlated Brain Images and Blood Supply.** R.M. Kriebel and M.J. Patney. Dept. of Anatomy, Philadelphia College Osteopathic Medicine, Philadelphia PA 19131.

This Computer Based Learning(CBL) program is designed to provide a concise instructional program which correlates cerebrovascular distribution with images obtained with modern MRI/CAT scan technologies. Most medical neuroscience courses emphasize these two important aspects of the central nervous system (CNS) because of the prevalence of cerebrovascular disorders. Regardless of the individual's final specialty choice, all physicians in training will encounter patients with insufficient cerebrovascular supply and resultant neurological deficits. Non-invasive brain imaging techniques have dramatically assisted the physician in prognostication and management of individuals with cerebrovascular problems. By progression through a layer upon layer window format, the user can review basic brain structure, brain image anatomy, correlated regional blood supply, and exercise this knowledge with a case study problem solving segment. This CBL program is intended to provide opportunity for review for national boards and may have usefulness to those allied health students preparing for careers in neurologically related nursing, therapy, counseling, and rehabilitation. The program was developed for use on an IBM or compatible platform by using a hypermedia authoring program, The Knowledge Engine™, a product of Software Artistries Inc. This project was supported by a Pew Foundation grant to RMK.

203.10

INTERACTIVE USE OF VIDEODISC ANIMATIONS OF COMPUTER-RECONSTRUCTED HUMAN BRAIN. J.W. Sundsten*, J.F. Brinkley*, K. Eno*, R.M. Harris, K. G. Kastella*. Dept. of Biological Structure, University of Washington School of Medicine , Seattle, WA 98185.

We are using 3-D computer graphic animations of brain components in neuroanatomy tutorials. Brain serial sections are digitized and the x-, y-, z-coordinates transferred to a computer graphic work station. The data are reconstructed and the images recorded on videodisc. (Sundsten, J.W. et al., J. Biocommun., 18:45-49, 1991). Objects include: amygdala, anterior commissure, brainstem, caudate, cerebellum, cerebral cortex, choroid plexus, claustrum, corpus callosum, fornix, globus pallidus, hypothalamus, hippocampal formation, internal capsule, olfactory tract, optic nerve and tract, putamen, thalamus, ventricles, and white matter.

The disc is divided into chapters. Useful animations include: structures seen through translucent cerebral cortex; slicing the forebrain with or without objects protruding; removal or addition of structures to show relationships to the cerebral ventricles; and selected functional pathway presentations. Each object can be viewed alone or in combinations with other objects in different structural contexts.

The disc is controlled by a Macintosh computer that runs our Anatomy Browser software. The system contains serial sections of brainstem and forebrain that provide a base for quizzing, browsing and accessing videodisc 3-D animations of objects seen in the cross section. The Browser has authoring tools that readily enable expansion and updating of material. Its neuroanatomical "knowledge base" adds textual information including structural definitions and neuroanatomical subdivisions. The Browser is currently in use for courses in neuroanatomy. We will present examples of the material seen on the videodisc and on the Browser.

Supported in part by a grant from the National Library of Medicine, LM04925.

203.11

IMPLEMENTATION OF A HIGH-RESOLUTION, DIGITAL COLOR IMAGE AUTHORING SYSTEM FOR THE TEACHING OF HUMAN NEUROANATOMY. U. J. Balis* and S. Saporta. Department of Anatomy, Univ. S. Florida College of Med., Tampa, FL 33612.

Current trends in neuroanatomy education clearly highlight the importance of audio-visual materials as a teaching adjunct. Typically, such materials are prepared and distributed by commercial educational publishers. As a result, the educator is constrained to presentation of materials found within the selected product.

Authoring systems, conversely, are computer programs which allow for an educator to directly assemble textual and graphic teaching materials, which may later be replayed on student computer workstations, as an adjunct in the teaching of a given course. These systems have become increasingly widespread in their use for medical education but remain limited in their application to image-intensive topics, due to the high expense of equipment needed to realize high quality digital color images.

We have developed an authoring system which has the capability of rendering digital, high-resolution color images, with minimal additional expense, thus making it ideal in educational settings where multiple student workstations are needed. The system is made possible by the availability of a new series of recently designed semiconductors, originally used in the imaging aspects of the defense industry. With these devices, it has become possible to fabricate hardware that is essential to the digitization process and rendering of color images. This hardware allows for a personal computer to render digital images in true, high-resolution color (1000x800 pixels @ 18 bits/pixel). Previously, this degree of resolution and colorspace was available only in digital video processors with prohibitively expensive image storage buffers. Using the above technology, the authors have successfully designed a neuroanatomy authoring system as a prototype teaching adjunct.

203.12

A COMPUTER MODEL OF THE CEREBELLUM. K. R. Dunleavy* and S. J. Velez. Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

A computer model of the cerebellum was developed for the Macintosh II using Object-Oriented Pascal and tested with undergraduate neurobiology students. The program allows the user to asses four different areas: Anatomy, Physiology, Pathology and Experimental. The Anatomy area allows the user to view in detail particular anatomical structures. In the Physiology area, the user is provided with a model which maintains cell geometry, the number and ratios of cells in cerebellar microzones, and the number and strength of contacts per cell in order to calculate cell locations and innervations. By clicking on selected cells, the user can then mimic the progress of climbing or mossy fiber activation in a microzone array of Purkinje cells. Snapshots of cell activity at different time intervals may be taken for later analysis. The Pathology area makes available different libraries of known conditions that alter the microzones, allowing the user to study the physiological manifestations generated by the pathological changes. In the Experimental area, changes in all of the aforementioned characteristics can be made in order to study their effects on the processing of information in cerebellar microzones. Help windows throughout the program provide information about the cells and the processes being observed. The level of difficulty of the program can be tailored to the user's knowledge of cerebellar function.

203.13

CATECHOLLISION: A GAME OF CATECHOLAMINE SYNTHESIS FOR IBM-PCs AND COMPATIBLES. W. Jeffrey Wilson' & Jennifer A. Cook"; 'Dept. of Psychological Sciences, Indiana University - Purdue University at Fort Wayne, & "Dept. of Anatomy, Indiana University School of Medicine, Fort Wayne, IN 46805

CATECHOLLISION is an arcade-style game based on catecholamine synthesis. Tyrosine and the enzymes involved in the synthesis fall from the top of the screen. The player must combine them to produce DOPA and the catecholamines (dopamine, norepinephrine, and epinephrine). For example, dropping tyrosine on tyrosine hydroxylase produces DOPA, and dropping dopamine beta-hydroxylase on dopamine produces norepinephrine. As play progresses, the screen fills with unused chemicals until too much has accumulated and the game ends. Late in the game monoamine oxidase falls, destroying the catecholamines and helping to clear the screen. Successful synthesis of catecholamines causes higher scores, increases the level of difficulty of the game, and allows the game to last longer as the screen does not fill as quickly. Users quickly become familiar with the synthetic pathway.

CATECHOLLISION has a musical score and sound effects (which can be disabled), saves high scores to disk, offers four levels of difficulty plus hints for the novice, and runs on any IBM-PC or compatible with graphics capabilities (CGA, EGA, VGA, or Hercules). CATECHOLLISION is distributed as shareware; a copy is free upon receipt of a disk and self-addressed, postage-paid mailer. A registration fee of $20 is requested if you like the program.

203.14

COMPUTER PROGRAM TO ILLUSTRATE PROPERTIES OF SEMIPERMEABLE MEMBRANES. P. J. Best and R. Fessler. Department of Psychology and Systems Analysis, Miami University, Oxford, OH 45056.

A computer program was developed to illustrate the interaction between membrane potential and ion concentration across a semipermeable membrane. The program displays a two-chambered bath with a semipermeable membrane stretched vertically across it. Individual ions move with constant velocity in the bath and cross the membrane with a specific probability determined by the membrane's permeability to that ion species. Below the pictorial representation of the bath, a number of values are displayed; ion concentrations, resulting equilibrium potential, membrane potential. The concentration of the ions and membrane permeability can be altered. A pump can be inserted in the membrane, and a voltage clamp can be imposed across it. By varying the basic parameters of the membrane, students master the relationship of ion concentration gradient to equilibrium potential. Thereafter, additional ions can be added to the bath with varying valences, permeabilities, and pump properties. Thus the program can be used to help students understand basic membrane biophysics including the Nernst Equation and the Goldman Equation at a conceptual level.

203.15

BRINGING MOLECULES TO LIFE: AN ANIMATED HYPERCARD TOUR OF NEURONS AND MUSCLES. W.H. Watson III and J.J. Sasner*. Dept. of Zoology, University of New Hampshire, Durham, N.H. 03824

During the last decade there has been a revolution in our understanding of biological phenomena so that it is now possible to explain events such as action potentials and muscle contraction at the molecular level. One of the most difficult challenges biology professors face is communicating our understanding of these molecular events to our students. In particular, we must devise ways to help them: (1) understand the relationships between molecular processes and overt behavior and; (2) visualize the dynamic interactions between molecules which are too small to capture on film.

We have developed a series of animations, using the program Macromind Director, which depict processes such as transmitter release and muscle contraction. These animations are integrated into a Hypercard Stack so students can take a tour of different physiological systems. Thus, simply by clicking on different images, students can work their way into an organism and see, for instance, how interactions between actin and myosin give rise to muscle contractions. The processes that are difficult to conceptualize are illustrated in a dynamic way through animations. This work was funded, in part, by grants from the U.N.H. Discovery Program and Apple Computer Corp.

203.16

THE SECOND MESSENGER SYSTEM. S.S. Ball* and V.H. Mah. Dept. of Pathology, Univ. of Mississippi, Jackson MS 39216 & Div. of Neuropathology, Thomas Jefferson Univ., Philadelpia, PA 19107.

The long range objective of this project is to provide an interactive computer workstation for students, educators, and research investigators in the molecular neurosciences. The workstation will allow an individual to ask a sequence of questions in a single interactive session, thereby facilitating the development and testing of several hypotheses in a short period of time. Graphical representations of molecular events will enable investigators to grasp the spatial, temporal and functional relationships among molecules, cellular compartments, and cell regions. Limited reasoning capabilities will allow an individual to ask sophisticated questions and to predict novel molecular events or pathways. A prototype system, the SECOND MESSENGER SYSTEM (SMS), is under development. SMS contains information about: 1) the molecules of signal transduction processes and the motifs that impart function to these molecules; 2) the molecular events of signal transduction processes; 3) cell-specific expression of genes critical to signal transduction processes. Signal transduction is a particularly appealing area to develop a prototype neurosciences information system for two reasons: 1) the complexity of regulatory cascades that result from numerous but relatively simple interactions provides a system relatively straightforward to represent, and at the same time, exploits something computers do well, keep track of details; 2) the central role that abberrations in signal transduction play in disorders of development, aging, and mental health. SMS is being developed through object-oriented programming in COMMON LISP for remote access on any computer terminal that can run X windows.

203.17

A GENERAL SIMULATION PROGRAM FOR DESCRIBING NEURONAL IONIC SIGNALS. K. Miller and P. Pennefather. Faculty of Pharmacy and MRC Nerve Cell and Synapse Group, University of Toronto, M5S 2S2.

In recent years there has been an explosion of quantitative information concerning the influence of independent variables on the processes that govern ionic signal formation in neurons. Such processes include: gating of ion channels mediated by membrane potential, ions, and chemicals; diffusion and buffering of ions inside neurons; and transport of ions across cell membranes. Computer simulations are extremely useful in helping the student appreciate how these disparate processes and influences interact to generate ionic signals. We have developed a general and open ended simulation program that allows the student to define or consider as many steps as are thought to be needed to describe the system under consideration. A system of ordinary differential equations (ODE's) describing trans membrane ion fluxes and the dynamics of gating and transport mechanisms is solved explicitly with systems of partial differential equations describing buffered diffusion of ions and diffusion of buffers defined to be diffusible. These systems are defined from an intuitive user interface that facilitates definition of the number of states of the channel and transport mechanisms and the influence of ions, chemicals and voltage on the transition rates between states. A numerical integrator for stiff systems of ODE's (Gear method) is included to allow rates to span many orders of magnitude. The program allows voltage clamp and current clamp experiments to be simulated and I/V and gating/V relations to be calculated. A multitude of variables can be plotted or stored in a file. The program runs on the IBM PC platform and is written in the C programming language. On an 80386 machine equipped with a math coprocessor even very complicated systems are simulated at 50-1000x real time. Results of the simulation compare well with other less versatile simulation programs and match the results of analytical solutions for systems where such solutions are possible.

203.18

COMPUTER SIMULATION IN NEUROPHYSIOLOGY INSTRUCTION FOR UNDERGRADUATES. J.G. New and R.R. Fay*, Depts. of Biology and Psychology and Parmly Hearing Inst., Loyola University of Chicago, Chicago, IL 60626.

Teaching neurophysiological principles in a laboratory setting to undergraduate students is often difficult due to limited and expensive equipment, large numbers of students, and the relative difficulty of neurophysiological manipulations. As an alternative to traditional labs, we have been using interactive software as an instructional tool to introduce neurophysiological principles to our undergraduate classes.

We have been using the *Loligo electronicus*™ (EDUCE) and Sensory Neurons (Hill-Fay Assoc, Inc) software in our Neurobiology and Psychobiology courses taught primarily to undergraduates. The *Loligo* program is a "laboratory environment" program that introduces students to both the tools of neurophysiological investigation, as well as principles of cellular physiology. The Sensory Neurons program models the responses of peripheral and central sensory neurons to applied stimuli and teaches principles of sensory coding and processing with an emphasis on the auditory system.

Experiments are performed individually and independently by students with periodic meetings with faculty. The principle advantage of using computers in this type of instruction is that they make each student an "independent investigator", extracting relevant principles of neurophysiology in a "failsafe" laboratory setting in which they become familiar with the tools, strategies and principles of experimental design employed by researchers. Additional advantages include the reduction of costs for acquisition and maintenance of equipment and specimens, avoidance of issues associated with the ethical use of living animals in teaching laboratories, and minimizing student frustration in attempting complex experiments that sometimes fail after considerable effort. Although by no means a total substitute for traditional "wet" labs, computer simulation is a valuable alternative in providing undergraduate students with an investigative experience in neurophysiology.

203.19

COMPUTER SIMULATIONS AS A TEACHING AID IN NEUROSCIENCES. Enrique Soto. *Departamento de Ciencias Fisiológicas, Universidad Autónoma de Puebla, P.O. Box 406, Puebla 72000, México*

Computer simulations seem particularly suited as an aid for teaching some elementary physiological phenomena. A set of programs for this purpose has been develop; membrane potential experiments, Lorente de Nó's classical demonstration of nerve field potential and osmotic phenomena.

Programs are written in *Turbo Pascal*™ to operate in IBM-PC or compatibles. Continuous interactive display of results, and freedom to select display characteristics maintains the user's interest. An introductory tutorial and a users guide, allows usage of the programs without the mediation of a teacher. Programs are fully debugged and protected, and no computer expertise seems necessary to use them.

The programs have been used in several graduate general physiology courses taught at our University. Students acquainted with the required experimental setup, and the type of results that should be expected from an experiment. These turn into a significant increase in their experimental success.

Students were encouraged to understand who the simulation programs are constructed introducing this as a new teaching element. This provides students with a deeper comprehension of the parameters which determine the dynamics of physiological processes.

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203.20

TRION MODEL OF CORTEX AND MUSIC COMPOSITION: AN INTERACTIVE EXHIBIT FOR SCIENCE MUSEUMS Xiaodan Leng* and Gordon L. Shaw. Center for the Neurobiology of Learning and Memory, Univ. of California, Irvine, CA, 92717.

The trion model [Shaw et al. (1985) PNAS, 82, 2364] is based on the Mountcastle columnar organizational principle of cortex. This model of memory and information processing is highly structured in time and spatial connections. "Translating" the probabilistic spatial-temporal firing pattern evolutions of the model onto music [Leng et al. (1990) Music Perception, 8, 49] gave results having the "flavor of a minuet, a waltz, or styles of specific periods of Western art music. A theme can be learned. Further, such musical styles are controllable and are done in real time. Such special features of the model integrate brain theory, music and science into an unique system that can be accessed and learned through hands on experience by people from various background and ages. An interactive trion music exhibit is now being setup at the Science Museum in the Stanford Univiversity Physics Department. Supported in part by the National Asssociation of Music Merchants.

203.21

GENESIS: USE OF A COMPUTER SIMULATION ENVIRONMENT FOR UNDERGRADUATE AND GRADUATE INSTRUCTION IN NEUROBIOLOGY. D. Beeman and J.M. Bower. DIvision of Biology, California Institute of Technology, Pasadena, CA. 91125

Over the last six years we have developed a computer simulation platform for the construction of realistic numerical simulations of different nervous system components. This simulation system, called GENESIS (GEneral NEural SImulation System), is designed to support a wide range of neural models from parts of single cells to large scale network reconstructions. While developed as a research tool, from the beginning we have been interested in the instructional use of the GENESIS system. Accordingly, GENESIS has been used at Caltech and in the course on "Methods in Computational Neurobiology" co-directed by J.M. Bower and C. Koch each summer at the Marine Biological Laboratory in Woods Hole, MA.

Recently, we have begun to make GENESIS available to other universities for both research and educational purposes. Associated with this activity, we have been developing tutorials within the system based on different neurobiological subjects. Currently, for example, tutorials exist for neuronal cable theory and for the dynamics underlying the Central Pattern Generator found in Tritonia. These and other existing tutorials are already being used in several locations around the country. Tutorials provide on screen support for student use and include a series of exercises performed by the student using the simulation. Because GENESIS is in addition a research tool, these tutorials also serve as a mechanism to start the process of building new research related simulations. Supported by NSF grant DIR-9017153.

203.22

MACINTOSH PLATFORMS FOR TEACHING LABORATORY COMPUTING, BIOMECHANICS AND NEUROETHOLOGY J. Ayers, G. Fletcher and K. Hoff, Marine Science Center, Northeastern Univ., East Point, Nahant, MA 01908

We have developed a set of programs which are used in undergraduate and graduate instruction in neuroscience classes. (1) The *Laboratory Toolbox* Hypercard™ stack implements an object-oriented "Laboratory Notebook" structure for the acquisition and management of sensor acquired data with NUBus, SCSI and serial line peripherals. It supports interactive control of consumer-grade VCRs, as well as acquisition and display from frame grabbers and a variety of GWI™ analog interfaces. It provides XCMDs and XFCNs for an interactive instrument programming environment which students can adapt to different experimental paradigms while maintaining records of their activities and acquired data. (2) *Color Image* is an extension of *NIH Image* which supports color acquisition and segmentation of video images as well as interactive control of VCRs. *ColorImage* generates spreadsheet tables of numerical parameters of segmented objects as well as time-series data sets of the motion of objects from digital "movies" acquired from videotape sources. In addition *ColorImage* supports the correlated acquisition of kinematic and electrophysiological data by synchronizing the acquisition of digital "movies" with analog acquisition using A/D converters. (3) *MacGraph* generates parametric graphs from data generated by *ColorImage* including histograms, scattergrams and time-series graphs. (4) *Spike Train* processes analog files generated by the *Laboratory Toolbox, ColorImage* or the MacRecorder™ to segment multi-unit neuronal recordings into the activity patterns of individual units using a clustering algorithm. It generates raster diagrams of the activity patterns of individual units as well as graphical representations of the cluster distributions. The programs generate MacPaint™ files for report generation.
Supported by NSF Grant DIR-8917532

203.23

APPLICATION OF COMPUTER-GENERATED GRAPHICS AND ANIMATION TO TEACHING NEUROPHYSIOLOGY. G.E. Lucier and R. Egizii*. Dept. of Med. Physiol., Univ. of Calgary, Calgary, AB. T2N 4N1.

With the advent of low-cost computer and video systems, the ability to produce sophisticated low-budget audiovisual material is becoming a realistic tool for the teacher. This presentation will highlight the production of a fully animated 5-minute teaching video reviewing the neurophysiological control of orthostatic hypotension. Techniques employed in the production involved photographing cut-out paper characters moved under a single-frame 35mm film camera, as well as computer-generated graphics filmed directly from a CRT screen. All computer graphics were generated on a personal computer and were created by tracing outline material with a stylus on a digitizing pad and then utilizing a drawing and paint program to add color, shading, etc. Each graphic component was stored as a separate file and was recalled and manipulated for assembly of a final screen, which could consist of several files. This provided a stored data base of figures and shapes which could be used for several different composites. The files can be manipulated by use of available word-processing packages such as WordPerfect. Although specific equipment used in this particular production may not be available to every teacher, similar equipment could be easily assembled. The technique of creating a data base of physiological pictographs can not only be used to generate videos, but also for the production of 35mm slides, overheads and other visual material. Once assembled and stored, the data becomes a library of visual information with a variety of uses and applications.

203.24

NEURODYNAMIX: A GRAPHICAL SYSTEM FOR SIMULATING NEURONAL ELECTROPHYSIOLOGY. W.O. Friesen and J.A. Friesen*. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

We have developed a computer-based neuronal modeling system as an aid for teaching electrophysiological concepts to advanced undergraduate and graduate students. This hierarchical system incorporates an integrated set of models that simulates neuronal function at four levels of analysis: the ionic channels observed in membrane patches; the macroscopic membrane currents in nerve-cell compartments (voltage, but not time-dependent currents in passive membrane, and time- and voltage-dependent currents that underlie impulse generation in axons); longitudinal currents in spatially distributed neurons; and synaptic currents that generate excitatory and inhibitory synaptic potentials in neuronal networks.

NeuroDynamix employs a custom-designed graphical user interface that underlies all aspects of the modeling system. This interface generates multiple time-series and phase-plane graphs of model variables. In addition, windows are used to set parameter values, to control a stimulator and to activate the measurement cursor. These windows are available at any time, allowing for instant visualization of the effects of experimental perturbations. The models that comprise *NeuroDynamix* include configuration files with which the user can select (and save) specific experiments for simulation. A set of modeling exercises developed for *NeuroDynamix* are an integral component of our 'Cellular and Molecular Neurobiology' course at the University of Virginia. Students spend class and individual time performing model exercises that are described in a workbook that accompanies the *NeuroDynamix* software.

203.25

HYPERBRAIN, AN INTERACTIVE, MULTIMEDIA SOFTWARE PROGRAM. Suzanne S. Stensaas, Department of Pathology, Cornell University Medical College, 1300 York Ave., New York, NY 10021

HyperBrain was designed to supplement or substitute for neuroanatomy labs (25 hrs). The decreased number of TAs and faculty, scarcity of brains from autopsies, and drop in price of videodisc players and computers prompted a search for alternatives. The program uses an authoring environment, HyperCard, and is based on a syllabus by O. E. Millhouse and a videodisc, **Slice of Life.** The hypertext feature links diagrams, text, glossary, and atlases with images, animations, pathway quizzes as well as graphics from Haines' Neuroanatomy: an Atlas of Structures, Sections and Systems.

Traditional wet labs and 14 VHS videotapes provide other alternatives for students to learn the same material. The 80 hr course (32, lectures; 25, labs, video or computer; 6, small group case studies; 12, neurology correlations; 3, exam) is integrated in time with neurophysiology and psychiatry. Course examinations include neurology cases with multiple short answers, and a traditional practical using kodachromes, radiographs, gross specimens, models, and diagrams. The results of student surveys at the University of Utah during its three years of development will be presented as well as a demonstration of the program. The software is available through the Univ. Utah Medical Library; it can be modified to link or launch other programs. Modules for neurology, head and neck anatomy, ear, eye, neurosurgery, neuropathology, neuroradiology developed at the University of Utah or elsewhere have been linked to the program providing a software shell for medical and graduate students using Apple Macintosh Hardware (Stensaas, S. S. and D. K. Sorenson, 1988. 12th Symposium Computer Applications in Medical Care:p415-420).

203.26

PATHMAC: USING DIGITAL IMAGING AND HYPERTEXT FOR MEDICAL EDUCATION.
Dana Brooks* and Steven M. Erde, Depts. of Cell Biology and Anatomy, and Pathology, Cornell Univ. Medical College, New York, NY 10021.

The computer based teaching lab at Cornell University Medical College originally grew out of a need to help medical students review for the second year pathology course. The first pass, developed 5 years ago, was based on a "user-friendly" computer interface (using Apple Macintosh) and recordable videodisks (Panasonic OMDR). This techology was immediately adapted for the teaching of neuroscience, initially with the creation of a self-paced neuroanatomy tutorial called "MacBrains".

Three years ago, digital imaging and hypertext systems became available on the Macintosh platform. This enhancement in imaging technology allowed an instructor, working at the desktop, to retouch, crop, color correct, and add overlays to neuroscience images. The hypertext system permitted migration away from the linear "frame-oriented" system (too constraining for purposes beyond review of images) to a far more versatile "electronic textbook".

We will demonstrate this improved technology, as currently utilized by medical students at Cornell University Medical College for part of the first-year Neuroscience course. Additional materials, including computer-based simulations developed at our institution (Gardner and Abato 1991, this volume) as well as those produced at other medical schools, are being used to enhance the neurophysiology component of the Neuroscience course.

Supported by Cornell's PATHMAC Project.

203.27

STOCHASTIC REAL-TIME SIMULATION REPRODUCES BIOLOGICAL VARIABILITY AND TIME COURSE.

Daniel Gardner and Michael Abato, Dept. of Physiology and Project PATHMAC, Cornell Univ. Medical College, New York, NY 10021.

VIRTUAL CAT: In an early attempt to simulate a first-year Neuroscience laboratory in plasticity and pharmacology of neuromuscular transmission in the cat, the VIRTUAL CAT was developed in 1986 for the PC/AT. Four classes of students utilized the program in standard teaching labs, often alongside the experiment being modeled. On-screen and hardcopy chart records plot muscle twitch and tetanic tension to nerve stimulation, as modified by each of four neuromuscular drugs. Two design features distinguish this program from other educational software: 1) To accommodate biological variability, the non-deterministic simulation, based on arrays of real data, uses more than 60 pseudo-random parameters to calculate simulated responses within appropriate limits. 2) Delays based on physiological timings stress the time course of drug and posttetanic effects. A goal was for the simulation to be able to pass a modified Turing test: students should not be able to tell from the responses whether they resulted from a live preparation or a simulation.

ALARUM: The main-event-loop based structure of VIRTUAL CAT facilitated implementation of a modified version of the simulation on the Apple Macintosh II, as ALARUM: A LABORATORY ANIMAL REPLACEMENT USING MACINTOSH. Additional advantages include a user interface uniform for Mac applications, a scrollable virtual 'chart record' of the complete experiment, the ability to instantiate multiple virtual cats simultaneously, and integration with other Neuroscience teaching software. This version, which has been in use for two years, will be demonstrated.

Supported by an AEP grant from IBM and by Cornell's PATHMAC Project.

203.28

MacRETINA: SIMULATED MAPPING OF RETINAL GANGLION CELLS.

Richard F. Olivo. Dept. Biol. Sci., Smith College, Northampton, MA 01063.

MacRetina is a Macintosh simulation of an experiment to map the receptive fields of ganglion cells in the cat retina. MacRetina was designed with two principal goals: to provide rapid responses that mimic the "look and feel" of a real experiment, and to represent accurately the dynamic on- and off-responses of ganglion cells and the center-surround characteristics of their receptive fields. Many neuroscience textbooks describe the receptive fields of retinal ganglion cells, but students find the concept difficult to grasp because static illustrations do not readily convey dynamic properties. MacRetina simulates the ganglion cells' dynamic properties, and allows students to conduct experiments that would not otherwise be feasible.

MacRetina's screen has three areas: an oscilloscope trace that displays spikes (which also are heard as clicks), a projection screen where a spot of light is moved and flashed by moving the mouse, and a control zone with buttons to select drawing tools and single or repeated sweeps. Additional controls are available in dialog boxes. By "positioning" an extracellular electrode near different cells, a student can map sustained (X) or transient (Y) cells with on- or off-centers and antagonistic surrounds. The simulated spike trains are remarkably realistic in their appearance and sound, and the receptive field sizes and positions are based on published accounts of retinal anatomy. Help screens provide guidance and background information. The help files are written in MacWrite's simple text format, and can be customized or replaced by instructors who wish to do so.

Supported by a grant from NECUSE, the New England Consortium for Undergraduate Science Education.

203.29

INSIGHT 2 - A SERIES OF FULL-COLOR, INTERACTIVE DEMONSTRATIONS IN VISION SCIENCE FOR THE MACINTOSH COMPUTER.

J.A. Baro* & S. Lehmkuhle. School of Optometry, University of Missouri-St. Louis, St. Louis, MO 63121.

InSight 2 is designed for use in educational laboratories to supplement classes in experimental psychology, experimental methods, sensation and perception, or vision science. InSight 2 provides an interactive, dynamic environment in which students actively participate and can fully appreciate visual phenomena that are not well conveyed in a static medium (e.g., textbooks or slides). As such, InSight 2 is an alternative to traditional educational software, much of which is based on the database/flashcard concept.

All programs have a consistent, easy-to-use HyperCard interface, and also make use of external commands to take full advantage of the Macintosh's graphics capabilties for presenting gray scale/color images and animation. The programs can be divided into two general categories: experiments, which present stimuli, record responses, and graph/analyze data; and interactive demonstrations, which present visual phenomena and permit a variety of student interactions. Each program includes an introduction to the topic, instructions, references, and editable questions. Experiments include Spatial Contrast Sensitivity, Stereopsis and Depth, and Color Vision Test. Demonstrations include Random-Element Stereograms, Additive Color Mixing, Brightness Effects/Lateral Inhibition, Spatial Filtering/Subsampling, and Illusions and Aftereffects.

TUESDAY PM SYMPOSIA

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SYMPOSIUM. MOLECULAR MECHANISMS OF NEUROTRANSMITTER SECRETION. G. Augustine, Duke Univ. (Chair); P. De Camilli, Yale Univ.; R. Llinás, NYU; T. Südhof, HHMI, Univ. Texas; W. Almers, Univ. Washington; H. Betz, Max Planck Institute.

The objective of the symposium is to bring together recent information on the properties of molecules found in presynaptic terminals and the possible functions of these molecules in the secretion of neurotransmitters. To achieve this objective, the symposium will present pairs of speakers who will address putative molecular mechanisms from either a molecular biological or physiological standpoint. Three molecular mechanisms will be considered: (1) *Synapsin I*, which may serve as a phosphorylation-sensitive link between synaptic vesicles and the cytoskeleton (De Camilli, Llinás). (2) *GTP-binding proteins*, which may be involved in vesicle trafficking within the presynaptic terminal (Südhof, Augustine). (3) *Fusion pores*, which may serve as the site of exit of materials from within vesicles and may be produced by synaptophysin, an integral protein of the vesicle membrane (Almers, Betz).

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SYMPOSIUM: THERAPEUTIC POTENTIAL OF NEUROTROPHIC FACTORS K. NIKOLICS, Genentech, Inc. (Chairperson); R. M. LINDSAY, Regeneron Pharmaceuticals; J. A. KESSLER, Albert Einstein College of Medicine; F. HEFTI, Univ. Southern California; D. F. PRICE, Johns Hopkins Univ. Sch. Med.

Molecular and biological properties of neurotrophic factors and their receptors will be discussed. Neurotrophins and other trophic factors have differential effects on specific neuronal subpopulations *in vitro* and *in vivo* that provides the basis of their potential therapeutic applications. Neurotrophic factors stimulate regeneration of mechanically or toxically lesioned peripheral nerves. They also stimulate the survival of central neurons following mechanical lesions and they may prevent toxin-induced neurodegeneration of specific subpopulations of central nerve cells. Rodent and primate animal models of neurodegenerative diseases and attempts of rescuing specific nerve cells by neurotrophic factors will be discussed. Morphological and functional aspects of recovery from lesions as a result of neurotrophic factor treatment will be discussed.

208.1

OSCILLATORY NEURONAL ACTIVITY IN THE SUPERIOR TEMPORAL SULCUS OF MACAQUE MONKEYS. Andreas K. Kreiter and Wolf Singer, Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, 6000 Frankfurt 71, F.R.G.

It has been proposed that synchronization of temporally structured neuronal responses in the cat visual cortex could serve as a mechanism to bind spatially segregated features of visual objects. To further explore this possibility we performed single and multiple electrode recordings in STS of one awake behaving monkey. The animal was trained to fixate a dimming light spot while moving stimuli were passed over the receptive fields of the recorded units. At many recording sites, neurons tended to discharge rhythmically with frequencies between 30 and 60 Hz, epochs with regular rhythmicity lasting about 100 to 300 ms. During these epochs the different neurons recorded from the same electrode discharged in synchrony but these coherent patterns were not phase locked to the stimulus. Response synchronization occurred also between groups of cells recorded from different electrodes separated by 0.3 to 1.3 mm, and again, the synchronous events were not locked to the stimulus. In comparison to the anesthetized cat epochs of patterned and correlated activity were shorter and frequency variations larger. These results suggest that the basic requirements for temporal coding, the quasi periodic time structure of responses and the synchronization of responses of spatially distributed neurons are met in the STS of behaving monkeys.

208.2

ARCHITECTURE OF PRIMATE AREA MT, R. B. H. Tootell and R. T. Born, Dept. Neurobio., Harvard Med. Sch., Boston, Mass. 02115.

To investigate how motion information is extracted and organized, we studied the functional and anatomical organization of area MT. It has an elaborate and interesting architecture with at least three columnar systems.

Studies were done in both Aotus (owl) and Macaque monkeys, using deoxyglucose (both single- and double-labelled: DG and 2LDG) and single unit mapping, microstimulation, histological staining and tracer injections. Among our findings are the following: 1) In 2LDG tests, random-dot arrays drifting in opposed (180^{o}) directions produced interdigitated bands of columns [spacing=800-600 um (macaque); 400-600 um (Aotus)]. This is evidence for a 360^{o} map of direction in area MT. 2) We see no 2LDG evidence of an organization for velocity nor binocular disparity. 3) The 2LDG-labelled retinotopy in macaque MT is patchy and asymmetric, but not diffuse. 4) Microstimulation at parameters used by Newsome and colleagues (Salzman et al, 1990) produced very discrete regions of high DG uptake at the electrode tip (half-width at half-height = 120 um), with minimal activation away from the tip. This supports the idea that microstimulation can preferentially activate cells within a "single" direction column in MT. 5) Extensive DG and single unit mapping indicate a prominent columnar grouping of cells into "bands" and "interbands". Band cells respond well to large-field stimuli such as random dot arrays, and interband cells show surround inhibition. 6) Cytochrome oxidase patchiness is present in MT layers 1-4 in both the New World Aotus monkey (Tootell et al, 1985) and the Old World green monkey (Cercopithecus aethiops). However, the topography and laminar profile of the cytochrome oxidase "patch/interpatch" architecture are different from that of the DG-labelled "band/interband" architecture. In addition, the specific inputs to the patches appear different from those to the bands. This work was supported by EY07980 and T32NS07112.

208.3

TRANSPARENCY AND MOTION INTEGRATION: RESPONSES OF AREA MT NEURONS. G.R. Stoner and T.D. Albright The Salk Institute, La Jolla, CA 92037

Mounting evidence indicates that there are at least two stages of cortical motion processing. Local motion signals detected by the first stage are integrated by the second to yield a representation of 2D object motion. Since local motion signals in close proximity may or may not arise from a common object, factors affecting image segmentation should contribute to the integration process. Using "plaid patterns", we showed previously that perceptual transparency, a reliable image segmentation cue, exerts strong influence over perceptual integration of motion signals.

The two stages of motion processing are manifest by two types of directionally selective neurons in macaque visual cortex. Cells have been classified as component (stage 1) or pattern (stage 2) type based on responses to *perceptually coherent* plaid patterns. A thorough understanding of the integration process, however, requires knowledge about the behavior of both classes of cells under *non-coherent* conditions.

We have compared responses of MT neurons to coherent and non-coherent plaids, using transparency to manipulate coherence. Seventy neurons have been studied in two alert fixating macaques. Approximately 50% of the cells in this sample responded differentially to coherent and non-coherent plaids. When compared to coherent plaids, non-coherent plaids typically yielded enhanced responses to motion of the component gratings and decreased responses to pattern motion. This increase in "component-like" activity was observed for neurons of component, pattern and "unclassifiable" types, although the most striking changes were observed for pattern neurons. As a result, a net increase in component vs. pattern responses was observed for "transparent" plaids.

Thus motion signal integration by MT neurons is "gated" by stimulus factors that affect perceptual integration. As a consequence, the population ratio of component/pattern responses is a good predictor of perceptual coherence. This finding strengthens links between perceptual motion integration and neural processing in area MT, providing further evidence that transparency is used in the coherence "decision".

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208.4

THE USE OF COLOR- AND LUMINANCE-DEFINED EDGES FOR MOTION CORRESPONDENCE. KR Dobkins and TD Albright. Salk Institute, La Jolla, CA

Previous studies from our laboratory have shown that neurons in macaque area MT detect motion of drifting chromatic edges that undergo repetitive contrast reversal (Dobkins and Albright, 1991). This property is predicted by the presence earlier in the magno pathway of neurons that detect chromatic contrast but are unselective for sign. To further explore mechanisms underlying this phenomenon, we have studied motion detection for *achromatic* gratings that undergo repetitive contrast reversal.

In human psychophysical experiments we used chromatic (red/green) and achromatic sine-wave gratings moved by repetitive phase shifts of < 30°. With each displacement, gratings underwent contrast reversal. When presented with isoluminant chromatic gratings, Ss reported motion in the direction of contrast-reversing edges. Away from isoluminance, perceived direction reversed; Ss reported motion in the direction that preserved luminance and color correspondence. When presented with achromatic gratings, Ss reported motion in the direction that preserved luminance correspondence, regardless of luminance contrast.

We used identical stimuli to study MT neurons in fixating macaques. Results were congruent with the psychophysics. For chromatic stimuli, cells selected the direction of contrast-reversing edges at isoluminance but reversed selectivity at non-isoluminance to follow the direction of luminance and color correspondence. When presented with achromatic gratings, however, cells responded in the direction of luminance correspondence. We observed a similar phenomenon using *aperiodic* contrast-reversing patterns (random textures). This result reveals a neural correlate of the "reversed phi" motion percept (Anstis, 1970).

These data demonstrate that under certain conditions the motion system uses information about edges defined by color at the expense of disregarding information about the colors that make up those edges. By contrast, for achromatic stimuli, luminance polarity dictates direction of motion.

208.5

CHROMATIC PROPERTIES OF NEURONS IN MACAQUE MT. J. Anthony Movshon, Daniel Kiper, Jack Beusmans, Karl Gegenfurtner, Qasim Zaidi* and Matteo Carandini. Howard Hughes Medical Institute, Center for Neural Science, New York University, New York 10003, and *Department of Psychology, Columbia University, New York 10027.

The role of chromatic signals in visual motion processing is much debated. Some aspects of motion perception are altered or impaired when isoluminant colored targets are used, but it is clear that chromatic signals can support both motion perception and pursuit eye movements. In macaques, extrastriate visual area MT is thought to be of unique importance in visual motion processing. We have therefore studied the responses of MT neurons to moving grating targets modulated in luminance and chromaticity. We describe our stimuli using the color space of Derrington *et al.* (1984), in which the three cardinal axes correspond to luminance, red-green, and blue-yellow modulations.

Most MT neurons responded briskly and with high contrast sensitivity to targets whose luminance was modulated, with or without added chromatic contrast. When modulation was confined to the isoluminant plane, the responses of all MT neurons were attenuated. Most could in fact be completely silenced by stimulation with targets whose modulation lay within a "null" plane in color space; these null planes varied from neuron to neuron, but all lay within 5° of the canonical isoluminant plane. For a minority of neurons, we could find no color direction at which responses are abolished; these neurons had a definite minimum response for modulation near the isoluminant plane. Responses to isoluminant targets were generally inconsistent, and only intensely modulated targets were effective. The best sensitivity of MT neurons to luminance targets was close to behavioral contrast threshold, but the best sensitivity to chromatic modulation was many times worse.

We conclude that MT neurons can carry weak chromatic signals. But because they respond only at the highest chromatic contrasts, they do not seem to be the source of the chromatic motion signals revealed behaviorally.

208.6

MT NEURONS ENCODE THE SIGNAL-TO-NOISE RATIO OF SIGNALS IN A STOCHASTIC MOTION DISPLAY. Kenneth .H. Britten*, William T. Newsome, and J. Anthony. Movshon§. Dept. of Neurobiology, Stanford University, Stanford, CA 94305 and §Howard Hughes Medical Institute, Center for Neural Science, NYU, NY 10003.

We have previously shown that dynamic random dot stimuli can be used to explore the sensitivity of directional signals in monkey extrastriate area MT. These stimuli, in which a portion of dots carry a correlated motion signal while the remaining dots move randomly, are also well suited for studying the input-output characteristics of these neurons. Increasing the proportion of dots in correlated motion produces a proportional increase in the signal-to-noise ratio of the motion signal across a wide range of correlations. We have explored the effect of this signal-to-noise ratio on the responses of 217 MT neurons by presenting motion in the neurons' preferred and null directions over a range of correlations.

The majority of MT response functions were linear with stimulus correlation; a minority showed nonlinearities, with roughly equal numbers showing positively and negatively accelerating responses. Analysis of the quadratic term of polynomial fits to the data showed that the distribution of this acceleration was unimodal and centered about zero for both preferred and null direction stimuli. Thus most MT cells are dominantly linear in their responses to stimuli of increasing signal-to-noise ratio; this is quite different from their responses to increasing stimulus contrast, which generally show strong saturation (negative acceleration). We are presently exploring the relationship between these data and the predictions of motion energy models of the sort proposed by Adelson and Bergen (1985).

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208.7

DO CELLS IN AREA MT CODE THE ORIENTATION OF A KINETIC BOUNDARY? V.L. Marcar*, S.E. Raiguel, D. Xiao*, H. Maes* & G.A. Orban. Laboratorium voor Neuro- en Psychofysiologie, K.U.Leuven, GHB, B-3000 Leuven, Belgium.

The cells in the area MT of the owl monkey appear to be suited for detecting kinetic boundaries (Allman *et al*, Perception, 1985). Lagae *et al* (Brain Research, 1989) reported that the different layers within area MT of the macaque monkey process motion stimuli of increasing complexity. Does this hierarchical processing within MT include cells, that code the orientation of a kinetic boundary? We investigated this question using the macaque monkey prepared for acute electrophysiological recording.

Our stimuli consisted of 16 random dot fields moving coherently in directions differing by 23° and 8 kinetic boundaries, differing in orientations by 23°, generated by combining two random dot fields moving in opposite directions. Two types of motion attributes were used to define a kinetic boundary; motion parallel and motion orthogonal to the orientation of the boundary. We also used two types of kinetic boundary stimuli, kinetic edges and kinetic gratings.

None of the cells located within area MT showed such selectivity for the orientation of kinetic boundaries. Cells shifted their apparent 'orientation preference' by 90° when the motion attributes changed from parallel to orthogonal to the orientation of the kinetic boundary. The response level of cells to their preferred kinetic edge was 30%-50% of their response level to a random dot field moving in their preferred direction and response levels to a kinetic grating was lower still. The consecutive reversal between 'Preferred' and 'Null' direction of motion of a kinetic grating was therefore much more effective in inhibiting the cell than the single reversal of a kinetic edge stimulus, confirming that cells in MT respond to the local motion only.

Supported by EBRA/Insight

208.8

MICROSTIMULATION OF MT DURING AN EIGHT-ALTERNATIVE MOTION DISCRIMINATION: DIRECTIONAL TUNING OF THE BEHAVIORAL EFFECT. C. Daniel Salzman and William T. Newsome. Department of Neurobiology, Stanford University, Stanford, CA 94305.

Previously, we reported that microstimulation in visual area MT can bias a monkey's perceptual judgements of motion direction towards the preferred direction of neurons at the stimulation site (*Nature* 346: 174-177). Since in those experiments we required the monkey to discriminate between two directions of motion 180° apart, we did not measure the directional tuning of the microstimulation effect. MT neurons, though direction selective, generally respond to motion over a broad range of directions near the preferred (mean bandwidth at half-height = 83°; Albright, 1984). We therefore sought to determine whether activation of such neurons would bias a monkey's judgements towards all directions within the tuning bandwidth of neurons at the stimulation site, or only towards the neurons' preferred direction.

To measure the directional tuning of the microstimulation effect, we trained a monkey on an eight-alternative, forced choice motion discrimination in which each of the eight possible directions of motion was separated by 45°. Thus the monkey's choices spanned 360°. We then applied microstimulation (10 μA, 200 Hz, biphasic pulses) to selected sites in MT while the monkey performed the eight choice discrimination. In 25 experiments, microstimulation induced a choice bias towards a direction within the tuning bandwidth of neurons at the stimulation site. Moreover, the bandwidth of the behavioral effect was typically much narrower than that of the neurons, frequently being restricted to a single direction. This narrow bandwidth suggests that the cortex computes a single, most likely direction of motion consistent with the activity in a population of broadly tuned neurons.

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208.9

MICROSTIMULATION OF VISUAL AREA MT: EFFECTS ON CHOICE BEHAVIOR IN THE ABSENCE OF MOVING VISUAL STIMULI. Chieko M. Murasugi, C. Daniel Salzman and William T. Newsome, Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

We previously reported that electrical microstimulation of MT during a two-alternative, forced choice direction discrimination biases a monkey's perceptual judgments in favor of the preferred direction of neurons at the stimulation site (*Nature* 346, 174-177). In those experiments, microstimulation was applied during the presentation of a dynamic random dot motion stimulus within the receptive field of the stimulated neurons. In a continuing attempt to describe the necessary and sufficient conditions for eliciting the effect, we examined the influence of microstimulation on choice behavior in the absence of moving visual stimuli.

Our methods were the same as in the original experiments except that a small number of "no motion" test trials were randomly interleaved among the usual range of dynamic random dot stimuli. The test trials were of two types: 1) static random dot patterns, and 2) no visual stimulus at all. Microstimulation occurred on half of the test trials. Since there was no "correct" answer on these test trials, the monkey was randomly rewarded with a probability of 0.5. Across 48 experiments, microstimulation in both "no motion" conditions induced a mean increase (t-test, $p < .01$) in the number of choices favoring the preferred direction of the neurons at the stimulation site. On average, the size of the effect on the "no motion" trials was 1/3 - 1/2 of that observed on the randomly interleaved dynamic random dot trials. Thus, microstimulation has its largest influence on the monkey's choice behavior in the context of the normal motion discrimination task on which the monkey was trained. Microstimulation can, however, elicit smaller choice biases in the absence of moving visual stimuli.

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208.10

LIMITATIONS OF THE OPTIC FLOW SELECTIVITY IN MST NEURONS. G.A. Orban, L. Lagae*, D. Xiao*, S. Raiguel and H. Maes*. Laboratorium voor Neuro- en Psychofysiologie, K.U.Leuven, B-3000 Leuven, Belgium.

We previously reported on the selectivity of MST cells for optic flow components (rotation, expansion, deformation). We showed that position invariance of the directional responses for the optic flow components indicates genuine optic flow selectivity (Lagae *et al.*, Inv.Opht.Vis.Sc.,1991). We now report on the limitations of this selectivity.

If these units encode optic flow components, then it is expected that they can extract the optic flow component for which they are selective from a more complex motion stimulus. Therefore, we tested the selectivity of these units for combinations of the selective component which remained constant and another optic flow component or translation which changed. It was found that the majority of the cells tested ($n=14$) remained direction selective only for combinations in which the fixed component was stronger than the variable component. These are combinations in which the direction offset of the local vectors is less than 45 degrees on either side. These units are thus extracting the optic flow component only over a limited range of combinations.

To test the receptive field organization, we compared a normal area summation test with an inverse summation test for a number of speeds. In the former, stimuli of increasing area were presented, while in the latter one, masks of increasing size covered the centre of the receptive field. While the responses increased with increasing stimulus size, masking the centre of the receptive field did not change the response for the optic flow component. This indicates a silent inhibitory discharge region under the excitatory discharge region and correlates with the fact that the centre of an optic flow component is not necessary for the detection of that component.

Supported by EBRA Insight

208.11

MAGNOCELLULAR AND PARVOCELLULAR CONTRIBUTIONS TO THE VENTRAL EXTRASTRIATE CORTICAL PROCESSING STREAM. T.A. Nealey, V.P. Ferrera and J.H.R. Maunsell. Department of Physiology and Center for Visual Science, University of Rochester, Rochester, NY 14642.

The magnocellular and parvocellular subdivisions of the lateral geniculate nucleus (LGN) relay different types of visual information and terminate in different sublaminae in striate cortex (V1). Several lines of evidence support the idea that these channels remain separate in cortex and contribute differentially to the dorsal and ventral extrastriate streams of processing. We have previously used reversible inactivation of LGN laminae to show that area MT in the dorsal cortical pathway is dominated by magnocellular input. We now report the results of similar experiments examining magnocellular and parvocellular contributions to elements in the ventral cortical pathway. We reversibly blocked individual LGN layers with small injections (75-150 nl) of either lidocaine (2%) or GABA (25 mM) while recording from area V4 or the superficial layers of V1. We tested 90 single or multiunit sites in V4 during either magnocellular or parvocellular blockade. As expected, parvocellular blockade reduced V4 responses (mean reduction of 28%). However, magnocellular blockade unexpectedly produced comparably strong effects on the V4 responses (mean reduction of 38%). In V1, 19 sites in the superficial layers of V1 were recorded during magnocellular blockade. Many showed reduced responses, with a mean reduction of 42%. Responses of V1 sites in the interblob regions of V1 showed somewhat stronger effects of magnocellular block than those in the blobs. Thus it appears that elements in the ventral extrastriate cortical processing stream receive substantial excitatory drive from both magnocellular and parvocellular LGN.

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208.12

RESPONSES OF CELLS IN AREA V4 OF MACAQUE VISUAL CORTEX TO SIMULATED 3D SURFACES. Jack L. Gallant, Jochen Braun, and David C. Van Essen. Div. Biology, Caltech, Pasadena, CA.

We have examined neural responses to texture patterns that simulate flat surfaces oriented in 3-dimensional space, in order to ascertain how 3D objects are represented in primate visual cortex. The surfaces were computer-graphic representations composed of circular texture elements, transformed so as to appear to be oriented in 3D space. The flat surfaces we simulated can be parameterized by the slant of the surface normal away from the image plane, and the tilt of the surface normal around the line of sight. Changes in slant and tilt produce changes in the distribution of their 2D Fourier energy, so we also collected data on 2D spatial frequency and orientation tuning.

We studied 106 cells in detail in area V4 of anesthetized, paralyzed macaque monkeys. In 72 cells quantitative analyses were obtained for both 2D grating and 3D texture patterns. About one-third of the cells were tuned for 3D slant and/or tilt, and about half were tuned for 2D orientation and/or spatial frequency. Only a minority of cells responded well to both gratings and textures, but for this subset the optimal 3D and 2D parameters were correlated: the best slant contained textures with high Fourier energy near the optimal spatial frequency, and the best tilt corresponded to the optimal grating orientation. Many cells responded well to gratings but poorly to all textures tried, and some cells responded well to textures but poorly to all gratings, suggesting that these cells are spatially nonlinear. Altogether, we find that many cells in V4 appear to be tuned for the 3D orientation of surfaces, but that for most (but perhaps not all) cells this tuning is a result of the correspondence between their 2D spatial and orientation tuning and the 2D distribution of energy in images of surfaces differing in their 3D orientation.

209.1

GABA RECEPTOR FUNCTION IS ALTERED IN THE C. ELEGANS MUTANT UNC-49. J. R. Mancillas[1,3], A. Ruiz-Morales[1] and R. Olsen[2]. [1]Depts. of Anatomy and Cell Biology and [2]Pharmacology, UCLA School of Medicine and [3]Molecular Biology Institute UCLA, Los Angeles, CA 90024.

Unc-49 is a genetic locus identified by mutation which causes a "shrinker" phenotype. Worms attempting to move backward shrink by simultaneous contraction of dorsal and ventral mucles suggesting a defect in the system of cross-inhibitor GABAergic motorneurons, the DDs and VDs. While DD and VD morphology and axonal trajectories are normal, GABA receptor activity is impaired. Binding studies reveal a 3 fold reduction in receptor-specific GABA binding in unc-49 as compared to wild type. Unc-49 displays a 10 fold increased delay in responses to muscimol [8mM], which inhibits motility measured either as rate of locomotion on an agar plate or as frequency of swimming motions. Analysis of muscle responses to GABA and muscimol in cut worm assays also reveals altered responses in unc-49.

Unc-49 has been cloned by transposon tagging. 2.9 kb of genomic sequences flanking a TC1 insertion were cloned and used to isolate 3 cDNAs (1.5, 2.8 and 3.5 kb) and to identify two genomic YAC clones, Y40C2 and Y52C5, as containing the unc-49 coding region. These YACs overlap and are located in a region of the C. elegans physical map predicted to include the unc-49 genetic locus. We are currently sequencing unc-49, and performing Northern blots of staged mRNA and in situ hybridization to establish its temporal and spatial patterns of expression. Preliminary results using in situ hybridization show the presence of a putative unc-49 message in the body wall musculature.

209.2

CLONING AND SEQUENCING OF GENOMIC DNA FOR THE HUMAN β1 SUBUNIT OF THE $GABA_A$/BENZODIAZEPINE RECEPTOR S.I. Russek* and D.H. Farb. Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA, 02118.

The $GABA_A$ receptor is the major inhibitory neurotransmitter receptor in the CNS. Molecular biological studies demonstrate that the pharmacology of the $GABA_A$ receptor is critically dependent on its subunit composition. *In situ* hybridization reveals a differential distribution of subunit specific mRNAs in different brain regions. To study regulation of $GABA_A$ receptor expression in the nervous system we are identifying the regulatory elements of this gene family. Polymerase chain reaction (PCR) with human genomic DNA was carried out using primers made to the human β1 cDNA. A probe was prepared from the first 133 bp of the β1 cDNA and was used to identify, by Southern hybridization, the relevant PCR products. Subcloning and sequencing of a 1 kb PCR product revealed sequence identity with the 5' end of the human β1 cDNA, and several intron/exon boundaries have been mapped. Using inverse PCR and ligation mediated PCR, we have identified the 5' flanking region of the human β1 gene. To study the cellular regulation of the β1 gene, conditions have been established for the optimal transfection of embryonic chick neurons in primary cell culture. Our previous studies have demonstrated that GABA downregulates receptor number and α subunit specific mRNA levels (Roca et al. Mol. Pharmacol. (1990) 37:37; Montpied et al. J. Biol. Chem. (1991) 266: 6011). Studies suggest that, as for the muscle nAcCh receptor, the 5' flanking region of the β1 gene may contain the regulatory elements necessary for the control of this gene's expression in the nervous system.

209.3

ACTIVATION OF $GABA_A$ RECEPTORS BY ENDOGENOUS GABA RELEASE IN DEVELOPING RAT OPTIC NERVE. K. Sakatani, J.A. Black, and J.D. Kocsis Dept. of Neurosurgery, NYU Medical Center New York, NY 10016, and Dept. of Neurology, Yale University, New Haven, CT 06510

A number of reports indicate that GABA can modulate the excitability of some CNS white matter tracts where synapses and neuronal cell bodies are not present. We studied immunolocalization of GABA and sensitivity of GABA receptors in the rat optic nerve during development by immunohistochemical and electrophysiological techniques.

We report that transient endogenous GABA is present in situ in rat optic nerve and localized to astrocytes and premyelinated axons during the first few weeks of postnatal development; GABA immunoreactivity of the optic nerve is markedly reduced in the adult. Electrophysiological results, obtained by a modified sucrose gap technique, demonstrate that the optic nerve axons are depolarized by the activation of $GABA_A$ receptors and the inhibition of GABA-uptake with nipecotic acid, indicating that functional endogenous GABA and the GABA-uptake mechanism are present in the neonatal optic nerve. Baclofen, a $GABA_B$ receptor agonist, did not result in membrane potential change. Furthermore, the GABA-induced depolarization is extremely attenuated in the adult.

Although the biological role of GABA and $GABA_A$ receptors in the optic nerve is not certain, it is clear that GABA and $GABA_A$ receptor interactions can occur at a nonsynaptic region in CNS white matter in an age dependent manner. The transient nature of the GABA and $GABA_A$ receptor expression is in agreement with other developmental studies suggesting a trophic role of GABA. Alternatively, this interaction may provide for a nonsynaptic signal pathway between glia and neurons. Supported by the NIH, the NMSS, and the VA.

209.4

THE POSTSYNAPTIC ACTIONS OF SPONTANEOUSLY RELEASED GABA. I. Mody and T.S. Otis, Dept. of Neurology & Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA.

Spontaneous miniature postsynaptic currents (sIPSCs) were recorded in dentate gyrus granule cells of 400 μm thick hippocampal slices maintained at 34±1°C in the presence or absence of 1-5 μM tetrodotoxin (TTX). These inhibitory currents generate an average conductance ranging between 200-600 pS (in TTX) which may effectively control the excitability of CNS neurons by providing a filtering mechanism for various inputs reaching the cells.

The sIPSCs were solely mediated by $GABA_A$ receptor activation, since they reversed at the Cl^- equilibrium potential, were blocked by bicuculline methiodide but were unaffected by the $GABA_B$ receptor antagonists 2-OH-saclofen (200 μM) or CGP 35348 (1 mM) while the stimulus-evoked IPSCs (in the presence of CNQX and APV) displayed a prominent $GABA_B$ receptor mediated component. The decay time constant (τ_D, range: 4.2 to 7.2 ms) of the sIPSCs was mostly monoexponential and was dependent on the membrane potential and temperature. A 133 mV change in membrane potential produced an e-fold change in τ_D while the Q_{10} (measured between 22-37°C) was 2.0. As expected, the τ_D of sIPSCs were prolonged in a dose-dependent manner by pentobarbital and the benzodiazepine (BZ) midazolam. The midazolam effect was antagonized by the BZ receptor antagonist flumazenil (RO 15-1788), which by itself had no effect on the τ_D of sIPSCs indicating no endogenous BZ receptor activation. The τ_D of sIPSCs was also modulated by loading the neurons with various concentrations of Ca^{2+} through the patch pipette. Elevating intracellular Ca^{2+} concentration produced a gradual prolongation of the τ_D without a significant change in the rise-time or amplitude of the events. Supported by NIH grants NS-27528 and the Klingenstein Foundation (I.M.) and a Howard Hughes Predoctoral Fellowship (T.S.O.)

209.5

DIFFERENTIAL MODULATION OF STIMULUS-EVOKED AND SPONTANEOUS GABA RELEASE BY PRESYNAPTIC $GABA_B$ AUTORECEPTORS. T.S. Otis and I. Mody, Dept. of Neurology & Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA.

Spontaneous miniature inhibitory postsynaptic currents (sIPSCs) and monosynaptically evoked inhibitory postsynaptic currents (evIPSCs) were recorded in the whole-cell mode in dentate gyrus granule cells of 400 μm thick hippocampal slices maintained at 34±1°C in the presence of CNQX (5-10 μM) and APV (20-40 μM). The evIPSCs, elicited by stimulating in the molecular layer, showed marked paired-pulse depression (30-60%), which peaked at approximately 200 ms inter-stimulus interval, and lasted over 3 s. This depression was sensitive to the $GABA_B$ antagonist CGP 35348 (1 mM). The evIPSCs were also depressed (86±5%) by 1 μM (-)baclofen. To determine the effect of evoked GABA release on sIPSCs, we performed a peri-stimulus analysis, comparing sIPSCs occurring in a 500 ms epoch beginning 100 ms after a stimulus to an identical epoch preceding the stimulus. Post-stimulus, despite the marked depression of evIPSCs, the amplitudes and inter-event intervals (IEIs) of the sIPSCs were statistically indistinguishable from those in the pre-stimulus category. Consistent with this effect, 1 μM (-)baclofen had minimal effects on the average sIPSC amplitude (18±6% depression) and IEI (17±23% increase).

These results strongly suggest that the stimulus-evoked release of GABA causes a presynaptic autoinhibition of subsequent evoked release but it has little effect on the spontaneous GABA release. We conclude that spontaneously active presynaptic GABAergic terminals do not contribute substantially to the evIPSC, and that these terminals have few, if any, functional $GABA_B$ autoreceptors. Supported by a Howard Hughes Predoctoral Fellowship (T.S.O.), and the Klingenstein Foundation (I.M.)

209.6

SINGLE CHANNEL KINETIC PROPERTIES OF NEUROSTEROID REGULATION OF $GABA_A$ RECEPTORS. R.E. Twyman+ and R.L. Macdonald+#. Depts. of Neurology+ and Physiology#, Univ. of Michigan, Ann Arbor, MI.

Neurosteroids have been shown to be potent regulators of $GABA_A$ receptors. The mechanism of enhancement of GABA-evoked current remains unclear, but has been suggested to be 'barbiturate-like'. However, steroids and barbiturates appear to bind to separate sites on the GABA receptor. To determine the mechanism of action on GABA receptor currents, the gating (opening and closing) kinetics of single channel GABA receptors regulated by anderosterone (10 nM - 10 μM) and pregnanolone (100 nM - 10 μM) were analyzed.

Excised outside-out patches were obtained from mouse spinal cord neurons in culture and voltaged clamped at -75 mV in symmetric chloride solutions. Average GABA- (2 μM) evoked current was increased by the steroids, but single channel conductance was unchanged. There were concentration-dependent increases in average channel open and burst durations and channel opening frequencies. Three open and burst duration and two brief intraburst closed duration time constants were unchanged by the steriods. There was a concentration-dependent increase in the relative fraction of the longest open and burst duration time constants which accounted for the increased average open and burst durations. This mechanism of potentiation of single GABA-receptor currents was similar to that previously described for barbiturates and suggested that although their bindings sites may be different, both had a common effector mechanism for enhancement GABA receptor current. The increased opening frequency observed with these steroids, however, has not been described as a consistent finding for barbiturates.

209.7

ACTIONS OF AN ENDOGENOUS STEROID ON CHINESE HAMSTER OVARY CELLS TRANSFECTED WITH THE α1β1 GABA SUBUNIT COMBINATION. C. Hill-Venning, J.A. Peters* and J.J. Lambert. Department of Pharmacology, Ninewells Hospital, Dundee, DD1 9SY, Scotland, U.K.

Some endogenous steroids e.g. 5β-pregnan-3α-ol-20-one (5β3α) are potent positive allosteric modulators of the $GABA_A$ receptor. In an attempt to better understand the molecular basis of this interaction, we have used the whole-cell mode of the patch-clamp technique to examine the actions of 5β3α on GABA-evoked responses in a stable Chinese hamster ovary cell line. These cells have been initially transfected with an expression vector for the α1 and β1 subunits cDNAs of the bovine $GABA_A$ receptor, and transcription was achieved using a dexamethasone-inducible promoter (Eur. J. Pharmac. 189, 77-88, 1990). GABA (50μM) induced an inward current on 44% of cells tested (n = 169) (i.e. successfully transfected cells), while voltage clamped at -55mV. The GABA-evoked current varied considerably from approximately 50pA to 3nA. 5β3α (30nM-300nM) enhanced such currents in a dose-dependent manner. Additionally, application of the steroid alone induced a dose-dependent inward current which was antagonized by bicuculline (10μM), and potentiated by pentobarbitone (10μM). Collectively, these observations demonstrate that the α1β1 $GABA_A$ receptor subunit combination is sensitive to both steroids and barbiturates, and additionally supports the proposal that these agents bind to different sites. We thank E.A. Barnard for the cells and T.G. Smart for technical advice.

209.8

$GABA_A$ RECEPTOR-ACTIVE NEUROSTEROIDS IN THE RAT BRAIN. R.H. Purdy[1], P.H. Moore, Jr.[1], N. Hagino[2*], A.L. Morrow[3], and S.M. Paul[4]. [1]S.W. Found., San Antonio, TX 78228, [2]Univ. Tex. Hlth. Sci. Ctr., San Antonio, TX 78284, [3]Univ. N.C. Sch. of Med., Chapel Hill, NC 27599, [4]NIMH, Bethesda, MD 20892.

Electrophysiological and biochemical studies have shown that the neuroactive steroids 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, AP) and 3α,21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone, alloTHDOC) are the most potent known ligands of the $GABA_A$-receptor complex in the CNS. These endogenous steroids allosterically augment $GABA_A$ receptor-mediated Cl^- ion conductance in a manner similar to that of the anesthetic barbiturates. Using specific radioimmunoassays to measure these steroids in extracts of brain tissue after purification of the steroids by HPLC, we have recently demonstrated that AP is low and alloTHDOC undetectable in the cerebral cortex of male rats, but increased after acute swim stress to about 5-6 ng/g and 1-2 ng/g, respectively. Because of the cyclic nature of progesterone in female rats, we have examined the role of age, the estrus cycle and pregnancy on AP levels in the brain of females. In juvenile and diestrus rats, low but detectable levels of AP (2 ng/g) were found in cortex. A relatively uniform regional distribution was found throughout the female rat brain in proestrus and estrus, ranging from 4-7 ng/g. Aged female rats had cerebral cortex levels of about 3 ng/g. The level of AP reached 12 ± 1.0 ng/g in cerebral cortex of pregnant rats (gestational day 15), where plasma levels were also elevated to 17 ± 2.0 ng/ml. These findings suggest that $GABA_A$ receptor-active steroids may modulate CNS excitability in various physiological states.

209.9

MODULATION OF $GABA_A$ RECEPTOR BINDING BY PREGNANE STEROIDS IN RAT BRAIN. E.H.F. Wong, H.I. Wu*, P. Nelson* and R.M. Eglen*. Institute of Pharmacology and Chemistry, Syntex Research, Palo Alto, CA 94303.

Radiolabel binding studies were done to characterize steroid modulation of binding at the picrotoxin site and the benzodiazepine site of the $GABA_A$ receptor complex. Crude P_2 fractions of rat brain tissue were incubated with selected steroids and either [35S]TBPS or [3H]flunitrazepam for 90 min at 25°C. In rat cortical membranes, the following compounds were shown to inhibit [35S]TBPS binding: 5β-pregnan-3α-ol-20-one (pIC_{50} = 7.01), 5α-pregnan-3α-ol-20-one (6.66), 5α-pregan-3α21-diol-20-one (6.50), 5β-pregnan-3α20α-diol (6.33), 5β-pregnan-3α-20β-diol (6.26), 5β-pregnan-3α-ol-11,20-dione (6.05), 5α-androstan-3α-ol-17-one (5.79), 5β-pregnan-3β-ol-20-one (4.95), 5α-pregnan-3β-ol-20-one (<4), 5β-pregnan-3,20-dione (<4), 5α-pregnan-3,20-dione (<4), and 5β-pregnan-3α,17α,21-triol-11,20-dione (<4). Enhancement of [3H]flunitrazepam binding was demonstrated by these compounds with similar potencies and rank order. Two stereoisomer pairs were chosen to evaluate modulation of binding in the following additional rat brain regions: cerebellum, hippocampus, striatum, midbrain, and brain stem. For both [35S]TBPS and [3H]flunitrazepam binding, the 3α-OH analogs of 5α or 5β pregnan-20-one were approximately 30-fold greater in potency than the corresponding 3β-OH pair. The same results were obtained for all brain regions. While different steroid compounds demonstrated a dose-dependent and stereoselective modulation of binding at the picrotoxin and the benzodiazepine sites of the $GABA_A$ receptor complex in rat brain, no differences among brain regions have been observed so far.

209.10

ENDOZEPINE-2: IDENTIFICATION AND PURIFICATION OF AN ENDOGENOUS LIGAND FOR THE BENZODIAZEPINE RECEPTOR.
JD Rothstein, W Garland[1], G Puia[2], A Guidotti[2], E Costa[2]. Johns Hopkins Univ, Dept. Neurology, Baltimore, MD, 21205; [1]Hoffman-La Roche, Nutley NJ, 07110; [2]FGIN-Georgetown Univ, Washington D.C., 20007.

Recently, we demonstrated the existence of multiple benzodiazepine-like substances (endozepines) in human and animal brains. We have now purified one of these chemicals to homogeneity. Endozepine-2 was purified from rat (1 kg) or bovine (~20 kg) brain using tissue acidification, chloroform/acid extraction, and tandem reverse-phase - silica/organic HPLC. This material appears to be identical to "peak 2" from our previous HPLC purification (J Neurochem 55:2015). Using this protocol, microgram quantities have been purified. The final purified chemical is a non-peptide, weak base with a molecular weight of less than 700. Although the chemical structure is not yet known, gas chromatography/negative chemical ionization mass spectroscopy revealed that endozepine-2 is not halogenated, in contrast to reports by others of halogenated 1,4 benzodiazepines in brain (PNAS 87:5263). Endozepine-2 specifically binds to the benzodiazepine receptor with an apparent nM affinity and a unitary Hill coefficient. Its physiological properties were studied on patch clamped human embryonic kidney cells transfected with cloned GABA receptor subunits $\alpha_1\beta_2\gamma_2$ and in whole cultured rat cortical neurons. Endozepine-2 potentiated GABA mediated chloride currents in a dose response manner ranging from 10^{-8} to 10^{-5} M(diazepam equivalents). Its actions were readily blocked by flumazenil. Endozepine-2 has also been found in microdialysis dialyzates from rat brain. Furthermore endozepine-2 and another endozepine, yet to be characterized, are released by potassium depolarization in microdialysis preparations. The exact molecular weight and chemical structure of endozepine-2 is under investigation.

209.11

BENZODIAZEPINES ACTING AS FULL AND PARTIAL MODULATORS ON NATIVE AND RECOMBINANT $GABA_A$ RECEPTORS
G. Puia, G.Mereu, S. Vicini, and E. Costa. FIDIA-Georgetown Institute for the Neuroscience, Washington DC.

Pharmacological studies (Haefely,W., 1990 TIPS, 11:452) indicated that *in vivo* the positive allosteric modulator of $GABA_A$ receptor, Bretazenil, is endowed of an intrinsic activity much lower than that of other structurally related positive modulators such as diazepam, clonazepam etc. This action could be due to a selective intrinsic activity on a small number of $GABA_A$ receptor subtypes or to a reduced intrinsic activity on every $GABA_A$ receptor subtypes. To investigate which of the two alternative hypothesis was valid, we employed whole-cell recordings of GABA-activated Cl^- currents in human kidney tumoral cells transiently transfected with cDNAs encoding different molecular forms of α, β, and γ subunits of the $GABA_A$ receptor. Bretazenil is a positive allosteric modulator with partial intrinsic activity in each receptor assembly we tested that contains a γ subunit. Dose-response study indicates that bretazenil has lower efficacy but a potency similar to that of diazepam. When we applied Bretazenil together with a high intrinsic activity full positive allosteric modulator that acts on GABA-gated Cl^- currents such as diazepam, the efficacy of the latter was considerably curtailed. The clinical use of a low intrinsic activity allosteric modulator of GABA action may be of interest because its regulated efficacy appears to reduce unwanted collateral effects such as sedation and tolerance that are typical of high intrinsic activity.

209.12

LONG-TERM INFLUENCE OF TETRODOTOXIN AND HISTAMINE ON $GABA_B$ RESPONSES IN THE CNS OF NEWBORN OPOSSUM IN CULTURE. J.M. Treherne, D.-J. Zou*, R.R. Stewart, J.G. Nicholls and N.R. Saunders* Pharmacology Dept., Biocenter Basel, Switzerland and Clinical Neurological Sciences, Southampton University, UK.

The CNS of the newborn opossum, *Monodelphis domestica*, has been isolated and maintained in culture to study neurotransmitter action. Immediately after isolation and after 7 days culture GABA reversibly inhibited synaptic transmission in the spinal cord. Inhibition involved both $GABA_A$ and $GABA_B$ receptors as determined by selective agonists (muscimol and baclofen) and antagonists (bicuculline and CGP 35348). Dose-response curves for both $GABA_A$- and $GABA_B$-mediated responses remained virtually unchanged over 7 days in culture. $GABA_B$-mediated responses to baclofen, however, were virtually abolished by chronic exposure to L-histidine (150 μM). Modulation of $GABA_B$ receptors was dependent on electrical activity, since TTX (which reversibly blocked all action potentials) protected preparations from the actions of L-histidine. Further experiments suggested that L-histidine acted indirectly and was converted to histamine. These results demonstrate the potential of this preparation for the study of receptor regulation and synaptic plasticity during development.

210.1

EVIDENCE THAT PNMT-CONTAINING NERVE TERMINALS IN RAT SPINAL CORD ARE NORADRENERGIC. A.F. Sved, C.T. Hasselman*, M.G. Backes, J.N. Salter*. *Department of Behavioral Neurosciences, University of Pittsburgh, Pittsburgh, PA 15260.*

We have previously reported that, although the intermediolateral cell column (IML) of the rat spinal cord is densely innervated by the C1 PNMT-containing catecholamine cell group in the ventrolateral medulla, the IML does not contain epinephrine. In the present study, two approaches were used to address the hypothesis that the terminals in the IML arising from the C1 neurons are instead noradrenergic. First, the effect of destruction of the A5 cell group on norepinephrine (NE) level in the IML was determined, since the A5 cell group in the caudal pons is the only classical noradrenergic cell group to innervate the IML. Two weeks following complete bilateral lesions of the A5 cell group (less than 5% of the A5 cells remaining) by local administration of 6-hydroxydopamine, NE levels in the IML were reduced but not eliminated (2.17±.22 pg/mg protein compared to 5.30±.49 in control rats; n=6; p<0.01). Next, the effect of electrical stimulation of the region containing the C1 cell group on NE levels in the IML was determined in anesthetized rats (urethane, 1.5g/kg i.p.). Stimulation of the C1 region (0.2 msec. pulses at 100 Hz, 100uA, 1 sec. trains at 0.5 Hz for 45 min.) in rats treated with a tyrosine hydroxylase inhibitor (alpha-methyltyrosine methyl ester, 400 mg/kg i.p.) produced a significant decrease in NE level in the IML (6.40±.35 compared to 7.96±.33 in non-stimulated controls; n=6-8; p<0.01). These results support the hypothesis that terminals in IML arising from the C1 neurons are noradrenergic.

210.2

GLUCOCORTICOID CONTROL OF ADRENERGIC FUNCTION IN VIVO. D. L. Wong, A. Lesage*, B. Siddall* & J. W. Funder*. Dept.Psychiatry & Beh.Sci., Nancy Pritzker Laboratory, Stanford Univ.Sch.Med., Stanford, CA 94305

A glucocorticoid responsive element (GRE), present in the 5' regulatory region of the rat PNMT gene, acts classically as a transcriptional enhancer in vitro. However, in mature rats, glucocorticoids (GC) control adrenal medullary PNMT by regulating its degradation through the cosubstrate, S-adenosyl-methionine. To examine the in vivo role of the GRE, the effects of RU28362, a type II GC agonist, and dexamethasone (DEX), a mixed type I and type II GC agonist, on PNMT activity and mRNA were compared. Drugs were administered over 3 log doses (1-1000 µg daily i.p.). RU28362 reduced PNMT activity in a dose dependent fashion, with a plateau at 68% of control (≥100 µg). While low doses of DEX (≤30 µg) similarly decreased enzyme activity, high doses reversed this suppression, so that activity was again normal. For both drugs, PNMT mRNA is not significantly different from normal at low doses, but increases 6-10 fold at high doses, with DEX again showing greater potency. Thymus weight, an indirect measure of plasma corticosterone (CORT), and adrenal weight reached a plateau of 9% and 50-60% of normal weight respectively after steroid treatment. When the two steroids were administered to hypophysectomized animals, both replenished PNMT activity, but to no greater than 30% of normal values, despite the fact that PNMT mRNA was substantially elevated. Again, DEX was more effective, but less effective than ACTH at reversing PNMT depletion. To examine the involvement of a GC type I receptor (classical mineralocorticoid receptor), aldosterone was elevated by feeding rats a diet of rice and administering the diuretic lasix (4 mg/day i.p.). Neither PNMT activity or mRNA were significantly altered. Thus, while corticosteroids may control PNMT transcriptional activity in vivo, they or unidentified factors also exert posttranscriptional controls on PNMT expression. Moreover, these translational/posttranslational regulatory mechanisms seem to determine the limits of adrenergic function.

210.3

GLUCOCORTICOID-MEDIATED TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE. A. Lesage*, S. Levine and D.L. Wong. Dept of Psychiatry & Beh. Sci., Nancy Pritzker Laboratory, Stanford Univ. Sch. Med., Stanford, CA 94305.

To examine the role of corticosteroids in the in vivo regulation of phenylethanolamine N-methyltransferase (PNMT) transcription and translation, steady-state levels of PNMT activity and mRNA were analyzed in the adrenal glands of intact and hypophysectomized rats, following administration of ACTH (4 I.U. s.c.) or dexamethasone (DEX, 1 mg i.p.) for 7 days. In intact animals, PNMT activity is depressed to 85% and 78% of control values by ACTH and DEX respectively, while PNMT mRNA is not significantly altered. Hypophysectomy also reduces PNMT activity, but more markedly, to 13% of control levels. ACTH and DEX administration reverses this depression and restores activity to 32% and 27% of normal values. However, changes in PNMT mRNA are not coincident; while hypophysectomy decreases PNMT mRNA to 15% of control, ACTH only restores message to 22% while DEX increases message to 48% or twice the restoration observed for PNMT enzymatic activity. Effects of dexamethasone were further investigated in hypophysectomized rats by administering a range of doses (0.2-3.0 mg daily i.p.). As previously, hypophysectomy reduced PNMT activity and DEX reversed this effect; maximum restoration was comparable to the 1 mg daily dose, and doses ≥1 mg daily showed no significant differences in their restorative capacity. PNMT mRNA was again elevated to a greater degree than PNMT activity. These results are consistent with independent regulatory mechanisms for PNMT transcription and translation. Transcriptional control may be mediated via the glucocorticoid responsive element (GRE) identified in the 5' regulatory region of the rat gene, which has demonstrated in vitro functionality. In addition, however, glucocorticoids or other factors appear to regulate PNMT translation and hence, are enzyme limiting.

210.4

COOPERATIVE INTERACTION BETWEEN TRANSMITTER RECEPTOR FUNCTION AND STEROID HORMONES ON *NUR/77* (*NGFI-B*) GENE EXPRESSION IN ASTROCYTOMA CELLS. A.M.Szekely, D.R.Grayson and E.Costa. FGIN, Georgetown University, Washington D.C. 20007.

In glial cells transmitters, hormones and other extracellular signals change the expression of specific genes encoding for growth factors or cytokines. These changes presumably are mediated by cooperative interactions among various classes of specifically targeted transcription factors (nuclear "third messengers"). In the C6-2B rat astrocytoma cell line, β-adrenergic receptor stimulation by isoproterenol (1 uM), in a manner sensitive to propranolol (10 uM), causes a transient induction of *c-fos* and of *nur/77* mRNAs by 5-6 and 7-9 fold, respectively. *Nur/77* mRNA encodes a protein highly homologous to members of the ligand-dependent transcription factor steroid/thyroid receptor superfamily. Dexamethasone (100 nM) also increases *nur/77* mRNA content by 3 fold, which increase is sensitive to RU-486 (5 uM), an antagonist of the glucocorticoid/progesterone receptor. Co-treatment of the cells with dexamethasone and isoproterenol leads to a synergistic increase of *nur/77* mRNA content, but to an antagonism of *c-fos* mRNA induction, suggesting a differential regulation of these genes. Progesterone, testosterone, 17-β-estradiol (100 nM), as well the peptide hormone ACTH (10 nM) fail to change *c-fos* or *nur/77* mRNA expression. However, when progesterone receptor expression is amplified by estrogen pretreatment, progesterone (100 nM) results in a 2.5 fold increase of *nur/77* mRNA content. This astrocytoma cell line, similarly to certain glia cells, synthesizes steroid hormones in a manner facilitated also by "mitochondrial" benzodiazepine receptors (MBR) agonists. Therefore it is of significance to further elucidate the mechanisms, whereby steroid hormones modulate the pattern of gene expression elicited by transmitter receptor-mediated changes of nuclear third messengers.

210.5

COMPARISON OF THE D1 DOPAMINE AGONISTS SKF-38393 AND A-68930 ON BEHAVIORAL RESPONSES AND C-FOS PROTEIN INDUCTION IN 6-OHDA-LESIONED RATS. K.B. Johnson, K.F. Jensen, H.E. Criswell, R.A. Mueller, and G.R. Breese. Neurobiology Curriculum, and Brain and Development Research Center, UNC School of Medicine, Chapel Hill, NC 27599.

In order to examine the specificity of c-fos induction as an indicator of D1-dopamine receptor supersensitivity, SKF-38393-induced behavior and c-fos responses were compared with changes induced by A-68930, a new D1 dopamine agonist, in 6-OHDA-lesioned rats. The two agonists produced similar behavioral responses, but the behavioral activity induced by SKF-38393 was greater than that for A-68930. The time courses for FLI by both agonists were found to peak at 2 hrs. after drug administration, decreasing to baseline levels after 8 hrs. Dose-response relationships for both agonists were observed on FLI. Even though activity with quinpirole, a D2 agonist, was greater than that for D1 agonists, quinpirole produced no increase in FLI, eliminating activity as a factor in the FLI increase seen with D1 agonists. Further, the FLI induction by both D1 agonists could be effectively blocked with SCH-23390, a D1 dopamine antagonist. The NMDA antagonist MK-801, at a dose of 1 mg/kg, failed to block D1 agonist-induced FLI, indicating that an NMDA link is not involved in this response, as it is in the development of D1-dopamine receptor sensitivity (i.e. priming). These results indicate that c-fos induction may be a specific and sensitive indicator of D1-dopamine receptor supersensitivity. (Supported by HD23042).

210.6

NEW CATECHOLAMINERGIC BRAIN AND ADRENAL CELL LINES FROM TRANSGENIC MICE. C. Suri, B. Fung and D. M. Chikaraishi. Neurosci. Prog., Tufts Univ. Sch. of Med., Boston, MA 02111.

New catecholaminergic cell lines were derived from adrenal and brain tumors in which SV40 T antigen (Tag) was under the control of 773 bp of the rat tyrosine hydroxylase (TH) 5' flanking sequences. CNS tumors spanned the midbrain and anterior brain stem regions on the ventral surface of the brain, whereas a single massive adrenal tumor in a separate founder occupied almost half of the upper abdominal cavity. Immunohistochemistry revealed the presence of both TH and Tag within the tumors.

Cell cultures were derived using standard techniques. Various subclones differing in morphology and TH expression have been selected from the adrenal culture. Their TH specific activities range from .05-2.02 nmoles DOPA/mg protein/min. The parent culture produces dopamine (DA, .28 nmoles/mg protein) and norepinephrine (NE, .35 nmoles/mg protein). Earlier passages also produced epinephrine (E). Subclones are also being isolated from two brain cell cultures which express both TH and Tag. These cells extend varying lengths of processes without treatment. Their TH specific activities are .11 and .21 nmoles DOPA/mg protein/min. and both synthesize DA and NE. Adrenal and brain cell cultures stain positively for NF and are negative for GFAP. Further characterisation of individual subclones with respect to catecholamine uptake, regulated secretion and expression of receptors for NGF, ACh, GABA is underway.

210.7

UNBIASED ESTIMATION OF TOTAL NUMBER AND MEAN CELL VOLUME OF PIGMENTED NUCLEUS LOCUS COERULEUS NEURONS IN THE HUMAN AND MONKEY BRAINSTEM: P.R. Mouton*, B. Pakkenberg, R.S. Burns, and H.J.G. Gundersen. Neurological Research Lab., Bartholin Institute, Kommune Hospital, 1399 Copenhagen K; Stereological Research Lab., Dept. Anatomy, Århus Univ., 8000 Århus, Denmark.

Nucleus locus coeruleus (LC) is a compact group of densely pigmented neuron cell bodies in the dorsal mammalian brainstem. Projections from LC provide the primary noradrenergic innervation to the entire neural axis. Functionally, LC appears to regulate attentional and motivational states, and may exert an inhibitory influence on impulse flow in the neocortex. The importance of quantitating LC parameters is underlined by a general age-related disruption in noradrenaline levels, in addition to reports of severe changes in LC neuron number and noradrenaline transmission in dementia conditions, including Alzheimer and Parkinson diseases. Innovative and unbiased neurostereological methods developed within the last decade, the optical disector/Cavalieri combination for total number and the rotator method for mean cell volume, were used to estimate total number and volume of LC pigmented neurons in aged, Alzheimer, and Parkinson diseased brains, and total LC number in aged and young monkey brains. In addition to the methods for these highly efficient, precise, and inexpensive estimations, results will be presented which are available as far as codes have been broken in these continuing studies.

210.8

CHOLINOLYTIC SEIZURES: POSSIBLE ROLE OF NUCLEUS LOCUS COERULEUS (LC). M. El-Etri, M. Ennis, M.T. Shipley, and W.T. Nickell. Dept. Anat., Univ. Cinti. Coll. Med., Cinti, OH. 45267.

We are determining the earliest genetic, cellular and neurochemical changes during organophosphate-induced seizures. We have reported that systemic soman injection in rats produces rapid (1-2 hr), profound depletion of forebrain norepinephrine (NE) and induces a wave of early (30-45 min) c-fos expression in only two areas: piriform cortex and LC. Soman microinjection into LC rapidly and tonically increases LC discharge by 3-7 fold (blocked by iv scopolamine, and induces c-fos in LC cells. Thus, soman inhibition of AChE appears to cause tonic hypercholinergic stimulation of LC, leading to depletion of forebrain NE. It is possible, however, that seizures, and not cholinergic hyperstimulation, causes LC activation during soman-induced convulsions.

New results suggest that seizures are not necessary for LC activation. Systemic soman injection increased the discharge rate of LC neurons in anesthetized rats by 3-7 fold and caused desynchronization of cortical EEG, but not seizures. Systemic pilocarpine (380 mg/kg, im) also activated LC neurons in the absence of seizures. By contrast, the GABA antagonist picrotoxin caused only a transient (30-60 sec), 0.5 - 1.0 Hz increase in LC activity, however, LC neurons discharged in their characteristic regular pattern during prolonged seizures.

These findings indicate that cholinolytic agents potently activate LC neurons in the absence of cortical seizures. Moreover, LC neurons can discharge in a normal manner during intense seizures. Thus, depletion of forebrain NE induced by soman may be due to cholinergic hyperstimulation of LC rather than to seizures. (US Army DAMD17-86-C-6005 and PHS Grant NS24698).

210.9

ARE DOPAMINE SYNTHESIS-MODULATING AUTORECEPTORS PRESENT IN PRIMATE BRAIN? J.D. Elsworth, D.E. Redmond Jr. and R.H. Roth Depts. Pharmacology and Psychiatry, Yale University Sch. Medicine, New Haven, CT 06510.

In the rodent brain, many dopamine (DA) neurons are equipped with synthesis- and impulse-modulating autoreceptors which regulate their biochemical and physiological activity. However, it is not known whether autoreceptors are present in primate brain, which hinders the logical development of pharmacological treatments for conditions or diseases that involve central DA dysfunction. Thus, in the monkey we have applied an *in vivo* paradigm, which was developed in the rat, to identify those DA neuronal systems in the primate that possess synthesis-modulating autoreceptors. This method is based on the observation that DA neurons with autoreceptors respond to cessation of impulse flow by increasing tyrosine hydroxylase activity, measured by the short-term accumulation of DOPA after inhibition of DOPA decarboxylase activity.

Under light pentobarbital anesthesia, vervet monkeys were treated with gamma-butyrolactone (GBL; 400 mg/kg i.v.) to interrupt impulse flow in DA neurons, or saline 20 min before sacrifice. Five min later all animals received NSD-1015 (100 mg/kg i.v.), a DOPA decarboxylase inhibitor. Under deep anesthesia, the monkeys were killed. Brain regions were removed and later analyzed for DOPA concentration by HPLC. In several subcortical brain regions (eg. putamen, caudate, accumbens) DOPA levels were increased by GBL treatment. However, preliminary data suggest that in other regions (eg. cingulate cortex) this does not occur. Thus, this model provides evidence for the presence of autoreceptors in some regions of primate brain.

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210.10

EFFECT OF NEUROPEPTIDE Y ON THE RELEASE OF DOPAMINE IN FREELY MOVING CONSCIOUS RATS USING MICRODIALYSIS. T.C. Westfall, V. Tseng* and L.E. Vickery* Dept. Pharmacol. & Physiol. Science, Saint Louis Univ. Sch. of Med., St. Louis, MO 63104

We have previously observed that neuropeptide Y (NPY) decreased the nerve stimulation induced release of NE from peripheral sympathetic neurons and the K^+-evoked release of NE from hypothalamic slices (Synapse 2:299, 1988). NPY neurons are also known to be present in the striatum in close juxtaposition to dopamine (DA) neurons. The present studies were designed to examine the effects of NPY on the evoked release of dopamine using in vivo microdialysis. Rats were anesthetized with ketamine/acepromazine and a guide cannula stereotaxically implanted into the striatum. The microdialysis probe was introduced into the guide cannula after 5-7 days and the experiment carried out in conscious freely moving rats. The microdialysis probe was perfused with Ringer's solution (2 μl/min). Fractions were collected at 20 min intervals for analysis of DA and metabolites by HPLC-EC detection. K^+ (30 mM) was administered twice 120 (S_1) and 250 (S_2) min after the start of perfusion. In other experiments NPY (10^{-7}M) was introduced 20 min before the second stimulation with K^+. Basal DA levels could be readily measured in the dialysate from microdialysis probes placed in the striatum. Removal of extracellular Ca^{+2} resulted in a marked lowering of basal DA levels. Introduction of K^+ produced a marked increase in the overflow of DA. With S_1 resulting in a 861 $\pm$ 230 and S_2 639 $\pm$ 232 % increase over basal. The presence of NPY during S_2 resulted in a significant attenuation of the K^+-evoked increase in overflow with a 212 $\pm$ 39% increase over basal ($p < .01$). These results are consistent with NPY exerting an inhibitory effect on DA transmission in the striatum (Supported by HL26319, 35202 and DA02688).

210.11

EXTRACELLULAR INOSITOL HEXAKISPHOSPHATE ($InsP_6$, PHYTIC ACID) AND ADRENAL CHROMAFFIN CELLS: BINDING AND EFFECT ON Ca^{2+} FLUXES AND CATECHOLAMINE SECRETION. D.J. Reis, S. Regunathan and C. Wahlestedt. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

In brain $InsP_6$ possesses extracellular binding sites (Hawkins et al., *BBRC* 167:819, 1990). To establish whether such $InsP_6$ is biologically active, we have investigated the possibility that $InsP_6$, which is contained in adrenal chromaffin cells (Sasakawa et al., *JBC* 265:17700, 1990), (a) binds to specific membrane sites; (b) elicits Ca^{2+} influx; and (c) modulates catecholamine secretion in primary culture of bovine adrenal chromaffin cells. ^3H-$InsP_6$ bound specifically to a standard membrane preparation with a K_d of 90 nM and a B_{max} of 700 fmol/mg protein. The Hill coefficient was not significantly different to unity. Association of ^3H-$InsP_6$ was relatively slow, with equilibrium binding being reached within 10 min. Dissociation of ^3H-$InsP_6$ showed monophasic kinetics. In intact cells, $InsP_6$ elicited a concentration-dependent facilitation of $^{45}Ca^{2+}$ influx and release of epinephrine and norepinephrine, the responses being slower and longer-lasting than those elicited by depolarization by nicotine and K^+. Catecholamine release by $InsP_6$ required extracellular Ca^{2+}. Reduction in number of phosphorylated sites on inositol resulted in gradual loss of binding and secretagogue potency. In conclusion, adrenal chromaffin cells contain specific $InsP_6$ binding sites functionally coupled to the regulation of Ca^{2+} influx and catecholamine secretion. Conceivably, IP_6 may act as a neurotransmitter/hormone in adrenals and possibly brain.

210.12

HETEROGENEITY OF THE PREFRONTAL CORTICAL DOPAMINE SYSTEM IN RESPONSIVENESS TO STRESS. Ariel Y. Deutch. Yale University School of Medicine, New Haven, CT 06510.

We have recently demonstrated that stress evokes Fos protein expression in a distinct subpopulation of midbrain dopamine (DA) neurons which project to the medial prefrontal cortex (PFC). This observation suggests that the dopamine innervation of the PFC may be biochemically heterogeneous is response to stress. In order to determine if there are differences in the magnitude of stress-elicited changes in DA utilization in various PFC regions, animals were subjected to mild stress and different regions of the PFC dissected for biochemical analyses. Low intensity footshock stress or alternatively short duration immobilization stress resulted in differences in the degree to which DA utilization was augmented in the PFC. The magnitude of stress-induced increases in utilization was prominent rostrally within the medial cortical region. We also demonstrated differences in the susceptibility of the DA innervations of different nucleus accumbens compartments (core and shell) to stress. Ongoing analyses will determine the relationship between the precise PFC sites responsive to stress and the subcortical targets of these areas. These data further illustrate the functional heterogeneity of the PFC DA innervation, and suggest that specific DA innervations of the cortex may be in register with specific corticofugal pathways to form a stress circuit. Supported by MH-45124 and the National Parkinson Foundation Center at Yale University.

211.1

BOVINE TYROSINE HYDROXYLASE GENE - REGIONS MEDIATING ACTIVATION BY PMA AND C-FOS/C-JUN. M.K. Stachowiak, A. Goc., E. Puchacz*, E.K. Stachowiak*, Barrow Neurol. Inst., Phoenix AZ 85013

Previous studies in our laboratory demonstrated regulation of tyrosine hydroxylase (TH) gene expression by protein kinase C (J. Biol. Chem. 1990, 265, 4694). In this study we examined whether the 5'flanking region of the TH gene contains elements that mediate this regulation. SH-SY5Y human neuroblastoma cells were lipofectin-transfected with plasmids containing upstream sequences of the bovine TH gene fused to CAT reporter gene. Subsequently, cells were treated with 0.1 uM PMA or incubated in drug-free control medium. PMA treatment induced a 6.3 fold increase in CAT expression directed by the -245/+21 fragment of the TH promoter. CAT expression controlled by longer -1523/+21 fragment was less affected by PMA (1.6 fold increase). Deletion of sequences between -265 and -52 nt abolished PMA stimulation. These observations indicate that main PMA regulatory element is located in the -245/-52 region and that additional negative modulatory element(s) exist in more distal regions of the TH promoter.

Our previous experiments demonstrated binding of AP1/c-Fos-like factors to multiple sites in the TH promoter and its stimulation by protein kinase C-activating agents (Mol. Cell. Neurosci. 1990, 1, 202). In the present study the -245/-52 region of the TH promoter conferred activation of TH-CAT gene by c-Fos and c-Jun expressed from cotransfected plasmids. In contrast to PMA effects, this activation was enhanced by sequences upstream from -245. In conclusion, PMA activation of the TH gene could be mediated by c-Fos and c-Jun, however additional inhibitory factor(s) appear to participate in the regulation by PMA.

211.2

FUNCTIONAL DISSECTION AND MOLECULAR ANALYSIS OF THE UPSTREAM SEQUENCE OF TYROSINE HYDROXYLASE (TH) GENE. K.S. Kim, J.M. Carroll, M.K. Lee*, D.H. Park and T.H. Joh. Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605.

TH, the first and rate-limiting enzyme in catecholamine biosynthesis, is expressed in neurons which synthesize and release catecholamines. Sequencing of the 5' flanking region of the rat TH gene revealed some putative regulatory sites (AP1, AP2, POU/Oct, Sp1 and CRE) within 250 base pairs (bp) as well as a 35 GT pair repeat at around 2.5 kb from the transcriptional start site.

To investigate the underlying molecular mechanisms of TH gene regulation, the model systems used were two human neuroblastoma cell lines which are either adrenergic (SK-N-BE; TH-expressing) or cholinergic (SK-N-MC; TH-nonexpressing). Different lengths of upstream sequence of the rat TH gene were fused to the bacterial CAT gene. Transient transfection analysis of these constructs showed that the 150 bp flanking region retains sufficient information for full transcriptional activity in the SK-N-BE cell line. In contrast, none of these constructs derived any significant level of expression in the SK-N-MC cell line. Furthermore, site-directed mutagenesis of the putative regulatory motifs demonstrated their functional importance in TH expression. These data suggest that the utmost 5'flanking sequences play a critical role in TH gene regulation.

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211.3

BOVINE TYROSINE HYDROXYLASE GENE - REGIONS CONTROLLING EXPRESSION IN NEURAL CELLS. A. Goc, E. Puchacz*, E.K. Stachowiak*, M.K. Stachowiak, Barrow Neurol. Inst., Phoenix AZ 85013.

In this study we examined whether the 5'flanking region of the bovine tyrosine hydroxylase (TH) gene contains genetic elements that determine its cell specific expression in the nervous system. Chimeric TH-CAT genes were prepared by ligating a -1523/+21 nt fragment of the TH gene to the coding region of the CAT reporter gene. TH promoter mutants were derived by deleting sequences upstream from -245 nt [pTH(dP)CAT] or between -265 and -52 nt [pTH(dA)CAT]. As a control, promoterless pCATbasic plasmid and pCATcontrol containing the SV40 promoter were used. Plasmids were lipofectin-transfected into neuron-like neural crest-derived cell lines, human SH-SY5Y and rat PC12, and into glia-like SF-763 glioma cells. In PC12 and SH-SY5Y cells CAT expression from the wild type TH promoter occurred at the level 12-15 times higher than from the SV40 promoter and 2-3 higher than from mutant pTH(dP)CAT and pTH(dA)CAT plasmids. No expression from promoterless pCATbasic was detected. In glioma cells the levels of CAT expression from wild-type pTHCAT did not differ from the levels expressed from promoterless pCATbasic and were 100 times lower than expression from the SV40 promoter. In contrast to SH-SY5Y, SF-763 cells transfected with pTH(dP)CAT or pTH(dA)CAT expressed CAT activity at the levels 11-14 times higher than from the wild type TH promoter. In conclusion the 5'flanking region of the TH gene contains elements that control its cell specific expression. Regions that support TH promoter function in neural crest-derived cells also contain elements that repress expression in the glia-derived cells. Supported partially by APDA and Women's Board of BNI.

211.4

TISSUE SPECIFIC EXPRESSION DIRECTED BY UPSTREAM SEQUENCES FROM THE RAT TYROSINE HYDROXYLASE GENE IN TRANSGENIC MICE. S.A. Banerjee and D.M. Chikaraishi, Dept. of Microbiology and Molecular Biology and Neuroscience Program, Tufts Univ. Schl. of Med., Boston, MA 02111.

To identify sequences that direct tissue-specific expression of tyrosine hydroxylase (TH) in vivo, we generated 4 lines of transgenic mice with 4.8 kb of 5' flanking TH sequences linked to a chloramphenicol acetyl transferase (CAT) reporter. Only one line expressed the reporter and it did so in a tissue-specific manner. CAT activity was detected in lysates from the olfactory bulb, brain, superior cervical ganglia (scg), and adrenal, tissues containing TH expressing cells. However, CAT was expressed to higher levels in the bulb and brain than the adrenal or scg, suggesting preferential expression in the CNS. In contrast, all non-TH expressing organs (liver, kidney, spleen, heart) lacked CAT activity.

We also studied the regulation of CAT in the olfactory bulb where others have shown that TH is transsynaptically regulated; deafferentation induces loss of TH. Denervation of the bulb by $ZnSO_4$ lavage induced a parallel loss of CAT and TH activity in transgenic mice. During development, CAT expression increased linearly after birth in the bulb, similar to the postnatal rise in TH levels, but dropped slightly in the adult. We have thus obtained transgenic mice in which TH regulatory sequences direct correct tissue-specific, developmental and transsynaptic expression.

211.5

TRANSLATIONAL CONTROL OF POMC GENE EXPRESSION. CM Spencer & JH Eberwine. Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

In the rat anterior pituitary, proopiomelanocortin (POMC) gene expression is stimulated by corticotropin releasing hormone and inhibited by glucocorticoids. The time course of these responses is such that changes in peptide levels are apparent within minutes of treatment and hours before changes in POMC mRNA levels are detected. Translational control is therefore a potentially important aspect of POMC gene expression that may play an especially critical role in the acute responsiveness of the cell.

In order to evaluate the translational status of POMC in rat pituitary and AtT-20 cells, we have utilized *in situ* transcription followed by electrophoresis of the labeled newly-synthesized cDNAs. The banding pattern that is generated correlates to the sequence of the mRNA and reflects the progression of reverse transcriptase as it travels along the mRNA. The observed changes in the IST-generated banding patterns can be correlated to changes in translational status as assessed by polysome profiles. Furthermore, the banding pattern can be altered by pharmacological manipulations expected to influence translational status.

POMC mRNA is predicted to contain a stable (-45 kcal) stem-loop structure. We have observed this structure *in situ* using a 5'end-labeled oligonucleotide primer complementary to a region 35 bases 3' to the proposed stem-loop structure. This banding pattern can also be altered by pharmacological treatment. We observed in RNA gel shift assays the binding of at least four proteins from AtT-20 cytoplasmic extracts to a 60-nt riboprobe corresponding to the stem-loop structure. We are currently in the process of characterizing these RNA-binding proteins.

211.6

CHARACTERIZATION OF THE RAT PROOPIOMELANOCORTIN GENE SEQUENCES RESPONSIBLE FOR PITUITARY SPECIFIC EXPRESSION. B. Liu, G. Hammer* and M. J. Low, Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

The POMC gene is expressed at high levels in the anterior and intermediate lobes of the pituitary gland. Earlier data from our laboratory demonstrated that the 5' flanking region of the rat POMC gene (-706 to +64) targeted reporter gene expression specifically to pituitary corticotropes and melanotropes of transgenic mice. To define the minimal sequences required for pituitary specific expression, we produced new lines of transgenic mice with a series of fusion genes containing different lengths of 5' rPOMC gene sequences between -480 and -33, linked to the enhancerless viral thymidine kinase promoter (-109 to +51) and the KI mutant SV40 T antigen. 80% of the mice carrying the transgene with 5' rPOMC sequences from -480 to -33 expressed T antigen specifically in the corticotropes and melanotropes. 50% of the mice carrying the fusion gene with 5' rPOMC sequences from -323 to -33 had similar expression. Shorter promoter constructs, including -323 to -166 and -166 to -33, failed to express in the pituitary gland. To correlate potential DNA binding sites for transcription factors with our functional mapping of the rPOMC 5' flanking sequences, we performed gel-retardation and DNase I footprinting assays with AtT20 cell nuclear protein extracts. We observed at least 4 specific mobility shifted bands using the radiolabeled -323 to -33 DNA as the probe. Only minor differences were observed in the gel-shift pattern using nuclear protein extracts from GH4C1 cells and HeLa cells. Footprinting assays with the same DNA (-323 to -33) labeled at the 5' end revealed 2 sites obviously protected from DNase I, ranging from -98 to -168 and -183 to -218. With the 3' end labeled probe we detected the site of -98 to -168. These data indicate that DNA elements essential for pituitary-specific rat POMC gene expression are within the region from -323 to -33. The importance of the individual protected sites within this region is being investigated currently by further transgenic experiments.

211.7

MULTIPLE SIGNALS ACTIVATE A PURKINJE CELL-SPECIFIC PROMOTER. J. Oberdick*, K. Schilling, R.J. Smeyne, J. Corbin*, T.N. Sato, and J.I. Morgan. Dept. of Neuroscience, Roche Institute of Molecular Biology, Nutley, NJ 07110.

To identify mechanisms that orchestrate neurodevelopment we have investigated the expression of a Purkinje cell-specific transgene (L7βGal) in developing cerebellum and in dissociated cerebellar cultures. L7βGal *in vivo* is expressed in a dynamic parasagittal banding pattern during development. During late embryogenesis, when expression is first detected, this pattern correlates with birth date, a cell-intrinsic property. In Purkinje cell primary cultures, L7βGal is expressed roughly on schedule despite an absence of extracerebellar signals, also suggesting intrinsic regulation. However, depolarization with high extracellular K^+ in these cultures leads to an upregulation of both L7βGal and the cognate gene, indicating additional extrinsic mechanisms. Thus neuronal activity may be an underlying component of the banding pattern seen during development, a pattern which is reminiscent of parasagitally organized olivocerebellar projection zones. In addition, reduced activity could account for the gradual decline in L7βGal expression observed with age after 25 days postpartum. Successive 5' truncations of the promoter (from 4kb down to 0.25kb) retain Purkinje cell specificity but show subtle alterations in banding patterns; in all cases, however, bands give way to uniform distribution as animals age. The truncations also result in systematic reductions in the overall level of expression of L7βGal. This shows that multiple enhancer elements control the level of L7 expression. Since a TRE is bounded by one pair of truncations, and since its deletion results in a decrease in L7βGal, thyroid hormone may be one component of extrinsic regulation of this gene.

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211.8

MULTIPLE HOMOLOGS OF E12/E47 HELIX-LOOP-HELIX TRANSCRIPTIONAL FACTORS IN DEVELOPING MOUSE NERVOUS SYSTEM. T.Neuman, H.O.Nornes, M.X.Zuber*. Dept. of Anatomy & Neurobiology, *Dept. of Biochemistry, Colorado State University, Fort Collins, CO 80523.

Transcriptional factors that belong to the family of helix-loop-helix (HLH) proteins have been implicated in the regulation of differentiation of several tissues in several species of vertebrates and invertebrates. In order to study neuronal differentiation, cDNA libraries were prepared from different postnatal stages of development of the mouse cerebellum. Using PCR, several cDNAs with high homology to the HLH region of E12/E47 protein were isolated. RNAse protection analysis shows tissue and organ specific expression of these cloned genes during development. Furthermore, electrophoretic mobility shift analysis with different E motif containing oligonucleotides (binding site for E12/E47) gives a changing pattern of binding proteins in the developing nervous system. Two of these proteins, BrE2 and BrE3, are highly enriched in differentiating granular neurons. These data suggest that the E12/E47 family of transcriptional factors contains several members which may be important in regulating differentiation in a variety of organs.

211.9

ALTERNATIVE SPLICING GENERATES A VARIANT SNAP-25 PROTEIN DURING DEVELOPMENT. Christina Bark & Michael C. Wilson. Dept. of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

SNAP-25 (synaptosomal associated protein of 25 kD) is a neuron-specific protein enriched in presynaptic terminals. Its expression is widely distributed but discriminates between subpopulations of neurons. The 206 amino acid sequence of SNAP-25 is remarkably conserved throughout evolution (Risinger et al., 1990 Neurosci Abst. 154.14), and contains two domains that may play important roles for biological function and targeting of the mature protein. The amino terminus of SNAP-25 can form an amphipathic helix and a cluster of cysteine-residues is substrate for palmitoylation.

SNAP-25 is a component of fast axonal transport. However, in rat it has been shown that the sub-localization of SNAP-25 protein changes from axons to presynaptic terminals during development and translocation of SNAP-25 immunoreactivity to distal ends of neurites in induced PC12 cells appears to be coupled to new RNA and protein synthesis. Taken together, this might imply the presence of alternative SNAP-25 proteins during development and/or differentiation, or that auxiliary protein(s) are synthesized to target SNAP-25 to the presynaptic membrane.

In order to investigate the first possibility, SNAP-25 cDNA-clones from a human fetal 17-18 week brain library were isolated and analyzed by DNA-sequencing. The majority of clones correspond to a SNAP-25 variant, with an alternative exon 5, as inferred from the exon organization of the chicken gene. Although the alternative splicing of exon 5 only changes 8 out of 39 amino acids, the biological significance might be dramatic since the evolutionary conserved domain spanning the cysteine-residues is replaced.

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211.10

DEVELOPMENTALLY REGULATED ASTROCYTIC EXPRESSION OF A THY-1.2/*lacZ* FUSION GENE IN THE CNS OF TRANSGENIC MICE. K.A. Kelley, V.L. Friedrich Jr. and K. Herrup. The Fishberg Research Center for Neurobiology, The Mount Sinai School of Medicine, New York, NY 10029 and The E.K. Shriver Center, Waltham, MA 02254.

A transgenic mouse strain has been prepared from a Thy-1.2/*lacZ* hybrid gene. As previously reported (Soc. Neuro. Abst., 14: 622; Soc. Neuro. Abst., 15: 957), the transgene is expressed in CNS neurons of adults, and in neuroblasts and neuroepithelial cells during embryogenesis. Subsequent studies have revealed that expression also occurs in neuroglial cells during early postnatal life. Double immunofluorescence with bacterial ß-galactosidase and glial fibrillary acidic protein (GFAP) antibodies revealed substantial transgene expression in astrocytes from birth through postnatal day 20 (P20). In cerebellum, the double labeled cells included radially oriented Bergmann glia as well as astrocytes in granular layer and subcortical white matter. By P25, astrocyte expression was significantly reduced, and at later stages was barely detectable. The same developmental pattern prevailed in many other CNS areas. An exception was the olfactory bulb where astrocytic expression persisted throughout adulthood. These results suggest that the Thy-1 promoter can be expressed and developmentally regulated in non-neuronal as well as neuronal CNS cell types, which may reflect factors associated with the structure of the transgene or its site of integration. Alternatively, the endogenous Thy-1 gene may be transcribed in young astrocytes at previously undetected levels.

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211.11

EXPRESSION OF MURINE NCAM IN CELLS AND TRANSGENIC MICE. W. Wille, E. Heider*U. Hartmann*H. Cremer*R. Kehlenbach* & D. Barthels Inst. f. Genetik, Univ. Köln (Cologne), Zülpicher Str. 47, D-5 Köln 1, FRG

The neural cell adhesion molecules are a class of cell surface glycoproteins which mediate autoadhesive cell-cell interactions. In addition, the molecules carry a functional heparin-binding site and a fibronectin-like domain in the extracellular portion of the protein. Besides the three major isoforms, NCAM-120, -140 & -180, alternative splicing generates almost 200 diversity forms. These types are due to small extra-sequences, which can be introduced into the Ig-like domain IV and into a site between exons 12 and 13. An alternative acceptor site at the 5'-end of exon 14 contributes to the high number of diverse NCAMs in brain and muscle. In order to study the function of NCAMs *in vivo* and to analyze the biological relevance of various diversity forms, we established different *in vitro and in vivo* systems.

On the one hand, we obtained transgenic founder mice carrying 2 to 10 copies of a pUC derivative with NCAM-120 cDNA-clone N1 cloned into the 3'-end of exon 1b of the Thy-1 gene. This construct is transcribed in the CNS under the control of the Thy-1.2 promoter causing levels of vector-derived NCAM messengers 30-95 fold higher than in control tissues. Transgenic mice with this construct carrying *lac* Z instead served as controls.

On the other hand, fibroblast cell lines have been transformed with pMV7-NCAM-constructs with various iso- and diversity forms. These NCAM-synthesizing fibroblasts have been applied to four adhesion assays: i) cell adhesion in suspension, ii) adhesion of single cells to monolayers, iii) gap junction formation, and iv) out-growth of chicken retina neurons on alternating stripes containing different NCAM-forms ("stripe assay" after Bonhoeffer et al.). Data on *in vitro* and *in vivo* systems and the impact of ectopic NCAM-expression on brain development will be presented.

Grants: DFG: SFB 243-B5 & SPP Wi 563/4-1 and the Fonds der Chemischen Industrie.

212.1

VIDEO MICROSCOPY OF GROWTH CONES EXTENDING IN THE CORPUS CALLOSUM OF A HAMSTER CORTICAL BRAIN SLICE. M.C. Halloran and K. Kalil. Neuroscience Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

Previous observations in fixed tissue (Norris and Kalil, 1990, J. Comp. Neurol. 293:268-281) showed that populations of axonal growth cones in the developing hamster corpus callosum assumed a variety of complex lamellar and filopodial morphologies within the callosum. We wished to determine whether shape changes of individual growth cones are associated with specific behaviors, such as rapid extension or changes in direction. We therefore developed a callosal slice preparation through the newborn hamster sensorimotor cortex, in which growing callosal axons and their growth cones were labeled with DiI injected in vivo. Using a SIT camera under low light level conditions, and an image processing and storage system, we were able to make real time video observations of callosal growth cone behaviors within the living brain slice. Preliminary results from video recording sessions lasting up to 10 hours showed that rates of outgrowth for populations of callosal axons were consistent with those estimated in fixed tissue (i.e. about 50 μm/hour). However, rates of extension of indivdual growth cones were highly variable, and low power observations suggested an overall saltatory motion of developing callosal axons. Individual growth cones continually changed shape during bouts of rapid extension, and often changed direction along their callosal trajectory, observations consistent with those made in fixed tissue. Preliminary analysis of video images suggests a correlation between growth cone expansion and rapid axon outgrowth. These results in vitro suggest that the living slice preparation will be useful in understanding how changes in growth cone shape relate to guidance decisions by callosal growth cones in vivo. Supported by NIH Grant NS14428 (K.K.) and Training Grant T32 GM 07048 (M.H.).

212.2

A STEP GRADIENT OF CHONDROITIN SULFATE PROTEOGLYCAN CAN MODULATE THE TIMING OF NEURITE ELONGATION. D. M. Snow and P. C. Letourneau. The University of Minnesota, Minneapolis Minnesota, 55455.

Although chondroitin-sulfate proteoglycan (CS-PG) has been shown to repel elongating neurites in vitro, there exist regions of the developing nervous system where CS-PG expression coincides with growing axons, e.g. rodent forebrain. Relevant to this observation is our previous finding that CS-PG can be modified to become growth-permissive if present in conjunction with an appropriate concentration of laminin. We hypothesized that the function of CS-PG in regions of the developing brain where axons grow may be not to prevent their forward progress, but rather to slow their rate of elongation, thereby regulating the timing of axonal outgrowth.

We have developed a technique to produce an overlapping step gradient of CS-PG and laminin in a culture dish. Within the step gradient, concentration changes occur over a distance of 20-30 μm. Quantitative fluorescence of immunocytochemically-labeled CS-PG and laminin was used to determine the concentration of each molecule at positions along the gradient. Neuronal explants from a variety of sources were placed on a laminin-coated substratum facing the CS-PG gradient. As neurites grew along the gradient, they traversed an increasing concentration of CS-PG. Analysis with time-lapse video microscopy showed that growth cones were not repelled by concentrations of CS-PG that were repellent in previous paradigms where growth cones were confronted by sharp concentration changes (Snow et al., J. Exp. Neurol. 109, 1990). Importantly, as the growth cones grew along increasing concentrations of CS-PG in the gradient, the rate of outgrowth decreased.

These data combined with previous results indicate that CS-PG may be a multifunctional molecule in vivo. When present in high concentrations and lacking significant contributions from growth-promoting molecules, CS-PG may prevent neurites from entering forbidden territories. Alternatively, when present in a gradient and modified by growth-promoting molecules such as laminin, CS-PG may merely reduce the rate of neurite outgrowth to precisely regulate the timing of axonal elongation.

212.3

REGULATION OF AFFERENT GROWTH BY TARGET NEURONS *IN VITRO* IS AFFECTED BY TTX, HIGH MAGNESIUM OR A GLUTAMATE ANTAGONIST. D.H. Baird Dept. of Pathology, College of Physicians and Surgeons of Columbia University, New York, N.Y. 10032.

The outgrowth of axons from pontine nuclei explants, a source of cerebellar mossy fibers, is arrested upon contact with their target neuron, the cerebellar granule cell (Baird *et al. Soc. Neurosci. Abstr.* 16: 1127). After three days *in vitro*, explants grown on laminin, poly-lysine or cerebellar astroglia extend axons greater than 300 μm in length. When explants are added to granule cells plated at high density on any of these substrates, explants produce a short fringe of axons, less than 200 μm in length. This "stop-growing" signal is afferent specific, as climbing fibers (Purkinje afferent) grow to comparable lengths on granule cells or laminin. To investigate if perturbing electrical and synaptic activity affects the stop-growing signal, murine pontine nuclei explants were co-cultured with granule cells in the presence of 1 μM TTX, 4 mM magnesium (5X normal), or an antagonist of glutamate receptors (on granule cells), kynurenic acid (1 mM). All of these agents interfered with the ability of granule cells to arrest mossy fiber outgrowth. In treated co-cultures, axons extended over 300 μm from explants on granule cell monolayers. The agents have little or no effect on outgrowth from explants cultured in the absence of granule cells, the survival of granule cells, or their morphology during the three day culture period.

During development, the stop-growing signal may help determine the target neuron specificity of axons or regulate the size of afferent arbors. These experiments suggest that electrical and synaptic activity play a role in the regulation of axonal growth by target cells. The cerebellar afferent-target co-cultures provide an accessible system to further investigate the activity dependence of axon-target interactions. Supported by NS 08761 to D.B and NS16951 to C.A. Mason.

212.4

CONTACT-DEPENDENT MECHANISMS OF MOTOR AXON SEGMENTATION R.A. Oakley and K.W. Tosney. Neuroscience Program & Biology Dept., Univ. of Michigan, Ann Arbor 48109.

Motor axons become segmented in the chick by preferentially invading the anterior half of each somite and interacting with sclerotome. Since motoneurons grown on explanted somites exhibit contact-mediated avoidance of posterior sclerotome, contact-mediated interactions contribute to segmentation. To elucidate mechanisms at the cellular level, we used videomicroscopy to compare the interactions of identified motoneurons with identified somite cells in culture.

Motor growth cones do not cross posterior sclerotome cells but will cross cells from the anterior somite. Preliminary analysis suggests that such differential behavior results from very local differences in growth cone activity that can be initiated when a single filopodium contacts either cell type. Filopodia form persistent contacts with *posterior* sclerotome cells and exert obvious tension, but only poorly support the distal progression of veils. Since this inhibition of veil activity is local, it is not sufficient to fully impede the forward advance of the growth cone until the entire leading edge makes contact. Veils at the leading edge that contact the cell ultimately retract while veils at the lateral edges remain active. In contrast, filopodia that initially contact *anterior* somite cells are less persistent, appear to generate less tension, and support veil activity. These filopodia gradually thicken and consolidate to form a nascent neurite. We suggest that a local inhibition of veil formation by posterior cells in combination with an enhancement of neurite formation by anterior cells contributes significantly to the preferential advance of motor axons into the anterior somite. Supported by NS-21308.

212.5

STROBOSCOPIC ILLUMINATION ALLOWS TIME-LAPSE OBSERVATION OF GROWING AXONS IN LIVING MOUSE MUSCLE. P. van Mier, S.G. Turney, and J.W. Lichtman. Department of Anatomy & Neurobiology, Washington University School of Medicine, St.Louis, MO 63110.

To better understand the behavior of growing axons in living animals, we studied the re-growth of crushed motor axons and the formation of terminal sprouts in the mouse sternomastoid muscle *in situ*. In order to do this, many views of single vitally stained axons were obtained over intervals of minutes. It was necessary to drastically shorten the time that each growing axon was exposed to light. For this purpose we used intermittent illumination during imaging from a Xenon-arc stroboscope coupled by fiber optics to a standard epifluorescence microscope. Typically, 4-7 days after nerve crush, an anesthetized mouse was placed on the microscope stage, mechanically ventilated and its left sternomastoid muscle exposed. A vitally stained axon was chosen, and with 5-15 stroboscopic light flashes (16 μsec each) and the use of a SIT camera connected to an imaging system, a digitized image was obtained. During averaging, the flashing of the stroboscope was triggered by the stroke of the ventilator to eliminate respiratory movements. At short intervals repeated images were taken of the same axon.

Preliminary results show that sprouting axons no longer stop growing or retract as was seen in experiments using either continuous or prolonged intermittent illumination (1-15 sec). Sprouts were observed to grow tens of microns over periods of several hours, and in some cases we observed branch formation near the growing tip. Movement of organelles along the length of the sprouts was also observed. Thus stroboscopic illumination offers the possibility of following dynamic events in real time in living animals.

212.6

DEVELOPMENT OF MOSSY FIBER AXONS AND SYNAPSES IN RAT HIPPOCAMPAL SLICES EXAMINED WITH A SCANNING LASER CONFOCAL MICROSCOPE. M.E. Dailey & S.J. Smith*, Dept. of Molecular & Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305.

Little is known about the initial interactions of afferent axons and target cells leading to synapse formation in developing mammalian CNS tissue. We are examining the growth and cellular interactions of dentate granule cell mossy fibers (MF) that extend into hippocampal area CA3 and form giant *en passant* synapses on apical dendrites of pyramidal cells. To examine growing MF directly, we made focal injections of a lipophilic fluorescent dye (DiO or DiI) into the dentate hilus of live hippocampal slices from early post-natal (P3-P5) rats, when many MF are first contacting their targets. MF were labeled to their tips within 4-6 hr. A scanning laser confocal microscope was used to follow labeled fibers over time. When images were acquired at 30-120 sec intervals, extended (>6 hr) time-lapse observations could be made with no evidence of tissue rundown. We were able to observe highly motile growth cones with distinct filopodia and lamellipodia at the tips of growing axons 20-80 um below the slice surface. Some of these axons extended at rates of up to 30 um/hr, and occasionally individual growth cones would abruptly stop and remain relatively quiescent for extended periods of time while adjacent fibers continued to extend. Active filopodia-like structures were also found along the length of many axons. Some slices were counter-stained with DiO or Fluo-3, a fluorescent Ca-sensitive dye, permitting simultaneous imaging of afferent growth cones and candidate target cells. In conjunction with post-fixation immunostaining with Synapsin I antibodies (gift of P. DeCamilli, Yale), which has provided a suitable method for morphological detection of developing synapses, these methods hold promise for direct observation of synaptogenic interactions *in situ*. Supported by NIH grants NS09027 & NS28587.

212.7

***IN VIVO* TIME-LAPSE VIDEO MICROSCOPY OF COMMISSURAL GROWTH CONES IN THE GRASSHOPPER CNS.** Paul Z. Myers and Michael J. Bastiani. Dept. of Biology, University of Utah, Salt Lake City, UT .

We have used time-lapse video microscopy to observe the behavior of a neuron, Q1, that pioneers the posterior commissure of the grasshopper. Our goal is to discover the significant interactions that play a role in Q1's pathfinding by identifying the dynamic and transient associations that the Q1 growth cone makes during its outgrowth.

We labeled individual cells by iontophoresing a 0.5% solution of the lipophilic dye Di-I in DMSO onto the surface of the soma. The labeled neurons were then imaged at regular intervals (typically every 3 minutes) at low levels of illumination using a video intensifier and image processing software to enhance the signal, and computer-driven shutters to minimize exposure to light. Our system has sufficient spatial resolution to detect individual filopodia. The temporal resolution is more than adequate to follow the migration of growth cones, which move at 4-5 μm/hr, and has also allowed us to observe the behavior of single filopodia, which extend and retract at rates of up to 250 μm/hr.

We have found that the Q1 growth cone often (50% of the time) retracts or halts in its growth at the time its filopodia first touch the midline. In cases in which we have labeled both Q1 and its contralateral homolog, we have seen that this cessation of growth does not occur when filopodia from both growth cones meet at the midline; instead, growth is accelerated and the growth cones rapidly translocate by expanding into the contacting filopodium, a behavior previously described in the grasshopper limb by O'Connor *et al.* (J.Neurosci. 10:3935,'90). Our results suggest that the Q1 growth cone may be inhibited by contact with unidentified and possibly non-neuronal elements at the midline, but that this inhibition can be overcome by a strong preferential interaction with filopodia of the growth cone of the contralateral Q1.

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212.8

TIME-LAPSE IMAGING OF MIGRATING CELLS IN CORTICAL SLICES. Nancy A. O'Rourke, Michael E. Dailey, Stephen J Smith* & Susan K. McConnell, Depts. of Bio. Sci. & Molec. Cell. Physiol., Stanford Univ., Stanford, CA 94305

We are using cultured slices of developing cerebral cortex as a model system for studying neuronal migration in organotypic, three-dimensional environments. Small injections of the fluorescent vital dye DiI were made in the ventricular zone of ferret cortical slices and labeled cells were followed using time-lapse imaging with a laser-scanning confocal microscope. We observed elongated, bipolar cells with thickened leading processes oriented toward the pial surface and thinner trailing processes extending toward the ventricular surface. This morphology strongly resembles that of migrating neurons described by Rakic in EM studies (1972, J. Comp. Neurol, 145:61). Time-lapse analysis of these cells revealed migratory movement toward the pial surface at rates up to approximately 18 um/hr. The leading processes of these cells continually extended and retracted pseudopodia during migration. The cell soma often moved independently of the leading process, as has been shown in neuron-glia co-cultures by Hatten and colleagues (1987, J. Neurosci. 7:1928). However, in contrast to such co-cultures, migrating cell bodies in slices often showed sudden, rapid translocations, as if squeezing past an obstruction in the three-dimensional environment. It is important to directly confirm the neuronal identity of these migrating cells. Toward this end, we have labeled newly-generated neurons with BrdU in the same group of slices; labeled cells moved from the ventricular zone toward the cortical plate over several days in culture, a pattern suggestive of the migration of neurons in the cortex. Consistent with the possibility that migration in slices is guided by radial glia, anti-vimentin immunohistochemistry and DiI labeling revealed radial glia in the cultured slices. In one case, a migrating cell switched from one migratory path to another directly adjacent to it, as if jumping from one radial process to another. Such behavior has been observed in neuron-glia co-cultures and implied in EM studies. Thus, these slice cultures provide a promising system for examining the dynamic behavior of migrating neurons in the developing cortex.

212.9

THE *WEAVER* GENE ACTS NON-AUTONOMOUSLY IN GRANULE NEURON DIFFERENTIATION *IN VITRO*. W.-Q. Gao, X.-L. Liu* and M.E. Hatten. Department of Pathology and Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, NY 10032

In the neurological mutant mouse *weaver*, although generation of the cerebellar external granule cell layer occurs normally, differentiation of granule cell precursors via the extension of parallel fiber axons and migration along the Bergmann glia fails. In a reaggregate culture system (Gao *et al*, 1991, Neuron 6, 1-11), the proliferation of P5-P7 B6CBA-A^{w}-*J*-*wv* (wv/wv) granule cell precursors, as measured by ^{3}H-thymidine incorporation and BrdU labelling assays, was comparable to that of wild-type cells. By contrast, several aspects of neural differentiation were impaired in *weaver* cells. Whereas wild-type cells extended neurites 300 μm in length in the reaggregate culture system, *weaver* neurite extension was limited to 5-25 μm. Moreover, expression of the axonal glycoprotein TAG-1 and the neuron-glia ligand astrotactin was reduced in *weaver* cells compared to wild-type cells.

To determine whether the *weaver* gene acts autonomously or non-autonomously in neural differentiation, we labeled *weaver* precursor cells with a fluorescent dye (PKH2), intermixed them with wild-type cells in reaggregate cultures, and measured neurite extension of labelled *weaver* cells. In co-culture with wild-type cells, *weaver* granule cells extended neurites 200-400μm in length, a value that was indistinguishable from wild-type cells. These results suggest that the *weaver* gene acts non-autonomously and that granule cell precursors produce a ligand which induces CNS neural differentiation.

212.10

ANTIBODIES AGAINST A ~300 kD *HELISOMA* ECM PROTEIN CAUSE GROWTH CONE COLLAPSE AND NEURITE RETRACTION FROM CULTURED NEURONS. J. D. Miller and R. D. Hadley, Dept. Anat. & Cell Biol., Med. Univ. of SC, Charleston, SC 29425.

We have previously identified a ~300 kD protein in the extracellular matrix (ECM) of *Helisoma* ganglia that is recognized by anti-laminin antibodies. *Helisoma* brain-conditioned media (CM) contains the ~300 kD ECM protein and supports outgrowth from cultured *Helisoma* neurons. Addition of anti-~300 kD Fab fragments to CM (100 μg/ml) inhibits neurite initiation from identified neurons B5 and B19. We have extended our observations to assess the effects of anti-~300 kD Fab fragments on neurites that have already initiated. Addition of anti-~300 kD Fabs (200 μg/ml) to cultures of actively growing B5 and B19 neurons induces growth cone collapse and neurite retraction within 2 hrs of Fab addition. Retraction continued for up to several hours, culminating in the formation of a static retraction bulb. Neurons treated with anti-~300 kD Fabs were fixed either during initial retraction of neurites or after retraction had occurred. Immunofluorescence of microtubules during early phases of neurite retraction revealed a tortuous, sinusoidal distribution of microtubules within collapsed growth cones. Conversely, retracted stumps were uniformly and intensly labeled, with no discernable microtubular structures. These results suggest the ~300 kD ECM protein functions in the adhesion between the culture substrate and growth cones, and that loss of this adhesion results in rapid growth cone collapse and retraction. The transient appearance of disorganized microtubules within actively retracting neurites may reflect a specific interaction between the ~300 kD protein and the cytoskelton, or may be a general feature of neurite retraction phenomena. At later stages of retraction, the uniform distribution of label is likely due to microtubular depolymerization.

212.11

RAPID DISAPPEARANCE AND GRADUAL REAPPEARANCE OF ACETYLATED MICROTUBULES FOLLOWING AXOTOMY AND REGROWTH OF *HELISOMA* NEURONS. R. D. Hadley and J. D. Miller, Dept. Anat. & Cell Biol., Med. Univ. of SC, Charleston, SC 29425.

When a neuron is axotomized and induced to regrow its axon, its cytoskeleton must undergo rapid and dramatic changes. One obstacle that the neuron must overcome is that the cytoskeleton is stabilized by a variety of factors. One stabilizing factor that contributes to the maintenance of cellular form is the acetylation of microtubules on the α-tubulin subunit, conferring a resistance to the depolymerizing actions of antimitotic drugs. We used a monoclonal antibody against the acetylated epitope of α-tubulin (kindly provided by G. Piperno, Rockefeller Univ) to assess the distribution of acetylated microtubules (Ac-MT) in cultured *Helisoma* neurons. Shortly (~2 hr) after plating, axonal remnants of neuron B5 contained a large number of Ac-MT's distributed in bundles along the length of the axon. These MT's constituted a minority (~10-20%) of the total MT's. Membrane veils at the distal attachment point of the axonal stump never contained any Ac-MT's, although they contained a distribution of total MT's consistent with an incipient growth cone. In the early stages of neurite outgrowth, neurites less than 200-300 μm long never contained any Ac-MT's, although they were filled with MT's as they grew out. With longer culture times, and consequently longer lengths of neurites, there was a reappearance of Ac-MT's within the neurites. These Ac-MT's did not necessarily exist in bundles, but often as individual MT's within a matrix of many other non-acetylated MT's. Neither did the Ac-MT's begin at the soma and extend distally. Rather, Ac-MT's often existed as isolated short segments. At no stage did Ac-MT's appear in growth cones or membrane veils. Thus, Ac-MT's are rapidly deacetylated or depolymerized during axonal resorption and re-expressed only in stabilizing neurites.

212.12

DEVELOPING LEECH NEURONS EXCHANGE CALCIUM VIA CNS GAP JUNCTIONS *IN VIVO*. L.R. Wolszon, V. Rehder, S.B. Kater and E.R. Macagno. Columbia Univ. Biology Dept., New York, NY 10027; Colorado State Univ. Dept. of Anatomy & Neurobiology, Fort Collins, CO 80523.

Communication among particular leech neurons during their initial phases of outgrowth is important for establishing their correct branching patterns. This is true for the Anterior Pagoda (AP) cells, which are found as bilateral pairs in 20 of the 21 segmental ganglia of *Hirudo medicinalis*. Gao and Macagno (*J. Neurobiol.* **18**:295 [1988]) found that developing AP's in adjacent ganglia extend transient axons toward each other, that overlap for a few days before being retracted. These axons actually inhibit each other's growth during this period, because if one AP cell is deleted *specifically during the time of overlap*, its neighbor does not retract, but instead resumes growth and takes over the vacated territory. The overlapping axons therefore communicate the existence of each cell to the other.

Since preliminary data indicate that the overlapping AP axons are electrically coupled, we are looking for small signalling molecules or ions that might cross these gap junctions. To test whether Ca^{2+} can cross, we injected Fura-2 into two adjacent AP cells of an E11 embryo. We report here that locally elevating the Ca^{2+} level in one AP, by briefly focussing a UV beam on part of the cell, led to a Ca^{2+} wave *that crossed into the adjacent AP*. Both cells remained healthy during this procedure. Two observations indicate that the Ca^{2+} rose in the receiving cell via direct transfer across gap junctions, rather than from depolarization or receptor activation: 1) The increase in Ca^{2+} occurred after a long delay (20-60 min), and 2) We found no evidence of voltage-dependent Ca^{2+} channels in these cells. These data suggest that Ca^{2+} may play a role in conveying information about the presence of homologous neurons during development.

212.13

Properties of *Hirudo* Retzius neurons cultured in isolation and with reproductive ducts. K.A. French, S.M. Jordan*, L. Szczupak*, and W.B. Kristan, Jr. Department of Biology, U.C.S.D., La Jolla, CA 92093-0322.

Although all Retzius (Rz) neurons begin development identically, by adulthood Rz neurons in the segments containing the male (segment 5) and female (segment 6) reproductive ducts differ from their segmental homologs in several respects, including their response to ACh. Normally, Rz neurons in the reproductive segments [Rz(5,6)] innervate the reproductive ducts (RDs), while Rz neurons in standard mid-body segments [Rz(X)] diffusely innervate muscles and glands in the body wall; the distinctive properties of Rz(5,6) arise only if their normal targets are present during development. To understand the interaction between Rz(5,6) and the RDs, we have begun to study Rz neurons when they are cultured in isolation or in association with juvenile RD tissue.

Rz(5,6) and Rz(X) from adult leeches were cultured on concanavalin A substrate, using previously described techniques (Chiquet and Acklin (1986) *P.N.A.S.* **83**:6188). These conditions also supported slices of juvenile male RD. Both Rz(X) and Rz(5,6) neurons that were co-cultured with RD slices were more likely to sprout neurites than were isolated Rz neurons, their total neurite length was greater, and the neurites had a more complex branching pattern. Outgrowth occurred primarily when the Rz axon hillock was placed directly in contact with the duct. Rz neurons cultured with, but not touching, RD slices grew like isolated Rz neurons. If embryonic RDs are transplanted into standard mid-body segments of embryonic leeches and the local Rz neurons innervate the ectopic tissue, these Rz(X) neurons similarly increase their structural complexity (French, et al., in press). Preliminary results indicate that co-culturing Rz neurons with flattener muscle less effectively stimulates neurite outgrowth, suggesting that at least part of the increased growth depends on properties specific to RDs.

Rz neurons cultured in isolation retained their responsiveness to exogenous ACh for at least 5 days, and the sign of the ACh response depended upon the segment of origin. We have now begun to study the ACh response of Rz(5,6) and Rz(X) that have been co-cultured with male RD slices to determine whether this physiological property responds to contact with the target, as does the pattern of neurite outgrowth. Supported by the March of Dimes and by NIH grant #25916 to WBK.

NEURAL PLASTICITY II

213.1

A NETWORK MODEL OF THE EFFECTS OF DEPRIVATION AND PHARMACOLOGICAL AGENTS ON THE RESPONSE PROPERTIES OF KITTEN VISUAL CORTEX. C. Law*, Leon N. Cooper. Center for Neuroscience, Brown Univ., Providence, RI 02912.

The Bienenstock, Cooper, Munro (BCM) model of synaptic plasticity has been successful in simulating the response properties of neurons in kitten visual cortex under various rearing conditions. This model has been extended from the single cell mean field approximation to a network of neurons with intracortical inhibition without altering the overall results. However, there are some modifications. For example, in network simulations of reverse suture, the length of time neurons were unresponsive to either eye varied. This is in agreement with experimental results. We are currently in the process of modeling the effects of various pharmacological agents on the plasticity of kitten visual cortex. The infusion of bicuculline (BIC) into the cortex was successfully modeled by a reduction of the efficacy of inhibitory connections. In agreement with experimental results BIC caused a reduction of orientation selectivity in modeled neurons, and blocked the ocular dominance shift usually seen during monocular deprivation.

213.2

Functional Rorganization of the Primary Auditory Cortex in Adult Owl Monkeys Parallel Improvements in Performance at an Auditory Frequency Discrimination Task. G.H.Recanzone,C.E.Schreiner, G.T. Hradek*, M.L.Sutter, R.E.Beitel*, and M.M. Merzenich. Coleman Laboratory and Keck Center for Integrative Neuroscience. Departments of Otolaryngology and Physiology. University of California at San Francisco. San Francisco, CA 94143

Electrophysiological experiments in several neocortical areas have shown that the representational 'maps', for example of sensory surfaces, are alterable in the adult mammal. Here we report representational changes in primary auditory cortex (AI) recorded in adult monkeys that were trained in a frequency discrimination task.

Five monkeys were trained to detect an increase in the frequency of the second tone pip of stimulus pairs presented sequentially in the free field. Stimuli both above and below discrimination threshold were presented on 20-50 trials on daily sessions. Different monkeys were trained at different comparison frequencies: 2.5, 3, 5, or 8 kHz. The behavioral performance of all monkeys progressively improved during several weeks of training. Unattended auditory stimuli matching those in the 5 kHz and 8 kHz task were presented to two control monkeys trained to perform a tactile task.

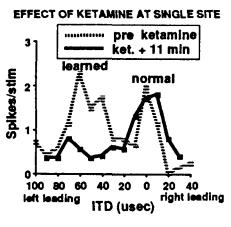
Three experimental and both control monkeys were then anesthetized with nembutal and the frequency representation in AI was defined by multiple unit recording in the middle cortical layers at locations separated by 200 µm. The characteristic frequency (CF), Q10, Q40 and latency of the response at CF was defined from the neural responses to 675 tonal stimuli presented through ear bars. The location of each penetration was determined histologically. The cortical area at which neurons had a CF within the range of frequencies presented in the behavioral task was 2-8 times greater in monkeys that had been trained at that frequency range when compared to either the monkeys trained at a different frequency range or to the passive stimulation control monkeys that had received an equivalent number of auditory stimuli. These results demonstrate that the functional organization of AI in the adult primate is mutable and parallels an improvement in behavioral performance following an extended period of training. Funding provided by NIH grants NS-10414, GM-07449, Hearing Research and the Coleman Fund.

213.3

LEARNED AUDITORY RESPONSES IN THE BARN OWL'S OPTIC TECTUM ARE PREFERENTIALLY SUPPRESSED BY THE NMDA RECEPTOR BLOCKER KETAMINE. M.S. Brainard and E.I. Knudsen. Dept. Neurobiology, Stanford University, Stanford, CA 94305.

Space-specific neurons in the barn owl optic tectum (superior colliculus) are normally tuned to the interaural timing differences (ITDs) which are produced by sounds at the locations of their visual receptive fields. Neurons in owls raised with laterally displacing prisms become tuned for abnormal ITDs corresponding to the locations of their optically displaced visual receptive fields. We have found that these learned responses to abnormal ITDs are strongly suppressed by ketamine, a blocker of the N-methyl-D-aspartate (NMDA) type of glutamate receptor.

Owls were raised with prismatic glasses that displaced the visual field by 23° to the left or right. Beginning at 60 days of age, extracellular recordings were used to measure unit tuning for the ITD of sounds delivered via earphones. At some recording sites, units responded both to the ITD which was normal for that location in the tectum ("normal ITD"), and to the ITD which corresponded to the prismatically displaced visual receptive field ("learned ITD"). At those sites, responses to repeated presentations of normal and learned ITDs were monitored for a 20 minute control period, and then for up to 100 minutes following injection of ketamine HCL (10-25 mg/kg; IM).



Ketamine suppressed responses to learned ITDs by 40 to 90% during the 20 minute period following injection, with gradual recovery over the next hour. In contrast, ketamine had relatively little effect on the responses of units to normal ITDs (see Fig.). These results suggest that NMDA receptors participate differentially in mediating the response to the learned versus normal range of ITDs. Supported by NIH: RO1 DC00155-11 and NSF: RCD 8758111.

213.4

WITHDRAWN

213.5

ADULT RATS EXPOSED TO COMPLEX ENVIRONMENTS HAVE A GREATER NUMBER OF SYNAPSES PER NEURON THAN INDIVIDUALLY HOUSED RATS. V.L. Kilman, A.M. Sirevaag & W.T. Greenough. Beckman Inst., Neurosci. Prog., Dept. Psych. & Cell & Struct. Biol., University of Illinois, Urbana-Champaign, Urbana IL 61801.

Synaptic and neuronal densities (Nv) were estimated in Layer IV of the Occipital cortex of adult male rats exposed to either a complex (EC), social (SC) or individually caged (IC) environment. Nv neurons has been reported previously (Hess et al., 1990 SFN abstract) and was estimated by an unbiased method using the nucleator and Vv. Nv synapses is now reported for the same animals and was estimated by both the unbiased method of the disector and the biased formula Na/D. For both the disector and the Na/D methods Nv synapses did not differ among EC, SC and IC rats. The number of synapses per neuron was estimated by the ratio of Nv synapses to Nv neurons. EC rats had an approximately 40% greater number of synapses per neuron than IC rats and 35% more than SC rats regardless of the method used to estimate synaptic density. These results are similar to the results for weaning age animals where EC rats had a 25% greater number of synapses per neuron than IC rats (Turner and Greenough, 1985). Despite the fact that the conclusion (EC rats have more synapses per neuron than IC rats) is identical for both the disector and the Na/D method, we do not recommend the use of the biased Na/D method. The influence of size, shape and orientation biases on Nv can not be reliably predicted in the Na/D method and it can not be assumed that identical conclusions will always arise when the two different methods are used. Furthermore, the amount of effort and time involved in obtaining the biased estimate is much greater than effort needed to obtain the unbiased disector estimate. Supported by HD-07333 and MH-35321.

213.6

QUALITATIVE CHANGES FOLLOWING HIPPOCAMPAL LESIONS IN INFANT RATS. E.J. Holmes. Lab. for Neurobehavior, Dept. of Psychology, Hampton University, Hampton, VA 23668.

Adult rats which received large, bilateral aspiration lesions of the hippocampal complex (HPC) in infancy have been reported by this author (EPA Proceedings, 1991, 62, p.53) to be significantly less impaired on a radial arm maze task than rats which received HPC lesions as adults and given equal periods of recovery time from surgery. To address the question of the basis for the "sparing of impairment," the brains of the animals receiving HPC lesions in infancy were examined for anatomical patterns of restructuring or reorganization to explain the phenomenon. Most surprising was the noted degree of absence of qualitative changes in the brain material (~5 mos. after surgery) which could be used to explain the behavioral sparing. Moreover, no common pattern of change existed which could be attributed as an underlying factor. In many cases, however, there was an obvious migration of CA1 cells in the dorsal hippocampus to "fill in" the cortical lesion site and a proliferation of glial supportive material within the damaged HPC area. In addition, changes in associated "storage" areas which receive projections from the hippocampus, e.g., the mammillary nuclei, were notably unaffected. Thus, a sufficient qualitative anatomical explanation for this sparing of impairment following HPC lesions in infancy is still obscure and the search for a satisfying explanation is in progress.

213.7

CHANGES IN SHORT-TERM PLASTICITY OF RAPHE NEURONES AFTER MICROCUT LESIONS AND TRANSMITTER INJECTIONS. P. Hinckel, Ch. Rüsing* and M. Rüsing*. Physiologisches Institut, Universität Giessen, Aulweg 129, D-6300 Giessen, Federal Republic of Germany.

Neurones in the nucleus raphe magnus (NRM) have been shown to belong to the thermoafferent system. Beside thermal long-term adaptation (Hinckel et al., Soc. Neurosci. Abstr., 14, 606, 1988; 16, 344, 1990) during cold acclimatization (within weeks) warm-responsive NRM neurones show short-term adaptive behavior in the order of minutes.

In 30 guinea-pigs basic spike rate as well as peak activity of 34 warm-responsive NRM neurones increased within 10-40 min during constant cold stimulation of the receptive field of the cells. After interruption of the dorsal and rostral connexions of the NRM by microcuts the basic rate was not changed during the thermal skin stimulation, whereas the maximum spike rate increased again. Control lesions of lateral pathways did not show any effect.

Microiontophoretic application of serotonin and/or substance P on warm-responsive NRM neurones caused similar effects.

It is concluded that the thermal short-term plasticity of NRM neurones has two components. The cold-adaptive increase of peak activity is generated at a lower level, whereas the increase of tonic basal spike rate is mediated by higher pathway loops.

213.8

L-LYSINE DEFICIENCY MAY INDUCE PLASTICITY IN THE NEURONS OF THE LATERAL HYPOTHALAMIC AREA. K. Torii[1*], K. Hanai[2*], T. Ono[3] and C.J. Wysocki[4], [1]ERATO, R&D Corp. of Japan, Yokohama; [2]Shiga Med. Univ., Shiga; [3]Toyama Med. Pharm. Univ., Toyama, Japan; [4]Monell Ctr., Phila., PA, 19104.

When a L-lysine-deficient diet was offered to rats, the concentration of L-lysine (Lys) declined in plasma and brain. Subsequently, when offered Lys solution and solutions of other amino acids (AA), they specifically selected the Lys solution and their food intake and growth normalized. The present study determined both neural activity of the lateral hypothalamic area (LHA) to AA feeding and release of growth factors in serum during ingestion of a diet deficient in Lys followed by one replete in Lys. Physiological factors were bioassayed using Hydra japonica, under varying methylglutathione levels. A multi-barreled microelectrode was implanted into the LHA stereotactically. After achieving Lys-deficiency, rats were given access to various AA in a licking apparatus constantly monitored for lick rate and volume. Within one day they showed a marked preference for Lys. The single unit activity of LHA neurons was recorded while the animal consumed fluids containing taste stimuli or AA. During Lys deficiency, LHA neurons became more sensitive to AA intake. Some neurons specifically responded only to Lys, suggesting that some neural plasticity was elicited by dietary treatment. A thousand-fold increase in serum inhibin was observed in rats fed a normal diet compared with the fasted state. Serum actibin levels increased just after feeding of a non-protein diet. However, levels of both peptides did not vary under Lys-deficiency. These facts indicate that ingestion of Lys-deficient or non-protein diets caused a change in serum (and possibly brain) levels of physiological factors, including actibin and inhibin. This release may elicit plasticity in the sensitivity of some LHA neurons to AA that could selectively drive ingestive behavior for particular AA, e.g., Lys, to maintain AA homeostasis.

213.9

DIFFERENTIAL RETRACTION OF SNB DENDRITIC ARBORS WITH UNILATERAL TESTOSTERONE TREATMENT OF BC/LA MUSCLES. *Mark N. Rand, Billie F. Loftus*, and S. Marc Breedlove. Psychology Dept., U.C. Berkeley, Berkeley, CA 94720.*

We previously reported that local, lateralized administration of androgen to the bulbocavernosus/levator ani complex (BC/LA) results in a local anabolic effect on these muscles (Rand & Breedlove, Soc. Neurosci. Abstr. 13:54, 1987), and that the dendritic arbors of motoneurons innervating androgen-treated muscles are 40% longer than those of motoneurons innervating BC/LAs given anti-androgen treatment ($p < 0.04$; Rand & Breedlove, Soc. Neurosci. Abstr. 15:377, 1989). The present study examined the effects of local steroid manipulation at the BC/LA on differential retraction in the three primary projection fields of SNB dendrites: ipsilateral, contralateral, and dorsal.

Adult male rats 90-120 days of age were castrated and implanted with two capsules, one containing testosterone (T), and the other hydroxyflutamide (hFl), an anti-androgen. The capsules had a single diffusable surface and were sutured one on each BC/LA side so that the diffusible surface faced the muscle. After 30 days the capsules were removed and the lateral BC on one side was injected with 1 µl of 2% cholera-toxin HRP (CT-HRP, List Labs). Four days later the animals were sacrificed; the lumbar spinal cord was frozen-sectioned at 50 µm and reacted with TMB to visualize the CT-HRP-filled motoneurons and their processes. These were then photographed, traced, and scanned into a microcomputer for analysis, all without knowledge of hormone treatment at the muscle. We found a significant effect by treatment in the contralateral projection field; dendritic arbors of motoneurons innervating hFl-treated muscles were 44% shorter than those of T-treated muscles ($p < 0.03$, Scheffe's F-test). Differences in the dorsal and ipsilateral fields did not achieve significance. The percentage of dendritic retraction in the contralateral field was significantly greater than that of the ipsilateral field (29%; $p < 0.02$), demonstrating that unilateral T administration at the BC/LA results in uneven dendritic retraction in the SNB. These results are not consistent with the hypothesis that androgens increase the retrograde transport rate of CT-HRP.

213.10

GENE TRANSFER INTO *APLYSIA* NEURONS SHOWS SEROTONIN ACTIVATES TRANSCRIPTION VIA cAMP RESPONSE ELEMENTS (CRE): A POSSIBLE ROLE FOR CREB AS A SWITCH FOR THE LONG-TERM PROCESS. B.-K. Kaang, E. R. Kandel and S. G. N. Grant. HHMI & Columbia, N.Y., N.Y. 10032.

A major weakness in the molecular biological studies of mature neurons is the lack of systems for expressing cloned genes. We have devised a general neuronal expression plasmid (pNEX) that after microinjection provides high level expression of cloned genes in all neurons of adult *Aplysia*. With this DNA expression system, reporter genes (β-galactosidase and CAT) and *Aplysia shaker* K^+ channel (see Furakawa et al., this meeting) were expressed in over 80% of injected neurons in ganglia and primary culture.

Different DNA constructs allow study of either constitutive or inducible expression. We have tested the hypothesis that serotonin can activate transcription through a CREB (cAMP responsive element binding protein) dependent pathway in sensory neurons of *Aplysia*. We first injected plasmids that contained the CREB binding site (CRE) driving the lacZ reporter into sensory neurons and found a dramatic increase in levels of lacZ expression following application of serotonin. This induction appeared to be at the transcriptional level and mediated by the CRE since: i) coinjected competitor wild-type CRE oligonucleotides, but not mutant oligonucleotides, blocked the induction, and ii) no induction was seen with a control plasmid that expressed lacZ from a constitutive promoter. These data, together with studies using inhibitors of macromolecular synthesis and injected CRE oligonucleotides (Dash et al., 1990), indicate that transcriptional induction, involving CREB, is necessary for long-term synaptic facilitation and that CREB may serve as a switch for turning on the long-term process.

The ability to efficiently express cloned genes and regulate reporter genes in *Aplysia* neurons now allows assessment of a range of neurobiological problems.

213.11

STRUCTURAL CORRELATES OF ASSOCIATIVE MEMORY IN *HERMISSENDA* NEURONS: THE ROLE OF SMALL G-PROTEINS. C. Collin, M. Sakakibara, D. McPhie, J.V. Sanchez-Andres, T.J. Nelson and D. L. Alkon. Neural Systems Section, NINDS, NIH, Bethesda MD, 20892

Associative conditioning causes functional and structural modifications of the type B photoreceptors of the sea snail *Hermissenda*, that lead to enhanced excitability and a reduction of the enclosing volume of the terminal dendritic arborization. Here we studied the signal transduction pathway that mediates the structural modifications. A small G-protein, cp20, that is phosphorylated by conditioning, has recently been purified from *Hermissenda* brain, and shown to regulate potassium currents when microinjected back into neurons.

We report that iontophoresis (-2 to -3 nA, 500 msec, 1 Hz pulses for ten minutes) or pressure injection of cp20 has also structural effects on lucifer yellow filled B photoreceptors analyzed with confocal microscopy. These changes are taking place in a post-mitotic and differentiated neuron and have a fast time course. Two hours after cp20 microinjections, a reduction of the dendritic enclosing volume was observed in more than 70% of the injected cells. This effect was not prevented by 10 μM staurosporine pretreatment, a PKC blocker. Experiments with colchicine, an axonal transport blocker, did not interfere with the lucifer yellow labelling of the axons, indicating that under these injection conditions cp20 effects were not mediated by a block of axonal transport.

The results suggest the existence of a PKC dependent signalling pathway, that can be activated during classical conditioning, and causes the early regulation of potassium channels. Structural- and long term modifications involving this and other pathways, including cp20, may induce more permanent structural re-arrangements of the dendritic area.

NOCICEPTION

214.1

DID MECHANISMS OF HYPERALGESIA EVOLVE FROM PRIMITIVE ADAPTIVE RESPONSES TO AXONAL INJURY? CLUES FROM NOCICEPTIVE PLASTICITY IN AN INVERTEBRATE, APLYSIA . E.T. Walters. Dept. of Physiology & Cell Biology, Univ. of Texas Medical School, Houston, TX 77225.

Although hyperalgesia and allodynia are defined in human terms, various vertebrates and invertebrates display similar hypersensitivity near a wound. Nociceptive sensory neurons in Aplysia (which combine integrative functions that in mammals are distributed across primary sensory neurons and dorsal horn neurons) show increased excitability, receptive field expansion, and synaptic enhancement after injury to their receptive fields. It was shown previously that these effects can be produced by activity-dependent actions of extracellular modulators (ADEM) released during injury. Several arguments suggest that some mechanisms of nociceptive sensitization may have descended from mechanisms of cellular repair in sensory neurons of early common ancestors of existing phyla. To begin to test this idea, long-term plasticity within the VC sensory population was compared after noxious cutaneous stimulation (maximizing ADEM) and after sensory axons were crushed under complete anesthesia (minimizing ADEM). Although delayed in onset, axon injury caused the same set of central changes as nociceptive ADEM did: significant a) decreases in spike threshold, accommodation, and AHP, and b) increases in spike duration, synaptic efficacy, and afterdischarge.

214.2

PHYSIOLOGICAL AND PSYCHOPHYSICAL STUDIES OF THE EFFECT OF CALCIUM CHANNEL BLOCKERS ON CORNEAL SENSATION R. W. Beuerman, H.W. Thompson*, A. Snow*, C. Breen*, LSU Eye Center, New Orleans, LA 70112.

The cornea supports the most densely innervated surface epithelium in the body. Stimulation of these free nerve endings evokes only sensations of irritation or pain. Ionic mechanisms of the electrical events following stimulation are not understood for free nerve endings. We investigated the decrease in neural stimulation in a rabbit model using hyperosmotic concentrations of sodium chloride. The amplitude of the integrated neural response showed a linear decrease for 0.5 M NaCl combined with caffeine (1mM-50mM) or pretreatment with verapamil (1μM-100μM). Theophylline, another xanthine, was only 50% as effective in suppressing the response. Psychophysical assessment of stimulus magnitude and duration was performed in a series of consenting volunteers using a visual-analog scale. In 11 subjects the pain response to 10^{-7}M capsaicin was significantly ($P=0.0012$) decreased by combining caffeine (50mM) with the capsaicin. In 20 subjects, pretreatment with verapamil (0.1mM) decreased the pain response (43.6 ± 5.73 to 36.9 ± 4.91). Although the duration of the sensation decreased 20-60%, the difference was not significant. These results suggest that calcium channels may be involved in the transducer action of the free nerve endings and that calcium channel blockers may be useful as topical analgesics. (NEI EY04074)

214.3

DIFFERENTIAL EFFECTS OF LASER IRRADIATION ON AXOPLASMIC TRANSPORT IN SENSORY NEURONS AS COMPARED TO MOTOR NEURONS. U. Wesselmann# and W. Z. Rymer. Dept. of Physiology & Rehabilitation Medicine, Northwestern University Medical School, Chicago, IL 60611.

In the quest for a reliable physical method to alter nerve function selectively and differentially a number of radiation sources have been studied over the last 30 years (ultrasound, microwaves, radiofrequency current). We have demonstrated recently that Nd:YAG laser irradiation selectively impairs neural impulse propagation in small slow conducting fibers, as compared to large diameter afferents (Wesselmann et al., Physiol. Chem. Phys. & NMR, 23, 1991, in press). In an attempt to clarify the ultimate fate of sensory and motor neurons after laser application to their peripheral axons, we have used HRP to retrogradely label DRG cells and motor neurons 7 days after laser irradiation (Nd:YAG laser, 70 mJ/pulse x 5 min) of their peripheral axons. The number of labeled DRG cells was significantly ($P<0.003$) decreased on the laser-treated side (laser-treated: 2183 $\pm$ 513 vs control: 3937 $\pm$ 225, n=7). Dimension analysis of the labeled DRG cells showed that the cell deficit on the laser-treated side was mainly due to a decrease in small neurons of the A-delta- and C-group (Wesselmann et al. , Exp. Neurol., 111, 251-262, 1991). In contrast the number of HRP labeled motor neurons was not altered to a significant ($P>0.05$) extent (laser-treated: 767 $\pm$ 10 vs control: 808 $\pm$ 19, n=5). Since the majority of A-delta and C-fibers have nociceptive function, the observed selective effects of laser irradiation on this fiber class, when radiating the whole nerve, might lead to potentially useful applications for the treatment of pain.

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214.4

Delayed Activation of C-Fiber Nociceptors: A Novel Mechanism of Nociception Deborah M. White and Jon D. Levine Dept. of Anaesthesia & Pain Mgt, University of Sydney, St. Leonards, N.S.W., 2065 and Division of Neuroscience, UCSF, San Francisco, CA 94143.

We evaluated responses of C-fiber mechano-heat nociceptors (C-MH) to sustained mechanical stimuli and compared this response to sensitization of C-MH by prostaglandin E_2 (PGE_2). Action potentials of single C-MHs were recorded from the saphenous nerve of anaesthetized rats (pentobarbital; 50mg/kg; i.p.). Calibrated von Frey hairs (VFH) of threshold and subthreshold force were applied for 5min. While threshold force VFHs activate C-MH immediately, subthreshold stimuli activate C-MHs (n=36) with a delayed onset which increases exponentially with decreasing force. We found that the delayed activation of C-MHs differs from sensitization in at least three aspects. Firstly, the delayed activation of C-MHs does not involve a decrease in mechanical threshold (n=11). Secondly, an i.d. injection of PGE_2, which decreased the mechanical threshold of C-MHs (n=7), had no effect on the latencies of the delayed activation. Also, the delayed activation of C-MHs is Ca^{2+}-dependent since latencies were increased by Quin 2 (n=6), a calcium chelating agent, and decreased by A23187 (n=6), a calcium ionophore. We propose that the delayed activation of C-fibers is a novel mechanism of nociception that may explain the delayed sensations of pain induced by sustained mechanical stimuli.

214.5

A DOMINANT ROLE OF ACID PH IN INFLAMMATORY EXCITATION OF NOCICEPTORS IN RAT SKIN. P.W. Reeh*, K.H. Steen* and A.E. Hanisch*. Institute of Physiology and Biocybernetics, D-8520 Erlangen, FRG

A major role of local acidosis in long lasting excitation and sensitization of cutaneous nociceptors has recently been demonstrated (Steen et al. 1991, J. Neurosci. submitted). In inflamed tissue, acid pH meets with a mixture of inflammatory mediators. We have mimicked this condition in a rat skin-saphenous nerve preparation in vitro which allows direct application of chemicals to the isolated receptive fields at the corium side. Stimulant solutions used were: CO_2 saturated "synthetic interstitial fluid" (CO_2-SIF, pH 6.1), an "inflammatory soup" (IS) in submaximal concentration containing bradykinin, serotonin, histamine, prostaglandin E_2 (all 10^{-6} M) in neutral SIF, and a combination made of CO_2 saturated IS (CO_2-IS, pH 6.1). Mechano-heat sensitive C-fiber terminals (n=36) were treated for 5 min at 10 min intervals with these solutions: 20 units responded to CO_2-SIF, 12 to IS, 11 to both, whereas 27 (77%) units were excited by CO_2-IS. Thus, 7 of 16 units insensitive to either of the two solutions were stimulated by their combination. This increased effect of CO_2-IS was also expressed in a larger mean response magnitude: 152 spikes with the combination versus 45 spikes evoked by IS and 93 spikes by CO_2-SIF (n=25; $p<0.002$ resp. <0.02, Wilcoxon-test). During long term nociceptor stimulation (30 min) by either CO_2-SIF or IS, the discharge regularly and markedly increased when CO_2-IS was applied during the middle 10 min. There is a strong algogenic interaction between acid pH and inflammatory mediators. At equal and pathophysiologically relevant concentration, however, hydrogen ions play a dominant role. (DFG grant RE 704/1-5)

214.6

ATP-SENSITIVE K^+ CHANNELS MEDIATE THE PURINERGIC IPSP IN NOCICEPTIVE DORSAL HORN NEURONES IN THE CAT SPINAL CORD. M.W. Salter, Y. DeKoninck[1] and J.L. Henry[1,2]. Div. Neurosci., Hosp. for Sick Children, Toronto, Ont. M5G 1X8. Depts. [1]Physiol., [2]Res. Anaes. and [2]Psychiat., McGill Univ., Montréal, PQ H3G 1Y6.

Input from vibration-sensitive, large diameter primary afferent fibres evokes an inhibitory postsynaptic potential (IPSP) in nociceptive neurones in the dorsal horn. This IPSP results from a K^+ conductance activated by adenosine acting through P_1-purinergic receptors. Recently, it has been reported that the K^+ channels activated by adenosine in cardiac muscle are ATP-sensitive K^+ channels (Kirsch et al. *Am. J. Physiol.* 259:H820, 1990). Therefore, we examined whether these channels might mediate the adenosine IPSP in nociceptive dorsal horn neurones. Intra- and extracellular recordings from neurones in the lumbar spinal cord were made using adult cats anaesthetized with α-chloralose. It was found that iontophoretically applied glibenclamide, a blocker of ATP-sensitive K^+ channels, attenuated the inhibition of nociceptive neurones by vibratory stimulation. Glibenclamide also blocked this inhibition when administered *i.v.*; this effect was dose-dependent and 20 mg/kg caused about 50% blockade. In addition, the IPSP evoked by vibratory stimulation was blocked by injecting ATP directly into neurones during intracellular recording. These results indicate that the purinergic IPSP in nociceptive neurones is mediated by ATP-sensitive K^+ channels. As this IPSP may be the physiological substrate for the analgesia produced by vibratory stimuli, activation of ATP-sensitive K^+ channels in nociceptive dorsal horn neurones may be necessary to produce this analgesia. (Supported by the Canadian MRC, the FRSQ and the Nicole Fealman Fund.)

214.7

ANALYSIS OF SYNAPTIC TRANSMISSION IN THE SPINAL CORD EVOKED BY ACTIVATION OF DORSAL ROOT GANGLION NEURONS WITH CHEMICAL IRRITANTS. S. Jeftinija and L. Urban. Department of Vet. Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011, USA, Sandoz Institute for Medical Research, Gower Place, WC1E 6BN, London, UK.

The synaptic activation of dorsal horn (DH) neurons evoked by stimulation of dorsal root ganglion (DRG) neurons with high potassium and capsaicin was studied in a spinal cord slice - DRG preparation. Brief (1 min) perfusion of 50mM potassium to the DRG depolarized all sensory neurons (29±2.2 mV, mean ±SEM, n=32). In spite of the large depolarization, firing of action potentials was recorded in 11 of the neurons. By simultaneous intracellular recording from DRG and superficial DH neurons we established that all spinal cord neurons (n= 30) that had a C-fiber input were synaptically activated when DRG neurons were stimulated by high K or capsaicin. Effect of capsaicin was dose dependent and when applied in 0.2 and 0.5μM repeatable excitatory effects were observed. In concentration of 10μM capsaicin effects were very long lasting (up to 45 min) and while in 3 cases neurons responded to repeated application in 2 no response was obtained when capsaicin was repeated. Following capsaicin-evoked desensitization, perfusion of high K to DRG depolarized DRG neurons but failed to synaptically activate DH neurons (n=5). High capsaicin failed to depolarize large DRG neurons (n=8). Perfusion of 0.5μM TTX to the DRG selectively blocked the Na spikes in large fibers (n=7) but even at 10μM concentration failed to block synaptic activation of DH neurons evoked by high K or capsaicin applied to DRG-compartment. In summary, our data demonstrate that primary afferents are depolarized by KCl and capsaicin. In addition, our data suggest that high potassium evokes synaptic responses in DH neurons by activating small unmyelinated fibers in TTX insensitive fashion. Work was supported by NIH Grant NS27751 and USDA Grant PL95-113.

214.8

PERIPHERAL INFLAMMATION INDUCES EXPRESSION OF THE NERVE TERMINAL PROTEIN NT75 IN SPINAL NEURONS. S.S. Kanekar, T.C. Ritchie and J.D. Coulter, Neurosci. Prog. & Dept. of Anat., U. of Iowa, Iowa City, IA 52242.

In adult spinal cord, staining for NT75 with the S-7B8 antibody is restricted to nerve terminals concentrated in the superficial dorsal horn. Study of the origin of NT75 immunoreactivity (NT75ir) indicates that most neurons with terminations in the spinal cord are capable of expressing NT75ir, but that only certain populations, such as primary afferents, maintain high levels in their terminals. This implies some form of regulation of the level of NT75ir in different neural systems. To explore factors that affect NT75ir in rat spinal cord, Complete Freund's Adjuvant (CFA) was injected into the hindfoot to induce a small, localized area of inflammation. A strong hyperalgesic response to radiant heat was seen in the injected paw from 4hrs to 4 days, then declined and was abolished by 2 weeks. Rats were perfused 12hrs, or 1, 2, 4, 10 or 14 days after injection, and the spinal cord and dorsal root ganglia (DRG) were stained for NT75. No change was seen in NT75ir in spinal nerve terminals or in DRG cells. However, NT75ir was induced in a small number of spinal neurons. NT75 labeled neuronal perikarya, clustered in laminae IV-VI ipsilateral to CFA, were detectable by 12hrs after injection, peaked in number at 2 days, and were still evident at 14 days. NT75ir cells (1-15 cells /100 tissue sections) were present from L1 to S1 but were most dense at L3-L5. In contrast, staining for synaptophysin or substance P was not affected by CFA treatment. The change in NT75ir may be related to increased neural activity resulting from inflammation-induced hyperalgesia. However, NT75ir is modulated in spinal neurons, but not in DRG cells, implying that neuronal activation alone is not a sufficient stimulus to alter NT75, but that synaptic activity may be required. During development, NT75 expression corresponds closely to the formation of synaptic connections. Plasticity in expression of NT75ir in adults and the timing of its expression during development may imply a role in active nerve terminals. Supported by NS23783.

214.9

RESPONSES OF SPINAL DORSAL HORN NEURONS IN RATS WITH AN EXPERIMENTAL PERIPHERAL MONONEUROPATHY Jennifer M.A. Laird & Gary J. Bennett Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892 U.S.A.

Rats with a constriction injury of one sciatic nerve show evidence of hyperalgesia, which is maximal 10 days after surgery (Bennett & Xie *Pain* 33:87, 1988). We have examined the responses of dorsal horn neurons at this time in 6 rats with a constriction injury of one sciatic nerve and in 3 rats in which the nerve was exposed but not ligated (sham-operated). In the acute experiments, extracellular recordings were made from all dorsal horn neurons encountered in the ipsilateral L_4-L_5 segments of the spinal cord of rats anesthetized with pentobarbital. Neurons with input from the sciatic nerve were located by stimulating the nerve proximal to the site of injury, or at a comparable location in the sham-operated animals.

In the nerve-injured animals less than half (46%; 31/67) of the neurons had a peripheral receptive field (RF) as compared with 98% (48/49) of neurons in the sham-operated animals. The proportions of cells with low, high and both low and high threshold inputs was significantly different in the two groups. Half of the neurons in the nerve-injured animals responded to tapping the injury site on the nerve (49%; 33/67); this response was rapidly adapting. Some of these neurons also responded to stimulation of a RF. None of the neurons recorded in the sham-operated animals responded to tapping the nerve. A small number (<5%) of neurons in the nerve-injured animals had abnormally prolonged stimulus-evoked discharges and high spontaneous activity; such cells were not seen in the sham-operated animals. The loss of peripheral RFs and responses to mechanical stimulation of the injury site are consistent with the known effects of the constriction injury on primary afferent neurons. However, substantial spontaneous activity is seen in the injured nerve, yet this was seen in surprisingly few dorsal horn neurons.

214.10

NOXIOUS SKIN HEATING OR SUBCUTANEOUS FORMALIN INJECTION INDUCE SEQUENTIAL TRANSCRIPTIONAL OPERATIONS IN RAT SPINAL NEURONS. **M. Zimmermann, T. Herdegen, T. Tölle, W. Zieglgänsberger, R. Bravo, II. Physiologisches Institut der Univ. Heidelberg, FRG, Max-Planck-Institut für Psychiatrie, München, FRG, The Squibb Institute, Princeton, USA.**

Long-lasting expression of immediate-early gene (IEG) encoded proteins was previously observed after electrical stimulation of sciatic A delta- and C-fibers (Herdegen et al., Eur. J. Physiol. 415, R93, 1990). We have investigated the induction of IEGs following stimulation of nociceptors. In anesthetized rats one hind paw was immersed in 52^0 C hot water. Formalin (2 %) was injected in one hind paw of unanesthetized rats. The expression of c-JUN, JUN B, JUN D, c-FOS, FOS B and KROX-24 proteins was visualized by immunocytochemistry. The temporo-spatial patterns were similar in both experiments. Numbers of neurons labelled for c-FOS, c-JUN, JUN B and KROX-24 were at maximum after 2 h and then declined to basal levels between 8 h and 24 h. FOS B labelled neurons showed maximum numbers between 4 and 8 h and were still present after 24 h. JUN D had a delayed onset after 4 h, a maximum after 8 h and remained on this level for up to 24 h. Expression was most pronounced in the superficial dorsal horn, except for c-FOS and JUN D. Frequency of labelled neurons showed falling order for KROX-24, c-FOS, JUN B, JUN D, FOS B and c-JUN. This different expression may indicate that a limited number of AP-1 transcription complexes had formed. The sequential expression of IEG proteins may be meaningful in relation to long-term alterations of neuronal programs which could be a new dimension of neuronal processes of pain. **Supported by Deutsche Forschungsgemeinschaft.**

214.11

NMDA RECEPTOR INVOLVEMENT IN MECHANICALLY AND ELECTRICALLY EVOKED SPINAL REFLEXES IN NORMAL AND MONO-ARTHRITIC RATS. Nick A. Hartell and P. Max Headley. SPON: Brain Research Association. Department of Physiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD. U.K.

NMDA receptors may mediate increased central excitability following peripheral tissue injury. We have used ketamine to asses this in 5 arthritic and 19 normal male Wistar rats. Rats were innoculated with Freund's Complete Adjuvant (50 µl s.c. into one hind paw) 4 days before the experiment. Under halothane anaesthesia, animals were spinalized at a low thoracic level and anaesthesia switched to α-chloralose. Percutaneous needle electrodes were used to record single motor unit responses to controlled noxious mechanical stimuli (15 sec), cycled every 3 min with percutaneously applied electrical stimuli, delivered at 2 and 0.3 Hz (1 msec duration; 5xT for evoking reflex spike). Prior to drug administration, similar strength mechanical stimuli evoked higher mean (± s.e.) motor unit firing rates in the arthritic (25.5 ± 1.7 Hz; n=6) than in the normal group (21 ± 3.1 Hz; n=11; $P<0.05$). There was no significant difference, however, in the potency with which ketamine (1-8 $mgkg^{-1}$) depressed the responses to the noxious stimuli. In normal animals, 2 Hz electrical stimulation evoked 40% more spikes over 16 stimuli than did 0.3 Hz. No frequency-dependent increase in responses was observed in the arthritic rats; moreover, the response size at 0.3 Hz was only 40% of that in normals. Ketamine was similarly effective in depressing the responses to low and high frequency stimulation in arthritic rats, low frequency stimulation in normal rats and to mechanical stimulation in both normal and arthritic rats (52-60% control with 4 $mgkg^{-1}$ i.v. ketamine). In normal animals, however, the wound up component of the response was reduced to 20% of control by 4 $mgkg^{-1}$ i.v. ketamine. The results indicate that NMDA-mediated wind-up phenomena do not contribute to the changes of excitability seen in mono-arthritis. The disparity between increased mechanical and decreased electrical responsiveness in mono-arthritic rats remains to be examined.

214.12

NITROARGININE, A NITRIC OXIDE SYNTHASE INHIBITOR, ATTENUATES MORPHINE WITHDRAWAL. A.S. Kimes, D.B. Vaupel, M. Bruckner*, and E.D. London. Neuropharmacology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224.

Antagonists of the N-methyl-D-aspartate subtype of the glutamate receptor (NMDA-R) recently have been shown to reduce opioid withdrawal behavior (Rasmussen et al., *Eur. J. Pharmacol.* **197**:9, 1991; Trujillo and Akil, *Science* **251**:85, 1991). Recent studies have implicated nitric oxide (NO•) as a mediator of some NMDA actions in the nervous system (Garthwaite, *TINS* **12**:60, 1991). N^{ω}nitro-L-arginine (N-Arg) is a potent inhibitor of NO• synthase in brain (Hecker et al., *Biochem. Biophys. Res. Comm.* **167**:1037, 1990). To determine if NO• plays a role in NMDA-R involvement in opioid dependence, we studied the effect of N-Arg on naloxone-precipitated morphine withdrawal (NPMW). Four groups of rats received one 75 mg morphine pellet on day 1, and two more pellets on day 4; two groups received placebo pellets on the same schedule. On days 4-7, morphine groups received either 0, 1, 3 or 7.5 mg/kg N-Arg (i.p., b.i.d.); placebo groups received either 0 or 7.5 mg/kg N-Arg. On day 8, signs of NPMW were assessed for 15 min after an injection of naloxone (0.5 mg/kg, s.c.). N-Arg (7.5 mg/kg) significantly reduced jumping and hyperactivity and tended to decrease wet dog shakes, weight loss due to diarrhea and irritability in morphine-dependent rats, but had no effect in placebo-treated rats. Grooming, abnormal posturing, ptosis, erections and ejaculations were unaffected by N-Arg treatment. The results of this study support a role of NMDA-R in NPMW and suggest that the effect is mediated, at least partially, through NO• synthase.

MOLECULAR AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT I

215.1

INDUCIBLE EXPRESSION OF A HOMEODOMAIN PROTEIN, HOX 1.3, IN ADULT AND EMBRYONIC TRANSGENIC MICE. P. Vos, R. De Santo*[1], S.-D. Zhang*, H. Arnheiter*[1], W.Odenwald* Lab. of Neurochem., NINDS, NIH; [1]Lab. of Viral & Molec. Pathogenesis, NINDS, NIH

Homeotic genes, a class of genes that code for DNA binding proteins, have been implicated as regulatory elements that control and coordinate the expression of other subordinate genes. Our inability to selectively control the expression of individual homeotic genes has made it difficult to identify candidate targets for these homeotic gene products in vertebrate systems. To test the *in vivo* function of Hox 1.3, a murine homeodomain protein, we have generated transgenic mice in which the levels of Hox 1.3 protein can be controlled by injecting interferon (IFN). These mice contain in their genome a transgene consisting of Hox 1.3 cDNA linked to an IFN inducible promoter, the murine Mx1 promoter.

In the absence of deliberate induction, the transgenic mice appeared normal. Baseline transgene expression was undetectable by Northern analysis in many organs of the transgenic mice. However, IFN induction led to a rapid accumulation of Hox 1.3 RNA in many organs (e.g., CNS, lung, liver). Furthermore, IFN injections of nontransgenic pregnant dams also induced transgene expression in embryonic mice pups. Fetal induction was detectable as early as day 11.5 of gestation using Northern analysis. The distribution of Hox 1.3 transgene expression in fetal mice was examined using *in situ* hybridization. The tissue distribution of induced transgene expression in embryos paralleled that seen in adults. Current efforts are directed towards using these transgenic mice as tools to probe the biofunction of Hox 1.3 during development and in adulthood. Tissue-specific cDNA libraries from transgenic and nontransgenic animals are being screened for potential targets of Hox 1.3 using subtractive hybridization methodologies.

215.2

THYMOSIN ß10 GENE EXPRESSION IN TRANSGENIC MICE AND ITS REGULATION BY RETINOIC ACID. S.-C. Chen*, D. I. Lugo, R. J. Smeyne, J. G. Corbin*, J. L. Hempstead*, V. R. Albert and J. I. Morgan, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

In previous studies, levels of thymosin ß10 have been shown to be high in the embryonic nervous system but to decline dramatically after birth. In addition, the expression of this gene is up-regulated by the morphogen, retinoic acid, in a variety of neuroblastoma cell lines eg., B104. Using a thymosin ß10 cDNA as a probe, we have investigated the levels of ß10 mRNA in B104 cells treated with retinoic acid, either in the presence or the absence of cycloheximide. The results indicate that protein synthesis is required during the first 5 hours of retinoid treatment for the increased expression of thymosin ß10 mRNA. This suggests the involvement of intermediate transcription factors eg., immediate-early genes, in the regulation of thymosin ß10 gene expression by retinoic acid.

Using lacZ as a reporter, we have constructed transgenic mice that carry a ß10-lacZ fusion gene and examined its expression during development. We observe high levels of expression of this construct in CNS, including hippocampus, spinal cord, cerebellum, olfactory bulb and olfactory neuroepithelium. Several other constructs, with different lengths of the thymosin ß10 promoter, have also been used. These studies will help to dissect out the regulatory elements responsible for the developmental expression of the thymosin ß10 gene and the modulation by retinoic acid.

215.3

BRAIN-SPECIFIC INCREASED EXPRESSION OF GLIA DERIVED-NEXIN IN TRANSGENIC MICE ALTERS BRAIN FUNCTIONS. F.M.Botteri*[1], I.Mansuy*[1], C.Mondadori[2], G.Sansig*[3], H. van der Putten*[3], and D. Monard[1]. [1]Friedrich Miescher-Institute, P.O.Box 2543; [2]Pharmaceutical Research Department, Ciba-Geigy Ltd.; [3]Biotechnology, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland. Glia derived-nexin (GDN), also known as protease nexin I, is a serine protease inhibitor, that in vitro promotes neurite outgrowth from neuroblastoma cells, sympathetic and hippocampal neurons. In vivo, GDN is constitutively expressed in the olfactory system, where axonal regeneration and neurogenesis occur continuously throughout life, and it is also upregulated following lesion of the rat sciatic nerve. Therefore GDN could serve crucial roles in the process of axonal regeneration in vivo. To investigate biological relevance of GDN in vivo, we generated several strains of transgenic mice carrying a chimeric Thy-1-GDN gene, which directs the expression of high levels of rat GDN in the brain. Transgenic mice of one strain went through a whole series of behavior tests. In any of the observation tests used (global behavioral assessment, rotarod, motility) no differences were noted between transgenic mice and non-transgenic littermates. The transgenic mice, however, tended to have a slower acquisition rate in the Morris water maze, although this effect was observed in only 6/40 animals and might be attributed to differences in GDN expression among individuals of the same strain. A second strain is being tested to exclude a role for the specific chromosomal integration site of the transgene influencing the learning performance of the first strain. Immunohistochemistry and in situ hybridisation analyses are in progress to determine possible consequences of the overexpression of GDN on brain organisation.

215.4

IN VIVO TISSUE-SPECIFIC EXPRESSION OF A CHIMERIC CONSTRUCT CONTAINING 3.5 KB OF THE 5' FLANKING DNA FOR THE MOUSE TYROSINE HYDROXYLASE GENE. W.W. Morgan and Z.D. Sharp*. Dept. Cellular and Structural Biology and the Institute for Biotechnology, Univ. Texas Hlth. Sci. Ctr. at San Antonio, TX 78284-7762.

The expression of tyrosine hydroxylase (TH) is limited to catecholaminergic neurons located within the brain, the sympathetic chain ganglia and paraganglia and to adrenal medullary cells. This limited distribution suggests that the activation of the gene which encodes TH is regulated by very precise molecular genetic mechanisms. To investigate these processes, over 3.5 kb of the 5' flanking region of the mouse TH gene was cloned and sequenced. A chimeric construct was made by attaching this upstream DNA including the +1 initiation site to a reporter gene consisting of the coding sequence for bacterial beta galactosidase plus polyadenylation signals. The construct was introduced into fertilized zygotes of C57BL/6 mice, and transgenic mice were identified by PCR analysis of mouse DNA. Bacterial beta galactosidase enzymatic activities in the medulla oblongata were 2.6 and 0.6, in the adrenal 120.6 and 13.5 and in the liver 0.2 and 0.2 picomoles/ml/min/mg protein in transgenic versus control animals, respectively. These results show that the DNA sequences required for the *in vivo* tissue-specific expression of the TH promoter are located within the 5' region of the gene and are all within 3.5 kb of the +1 initiation site. Supported by DA00755 and GM43763 to WWM and DK38546 to ZDS.

215.5

ONTOGENY OF mRNA FOR DOPAMINE RECEPTOR SUBTYPES (D_1,D_2,D_3) IN THE RAT BRAIN. U.B. Schambra, G.E. Duncan, G.R. Breese, M.G. Fornaretto*, M.G. Caron and R.T. Fremeau, Jr. UNC, Chapel Hill, NC 27599 and Duke Univ. Med. Ctr., Durham, NC 27710.

The ontogeny of the expression of D_1, D_2, and D_3 dopamine receptor mRNA in the rat brain was assessed at 16 time points between gestational day (GD) 14 and postnatal day (PD) 130-140 by in situ hybridization. A differential developmental pattern of expression of the 3 dopamine receptor subtypes was observed. X-ray film autoradiography revealed that the D_1 receptor message was present at GD 14 whereas the D_2 receptor mRNA was first evident at GD 18. In contrast D_3 receptor mRNA was detectable at PD 5. At GD 14, the D_1 message was observed in the area of the developing striatum/olfactory tubercule, cingulate and pyriform cortex in both ventricular and intermediate zones. At GD 18, D_2 mRNA was initially observed in the area of the developing striatum/olfactory tubercule and substantia nigra whereas at PD 0, cells in layer V of the cingulate, parietal, and pyriform cortices were labelled. D_3 mRNA was first observed at PD 5 in the olfactory tubercule and island of Calleja with weaker signals in the parietal cortex and nucleus accumbens. Maximal levels of receptor mRNA were observed at PD 14 for both D_1 and D_3 and PD 10 for D_2 and thereafter all three mRNAs declined significantly with age. Our observation that D_1 receptor mRNA is expressed in the ventricular zone, a region of dividing neuroblasts and the intermediate zone, a region of migrating neurons, is consistent with previous studies suggesting that D_1 receptors regulate neurite outgrowth and growth cone motility. Supported by Training Grant #5-39056, and, grants HD-23042 and NS-19576.

215.6

EXPRESSION OF BC1 RNA IN DEVELOPING HIPPOCAMPAL NEURONS IN CULTURE. H. Tiedge*, G. A. Banker[1], and J. Brosius. Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029. [1]Department of Neuroscience, University of Virginia, Charlottesville, VA 22908.

BC1 RNA is a short non-translatable polymerase III transcript that is selectively expressed in the somata and dendrites of a subset of neurons in the adult rat nervous system [Tiedge et al. (1991) Proc. Natl. Acad. Sci. USA 88, 2093-2097]. The expression pattern and subcellular location of the RNA have been interpreted to indicate a possible functional role in postsynaptic domains of neurons, conceivably in the context of localized protein biosynthesis. In the present study, we have analysed the expression of BC1 RNA in developing hippocampal neurons over a period of up to 4 weeks in culture. Neurons were isolated from 18 day-old rat embryos and maintained in culture as described earlier [Bartlett and Banker (1984) J. Neurosci. 4, 1944-1953]. Cultures were fixed at various time points and probed for selected target RNAs. Significant amounts of BC1 RNA were not detectable in hippocampal neurons until the end of the first week in culture. During this time, hippocampal neurons in culture establish their polarity: they extend first axonal, then dendritic processes [Dotti et al. (1988) J. Neurosci. 8, 1454-1468]. In contrast, SRP RNA, the 7SL RNA component of the ubiquitous signal recognition particle, was detectable after only 2 days in culture, the first time point taken, and continued to be expressed at substantial levels during all phases of development. Levels of BC1 RNA rose sharply at the beginning of the second week in culture, at a time when synaptic contacts become numerous. In contrast, no abrupt changes were observed in expression levels of SRP RNA. By the end of the second week in culture, high levels of BC1 RNA were detectable in the somatic and dendritic domains of most neurons, a pattern that persisted in mature neurons. SRP RNA, on the other hand, was predominantly localized to the perikarya of hippocampal neurons at all stages during development in culture. Our results indicate that BC1 RNA is not required for initial outgrowth of either dendrites or axons by hippocampal neurons in culture; however, the time course of BC1 expression may correlate with the formation, maturation or maintenance of synaptic connections.

215.7

ELECTRICAL ACTIVITY REGULATES EXPRESSION OF mRNAs CODING FOR MYOD-RELATED TRANSCRIPTION FACTORS AND ACETYLCHOLINE RECEPTORS. A.Buonanno, R.Beers,* H.Brenner* and R.Eftimie,* Unit on Mol. Neurobiol., LDN, NICHD, NIH, Bethesda, MD 20892

Electrical activity represses the expression of a repertoire of muscle genes during innervation; i.e., genes coding for nicotinic acetylcholine receptors (nAChR). We have analyzed the expression of members of the myoD family of trans-acting factors during muscle development, to determine if they may regulate transcription of genes down-regulated by innervation. Using Northern blot analysis, we found that initially myoD, myogenin and myf-5 mRNA levels decrease during a period that coincides with innervation (E17 to P7), and is followed by a diminution in nAChR mRNA levels that occurs after birth (P7). In contrast, mrf-4 levels begin to accumulate around birth. Denervation reverses the effects of innervation and leads to the accumulation of transcripts coding for the myogenic factors and receptor. Myogenin and myoD mRNAs increase dramatically (40 and 15-fold) 8h after denervation; the increase precedes the accumulation of nAChR α and γ subunit mRNAs which begins approximately 1d after denervation. Increases in myf-5 and mrf-4 mRNA levels, however, are small and occur after the accumulation of receptor transcripts. The effects of innervation are due largely to electrical activity because stimulation of denervated muscle with extracellular electrodes prevents the accumulation of mRNAs coding for myogenic factors and receptor. Next, we analyzed if the myogenic factors can regulate transcription of receptor genes. Using gel-shift assays and DNA footprinting we found that myogenin and MyoD bind to the nAChR α and γ subunit enhancers which contain two consensus "myoD binding sites". Functional studies showed that myogenin and myoD activate transcription from constructs containing either the α or γ subunit enhancer linked to the CAT reporter gene. Point mutations in the myoD binding sites of either enhancer abolish expression of the reporter gene in transfected C2C12 myotubes. It is interesting to speculate from our results that myogenin and/or MyoD coordinately activate transcription of nAChR genes during myogenic differentiation, and may also modulate transcription of a repertoire of skeletal muscle genes down-regulated by electrical activity.

215.8

SUBTRACTIVE HYBRIDIZATION TO IDENTIFY GENE PRODUCTS EXPRESSED DURING THE CRITICAL PERIOD FOR VISUAL CORTEX DEVELOPMENT. Shiv Prasad and Max Cynader, Department of Ophthalmology, University of British Columbia, 2550 Willow Street, Vancouver, B.C., Canada. V5Z 3N9

The developmental patterns of expression in the visual cortex for some known molecules have been previously examined by ligand binding autoradiography, immunocytochemistry and in situ hybridization. The results of these studies have indicated that such molecules can be expressed transiently in high abundance during the critical period for developmental plasticity of visual cortex .

We have isolated and characterized cDNA clones of mRNAs whose levels change in the kitten visual cortex during the critical period. Our approach has been to construct and analyse a subtractive cDNA library for the visual cortex of a 30 day old kitten subtracted with an adult cat. In total approximately12,000 clones were screened, 200 of which hybridized to the subtracted probes. The positives were screened with individual clones to determine how many represented copies of each other. About 75% of the positive clones were found to be single copy and the rest represented either two or many copies. One of these represented 20% of the positives.

The individual clones from the subtractive library are being examined by Northern blot hybridizations to mRNAs from various tissues and developmental ages. The individual clones are also being sequenced to identify the primary structure. The inferred amino acid sequence of some of these clones have shown identity to some functionally identified molecules in the EMBL nucleotide and SWISS-protein data base. *In situ* hybridization using the individual cDNAs as probes will facilitate the exact localization of these transiently expressed transcripts in the developing visual cortex.

215.9

DEVELOPMENTALLY-REGULATED mRNA CLONED FROM AN EMBRYONIC NEURONAL CULTURE

Y. Aizenman, R.J. Milner, Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

To gain insight into brain development, we have used molecular cloning techniques to identify mRNAs that are expressed at particular stages of neural development. Primary neuronal cultures were prepared from 17-18 days old embryonic rat cortices in a defined medium. A cDNA library was constructed from cultures that were induced for 2-3 hours with thyroid hormone, a crucial hormone for brain development, in the presence of cyclohexamide. Several clones of interest were selected from this library by a subtractive hybridization procedure. Three of these clones hybridized to mRNAs that displayed regulated patterns of expression during development, with increased expression in RNA samples from embryonic brain as compared to adult. Two of these clones were expressed at a constant amount from embryonic day 14 to postnatal day 10. Their expression declined on postnatal day 15 and was further decreased on day 20. This time course, taken with the fact that these mRNA were cloned from a rapidly growing neuronal culture, suggests that the mRNAs might encode proteins involved in the process of neural development, such as neurite outgrowth. Partial DNA sequence analysis of these clones revealed that one of the clones encoded Tα1 tubulin, whereas the other two clones, which hybridized to mRNAs of 4500 bases and 2200 bases, were not similar to any known molecules contained in the NBRF and GenBank databases. Supported by NIH grant NS22347.

215.10

RAPID GLUCOCORTICOID MODULATION OF ASTROCYTE mRNAS AND PROTEINS. M. K. O'Banion*, D. A. Young*, E. W. Howard*, and M. C. Bohn, Departments of Medicine, Biochemistry, and Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

Glucocorticoids (GC) affect the developing nervous system and are important to homeostasis in the adult brain. Glucocorticoid receptors are expressed in astrocytes and oligodendrocytes, as well as in neurons. This study was undertaken to begin to identify genes that are regulated by glucocorticoids in the brain.

The influence of GC on gene expression in primary cultures of type I astrocytes was studied using ultra-high resolution giant two-dimensional (2D) gel electrophoresis of the ^{35}S-labeled *in vitro* translation products of poly-A RNA isolated from cells treated for 3 h with corticosterone (10^{-6} M; CORT). Quantitative analysis of over 1500 *in vitro* translation products reveals 7 that change by more than 2-fold (6 increases, 1 decrease). Four of 6 increases are observed in cells treated with cycloheximide, suggesting that these arise by direct transcriptional activation. Two of these inductions are also observed in mouse fibroblasts treated with dexamethasone. These have been identified as glucocortin (17 kDa, pI 4.8) and glutamine synthase (43 kDa, pI 7.0).

To determine whether the changes observed in levels of mRNAs correspond to changes in protein expression, 2D gels of ^{35}S-labeled whole cell proteins were also examined. Five proteins induced within 3 h by CORT correspond to the changes seen in *in vitro* translation products.

These data demonstrate that type I astrocytes express functional GC receptors that mediate changes in gene expression within 3 hours. These changes appear to include both primary inductions and secondary inductions, requiring protein synthesis. The future identification of these GC-regulated genes will help elucidate the role of glucocorticoids in the brain. [Supported by the J. P. Wilmont Foundation and NIH grants NS20832 and RRO4968.]

215.11

CHARACTERIZATION OF NOVEL DEVELOPMENTALLY-REGULATED PROTEINS IN EMBRYONIC RAT SPINAL CORD. D.H. Geschwind and S. Hockfield, Section of Neurobiology, Yale School of Medicine, New Haven, CT 06510.

We have previously used 2-dimensional electrophoresis to identify several proteins (DRPs) whose synthesis is regulated during cortical neurogenesis in the rat (J. Neurosci. 9:4304). To further characterize some of these proteins, 2-D electrophoresis was used to study their regulation in the spinal cord at times (E12 v. E21) roughly comparable to those used in the previous study of developing cortex. Experimental procedures were similar to those previously described (*ibid*). Several of the proteins that were down-regulated in the cortex between E14 and E21, demonstrated significant down-regulation in the spinal cord between E12 and E21. Several of these do not appear in the liver, lung or heart and thus appear to be CNS-enriched. One of the previously up-regulated proteins, 407, was up-regulated as seen in cortex. Two additional proteins that were up-regulated in spinal cord were not observed in the analysis of cortex. Also intriguing is a set of proteins that were up-regulated more than 100-fold in cortex, but that are not seen in spinal cord, lung, heart or liver and thus may be cortex-specific.

One of the cortical up-regulated proteins, 310, was up-regulated 4-fold in spinal cord, but partitioned primarily in the soluble fraction in spinal cord(>90%), while being more evenly distributed between particulate and soluble fractions in the cortex. We have further characterized 310 by protein microsequencing. Five stretches of sequence ranging from 7-18 amino acids indicate no significant homologies with other proteins in several databases, indicating that protein 310 is a previously uncharacterized, neural-enriched protein whose abundance increases during neurogenesis in cortex and spinal cord. The apparent neural-specific expression protein 310 and other DRPs, coupled with their regulation during the period of neurogenesis in spinal cord and cortex suggests that they may be involved in neurogenesis. Experiments are currently underway to further characterize them using antibody and oligonucleotide probes. *Supported by the NSF.*

BRAIN METABOLISM AND BLOOD FLOW III

216.1

A NETWORK-SIMULATION MODEL FOR STUDYING INTERREGIONAL CORRELATIONS BETWEEN REGIONAL RATES OF CEREBRAL BLOOD FLOW (rCBF). B. Horwitz, Lab. Neurosciences, Natl. Inst. on Aging, NIH, Bethesda, MD 20892.

Correlations between pairs of cerebral metabolic rates, obtained by positron emission tomography (PET), have been used to study patterns of functional associations among brain regions in humans. Recently, a computer simulation model was developed for partial validation of the correlation method (Horwitz, Int J Biomed Comput, 26:149-170, 1990). The current study presents a generalization of the model for simulating (O-15)water-cerebral blood flow PET studies, where multiple tasks can be performed within a single scanning session on a single subject. The model generates a set of simulated rCBF data upon which correlational analysis is performed. The model consists of n regions, in which blood flow in one region is a sigmoidal function of blood flow in some or all of the other regions, with the linking parameter called a functional coupling coefficient. The pattern of functional coupling coefficients is specified for each simulation. Random numbers provide the within-subject and between-subject variabilities seen in experimental data. It is shown how the model parameters can be determined using existing experimental data. Simulations are presented that demonstrate how correlational analysis provides information about neural relations that cannot be determined by analysis of changes in rCBF values alone, and how models like this can provide a link between PET data and cognitive neuroscience hypotheses of brain function.

216.2

AN ALTERNATIVE OPERATIONAL EQUATION FOR SEQUENTIAL DOUBLE-STUDY METHOD USING RADIOLABELED DEOXYGLUCOSE. J.Y. Chang, M.D. Ginsberg. Cerebral Vascular Disease Research Center, University of Miami, Miami, FL 33101.

We have previously described a sequential double-study method with autoradiography and radiolabled DG(Chang et al., Soc Neurosci Abstr 16:23,1990). The success of this method depends upon how accurately RC can be estimated. RC is defined as the ratio of the tracer concentration at the end of the first study to its remnant at the end of second study. Recently we have realized that the limited information embedded in the history of tracer concentration prohibits us from estimating all five rate-constant values accurately when the least-square best-fit method is used. Thus, RC may have been estimated incorrectly using the average rate-constant values. Results of our computer simulation suggest that the error of RC, estimated through the use of average rate-constant values, is 10.7, 7.0, 4.4, and 1.8% with the study length for rate-constant estimation is 120, 180, 240, and 360 min, respectively. Individual RC, however, can be easily estimated correctly, within 0.5%, from a set of tracer concentration values obtained from a 120-min study. Thus, to take the advantage of better RC measurement from each data set, we devised an alternative operational equation using the *average RC* for the sequential double-study method as

$$lCMRglc1 = \frac{Cp}{LC}\left(\frac{\overline{k_1^* k_3^*}}{k_2^* + k_3^*}\right)\frac{\overline{RC}_{(k_1^*,k_2^*,k_3^*,k_4^*,k_5^*)}[C^{*1}(T_2) - C_s^{*1}(T_2 : T_1)]}{C_{e+m}^{*1}(T_1)}$$

instead of obtaining *RC from the average rate-constant values* as

$$lCMRglc1 = \frac{Cp}{LC}\frac{\bar{k}_1^* \bar{k}_3^*}{\bar{k}_2^* + \bar{k}_3^*}\frac{RC_{(\bar{k}_1^*,\bar{k}_2^*,\bar{k}_3^*,\bar{k}_4^*,\bar{k}_5^*)}[C^{*1}(T_2) - C_s^{*1}(T_2 : T_1)]}{C_{e+m}^{*1}(T_1)}$$

216.3

IN VIVO MAPPING OF BLOOD OXYGENATION LEVELS IN RAT BRAIN BY MAGNETIC RESONANCE IMAGING. S. OGAWA AND T.M. LEE* AT&T Bell Labs, Murray Hill, NJ 07974

With high field MRI (magnetic resonance imaging), Blood Oxygenation Level Dependent (BOLD) contrast has been observed in rat brains. BOLD contrast with numerous dark lines in these images reflects the venous blood micro-vasculature of the brain under normoxic conditions. The contrast is sensitive to the physiological condition of the brain (1). By changing the depth of anesthesia with 0 %to 3% halothane, the venous blood oxygenation was varied and the level at the sagittal sinus was measured from the blood water relaxation time in MRI. BOLD contrast as measured by image signal intensities in the cerebral cortex region showed linear (rather than exponential or gaussian) dependencies on the sagittal sinus oxygenation level. Image signals from the midbrain region did not necessarily fit to the linear relation. CFS signals in ventricles showed opposite correlations to the depth of anesthesia (the lower the dose of halothane, higher the signal intensity), indicating the more CSF movement in awake states than in anesthetized states. These relationships between the image contrast and the venous blood oxygenation can be used to calibrate changes in BOLD contrast, responding to changes in the physiological condition, in terms of local blood oxygenation and further to correlate with local oxygen consumption and /or CBF.

(1) S. Ogawa, T.M. Lee, A. Kay & D. W. Tank, Proc. Natl. Acad. Sci. USA 87 9867

216.4

CONTINUOUS CEREBRAL BLOOD FLOW MEASUREMENT BY A THERMAL METHOD. Datong Wei*, Mary Shea*, Gerald M. Saidel*, and Stephen C. Jones. Cerebrovascular Res. Lab., Cleveland Clinic Foundation, Cleveland, OH 44195 and Dept. of Biomed. Eng., Case Western Reserve Univ., Cleveland, OH 44106.

A continuous thermal perfusion measurement system was developed to study temporal variations of cerebral blood flow (CBF) with 0.5 mm diameter thermistors placed on the cortical surface. The system is optimally designed based on a distributed heat transfer model under requirements of probe size, sensitivity, resolution, and frequency response (IEEE Trans. Biomed. Eng. 37:1159-1172, 1990). *In vitro* tests indicate that the long-term drift of the system is less than the equivalent of 0.001°C, an estimated temperature change caused by perfusion change of about 5 ml/100g-min. The system can compensate for baseline temperature changes during measurement.

In vivo tests were performed on Nembutal anesthetized, artificially ventilated Sprague-Dawley rats both to refine the probe design and to investigate its use for measuring dynamic changes in CBF. Norepinephrine (0.1 - 0.5 mg) was injected as a bolus into the femoral vein repeatedly to cause transient CBF changes in response to changes in arterial blood pressure (ABP). The results from 8 rats demonstrated a significant difference ($p<0.05$) between the flow response and the reference temperature changes. Five of these 8 rats demonstrated an autoregulatory response of CBF between 5 to 14 s after the onset of the ABP increase. The mean ratio of flow change to ABP change ranged from 0.0003 to 0.0015 °C/mmHg. Two rats showed temporal blood flow variations with a cycling pattern. Fourier transform analysis showed that the cycles had a distinct frequency peak component between 0.06 to 0.12 Hz and from 0.002 to 0.009 °C in magnitude.

These results indicate that the system can be used to study the initial response of CBF to ABP changes and temporal CBF cycling in different steady-state conditions. (Supported by USPHS NIH grant NS 21538 and NSF grant BNS 9022190.)

216.5

INTRACELLULAR ION CONCENTRATIONS AND CAPILLARY BLOOD FLOW WITHIN THE RAT BRAIN NEOCORTEX: AN IN VIVO IMAGING APPROACH. A Villringer, U Dirnagl, A Them*, G Sixt*, KM Einhäupl*, Dept. of Neurology, University of Munich, 8000 Munich, Germany

In order to investigate coupling between brain cells and microvascular blood flow, we established a model for the simultaneous assessment of intracellular ion concentrations and blood flow in surrounding microvessels within the outer layers of the rat brain cortex in vivo.

Methods: The parietal cortex (dura mater removed) of anesthetized Wistar rats was observed through a closed cranial window using a Bio-Rad MRC 600 confocal laser scanning microscope (Ar/Kr Laser) attached to a Nikon Optiphot microscope with a x40 water immersion objective. Brain cell loading with AM-esters of ion sensitive fluorescent dyes was achieved by superfusion for 30 min (10μM in artificial CSF, washout 30min). To label the intravascular space dyes that do not pass the blood brain barrier were injected i.v.. Dyes with differing optical characteristics were used for labeling of brain cells and intravascular space, respectively. When brain cells were loaded with Ca-Crimson (Ca-sensitive dye, exc max 596nm, Molecular Probes) Na-fluorescein (exc max 488nm) was used as marker of plasma, when brain cells were labeled with BCECF (pH sensitive, exc max 488nm, Molecular Probes) Texas Red (exc max 596nm), respectively.

Results: Simultaneous imaging of brain cells loaded with ion concentration sensitive dyes and blood flow in surrounding capillaries was achieved in optical sections at different depths beneath the brain surface. Ion concentration dependent signal was derived from depths up to 200μm (BCECF) or 100μm (Calcium Crimson), individual cells could be differentiated up to 50μm or 30μm, respectively. Intracortical capillaries were seen up to 250μm (fluorescein) or 150μm (Texas Red), erythrocyte flow (erythrocytes were visualized as negative contrast within the labeled plasma) could be followed up to 150μm (fluorescein) or 100μm (Texas Red) beneath the brain surface, respectively.

Conclusion: The presented model is a promising approach to study the interaction of brain cells and surrounding vessels.

216.6

EVIDENCE THAT VASCULAR RECRUITMENT IS NOT IMPORTANT IN HETEROGENEITY OF CEREBRAL BLOOD FLOW (CBF). S. C. Jones, J. L. Williams, and M. Shea, Department of Brain and Vascular Research, Cleveland Clinic Foundation, Cleveland, OH 44195.

Recently, we hypothesized that recruitment of cerebral blood vessels might contribute to temporal cycling (3-10 cycles/min) and spatial heterogeneity of CBF (*Am. J. Physiol.* 257: H473, 1989). In the present study, we injected different fluorescent plasma tracers intravenously at 2 times before decapitation for examination of temporal and spatial relationships of cerebral vascular perfusion. In 7 anesthetized rats, either FITC- or RITC-dextran was injected 45s before decapitation. The other tracer and ^{14}C-iodoantipyrine were injected as a bolus 10s before decapitation. Immediately after decapitation, the head was immersed in chlorodifluoromethane (-40°C). While frozen, the brain was removed from the skull and sectioned. In each section, vascular fluorescence of both tracers was examined microscopically, and sections were placed on film for quantitative autoradiography and image analysis. CBF was calculated with the indicator fractionation technique. Maps of vascular fluorescence were overlaid on CBF autoradiograms.

In each rat, we examined 73±6 (mean±SE) large vessels (20-60 μm) in cerebral cortex. In every instance, both tracers were present in the lumens of large vessels. Large vessels were distributed equally in cortical areas of high and low blood flow (P and V, respectively; P/V = 1.1±0.2). In each rat, small vessels (≤12 μm) with fluorescence were counted in 25±2 randomly selected locations in cerebral cortex. The number of small vessels for tracer injected 45s and 10s before decapitation was similar (346±48 and 355±42 vessels/mm^2, respectively), and the selected locations were equally distributed in P and V. Our findings suggest that vascular recruitment is not an important mechanism in temporal cycling or spatial heterogeneity of blood flow in cerebral cortex. (Supported by NIH NS24343)

216.7

REMODELING OF PIAL BLOOD VESSELS IN MICE AFTER BIRTH. D-B. Wang*, C.M. Rovainen and T.A. Woolsey, Depts. Cell Biology and Neurosurgery, Washington Univ. Sch. Med., St.Louis, MO 63110

The aim of this work is to follow the changes in patterns, diameters, densities, and flow velocities of blood vessels on the developing barrel cortex of postnatal mice. Mice were anesthetized with 1-2mg/gm urethane IP or Metofane. Rhodamine- or FITC-dextrans and 1.3 µm YG latex beads were injected intracardially or intravenously. Pial blood vessels were imaged through closed cranial windows with an epifluorescence microscope, GenRad strobe lamp, and ICCD or SIT video cameras. In newborn mice pial capillaries formed a dense plexus over the surface of the cortex; venules were small, irregular and extensively connected with surface capillaries; arterioles had numerous anastomoses. By 7 days intraparenchymal capillary density increased (M.E. Spence); surface density decreased; surface venules and arterioles grew to accommodate the internal microcirculation. In adults few surface capillaries remained; pial venules were large and smooth-walled and drained internal radial venules; pial arterioles were large and lacked collaterals. At all ages the diameters of arterioles at branch points (N.Blocher) followed Murray's cube rule. Velocities were measured with strobe illumination of moving fluorescent beads at 400 Hz. Young (0-7 day old) mice and adults had the same distributions of Vmax in arterioles of the same sizes (6-50 µm), suggesting that peak wall shear rates were similar in developing and mature arterioles. These results show that pial vessels are dramatically remodeled during the growth and differentiation of the cortex. Flow and wall shear stress are hemodynamic factors that may help regulate the enlargement or regression of blood vessels during cortical maturation.

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216.8

DEVELOPMENTAL CHANGES OF ENERGY RELATED ENZYMES IN CULTURED ASTROCYTES, OLIGODENDROCYTES, SCWANN CELLS, AND NEURONS, by R.S.Rust, J.G.Carter*, D.Martin, J.M.Nerbonne, P.Lampe, M.E. Pusateri*, and O.H.Lowry*. Washington Univ. Sch. of Med., St. Louis, MO 63110.

Little information is available concerning developmental and cell-type variation in energy enzymes of nervous tissues. We studied 16 enzymes from 8 metabolic systems in cell culture. All enzymes increased as a function of time in culture (6-18d). Enzyme activity in mature astrocyte cultures (percentage of activity of brain homogenates) ranged from 9% (Glycerol-3PDH) to over 300% (G6PDH). Most astrocytic energy-yielding enzymes showed 25-100% of brain activity, but three NADPH generating dehydrogenases (G6PDH, 6PGDH, and ICDH) were more than two-fold enriched. Suprisingly, CPK activity was as high in mature astrocytes as in brain, half as high in oligos, but only 7% or less enriched in Schwann cells, cortical or cervical ganglion (SCG) neurons. Glycogen phosphorylase and PGM were also high in astrocytes, 130% and 90% of brain, respectively. SCG neurons were enriched in PGM and 6PGDH compared to cortical neurons. Oligodendrocytes and Schwann cells show remarkable enzymatic similarity to neurons in culture. Trypsinization affected the expression of LDH (+) and BOAC (-). These data support a role for astrocytes in provision of energy and production of reducing equivalents in brain.

216.9

STUDIES ON CEREBRAL O_2 LEVELS, FREE FATTY ACIDS, AND DIACYLGLYCEROL POOLS IN RAT HIPPOCAMPUS AND BRAIN CORTEX DURING SUSTAINED SEIZURES. [1]F. Visioli,* [2]L. Rihn,* [1]E. Rodriguez de Turco,* [2]N. Kreisman, and [1]Bazan N.G. [1]LSU Eye Center and Neuroscience Center and [2]Department of Physiology, Tulane University School of Medicine, New Orleans, LA 70112.

Cerebral blood flow (CBF) increases during early seizures but the increase progressively attenuates during late seizures, resulting in decreased cortical oxygenation at that time. Since changes in free fatty acids (FFA) and diacylglycerol (DAG) were shown during seizures, we hypothesized a correlation between FFA and DAG levels and cortical O_2. Male Wistar rats were anesthetized with pentobarbital, paralyzed, and ventilated. Seizures were induced by IV injections of pentylenetetrazol and relative changes in cerebral O_2 were monitored. Animals were killed by focused microwaves at different times and brain lipids were extracted and analyzed. There was an increase in both FFA and DG following the third seizure, and arachidonate (20:4) and stearate (18:0) showed the highest increase. Late seizures showed different responses: a rise in cerebral O_2 was associated with a lower release of FFA compared to early seizures, while a decrease in cerebral O_2 was associated with a higher production of FFA. DAG followed the same profile during late seizures, but they also were elevated during both ictal and interictal periods of early seizures. Accumulation of free 18:0 and 20:4, and 18:0- and 20:4-DAG indicates increased activity of phospholipases A_2 and C resulting in a higher turnover of inositol phospholipids. Either an enhanced acyl transferase activity and/or increased CBF decreases FFA during the interictal period, and a relative hypoxia during late seizures is associated with increased production and/or decreased reacylation of FFA and DAG. Supported by AHA 89-902 and NINDS NS23002.

216.10

MATURATION OF RAT BRAIN MITOCHONDRIAL CREATINE KINASE. D. Holtzman and T. Wallimann* Dept of Neurology, Harvard Medical School and The Children's Hospital, Boston, MA 02115; Inst. Cell Biol., ETH Honggerberg, Zurich, Switzerland.

Brain creatine kinase (CK)-catalyzed phosphorus flux from phosphocreatine to ATP was measured in the rat pup using the 31P-NMR saturation transfer (ST) technique. The results are compared to an in vitro measure of mitochondrial CK (MiCK) activity. As previously described in the mouse brain (Holtzman et al., Dev Brain Res, in press), rat brain CK-catalyzed phosphorus flux increases 3-4 fold between 12-18 days of age ($p<.05$). The MiCK isoform is not found in cerebral cortical tissue at 5 days. This isoform rises sharply between 12-17 days of age. This age period, in which the MiCK activity appears with in vivo and in vitro studies, is coincident with the appearance of a fraction of brain mitochondria which show contact sites between the inner and outer membranes (Holtzman et al.,J. Neurochem., 33:453, 1979). The capacity of cerebral cortical tissue to rapidly increase the rate of oxidative phosphorylation in response to increased energy demand also appears over this narrow age period (Holtzman et al.,J. Neurochem, 39: 274, 1979). These simultaneous developments in the physiology of brain ATP metabolism suggest that the MiCK isoform is important in closely coupling the rates of ATP synthesis to changes in ATP utilization in brain.

216.11

ION CHANNELS IN RAT MICROVASCULAR ENDOTHELIAL CELLS

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Endothelial cells play an obligatory role in mediating the response to endogenous vasodilators such as acetylcholine. However, no electrophysiological data are available on brain microvascular endothelial cells. We have therefore investigated the electrophysiological properties of cultured endothelial cells from intraparenchymal brain microvasculature by using the patch clamp technique. These cells appeared to express a variety of voltage- and receptor operated ion channels. Cell membrane depolarization failed to elicit any inward current even after full blockade of outward currents: the prominent feature was the activation of an outward almost ohmic potassium current. Whole cell and single channel recordings revealed an ATP-activated current inwardly rectifying in the depolarizing range. The ATP-activated channel appears to be permeant to cations, since its reversal potential was near 0 mV in symmetric KCl solutions. We also found evidence of ATP-sensitive potassium channels blocked by micromolar concentrations of glibenclamide. These electrophysiological features may play a role in the regulation of microvascular tone and blood-brain barrier permeability.
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216.12

ADVENTITIAL GANGLION CELL-LIKE STRUCTURES IN THE MAJOR CEREBRAL ARTERIES OF THE CAT: AN IMMUNOHISTOCHEMICAL STUDY.

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Adrenergic, cholinergic, GABAergic and peptidergic nerve fibers along cerebral blood vessels were found to originate from several sources such as the superior cervical, ciliary, sphenopalatine, otic and trigeminal ganglia. The present study aimed to elucidate the presence of adventitial ganglion cell-like structures (AGCLS) connected to perivascular nerve fibers in the cerebral pial arteries. Major cerebral arteries of adult cats weighing 2-3 kg were dissected and fixed by immersion in an ice-cold 3:1 mixed solution of Zamboni's and PLP fixative. The materials were processed for single or double labelling avidin-biotin peroxidase complex immunohistochemistry and observed under a light microscope. Two types of clusters of AGCLS immunoreactive (I) to choline acetyltransferase, glutamic acid decarboxylase (GAD) and calcitonin gene-related peptide (CGRP) were observed in the intracranial internal carotid arteries. Smaller ones (25-45 μm in diameter) composed of several ganglion cells were observed mostly along thick adventitial nerve fibers in the distal portion, and larger ones (150-250 μm in diameter) composed of 10-20 clusters of ganglion cells were more proximally located in the internal carotid artery. Some of GAD-I AGCLS were found to overlap with CGRP-I AGCLS. On the other hand, AGCLS in other cerebral pial arteries did not form clusters and were spindle in shape. The density of AGCLS was highest in the proximal portion of the middle cerebral artery. There were no AGCLS immunoreactive to tyrosine hydroxylase or vasoactive intestinal polypeptide in this study. The present results suggest a possiblity that AGCLS play some role in the regulation of cerebral blood flow as an origin of either cholinergic, GABAergic or CGRPergic nerve fibers. (Supported by NIH-HL27763, AHA/IHA, SIU Sch Med)

INGESTIVE BEHAVIOR: CCK, BOMBESIN AND NPY

217.1

INHIBITION OF SUCROSE INTAKE BY CHOLECYSTOKININ IN RATS WITH PYLORIC CUFFS.

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This experiment examined the effectiveness of cholecystokinin octapeptide (CCK-8) in reducing intake of 30% sucrose when gastric emptying was blocked. Prior research has suggested that cholecystokinin satiety is enhanced by (1) concurrent presence of sucrose or glucose within the small intestine and (2) greater gastric retention after CCK-8 administration than after saline; both of these factors should be eliminated by inflation of a pyloric cuff. Female (N=8) and male (N=2) adult Sprague-Dawley rats, with pyloric cuffs inflated or deflated, were allowed 20 min access to 30% sucrose beginning 10 min after intraperitoneal injection of saline or 1, 2, or 4 ug/kg CCK-8. When cuffs were deflated, these doses of CCK-8 reduced intake by 22%, 40%, and 45%, respectively. With cuffs inflated, corresponding reductions were 18%, 32%, and 49%. At each dose, the effect of CCK-8 did not differ significantly between tests with cuffs inflated or deflated ($P's > .05$). Thus, neither emptying of sucrose into the duodenum nor differential retention of ingested sucrose was necessary for the full expression of cholecystokinin satiety at the doses tested.

217.2

TRANSIENT, SELECTIVE ATTENUATION OF OLEATE AND CCK-INDUCED SUPPRESSION OF SHAM FEEDING BY INTRA-INTESTINAL CAPSAICIN.

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Previous work from our lab demonstrated that systemic capsaicin treatment causes long lasting attenuation of suppression of sham feeding by exogenous CCK and some intestinal nutrients. These effects are also produced by vagotomy, suggesting they are due to vagal sensory damage. Capsaicin, however, causes neuronal degeneration in nonvagal sensory nerves and at multiple central nervous sites. In addition it releases peptides from some enteric neurons. To assess the involvement of intestinal neurons and fibers in nutrient-induced suppression of sham feeding, we studied rats during and subsequent to low dose intraintestinal capsaicin infusion (500ug/ml; 10ml). Intraintestinal infusion of capsaicin suppressed sham feeding by 51.6±2.5%. Capsaicin-infused rats exhibited no signs of systemic capsaicin toxicity and were indistinguishable from controls except with regard to food intake. Intestinal capsaicin did not elevate plasma CCK concentrations. However, MK329, a CCK-A receptor antagonist attenuated capsaicin-induced suppression of sham feeding. Intestinal capsaicin attenuated oleate-induced suppression of sham feeding 48 and 96 hours after intestinal infusion. Reinstatement of oleate-induced suppression of sham feeding occurred by one week post capsaicin. Also, preliminary results indicate that intestinal capsaicin transiently attenuates CCK-8-induced suppression of sham feeding. These results suggest 1) that a capsaicin-sensitive substrate within or near the small intestine participates in suppression of sham feeding by nutrients and exogenous CCK and 2) that stimulation of the substrate may release endogenous, non-endocrine CCK.

217.3

BLOCKADE OF THE ACTIONS OF ENDOGENOUS CHOLECYSTOKININ (CCK) BY INTRAGASTRIC ADMINISTRATION OF THE TYPE A CCK RECEPTOR ANTAGONIST MK-329 SIGNIFICANTLY INCREASES FOOD INTAKE IN RHESUS MONKEYS.

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In order to determine the role of endogenously released cholecystokinin (CCK) in the control of daily food intake in rhesus monkeys, we examined the ability of various doses of the type A CCK receptor antagonist MK-329 to affect subsequent food intake. Four male rhesus monkeys weighing between 5 and 7 kg were maintained on a feeding schedule of 4 hr per day access to 1 gm food pellets delivered in response to lever pulls on an FR schedule. On test days, thirty minutes prior to the feeding period, monkeys received 5 ml intragastric infusions of various dosages (vehicle, 10, 32, 100 or 320 μg/kg) of MK-329. Subsequent intake was computer monitored so that the time that every pellet was taken was recorded. Administration of MK-329 resulted in dose related increases in food intake over the 4 hr feeding period beginning at 32 μg/kg and reaching a maximum increase of 42 % at the 100 μg/kg dose. Analyses of meal patterns revealed that blockade of type A CCK receptors resulted in significant increases in the size of the first meal (76 to 121 g) as well as decreased satiety ratios (intermeal interval / 1st meal size). These data demonstrate that endogenously released CCK acting at type A CCK receptors plays a significant role in both the patterning and amount of food intake in rhesus monkeys. (Supported by NIH grant DK19302.)

217.4

INVOLVEMENT OF CHOLECYSTOKININ IN THE PARABRACHIAL NUCLEUS (PBN) IN THE CONTROL OF FOOD INTAKE: ANATOMICAL AND BEHAVIORAL STUDIES.

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Research from our laboratory has been focused on the anatomy and function of CCK in the PBN. Three questions are currently being addressed: *(1) Does CCK act at the level of the PBN to suppress food intake?* Following recovery from implantation of chronic indwelling, unilateral cannulae terminating in the area of the PBN, each animal received local microinjections of saline, 4.4 and 22 picomoles of sulfated CCK-8 on three separate test days according to a counter-balanced design. Food intake was reduced by CCK-8. Ongoing studies are investigating the effects of receptor-specific CCK antagonists; *(2) What is the anatomical source of the CCK endogenous to the PBN which is likely to be involved in food intake control?* CCK in the PBN is known to arise from both intrinsic and extrinsic sources predominantly from cells in the nucleus tractus solitarius (NTS; e.g. Herbert and Saper, JCN 293:1990). To localize the precise site within the PBN of the terminus of this projection, we have conducted a series of retrograde pathway tracing/IHC experiments. Discrete injections of fluorogold (10-20 nl) were made into subregions of the PBN containing CCK fibers. Sections through the NTS were then processed using CCK immunohistochemistry (IHC). Results to date suggest that the effectiveness of the direct injections of CCK in suppressing food intake was due to activation of CCK receptors located either postsynaptic to axon terminals arising from non-NTS sources or in another adjacent brain area. These anatomical results are being confirmed using an antereograde tracer; and (3) *What is the anatomical relationship between CCK and CGRP cells in the NTS and their projections to the PBN?* Double label IHC for simultaneous visualization of CCK and CGRP indicates the existence of two distinct populations of cells containing these neuropeptides. Current studies are investigating the differential localization of afferent terminals from these cells. Supported by NS12311 (BKH); Hamline U. Lund Fnd. (RDH);.MH47189 (PLF);

217.5

INTRAVENTRICULAR INSULIN INCREASES SENSITIVITY TO CHOLECYSTOKININ (CCK) IN RATS. C.A. Riedy*, M. Chavez*, D.P. Figlewicz-Lattemann and S. C. Woods. Dept. Psychology, Univ. Washington, Seattle, WA.

Insulin acts at the CNS to reduce food intake and body weight. To determine if insulin might act by increasing sensitivity to satiety agents, we first determined a maximally subthreshold dose of insulin which, when infused via osmotic mini-pumps into the 3rd ventricle (IVT), has no effect on food intake or body weight. Doses from 1 mu/day and higher reduced food intake and body weight whereas lower doses did not. Naive male Long-Evans rats were given CCK-8 or vehicle ip at doses from 0.25 to 8 ug/kg. Rats were then infused with IVT insulin (0.5 mU/day) or its vehicle and all received the same doses of CCK-8 again. Rats receiving IVT insulin did not differ in 30-min meal size or body weight over the experiment. Rats receiving insulin were significantly more sensitive to ip CCK-8 than controls. 30-min meal size was reduced at a lower dose (0.5 uU/kg) in insulin than control (2 uU/kg) rats and the effect at 8 uU/kg was significantly enhanced (-72.8% with insulin vs. -30.2% with vehicle). These results demonstrate that insulin, while having no effect by itself, enhances the meal-suppression effects of CCK. This effect may account for some of insulin's ability to reduce body weight.

217.6

CHOLECYSTOKININ-INDUCED SATIETY DEPENDS UPON ACTIVATION OF 5-HT_1 RECEPTORS. B.Poeschla,* J.Gibbs, G.P. Smith, and C.Shamolan. E.W. Bourne Behavioral Research Lab., Cornell Univ. Med. Coll., White Plains, NY 10605.

To investigate the dependence of the satiety action of cholecystokinin upon serotonergic function we examined the effects of systemic pretreatment with 5-HT antagonists of varying selectivity for 5-HT_1, 5-HT_2 and 5-HT_3 receptor subtypes, on the suppression of solid food intake induced by systemic cholecystokinin octapeptide (CCK-8) in 3-h-fasted rats. We similarly tested a 5-HT_{1A} agonist (8-OH-DPAT) that decreases central serotonergic function via a presynaptic autoreceptor mechanism. On average, CCK-8 reduced food intake in the 30 minutes after food presentation by 51±4% at 4 μg/kg (n=11 experiments) and 73±3% at 6 μg/kg (n=2 experiments) compared to controls. 8-OH-DPAT (0.06 and 0.12 mg/kg), the 5-HT_1/5-HT_2 antagonist metergoline (0.06 and 0.10 mg/kg), and the 5-$HT_{1C/2}$ antagonist mianserin (5.0 mg/kg) significantly attenuated or blocked CCK-8-induced satiety, while the 5-$HT_{1A/1B}$ antagonist (-) pindolol had no effect. The 5-HT_2 antagonist ketanserin (1.0, 3.0 and 10.0 mg/kg) had no effect on the action of CCK-8, but reversed the anorectic action of the 5-HT_2 agonist DOI. The 5-HT_3 antagonists MDL-72222 (1.0 and 4.0 mg/kg), ICS 205-930 (2.5 and 5.0 mg/kg), and ondansetron (4.0 mg/kg) had no effect on CCK-8 induced satiety. None of the treatments with 5-HT antagonists or 8-OH-DPAT increased food intakes when given alone without CCK-8. These results indicate that the satiety action of exogenous CCK is dependent on activation of 5-HT_1 (possibly 5-HT_{1C}) receptors, probably at a central site. 5-HT_2 and 5-HT_3 receptors are not required.

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217.7

SEROSAL GASTRIC APPLICATION OF BENZALKONIUM CHLORIDE ENHANCES THE SATIATING POTENCY OF BOMBESIN. T.C.Kirkham, C.Walsh*, J.Gibbs and G.P.Smith. The NY Hospital-Cornell Medical Center, White Plains, NY 10605 USA.

Our previous work suggests that the stomach is an important site mediating the satiety actions of exogenous, peripherally administered bombesin (Kirkham et al, 1991). Bombesin (BN)-like immunoreactivity and BN receptors are located in intrinsic nerves of the stomach. To determine the importance of these neurons to BN satiety, we examined the satiating potency of BN following selective ablation of the gastric myenteric plexus as produced by the cationic surfactant, benzalkonium chloride (BAC), using the technique of Fox et al (1983).

Adult male Sprague-Dawley rats (n=10) were laparotomized and a 0.062% BAC solution was applied to the whole serosal surface of the stomach (by brushing at 5-min intervals over 30 min). At 8, 15 and 28 days after surgery, BN was injected i.p. (5 ug kg^{-1}), 5 min prior to 30-min feeding tests (40% v/v BioServ). A separate group of non-BAC treated rats (n=6) were similarly tested. Intakes were compared to those after i.p. saline at 7, 14 and 27 days.

In control animals, BN suppressed liquid food intake by 35-40% in each test. By contrast, at day 8, BAC-treated rats were considerably more sensitive to the intake-suppressing effects of the peptide: intake was reduced by approximately 87%. However, BN suppressed intake by 54% and 49% when tested at days 15 and 28, respectively. Thus, the response of BAC-treated rats to exogenous BN normalized over the course of this experiment.

The initial, 2-fold increase in responsivity to BN in the BAC-treated rats may be evidence of the induction of denervation supersensitivity. The mechanisms underlying this supersensitivity and the apparent recovery of normal sensitivity to BN in these animals remain to be determined.

Supported by USPHS grants DK33248 (JG) and RSA MH00149 (GPS).

217.8

POTENT BOMBESIN RECEPTOR ANTAGONIST D-PHE^6-BOMBESIN(6-13)-METHYL-ESTER STIMULATES FOOD INTAKE IN RATS. R.D. Reidelberger, G. Varga*, T. Castonguay, D.H. Coy*. VAMC and Dept. Biomed. Sci., Creighton Univ. Sch. of Med., Omaha, NE 68105; Dept. of Med., Tulane Univ., New Orleans, LA 70112.

Bombesin-like peptides (BN) inhibit feeding in several species when administered peripherally or centrally. We used the potent BN receptor antagonist D-Phe^6-bombesin(6-13)-methyl-ester (BME), which is highly selective for the pancreatic BN receptor subtype, to examine the role of endogenous BN in control of food intake. Effects of the C-terminal decapeptide of gastrin releasing peptide (GRP-10: .17, .5, 1.5, 4.5 nmol/kg-h iv), GRP-10 (4.5 nmol/kg-h iv) plus BME (400 nmol/kg-h iv), and BME (400 nmol/kg-h iv) alone, on solid food intake were determined in ad libitum fed rats (*n=9-11*) during the dark period. We have previously shown (Varga et al. *Peptides*, 1991) that in unanesthetized rats, 400 nmol/kg-h of BME completely blocks the maximal pancreatic amylase response to GRP-10 (0.5 nmol/kg-h). The minimal effective dose of GRP-10 for suppressing feeding was 4.5 nmol/kg-h; cumulative 1- and 2-h intakes were significantly decreased by 65 and 38%, respectively. BME completely blocked this effect of GRP-10. BME alone significantly stimulated 3-h cumulative food intake by 43%. ***Conclusions***: 1) BME is a potent antagonist of the effects of exogenous GRP-10 on food intake; 2) these results suggest that satiety is mediated in part by bombesin-like peptides acting at peripheral pancreatic-type BN receptors.

217.9

INVOLVEMENT OF THE NUCLEUS OF THE SOLITARY TRACT IN MEDIATING THE EFFECTS OF BOMBESIN ON FEEDING: BEHAVIORAL AND ELECTROPHYSIOLOGICAL EVIDENCE. F.W. Flynn. Psychology Dept. and Neuroscience Program, Univ. of Wyoming, Laramie, WY, 82071

The nucleus of the solitary tract (NST) is a possible site that mediates the behavioral effects of bombesin (BN)-like peptides based on the presence of BN-like peptide binding sites and immunoreactive neurons, as well as behavioral data. To further examine the role of the NST, bilateral cannula were aimed at the rostral (lateral) NST of male rats (n=10). Meal-related parameters and activity were monitored every 1 min for 1 hr following the injection. Rats were administered injections of saline (0.15 M), 0.5 ng, 1.0 ng, and 5.0 ng BN. Injections of 1 ng and higher reliably suppressed food intake and the reduction reflected a reliable decrease in meal size, p's < .05. Next, to begin to identify the neuronal mechanisms that relate to the behavior-controlling effects of BN, 2 tungsten microelectrodes were attached to a cannula with a removable injector. The assembly was positioned in the NST of Urethane anesthetized rats. Spontaneously active neurons were isolated from the multiunit signal and then BN (5ng in 0.3 μl) or isotonic saline was delivered through the injector into the NST. BN injections had an excitatory effect on single unit activity and in addition, units that were previously silent became active following the local BN injection. These results indicate that the NST is involved in mediating the effects of BN on food intake and that the behavioral effects of BN involves a change in the excitability of NST neurons. (Supported by RO1 NS-24879)

217.10

POST-PRANDIAL CHANGES IN BOMBESIN-LIKE IMMUNOREACTIVITY IN THE GASTROINTESTINAL TRACT AND BRAIN

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Exogenously administered bombesin (BN) elicits a satiety-like state in experimental animals and in man. BN-like immunoreactivity (BLI) has been demonstrated in discrete regions of the brain and gut of the rat, dog, pig, cat, guinea pig and man. In order to explore whether endogenous BN-like peptide(s) are involved in the mediation/modulation of the satiety response, we monitored changes in endogenous BLI within the brain and/or gut, in response to meal ingestion. Seven pairs of male Sprague-Dawley rats (300-350 g) were food deprived for 12 hr and only one from the pair allowed to eat *ad libitum* for a period of 35 min. The animals were sacrificed, brains and gut segments rapidly dissected, weighed and processed for radioimmunoassay (RIA). Blunt brain dissection was employed to harvest the hypothalamus, cerebellum, medulla, pons, neocortex, striatum, olfactory bulbs, hippocampus, midbrain and pituitary. Gut tissue isolated included the oesophagus, fundus, antrum, duodenum, jejunum, ileum, colon and adrenal glands. RIA was performed using rabbit antibody specific for the C-terminal fragment of bombesin and cross-reacted with gastrin-releasing peptide (GRP_{18-27}). Within the gut, a significant increase in BLI was detected in the antrum ($p<0.05$). In the brain, the most notable changes were the post-prandial increases in BLI at the hypothalamus ($p<0.05$) and hippocampus ($p<0.05$). This finding is of interest as we have also found the greatest increases in BN-like binding sites at the hippocampus, in a parallel autoradiographic study. These rapid meal-related changes in the levels of BLI and their binding sites lend further support for BN's suggested role as a satiety peptide.

(Supported by MRC).

217.11

ANALYSIS OF NEUROPEPTIDE Y-INDUCED FEEDING: DISSOCIATION OF Y_1 AND Y_2 RECEPTOR EFFECTS ON MEAL PATTERNS. J. T. Alexander*, C. B. Dietz*, G. Brennan and S. F. Leibowitz. The Rockefeller Univ., N. Y., N. Y. 10021.

Computer-assisted, minute-to-minute analyses of meal patterns were performed on rats after paraventricular nucleus (PVN) injection of neuropeptide Y (NPY), the selective Y_1 receptor agonist [Leu^{31},Pro^{34}] NPY, and the selective Y_2 receptor agonist NPY 13-36, at a dose of 100 pmol. Analyses of 12-hour nocturnal feeding patterns after PVN peptide or saline injections, in rats maintained on pure diets of protein (P), carbohydrate (C) and fat (F), revealed: 1) with NPY, a selective increase in C intake (+13.1 Kcal) during the first meal (increase in feeding time, not rate); little change in F intake; and a significant decrease in P intake (-3.2 Kcal) during the second meal. With no subsequent changes apparent, this pattern of effects, increased C and decreased P in the diet, can be seen for up to 9 hours after NPY injection with no change in total meal number; 2) with [Leu^{31},Pro^{34}] NPY, a selective, although somewhat smaller (+7.7 Kcal), increase in C intake and feeding time (not rate) during the first meal after injection as well as 9 hours later, with little change in P or F intake; and 3) with NPY 13-36, a significant decrease in P intake (-6.7 Kcal), feeding time but not rate, during the first meal as well as after 9 hours; a small transient decline in C intake (-3.2 Kcal) during meal 1; and no change in F intake. These findings indicate that the effect of the complete NPY molecule is on meal size and feeding time, rather than on feeding rate or meal number, and it is characterized by a selective increase in C and a decrease in P intake. The results obtained with the selective receptor agonists suggest that this stimulatory effect on C intake is mediated via Y_1-type receptors, whereas the suppressive effect on P intake may be mediated via Y_2-type receptors.

217.12

EATING ELICITED BY PERIFORNICAL HYPOLALAMIC NEUROPEPTIDE Y INJECTION IS ABOLISHED IN STREPTOZOTOCIN-DIABETIC RATS. B.G. Stanley, S.M. Yee*, M.J. Rosenthal[1]* & M.W. Gunion[1]. Dept. of Psychology, Univ. of California, Riverside, CA 92521; [1]GRECC, Sepulveda VAMC, Sepulveda, CA 91343.

To determine whether the powerful eating response elicited by perifornical hypothalamic (PFH) injection of neuropeptide Y (NPY) might be dependent upon circulating insulin, NPY's effectiveness was tested in streptozotocin (STZ)-diabetic rats. Groups of rats were treated with either: 1) STZ (50 mg/kg, i.p.); 2) citric acid vehicle; or 3) STZ, followed by daily insulin injections (Lente, 1 U, s.q.). The induction of diabetes in the STZ group was confirmed by marked hyperglycemia, hypoinsulinemia, hyperphagia, polydipsia and body weight loss. These effects were partially normalized by daily insulin injection. Subjects were subsequently given PFH injections of NPY (78 pM/0.3 μl) or artificial CSF vehicle, and food intake was measured 1, 2 and 4 hr postinjection. The results confirm that in the control group, PFH injection of NPY elicits eating (e.g., 1.1 g for CSF versus 6.5 g for NPY, one hr postinjection), and demonstrate that this eating response is abolished in diabetic rats (e.g., 2.2 g for CSF versus 2.3 g for NPY), as well as in diabetic rats with partial insulin replacement (e.g., 1.2 g for CSF versus 2.8 g for NPY). These findings suggest that circulating insulin and/or glucose may play a critical role in the eating response elicited by PFH injection of NPY.

OCULOMOTOR SYSTEM III

218.1

CHANGES IN THE HEAD VELOCITY SENSITIVITY OF VESTIBULAR NEURONS DURING VOLUNTARY CANCELLATION OF THE VESTIBULO-OCULAR REFLEX. K. E. Cullen, C. Chen-Huang and R. A. McCrea. Comm. on Neurobiol., Univ. of Chicago, Chicago, Ill. 60637.

In this study, we recorded single unit activity in the vestibular nuclei of alert squirrel monkeys who had been trained to generate smooth pursuit eye movements (SP) and to cancel their vestibulo-ocular reflex (VORc) by fixating a visual target.

Vestibular neurons were first classified on the basis of their behavior during spontaneous eye movements and sinusoidal SP, VOR and VORc. Secondary vestibular neurons were identified by short latency (0.9-1.3ms) activation following stimulation of the ipsilateral vestibular nerve. Two types of ipsilateral head movement sensitive, secondary neurons were recorded 1) neurons that were only sensitive to head movements (*vns*) and 2) neurons which were also sensitive to contralateral eye movements and that paused during saccades, position-vestibular pause neurons (*PVPs*). Although there was no difference in the head velocity sensitivity of the *vns* during VOR and VORc, the head velocity sensitivity of most *PVPs* **decreased** during VORc. Two other types on non-secondary neurons were also studied, 1) neurons whose behavior mirrored that of the *PVPs* (*type IIs*) and 2) gaze velocity type neurons whose eye and head movement sensitivities were in the same direction, vestibular smooth pursuit neurons (*spns*). The modulation of both of these types of neurons was greater during sinusoidal VORc than during VOR.

The responses of these four types of neurons to a step in head acceleration (400°/s²) was also studied. The head velocity sensitivity of the *PVPs* was attenuated at short latencies (>40 ms) in trials during which the monkeys had been cancelling their VOR. In contrast the head velocity sensitivity of the *vns* and *type IIs* remained constant. Surprisingly, most *spns* did not respond to the head velocity transient for > 80 ms. Since the *PVPs* are likely 2° VOR neurons, it is likely that direct VOR pathways can mediate non-visual suppression of the VOR. Furthermore it appears that the *spns* specifically generate a smooth pursuit signal which can aid to cancel the VOR during predictable head movements.

218.2

SMOOTH EYE MOVEMENTS ELICITED FROM THE MONKEY PERI-ARCUATE AREA: TOPOGRAPHY AND RELATION TO SINGLE UNIT RESPONSES J.P. Gottlieb, M.G. MacAvoy and C.J. Bruce, Section of Neurobiology, Yale Univ. Sch.of Med., New Haven, CT 06510

We studied the peri-arcuate representation of smooth-pursuit eye movement (SPEM) using microstimulation and single unit recording in three adult unanesthetized rhesus monkeys. Smooth eye movements (SEM) were elicited at 67 sites in 6 hemispheres, commonly with currents of 100μA or less. Penetrations in which SEM were elicited entered the cortex in a small area (at most 6 mm2) slightly lateral to the spur (or bend) of the arcuate sulcus. Individual sites were localized in the fundus or in the posterior bank of the arcuate sulcus. Sites located in the fundus had lower thresholds (e.g., 25-50μA) and yielded predominantly movements directed ipsilaterally. In contrast, sites in the posterior arcuate bank had higher thresholds (e.g., 75-100μA) and yielded a larger proportion of movements directed contralaterally and downward.

Single unit activity was recorded at 47 of the stimulation sites. Neuronal responses during SPEM were excitatory at 31 sites and inhibitory at 2 sites. Neurons at the remaining locations responded in relation to the angle of gaze (2 sites), to foveal visual stimulation (3 sites) or did not respond to the tasks we used (9 sites). The tracking direction correlated with the best neuronal response was determined for 22 of the neurons with excitatory SPEM-related activity. There was a high correlation between the neurons' best tracking direction and the direction of the elicited SEM at their sites (R=0.93), suggesting that this activity helps elicit SPEM under physiological conditions. SPEM-related neurons often have responses to visual motion presented during fixation (Gottlieb et al., Soc. Neurosci. Abstr. 15:1203,1989). Neurons with excitatory SPEM-related responses at SEM sites had ratios of visual motion to pursuit responses between 0.0-2.6 (median=0.4; n=10). We found no relationship between these ratios and the velocity or threshold of the elicited SEM at the site. The activity of SPEM-related neurons at SEM sites is thus not obligatorily related to pursuit movements, but can be interpreted in several ways by downstream oculomotor structures. Supported by PHS grants EY04740 and MH44866.

218.3

CHANGES IN THE EYE POSITION SENSITIVITY OF BRAINSTEM NEURONS DURING SACCADIC EYE MOVEMENTS, SMOOTH PURSUIT AND THE VESTIBULO-OCULAR REFLEX. R. A. McCrea, K. E. Cullen and C. Chen-Huang. Comm. on Neurobiol., Univ. of Chicago, Chicago, Ill. 60637.

Single unit recordings were obtained from neurons in the vestibular, prepositus and abducens nuclei in alert squirrel monkeys who had been trained to generate sinusoidal smooth pursuit eye movements (SP) and to cancel their vestibulo-ocular reflex (VOR) by fixating a small visual target. The eye position sensitivity (EPS) of a neuron was assessed by measuring the firing rate (FR) during periods of steady fixation between saccades or by measuring the amplitude of the component of a units FR that was in phase with SP eye position.

In most of the brainstem neurons recorded, the relationship between FR & eye position was different during smooth pursuit and steady fixation. In the case of abducens motoneurons and prepositus neurons, and secondary vestibular neurons the estimates of EPS were usually similar, although EPS tended to be greater during SP.

The EPS of many type II vestibular neurons and gaze velocity type vestibular neurons was dramatically increased or decreased (> 2X) during SP, compared to steady fixation. In fact, some did not show changes in FR related to eye position during saccades, but were strongly related to eye position during smooth pursuit. The SP EPS of these cells was also studied during eye movements evoked by transient steps in head acceleration during target fixation that typically evoked an eye movement. Their FR was not related to the vestibularly evoked changes in eye position during the first 80 ms after the initiation of the head acceleration step.

These data suggest that the neural substrate for the velocity-position integrator operating during smooth pursuit eye movements is not identical to that which operates during the VOR or saccades.

218.4

DISRUPTION OF VISUAL FIXATION FOLLOWING INJECTION OF GABAERGIC DRUGS INTO THE FIXATION ZONE OF THE PRIMATE SUPERIOR COLLICULUS. D. P. Munoz and R. H. Wurtz. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Cells in the rostral pole of the superior colliculus (SC) are tonically active whenever an animal fixates a target of interest and pause immediately prior to the initiation of saccadic eye movements (Munoz et al., 1990, *Neurosci. Abstr.*, 16: 1084; Munoz and Guitton, 1989, *Rev. Neurol.*, 145: 567-579). This brain region may therefore play an important role in the control of visual fixation. To test this hypothesis, we altered the activity of cells in the rostral pole of the SC by injecting micro quantities of muscimol (a GABA agonist) or bicuculline (a GABA antagonist) while the alert monkey performed fixation and saccade tasks. Unilateral injections of muscimol (1-4 μg) led to: 1) an increase in the frequency of saccadic eye movements; 2) loss of the ability to suppress the initiation of a saccade following the sudden appearance of a visual stimulus in the peripheral visual field; 3) higher speed and shorter duration saccades directed toward visual stimuli; 4) decreased latency to initiate visually guided saccades; and 5) increased frequency of express saccades (latency < 100ms). Unilateral injection of bicuculline (0.25 μg) led to: 1) slower visually guided saccades with longer durations; and 2) increased latency to initiate voluntary saccades. Thus, muscimol-induced deactivation of cells in the fixation zone of the rostral SC made it harder for the monkey to suppress the initiation of saccades, while activation with bicuculline made it harder to initiate saccades.

These results are consistent with the hypothesis that activation of fixation-related cells in the rostral SC is necessary to maintain visual fixation, while a pause in the discharge of these cells is a prerequisite for the initiation of a saccade. Furthermore, the occurrence of express saccades may depend critically upon decreased activity of collicular fixation cells.

218.5

ATTENTION-RELATED RESPONSES IN THE SUPERIOR COLLICULUS OF THE MACAQUE. R. Gattass and R. Desimone. Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

Previous studies have reported that superficial layer cells in the superior colliculus (SC) give an enhanced response to a stimulus when it is the target for an eye movement. However, in a peripheral detection paradigm, no such enhancement was found when a stimulus was attended, in the absence of an eye movement (Mohler and Wurtz, '76). Because behavioral studies have found attentional deficits in the absence of eye movements following SC lesions or deactivation, we investigated this issue in a paradigm that is very sensitive to effects of attention. In a matching-to-sample paradigm, a sample was presented at one location, followed by a brief test stimulus at that (relevant) location and a distractor at another (irrelevant) location. While maintaining fixation, the monkey indicated whether the sample and test stimulus matched, ignoring the distractor. The relevant and irrelevant locations were switched from trial to trial. SC cells in the superficial layers tended to give enhanced responses when the attended test stimulus was inside the receptive field compared to when the (physically identical) distractor was inside the field. Thus, responses of SC cells appear to be modulated by directed attention, even in absence of eye movements, probably reflecting the properties of cortical cells projecting to the SC.

218.6

VELOCITY AXES OF EYE ROTATION DURING VESTIBULAR AND OPTOKINETIC STIMULATION IN THREE DIMENSIONS IN THE MONKEY. V Henn, N Iida*, D Straumann, Th Haslwanter*, BJM Hess. Neurology Dept, University Hospital, CH-8091 Zürich, Switzerland.

Chronically prepared rhesus monkeys were seated in the center of a three-axes turntable surrounded by an optokinetic sphere. The turntable or the sphere were given velocity steps about an earth-vertical axis. Animal position was systematically shifted in 15 deg steps from upright to supine or side down. Three-dimensional eye position was measured with an implanted magnetic search coil. Angular eye velocity was found to be collinear with the stimulation axis for horizontal, vertical or torsional nystagmus. However, for oblique directions, systematic deviations occurred, with axes tilted away from the torsional towards the horizontal or vertical directions. In oblique directions, dynamics of OKN or VOR (gain and time constants) were similar for the different directional components. Neither the direction nor the dynamics of nystagmus could be accurately predicted from vector additions of horizontal or vertical and torsional components. This suggests that the velocity storage mechanism works in three dimensions with anisotropic directional characteristics.

218.7

OCULOMOTOR SHORT-LEAD BURST NEURON POPULATIONS GENERATE EYE VELOCITY VECTORS IN LISTING'S COORDINATES. J.D. Crawford and T. Vilis. Depts. of Physiology and Ophthalmology, University of Western Ontario, London, Canada, N6A 5C1.

3-D eye velocity vectors and positions were recorded in 4 alert monkeys while the midbrain riMLF burst region was explored with single unit recording, microstimulation, and injection of the GABA agonist muscimol. This confirmed that torsional-vertical quick phase components are generated by 4 riMLF neuron populations (Vilis et al. 1989), but these populations also had a small influence on horizontal rotation. The directions of rotation generated by the 4 populations were: clockwise-up-left and clockwise-down-right in the right riMLF and counterclockwise-up-right and counterclockwise-down-left in the left riMLF. Thus, various combinations of activity in these four populations could potentially produce eye rotations about any axis.

During riMLF inactivation the vertical axes for horizontal saccades, presumably generated by the intact pontine PPRF, lost their normal torsional tilts, suggesting that each population encodes a component of velocity rather than rate of position change (Tweed and Vilis 1990). The remaining vertical axis was abnormally confined to Listing's plane. During unilateral riMLF stimulation intermingled vertical and horizontal components cancelled, and the eye rotated torsionally about an axis aligned with the primary gaze direction orthogonal to Listing's plane. These stimulation and inactivation axes did not align with the anatomical coordinates of the semicircular canals or eye muscles. This suggests that although burst neuron populations are not organized into torsional, vertical and horizontal pairs, they individually produce eye velocity vectors that are symmetrical about Listing's coordinates.

218.8

A MODEL OF SMOOTH PURSUIT PERFORMANCE ILLUSTRATES THE RELATIONSHIP BETWEEN GAIN, CUS AMPLITUDE, AND CUS RATE. L. Friedman, J.A. Jesberger*, and H.Y. Meltzer. Lab. of Biological Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

During smooth pursuit tracking, if gain is less than 1.0, position error accumulates. Catch-up saccades (CUS) correct for this position error. Two general strategies are possible: either to make many small CUS or to make few large CUS. The relationship between gain (G), CUS amplitude (A) and CUS rate (R) can be expressed as follows:

$$G = 1 - (AR/Vt)$$

where Vt = target velocity. The fit of this model was tested in 45 normal controls and 39 schizophrenics. Eye movements were recorded with infrared oculography, and digitized for offline analysis. Target velocity was 5°/sec. The fit of the model to empirical data, as assessed graphically and with non-linear regression (NLR) techniques, was excellent. For example, the model R^2 for predicting G from A and R was 0.87 for all subjects combined. The model was linearized [ln(1-G) = ln(A) + ln (R) - ln(Vt)] and tested with linear regression techniques. The model fit was highly significant ($F=203$, $df=1,82$, $p < 0.00005$). When NLR was allowed to fit the constant (Vt) the optimal value was 4.85 (conf.lim=4.65-5.05). The model also applies when gain > 1.0, when backup-up saccades (-A) correct.

218.9

OCULOMOTOR INTERNUCLEAR NEURONS: THEIR ROLE IN HORIZONTAL EYE MOVEMENTS R.A. Clendaniel,* L.E. Mays and P.D.R. Gamlin. Departments of Psychology and Physiological Optics; University of Alabama at Birmingham; Birmingham, AL 35294

Antidromically identified oculomotor internuclear neurons (OINs) that project to the contralateral abducens nucleus were recorded in monkeys (*Macaca mulatta*) trained to make saccadic, smooth pursuit, and vergence eye movements. The identified OINs have all been located in or near the oculomotor nucleus, and the cells in the vicinity of the OINs display behavior which is consistent with that of medial rectus motoneurons. The OINs also have firing characteristics similar to the putative medial rectus motoneurons. OINs increase their activity for adduction of the ipsilateral eye, and they also increase their firing rate for convergent eye movements. The conjugate gain (k_c) of these cells ranged from 2.29 to 8.35 spikes/sec/deg (mean 4.83); similarly the vergence gain (k_v) of these cells ranged from 1.13 to 8.51 spikes/sec/deg (mean 4.46). These values are similar to those of putative medial rectus motoneurons.

A series of stimulation and reversible inactivation studies have been used to assess the effect OINs exert on the contralateral abducens neurons. Microstimulation in the region of identified OINs produces a small abducting twitch in the contralateral eye. Injections of 0.1 µL of 10% lidocaine HCl result in hypometric abducting saccades and hypermetric adducting saccades for the eye contralateral to the injection site. These findings are consistent with the hypothesis that OINs provide an excitatory input to the contralateral abducens nucleus. Thus, OINs appear to send a signal to the abducens nucleus which is appropriate for conjugate eye movements, but inappropriate for disjunctive movements. (Supported by EYO3463 and P30 EYO3039)

218.10

SMOOTH PURSUIT AND OPTOKINETIC DEFICITS FOLLOWING CHEMICAL LESIONS TO THE CEREBELLAR UVULA S.J. Heinen and E.L. Keller. Smith-Kettlewell Eye Res. Inst., San Francisco CA, and Dept. of Elect. Eng. and Comp. Sci., Univ. of California, Berkeley, CA 95720.

The uvula (lobule IX of the cerebellar vermis) receives indirect input from the superior temporal sulcus and pretectum via the dorsolateral pontine nucleus, areas all involved in visual motion processing. Although previous studies had implicated the uvula in visual alterations of the velocity storage mechanism of optokinetic eye movements, we reported last year that some uvular cells respond during pursuit eye movements as well. The deficits produced by chemical lesions placed in this structure now provide further evidence for a role of the uvula in the execution of pursuit. Three monkeys were trained to smoothly track a small ramping target spot (randomly selected from 10, 20 or 40 deg/sec) and to suppress optokinetic following movements by fixating a stationary spot during the sweep of a large-field, textured background (moving over the same range of velocities). Lidocaine and ibotenic acid were used to temporarily disrupt neural activity and to destroy Purkinje cells respectively. We found that initial eye acceleration to the ramping spot in some cases was increased by 200% due to infusion of these agents. This increase was sometimes accompanied by an inability of similar magnitude to suppress optokinetic following movements. Lesions placed in other cortical, brainstem and cerebellar areas have all been reported to produce markedly lower pursuit responses, but the results of uvular lesions are unique in producing accentuated pursuit. This effect is probably a reflection of the removal of inhibitory Purkinje cell activity either directly or indirectly from the pursuit pathway.

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218.11

EYE POSITION DEPENDENCY OF UNITS IN THE DORSOMEDIAL FRONTAL CORTEX. K. M. Lee and E. J. Tehovnik. Department of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

It has been discovered by electrical stimulation that the dorsomedial frontal cortex (DMFC) contains a systematic representation of orbital eye position. Not only do evoked contraversive eye movements go to a specific orbital position but also the position is mapped in the antero-posterior dimension of this area of the brain.

Electrical stimulation, however, cannot localize neuronal elements for this representation, since collateral fibers from cells in other regions of the brain or fibers of passage are likely stimulated. Therefore, in this study we used single-unit recording to find out if this map of eye position is indeed represented by the activity of units in the DMFC. Using a prolonged fixation paradigm where behaving monkeys fixate a target for up to 2000 msec, we found units whose firing frequency show eye position dependency. Most of these cells are more active when the animal is fixating at contralateral targets in head-centered coordinates than ipsilateral targets. This eye position dependency of firing is most prominent for up to several hundred msec after the animal has acquired the target. Furthermore, units in the posterior sites within the DMFC have their optimum eye position closer to the midline than those in the anterior sites do, which is consistent with the electrical stimulation result.

The units found in this study along with the electrical stimulation results suggest that the DMFC is an area of the brain where visual-target position in head-centered coordinates is computed for visuomotor transformation in making eye movements.

(Supported by NIH EY00676 and McDonnell-Pew)

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION V

219.1

NOVEL STRUCTURES IN THE GONADOTROPIN-RELEASING HORMONE FAMILY. D.A. Lovejoy*, S. Ngamvongchon*, N.M. Sherwood Biology Dept., University of Victoria, Victoria, B.C. Canada, V8W 2Y2. W.H. Fischer*, A.G. Craig*, J.E. Rivier* The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla CA 92037.

The structural characterization of two novel forms of gonadotropin-releasing hormone (GnRH) is reported here. These structures expand the GnRH family of neuropeptides to seven members. In addition, confirmation of His^5,Trp^7,Tyr^8-GnRH (chicken GnRH-II) by sequence analysis in cartilaginous fish, bony fish, and reptiles indicates that this neuropeptide is present in all major vertebrate groups and has been conserved for over 400 million years.

Each form of GnRH was purified by reverse-phase HPLC from extracts prepared from spiny dogfish (*Squalus acanthias*), Thai catfish (*Clarias macrocephalus*) and American alligator (*Alligator mississippiensis*) brains. The sequence was determined by automated Edman degradation after cleavage of the pGlu residue using bovine pyroglutamyl aminopeptidase. Confirmation of the sequence and presence of the amidated Gly^{10} residue was established by mass spectral analysis.

Sequence comparisons of the six forms of GnRH present in the brains of jawed vertebrates suggest the molecule evolved along two lines. One lineage is characterized by Trp^7 and hydrophobic^8 residues whereas the other possesses Leu^7 and hydrophilic^8 residues. We suggest that this structural dichotomy in GnRH may be the basis of functional differences.

219.2

GASTRIN-RELEASING PEPTIDE (GRP) IS NOT THE MAMMALIAN HOMOLOG OF BOMBESIN. S.R. Nagalla*[1], B.W. Gibson*[2], J.R. Reeve Jr.*[3], and E.R. Spindel[1]. [1]Div of Neurosci, Oregon Rgl Primate Res Ctr, Beaverton, OR 97006, [2]Dept of Pharmaceutical Chemistry, UCSF, San Francisco, CA 94143, [3]Ctr for Ulcer Res and Education, UCLA, Los Angeles, CA 90073.

Bombesin-like peptides are potent growth factors, paracrine hormones and neurotransmitters. Based on amino acid homology, the 27-amino acid gastrin-releasing peptide (GRP) and its C-terminal decapeptide (GRP-10) since their discovery have been considered the mammalian homologs of amphibian bombesin. HPLC analysis of extracts of frog (*Bombina orientalis*) stomach revealed the presence of two peaks of bombesin-like immunoreactivity, consistent in size with GRP and GRP-10. The amino acid sequence of the smaller immunoreactive peak was determined, in part, by liquid secondary ion mass-spectrometry. Mixed oligonucleotides probes complementary to this sequence were used to screen a *B. orientalis* stomach cDNA library. Sequence analysis of hybridizing clones revealed a 970 bp cDNA encoding an amphibian GRP. Homology between frog and mammalian GRPs was high and frog GRP-10 is identical to rat GRP-10. Northern blot analysis showed high levels of GRP mRNA present in frog stomach and brain. Northern blot and *in situ* hybridization also showed the presence of frog bombesin mRNA in stomach and brain but with a different distribution than that of the frog GRP mRNA. Thus in *B. orientalis*, GRP and bombesin are separate entities encoded by distinct mRNAs with different patterns of expression. Analysis of nucleic acid and amino acid homologies suggests that bombesin and GRP diverged prior to the vertebrate radiation. This evidence raises the possibility that mammals may also have an as yet undiscovered gene encoding a "true" mammalian bombesin distinct from the well characterized gene for GRP.

219.3

USE OF A RETROVIRAL VECTOR FOR EXPRESSING ANTISENSE RNA OF PROOPIOMELANOCORTIN IN AtT-20 CELL LINE. S. Spampinato, M. Canossa* and S. Ferri*. Dept. of Pharmacology, University of Bologna, 40126 Bologna, Italy.

Recent studies have demonstrated that gene expression in mammalian cells can be suppressed by nucleic acid sequences complementary to endogenous transcripts. This approach is based on the assumption that these "antisense" sequences can hybridize to primary RNA transcripts and prevent normal gene expression. However, one of the major problems emerged concerns the use of techniques that allow an efficent transfer of antisense constructs. Recently, recombinant retroviruses have been employed as vectors to stably transfer genes into a variety of cells. Therefore, with the aim to investigate their ability to produce antisense RNAs of neuropeptide genes we have examined their possible expression in cell lines. In this study we have evaluated the expression of an antisense RNA sequence of 940 bp, complementary to mouse proopiomelanocortin (POMC) mRNA, in a murine neuroendocrine cell line (AtT-20) that highly expresses this gene. A cDNA of mouse POMC (940 bp) was ligated in "antisense" orientation to the promoter in a retroviral vector (pJDOL$^-$) and transfected into a cell line of helper cells (ψ cre). Mature virions, collected in the medium, were titrated and used to infect AtT-20 cells. We found that infected cells express POMC antisense RNA by a sensitive solution assay. The values obtained from six different plates were between 5 and 20 pg/μg total RNA. Therefore, retroviral vectors may represent an useful tool to introduce and express antisense sequences to neuropeptide genes in cell lines.

219.4

EXPRESSION OF A HUMAN PROENKEPHALIN ß-GALACTOSIDASE FUSION GENE IN TRANSGENIC MICE. David Borsook, Haim Rosen*, Karl Herrup, Holly Dressler*, Michael Comb, and Steven Hyman. Molecular Neurobiology Laboratory, Massachussets General Hospital, Harvard Medical School, Boston MA 02114

Peptide gene expression is thought to occur via cis regulatory elements contained within the gene. However for genes with complex patterns of expression such as the human proenkephalin gene, it has proved difficult to map such elements by mutagenesis and transfection into tissue cells, perhaps because no available cell line can adequately model the variety of neurons in which the gene is expressed. Using fusions expressing the easily visualized histochemical marker ß-galactosidase, we are in the process of mapping regulatory elements required for cell type specific expression of the proenkephalin gene. We present data of the expression of a proenkephalin ß-galactosidase fusion gene in various brain regions of the mouse. The initial transgenic mice were made by injecting a construction containing 3 kilobases (kb) of human proenkephalin 5' flanking sequence fused to the E. coli lac-z transcription unit and 1 kb of proenkephalin 3' flanking sequence including sequences required for polyadenylation. The transgenic animals appeared morphologically and behaviorally normal. To determine expression of the fusion gene, brains from animals were stained for ß-galactosidase activity using the chromogenic substrate X-gal, and then counterstained with neutral red. In the founder lines produced, levels of expression were seen in most brain regions corresponding with the expected distribution of the endogenous gene. However, no expression was seen in the basal ganglia. Furthermore, correct expression was seen in the reproductive organs of male (testis and epididymus) and female (ovary, oviduct and uterus) with high levels of expression were seen in pregnant or superovulated animals as well as at the utero-placental junction in pregnant females. Examination of other somatic tissue was negative for ß-galactosidase staining with X-Gal. Examination of further constructs to explain these discrepancies and to map tissue specific regulatory elements is in progress.

219.5

DETECTION OF NEUROPEPTIDE HETERONUCLEAR RNA's BY IN SITU HYBRIDIZATION USING SINGLE STRANDED DNA PROBES PRODUCED BY ASYMMETRIC PCR. P.J. Brooks, S.P. Kleopoulos*, C.V. Mobbs, and D.W. Pfaff The Rockefeller University, New York, NY

In situ hybridization using intron derived probes is a powerful method to examine changes in gene expression at the single cell level. Because of the low abundance of heteronuclear (hn) RNA, previous authors (Fremeau and Roberts, 1986, Herman et al, Soc. Neurosci. Abst. 526.5, 1990) have used single stranded RNA probes for such studies. We have explored the use of single stranded, internally labelled DNA probes produced by a modified asymmteric PCR procedure to detect low abundance hnRNAs.

Small (200-300 bp) regions of introns from the preproenkephalin (PPE) and oxytocin (OXY) genes are amplified from plasmids or genomic DNA by conventional PCR. Probe labelling is carried out by 50 cycles of linear amplification of the antisense strand of the PCR product in the presence of the C-terminal PCR primer and labelled dNTPs, producing internally labelled copies of the antisense DNA strand. Typically, between 40-85% of the radiolabel (we have used either H3, 125I, or 32P) is incorporated into DNA using these conditions. Probe specificity is confirmed by Southern blot hybridization. Our initial results indicate that these probes retain all of the features of single stranded RNA probes (high sensitivity, choice of specific activity) but are easier to produce and use. Using *in situ* hybridization with high specific activity 32P labelled probes, both PPE and OXY hn RNAs are detectable with appropriate regional distributions after overnight exposure to X-ray film. With H3 labelled probes, cell nuclear localization of OXY hn RNA can be detected in magnocellular neurons of the PVN and SON after 3 weeks exposure to nuclear emulsion. We are presently using this approach to study the regulation of both mRNAs under different experimental conditions.

219.6

PROCESSING AND SORTING OF THE ELH PROHORMONE IN AtT-20 CELLS P. A. Paganetti*, L. J. Jung* and R. H. Scheller. Howard Hughes Medical Institute, Stanford University, Stanford CA 94305

In the bag cells of *Aplysia*, proteolytic processing and sorting of the egg-laying hormone (ELH) precursor is highly regulated. Following the first endoproteolytic cleavage at a unique tetrabasic site, the two intermediates are packaged in distinct sets of vesicles. Within the vesicles further processing results in multiple physiologically active products. These two vesicle classes are then sorted to distinct targets in the cells. Atrial gland cells express the peptide A precursor which is 88% homologous at the nucleotide level with the ELH precursor.

Transfection studies suggest that similar processing and sorting events occur in the bag cell neurons and pituitary cells (Jung and Scheller, Science 251: 1330-35. 1991). Pulse/ chase experiments indicate that the first cleavage of the ELH precursor in AtT-20 transformants occurs at the tetrabasic site in the late Golgi, similar to the bag cells. Mature ELH is colocalized in DCVs with endogenously expressed ACTH. In contrast to bag cells, no staining is observed with antibodies directed against the NH_2-fragment, possibly indicating the involvement of a degradative pathway. All these results suggest that much of the information required for correct processing and sorting of ELH precursor is contained within its structure.

To further study the details of these sorting events, we have constructed deletions of the ELH cDNA at the tetrabasic site. We are currently analyzing the processing of mutant ELH and peptide A precursors in AtT-20 transformants.

219.7

IDENTIFICATION AND CHARACTERIZATION OF ENKEPHALIN-DEGRADING AMINOPEPTIDASE AND NEUTRAL ENDOPEPTIDASE IN APLYSIA CALIFORNICA. W. Bawab', P. Crine' and L. DesGroseillers. Department of biochemistry, University of Montreal, Montreal, Canada, H3C 3J7.

We are investigating the role of enzymes in the inactivation of neuropeptides in Aplysia. Using [^{3}H]leu-enkephalin (YGGFL) as substrate, we have identified two different neuropeptide degrading enzymes in kidney, heart, ovotestis, central nervous tissues and hemolymph. The most abundant enzyme in these preparations cleaves leu-enkephalin at the Tyr^1-Gly^2 bond, as determined by HPLC analysis of metabolites. In the kidney, this aminopeptidase activity was detected both in membrane-bound and soluble fractions. The Km value of this aminopeptidase in kidney plasma membranes for the leu-enkephalin substrate is 2.4 μM. Using specific enzyme inhibitors, we showed that this enzyme is a metalloprotease, with a profile of inhibition similar to the one of mammalian aminopeptidase N: amastatin was the most potent inhibitor, while bacitracin, bestatin and puromycin were about 100 to 1000 times less potent. However, the aminopeptidase activity was unaffected by thiol-, serine-, and acid protease inhibitors, as well as by neutral endopeptidase and angiotensin converting enzyme inhibitors. Inhibition of aminopeptidase activity by amastatin allows us to detect a second metabolite, Tyr-Gly-Gly, suggesting that an endopeptidase cleaves the Gly^3-Phe^4 bond of Leu-enkephalin. The presence of this metabolite was abolished by neutral endopeptidase (NEP) inhibitors HACBO-Gly, thiorphan and phosphoramidon, and by the divalent cation chelating agent 1-10 phenanthroline, suggesting that this enzyme is similar to the mammalian NEP. The presence of both the aminopeptidase and the NEP suggests that these enzymes may contribute to the extracellular degradation of Aplysia neuropeptides.

219.8

REGULATION OF PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM) ACTIVITY BY DIETARY COPPER. G.P. Mueller, T.A. Ford* and M.L. Failla*. Dept. of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and Vitamin and Mineral Nutr. Lab., USDA, Beltsville, MD 20705.

PAM (EC1.14.17.3) is the copper (Cu) dependent enzyme catalyzing the two-step formation of bioactive α-amidated peptides from their inactive glycine-extended precursors. We previously observed that Cu deficiency dramatically decreased body Cu stores yet failed to alter concentrations of amidated peptides in brain, pituitary or intestine. Here we examined the effects of dietary Cu deficiency on PAM activity in male and female rats. Sex difference and the effects of Cu deficiency on PAM activity (assayed in vitro under optimal conditions) were most clearly evident in the anterior pituitary (AP). In control animals, PAM activity in the AP of males was nearly double that observed in females (2.8 ± 0.2 vs 1.8 ± 0.1 pmol/μg protein/hr). Severe Cu deficiency (depressed growth, enlarged hearts, low tissue Cu) induced by feeding low Cu diet (0.7 μg/gm) to dams beginning at 13 days gestation and weaning pups to the same diet, increased PAM activity in the AP nearly 2-fold in both sexes at 7 weeks of age. Similar though less dramatic differences in PAM activity were observed in atrium and hypothalamus. High levels of PAM activity in serum were not affected by Cu status or gender. These findings suggest the expression of PAM is influenced by gender and increased to maintain peptide amidation in Cu deficient states.

219.9

GENE EXPRESSION OF PROHORMONE CONVERTASES IN THE CNS AND PERIPHERY. R. Day, M.K.-H. Schafer, S.J. Watson, M. Chrétien, N.G. Seidah*. Clinical Research Institute of Montreal, Montréal, Québec, Canada, H2W 1R7 and Mental Health Research Institute, University of Michigan, Ann Arbor, MI, USA, 48109.

Recent advances in the molecular characterization of processing enzymes have demonstrated a subtilisin-like enzyme family in mammalian tissues to be responsible for the endoproteolytic processing of inactive precursors to biologically active peptides, via selective cleavage of basic residues. The neuroendocrine tissue distribution of two of these enzymes, PC1 and PC2, has been demonstrated (Seidah et al, Mol Endo, 5, 111, 1991). The cellular expression of Furin, a structurally related enzyme carrying a putative transmembrane domain, (like the yeast enzyme Kex2), is unknown in rat brain. The present study sets 3 objectives: (1) defining the cellular expression pattern of Furin, PC1 and PC2 in the rat CNS and select endocrine tissues, (2) identifying neuropeptide precursors as specific candidates for processing, (3) determination of the codistribution of PC1, PC2 and Furin with the other enzymes known to be involved in processing, i.e., carboxypeptidase E (CPE) and peptidy-glycine α-mono-oxygenase (PAM). In situ hybridization studies using ^{35}S-labelled cRNA probes were carried out on frozen rat brain, pituitary and adrenal tissue sections. In the brain PC2 is more abundant and more widely distributed than PC1. PC1 and PC2 were preferentially expressed in neural and endocrine tissues, and were always coexpressed with CPE, however, coexpression with PAM was not always observed. For example, PC1, PC2, CPE and PAM are coexpressed in the supraotic and paraventricular nuclei, but only PC1, PC2 and CPE are expressed in the cerebellum. Furin's distribution was ubiquitous, however, surprisingly high levels of Furin mRNA were expressed by non-neuronal brain tissues such as ventricular eppendyma and choroid plexus. CPE was coexpressed in these cells with Furin. Other examples showing the distinct nature of PC1, PC2 and Furin will be shown. We conclude that Furin is a processing enzyme important to all cells and is most likely unique to the constitutive secretory pathway, whereas PC1 and PC2 are expressed in cells with a regulated secretory pathway.

219.10

REGULATION OF STRIATAL CCK mRNA BY GLUTAMATE. X.Z. Ding*[1,2], M.Olasmaa[1], E.Costa[1] and I.Mocchetti[2]. [1]F.G.I.N. and [2]Dept of Anatomy and Cell Biology, Georgetown Univ. Washington,DC20007

Stimulation of dopaminergic (DA) D_2 but not D_1 receptor increases striatal CCK mRNA content. In the striatum, D_2 receptors are located on dendrites and cell bodies but also on the glutamatergic axon terminals of corticostriatal afferent neurons. Therefore, the release of glutamate could be modulated upon D_2 receptor stimulation, and this transmitter could in turn regulate striatal CCK mRNA. This hypothesis was tested by measuring changes in the striatal CCK mRNA elicited by specific glutamatergic and DA receptor agonists and antagonists. The activation of NMDA receptor by a single injection of NMDA (10nmol, i.c.v.) elicited a 70% increase in striatal CCK mRNA content. A single injection of MK-801 (1mg/kg, i.p.), a selective non-competitive NMDA antagonist, produced a 30% decrease in striatal CCK mRNA content 8 hours after the injection. This decrease was time and dose-dependent. A 50% decrease was observed after 7 daily MK-801 injections. To better characterize the interaction between glutamatergic and D_2 receptors in the regulation of CCK mRNA, MK-801 was injected concomitantly with benztropine, an indirect DA receptor agonist which increases DA content in synaptic clefts by blocking DA reuptake, or BHT-920, a specific D_2 agonist. Both DA agonists induce a two fold increase in CCK mRNA, which is partially reversed by MK-801. Hence the regulation of striatal CCK mRNA content might be modulated by both DA and NMDA receptors. This hypothesis is supported by the finding that the lesion of DA striatal afferent by 6-OHDA, which per se decreases striatal CCK mRNA, prevents the increase of CCK mRNA induced by NMDA.

219.11

METRAZOLE (MTZ) SEQUENTIALLY ACTIVATES C-FOS, PROENKEPHALIN (Penk) AND TYROSINE HYDROXYLASE (TH) GENE EXPRESSION IN RAT ADRENAL. Y.S. Zhu, S.O. Franklin*, T. Huang* and C.E. Inturrisi, Dept. of Pharmacology, Cornell Univ. Med. College, New York, NY 10021.

It has been postulated that the immediate-early genes, e.g., c-fos, may function as "third messengers", which act on late-effector genes to modify gene expression. In the present report, we have studied the effects of MTZ on c-fos, preproenkephalin (PPenk) and TH mRNA levels in rat adrenal gland. One hour after MTZ, adrenal c-fos mRNA levels increased about 5-7 fold and return to control by 6 hr. At 1 hr after MTZ, adrenal PPenk mRNA was increased 2-fold and TH mRNA doubled. In contrast to c-fos mRNA, both PPenk (5-fold) and TH (2-3 fold) mRNA were significantly increased at 6 hrs. These MTZ-induced increases in c-fos, PPenk and TH mRNAs were significantly reduced (by 41%, 76% and 37%, respectively) after hypophysectomy (hypox). ACTH treatment of hypox rats (for 7 days) failed to restore the MTZ-induced c-fos mRNA levels, while PPenk and TH mRNA levels were partially restored in these animals. Furthermore, ACTH treatment completely blocked the MTZ-induced increase in c-fos mRNA but not the increase in PPenk and TH mRNA in control (sham) rats. These results suggest (1) there is a linkage between neuronal excitation by MTZ and c-fos, Penk and TH gene expression in the adrenal; (2) glucocorticoid may, at least in part, modulate this system; (3) ACTH treatment can differentially affect expression of these genes. (Supported in part by DA01457.)

219.12

DIFFERENTIAL EFFECTS OF TYPICAL AND ATYPICAL NEUROLEPTICS ON NEUROTENSIN GENE TRANSCRIPTION. K.M. Merchant, P.R. Dobner and D.M. Dorsa. GRECC, VA Medical Ctr; Depts. of Medicine, Pharmacol., and Psychiatry, Univ. of Washington, Seattle, WA 98195; Dept. Mol. Genetics & Microbiol., Univ. of Mass., Worcester, MA 01655.

The present study characterized and compared the effects of a classical (haloperidol) and an atypical (clozapine) neuroleptic on the expression of neurotensin/neuromedin N (NT/N) mRNA in rat neostriatum using *in situ* hybridization analysis and quantitative solution hybridization. NT/N mRNA levels in the dorsolateral striatum were rapidly (within 30 min) and reversibly increased by a single dose of haloperidol (1 mg/kg, i.p.) but not clozapine (20 mg/kg, i.p.). Maximal induction following haloperidol occurred at 7 hr after drug administration, at which time NT/N mRNA levels were an order of magnitude higher than basal levels. Hybridization with an intron-derived probe showed that the increases in mature NT/N mRNA levels were preceded by an increase in intron-containing NT/N gene transcripts located in neuronal nuclei of the striatum. These data strongly indicate that haloperidol increases the transcription of NT/N gene and/or stability of its primary transcripts in the dorsolateral striatum. Unlike the effects in the caudate-putamen, both clozapine and haloperidol induced a small but significant increase (40-45%) in NT/N expression in the shaft region of the nucleus accumbens. These results suggest a functional diversity in striatal NT neurons. (Supported by Washington Institute for Mental Illness and NS 20311)

INVERTEBRATE LEARNING AND BEHAVIOR I

220.1

RESPONSE-SPECIFIC LEARNING IN APLYSIA: FREQUENCY-DEPENDENT EFFECTS OF DIRECTIONAL SIPHON MOTOR NEURONS. C.Hickie and E.T. Walters. Dept. of Physiology & Cell Biol., Univ. of Texas Medical School, Houston, TX 77225.

Six identified siphon motor neurons account for the flaring response of the siphon to tail stimulation (4 LFSB cells) and constricting response to head stimulation (RDS and LDS1). Each motor neuron is activated at high frequency by stimuli used in conditioning, and the LFSB cells show a prolonged increase in background firing after conditioning. We examined the effects of motor neuron firing on siphon response plasticity and found: (1) Tonic background firing facilitates siphon movements produced by high frequency test bursts (1 sec, 12-30 Hz, normalized to motional threshold) in each motor neuron (cf. Frost et al. J. Neurobiol. 19:297, 1988); (2) In the absence of background activity, movements produced by motor neuron test bursts were facilitated by a preceding test burst, but the LFSB cells showed greater and longer-lasting interburst facilitation than LDS1, or RDS which showed the least facilitation; (3) Following a single 2 sec, 40 Hz conditioning burst, LFSB neurons could produce enhanced movements for at least 10 min. These results suggest that patterns of motor neuron firing may contribute to both the induction and expression of response plasticity. We are now examining response plasticity induced by redriving motor neurons with activity patterns recorded during conditioning.

220.2

LONG-TERM HYPEREXCITABILITY OF CEREBRAL MECHANOSENSORY NEURONS IN APLYSIA INDUCED BY AXON INJURY. A.L. Clatworthy and E.T. Walters. Dept. of Physiology & Cell Biol., Univ. of Texas Medical School, Houston, TX 77225.

Walters, Alizadeh and Castro (submitted) found that crush of pleural (VC) sensory neuron axons, under conditions in which synaptic transmission and spike activity were severely reduced, induced a delayed but persistent decrease in sensory spike threshold, accommodation and afterhyperpolarization, and an increase in spike duration, synaptic transmission and afterdischarge. To test the generality of these effects we crushed all cerebral (and pedal) nerves on one side of the animal under ice-cold $MgCl_2$ anesthesia. Six to 8 days later, cerebral (J,K) sensory neurons on the crushed side (52 pairs, 9 animals) displayed a decrease in spike threshold (0.7 vs 1.3 mA, $p<.05$), accommodation (5.3 vs 1.8 spikes in 1 sec, $p<.05$), and an increase in spike duration (2.9 vs 2.5 ms, $p<.05$). No differences in sensory neuron excitability were observed among neurons with uncrushed axons in these animals, sham-operated animals and naive animals, suggesting that axonal injury is required to induce the plasticity. Similar results were obtained for 25 pairs of pleural sensory neurons from 5 animals, although the magnitude and number of alterations tended to be greater in the pleural cells. We are currently examining whether any synaptic or morphological changes are induced in cerebral cells by axonal injury.

220.3

NEURONS IN THE J CLUSTER OF THE CEREBRAL GANGLION PRODUCE MODULATORY EFFECTS ON SENSORY NEURONS IN THE PLEURAL GANGLIA OF *APLYSIA*. J.L Raymond and J.H. Byrne. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

Changes in the biophysical and biochemical properties of sensory neurons (SNs) in the pleural ganglia of *Aplysia* have been correlated with behavioral plasticity of the tail-withdrawal reflex. Here we report that neurons in the J cluster of the cerebral ganglion produce effects in the SNs that mimic changes that have been associated with modulation of the reflex. We refer to these cells as J_m neurons.

Brief stimulation of J_m neurons produced hyperpolarizing and depolarizing responses in SNs in the pleural ganglion. In some preparations, a slow EPSP was produced in a SN by stimulation of a J_m cell. These EPSPs were associated with an increase in the excitability of the SNs. The effect on excitability generally persisted for less than 2 min, but outlasted the EPSP. In other preparations, IPSPs or mixed (E-I or I-E) responses were observed. Often a single action potential in a J_m neuron was sufficient to produce a PSP in a SN, and, in one case, a discrete fast IPSP was observed, indicating that the connections may be monosynaptic.

The J cluster contains mechanoafferent neurons innervating the head and neck regions of the animal (Rosen et al., 1979). The properties of the J_m neurons resembled those of the mechanoafferent neurons in the J cluster and in other ganglia. Specifically, they were not spontaneously active, had little spontaneous input, and exhibited rapid accommodation. Moreover, the efficiency of their connections to the pleural SNs depressed rapidly. The J_m cells generally received fast and slow excitatory input from cerebral nerves C1-C3 and from the cerebral-buccal connective and received inhibitory input from pedal nerves P7-P9.

These data suggest that the J_m neurons may be elements of a modulatory pathway involved in the behavioral plasticity of the tail-withdrawal reflex. In particular, they may contribute to modulation of the reflex produced by tactile stimulation of anterior parts of the animal.

220.4

EFFECTS OF HABITUATION ON NEURONAL ACTIVITY IN APLYSIA ABDOMINAL GANGLION DURING THE GILL-WITHDRAWAL REFLEX. Chun X. Falk, Jian-young Wu, Larry B. Cohen. Dept. of Physiology, Yale U. Sch. of Med., New Haven, CT 06510.

Previous experiments indicated that habituation results in a decrease in the number of active neurons responding to the siphon touch. In the present experiments we made optical recordings during the 1st, 4th, 10th, 20th, 30th and 40th habituation trials and during control trials before habituation and after a 20 min recovery period. Using control trials, neurons were grouped into those that were activated by the touch, those that were inhibited by the touch and those with activity apparently unrelated to the stimulus. During habituation, the activity of almost all the neurons in the activated group decreased, but at different rates. Some neurons stopped responding to the stimulus by the 4th habituation trial, while others in this group continue to be excited in the 40th trial. Some of the inhibited neurons were less inhibited after habituation. In about half of the preparations there was increased activity among the cells that did not respond to touch. Thus, when the responses to habituating stimuli are examined in many neurons, there is substantial diversity. Supported by NIH grant #NS08437.

220.5

COMPARISON OF NEURONAL ACTIVITY IN THE APLYSIA ABDOMINAL GANGLION DURING RESPIRATORY PUMPING AND THE GILL WITHDRAWAL REFLEX. Jian-young Wu, Larry B. Cohen and Chun X. Falk. Dept. of Physiology, Yale Univ. School of Medicine, New Haven, CT.

We used optical recording to monitor the neuronal activity during spontaneous gill contractions (respiratory pumping) and during the gill-withdrawal reflex elicited by a light touch to the siphon skin. We estimate that about 300 neurons are active during spontaneous contractions and that approximately 70% of these neurons are also active during the gill-withdrawal reflex. Many neurons had different activity patterns during spontaneous contractions and the gill-withdrawal reflex. In addition, when the gill-withdrawal reflex was elicited just after a spontaneous contraction, the evoked neuronal activity was altered in comparison with the activity elicited between spontaneous contractions. In different preparations we have seen both dramatic suppression or enhancement of activity. These results showed that complex neuronal events with some shared neurons are associated with both kinds of gill withdrawal behaviors. Thus further efforts to distinguish the neurons which generate or coordinate behaviors with gill movements remain interesting. Supported by NIH grant NS08437.

220.6

Dissecting the Memory for Sensitization in *Tritonia*. G. D. Brown and A. O. D. Willows. Friday Harbor Laboratories, Friday Harbor, WA 98250.

Previous experience with noxious chemical stimuli produces sensitization of the *Tritonia* escape swim. Sensitization is manifest in behavioral changes such as a decrease in the latency to swimming, an increase in the strength of swim movements, and an increase in the number of cycles per swim episode. Because the neural circuitry which underlies escape swimming is comparitively well understood and the memory for sensitization can be traced to these circuit elements, *Tritonia* can be used to study how a memory codes for a complex set of behavioral changes.

Using a head-attached preparation we have shown that the stimuli which produce sensitization in *Tritonia* also produce an increase in the firing rate of dorsal swim interneurons (DSI's). Double labeling experiments in which DSI's were labeled with an intracellular dye and tagged 5-HT antibodies support earlier evidence that DSI's contain serotonin, a neurotransmitter thought to be important for sensitization in other animals. Increasing the rate of DSI firing by intracellular current injection in reduced preparations results in a strengthening of functional connections made by the interneuron C2 onto its followers. [Brown and Getting, unpublished] We have found that increasing DSI activity also caused an increase in the spontaneous firing of putative motor neurons in the swim neural circuit. Neural correlates of the latency change occuring as a result of sensitization have not been found in the isolated brain preparation indicating that peripheral sensory systems may be necessary for sensitization of latency.

In a tail-attached preparation, stimulating a DSI before application of a swim stimulus causes changes in the swim pattern comparable to those seen in behavioral experiments, thus increased DSI activity is a sufficient condition for sensitization.

220.7

PAIRING-SPECIFIC TRANSFORMATION OF GABA-MEDIATED SYNAPTIC INTERACTION IN HERMISSENDA. D.L. Alkon, J.V. Sanchez-Andres, C. Collin. Neural System Section, NINDS, NIH, Bethesda MD 20892.

Previous experiments have demonstrated that GABA is the likely transmitter released by presynaptic hair cells into post-synaptic type B photoreceptors. Other observations have shown that a single pairing of depolarization of the B-cell with GABA iontophoresis produces long-lasting (>10 min) depolarization and increased resistance of the B-cell (Matzel and Alkon, Brain Res. 1991). Here we examine the ionic and pharmacological basis of these GABA-mediated responses. Current-clamp and two microelectrode voltage-clamp experiments demonstrated that 1) GABA-A receptors on the B-cell, in response to hair cell release of GABA open Cl^- channels, 2) GABA-B receptors briefly open Na^+ channels and open K^+ channels over a prolonged time course. If GABA applied iontophoretically or released by depolarization-induced firing of the presynaptic hair cell is paired with a depolarizing light stimulus to the B-cell, the GABA response thereafter remains transformed (for hours) as follows: 1) the GABA-A and GABA-B induced hyperpolarization are markedly reduced, 2) GABA-B mediated *reduction* of K^+ current and depolarization now predominate in the B-cell response. The net effect of pairing is to transform a predominantly hyperpolarizing to a predominantly depolarizing GABA-mediated synaptic effect received by the postsynaptic B-cell from the presynaptic hair cell. This is a new type of synaptic function which seems ideally suited for associative learning.

220.8

ROLE OF IDENTIFIED SEROTONERGIC NEURONS IN GENERATING ROTATIONAL BEHAVIOR IN *HELISOMA* EMBRYOS. T.J. Diefenbach*, N.K. Koehncke* and J.I. Goldberg. Dept. of Zoology, Univ. of Alberta, Edmonton, Alta., Canada T6G 2E9.

Embryonic neurons C1 differentiate early in *Helisoma* embryogenesis and have been implicated as regulators of neural development. Since these neurons first express serotonin at a stage corresponding to the onset of cilia-driven rotational behavior, we tested the hypothesis that neuron C1 also plays a role in the generation of this embryonic behavior. Embryonic neurons C1 were localized in live embryos using the fluorescent serotonin analogue 5,7-dihydroxytryptamine and sequential fluorescence-DIC microscopy. Intracellular recordings revealed spontaneous action potential activity in these neurons as early as stage E20. Together with data on serotonin immunoreactivity, functional serotonin uptake and neurite projections, these electrophysiological data suggest that embryonic neuron C1 releases serotonin. Using time-lapse video microscopy, embryonic rotational behavior was characterized in terms of its behavioral components, developmental changes and responses to exogenous serotonin. Rotation consisted of a slow tonic component that was interrupted by brief periods of accelerated rotation (surges). Quantitative analysis of rotation rate and surge frequency revealed that both of these parameters were maximal at stage E25. Application of serotonin induced a dose-dependent, reversible increase in rotation rate that was blocked by the serotonin antagonist, mianserin. Furthermore, application of mianserin alone abolished the expression of rotational surges, leaving the slow tonic component intact. These results support the hypothesis that embryonic neuron C1 not only regulates neural development, but also acts as a cilio-excitatory motor neuron which generates rotational surges.

Supported by NSERC of Canada.

220.9

IDENTIFYING THE NEURONS INVOLVED IN *C. ELEGANS* MALE MATING BEHAVIOR. K. Liu and P. Sternberg*, HHMI, Division of Biology, Caltech, Pasadena, CA.

Mating behavior in *C. elegans* is a male specific behavior and mediated at least in part by the 79 male-specific neurons, most of which reside in a set of copulatory structures which comprise the male tail. As this system is not amenable to electrophysiology, we have set out to examine the role of these neurons via the successfully used technique of cell ablation. And since cells comprising a common copulatory structure are often generated by a common precursor, we have chosen to follow a lineal approach. That is, male specific post embryonic blast cells which give rise to male copulatory structures and/or neurons are ablated and the resulting phenotype (mating defect), if any, is determined by observation. Given a defect, the progeny of that blast cell are then systematically ablated until the neuron(s) responsible for the phenotype are identified.

Using this approach, we have identified the copulatory structures underlying each of the previously reported steps in male mating behavior:

structure ablated	resulting defect
V rays	response to hermaphrodite
T rays	turning around hermaphrodite
hook	location of vulva
spicules	insertion of spicules into vulva

We are currently trying to identify the individual neurons which control these behaviors. For example, using this approach we have determined that the hook sensory neurons HOA and HOB are required for vulval location while the closely related interneuron PVZ is not. Similarly, the sensory/motor neurons SPCL/SPCR are required for spicule insertion while the sensory neurons SPVL/SPVR are not.

220.10

MALE MATING FACTORS MEDIATE BLOCKING OF SEX PHEROMONE PRODUCTION AND SEXUAL RECEPTIVITY IN FEMALES OF A MOTH, *HELICOVERPA ZEA*. T.G. Kingan[1], A.K. Raina[1*], W. Bodnar[2*] and D.F. Hunt[2*]. 1, USDA ARS, Insect Neurobiology and Hormone Lab, BARC-East, Bldg. 225, Beltsville, MD 20705; 2, Dept. of Chemistry, Univ. of Virginia, Charlottesville, VA 22901.

The production of sex pheromone in the females of several moths has been shown to be under the positive control of a neuropeptide, termed pheromone biosynthesis activating neuropeptide (PBAN; Raina et al., Science, 244:796, 1989) The production of pheromone is followed by the "calling" behavior associated with the release of pheromone, attraction of a male moth, and mating. Similarily, mating in several moths has been shown to lead to a precipitous decline in pheromone production and sexual receptivity for varying periods of time. This decline could be associated with mechanical stimulation or with chemical factors transferred during mating. In earlier studies, we showed that the decline in pheromone after mating of the corn earworm moth *Helicoverpa zea* (formerly *Heliothis*) is likely to be associated with soluble factors from the male accessory gland, sites of production of the seminal secretions, that are transferred during mating. In order to understand the mechanisms by which male-derived factors cause depletion of sex pheromone and loss of sexual receptivity in females, we have purified and begun to characterize two, apparently related accessory gland factors, that when injected into females cause a depletion of PBAN-evoked pheromone accumulation. The factors are basic peptides, each is ca. 6609 Daltons in molecular weight (determined by electrospray tandem mass spectrometry) and contains a disulfide linkage. We refer to these peptides as pheromonostatic factors (PSFs). Partially purified extracts of accessory glands, containing the PSFs, have been tested in a "mating assay" and shown to render the females "unattractive" as well as to block receptivity to the sexual advances of pheromone-primed males.

220.11

EVIDENCE FOR A PROPOSED COMPUTATIONAL PROPERTY OF REMOTE DENDRITIC INHIBITION OBTAINED DURING INHIBITORY CONTROL OF CRAYFISH ESCAPE BEHAVIOR.
E.T. Vu and F.B. Krasne. Neuroscience Program, Department of Psychology, and Brain Research Institute, University of California, Los Angeles, CA 90024.

The functional consequences of placing inhibition remotely from the axon spike initiation zone have been a subject of increasing interest. While studying inhibitory control of the lateral giant command neurons for crayfish tail flip escape behavior, we have come to appreciate a novel and generally important reason for such inhibition: When inhibition must interact with excitation in a "competitive" fashion, with each process being able to override the other by an increase in strength (as is the case for many processing tasks in the nervous system), then inhibition should be distal. In contrast, when computational requirements dictate that inhibition should be absolute, inhibitory synapses should be placed proximally.

Biological evidence for this notion was obtained. In crayfish, a form of inhibition that is active when lateral giant escape must be absolutely prevented was shown to produce an entirely proximal shunt of excitation to the command neurons for this behavior, while a different form of inhibition used to down-modulate but not eliminate this behavior was found to produce an exclusively distal shunt in the same neurons.

Supported by USPHS grant NS08108 (FK) and a Predoct. NSF Fellowship (EV).

220.12

POSTEMBRYONIC GROWTH OF THE CRAYFISH ESCAPE CIRCUIT.
D.H. Edwards, R.A. Fricke*, L. Barnett* and P.R. Nagappan*. Department of Biology, Georgia State University, Atlanta, GA 30303.

Both 1 cm juvenile and 10 cm adult crayfish employ the tailflip response triggered by the lateral giant (LG) interneuron to escape from attacks directed at the abdomen. The response is reliable in small crayfish, whereas larger crayfish habituate to repeated stimuli. LG in both animals receives monosynaptic inputs from primary afferents and disynaptic inputs from mechansensory interneurons through depression-prone chemical synapses from the same afferents. LG is reliably excited by the monosynaptic electrical input in juvenile crayfish, whereas that input is insufficient in adults, and so defers to the later, depression-prone input. We asked why the relative strengths of these inputs change during growth.

The soma is twice as large in 10 cm adults than in 1 cm juveniles, while the major dendrite's length and diameter both increase by 3 times. The axon grows 10-fold in length and 5-fold in diameter. Although the membrane time constant increases from 8.6 ms to 21 ms, the input resistance falls from 742 k-ohms to 167 k-ohms at the initial axon segment. Simultaneous recordings in adult LG neurons show that whereas both mono- and disynaptic PSPs have similar amplitudes in the dendrite, the phasic monosynaptic PSP is much smaller in the initial axon segment. In juvenile crayfish, the monosynaptic PSP is usually larger than the disynaptic PSP in the initial axon segment. Electrical models of large and small LGs show that LG becomes a low-pass filter for synaptic inputs during postembryonic growth. Supported by NIH Research Grant NS26457.

NEUROGLIA AND MYELIN III

221.1

CHARACTERIZATION OF IMMORTALIZED SCHWANN CELL LINES EXPRESSING MYELIN PROTEINS.
J.A. Small*, K. Toda*, E. Yavin, and R.H. Quarles*. Laboratory of Molecular and Cellular Neurobiology, NINDS, NIH, Bethesda, MD 20892.

Cell culture systems for studying the expression of myelin proteins are complex. Primary cultures of rat Schwann cells do not express myelin components in the absence of axonal contact. Our laboratory has recently described a Schwann cell line, immortalized by continuous passage, that expresses myelin-associated glycoprotein (MAG) at levels found in adult sciatic nerve (Goda, et al., 1991, J. Neurochem., 56: 1354-1361). The MAG expressed in these cells had biochemical properties similar to MAG in sciatic nerve.

This cell line and similarly derived ones have been further characterized. Myelin-specific gene expression was determined at the RNA and protein levels. Cell lines S16 and S42 were found to express MAG and PO at both the RNA and protein level. The level of MAG was greater than or equal to that found in sciatic nerve, while the level of PO was much lower. Myelin basic protein (MBP) or its RNA could not be detected. Proteolipid protein (PLP) RNA was found, but no protein was detected. In addition, glial fibrillary acidic protein (GFAP), normally expressed in nonmyelinating Schwann cells, was expressed in these cell lines. Cell line S16Y does not express myelin proteins or GFAP.

The cell lines have been tested for their transfection efficiency. Plasmids containing the chloramphenicol acetyl transferase (CAT) gene driven by a variety of strong eukaryotic promoters were used. Transfection efficiency was high for all three cell lines in transient expression assays. Cells could also be stably transfected with the bacterial neo gene, using Geneticin (G418) eukaryotic antibiotic selection. Transfected cell colonies maintained the myelin expression pattern of the parental cell line.

These cell lines will be very useful tools for studying regulation of gene expression of myelin proteins.

221.2

DIFFERENTIATED IMMORTAL CELL LINES DERIVED FROM RAT BRAIN ASTROCYTES.
C.F. Deschepper[1], E. H. Radany*[2], V. Bigornia*[1], K.R. Zahs[1], J.M. Bishop*[2], F. Besnard[3] and M. Brenner*[3]. [1]Dept of Physiology and [2]J.W. Hooper Foundation, U.C. San Francisco, CA 94143 and [3]Laboratory of Molecular Biology, NINDS, Bethesda MD 20892

A common strategy to immortalize cells from rodent brains has been to infect primary cell cultures derived from embryonic animals with retroviral constructs. Apparently, this approach was mostly successful in immortalizing the more proliferative and less differentiated cells. As an alternative approach, we constructed a plasmid where a 2 kb fragment from the 5' region of the human GFAP gene was placed in front of oncogenic sequences from the SV40 early region. Using calcium-phosphate precipitation, this plasmid was cotransfected with a plasmid conferring resistance to G418 into astrocyte primary cultures derived from neonatal rats. After drug selection, 16 individual clones were isolated and maintained in culture. At least 6 of these cell lines expressed GFAP at the time of their 10th passage in culture, as determined by ICC and Western blot analysis. Pulse-chase analyses determined that these cells synthesized and secreted some of the proteins normally secreted by type I astrocytes, including transferrin, angiotensinogen and α_2-macroglobulin. These results indicate that this approach may be useful to generate immortal astroglial cell lines with the phenotypic characteristics of mature astrocytes. Supported in part by HL29714 and HL 38774.

221.3

PURINE NUCLEOSIDES AND NUCLEOTIDES STIMULATE ASTROCYTE PROLIFERATION *IN VITRO* AND ASTROGLIOSIS *IN VIVO*.
M.P. Rathbone, S. Hindley*, J.W. Gysbers*, B. Gabel*. Depts. Biomed. Sci. and Medicine, McMaster Univ. Health Sci. Center, Hamilton, Ont., Canada L8N 3Z5

Extracellular adenosine (Ado), guanosine (Guo), ATP and GTP stimulate the proliferation of astrocytes, microglia and brain capillary endothelial cells *in vitro* [1,2,3]. The mitogenic effects of Ado and Guo are mediated through adenosine A_2 receptors and increased intracellular cAMP [1], whereas ATP and GTP activate purinergic P_{2Y} receptors and increase phosphoinositide metabolism. Hypoxic, "sick" and dying cells release high concentrations of soluble nucleosides. Do these stimulate the reactive proliferation of microglia, astrocytes and blood vessels *in vivo*? Ten µL of Guo, Ado, (both 1 mM), ATP and GTP (both 500 µM) were each infused into one cerebral hemisphere of rat brains over 2h. Simultaneously the contralateral hemisphere was infused with saline (10 µL). The purines, but not control injections, stimulated marked reactive astrogliosis within 48 h. Astroglial reaction was also present in sub-pial and perivascular regions characteristic of Scherer's secondary structures observed around malignant astrocytomas. Microglia, which proliferate early and secrete IL-1, may augment the purinergic effects. Purines released in brain injury or neuronal death may stimulate glial response to injury. [1]Rathbone, M.P. et al., (1990) Soc. Neurosci. Abs. 16:1000; [2]Norenberg, M.D. et al., (1990) Soc. Neurosci. Abs. 16:667; [3]Kim, J.-K. et al., J. Neurosci. Res. (1991) 28:442. Support: The Hospital for Sick Children Foundation, Rick Hansen Man in Motion Legacy Fund and The Cancer Research Society, Inc.

221.4

ASTROCYTE CELL CYCLING IN VITRO: G1/S TRANSITION REQUIRES MULTIPLE ISOPRENOIDS.
T.J.Langan and M.C.Slater, Dept. of Neurology, SUNY Sch. Med., Buffalo NY 14222

Astroglial cultures were used to compare the relationships to cell cycling of dolichol-linked glycoprotein synthesis, and of availability of mevalonate, the precursor of cholesterol, dolichol and other isoprenoid lipids. With shift-up to 10% serum (time 0) after 48 h of serum depletion, the proportion of cells in S phase (bromodeoxyuridine immunofluorescence) remained under 15% for 12 h, then increased by 20 h to 72 ± 10 %; DNA synthetic rates (thymidine incorporation) increased 5-fold. S phase transition was prevented by addition at 10-12 h of tunicamycin, an inhibitor of transfer of saccharide moieties to dolichol. Mevinolin, an inhibitor of mevalonate biosynthesis, also blocked cycle progression at this time, but had no effect on net glycoprotein synthesis. Removal of mevinolin after a 24 h exposure delayed S phase until 48 h, following recovery of sterol synthesis, even though kinetics of glycoprotein synthesis were unaffected. Tunicamycin removal after 24 h spared sterol synthesis, but caused delay of S phase until 72 h, following recovery of glycoprotein synthesis. The G1/S transition occurring after hydroxyurea release was unaffected by mevinolin, tunicamycin or cycloheximide. Thus, in these cultured developing astroglia, mevalonate and its isoprenoid derivatives have at least two cell cycle-specific roles: dolichol-linked glycoprotein synthesis is required before the G1/S transition, at a time when a distinct mevalonate requirement is also apparent.

221.5

A COMPARISON OF OLIGODENDROCYTE AND SCHWANN CELL PROTEINS AND THE FUNCTIONAL CIS-ACTING ELEMENTS WHICH THEY BIND IN THE MBP PROMOTER REGION . L.G. Wrabetz*, S. Shumas*, M.L. Feltri*, J. Grinspan, D. Bozyczko*, F.A. McMorris, D. Pleasure, and J. Kamholz. Department of Neurology, University of Pennsylvania School of Medicine, and The Wistar Institute, Philadelphia, PA 19104.

During myelination, both oligodendrocytes(OL) and Schwann cells(SC) coordinately express a set of myelin-specific proteins including myelin basic protein(MBP). Others have shown that in development, MBP gene expression is regulated at the level of initiation of transcription. We have previously shown that the start site of MBP transcription is identical in OL and SC, suggesting that the MBP promoter region is the same in both cell types. MBP promoter-CAT fusion transient transfections demonstrate that the 150 base pair region upstream of the MBP gene contains sequences which are sufficient to promote CAT expression in a tissue-specific and orientation-dependent manner in both cell types. However, the 5' extent of cis-acting elements necessary for this expression differs in OL and SC, and therefore, the cis-acting regions necessary for MBP gene expression in OL and SC are also different.

We now extend these observations using enhancer trap experiments to examine the function of oligonucleotides spanning these OL and SC cis-acting regions. These experiments confirm that a different cis-acting element within this 150 base pair region enhances basal promoter activity in OL as compared to SC. Secondly, band-shift assays of OL and SC nuclear extracts with the oligonucleotides containing one or the other of these cis-acting elements demonstrate that each oligonucleotide binds proteins from both OL and SC. However, the oligonucleotide/protein complexes differ in mobility from OL as compared to SC. These results suggest that both the cis-acting elements and the trans-acting factors which bind them during initation of MBP gene transcription differ in OL and SC.

221.6

EFFECT OF PDGF TREATMENT ON GALACTOCEREBROSIDE AND PLP MRNA EXPRESSION IN CULTURES OF RAT OLIGODENDROGLIA. J.B. Grinspan, L.G. Wrabetz* and J. Kamholz. Dept. of Neurology, University of Pennsylvania, Philadelphia, PA 19104.

We have previously demonstrated that platelet derived growth factor (PDGF) causes proliferation of O2A cells and accumulation of galactocerebroside (GalC) positive oligodendroglia (OL) in cultures of rat cerebral white matter (CWM) (Grinspan et al, J.Neurosci. 10: 1866, 1990). In order to extend these studies, we have compared the expression of PLP mRNA with that of GalC and A2B5 in these rat CWM cultures in the presence and absence of PDGF using in situ hybridization and immunhistochemistry. At 3, 7 and 14 days after plating in the absence of PDGF, the number of GalC positive OL in these cultures are approximately the same as the number of cells expressing PLP transcripts. Grain counts of PLP expressing cells demonstrate that each cell contains less than 10 grains. The number of A2B5 and GalC positive cells increases in cultures grown for either 3 or 10 days in the presence of PDGF, although the number of GalC positive cells is still approximately the same as that of the PLP expressing cells at both times. Grain counts of the PLP expressing cells demonstrate, however, that most cells contain many more than 10 grains after 3 days of PDGF treatment and more than 50 grains after 10 days in PDGF. These studies show that GalC expression, as detected by suface labelling, appears at the same time as that of PLP mRNA expression, as detected by in situ hybridization, suggesting that OL maturation and PLP gene expression occurs contemporaneously in vitro. Furthermore, OL that mature in the presence of PDGF accumulate large amounts of PLP transcripts per cell. These studies, taken together with our previous data, suggest that PDGF promotes the survival of GalC positive OL in these cultures, and that subsequent OL maturation is associated with the accumulation of myelin transcripts.

221.7

Regulation of Myelin Gene Expression in C6 Cells. R. C. Wiggins and G. W. Konat.* Dept. Anatomy, West Virginia University School of Medicine, Morgantown, WV 26506.

A model system of rat glioma C6 cells grown in defined medium was used to investigate the mechanisms involved in the regulation of myelin proteolipid protein (PLP) and myelin associated glycoprotein (MAG) genes. Treatment of cells with cAMP-stimulating agents or ascorbic acid upregulated both the PLP and MAG genes, although the timing and extent differed. Retinoic acid upregulated the expression of PLP gene, but downregulated the expression of MAG; whereas, SV40 T antigen downregulated PLP and upregulated MAG. The dissimilarity of PLP and MAG gene responses to these agents indicates the existence of different regulatory mechanisms for the activation of these genes. Thus, the expression of myelin genes and their modulation in C6 cells extends the applicability of these cells as a convenient *in vitro* model system for study of the elementary mechanisms of myelin gene regulation. This research was supported by PHS grants NS13799 and DA04072 and the WVU Medical Corp and NIH BRG 2S07RR05433.

221.8

CHARACTERIZATION OF A MAJOR ASTROCYTIC PHOSPHOPROTEIN SUBSTRATE FOR CALCIUM-DEPENDENT REGULATIONS: PEA-15.

H. Chneiweiss*, H. Araujo*, J. Cordier*, A. Sobel and J. Glowinski*. INSERM U114, 75231 PARIS Cedex 05, FRANCE.

Communication between cells involves phosphorylation as an important molecular mechanism. Astrocytes express membrane receptors capable of responding to extracellular signals. Activation of receptors induces intracellular reactions leading to phosphorylation of specific proteins. Thus, search for major phosphorylated proteins may lead to the discovery of unknown regulatory processes.

We have characterized a novel cytosolic astrocytic phosphoprotein with an apparent molecular mass of 15KDa. It is a major phosphorylation substrate, binding 1.5% of 32P incorpored onto proteins in astrocytes grown in primary culture. Two-dimensional gel electrophoresis of PEA-15 (phosphoprotein enriched in astocytes of 15KDa) allowed to recognize three isoelectric variants between pI 5.3-5.4, the more basic, unphosphorylated, form yielding the two more acidic forms by phosphorylation in a Ca^{2+}-dependent manner.

Phorbol esters and Ca^{2+} ionophores increase the phosphorylation of PEA-15, whereas specific inhibitors of Ca^{2+}-dependent kinases such as staurosporine antagonize this effect. In brain slices PEA-15 is observed throughout all post-natal ages analyzed without great variations of its expression. PEA-15 can also be found in other cellular populations, although the main contribution remains astrocytic.

These results suggest a major involvement of PEA-15 in Ca^{2+}-dependent regulatory processes in the astrocyte.

221.9

INSULIN-LIKE GROWTH FACTOR I GENE EXPRESSION IS INDUCED IN ASTROCYTES DURING DEMYELINATION.

S. Komoly*, H.deF. Webster, and C.A. Bondy*. NINDS and NICHD, NIH, Bethesda, MD 20892.

We have studied the effects of experimental demyelination and remyelination on IGF-I and IGF-I receptor expression in the mouse brain using in situ hybridization and immunocytochemistry. Weanling male mice were fed diets containing 0.5% cuprizone for 8 weeks. In this model, myelin loss is restricted to specific white matter areas and remyelination occurs following cessation of treatment. In control animals IGF-I mRNA was undetectable in white matter, but in treated mice high levels of both IGF-I mRNA and peptide were expressed by astrocytes in the areas affected by myelinolysis. During recovery, IGF-I expression decreased rapidly as oligodendroglial expression of myelin-related genes increased. During early recovery there also was a transient increase in IGF receptor mRNA and peptide immunostaining of oligodendroglia-like cells in the affected areas. This highly specific pattern of IGF-I induction in astrocytes localized in demyelinating areas supports a physiological role for IGF-I in the regulation oligodendrocyte and myelin metabolism in vivo.

221.10

AXON-REGULATED SCHWANN CELL PROTEIN HOMOLOGOUS TO A GROWTH ARREST-SPECIFIC GENE. H.W.Müller, P.Spreyer*, G.Kuhn*, O. Hanemann*, C.Gillen* and H.Schaal*. Molecular Neurobiol. Lab., Dept. Neurology, Univ. of Düsseldorf, D-4000 Düsseldorf, FRG.

A cDNA-library from crushed rat sciatic nerve was differentially hybridized with mRNA expressed in regenerating and intact nerve (Spreyer et al., EMBO J. 9, 1990, 2479). We have isolated a 1.8kb cDNA clone which codes for a 17kD protein with 91.7% homology to the growth arrest-specific membrane protein gas3 (Manfioletti et al., Mol. Cell Biol. 10, 1990,2924). Steady state mRNA levels are very much higher in sciatic nerve than in other tissues, and expression in sciatic nerve is confined to Schwann cells. After nerve injury, transcript levels rapidly declined in nerve segments distal to the site of lesion, but recovered upon nerve regeneration. In contrast, in distal stumps of permanently transected nerves, the mRNA level remained very low. Substantial amounts of mRNA could be reinduced only upon anastomosis of the transected nerve stumps. Reinduction of the transcript correlated with the spatio-temporal pattern of axonal elongation through the distal segment.

Our results indicate an intriguing axonal regulation of the mRNA encoding a membrane-bound "gas" protein in peripheral nerve with putative functions related to Schwann cell growth and differentiation.

Supported by BMFT.

221.11

SCIP MARKS A PROLIFERATIVE SCHWANN CELL PROGENITOR AND ACTS AS A TRANSCRIPTIONAL REPRESSOR OF THE MYELIN GENES. E.S. Monuki, R. Kuhn*, and G.E. Lemke. Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

SCIP is a member of the POU domain transcription factor family that is transiently expressed during a narrow window of glial development in both the central and peripheral nervous systems. In developing rat Schwann cells (SC), SCIP mRNA is expressed at high levels prior to peak myelin gene expression, during the proliferative period that occurs as pre-myelinating SC differentiate into myelinating SC. SCIP mRNA is not expressed at high levels in fully-differentiated myelinating SC, but is transiently induced following peripheral nerve transection, during the proliferative period that occurs as these cells dedifferentiate back into pre-myelinating SC. Thus, elevated SCIP expression appears to mark specifically these proliferative transition periods in SC differentiation. In cultured SC, agents that raise intracellular levels of cAMP are strong inducers of this proliferative progenitor phenotype characterized by elevated SCIP expression.

In transient cotransfection assays, SCIP acts as a repressor of myelin-specific genes. This repression appears to be the function of two distinct domains in the SCIP protein: 1) the POU domain, encoding the DNA binding domain, and 2) an amino-terminal repression domain. Mutations that inactivate either of these domains inhibit the ability of SCIP to repress the P0 promoter, a myelin-specific gene promoter that contains multiple SCIP binding sites as detected by gel shift and DNaseI footprinting analyses. These data indicate that the transcriptional regulatory properties of SCIP are distinct from those of previously-characterized POU proteins, and suggest that SCIP acts as a transiently-expressed repressor of the myelin genes during proliferative periods in SC differentiation.

221.12

A NOVEL MYELIN PROTEIN IS ENCODED BY THE HOMOLOG OF A GROWTH ARREST SPECIFIC GENE. U. Suter*, A. A. Welcher, M. De Leon*, G. J. Snipes* and E. M. Shooter. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305-5401

We have isolated a rat cDNA, SR13, which is strongly downregulated in the initial phase after sciatic nerve injury. This cDNA encodes a glycoprotein which shares striking amino-acid similarities with a previously purified myelin protein and is specifically precipitated by a myelin-specific antiserum. Immunohistochemistry experiments using peptide-specific polyclonal antibodies localize the SR13 protein to the myelin sheath of the sciatic nerve. Northern and Western blot analysis of different rat tissues revealed that SR13 protein is predominantly expressed in the rat sciatic nerve while a weak signal is detectable in the brain. Computer-aided sequence analysis identified a pronounced homology of SR13 to a growth arrest specific mRNA (Gas-3) which is expressed in resting but not in proliferating 3T3 mouse fibroblasts. SR13 is similarly downregulated during Schwann cell proliferation in the rat sciatic nerve. The association of the SR13 as well as the Gas-3 mRNA with non-proliferating cells in two different experimental systems suggests a common role for these molecules in maintaining the quiescent cell state. ***Supported, in part, by the American Paralysis Association.***

NEUROGENESIS: TISSUE CULTURE MODELS

222.1

DEVELOPMENT AND CHARACTERIZATION OF A SECOND HUMAN NEURONAL CORTICAL CELL LINE, HCN-2. L.D. Hester, G.V. Ronnett and S.H. Snyder. Johns Hopkins University School of Medicine, Dept. of Neuroscience, Baltimore, MD 21205.

Previously, a cell line was successfully established from human cortical material obtained at the time of hemispherectomy (Science, 248:603-605, 1990). A similar methodology was employed to develop a cell line originating from material obtained from a patient with Rasmussen's encephalitis. This cell line, HCN-2, appears polygonal in shape and divides with a doubling time of approximately 36 hours. HCN-2 can also be induced to differentiate with the addition of nerve growth factor (NGF), the phorbol ester TPA, and isobutylmethylxanthine (IBMX). As with HCN-1, differentiation dramatically slows cellular division and induces the outgrowth of neurites over 3-4 days. In both undifferentiated and differentiated states, HCN-2 demonstrates immunoreactivity to neuron specific enolase (NSE), vimentin, neurofilament, and no immunoreactivity for glial fibrillary acidic protein (GFAP), and S-100 protein. These cells contain small amounts of somatostatin, vasoactive intestinal peptide, cholecystokinin, GABA, and glutamate. None or little immunoreactivity could be seen for neuropeptide Y, members of the enkephalin family, or substance P. HCN-2 represents a second line of human cortical neuronal cells established by our previously described methodologies.

222.2

BIOCHEMICAL AND IMMUNOCYTOCHEMICAL CHARACTERIZATION OF HUMAN FETAL ORGANOTYPIC CENTRAL NERVOUS TISSUE CULTURES W. C. Hatch*, M. Tricoche*, F.-C. Chiu*, I. Vincent, W. D. Lyman* Dept. of Pathology, Albert Einstein College of Medicine Bronx, NY 10461

Objective: CNS explant organotypic tissue cultures(OTC) derived from human fetal brain allow the study of complex interactions between CNS cell types which is not possible in dissociated tissue culture systems. This study describes the identification of CNS cell types present in OTC and the kinetics of gene expression as a measure of tissue growth and viability.

Methods: Human fetal OTC grown in 24-well Primaria plates were analyzed at 2, 7, 14, 21, 28 and 32 days in culture. Immunocytochemistry and histochemistry identified CNS cell types using antibodies and lectins to detect neurons(NF68, NGF receptor [NGFr]), astrocytes(GFAP), oligodendrocytes (GC), and microglia(RCA, EBM11, non-specific acetate esterase[NSAE]). Cultures harvested at each time point were analyzed for CNS specific protein expression using NF, GFAP, GC, and EBM11 antibodies by Western blotting.

Results: Immunocytochemistry and histochemistry has identified CNS cell types staining positive for NGFr, GFAP, EBM 11, GC, and NSAE in these CNS explant cultures up to 32 days in culture. RCA stained microglia like cells and blood vessel up to day 14, but detection decreased by day 21. Western blot analysis suggests that levels of NF and GFAP proteins increase up to 32 days in culture.

Conclusions: These immunohistochemical results suggest the CNS OTC model derived from human fetal brain is composed of all the major CNS cell types which undergo growth and protein expression for up to 32 days in vitro. This model may allow the examination of developmental and neuropathologic processes including the interaction of HIV-1 with the developing CNS.

Supported by USPHS grants MH 47667, MH 46815, and DA 05583.

222.3

NEUROTRANSMITTER EXPRESSION IN DIPLOID CELLS CULTURED FROM ADULT HUMAN BRAIN AT AUTOPSY. J.P. Blass, W.R. Markesbery,[1] J.T. Duffy, R.K-F. Sheu, C-S. Rhim, L. Ko, and R.S. Black. Burke Med. Res. Inst., Cornell Univ. Med. Coll., White Plains, NY 10605 and [1]Dept of Neurology, U of Kentucky, Lexington, KY 40536-0230.

The serial culture from adult human brain obtained at autopsy of diploid cells which contain neurofilaments (NF-H and NF-M) and neuron-specific enolase has been presented previously (Blass et al, Trans Amer Soc Neurochem 22:227, 1991). These cells also contain tau proteins and GFAP. We now report that these cells also contain neurotransmitter markers. Tyrosine hydroxylase was identified by immunocytochemistry with both a monoclonal and a polyclonal antibody, and by Western blotting. Immunocytochemical studies also demonstrated the presence of glutamic acid decarboxylase and probably of GABA, and of serotonin but not of choline acetyltransferase. Immunocytochemical studies indicated the presence of met-enkephalin, neurotensin, and neuropeptide Y, possibly of somatostatin, but not of substance P. Immunocytochemical reactivity to a neurotransmitter or marker enzyme, when present, was in essentially all cells and in essentially equal intensity. These studies extend evidence that these cells cultured from autopsy human brain synthesize molecules normally associated with neurons as well as with glia, and suggest that these cells are relatively undifferentiated compared to neurons expressing a single neurotransmitter phenotype.

222.4

ALTERED GLIAL CELL DEVELOPMENT IN THE TRISOMY 16 MOUSE. M.A. Barry, B.K. Krueger and P.J. Yarowsky. Depts. of Physiology and Pharmacol. & Exp. Ther., Univ. of MD Sch. of Med., Baltimore, MD 21201.

Trisomy of mouse chromosome 16 (MMU 16;Ts16) is a potential animal model for Down Syndrome (DS) because it contains several of the genes located on human chromosome 21 which is trisomic in DS. Studies of neuronal development in Ts16 indicate abnormal neurogenesis, a decrease in neuron density and premature neuron death. Gliogenesis and glial cell maturation was examined in primary cultures of the cerebral cortex from Ts16 and euploid mice. The development of oligodendrocytes ($GalC^+$) from glial progenitor (O2A) cells ($GFAP^-/A2B5^+$) was studied by immunofluorescence. Cells from E15-E16 Ts16 and euploid fetuses were maintained in DMEM/F-12 with 10% fetal calf serum for up to 30 days *in vitro* (div). During the first 12 div the numbers of both Ts16 and euploid O2As increased; however, the Ts16 cultures contained 2-3 times as many O2As as the euploid cultures. From 15-20 div the number of euploid O2As decreased whereas the number of Ts16 O2As continued to increase. Coincident with the decrease of O2As in euploid cultures, oligodendrocytes began to appear. In contrast, no oligodendrocytes were observed in the Ts16 cultures up to 30 div. The results suggest that overexpression of one or more genes on MMU16 alters the normal program for oligodendrocyte development from O2A progenitor cells. Supported by NIH and NSF.

222.5

GENERATION OF NEURONS AND ASTROCYTES FROM PROGENITORS OF THE ADULT MAMMALIAN CNS.
B.A. Reynolds and S. Weiss. Neuroscience Research Group, University of Calgary, Calgary, AB, Canada.

We report the presence of an adult CNS, epidermal growth factor (EGF) responsive multipotent progenitor which, in vitro, gives rise to neurons and astrocytes. Striata were dissected from adult mice (25-30gms), enzymatically dissociated and plated (300-500 cells/ml) in a defined serum-free media containing 20 ng/ml of EGF. Division of single progenitor cells (10-15/ml) was first observed after 2 days in vitro (DIV). By 5-7 DIV the proliferating cells had formed a sphere (neurosphere) which lifted off the plastic substrate and floated in suspension. Indirect immunocytochemistry of the neurospheres at 5-7 DIV revealed that the cells contained nestin-immunoreactivity (IR), an intermediate filament protein found in CNS neuroepithelial stem cells; neuron specific enolase (NSE)- or glial fibrillary acidic protein (GFAP)-IR was not detected. Single neurospheres (5-7 DIV) were transferred to poly-l-ornithine coated coverslips (in EGF-containing serum-free media). Attached neurospheres continued to proliferate and cells began to migrate from the sphere. After a further 14-21 DIV, neurofilament (160kDa)-, NSE- and GFAP-IR was present in the neurospheres and associated cells. These results demonstrate that the adult mammalian CNS contains progenitor cells which can be induced to proliferate and differentiate into neurons and astrocytes.

Supported by the Medical Research Council of Canada.

222.6

EMBRYONIC NEUROSPHERES: CONTINUAL PROLIFERATION OF CNS PROGENITOR CELLS IN SUSPENSION.
J.S. Williams, B.A. Reynolds and S. Weiss. Neuroscience Research Group, Univ. of Calgary, Calgary, Canada.

We describe the continual proliferation of undifferentiated CNS progenitor cells that can be subcloned, harvested and induced to differentiate into neurons and astrocytes. E14 striatal cells were plated (10^5 cells/ml) in defined, serum-free media containing epidermal growth factor (EGF; 20 ng/ml). EGF-responsive progenitors began to proliferate after 2-3 days in vitro (DIV) and by 5 DIV had formed a hollow sphere of cells (neurospheres) which lifted off the substrate and floated in suspension. Indirect immunocytochemistry of the spheres at 5-7 DIV revealed that the cells were nestin-immunoreactive (IR) characteristic of undifferentiated CNS stem cells; cells in the sphere were negative for markers of differentiated CNS cells (e.g. NFM (160kDa), NSE and GFAP). Neurospheres could be removed from suspension and plated intact onto poly-l-ornithine-coated coverslips where they readily attached and could be induced to differentiate into neurons and astrocytes. Alternatively, neurospheres could be mechanically dissociated into single cells and reseeded in defined EGF-media; 10-40% of the subcloned cells formed new neurospheres. Removal of EGF at any stage resulted in cessation of proliferation. This methodology may be applied towards generating large numbers of cells for neuronal transplantation from minimal fetal tissue.

Supported by the Medical Research Council of Canada.

222.7

GROWTH FACTORS INFLUENCE THE FATE OF EPIDERMAL GROWTH FACTOR-GENERATED EMBRYONIC PROGENITOR CELLS IN VITRO.
A.L. Vescovi*, B.A. Reynolds and S. Weiss. Neuroscience Research Group, Univ. of Calgary, Calgary, AB, Canada and *National Neurological Institute, Milan, Italy.

Neurotrophic factors may act in concert to regulate the proliferation and differentiation of progenitor cells of the CNS. E14 striatal cells were plated (10^5 cells/ml) in defined, serum-free media containing epidermal growth factor (EGF; 20 ng/ml). EGF-responsive progenitors began to proliferate after 2-3 days in vitro (DIV) and by 5 DIV had formed a sphere of cells (neurospheres) which lifted off the substrate and floated in suspension. Indirect immunocytochemistry of the spheres at 5-7 DIV revealed that the cells were nestin-immunoreactive (IR) characteristic of CNS stem cells; these cells were negative for neuronal and glial cell markers. Neurospheres were mechanically dissociated and reseeded as single cells in defined medium, in the absence or presence of EGF or basic fibroblast growth factor (bFGF). In the absence of growth factors, single cells rarely survived for more than 1-2 DIV. In the presence of EGF, 10-40% of the cells formed new neurospheres; remaining cells either differentiated into neurons or glia or died. In the presence of bFGF, a unique pattern of proliferation and differentiation of the progenitor cells was observed. These findings suggest that EGF and bFGF can influence the fate of embryonic, EGF-generated progenitor cells.

Supported by MRC (Canada), ARIN (Italy) fellowship (ALV).

222.8

NEURONAL DIFFERENTIATION OF EMBRYONIC STEM CELLS
K.S. O'Shea, Dept. of Anatomy & Cell Biology, Univ of Mich., Ann Arbor, MI 48109

Murine embryonic stem (ES) cells differentiate into a variety of cell types both in vitro as well as when injected into blastocysts to form chimeras. In the current study, a number of approaches were employed to examine the ability of ES cells to attain a neuronal phenotype in vitro. Briefly, ES cells (E14, M. Hooper) were cultured in bacteriological grade plastic in DMEM:F12 containing the N2 supplement. After 18h, aggregates were added to 24 well plates and differentiation monitored. For studies of the ability of chemical inducers, e.g., retinoic acid (RA) to promote differentiation, cell suspensions were added directly to 24 well plates. Medium was changed at 72h intervals. When maintained in complete medium also containing leukemia inhibitory factor (LIF), ES cells remain undifferentiated and proliferate rapidly. When removed from their substrate and aggregated overnight, then plated at low density in modified N2 medium, neurons were the predominate cell type present after a 3-4 week culture period. Culture in the presence of RA (10^{-6} - 10^{-7} M) produced a rapid shift (4-5 days) in morphology to a neuronal phenotype. On appropriate substrates there was extensive neurite outgrowth as verified by staining with neuron specific antibodies.

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222.9

EXPRESSION OF B-50 (GAP-43) DURING DIFFERENTIATION OF P19 EMBRYONAL CARCINOMA CELLS.
E.R.A. Jap Tjoen San, M. Mercken**, A.B. Oestreicher*, P. Schotman, S.W. de Laat* and W.H. Gispen Rudolf Magnus Institute and Hubrecht Laboratory*, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands and **Born Bunge Foundation, University of Antwerp and Innogenetics, Gent, Belgium.

The murine embryonal carcinoma cell (EC) line P19 is a pluripotent stem cell line, capable of differentiating into derivatives of each of the three primary germlayers, ectoderm, mesoderm or endoderm. B-50 (GAP-43) belongs to a set of neuronal specific proteins which expression is enhanced in early stages of neuron development. We have studied expression of the growth associated protein B-50 (GAP-43) in the development of P19 EC cells into neuronal or mesodermal structures.

Neuronal differentiation was induced by culturing P19 EC cells in suspension in the presence of 10^{-6} M retinoic acid (RA) for five days and subsequent plating of aggregates, resulting in heterogeneous cultures of neurons, glia and fibroblasts. Expression of B-50 was measured by Western blotting for protein and Northern blotting for mRNA levels. Undifferentiated cells express B-50 protein in low detectable amounts. Aggregation in the presence of RA, increased B-50 expression. During neuronal development, B-50 expression was further enhanced. In the heterogeneous cultures, B-50 immunofluorescence was highly restricted to neurons. Thus, B-50 appeared to be an useful neuronal marker in this system. The levels of expression followed the differentiation of P19 EC cells into neurons.

(Supported by the Center for Developmental Biology, Utrecht)

222.10

A CELL LINE DERIVED FROM THE NEUROEPITHELIUM EXPRESSES A NOVEL FORM OF HEPARAN SULFATE PROTEOGLYCAN (HSPG) WHICH IS DEVELOPMENTALLY REGULATED.
V. Nurcombe*, G. Binmore* and A.W. Goodwin. Dept. of Anatomy, University of Melbourne, Australia 3052.

We have been isolating and characterizing HSPGs from a cell line, designated 2.3D, derived from the immortalization of E10 mouse neuroepithelium with the *c-myc* oncogene (Bartlett et al., PNAS [1988] **85**:3255). 2.3D subcellular fractions containing HSPGs were isolated using anion-exchange and molecular sieve chromatography, and subjected to SDS-PAGE before and after treatment with nitrous acid or glycosaminoglycan lyases. This particular cell line appears to secrete approximately 90% of its PGs as a single species of HSPG of M_r = 460kDa. The core molecule has a M_r = 45kDa and significant homology at the amino acid level with another known basement membrane HSPG core molecule of much larger molecular weight (Noonan et al., JBC [1988] **263**:16379) as well as stretches of unique sequence. These data indicate that the nervous system expresses developmentally regulated sets of PGs in a neurotrophically-controlled fashion.

223.1

FINE-STRUCTURAL CHARACTERISTICS OF IDENTIFIED SEPTO-HIPPOCAMPAL NEURONS SURVIVING AXOTOMY. G. M. Peterson, T. Naumann and M. Frotscher. Inst Anatomy, Univ Freiburg, F.R.Germany and Dept Anatomy & Cell Biology, East Carolina Univ Sch Med, Greenville, NC, USA.

Previous studies have indicated that axotomized septohippocampal neurons undergo severe degeneration, presumably due to the loss of nerve growth factor supplied by the target region. Here we demonstrate that a substantial number of septohippocampal neurons survive axotomy and display fine-structural characteristics of intact nerve cells. Rats received intrahippocampal injections of Fluoro Gold to retrogradely label septohippocampal neurons. One week following tracer injection, the fimbria was transected bilaterally. After varying survival times of up to 10 weeks, the animals were fixed by transcardial perfusion and 100 μm thick sections were cut on a Vibratome. Retrogradely labeled septohippocampal neurons were visualized under a fluorescence microscope and were intracellularly injected with Lucifer Yellow (LY) to reveal the dendritic arbor of the cells. Following photoconversion of LY to an electron dense product, these identified septohippocampal neurons were subjected to light and electron microscopic analysis. Our results show that a large number of septohippocampal neurons survived even 10 weeks after axotomy. These cells displayed a normal endoplasmic reticulum and numerous input synapses on their cell bodies, but had severely shortened dendrites. These data show that septohippocampal neurons undergo some atrophic or degenerative changes following axotomy, but that many of them survive and maintain synaptic inputs for extended periods of time. (Supported by the Humboldt Foundation and Deutsche Forschungsgemeinschaft).

223.2

EFFECT OF T-3 SPINAL CORD HEMISECTION ON SURVIVAL OF TWO POPULATIONS OF SPINOCEREBELLAR NEURONS. T.M. Wallace*, D.S. Feldman, E.R. Feringa and R.L. McBride. VA Medical Center and Medical College of Georgia, Augusta, GA 30910.

We studied the relationship between distance from axotomy and survival of spinocerebellar neurons. In seven-week-old female rats, spinocerebellar neurons were retrogradely labeled by cerebellar injection of True Blue. Five days later, the spinal cords were hemisected at T-3. After 10 weeks, the rats were perfused. The cord caudal to the lesion was cut transversely into 30 μm sections. Based on survival, there appeared to be two populations of neurons in Clarke's column. Rostral to T-12 only 15% of the labeled neurons ipsilateral to the hemisection survived; caudal to T-12 77% survived. The transition zone between low and high survival was narrow, suggesting that factors other than distance were determining survival. Labeled ventral horn spinocerebellar neurons, found only in lumbar and sacral segments, were decreased by 48% contralateral to the hemisection. Neuron loss in both the ventral horn and Clarke's column at and caudal to T-12 was inversely proportional to the distance from the lesion. Supported by the VA and MCG.

223.3

EXTENT OF RETROGRADE DEGENERATION OF CORTICOSPINAL AXONS 20 WEEKS AFTER T-9 SPINAL CORD TRANSECTION. M.K. Garver, E.R. Feringa and R.L. McBride. VA Medical Center and Medical College of Georgia, Augusta, GA 30910.

We used the anterograde transport of tritiated protein to determine the extent of retraction of injured corticospinal axons proximal to a T-9 spinal cord transection. We transected the cord of 10 7-week-old female rats; 7 non-transected rats were controls. After 18 weeks, we injected 50 μCi of ^{3}H proline bilaterally into motor cortex and 14 days later perfused the rats with formalin. The cord was cut transversely into blocks and processed for liquid scintillation counting. Counts of adjacent blocks were not significantly different: 55±7 DPM/mg tissue (caudal border of sample 21 mm rostral to transection), 63±7 (13.5 mm), 59±7 (6 mm). Labeling increased slightly close to and within the glial scar: 72±8 (4 mm), 78±10 (2 mm), 85±9 (0 mm)). Since labeling did not decrease caudally, most axotomized corticospinal neurons incorporated proline into proteins and transported them down the axon. There was no evidence of substantial retrograde degeneration of the axons from the transection site at 20 weeks after lesion. Supported by the VA and MCG.

223.4

TOPOGRAPHY OF RETROGRADE HORSERADISH PEROXIDASE (HRP) LABELING OF CORTICOSPINAL NEURONS AFTER SPINAL CORD TRANSECTION. V.C. Livingston*, E.R. Feringa and R.L. McBride. VA Medical Center and Medical College of Georgia, Augusta, GA 30910.

Ten weeks after T-9 spinal cord transection, many rat corticospinal neurons do not retrogradely label with HRP; failure to retrogradely label with Fluoro-Gold occurs much later. To determine if the failure to label was due to the time interval between HRP placement and sacrifice, and if loss of labeling occurred in the same neurons as with Fluoro-Gold, the spinal cords of 27 7-week-old female rats were transected at T-9. Ten weeks later, a pellet of HRP was placed into a new transection at T-2 in these and 25 controls. Rats were sacrificed 1, 2, 3 or 5 days later. In both control and transected rats, labeling decreased as survival time increased (control vs transected, 1 day: 859±54 vs 565±53; 2 days: 689±111 vs 466±61; 3 days: 583±34 vs 353±52; 5 days: 546±76 vs 349±53). The decreased labeling in transected rats always occurred in the hindlimb area of motor cortex (as with Fluoro-Gold), and was not related to survival time after HRP placement. Supported by the VA and MCG.

223.5

THE DEVELOPMENTAL TIME COURSE OF AXOTOMY-INDUCED MOTONEURONAL DEATH IN THE HORMONE-SENSITIVE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS (SNB). J.L. Lubischer & A.P. Arnold, UCLA Brain Research Inst. and Dept. Psychol., Los Angeles, CA 90024

Motoneurons are more likely to die after axotomy early in development than after axotomy in adulthood. The developmental time course of this sensitivity to axonal injury roughly follows the time course of synapse elimination, suggesting that these cells are in a net regressive mode, incapable of an adult-typical response to axonal injury. Synapse elimination in the levator ani muscle (innervated by SNB motoneurons via the pudendal nerve) is delayed relative to other muscles, and can be altered by hormonal treatment. We asked whether susceptibility to axotomy-induced death is also delayed in the SNB, or is instead dissociated from synapse elimination in the normal development of this system.

The pudendal nerve was cut unilaterally in anesthetized male rats at various ages between postnatal day 1 (P1) and adulthood. Rats were perfused at P50 (or 3 weeks after axotomy for adults). The number of SNB motoneurons on the side of the axotomy was counted and divided by the contralateral number to determine percent cell survival. SNB motoneurons begin to show the ability to survive axotomy between P7 and P14, later than reported for other motoneurons (between P1 and P7). The adult pattern of survival is seen by P21:

	P1	P4	P7	P14	P21	P28	P42	adult
mean % survival	3.9	4.3	5.0	42.4	87.2	78.3	87.1	85.7
(SEM)	(1.5)	(1.8)	(1.0)	(5.1)	(3.6)	(6.3)	(3.6)	(4.3)

These results are consistent with a relationship between synapse elimination and sensitivity to axonal injury. Preliminary data, however, indicate that androgen treatment, which delays synapse elimination in the LA, does not alter the time course of axotomy-induced SNB cell death, suggesting that we can dissociate these developmental phenomena experimentally.

Supported by NIH grant HD15021 and an NSF Graduate Fellowship.

223.6

ORGANIZATION OF DORSAL ROOT GANGLION NEURON (DRGN) LOSS 20 WEEKS AFTER HINDLIMB AMPUTATION IN RATS. B.E. Smith*, J.K. Williams, Jr.*, E.R. Feringa and R.L. McBride. VA Medical Center and Medical College of Georgia, Augusta, GA 30910.

Twenty weeks after hindlimb amputation in rats, we compared the resulting loss of sciatic DRGNs with the loss of DRGNs supplying either the common peroneal or tibial nerves. DRGN somata were retrogradely labeled in 7-week-old female rats by soaking the proximal end of the cut right sciatic nerve (10 rats), common peroneal nerve (8 rats) or tibial nerve (7 rats) in True Blue for one hour. Five days later, we amputated the right hindlimb of half the rats in each group. Twenty weeks later the rats were perfused with formalin. Labeled DRGNs were counted on frozen sections. Amputation resulted in a loss of 49% of L-4, L-5 and L-6 DRGNs supplying the sciatic nerve; most of the loss was in L-5. The number of labeled tibial L-5 DRGNs decreased by only 29% while labeled common peroneal L-5 DRGNs decreased by 53%. Neurons supplying particular anatomical regions were more affected by injury than others. The determinants of the death are not clear. Supported by the VA and MCG.

223.7

MK-801 Inhibits Axotomy Induced Cell Death in the Clarke's Nucleus of the Rat C. Sanner and M.E. Goldberger. Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania, Phildelphia, PA. 19129.

It was shown in our previous studies that L1-S2 dorsal rhizotomy of the spinal cord prior to hemisection at T9, prevents axotomy induced cell death in Clarke's Nucleus (CN). This implied that dorsal root afferents may be cytotoxic to axotomized neurons since their removal promoted cell survival. In this study we have attempted a non-surgical deafferentation of CN neurons by administering MK-801 daily to T9-10 hemisected rats. The rats were divided into groups corresponding to five post-operative intervals. We compared each group of MK-801 injected rats with axotomized controls that received saline only. We found that the saline rats showed an ipsilateral cell loss of CN neurons that increased in magnitude over time until there was 30% or greater cell loss by 8 weeks. The MK-801 animals however, showed no cell loss over the same five time points. These results suggest that axotomy induced cell death in CN may be partially mediated by glutamate and its effect on CN NMDA receptors. Supported by grant NS24707.

223.8

PHOSPHORYLATION OF NEUROFILAMENTS (NF) IN THE AXOTOMIZED SPINAL DORSAL NUCLEUS OF CLARKE (DNC) NEURONS. S.Hong, P.J.Reier and G.Shaw. Depts. Neurological Surg. and Neuroscience, Univ. Florida Coll. Med., Gainesville, FL 32610.

It has been suggested that a persistence of cell body-associated (CB-A) NF phosphorylation within the CNS may be linked to the imminent death of injured neurons. To address this issue further, we have examined whether CB-A persistent phosphorylation is associated with retrograde cell death in the DNC. Previous studies have shown that these neurons undergo substantial degeneration within 5-10 weeks after spinal cord transection. Immunocytochemistry was performed using a specific monoclonal antibody (RT97, Boehringer) against the phosphorylated epitopes of the NF heavy subunit. DNC axotomy was performed by creating a hemisection cavity at the T_9-T_{10} spinal level. By 5 days post-injury (p.i.), reactive, swollen DNC neurons were seen; however, phosphorylated NF were only demonstrated in the cell bodies of a few axotomized DNC neurons caudal to the level of injury. By 2 weeks p.i. more cells in the DNC were stained by RT97. In addition, some contralaterally located neurons (e.g., Rexed lamina VII) exhibited intense RT97-immunoreactivity. By 10 weeks, when most DNC neurons have died, only a modest number of RT97-positive cells were seen in the DNC. These results suggest that phosphorylation of perikaryal NF may coincide with cell death in the DNC, though this process seems to be secondary to other neuronal responses to injury (e.g., chromatolysis). Hence, phosphorylation of CB-A NF may be a useful index of imminent cell death. Such a marker could be useful in demonstrating post-injury degeneration in other, more diffuse neuronal populations in the spinal cord, as well as elsewhere in the CNS. This work was supported by NIH PO1-NS 27511.

223.9

AXOTOMY ENHANCES GAP-43 mRNA LEVELS IN ADULT RAT RETINAL GANGLION CELLS. P.S. Jones and A.J. Aguayo. Center for Research in Neurosciences, Montreal General Hospital and McGill University, Montreal, Quebec, Canada H3G 1A4

Optic nerve (ON) transection near the eye leads to increased GAP-43 immunoreactivity in some but not all surviving retinal ganglion cells (RGCs) examined in retinal wholemounts (Doster et al., Neuron 6: 1-20, 1991). RGCs were immunoreactive from 6 to 21 days after injury and there was a marked increase in the levels of axonally transported GAP-43 in the ON. Neither of these changes were apparent when the ON was interrupted intracranially, approximately 8 mm from the eye. We have now used in situ hybridization to investigate GAP-43 mRNA in radial sections of the retina after axotomy near to and far from the RGC somata. A radiolabeled DNA probe corresponding to the first 500 base pairs of the rat cDNA clone pG43-1.2 (courtesy of Dr. J.H.P. Skene) was used for these studies. Retina were examined at 3, 5, 12, and 21 days after axotomy. We detected an increase in the hybridization signal over approximately 5% of the pre-axotomy cell number of RGCs. Similar increases occurred both after sectioning the ON intraorbitally (0.5 mm from the eye) and when the ON was cut 8-9 mm from the eye. With either of these axotomy locations, the enhanced signal was first observed in RGCs 3 days after injury and lost between 12 and 21 days. We conclude that: a) axotomy alone, without subsequent fiber elongation, can induce an elevation of GAP-43 mRNA in these CNS neurons; b) differences in GAP-43 immunoreactivity and axonal transport previously observed after intraorbital and intracranial axotomy do not reflect the changes in mRNA regulation that follow the injury of RGCs near and far from their somata.

223.10

RETROGRADE EFFECTS OF AXOTOMY BY CUT OR CRUSH OF THE ADULT RAT OPTIC NERVE. M. Berkelaar*, G.M. Bray and A.J. Aguayo. Centre for Research in Neuroscience, McGill University, 1650 Cedar Avenue, Montréal, Québec, H3G 1A4.

To investigate the anatomic effects of different types of axotomy on retinal ganglion cells (RGCs) and their fibers in the optic nerve (ON) and retina, we compared retrograde changes after cutting or crushing the ON intracranially, 8-10 mm from the posterior pole of the eye.

One week after ON cut, a central core of degenerating myelinating fibers (MFs) and macrophages extended 4-5 mm from the injury site towards the eye but at 1 mm from the eye, myelinated fibers (MFs) appeared normal in ON cross-sections examined by light microscopy (LM). At 4 weeks, however, many MFs had degenerated in the ON close to the eye. Within the retina, fiber bundles were decreased in size, although the extent of axonal loss was qualitatively less than in the ON, and RGC cell bodies expressing RT-97 immunoreactivity were prominent.

Crush injury 8-10 mm from the eye caused a slower, more homogeneous retrograde change. At one week, severe MF breakdown was restricted to the site of crush and extended only 1 mm towards the eye; no degenerative changes were observed by LM 3 mm proximal to the injury site. Four weeks after ON crush, there were few degenerating MFs in the ON 1 mm from the eye, axonal changes were not detectable in the retina, and only occasional RGC cell bodies expressed RT-97 immunoreactivity.

Thus, cutting the ON triggers different retrograde changes in the axotomized RGCs and their axons than interrupting the ON by crush injury. Conditions at the lesion site and substrates in the ON and retina may influence the extension of these changes towards the RGC somata.

223.11

EFFECTS OF PROTEINASE INHIBITORS ON RETROGRADE CHANGES IN AXOTOMIZED RETINAL GANGLION CELLS. G.A. Robinson and A.J. Aguayo. Center for Research in Neuroscience, Montreal General Hospital and McGill University, Montréal, Québec, Canada H3G 1A4.

Optic nerves (ONs) were unilaterally and intraorbitally transected in adult female Sprague-Dawley rats <1 mm from the eye. Immediately after axotomy, an Elvax pellet containing either leupeptin, an inhibitor of cysteine and serine proteinases, or E-64, an inhibitor of cysteine proteinases, was placed either in contact with the ON stump or under the sclera near the optic disk. Empty pellets or those containing leupeptin or E-64 were inserted into control eyes with intact ONs. Empty pellets were inserted into control eyes with cut ONs. One to four weeks after axotomy, control and experimental retinae were studied using a battery of antibodies to structural proteins and by electron microscopy (EM).

Neurofilament (NF) antibodies revealed that axotomy alone severely reduced the number of retinal ganglion cell (RGC) axons while inducing focal swellings in a small number of axons. Both leupeptin and E-64 greatly increased the frequency of these swellings and further reduced the number of axons that survived axotomy. EM analysis revealed that the axonal swellings contained large accumulations of NFs. Antibodies to NFs did not show differences in phosphorylated epitopes between swollen and not swollen segments of axons. These results suggest that both proteinase inhibitors may exacerbate cytoskeletal alterations in injured RGCs. It is not known if the NF accumulation in the swellings is related to the slowed transport of this and other proteins observed in the proximal stump of cut ONs (McKerracher et al., 1990, J. Neurosci. 10: 2834) or to an inhibition of NF breakdown in injured axons.

223.12

OLFACTORY BULB REMOVAL CAUSES INCREASED PROLIFERATION AND REDUCED SURVIVAL OF PRIMARY OLFACTORY NEURONS. S. Biffo*, M. Sassoe'Pognetto* and A. Fasolo. Dip. Biologia Animale. Univ. Torino. V. A. Albertina 17, 10123 Torino, Italy.

Olfactory neurons (ON) located in the olfactory epithelium project their axons to the olfactory bulb. Bulbectomy (BX) causes the death of ON followed by the restoration of their number from a stem cell compartment. However, in the absence of their target ON do not fully differentiate and retain an immature phenotype (expression of B-50/GAP43, absence of OMP).

In order to assess whether the effect of target deprival on ON was due to either a) block of differentiation and normal turnover or b) accelerated turnover and shorter survival, adult rats were unilaterally bulbectomized and 35 days later, when the number of ON recovered normal levels, injected with bromodeoxyuridine (BrdU). Rats were then sacrificed at various intervals following the injection and the expression of carnosine, calmodulin m-RNA (expressed both in immature and differentiated ON) and BrdU incorporation were analyzed by a novel technique combining in situ hybridization and immunohistochemistry.

In the BX side of the olfactory epithelium little or no expression of carnosine was seen. Conversely, ON expressing calmodulin m-RNA were abundant both in the control and in the BX side. At 1 day following BrdU injection ON that incorporated BrdU were much more abbundant in the BX side. Strikingly at 35 days following BrdU injection ON double labelled for both calmodulin m-RNA and BrdU incorporation were seen only in the normal side whereas no BrdU positive neurons were present in the BX side.

These data indicate that the OB is fundamental both for the survival and differentiation of ON. (Financial contribution from CNR, MURST).

224.1

ANALYSIS OF PRECEREBELLIN GENE EXPRESSION IN THE DEVELOPING MOUSE BRAIN. D.I. Lugo, Y. Urade*, J.D. Oberdick* and J.I. Morgan, Roche Institute of Molecular Biology , Roche Research Center, Nutley, NJ 07110.

The cerebellin precursor, precerebellin has been cloned from a human cDNA library. Previous immunocytochemical data had localized the hexadecapeptide primarily to Purkinje neurons of the cerebellum and to cartwheel neurons of the dorsal cochlear nucleus. In the present study we have utilized a ~1000 base cerebellin-specific riboprobe to study the spatial and temporal expression of cerebellin mRNA during mouse development by in-situ hybridization histochemistry. Surprisingly, within the cerebellum, cerebellin mRNA was confined to granule cells and neurons of the deep cerebellar nuclei and not to Purkinjie cells. Additional areas of cerebellin gene expression included various areas of the cerebral cortex, the thalamus , hypothalamus, parabigeminal nucleus, vestibular nuclei, dorsal and ventral cochlear nuclei and the reticular formation. Cerebellin transcripts can first be detected at postnatal day 2.5 in the external granular layer of the cerebellum and in thalamus. The relative levels continue to increase as the granule cells migrate to form the internal granular layer. In the adult, high levels remain in the cerebellum and the thalamus. Recently, a gene highly homologous to precerebellin (pseudocerebellin) has been cloned. In-situ hybridization experiments are underway to study its sites of expression and determine if the cerebellin peptide previously localized to Purkinjie cells, is the product of this homologous gene.

224.2

DIFFERENTIAL GENE EXPRESSION IN DEVELOPING AVIAN CEREBELLUM. P.L. Jeffrey, J.W. Sentry* and R.C. Henke*. Developmental Neurobiology, Children's Medical Research Foundation, P.O. Box 61, Camperdown NSW 2050, Australia.

To complement our immunological studies identifying and characterizing developmentally regulated neural antigens, we have constructed cDNA libraries from poly A^+ mRNA isolated from embryonic day 8 (ED8) and ED18 chick cerebellum in the plasmid vector pUEX1. (+/-) screening of these libraries with SScDNA from ED8 and ED18 cerebellum and liver poly A^+ mRNA have shown that 5.8% of the ED8 cerebellum cDNA clones are brain specific and developmentally expressed early in the cerebellum. Conversely 2.2% of the ED18 cerebellum cDNA clones are brain specific and expressed late in cerebellum. One late expressed brain specific clone 2352 bp in length hybridizes to a message of 2.4 kb. In cerebellum it is first detected at ED12 reaching maximum at ED20 and continuing through adulthood. In forebrain it appears later (ED16) peaks at postnatal day 7 and continues being expressed through to the adult. The translated sequence demonstrates 75% identity at the amino acid level to human, rat and mouse myelin basic protein, 18.5 Kd MBP (lacking exon 2). Other developmentally expressed clones are being characterized. By a combination of differential screening and screening the cDNA libraries with subtracted cRNA probes from directional libraries, we aim to isolate and characterize cerebellum specific, developmentally expressed genes.

224.3

A COMPARATIVE ANALYSIS OF THE POST-TRANSCRIPTIONAL REGULATION OF THREE CEREBELLAR MARKER mRNAS. L. Sangameswaran[1] ; Y. Urade[2]*; J. Oberdick[3]*; J.I. Morgan[3]. [1]Dept. of Pathology, Indiana Univ. Sch. of Med., Indianapolis, IN 46202-5120; [2]Towa City Corp.-Nishinotohin, Nishinotohin, Nakagyo-ku, Kyoto 604, Japan; [3]Dept. Neuroscience, Roche Inst. of Molec. Biol., Nutley, NJ 07110

Using rodent cerebellum as a model, we have isolated a set of markers, cerebellin, L7 and PEP-19, to address the question on the molecular and cellular mechanisms that play a role in the assembly of nervous system. All of them have been shown to be localised in the Purkinje neurons of the cerebellum. Previous studies indicated that cerebellin levels are diminished in the cerebella of the murine mutants, weaver and staggerer, suggesting that synaptic activity could regulate cerebellin biosynthesis. However, PEP-19 mRNA regulation seems to be under the influence of factors intrinsic to Purkinje neurons.

In the present study, a comparative analysis of the regulation of mRNA of the three markers has been attempted. Northern analyses were performed with tissues obtained from neurological mutant mice, with genetically determined cerebellar anomalies. Further, paradigms where either of the two inputs to the Purkinje neurons, climbing or parallel fibres, is impaired by chemical lesions were employed to investigate the levels of mRNA specific for each of the three markers. The results of these experiments with implications on the post-transcriptional regulation of these three polypeptides will be discussed.

224.4

GRANULE CELL ANTISERUM IDENTIFIES A BRAIN–SPECIFIC cDNA IN AN EXPRESSION LIBRARY DERIVED FROM NEONATAL HETEROZYGOUS WEAVER CEREBELLA. *M. Kambouris*, L. Sangameswaran, S.R. Dlouhy*, L.C. Triarhou, B. Ghetti & M.E. Hodes**. Dept. Med. Molec. Genetics, Dept. Pathol. (Neuropathol.) & Med. Neurobiol. Pgm, Indiana Univ., Indianapolis, IN 46202.

An antiserum was raised against a protein extract of cerebellar granule cells (GC). Western blot analyses showed that the antiserum cross–reacts with a number of different proteins with molecular weights ranging from 26 to 100 kDa. A 26 kDa protein detected by the antiserum is missing from *wv/wv* cerebella. Immunocytochemical analyses of normal cerebellum by light and electron microscopy showed positive immunoreactivity in the cytoplasmic compartment of GC and in neuronal processes of the GC layer neuropil, as well as in the molecular layer. The antiserum was used to screen a cDNA expression library made from postnatal day 0 *wv/+* cerebella. Twenty immunopositive clones were obtained. In order to determine which of these twenty clones is a GC marker, northern blot analyses were performed with *wv/wv* and +/+ cerebellar total RNA. At least one of the clones hybridized to RNA transcripts that appear to be missing in *wv/wv* but present in +/+ cerebellar RNA. This suggests that the insert may encode a GC marker. Another clone detects two RNA transcripts, indicating regulation by alternative splicing. Both transcripts are brain–specific. Further characterization of these clones, including sequence analysis, is in progress. (Supported in part by USPHS PO1–NS27613 and RO1–NS14426).

224.5

HB-GAM IMMUNOREACTIVITY IN THE DEVELOPING RAT CEREBELLUM K. Wewetzer*, H. Rauvala*, K. Unsicker Dept. Anatomy and Cell Biology, University of Marburg, Robert-Koch-Str. 6, 3550 Marburg, F.R.G. and Dept. Medical Chemistry, University of Helsinki, Finland

The heparin-binding growth associated molecule (HB-GAM) is a 18 kD protein which was recently purified from rat brain and promotes neurite outgrowth in vitro.
We have studied the distribution and function of HB-GAM in the postnatal cerebellum using a polyclonal antiserum raised against the native peptide. HB-GAM-immunoreactivity was found to be associated with cell processes rather than cell bodies. Through all stages examined, the molecular layer was labeled. Immunoreactivity was also associated with Bergmann glia-like processes located in the external granular layer. Immunoreactivity in the adult cerebellum was mainly restricted to the molecular layer. Together these results suggest that HB-GAM in the cerebellum might have a funtion in the context of cell migration and axon growth. In vivo studies aiming to perturb cell migration along radial glial cells, axon growth and fasciculation are under way.

224.6

DEVELOPMENTAL DISTRIBUTION OF MAP2 IMMUNOREACTIVITY IN THE CHICK EMBRYO SPINAL CORD. H.S. Keirstead, D.S. Henshel, D.M.S. Webster[1] and J.D. Steeves. Department of Zoology and [1]School of Rehabilitation Medicine, University of British Columbia, Vancouver, BC, V6T 1Z4.

Microtubule-associated protein 2 (MAP2) is a family of three cytoskeletal elements that have been localized to dendrites and certain somata in differentiated neurons. The relative abundance of these related forms changes during development, suggesting that MAP2 may be involved in the control of dendritic outgrowth and the stabilization and maintenance of dendrites. We have examined the developmental distribution of MAP2 immunoreactivity in chick embryo spinal cord from embryonic day 9 (E9) through to hatching (E21). At E9, MAP2 immunoreactivity appears in the neuropil at all levels of the spinal cord gray matter. Motor neuron cell bodies begin to be immunoreactive at E14, followed by interneuron cell bodies at later stages of development. The number of immunoreactive cell bodies at any given level of the spinal cord increases with development. By E21, virtually all motor neurons and many interneuron cell bodies are labelled. Furthermore, the proportion of cell body to neuropil labelling within the spinal cord increases in a rostral-caudal gradient. This developmental profile may reflect the stabilization of dendritic fields in the developing spinal cord.

Supported by the Medical Research Council of Canada and the Rick Hanson Man in Motion Legacy Fund.

224.7

AGE RELATED CHANGES IN PROTEIN SYNTHESIS IN THE MURINE LUMBAR SPINAL CORD. H.L. Stewart, S. Chaube*†, J.A. Birk*††, N.C. Mills*, M.H. Droge. Department of Biology, Texas Woman's University, Denton TX 76204. Department of Biological Sciences, University of North Texas, Denton TX 76203†, Department of Academic Computing, Texas Woman's University, Denton TX 76204††.

The objective of the current project was to characterize cell protein production in an effort to develop a valid assay for circuit viability as well as a biochemical assessment of development. To this end, samples consisting of200-300 µm thin transverse sections of lumbar spinal tissue were incubated in medium containing ^{35}S-methionine and examined for: 1) precipitable counts via scintillation counting and 2) protein patterns via electrophoresis using PAGE-gels containing SDS. Additional tissue samples were stained via acetylcholinesterase histochemistry for identification of cholinergic neurons. Peaks and valleys of protein production associated with specific ages were examined and increased synthesis of proteins with molecular weights was found. Antibody probes are being used to identify specific proteins that may contribute significantly to such peaks. Preliminary data suggest that changes in size and density of cholinergic neurons at the sampled intervals in culture are clearly reflected in total cell protein profile. Ultimately, we hope to correlate protein profiles with the morphological features and pattern generating capabilities of *in vitro* spinal circuitry.

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VISUAL SYSTEM: RETINA AND TRANSPLANTATION

225.1

EARLY DIFFERENTIATION OF NEURONS AND PHOTORECEPTORS IN THE EMBRYONIC ZEBRAFISH EPIPHYSIS. S.W. Wilson and S.S. Easter Jr. Dept of Biology, University of Michigan, Ann Arbor, MI 48109.

At 24h, the embryonic zebrafish brain contains a small number of discretely located neurons with axons that establish a simple scaffold of tracts. One group of the early projecting neurons is located in the developing epiphysis. The presence of this projection, long before the eyes assume visual function, suggests that the epiphysis may be involved in early light mediated behaviour. We are investigating the differentiation of cells within the epiphysis using immunocytochemistry and electron microscopy (EM). We find that by 22h, several cells are already immunoreactive for opsin on their medial surface; by 24h, these cells stain throughout; and by 48h, well developed outer segments are evident with EM. We have also screened monoclonal antibodies (FRets) raised against adult retina. By 24h one of these, FRet 43, labels a few cells that are coincident with the opsin containing cells in the medial region of the epiphysis. Retrogade labelling of the epiphysial projection neurons shows that they are laterally located. In addition, several of the epiphysial cells are GABA positive by 25h. We are currently double labelling embryos to resolve the exact relationship between the different cell types.

We thank R. Bremiller, K. Larison and R. Molday for antibodies. Supported by NIH EY00168 to SSE.

225.2

EFFECTS OF REDUCING MATURATIONAL DISPARITY ON SEGREGATION OF NORMAL AND ECTOPIC RETINAL INPUTS K.T. Yee and R.D. Lund Department of Neurobiology, Anatomy and Cell Science, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

A common feature in mammalian visual systems is the segregation of inputs from the two eyes. This can also be experimentally induced in the frog tectum following introduction of an ectopic eye. In contrast, segregation of normal and ectopic inputs does not occur when ectopic retinae are transplanted into neonatal rat hosts, even though specificity for normal optic target regions is exhibited.

We have examined whether this is due to maturational disparity of host and transplant inputs in two experiments: 1) pairs of stage-matched retinae (embryonic day 12 (E12) mouse and E13 rat retina) were co-transplanted into postnatal day 1 rat hosts and 2) E12 mouse retinae were transplanted into fetal hosts. Animals survived to 1 month of age. Transplant connectivity was revealed with antibodies specific for mouse tissue. In some hosts with double transplants, the projection from the transplanted rat retina was also labeled by tracer application to the transplant. In rats that received grafts as fetuses, the normal host projection was similarly labeled.

Our results show that projections from double retinal transplants overlap in the superior colliculus, and that at least the mouse retina innervates regions which do not receive input from single grafts. Transplant axons in fetal hosts integrate extensively with host optic axons, coursing through the superior colliculus in the stratum opticum where normal optic axons travel. In both experiments, projection patterns differ from those shown by single transplants placed into neonatal hosts. Therefore, reduction of maturational disparity alters the pattern of target innervation compared to postnatal hosts, but not does not produce segregation of optic inputs.

NIH HD 07343 and EY 05308

225.3

FETAL EYE AND HOST SUPERIOR COLLICULUS CONNECTED BY A PERIPHERAL NERVE BRIDGE: COMPARISONS OF FETAL AGE AND DONOR SOURCE. B.H. Hallas, L. Guarino*, M.R. Wells, M. LaCorte*, R. Cebelenski, B. Kim* and M.F. Zanakis. NY College of Osteopathic Medicine, NY and Veterans Administration, Northport, NY

Previous studies have shown that when fetal eye/peripheral nerve bridge complexes are used to "reconstruct" the damaged visual system, fetal retinal ganglion cells grow through the bridge and well into the host. The pattern of synapse formation is organized and quite extensive. The present studies expand on this technique to investigate the role of various fetal ages as well as the differences between homograft and heterograft nerve and eye donor animals. Sprague-Dawley or Wistar albino rats were used as donors and/or recipients (thereby establishing homografts or xenografts for the nerves). The immature eyes were also either from one or the other species. In all experiments, the eyes were removed from fetal or prenatal (3 day) rats and attached to a 3 cm segment of tibial nerve by use of gelled cyanoacrylate. The eye with attached nerve was inserted into the anterior eye chamber of an adult rat host (with optic nerve cut) while the free nerve end was inserted through a burr hole in the cranium into the contralateral superior colliculus. One month to one year post-implantation of the eye/peripheral nerve complex, either HRP or WGA/HRP was injected into the host eye. Twenty-four hours post injection, the eye/bridge complex and host brain was processed for HRP histochemistry. Bulk labelled axons and HRP filled cells were demonstrable in the contralateral superior colliculus in all animals. Homograft bridges with homograft eyes demonstrated the the best labelling (65%) while 60% of the heterograft bridges and 55% of heterograft eyes showed labelled axons and neurons. While PN3 eyes provided good results, E18 eyes generally demonstrated more labelling and pattern formation.

225.4

ALERTING AND ORIENTING RESPONSES MEDIATED BY INTRACEREBRAL RETINAL TRANSPLANTS. P.J. Coffey* and R.D. Lund. Department of Psychology, University of Sheffield, England and Department of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, Pittsburgh, PA.

Normal retinae project to a number of brain regions, each subserving a specific visual response. Retinae transplanted over the midbrain of neonatal rats connect with many of the same regions and these mediate pupilloconstriction, conditioned suppression and photophobic responses. The heaviest transplant input is to the superior colliculus, a region normally involved in alerting and orienting responses. In this series of experiments we examined the alerting and searching patterns in rats when a light was presented to their normal eye or retinal implant. When a visual stimulus is presented in a dark chamber, a rat typically inhibits its ongoing behavior and orientates toward the light source. Whereas presentation of the light in normal rats resulted in an inhibition of ongoing behavior often followed by a specific orientation towards the light, visual stimulation of the transplant resulted in the inhibition of ongoing behavior with undirected orientation behavior. These results suggest that the alerting response, thought to be mediated through the colliculus, can be driven by implant illumination. The orientating response may be confounded by the lack of topographic organization in the transplant-host projection.

This work was supported by grants from M.R.C. (U.K.), Royal Society, N.A.T.O. and N.I.H. (EY05283).

225.5

MORPHOLOGY OF THE TERMINAL ARBORS OF RETINAL TRANSPLANT-DERIVED PROJECTIONS IN EYELESS MUTANT AND NORMAL MICE. G.M. Horsburgh, R.D. Lund and M.H. Hankin, Dept of Neurobiology, Anatomy, and Cell Science, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We have previously shown that fetal retinae transplanted to the midbrain of neonatal rodents are capable of projecting to appropriate visual nuclei in the host brain where they form synapses and can mediate light-driven responses. In order to further understand the anatomical substrates for these specific projections and functional responses, the morphology of the retinal transplant-derived terminal arbors was examined using the anterograde neuronal tracers Biocytin and DiI.

In the present study we address two primary issues. First, how does the morphological development of retinal transplant-derived terminals in normal recipient mice compare with that of normal retinal terminals? Second, eyeless mutant mice (or^J) - in which the visual nuclei never receive retinal input - were used as recipients to determine whether the absence of prior optic innervation affects the development of transplant-derived terminal arbors.

Following large deposits of tracer in the transplant, the overall pattern of arborization within the visual nuclei appeared similar in normal and mutant hosts. However, transplant-derived projections tended not to occupy the full extent of the normal visual terminal fields. Following small deposits of tracer, the arbors of individual transplant-derived axons could be examined. The ramification of these arbors appeared similar in complexity to normal retinal terminals, and were comparable in genetically normal and mutant hosts.

These findings indicate that projections of transplanted retinae form morphologically normal terminal arbors despite the the ectopic locations of the transplants and the delayed arrival of their projections in the targets.

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225.6

LOCAL MYOPIA PRODUCED BY PARTIAL VISUAL-FIELD DEPRIVATION IN TREE SHREW. Thomas T. Norton and John T. Siegwart, Jr.* Department of Physiological Optics, School of Optometry/The Medical Center, Univ. of Alabama at Birmingham, AL 35294.

In many mammalian and avian species, deprivation of form vision during postnatal development produces axial elongation of the eye and a resultant myopia. This is mediated by direct retino-choroidal/scleral mechanisms; it occurs in tree shrew despite blockade of ganglion cell action potentials by TTX (Norton et al., Invest. Ophthalmol. Vis. Sci. 30: 32, 1989). To examine the local specificity of these mechanisms, we deprived tree shrews of form vision in either the nasal (N = 5) or temporal (N = 5) hemifield of one eye with a translucent half goggle. After 3 to 4 weeks the deprived region of the eye, measured 20°-30° off-axis, was significantly myopic (-8.7 ± 2.9 D [mean ± S.D.]) (streak retinoscopy) and elongated (0.14 ± 0.06 mm) (A-scan ultrasonography) compared to the control eye. In the non-deprived region, there was no significant refractive difference (-1.6 ± 3.2 D) or elongation (0.02 ± 0.06 mm). These results extend to mammals the previous reports of local myopia in avians (e.g. Wallman et al., Sci. 237: 73, 1987). Such a within-eye change implies that the neurobiological mechanisms which normally coordinate the axial length of the eye with its focal length are extremely local in their ability both to detect the absence of a clear retinal image and to rapidly alter ocular development. Supported by EY-05922, EY-03039, RR-05932.

225.7

EFFECT OF LESIONS OF THE OLIVARY PRETECTAL NUCLEUS ON THE DIRECT AND CONSENSUAL PUPILLARY REFLEXES M.J. Young, H. Klassen and R.D. Lund, Dept. Neurobiology, Anatomy & Cell Science, Univ. Pittsburgh Sch. Medicine, Pittsburgh, PA 15261.

It is well established that the olivary pretectal nucleus is central to the pupillary constriction response in rats. In order to determine the anatomical substrate of the consensual pupillary reflex in Long-Evans (L-E) rats, unilateral heat lesions of the olivary pretectal nucleus were performed, and the effect of these lesions on both the direct and consensual pupillary reflexes was determined.

Rats (aged 6 weeks) received unilateral heat lesions of the olivary pretectal nucleus. They were allowed to recover for 7-14 days. The degree of direct and consensual pupilloconstriction in response to a range of illumination intensities was then determined. After several trials, the animals received unilateral intravitreal injections of 30% HRP to verify the extent of the lesion. The rats were then perfused, and processed for HRP-TMB and Nissl stains.

It is apparent that unilateral lesions significantly increase the threshold of stimulus required to elicit both direct and consensual pupilloconstriction compared to controls. However, even complete unilateral lesions of the olivary pretectal nucleus fail to completely abolish either the direct or consensual pupillary reflex. This suggests that one component of the consensual reflex depends on bilateral innervation of the olivary pretectal nucleus, but not solely on this pathway.

Supported by NIH grant EY 05283.

225.8

CHICK EMBRYO RETINA CELLS METABOLIZE AND RESPOND DEVELOPMENTALLY TO RETINOIDS IN VITRO. **D. L. Stenkamp*, J. K. Gregory*, and R. Adler. Depts. Ophthalmology and Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.**

In addition to visual transduction, vitamin A appears to affect retinal development and differentiation through still poorly understood mechanisms. We are investigating these effects in RPE/glia-free cell cultures in which retinal precursor cells differentiate as either neurons or photoreceptors, the latter being opsin-immunoreactive and showing photomechanical responses to light. Our studies suggest that retinoids do not have a mitogenic effect, but do affect retinal cell survival and differentiation. While increases in neuronal numbers were small, retinoids (retinol >11-cis retinal >retinoic acid) caused marked increases in number of photoreceptors and also potentiated their photomechanical responses. HPLC analysis showed that cultures hydrolyze retinyl acetate and reduce exogenous retinal to retinol. Oxidation of retinol or retinal to retinoic acid was not detected. In vitro differentiation of retinal neurons and photoreceptors therefore includes a capacity for developmental responses to vitamin A, and expression of metabolic activities which may be important for these responses and for visual transduction. Supp. USPHS 04859; NSF Graduate Fellowship.

225.9

DEVELOPMENTAL REGULATION OF THE L7 GENE IN THE MOUSE CEREBELLUM AND RETINA. A.S. Berrebi, J. Oberdick*, R.J. Smeyne*, J.I. Morgan* and E. Mugnaini. Neuromorphology Lab, U-154, Univ of Conn., Storrs, CT. 06269 and *Dept. of Neurosci., Roche Inst. Mol. Biol., Nutley, NJ 07110.

A central issue in contemporary developmental neurobiology is the elucidation of the mechanisms that orchestrate the assembly of the nervous system. One approach to this problem is to study the transcriptional regulation of specific, informative, genes at the cellular and molecular levels. Previous studies have been aimed at identifying genes that show restricted expression in the CNS, particularly in Purkinje neurons of the cerebellar cortex.

The L7 gene is expressed only in cerebellar Purkinje cells and rod bipolar cells of the neural retina (Oberdick et al., 1989, 1990; Berrebi et al., 1991). In the present studies we examined the developmental pattern of L7 expression to determine whether the gene is coordinately regulated in these two structures.

In the cerebellum, Purkinje cell immunoreactivity (-IR) is first detected in late embryogenesis. At E17, two discrete parasagittal bands are present on each side of the midline. By P0, L7-IR is most evident in 3 bilateral clusters or bands of Purkinje cells. The number of sharp bilateral bands increases from 3 to 5 by P4. On P9, all Purkinje cells display strong L7-like -IR.

In retina, all rod bipolar neurons are completely immunonegative prior to P7. At P7, very faint staining of rod bipolar cell bodies is evident. However, by P8 all rod bipolars are densely immunostained, with no evidence of banding. At this stage, their fine radial processes, which traverse the inner plexiform layer (IPL), are also faintly labeled, as are their endings in the deepest regions of the IPL. By P9, intense immunolabeling of the radial processes and endings of rod bipolar cells is observed. The developmental sequence of L7 expression in the retina is essentially unchanged when animals are born and reared in complete darkness.

The disparity in both the time of onset and spatial progression of immunoreactivity in the cerebellum and retina suggests that induction of the L7 gene is controlled by different events in these two structures.

225.10

DEVELOPMENTAL EXPRESSION OF GLUTAMIC ACID DECARBOXYLASE (GAD 65) mRNA IN THE MOUSE RETINA. Vijay Sarthy. Dept. of Ophthalmology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Immunocytochemical studies show that GAD is present in horizontal cells in the developing rodent retina, although there is no evidence that these cells use GABA as a synaptic transmitter. We have used in situ hybridization to examine whether GAD 65 mRNA is expressed by horizontal cells during retinal development. In the adult retina, labeled cells were found in the inner nuclear layer (INL) and the ganglion cell layer (GCL). In the INL, all labeled cells were located close to the boundary of the INL and the inner plexiform layer (IPL) suggesting these are labeled amacrines. In the developing retina, lightly labeled cells were first detected at embryonic day 15 (E-15). Beginning at E-20, strong labeling was observed in the INL and GCL. The labeled cells in the INL formed a band at the interface of INL and IPL. In the GCL, the majority of labeled cells were present at the IPL/GCL boundary. At the postnatal stages examined (P1-P14), labeled cells were found at the INL/IPL and IPL/CGL boundary. In the GCL, there was a decrease in the number of labeled cells between P-1 and P-10. The intensity of cell labeling reached adult-like by P-7. In summary, we did not find GAD 65 mRNA-containing horizontal cells in either adult or developing mouse retina. Supported by EY-03664.

225.11

TAURINE IN DEVELOPING RABBIT RETINA. G. Battista* and N. Lake. Depts. Physiology & Ophthalmology, McGill University, Montreal, Canada H3G 1Y6.

The retina of a newborn rabbit is not fully mature in terms of morphology, chemical composition or capabilities. It has been suggested by Redburn et al [IOVS 31:1620A,1990] that the release of GABA from horizontal cells (HC) plays a trophic role in postnatal (PN) organization of cone terminals. GABA then disappears from HC after PN day 5, and their inhibitory transmitter remains unknown. We have observed that HC in the adult rabbit contain high levels of taurine, another amino acid with potent neuroinhibitory effects. The present study examined PN changes in retinal taurine levels and localized taurine immunoreactivity (T-IR). Taurine is present at birth (1.13 μmol/mg DNA) and increases four-fold by maturity. At PN1 to PN3 T-IR is in radial ventriculocytes /glia whose processes span the retina. Neurons also show T-IR, but apparently as an accompaniment to differentiation /maturation, since T-IR is not seen in neuroblasts, or relatively undifferentiated neurons, but tracks the maturation gradients: posterior pole to periphery, inner to outer retinal layers. Adult T-IR is in receptors, HC, amacrines, ganglion cells and the inner plexiform layer. From the earliest day examined, prominent T-IR is seen in HC and their lateral processes. These cells develop precociously compared to their neighbouring neuroblasts. In contrast to GABA-IR, T-IR persists in HC through to adulthood. Taurine may have roles related to development, and in adults, it may be a HC or amacrine inhibitory transmitter.

Supported by MRC Canada

225.12

HORIZONTAL CELLS IN THE MARSUPIAL RETINA A.M. Harman and L.D. Beazley. Dept. of Psychology, University of Western Australia, Nedlands, W.A. 6009, Australia.

We have previously shown that cells in the marsupial retina are generated in two separate phases (Harman and Beazley, Neuroscience 28: 219-232, 1989). Here we classify horizontal cells in the marsupial retina and determine in which phase they are generated. Adult retinae were examined from two species, the quokka wallaby and the brush-tailed possum. Many small crystals of HRP (Sigma) were pressed into the retinal surface and, following incubation in DMEM culture medium, retinae were processed with cobalt enhanced diaminobenzidine. Two types of horizontal cell are seen in the marsupial retina, one with and one without an axon. The cell type with an axon has many fine dendrites with an average dendritic length of about 100 μm and an axonal arborisation. Dendrites in the axonless cells are 300-400μm in length and each cell has 3-5 dendrites in the quokka and 8-10 in the possum. Immunohistochemical processing of other retinae revealed that calbindin immunoreactivity is restricted to the horizontal cells with axons. To establish birth dates of these two types of horizontal cell, animals were injected with tritiated thymidine during either the first or second phase of retinal cell generation. Animals were grown to maturity, retinae were processed as above to reveal horizontal cells and then sectioned horizontally in plastic (Historesin, Reichert-Jung) at 3μm and processed for autoradiography. Both types of horizontal cell were found to be produced in the first phase of retinal cell generation.

225.13

POSTNATAL DEVELOPMENT OF TYPE A HORIZONTAL CELL TOPOGRAPHY IN THE CAT RETINA. J.S. Tootle and J.W. Bledsoe, III*. Neurobiology Research Center and Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294.

The topography of type A horizontal cells (HCs) in the retina of the adult cat has been described (Wässle et al., Proc. R. Soc. Lond.B.,203:269,1978) but the ontogenetic events which give rise to the precise tiling observed at maturity have not been reported. In the present study we used a commercial (Sigma) monoclonal antibody to the 28 kD calcium binding protein calbindin to stain all the type A HCs in retinas taken from kittens at postnatal day 5 (P5), P7, P10, P27, P36 and in 2 retinas from adult cats. These data were supplemented by additional retinas taken at ages ranging from P0 to P33 for which significant fractions of the type A HCs were labeled. The general topographic features of this cell type in the adult retina were present at all ages studied and included: a peak density in the area centralis; a decline in density toward the periphery which was most rapid along the vertical meridian and slowest in nasal retina along the horizontal meridian. The densities of type A HCs near the area centralis did not differ markedly between ages but more peripherally there was a monotonic decrease in cell density with age. Nearest neighbor analysis showed that the regularity of the type A HC matrix was already adult-like at birth when the X/SD of nearest neighbor distances measured in the periphery ranged from 3.8-5.3. These results show that the mechanisms which generate the tiling of the retina by the type A HCs do so prenatally and that the general topography present at birth is preserved as cell densities are reduced during postnatal growth.

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225.14

CHOLINE ACETYLTRANSFERASE (ChAT) IMMUNOREACTIVITY IN THE EMBRYONIC OPOSSUM RETINA. [1]L. Camargo* & [2]J.N. Hokoç. [1]Dept. Histologia e Embriologia & [2]Instituto de Biofísica CCFº. UFRJ. BRAZIL.

Immunocytochemical localization of ChAT in the opossum retina revealed, as in other mammals, two major populations of cholinergic neurons. One with cell bodies in the inner nuclear layer and dendrites arborizing in sublamina 2, another with the soma in the ganglion cell layer and dendrites reaching sublamina 4 of the inner plexiforme layer (IPL). In this report we follow the time course of differentiation of these cells and compare this information with other events of development. Retinal sections from opossums 12-67 postnatal days were incubated in anti-ChAT serum (Hersh) and the reaction was visualized by the PAP method. The first ChAT(+) cell bodies were seen at P31 and immunoreactive processes and strata at P40. At P50 the ChAT (+) pattern is very similar to the adult. The onset of ChAT expression was the same at the inner nuclear and ganglion cell layers. In the north american opossum retina, the development of IPL is completed at P17 and the formation of conventional synapses at P30. Thus, in this animal ChAT phenotype is expressed after the establishment of synaptic contacts in the IPL.

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225.15

MORPHOLOGICAL CHARACTERIZATION OF TYROSINE HYDROXYLASE (TH) IMMUNOREACTIVE NEURONS IN THE DEVELOPING RABBIT RETINA. Giovanni Casini and Nicholas C. Brecha, Depts. of Anatomy & Cell Biology and Medicine, UCLA and VAMC-West Los Angeles, Los Angeles, CA.

TH cells represent a class of wide-field amacrine cells thought to exert modulatory functions in the processing of visual information. In the present study, retinas at different postnatal ages were processed as whole mounts for TH immunohistochemistry. In newborn retinas (**PD 0**), TH cells display very weak immunoreactivity. In most cases, one thick primary process, often with second-order ramifications, is present. Varicosities are not observed. At **PD 6**, variable characteristics are found, including third-order ramifications and a few varicosities, with no evident correlation between degree of maturation of TH neurons and their position in the retina. At **PD 12** (eye opening), TH cells have 2 to 5 primary processes, a complicated network of TH fibers begins to form in lamina 1 of the inner plexiform layer (IPL), and processes with small varicosities can be seen in lamina 3. Many growth cones are present. From **PD 12** to **PD 26**, the network of TH fibers attains a high degree of complexity (with varicosities arranged in ring-like structures), processes enter lamina 5 of the IPL and growth cones disappear. After **PD 26**, TH neurons are similar in appearance to those in the adult. These observations indicate that the transmitter phenotype of dopaminergic neurons is established before birth. Their maturation is well advanced at the time of eye opening, but mature characteristics are not seen until after the first month of age.

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225.16

POSTNATAL DENDRITIC MODIFICATION IN AN IDENTIFIED POPULATION OF HAMSTER RETINAL GANGLION CELLS. R.J.T.Wingate* and I.D.Thompson* (SPON: Brain Research Association).University Lab. of Physiology, Oxford University, Parks Road, Oxford OX1 3PT, U.K.

We have used a combination of fluorescent retrograde tracers and *in vitro* intracellular techniques to follow the dendritic development of retinal ganglion cells with an aberrant, uncrossed projection from nasal retina. These cells are characterised by a preferential postnatal elimination which leaves a small adult population of mainly sparse, type III dendritic morphologies. By making an injection of rhodamine latex microspheres into the ipsilateral optic pathway, cell bodies in this aberrant population can be labelled. After an appropriate survival time, retinae were lightly fixed, removed and placed under a compound microscope with a fixed stage and UV epifluorescence. Labelled cell bodies were selectively impaled with a glass micropipette and injected with Lucifer Yellow by iontophoresis to reveal their complete dendritic arbors. At postnatal day 1 (P1), arbors are uniformly small and sparse. Rapid dendritic growth and elaboration occur throughout the period of cell death so that by the end of this period (P13), diverse and complex cell morphologies are present. The adult morphology that characterises this population arises by a dramatic dendritic pruning coincident with eye opening (P16). This loss of complexity appears greater than the simple retraction of spines and transients that accompanies normal retinal ganglion cell maturation. The effect of an activity blockade on this regressive event was examined by the intraocular application of tetrodotoxin.

Supported by the Medical Research Council, U.K.

225.17

Developmental interactions between α and β cell dentritic arborizations in the cat retina. S Deplano*, G.M. Ratto* and S. Bisti, Lab. of Neurophysiol. CNR, 56100 Pisa, Italy and Inst.of Comparative Anatomy, University of Genova, 16126 Genova, Italy.

In the adult retina of the cat the dendritic fields of a well-defined physiological class of ganglion cells form a mosaic-like structure with little overlap between cell territories. It has been suggested that this pattern results from complex shaping interactions taking place during development between cell dendrites of the same class. No interactions seem to occur between cells of different classes. The aim of the present study was to test the possibility that also dendrites of different cell classes interact. We have analyzed the effect of a small lesion to the retina of a two days old kitten and observed that after degeneration of ganglion cells whose axons were severed, a restricted region of the retina remained depleted of cells. Cells located near the borders of the depleted zone showed an abnormal elongation of dendrites projecting towards the bare area. By means of a computer aided system we analyzed the whole population of cells at the two borders and, in agreement with previous data, we found that the effect was most prominent at the border and progressively decreased to eventually disappear while moving away to a distance of approximately 500 μm. The distance from the border, however, is not the only factor to influence the degree of asymmetry: with comparable distances, the vicinity of an α cell reduces the projection of the β cell dendrites toward the empty area. We suggest that the organization of the adult retinal pattern may be influenced also by interactions occurring between dendrites of different classes of ganglion cells.

Supported by ECC grant n° SC 1000224 and Strategic project CNR

225.18

AGE-RELATED CHANGES IN SPATIAL DENSITY OF RETINAL CELLS IN ALBINO RATS. W. Krebs and I. Krebs* . Dept. of Cell Biology and Anatomical Sciences, The City University New York Medical School, New York, NY 10031.

The spatial densities of retinal neurons have been determined in electron micrographs of tangential serial sections through the retina of laboratory albino rats 4 to 28 weeks of age. Retinal samples were taken 500 μm from the rim of the optic disk (see: Krebs, Krebs and Worgul: Rad Res 123:213,1990).

Between weeks 6 and 12, the cell density of the pigment epithelium dropped from 2,000 to 1,500 cells/mm^2 ; of photoreceptor cells, from 500,000 to 400,000 cells/mm^2; of horizontal cells, from 800 to 600 cells/mm^2; and of bipolar cells, from 70,000 to 60,000 cells/mm^2. The densities of amacrine cells (20,000/mm^2), ganglion cells (9,000/mm^2), and radial glial (Müller) cells (10,000/mm^2) did not change in the age range examined. The data imply that the retina of the laboratory rat may not be mature before the age of 6 weeks.

225.19

CELL GENERATION, DEATH, AND RETINAL GROWTH DURING THE PROTRACTED DEVELOPMENT OF THE CALIFORNIA MOUSE. A.A. Dos Santos and D.R. Sengelaub. Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

The California mouse (Peromyscus californicus) and the Syrian hamster (Mesocricetus auratus) are closely related cricetid rodents who differ dramatically in developmental duration and rate. Like all developmental features, retinal development in the California mouse is protracted, but the events and their consequences are strikingly similar to those seen in the hamster, resulting in virtually identical retinal morphologies. We are studying the protracted development of the California mouse to determine how generational and post-generational events interact in the development of retinal morphology.

Mice were pulse-labeled with tritiated thymidine on one of days 18 through 28 postconception (PC; day of birth=PC33), the period of retinal ganglion cell layer (RGCL) generation. Pups were killed prenatally (PC30), postnatally (PC36,41,46), or as adults (PC92) to assess the effects of cell generation, death and retinal growth. As in hamster, RGCL generation proceeded with both center-to-periphery and temporal-to-nasal progressions. Initial (PC30) distributions of labeled cohorts differed from their adult distributions, indicating that post-generational events sculpt individual cohorts. As in hamster, the distribution of labeled cells changed differently for each cohort during the period of cell loss. After cell death, the distributions of labeled cells changed similarly, suggesting an alteration by a mechanism common to all cohorts such as retinal growth. While similar to hamster, the protracted development of the California mouse allows a greater resolution of the temporal and spatial patterns of cell generation, death, and retinal growth that sculpt its hamster-like retinal morphology.

225.20

RETINAL TARGET GENESIS DURING THE PROTRACTED DEVELOPMENT OF THE CALIFORNIA MOUSE. D.A. Schaefer* and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

The California mouse (Peromyscus californicus) and the Syrian hamster (Mesocricetus auratus) are closely related cricetid rodents who differ dramatically in developmental duration and rate. Like all developmental features, retinal development in the California mouse is protracted, but the events and their consequences are strikingly similar to those seen in the hamster, resulting in virtually identical retinal morphologies. We are examining the neurogenesis of thalamic and midbrain retinal targets in the California mouse for similar evidence of protraction with a preservation of developmental patterns.

Mice were pulse-labeled with tritiated thymidine on one of days 9 through 30 postconception (PC; day of birth=PC33). Labeled cells were counted at adulthood (PC90) in the superior colliculus and the dorsal and ventral lateral geniculate. As in hamster, neurogenesis of the California mouse retinal targets commenced coincident with, or just prior to, retinogenesis (PC16-17). Cell generation extended over a significantly longer period than in hamster (11-12 days), with neurogenesis peaking at PC20-21 and declining rapidly thereafter. In all structures, large neurons were generated earliest and smaller, glial-like cells became more abundant after PC24. Generation began and ended earlier in the superficial gray layer of the colliculus than in the deeper laminae. Evidence of a lateral-to-medial pattern in cell generation was observed in the dorsal and ventral geniculate, consistent with the spatial pattern reported in hamster (Crossland and Uchwat,'82). Thus, as in retina, development of the retinal targets in the California mouse is protracted relative to the hamster, but the temporal and spatial patterns of neurogenesis are similar.

225.21

RADIALLY ASYMMETRIC GROWTH OF FISH RETINAS WITH SPECIALIZED AREAS. S. S. Easter, Jr. Dept. Biology, U. Michigan, Ann Arbor, MI 48109; and Vision, Touch, and Hearing Res. Centre, U. Qld., Brisbane, Australia.

The goldfish retina adds new ganglion cells at its margin, with the result that generations of cells occupy concentric retinal annuli, old centrally, young peripherally. Young and old retinas all subtend an optical field of about 180 degrees, so a cell's receptive field is initially peripheral and later moves centrally. The goldfish retina is homogeneous, but other fish have a small temporal area, with high cell density, for high acuity vision. How do they keep a specialized area in temporal retina while growing all around the retinal margin?

I have exploited the fact that each new generation of ganglion cells sends its axons together into the optic nerve. Retinas with attached optic nerves were obtained from sand perch, leather-jackets, and dragonets, which have a specialized area, and goldfish, gobies, and humbugs, which do not.They were fixed in paraformaldehyde. DiI was applied to 1-5 locations in the cross section of the nerve; it labeled generations of optic fibers and, via retrograde diffusion, their somata of origin, which were viewed in whole-mounts.

Labeled annuli in those retinas without specialized areas were roughly concentric around a nearly central optic papilla, consistent with radially symmetric growth. In those retinas with specialized areas, the labeled annuli were very asymmetric. The oldest annuli, labeled through fibers radiating from the central end of the elongate optic papilla, lay in temporal retina. The specialized area was within this early annulus, indicating that it was formed from the oldest retina. More peripheral annuli were skewed oppositely; they nested very close to the old annuli in peripheral temporal retina (indicating little late retinal growth temporally), but ballooned out widely in dorsal, ventral, and especially nasal retina (indicating substantial late retinal growth in these sectors).

In summary, radially asymmetric retinal growth maintains the specialized area in roughly the same part of the retina throughout life.

(Supported by EY-00168 to SSE and ARC support to J. D. Pettigrew.)

225.22

QUANTITATIVE ANALYSIS OF LOCAL REGENERATION IN THE RETINA OF THE GOLDFISH. **K. J. Lindsey and P. F. Hitchcock. Ophthalmology; Anatomy and Cell Biology; The Neuroscience Program, University of Michigan, Ann Arbor, MI 48105**

Local retinal damage in the goldfish is repaired by local regeneration (Hitchcock, P.F., [1990], Neuroscience abstracts). To quantitate this regeneration and gain insights into its control, we, 1) compared the planimetric density of cells (#/mm^2) in regenerated retina to adjacent, intact retina, and 2) taking advantage of the large changes in cell density with retinal growth (from high to low), compared the density of regenerated cells in a subset of animals: 5 small fish and 2 large ones. The data were expressed as the ratio of the planimetric densities.

Patches of retina, 0.5-1.5mm on a side, were excised using a trans-scleral surgical approach. Animals survived 5 months. Cells were counted in 5μm sections.

The regenerate:intact ratio (n=14) was 0.85. The small:large ratio for the intact retinas (excluding rods whose density doesn't change with growth; Johns, [1977] J. Comp. Neurol., 176:331) was 2.1. The small:large ratio for the regenerated patches was 1.4. The smaller small:large ratio for the regenerated patches is due to a relatively higher cell density within the large retinas. The data indicate that within each eye the density of the regenerated cells tends to approximate that of the surrounding, intact retina. EY07060; EY07003; T32 MH14279.

225.23

SYNAPTIC CONTACTS INCREASE AS MATURE DENDRITES GROW IN THE RETINA OF THE GOLDFISH. Peter F. Hitchcock, Departments of Ophthalmology & Anatomy and Cell Biology, The University of Michigan, Ann Arbor, MI 48105

As the goldfish retina expands, the dendritic arbors of 'mature,' large-field ganglion cells grow by a simple increase in the length and girth of individual dendritic segments, without a change in the dendritic architecture (Bloomfield and Hitchcock, [1991] J. Neurosci., 11: 910-917). These arbors are in a neuropil that continually adds new synapses, but maintains a constant afferent neuron:ganglion cell ratio. I sought answers to two questions: 1) are synapses added to arbors as they enlarge, and 2) is the addition of new synapses related to the changes in the arbor geometry?

Ganglion cells from large, old (n=2) and small, young fish (n=3) were intracellularly labeled with HRP and serially sectioned for electron microscopy. Synapses were tallied from a 1-in-10 series of sections for each arbor.

On average the large arbors were 1.7X longer, had 2.5X more surface area, and were 3.9X greater in volume than the small arbors. The larger arbors were contacted by 2.7X more bipolar synapses and 1.7X more amacrine-cell synapses than the small ones. This indicates that these arbors receive more synapses from each presynaptic neuron as they grow, and the addition of bipolar and amacrine-cell synapses may reflect different aspects of the arbor's changing geometry. Supported by EY07060; EY07003 (CORE).

225.24

EXPRESSION OF HOMEOBOX mRNAs IN ADULT GOLDFISH RETINA. E. Levine*, R. Druger* and N. Schechter. Departments of Biochemistry and Psychiatry, SUNY at Stony Brook, NY 11794.

Unlike visual systems of higher vertebrates, the goldfish visual pathway displays continuous growth and differentiation throughout life. Homeobox gene products have been implicated as potential regulators of embryogenesis and central nervous system development. We have begun studies to determine if homeobox gene products are involved with the regulation of gene expression in the goldfish retina. The PCR was used to obtain portions of the homeobox from cDNAs derived from adult retina. Clones were isolated and sequenced and their predicted amino acid sequences showed greater than 90% homology to four previously cloned homeobox genes. The pattern of expression of these clones in various tissues is being investigated by northern hybridization. Another clone had 60% amino acid homology to an invertebrate homeobox and less than 20% homology to any vertebrate homeobox. We suggest that this clone represents a novel vertebrate homeobox. Preliminary northern data shows this clone to be expressed in a tissue specific manner. Since the goldfish displays a remarkable capacity for optic nerve regeneration after injury, we are investigating the role of homeobox gene expression during regeneration. (Supported by NIH grant EY05212 to NS)

REGENERATION: MOLECULAR CORRELATES

226.1

TRANSIENT C-FOS EXPRESSION AND DENDRITIC SPINE PLASTICITY IN PARTIALLY DEAFFERENTED HIPPOCAMPAL GRANULE CELLS. D.E. Hillman and S. Chen Dept. Physiol. & Biophys. NYU Med. Ctr., New York, NY 10016.

A marked plasticity of dendritic spines and synaptic sites in the inner molecular layer occurs following partial deafferentation of dentate granule cells. (Soc Neurosci Abst.(1988) 14:1133). Within two hours of unilateral lesion to the entorhinal cortex or fornix of rats, the immediate-early gene, c-fos, was activated ipsilaterally in granule cell nuclei of the dentate gyrus. Beta adrenergic blocker, propranolol, completely negated the c-fos response indicating expression of this c-fos gene through norepinephrine. By 24 hours of deafferentation, the dendritic endoplasmic reticulum (normally extending into the spine neck as a single tube) elaborated into complex folds. The complex spine apparatus was associated with a widened spine neck and frequently cradled a multivesicular body with a coated patch on the surface As previously described, ribosomes lined the ER and were also free in the spine head and dendritic shaft (Steward, O & Reeves, TM, J. Neurosci 8:176-84). By 3 days, the undeafferented dendritic spines began to extend around boutons and the synaptic sites enlarged, became perforated and had irregular boundaries. We conclude that immediate-early genes, such as c-fos, are important links in signaling late-response genes for generating building blocks needed in plasticity and reorganization of remaining synaptic connections. Supported by USPHS NS-13742.

226.2

JUN, FOS, MYOD1, AND MYOGENIN PROTEINS ARE INCREASED IN SKELETAL MUSCLE FIBER NUCLEI AFTER DENERVATION. J. Weis, Inst. of Pathology, Aachen Univ., W-5100 Aachen, Germany

After denervation of skeletal muscle, mRNA levels of the c-jun and c-fos protooncogenes are increased (Bessereau et al., New Biol. 2, 375, 1990). The increase of c-fos was interpreted as part of a muscle fiber "dedifferentiation" process following denervation. This hypothesis was supported by the finding that fos represses myogenic differentiation of cultured cells induced by the myogenic factor MyoD (Lassar et al., Cell 58, 659, 1989).

We used immunohistochemistry to assess the expression of c-jun and c-fos as well as MyoD1 and the homologous factor myogenin in normal and denervated muscle. Rat diaphragms were denervated and analyzed after periods of 90 min to 8 d. An increase of fos, jun, MyoD1, and myogenin immunoreactivity was found after 2 - 2.5 d of denervation. MyoD1 and myogenin immunoreactivity was mostly confined to muscle fiber nuclei, whereas fos and jun antisera stained muscle fiber as well as some interstitial cell nuclei in denervated muscle. After 8 days of denervation, clusters of smaller cells, presumably satellite cells, showed strong staining for MyoD1 and myogenin.

These results suggest that a genetic program which includes expression of genes that are involved in proliferation (jun, fos) as well as myogenic differentiation (MyoD1, myogenin) is activated in skeletal muscle fibers after denervation.

226.3

EARLY MOLECULAR RESPONSES TO AXOTOMY AND DEAFFERENTATION: CHOLINERGIC NEURONS. M. Weiser, H. Baker, T. Wessel and T.H. Joh Lab. Molec. Neurobiol. Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605

Cholinergic neurons of the horizontal limb of the diagonal band (HLDB) have axons which travel rostrally to terminate within the olfactory bulb and are axotomized by olfactory bulb decentralization. Cholinergic interneurons within the corpus striatum (CS) receive afferent modulation from catecholaminergic neurons of the substantia nigra pars compacta (SNc). Removal of this dopaminergic input is achieved by transection of the medial forebrain bundle and results in partial deafferentation of the cholinergic interneurons in the CS. To compare the acute effect of axotomy versus deafferentation on gene expression for choline acetyltransferase (ChAT) and c-Fos mRNAs and proteins, Sprague-Dawley rats were examined by immunohistochemistry and *in situ* hybridization. Following unilateral olfactory bulb decentralization, no alterations in ChAT or c-Fos levels were observed in the axotomized neurons of the HLDB between 1 and 24 hours. In contrast, neurons in the piriform cortex which receive afferents from the olfactory bulb and are deafferented by this lesion, exhibited a large increase in c-Fos. In addition, cholinergic interneurons in the CS partially deafferented by a transection of the catecholaminergic neurons of the SNc showed a large induction of c-Fos between 1 and 2 hours. These data suggest that deafferentation, not axotomy, results in c-Fos induction in these cholinergic systems.
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226.4

EARLY MOLECULAR RESPONSES TO AXOTOMY AND DEAFFERENTATION: CATECHOLAMINERGIC NEURONS. T.H. Joh, M. Weiser, T. Wessel, and H. Baker Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605

A micro-knife lesion placed rostral to the substantia nigra transects the medial forebrain bundle (MFB) and axotomizes both the catecholaminergic neurons of the substantia nigra pars compacta (SNc) and deafferents the GABAergic neurons of the substantia nigra pars reticulata (SNr). In addition, catecholaminergic neurons in the locus ceruleus (LC), far removed from the site of injury, are also axotomized by this lesion. To determine the acute alterations in gene expression in response to axotomy and deafferentation, the distribution of and temporal changes in the mRNA and protein levels for tyrosine hydroxylase (TH) and c-Fos were examined in adult male Sprague-Dawley rats by immunohistochemistry and *in situ* hybridization. Following a unilateral lesion of the MFB, the axotomized neurons of the SNc exhibited no alteration in TH or c-Fos levels between 1 and 24 hours. In contrast, a dramatic increase in c-Fos protein occurred with maximum expression at 6 hours in the deafferented neurons of the SNr. Furthermore, a large increase in c-Fos protein was seen in the axotomized neurons of the LC also reaching a maximum at 6 hours. The transient expression of c-Fos in the LC was followed at 24 hours by an increase in TH mRNA. These data suggest that the neuronal response to axotomy and deafferentation is not stereotyped but is both region and phenotype specific.
Supported by MH44043 and AG08702.

226.5

THE EXPRESSION OF TGF-B1 MRNA INCREASES DURING WALLERIAN DEGENERATION IN THE PNS. S.S. Scherer* and S.B. Jakowlew, Dept. Neurol., Univ. Penn. Sch. Med., Philadelphia, PA 19104, and Lab. Chemoprevention, NIH, Bethesda, MD 20892.

Transforming growth factor (TGF)-B1 inhibits cellular proliferation of many cell types, but is mitogenic for Schwann cells (Ridley et al., J. Cell Biol. 109:3419). Since Schwann cells are well known to proliferate during Wallerian degeneration, we investigated whether the possibility that TGF-B1 might be involved in this proliferative response. The sciatic nerves of adult rats were either ligated and transected (to prevent axonal regeneration) or crushed (to allow axonal regeneration), and RNA was isolated from distal nerve-stumps at 1, 4, 8, 12, and 24 days post-lesion. Ten ug of total RNA from each time point was separated on agarose-formaldehyde gels, transferred to nylon membranes, and individual blots were hybridized with a cDNA probe for TGF-B1, then sequentially rehybridized with a cDNA probe for nerve growth factor receptor (NGFR) and the major myelin glycoprotein Po. In transected nerves, the levels of TGF-B1 and NGFR mRNA in the distal nerve-stump increased to a maximal extent by 4 days post-transection and remained at this level for at least 24 days, while the level of Po mRNA decreased in a reciprocal pattern. The steady state levels of TGF-B1 and NGFR mRNA increased in the distal nerve-stump of crushed nerves, too, but then decreased by 24 days post-crush, while the level of Po mRNA increased at 24 days post-crush in a reciprocal pattern. This decline in TGF-B1 and NGFR mRNA and increase in Po mRNA coincided with the remyelination of regenerated axons. We are currently performing immunocytochemistry to localize TGF-B1 in transected and crushed nerves.

226.6

GROWTH-ASSOCIATED GENE EXPRESSION DURING NEURONAL REGENERATION IS A FUNCTION OF THE AMOUNT OF AXON LOST. T.C. Mathew and F.D. Miller, Dept. of Anat. and Cell Biol., Univ. of Alberta, Edmonton, Alberta, CANADA.

We have previously demonstrated that the regeneration of sympathetic neurons is associated with increased expression of Tα1 α-tubulin mRNA. To elucidate the signals responsible for inducing a growth response following axonal injury, we have examined the expression of Tα1 mRNA following axotomy proximal to (a close cut) and distal to (a long cut) the superior cervical ganglion (SCG) of adult rats. The peak induction of Tα1 mRNA in the SCG occurred 5 days following either a long cut or a short cut, as indicated by Northern blot analysis. To determine the magnitude of this response on a per neuron level, we developed the following experimental paradigm. Sympathetic neurons that project from the SCG to the iris via the internal carotid nerve (ICN) were retrogradely labelled by injection of fast blue into the anterior chamber of the eye, while those that project via the external carotid nerve (ECN) to the pinna of the ear were retrogradely labelled with fluorogold. Following labelling, postganglionic axons that project to the iris were long cut by enucleating the eye, while those that project to the ear were short cut by transecting the ECN close to the ganglion. In situ hybridization analysis of fast blue versus fluorogold-labelled neurons in the same ganglia (done to minimize intersection variability) demonstrated that the peak level of expression of Tα1 mRNA was two- to three-fold higher in neurons axotomized close to their cell bodies as opposed to those transected distally. These results suggest that neurons are capable of monitoring the severity of the axonal damage, and of responding appropriately.

226.7

ANALYSIS OF THE RNA LEVELS OF SR13 (GAS-3) IN RAT SPINAL CORD AFTER SCIATIC NERVE TRANSECTION AND COMPLETE FREUND'S ADJUVANT (CFA) -INDUCED INFLAMMATION. M. M. Divish*, M. De León, R. Nahin and M. A. Ruda, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda,MD 20892

We are interested in studying the molecular changes associated with axonal injury and nerve regeneration. Previously we reported the isolation of a cDNA clone named SR (sciatic repressed) 13 from a rat sciatic nerve cDNA library that was found to be repressed in the sciatic nerve three days after nerve crush. Further characterization of this cDNA found that it is the rat homolog of the growth arrest gene 3 (gas-3) (Welcher et al, submitted). In the present study we analyzed the mRNA expression of SR13 in the rat spinal cord after two kinds of manipulation 1) sciatic nerve transection and 2) peripheral inflammation and hyperalgesia produced by unilateral hindpaw injection of CFA. Three days after treatment, the L4-5 segments of the spinal cord were collected and the RNA was extracted ipsilateral and contralateral. The RNA was fractionated in a formaldehyde agarose gel, northern blots were performed following standard techniques, and the membranes hybridized with 32P-labeled SR13 cDNA. RNA blot analysis demonstrated that the SR13 cDNA recognized a 1.8 kb mRNA species in the spinal cord similar (although less abundant) to that found in the sciatic nerve. We found that both nerve transection and CFA-induced inflammation triggered at least a two-fold induction of SR13 mRNA by day 3 after treatment on the ipsilateral side of the spinal cord as compared with the contralateral, untreated side. The mRNA level returned to normal by day 10. Our results suggest that both nerve transection and inflammation signal the regulation of SR13 (gas-3) in the central nervous system and that this response is uniquely different from that observed in the peripheral environment at the site of the injury.

226.8

IDENTIFICATION OF GENES TRANSCRIPTIONALLY REGULATED AFTER SCIATIC NERVE CRUSH. M. De León[1], A. A. Welcher[2], U. Suter[2], M. A. Ruda[1] and Eric M. Shooter[2]. [1]Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD, 20892 and [2]Department of Neurobiology, Stanford University, California, 94305-5401

The local environment at the sciatic nerve crush injury site may play an important role in modulating the neuronal response to axotomy. In order to characterize the molecular response present in this region, we have analyzed a group of genes that were found to be transcriptionally regulated after sciatic nerve crush. cDNA libraries were constructed from either the nerve segment distal to the crush site or the corresponding contralateral uninjured nerve of the same animal at day three after crush. These cDNA libraries were screened by differential hybridization and several transcriptionally repressed and induced sequences were isolated. In the contralateral segment library we found 30 colonies containing sequences that were repressed after sciatic nerve crush. Among these we found five sequences that were dramatically repressed and included creatine kinase (muscle type M), Myelin basic protein, Myelin P0 and a novel cDNA named SR -13, that after further characterization and sequencing , was found to be the rat homolog of growth arrest gene 3 (gas-3) (Welcher et al, submitted). Screening of the ipsilateral distal library resulted in eleven sequences found to be induced, including the rat homolog of vimentin. We also found a novel sequence called Distal Induced 12 (DI12) that showed homology at the nucleotide level with the Human FK506-binding protein. Northern blot analysis confirmed the regulation shown by these sequences after axonal crush. The observed pattern of complex regulation suggests that the local environment at the site of injury plays an important role during neuronal injury and regeneration.

226.9

RNA POLYMERASE ACTIVITY IN DORSAL ROOT GANGLION NEURONS AFTER NERVE CRUSH. M. R. Wells and U. Vaidya, Nerve Regeneration Research Laboratory, VA Med. Center, Northport, N.Y. 11768 and Depts. of Neurology and Psychiatry, SUNY, Stony Brook, N.Y. 11794.

After crush injuries to the sciatic nerve, dorsal root ganglion neurons undergo a complex series of metabolic changes which include phasic increases in RNA synthesis approximately one week apart. We wished to determine if these increases in RNA synthesis are accompanied by corresponding changes in RNA polymerase (RNAP) activity. Male, Wistar-Furth rats were subjected to unilateral crush lesions of the sciatic nerve at the level of the sciatic notch. At 12 hours, 1, 2, 3, 4, 5, 7, 8, 9, 11, 14, and 30 days after nerve crush, animals were perfused with buffered isotonic sucrose and the L5 lumbar ganglia removed bilaterally. RNA polymerase activity over nucleoli and nucleoplasm in neurons was measured autoradiographically on frozen sections by the method of Moore (Exp. Cell Res. 111: 317,1978) and compared the activity of sections from normal animals placed on the same slide. Compared to the unoperated side and normal controls, L5 ganglia from the side of the nerve crush showed a biphasic increase in RNAP activity consisting of two peaks between 1 to 5 days and 8 to 14 days after injury. Changes of similar magnitude occurred in both nucleolar (RNAP I) and non-nucleolar (RNAP II &III) RNA polymerase activity, although RNAP I appeared to peak first. There were no significant changes in RNA polymerase activity on the unoperated side compared to normal at any time point. The results suggest that RNA polymerase activity may be one mechanism through which the metabolism of injured neurons is controlled. Supported by the Veterans Administration.

226.10

CYTOSKELETAL GENE EXPRESSION FOLLOWING AXOTOMY IN THE PNS IN YOUNG VERSUS AGED ALBINO RATS. S.L. Cassar, C.A. Leonard* and W. Tetzlaff, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Peripheral nerves regenerate more slowly in aged versus young adult animals. We investigated whether or not changes in cytoskeletal gene expression in facial motoneurons following axotomy were less pronounced in aged versus young rats. Adult Sprague-Dawley rats aged 4, 16 and 23 months underwent a left-sided facial nerve transection. At 14 days post lesion the animals were sacrificed and cryostat sections through the facial nerve nucleus underwent *in situ* hybridization. We noted a consistent decrease in the expression of neurofilament mRNA, as well as a consistent increase in the levels of alpha-tubulin mRNA in axotomized facial motoneurons, as compared to undamaged contralateral control facial motoneurons. This response was quantitatively similar across all age groups.

These results demonstrate that, following axotomy, the gene expression of neurofilament and alpha-tubulins in aged mammalian motoneurons are similar to those seen in young adults. This indicates that the slower regeneration rate of injured peripheral nerves seen in aged animals is not due to a less vigorous gene expression response.

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226.11

DOPAMINERGIC RECOVERY FOLLOWING A UNILATERAL NIGRAL LESION IN THE RAT. J.S. Lamberti*, S.Y. Felten, S.B. Schwarzkopf, D.L. Felten. Dept. of Neurobiology and Anatomy, Univ. Rochester Sch. of Med., Rochester, NY 14642.

Unilateral injection of 6-hydroxydopamine (6-OHDA) into rat substantia nigra has been shown to produce degeneration of the nigrostriatal dopaminergic pathway. While recovery has been demonstrated, its mechanisms and time course remain unclear. In this study we examined striatal dopamine (DA) concentrations at various times following a unilateral nigral lesion.

69 eleven week old male Fischer rats received either a left nigral injection of 8 µg 6-OHDA, sham injection of 4 µL saline, or no treatment. All animals underwent rotometry following 5 mg/kg amphetamine infusion, with lesioned animals demonstrating at least 7 rotations/min. Animals were then sacrificed and left dorsal striatum was removed for analysis at 10, 30, or 90 days post lesion. DA concentrations in lesioned animals were 5.3%, 21.1%, and 10.0% of controls at those time periods respectively (t-test $p<.05$), while control and sham groups showed no significant differences. Anatomical studies are presently underway to determine the extent to which this pattern of recovery is due to metabolic regulation or regenerative sprouting. This work was supported by grants NIH AG06060 and NIMH MH40381.

226.12

REGULATION OF ß-PREPROTACHYKININ mRNA AND SUBSTANCE P IN RAT DRG NEURONS AFTER NEONATAL SCIATIC NERVE LESION. F. Nothias, A. Tessler, W. Battisti, M-F Chesselet[1] and M. Murray. Dept. of Anat. & Neurobiol., Med. Coll. of Penn., Philadelphia, PA 19129; [1]Dept. of Pharmacol., Univ. of Penn, Philadelphia, PA 19104.

Previous studies using sciatic nerve (SN) section have shown that in adult rat DRG neurons, SP and its m-RNA decrease and do not recover if regeneration is prevented. In neonates, large numbers of DRG neurons die after SN axotomy but recovery of SP-immunoreactivity (SP-IR) in the dorsal horn (DH), mediated by dorsal roots, is observed by 60 days, even if regeneration is prevented. We tested the hypothesis that this developmental effect is related to a permanent increase of SP synthesis and its gene expression in the remaining DRG neurons. Sixty days after neonatal SN axotomy, L4 and L5 DRG sections were used for *in situ* hybridization histochemistry using preprotachykinin (PPT)-^{35}S-cRNA probes, and immunocytochemistry for SP. Contralateral DRG served as controls. In the control and operated DRG, both PPT-mRNA hybridization and SP immunostaining were observed in medium and mostly small size neurons. SP-IR in both DH is comparable, but the total number of PPT-mRNA and SP-IR labelled neurons is lower in operated DRG than in control. The mean levels of hybridized PPT-mRNA and SP-IR per neuron in DRG ipsilateral to the lesion, were, however, comparable to the control. These data show that after neonatal sciatic axotomy, PPT-mRNA and SP-IR recover in DRG neurons but do not exceed that observed in controls, and suggest that both transcriptional and post-transcriptional regulation are different than when SN axotomy is made in the adult. *Supported by grants, NIH NS 24707, VA, VSAMRDC 51930002, and AFM.*

226.13

NEUROPEPTIDE CHANGES IN THE RAT LUMBAR DORSAL HORN AFTER SCIATIC DEAFFERENTATION INDUCED BY INJECTION OF THE SCIATIC NERVE WITH PRONASE. A. El-Bohy and C.C. LaMotte. Section of Neurol. Surgery, Yale Univ. Sch. of Med., New Haven, CT. 06510.

Sciatic deafferentation was induced by injecting the sciatic nerve of anesthetized rats with proteolytic enzymes (10-20mg Pronase). The effects of this chemical deafferentation were examined in two animal groups, i.e., short term (10-13 days) and long term (4-6 months) after injection, using a computerized densitometry analysis system. The L1-L5 segments and C5 control segments were removed, sectioned, and immunostained. The density of the label in the dorsal horn was measured for each section and the results were compiled for the segments L1-3 and L3-5. Using a paired t-test, we found a highly significant decrease in CGRP and SP on the pronase side at both the L1-3 and L3–5 levels in both the short term and the long term rats. In the long term group, the loss of CGRP staining was significantly less than that in the short term animals, indicating partial recovery. A similar trend was observed for SP. The results also indicated that recovery was greater in the L1-3 than that in the L3-5 levels for both peptides. The large decrease in CGRP and SP seen in short term animals reflects the large contribution of the sciatic nerve to the lumbar dorsal horn. The partial recovery of peptides may parallel sprouting of primary afferents from other nerves, such as the saphenous nerve, as we have demonstrated in previous studies. (Aided by grants from the Paralyzed Veterans of America Spinal Cord Research Foundation #880, the Eastern Paralyzed Veterans of America, and NIH Grant NS28876).

226.14

PUTATIVE ELECTRICAL SIGNALS ASSOCIATED WITH AXOTOMY **R.C. Berdan, J. Easaw, and R. Wang. Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.**

Recent molecular studies have found that neurons may undergo rapid changes in gene expression in response to axotomy. We are examining the possibility that changes in membrane potential associated with injury may represent one of the signals associated with axotomy that triggers regeneration. We have recorded intracellularly from identified neurons B5 from the mollusc, Helisoma, during axotomy. Axotomy was associated with a short injury discharge and a rapid membrane depolarization. Repolarization took place within 15 minutes. The magnitude of the depolarization recorded in the soma was a function of distance from the site of injury. Superfusion of saline containing elevated potassium onto buccal neurons mimicked the change in membrane potential associated with axotomy. Buccal neurons B5 that were axotomized, organ cultured for 5 days in the presence of elevated potassium (5-50 mM), and then filled with Lucifer yellow and examined by fluorescence microscopy, appeared to exhibit more extensive neurite outgrowth. Isolated neurons B5 cultured in defined culture medium (Liebovitz) containing elevated potassium (5-50 mM), however, did not extend neurites. Using the whole-cell patch clamp technique, both a low-voltage-activated (LVA) and a high-voltage-activated (HVA) calcium currents were detected in neurons B5 after 24 hours in culture. After culturing in depolarizing medium containing 25 mM potassium for 6 days the LVA channel could no longer be detected and the amplitude of the HVA channel current was suppressed approximately 10 fold. This observation indicates that expression of calcium channels may be altered following treatments that alter membrane potential. Together these results are consistent with the hypothesis that membrane depolarization may represent one of the signals associated with axotomy.

226.15

PREFERENTIAL GROWTH OF ADULT SENSORY NEURONS ON SECTIONS OF LESIONED PERIPHERAL NERVE IS NOT DEPENDENT ON NGF. (1) K.S. Bedi, (2) J. Winter(*), (3) M. Berry and (3) J. Cohen (*). (1) Dept. of Anatomy, University of Queensland; (3) Div. of Anatomy and Cell Biology, UMDS, Guy's Campus, London, and (2) Sandoz Inst. Med. Res., London.

In adult rats the axons of DRG neurons regenerate within damaged peripheral nerves. We have investigated whether cultured DRG neurons from adult rats could be induced to grow on various CNS and PNS tissue sections. Cryostat sections were prepared from freshly frozen rat tissues including lesioned and non-lesioned cerebrum, cerebellum, peripheral and optic nerves and picked up on coverslips. Dissociated adult rat DRG neurons were cultured with or without NGF on the tissue sections for 3-5 days. Neurites were visualized by immunostaining with antibodies to GAP-43 and neurofilaments. Only sections from lesioned peripheral nerves supported neurite outgrowth. This was independent of the presence of exogenous NGF. The neurite growth was largely parallel to the long axis of longitudinally-sectioned nerves. This tissue culture model system mimics the situation seen in regenerating sensory peripheral nerves in vivo and offers a unique opportunity to examine underlying mechanisms.

226.16

A SIGNAL SEQUENCE MEDIATES THE RAPID RETROGRADE TRANSPORT OF PROTEINS FROM THE AXON INTO THE NUCLEUS. R. Schmied,* M. Smedman,* and R. T. Ambron. Anatomy and Cell Biology, Columbia University, New York, N. Y. 10032

We have discovered a new feature of retrograde transport that may explain how external signals, e.g. growth factors, cause long-term changes at the presynaptic terminal. Since such changes require transcriptional activation in the nucleus, a signal must be conveyed from the periphery to the cell body. We searched for this signal using neurons of Aplysia. When a neuron is placed in culture, the cut axon seals to form a terminal swelling from which neurites emerge. Material injected into this swelling has direct access to the axon.

We coupled the nuclear import signal peptide of the SV-40 T antigen, and then rhodamine, to human serum albumin. The construct (rHSA-sp) was injected into the terminal of neurons *in vitro*. 24h later, all the rHSA-sp was found in the nucleus. Cells 20min to 3h after injection revealed that the rHSA-sp moved rapidly through the axon. Significantly, all transport was in the retrograde direction and none was toward neurites or growth cones. Injected rHSA did not move and rHSA-sp containing a single a.a. substitution in sp, which reduces its efficiency for nuclear import, was transported poorly. Thus, the nuclear import signal peptide is responsible for binding to the retrograde transport system. Microtubules are also involved, since nocodazole blocked transport of both forms of rHSA-sp.

To identify endogenous proteins that might use this transport system, we conjugated soluble proteins from adult nerves to biotin and exposed these proteins to isolated intact neuronal nuclei using a nuclear import system. The nuclei were washed, fixed, and probed with streptavidin-Texas Red. Many nuclei were fluorescent, indicating that they had imported nerve proteins. These findings show that a retrograde transport system in Aplysia neurons recognizes an evolutionarily conserved signal peptide that directs axonal proteins to the nucleus. This system may link the needs of the terminal to the biosynthetic machinery in the cell soma.

226.17

SEAL FORMATION IN EARTHWORM MEDIAL GIANT AXON FOLLOWING TRANSECTION STUDIED BY COMPLEX IMPEDANCE SPECTROSCOPY. T.L. Krause, H.M. Fishman[+] and G.D. Bittner. Univ of Texas, Austin, [+]Univ of Texas Medical Branch, Galveston.

The medial giant axon of the earthworm Lumbricus terrestris recovers from transection by reestablishing its resting membrane potential and DC cable impedance (input resistance) with a time course of about 1 hr. Complex (AC) impedance spectroscopy (0.5 to 1000 Hz) provides additional information about conducting structures in the current path prior to and following axonal transection. AC impedance data (400 freq pts) were rapidly acquired (1-2 sec) using two intracellular microelectrodes, 80-200 μm from the site of transection, in ion-channel blocked axons. Upon transection the impedance decreased dramatically, becoming largely ohmic in character. This was indicative of current flow through the low resistance path created by the transection. Subsequent impedance data acquired at regular intervals were well fitted with an RC model. These curve fits showed temporal changes in both resistance and capacitance consistent with formation of an electrical barrier. At 30 min or more, following transection, the AC impedance was found to be similar to that obtained prior to injury. Thus, the temporal changes in axonal complex impedance suggest that a membranous barrier (seal) forms during recovery from transection. Support: ONR (N00014-90-J-1137) TX ATP (004952011) and NSF (ECS-891-5178)

226.18

CHRONIC LEVODOPA AND RECOVERY OF NIGROSTRIATAL DOPAMINE NEURONS IN MPTP-TREATED YOUNG MICE. K. Steece-Collier, J.R. Sladek, Jr., S.Y. Felten, D.L. Felten. Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

The nigrostriatal dopamine (DA) system in young C57BL/6 mice can recover following damage from the neurotoxin MPTP. This is an excellent model for studying the potential toxicity of levodopa or DA itself on neuronal plasticity. Levodopa has been shown to stunt neurite outgrowth and reduce viability of embryonic nigral neurons. We investigated possible damage of recovering DA neurons by levodopa in MPTP treated mice. Two days after MPTP treatment (4 x 20mg/kg) mice were divided into 3 groups: MPTP/no injection; MPTP/ saline injection; and MPTP/levodopa injection. Levodopa (200mg/kg) or saline was injected twice daily for 5 weeks. HPLC analysis of nucleus accumbens and dorsal and ventral caudate revealed no differences in DA concentration between the 3 treatment groups. A trend toward a selective increase in DA turnover was observed following levodopa treatment in subcompartments of caudate but not in nucleus accumbens. It is possible that levodopa does not damage DA neurons recovering from MPTP in young mice. However, neurochemical analysis by itself is inadequate to assess the anatomical integrity of DA neurons, since upregulated metabolism in surviving cells could compensate for death or injury of neighboring DA neurons. We are assessing this possibility with quantitative morphological methods utilizing immunocytochemistry for tyrosine hydroxylase and GAP43. Supported by United Parkinson Foundation, PO1 NS24032, and R37 AG06060.

226.19

TESTOSTERONE EFFECTS ON AXONAL REGROWTH FOLLOWING SCIATIC NERVE INJURY. K.A. Kujawa and K.J. Jones. Dept. Cell Biology and Anatomy, The Chicago Medical School, North Chicago, IL 60064 and Depts. Physical Therapy, and Anatomy & Cell Biology, Univ. Il at Chicago, Chicago, IL. 60612.

Systemic administration of testosterone propionate (TP) accelerates both the rate of axonal regeneration and functional recovery from facial paralysis induced by facial nerve crush in the hamster. In this study, the hypothesis that TP affects the regenerative properties of spinal motoneurons as well was tested using fast axonal transport of radioactively labeled proteins to assess sciatic nerve regeneration. Adult male rats were castrated under anesthesia. 3-5 days postoperative (dpo), the animals were re-anesthetized and each subjected to a right sciatic nerve crush. One-half the animals immediately received subcutaneous implants of TP, with the others sham-implanted with blank capsules. Postoperative times ranged from 3-7 d. 18 h prior to sacrifice, stereotaxic injections of ^{3}H-amino acids into the ventral horn at spinal segments T13-L1 were done. Following sacrifice, the radioactivity in 1-mm segments of the right sciatic nerve was determined, and plotted as a function of postoperative time. The furthest distance point at which the radioactivity was $\geq$ 2 S.D. above background levels defined the outgrowth distance of the fastest growing axons. Thus far, the results indicate that at 5 dpo, the average outgrowth distances in the crush plus TP vs. crush alone groups were 10 mm $\pm$ 0.53 and 6 mm $\pm$ 0.6, respectively. This represented a TP-induced 40% increase in the outgrowth distance. Supported by NS28238 (KJJ).

226.20

TESTOSTERONE EFFECTS ON AXONAL REGENERATION FOLLOWING FACIAL NERVE CRUSH IN FEMALE HAMSTERS. E. Emeric*, K.A. Kujawa and K.J. Jones. Dept. Cell Biology and Anatomy, The Chicago Medical School, North Chicago, IL 60064 and Depts. Physical Therapy, and Anatomy and Cell Biology, Univ. Illinois at Chicago, Chicago, IL 60612.

We have previously demonstrated that testosterone propionate (TP) accelerates the rate of regeneration following facial nerve crush in castrated and intact male hamsters. In this study, we examined the effects of TP and the nonaromatizable form, dihydrotestosterone (DHT), on axonal regeneration in intact females following facial nerve crush. Following anesthetization, adult female hamsters were subjected to right facial nerve crush. One-half the animals immediately received subcutaneous implants of TP or DHT, with the others sham-implanted with blank capsules. Postoperative times ranged from 3-7 d. 18 h prior to sacrifice, stereotaxic injections of ^{3}H-amino acids into the right facial nuclear groups were done. Following sacrifice, the radioactivity in 1-mm segments of the right facial nerve was determined, and plotted as a function of postoperative time. The furthest distance point at which the radioactivity was $\geq$ 2 S.D. above background levels defined the outgrowth distance of the fastest growing axons. The results indicate that either TP or DHT accelerates the rate of axonal regeneration in females, albeit to a lesser extent than in the males (10% vs. 30%). This may be due to inherently faster regeneration rates in intact females when compared with intact males. Supported by NIH NS-28238 to KJJ.

226.21

CASTRATION OF ADULT MALE RATS INCREASES ASTROCYTE REACTIVITY (GFAP) IN THE MOLECULAR LAYER OF THE DENTATE GYRUS. J.R. Day, N.J. Laping, M. Lampert-Etchells*, T.H. McNeill, and C.E. Finch. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Increased glial fibrillary acidic protein (GFAP) mRNA and immunoreactivity (IR), as indices of astrocyte reactivity, are apparent in aging, neurodegeneration, deafferented regions and after brain injury. Recently, this laboratory showed that GFAP-IR and mRNA increase in the hippocampus 21 days after castration in unlesioned and entorhinal cortex lesioned (ECL) adult male rats (Day et al., Mol Endocrinol 4:1995). This study quantified the GFAP-IR increase with western blots and determined if gonadal steroid replacement would inhibit the increase in GFAP-IR in the hippocampus. Western blots confirmed that GFAP-IR in the hippocampus increased 21 days after castration (38%, $P < 0.0001$) and that the increase in GFAP-IR 2 days after ECL was enhanced by castration (54%, $P < 0.03$). Four groups of rats (n=3) were given subcutaneous silastic implants containing either testosterone (T), estradiol (E2), dihydrotestosterone (DHT), or an empty implant (CAST) on the day of castration. Additional rats served as intact controls. 21 days after castration, rats were perfused and brains were processed for GFAP immunocytochemistry. By videoimage analysis, the area occupied by GFAP-IR (including the soma) was determined within a fixed perimeter containing an individual astrocyte from the dentate molecular layer. This analysis revealed that the area of GFAP-IR increased 36% ($P < 0.03$) CAST vs intact controls. T, E2, DHT implants all suppressed this castration-induced increase to control values ($P < 0.01$). These results indicate that GFAP-IR in adult male rats is responsive to gondal steroids. We suggest that the increase in astrocyte reactivity after long-term castration is the result of astrocyte hypertrophy and not proliferation (Supported by USPHS Grant AG-07909).

226.22

MOTOR FUNCTIONAL RECOVERY AND ACHR CONCENTRATION REFLECT SEX DIFFERENCES IN REGENERATION. J. Kume and F.L. Strand Department of Biology and Center for Neural Science, New York University, New York 10003.

Sex steroids have been shown to affect the non-androgen sensitive muscle, the extensor digitorum longus (EDL) (Kume, J. and Strand,F.L. Soc. Neuroscience, Nov. 1990). The present study explored the relationship between acetylcholine receptor (AChR) concentration and motor recovery in the EDL, after nerve crush in castrated and normal rats. Male and female rats were divided into 3 groups: nerve crushed; castrated, nerve crushed; and castrated with subsequent LHRH antagonist administration (BIM-21009, Biomeasure, Inc., 5mg/kg, s.c.), nerve crushed. In a second experiment, castrated male and female rats were subcutaneously implanted with silastic implants filled with either testosterone propionate or estradiol benzoate. Under anesthesia, the peroneal nerve was crushed at the site of innervation. AChR concentration was found by radiolabelled binding of $I^{125}\alpha$-bungarotoxin. Motor tests were done on rats used for AChR assays. Digit Distance 1-5 (DD1-5), peroneal functional indices, and % toespread recovery were measured. We found previously that nerve crush increases AChR concentration especially in castrated animals (in this earlier study, LHRH antagonist was the castrating agent used for males only, females were surgically castrated). At 9 days post-crush, a sex difference between castrated groups appears, males having 28% higher AChR concentration than females. This difference is not seen in rats with normal steroid levels. When relating this difference to motor function recovery tests, male sex hormones appear to exert a positive effect on % toespread recovery at day 2 in males, whereas female sex hormones play a negative role in females. (Supported by NIMH grant # MH18882-03.)

227.1

COLLAGEN NERVE GUIDES PROMOTE PHYSIOLOGICAL RECOVERY OF A 2cm MEDIAN NERVE DEFICIT IN MONKEYS. [1]S.J. Archibald,[4]C. Krarup*, [1]L. Wrage*, [5]S-T Li* and [1,2&3]R.D. Madison, [1]Departments of Surgery (Neurosurgery) and [2]Neurobiology, Duke University Med. Ctr., Durham, N.C. 27710, [3]Research Service V.A. Hospital, Durham, N.C. 27710, [4]Department of Clinical Neurophysiology, Rigshospitalet, Copenhagen, Denmark and [5]Colla-Tec, Inc., Plainsboro, N.J.

We have recently shown that collagen nerve guide conduits permeable to macro-molecules up to 68kd support and maintain axon regeneration innerve deficits of 5mm in the median nerve of *Macacafasicularis*(Archibald et al., J. C. N.,306 (4):685-696, 1991.) Nerve deficits of more than 2cm clinically, are usually repaired with nerve grafting techniques. This study compares motor and sensory physiological recovery following the repair of a 2cm deficit of the median nerve in *Macaca fasicularis*. Adult male monkeys received bi-lateral median nerve section at the wrist and removal of a 2cm segment of nerve, followed by either: A) Sural nerve cable graft repair (n=8 nerves), B) Entubulation repair with 2mm I.D. nerve guide conduits (n=8 nerves). Serial evoked EMGs of the abductor pollicis brevis and sensory nerve conduction studies were performed bi-weekly up to 154 days following surgery and at monthly intervals thereafter.

Evoked EMG activity was first detected between 56-126 days and 98-182 days in the graft and entubulation repair procedures respectively. At 322 days average EMG amplitude of the graft repair and the entubulation repair procedures had returned to 55.5% (+/- st.dev.17.9) and 39.7% (+/- st.dev.22.8) of baseline respectively, no significant statistical difference can be demonstrated between the two procedures at this time point. Supported by NS-22404-06 and Colla-Tec, Inc.

227.2

An Artificial Nerve Graft Combines Schwann Cells & Acellular Components to Enhance Peripheral Nerve Regeneration. Rob Keeley*, Tanya Atagi*, Lawrence Eng, Joseph Rosen*.

A study was done to compare two artificial nerve grafts (ANG) in a rat model using the peroneal branch of the sciatic nerve. A 10 mm gap was created in one peroneal nerve and a graft composed of a porous conduit filled with a 0.8% collagen matrix implanted (Coll-ANG), while the contralateral nerve was repaired with an identical graft except for live schwann cells suspended in the matrix to support neurite outgrowth (SCC-ANG). Schwann cells were harvested from 2-day old syngeneic neonates. Fourteen rats were set up and evaluated bi-weekly with a rat walking test to measure functional recovery. After 4.5 months the animals were anesthetized deeply for electrophysiological analysis of the regenerated nerves, then sacrificed for qualitative and quantitative histological comparison. In addition, 15 control animals were implanted comparing SCC-ANGs to dead schwann cell-collagen grafts, to saline-filled conduits, and to sutured autografts, with results pending.

Walking pattern analysis demonstrated the SCC-ANG to enhance functional recovery from 60 to 110 days post-implantation over the Coll-ANGs ($p<.005$). Electrophysiological data demonstrated superior compound action potentials in the SCC-ANG repairs ($p<.05$). Quantitative histology revealed statistically higher axon counts for SCC-graft repairs ($p<.01$) and larger fiber diameters ($p<.05$). Qualitative histology demonstrated the SCC-ANG to have better organization ($p<.01$) than the Coll-ANG.

This study demonstrates that living schwann cells can be harvested, grown in cell culture, and incorporated into an ANG, and that this SCC-ANG improves nerve regeneration.

227.3

CARBON FILAMENT IMPLANTS SUPPORT AXONAL REGROWTH ACROSS TRANSECTED RAT SCIATIC NERVE. S. Sayers*, M. Dauzvardis*, and T. Khan. Rehab. R&D Center, Hines VA Hospital, Hines, IL 60141.

Current methods of peripheral nerve repair often result in unsatisfactory restoration of function. In some situations, a significant loss of nerve tissue may result in a gap too wide for end-to-end anastomosis. In this case, a nerve graft is necessary to bridge the gap. Although a nerve autograft would be the preferred method of repair, problems occur with obtaining donor nerve. An alternative is to use an artificial nerve graft as a bridge. In our laboratory, we have shown that small diameter carbon filaments can support and give directionality to growing rat fetal spinal cord explants (*Neurosci. Lett.* 118:172-176, 1990). We have also shown that carbon filaments implanted into the transected adult rat spinal cord can promote axonal growth across the transection site (*Brain Res.* 541:139-145, 1991). In the present study, we evaluated the feasibility of using carbon filament implants in the repair of peripheral nerve.

Young adult rats were anesthetized and the left sciatic nerve was cut. The proximal and distal ends were inserted into the ends of a silicone tube containing approximately 10,000 carbon filaments (5 µm diameter) 5 mm in length. The nerve stumps were held in place with a small amount of cyanoacrylate adhesive applied between the epineurium and the tubing. The right sciatic nerve served as a control. After 60 days, the rats were anesthetized and both the left and right sciatic nerves were crushed distal to the injury. WGA-HRP (0.3 µl of a 2% solution) was injected into the crush site of each nerve. After 24 h, the animals were sacrificed and perfused. The spinal cords were removed, fixed, sectioned, reacted for WGA-HRP, and segments L2-S5 were observed for labeled cells.

Preliminary results have shown labeled cells on the left half (injury side) of the spinal cord. Numerous labeled cells were always seen on the right (control side) of the spinal cord. These early studies suggest that carbon filament implants are capable of supporting axonal regrowth in peripheral nerve.

Supported by Rehab. R&D Center, Hines VA Hospital, Hines IL 60141.

227.4

CARBON FILAMENT IMPLANTS PROMOTE AXONAL REGROWTH IN THE TRANSECTED SPINAL CORD OF THE NEONATAL RAT. M. Dauzvardis*, T. Khan, and S. Sayers*. Rehab. R&D Center, Hines VA Hospital, Hines, IL 60141.

In the adult mammalian central nervous system (CNS), damaged axons fail to regrow to their former target sites largely due to influences of the CNS microenvironment. Glial scaring and the lack of appropriate trophic signals are among these factors. Recent evidence from *in vitro* and *in vivo* experiments in our laboratory indicate that small diameter carbon filaments are capable of supporting the growth of damaged spinal cord axons and directing these processes across a site of spinal cord transection. In the present study, we sought to determine whether carbon filaments implanted into the transected spinal cord of the neonatal rat would result in even greater trans-lesion growth than was observed in our previous adult transection model.

Three day old rat pups, sustaining complete spinal cord transection and recieving carbon filament implants (10,000 filaments, 0.5 cm long, 5 um diameter) (n=7), were compared to animals sustaining total spinal cord transections without implants (n=3).

Lesions were made at the T7-T8 spinal cord level under hypothermic anesthesia, following laminectomy, using a #11 scalpel blade and a fine wire hook. Both groups received bilateral injections of 2% WGA-HRP (0.05ul) in the somatosensory cortex 30 days after surgery.

While none of the sections taken from the spinal cords of surgical control animals demonstrated any label caudal to the lesion site, labeled axons were found below the lesion site in the spinal cords of 3 out of 7 of the animals receiving carbon filament implants. Regrowing axons were found to take a lateral or ventral course around the implant, while others appeared to travel through it.

Carbon filament implants appear capable of promoting significant axonal regrowth in the transected spinal cord of the neonate.

Supported by Rehab. R&D Center, Hines VA Hospital, Hines, IL.

227.5

CULTURED SYNGENEIC ADULT SCHWANN CELLS SEEDED IN SYNTHETIC GUIDANCE CHANNELS ENHANCE SCIATIC AND OPTIC NERVE REGENERATION. V. Guénard[1], T.K. Morrissey[2], N. Kleitman[2], R.P. Bunge[2], P. Aebischer[1]. [1]Division of Biology and Medicine, Brown University, Providence, RI 012912 and [2]The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL 33136.

Upon injury in the PNS, Schwann cells (SCs) within the distal nerve segment regain some of their developmental properties, including the ability to attract and guide regenerating axons. Peripheral and central nerve regeneration through permselective guidance channels could be enhanced by pre-forming an organized SC cable oriented along the longitudinal axis of the guidance channels. Permselective guidance channels were seeded with virtually pure cultures of SCs isolated from adult Fisher rat sciatic nerves and suspended in a 30% solution of Matrigel® in culture medium at a final density of either 40 or 80 x 10^6 cells/ml (SC-40, SC-80 channels). Matrigel®-filled channels were used as controls. Channels were implanted for 3 weeks in transected rat sciatic nerves across an 8 mm nerve gap. Matrigel®-filled channels and SC-80 channels were also implanted for 8 weeks in transected rat optic nerves across a 1 mm gap. In the sciatic nerve, cables with nerve microfascicles bridged the nerve stumps in all channels. Cables regenerated through SC-80 channels were larger, contained significantly more myelinated axons and showed less microfasciculation as compared to cables regenerated through Matrigel®-filled channels. The number of myelinated axons in SC-40 channels was between that in Matrigel®-filled channels and SC-80 channels. In the optic nerve, regenerated nerve cables bridged the nerve stumps in all animals. However, nerve microfascicles with myelinated axons were observed only in SC-80 channels. Axons originated from retinal ganglion cells as observed with fast blue retrograde tracing. These studies indicate that cultured syngeneic adult SCs can favorably influence nerve regeneration in both PNS and CNS structures.

227.6

VENTRAL ROOT REGENERATION THROUGH GLIAL CELL-SEEDED SEMIPERMEABLE GUIDANCE CHANNELS M.L. McCormack[1], T.K. Morrissey[2], N. Kleitman[2], R.P. Bunge[2], and P. Aebischer[1] Brown Univ.[1] and The Miami Project, Univ. of Miami[2]

Semipermeable guidance channels have been shown to be successful in supporting regeneration of ventral root axons across 4 mm gap injuries but insufficient when 10 mm gap injuries are involved. To determine intralumenal modifications that might enhance the supportive properties of these channels, glial cells seeded in a laminin-containing gel matrix were tested as potential outgrowth-promoting substrates for ventral root axons *in vivo*. Outbred early postnatal rat-derived astrocyte (AC) and olfactory bulb cell lines (ROB) (a gift of Dr. J. Jacobberger), outbred and inbred adult rat-derived primary Schwann cells (SC) were used as cellular substrates for regenerating axons. The various cell types were entubulated within the gel matrix so that a centrally-located cable of cells arranged longitudinally within the gel was formed down the length of an acrylic copolymer semipermeable guidance channel. The seeded channels were then used to bridge 10 mm gaps in the L5 ventral root of adult male albino rats. Eight animals received ROB implants, 9 received AC implants, 10 received outbred SC implants, and 3 received inbred SC implants. Regeneration was assessed at 4 weeks post-implantation. Successful regeneration (cables containing myelinated and nonmyelinated axons at the channels midpoint) was achieved only with the SC-seeded guidance channels. Furthermore, there was a marked difference in the success of the syngeneic grafts (100%) as compared to the allogeneic grafts (20%). This data suggests that Schwann cell-seeded semipermeable guidance channels can support regeneration of ventral root axons across large deficit injuries. In additon, syngeneic SC grafts are superior to allogeneic grafts for successful regeneration.

227.7

REGENERATION ACROSS PERIPHERAL NERVE ALLOGRAFTS WITH TEMPORARY OR CONTINUOUS CYCLOSPORIN A IMMUNOSUPPRESSION. R. Midha, S.E. Mackinnon and P.J. Evans. Peripheral Nerve Research Lab., University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

Peripheral nerve reconstruction would benefit from the use of nerve allografts, however life-long immunosuppression for non-vital organ transplantation is controversial. This study compares regeneration across peripheral nerve allografts in rats immunosuppressed with either temporary or continuous cyclosporin A. One hundred and fifty Lewis rats received 2 cm nerve grafts from allogeneic ACI or syngeneic Lewis donors, repaired to the sharply transected posterior tibial nerve of the hosts. Cyclosporin A (CyA) at 5 mg/kg/day subcutaneously is the sole immunosuppressive agent, given temporarily (12 weeks) or continuously from one day pre-engraftment to sacrifice. The graft recipients have been randomly allocated to the following groups: allogeneic grafts with continuous CyA for 12 (n=10), 18 (n=20) and 30 (n=20) weeks; allogeneic grafts with temporary CyA for 18 (n=20) and 30 (n=20) weeks; negative controls with allogeneic grafts and no CyA sacrificed at 12, 18 and 30 weeks (n=10, each); and positive controls with syngeneic grafts and no CyA sacrificed at 12, 18 and 30 weeks (n=10, each). Functional regeneration across the nerve grafts is being serially assessed by walking track analysis, performed every second week. Endpoint evaluations will include histologic, morphometric, and electrophysiologic determinations.

227.8

TOWARDS BANKING PERIPHERAL NERVES. P.J. Evans, D.C. Awerbuck, R. Midha, S.E. Mackinnon. Peripheral Nerve Lab., University of Toronto, Toronto, Canada, M5S 1A8.

We studied the possibility of storing peripheral nerves for later use in reconstructive procedures. The goal of preservation is to maintain an optimal nerve graft conduit through which proximal regenerating fibres can bridge and subsequently reinnervate distal muscle targets.

A 3.0 cm syngeneic (Lew/Lew) sciatic nerve graft model in the rat was used. Following harvesting, nerve grafts were stored at different *temperatures* (5°C, 22°C, 37°C) and for different *time periods* (6 hrs, 24 hrs, 3 weeks) in Belzer/UW CCS solution. Following preservation, nerve grafts were microneurosurgically interposed and repaired to the right sciatic nerve of recipient animals.

Recovery was assessed monthly by walking track analysis to generate a Sciatic Function Index. At sacrifice (14 months), electrophysiological and histomorphological studies of nerve regeneration, as well as gastrocnemius muscle contractile function studies were performed.

In nerves stored at 5°C no statistical difference was seen between storage times. There was a statistically decreased number of myelinated nerve fibres that regenerated across nerves stored at 22°C and 37°C. Nerves stored at 37°C had statistically slower conduction velocities than those at 5°C and 22°C when stored for 24 hours and 3 weeks.

Within this model, nerve autografts can be successfully stored at 5°C for up to 3 weeks for later use in reconstructive procedures. Regeneration was statistically better in 5°C stored nerves versus 22°C and 37°C stored nerves. It is likely that the 3 week stored nerves merely provide a nonviable conduit for proximal regenerating fibres.

227.9

TRANSPLANTS OF TARGET AND NON-TARGET NEURAL TISSUE SUPPORT REGENERATION OF AXOTOMIZED DORSAL ROOT AXONS IN NEONATAL RATS. E. Rinn, A. Adams*, J. Gomez*, and B.S. Bregman. Dept. of Anat. & Cell Biol., Georgetown Univ., Sch. Med., Washington, DC 20007

Transplants of fetal spinal cord tissue support the survival and growth of immature axotomized brainstem-spinal neurons in a target-specific manner. Although these descending axons initially grow into both target and non-target transplants, they subsequently withdraw from the non-target tissue. The aim of the current study was to determine the requirements of dorsal root ganglion (DRG) neurons, which are mature at birth, for axonal regrowth after central axotomy at birth. DRG neurons survive central axotomy at birth. Embryonic target (spinal cord (SC) E14) or non-target (cortex (CX) or hippocampus (HC) E18) transplants or non-neuronal extracellular matrix, (ECM, matrigel), were placed into hemisected lumbar spinal cord of neonatal rats (3 or 8 dpn). The L4 - L6 dorsal roots were cut and placed onto the transplant. Controls received no transplant. An antibody against calcitonin gene-related peptide (CGRP) was used to examine the regeneration of dorsal root axons at 1 week and 4 weeks after axotomy. Dorsal root axons regenerated into SC, CX, and HC transplants and ECM by 1 week and remained extended throughout the transplants at 4 weeks. The growth within the SC transplants was robust; highly branched fibers extended throughout the transplants. The regrowth of dorsal root axons into CX and HC transplants was less extensive; the fibers were thicker and less branched than in the SC transplants. ECM also supported regrowth of dorsal root fibers but the axons were disorganized, thick and mostly unbranched. Since both target and non-target transplants permitted the regeneration of dorsal root axons, non-specific growth cues may be provided. Mature neurons may require less precise growth cues for regeneration than immature neurons. Supported by NIH grants NS19259, NS27054 and NS01356 to BSB.

227.10

BIM 22015 ENHANCES MUSCLE FIBER DIAMETER IN PERONEAL NERVE CRUSH AND TRANSECTION MODELS. T.S. Lee, S.J. Lee, K.A. Williams and F.L. Strand. Department of Biology and Center for Neural Science, New York University, Washington Square, New York, New York 10003.

Sprague-Dawley male rats (175-200g) are subjected to peroneal nerve crush or nerve transection under ketamine (80mg/kg) and xylazine (5mg/kg) anesthesia. A #5 forceps is used for nerve crush, resulting in a 1mm wide lesion and a scissor is used for nerve transection. Starting at the time of surgery, ACTH 4-10 (10μg/48hrs i.p.) or an ACTH 4-10 analog (BIM 22015, 10μg/48hrs i.p.) is administered for 5 or 7 days for nerve crush, and 10 days for nerve transection studies.

Muscle fiber diameter was significantly increased with BIM 22015 treatment in both the nerve transection and nerve crush model. ACTH treatment resulted in muscle atrophy as compared to saline-crush at day 7. In the transected model BIM 22015 showed an enhancement of muscle fiber diameter in comparison to saline-crush. Previous studies have shown that the effects of these peptides on muscle fiber diameter is strikingly different. BIM 22015 affects muscle strength and speed whereas ACTH 4-10 affects muscle endurance.[1] ACTH 4-10 treated animals result in EDL muscle atrophy during regeneration whereas BIM 22015 treated animals do not. In addition, BIM 22015 seemed to enhance muscle fiber diameter by selectively affecting different muscle fiber types. This myotrophic property of BIM 22015 may have considerable clinical potential for diseases involving muscle atrophy.

[1] Saint-Come, C. and F.L. Strand. *Peptides* 9:215, 1988.

Supported by Biomeasure, Inc.

227.11

INSULIN-LIKE GROWTH FACTOR-II INCREASES REGENERATION OF MOTOR AXONS IN RATS. S.L. Near*[1], L.R. Whalen*[1], J.A. Miller*[3], J.F. Masken[2] and D.N. Ishii[2]. Dept. of Anatomy and Neurobiology[1], and Dept. of Physiology[2], Colorado State Univ., Ft. Collins, CO 80523; Amgen[3], Thousand Oaks, CA 91320.

Insulin-like growth factors (IGFs) can increase neurite growth in sensory, sympathetic and motor neurons in vitro, and IGF-II gene expression upregulates in denervated muscle during nerve regeneration (Ishii, Proc. Natl. Acad. Sci. USA 86: 2898, 1989). We tested the hypotheses that IGF-II can increase motor nerve regeneration in rats, and that endogenous IGF-II helps to support spontaneous regeneration.

Sciatic nerves were crushed (0.4 mm lesion), and miniosmotic pumps were implanted to continuously release either vehicle or IGF-II. After three days, the ventral roots at spinal cord level L4 were electrically stimulated, and evoked potentials were recorded to locate the most distal site of motor axon regeneration below the site of crush. The regeneration distance was statistically increased ($P < 0.05$) in the group treated with IGF-II. Moreover, this distance was reduced about 30% in rats treated with an anti-IGF-II antiserum. We conclude that exogenous IGF-II can increase the regeneration of motor axons, and infer that endogenous IGF-II plays a role in spontaneous regeneration. (Supported in part by NIH grant RO1 NS24787)

227.12

EFFECTS OF MIANSERIN AND FLUOXETINE ON THE AXONAL REGENERATION OF CENTRAL CATECHOLAMINERGIC NEURONS. S. Nakamura. Dept. of Physiology, Fac. of Medicine, Kanazawa Univ., Kanazawa 920, Japan.

We have reported that the antidepressants maprotiline and desipramine, which are potent noradrenaline (NA) reuptake blockers, induce regeneration of NA axons in the rat cerebral cortex (Neurosci. Lett., 111:64, 1990). In the present experiments, two different antidepressants mianserin (MIA) and fluoxetine (FLU), which reveal little or no effect on NA reuptake blockade, were examined. Female Sprague-Dawley rats, 8-16 weeks of age were used. Under chloral hydrate anesthesia, 6-hydroxydopamine (6-OHDA) was locally infused into the symmetrical sites of two hemispheres. One to 2 weeks after 6-OHDA pretreatment, the same cortical site of one hemisphere was infused with either MIA (gift from Dr. Watanabe) or FLU (gift from Eli Lilly) by means of an osmotic minipump, and the corresponding cortical site of the other hemisphere was infused with saline. After more than 2 weeks from the start of drug infusion, the density of glyoxylic acid-induced fluorescent catecholamine fibers was increased in the MIA- but not Flu-infused cortex. Since MIA is known to increase NA release by blocking presynaptic inhibition, the antidepressant-induced regeneration of cortical NA fibers may be associated in part with enhanced noradrenergic transmission. Supported by the fund for medical treatment of the elderly (Sclool of Medicine, Kanazawa University).

227.13

THE THERAPEUTIC POTENTIAL OF X-IRRADIATION IN LESIONED RAT SPINAL CORD. N.Kalderon and Z.Fuks+*. The Rockefeller Univ. and +Sloan-Kettering Cancer Ctr., New York, NY 10021.

The potential of x-irradiation, at clinical doses (e.g., eradicating malignant cells), to alter the cellular environment and events and thereby change the consequences of injury to adult mammalian CNS are being examined. We showed that a timed specific x-irradiation resulted in prevention of formation and/or elimination of reactive astrocytes which also enabled some of the regenerative processes in lesioned olfactory bulb to maintain their courses (PNAS 1990 87:10058). Presented here are the beneficial effects of x-irradiation on hemisectioned rat spinal cord (examined 2-3 mos postinjury) as studied based on the bulb data. Radiation (20Gy) of lesioned cord only when delivered at a critical period (15-18 days) postinjury led to elimination of gliosis at the wound, prevention of degeneration and cavitation, structural healing, and, most importantly, significant regeneration of the severed corticospinal (CS) axons beyond the cut. The number of severed CS axons growing past the lesion site is determined by the retrograde double-labeling procedure; dyes were applied: diI at lesion time into the cut, and fast blue after the recovery period 8-10mm distal to the cut. Degree of double-labeled CS neurons in irradiated (n=4) was 12-33% compared to 0-2% in unirradiated (n=5) animals. Axonal regeneration correlates with the degree of structural healing at the cord; hence an improved radiation regimen should enable regrowth of most of the severed axons beyond the cut, achieving one crucial step towards functional recovery.

227.14

ELECTROMAGNETIC FIELDS MODIFY NEW PROTEINS SYNTHESIZED IN RAT SCIATIC NERVE Betty F. Sisken, Martin Blank* and W. Kurtz*. Center for Biomedical Engineering and Dept. of Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY 40506; Dept. of Physiology, Columbia Univ., New York, NY 10032 and Bietic Research, Inc. Lyndhurst, NJ.

Stimulation of nerve regeneration in adult rats by pulsed electromagnetic fields (PEMF, 0.3 mT, pulsed for 20 msec at a repetition rate of 2 Hz, 4 hrs/day) after a crush or transection lesion has been reported. The mechanisms underlying the PEMF effects were investigated using transected rat sciatic nerves. The experimental animals were restrained and treated with PEMF for 4 hours/day for 5 days; the sham control animals were restrained but not treated. Two weeks later the transected and untransected segments of sciatic nerves from sham control and PEMF-treated rats were incubated in ^{35}S-methionine in methionine-depleted culture medium for 4 hours at 37^{o}C. The nerve sediments were analyzed by Protein Databases Inc., NY on 2D gels. The new polypeptides found were derived primarily from Schwann cells and fibroblasts found in the isolated nerves. Analyses of nerves from control and experimental animals indicated the presence or absence of new polypeptides in PEMF-treated nerves, and differences in the distribution of polypeptides based on molecular weight class as a function of transection and PEMF treatment.

227.15

APPLIED DC ELECTRIC FIELDS AND PERIPHERAL NERVE REGENERATION: AN ANALYSIS OF CRITICAL FACTORS. M. E. McGinnis. Center for Paralysis Research, Sch. of Veterinary Med., Purdue Univ., West Lafayette, IN 47907.

Several recent publications have reported an enhancement of peripheral nerve regeneration by application of low level DC currents. A variety of stimulation protocols, field strengths, and assay techniques have been used to obtain positive results. I have used a similar variety of techniques and have never detected a positive effect of applied fields on peripheral nerve regeneration. This led me to directly repeat several of the published experiments reporting positive results. Again I was unable to detect an effect of the applied fields (Abst # 479.2, 1990). Here I report an analysis of the factors that seem to be critical: current levels, current density, field strength, polarization effects, field patterns, and assay techniques. I compare my techniques yielding negative results with those that show an effect of fields. An example of the results is a possible explanation of why Román *et al.* (Exp. Neurology 98: 222-232, 1987) reported a 5-fold increase in the number of myelinated fibers regenerating through a silicone chamber with a 10μA cathode, and why we find a 2-fold decrease in the number of myelinated fibers under the same circumstances. I find there is an inhibition of regeneration through the tube due to electrode products produced by the cathode, specifically OH^-. This inhibition results in the formation of a neuroma-like structure just proximal to the cathode. Sections through the center of the tube just distal to the electrode show a lower number of fibers than controls, while sections 1 - 2 mm proximal have higher numbers than comparable control sections. Therefore, the apparent "positive" effect is dependent on the exact level of the histological sample and, ironically, is due to an inhibition of regeneration.

Supported by NIH grant NS 26251.

227.16

THIN-FILM MICROELECTRODE ARRAY FOR PERIPHERAL NERVE RECORDING AND STIMULATION

Gregory T. A. Kovacs*, Christopher W. Storment*, Carl R. Belczynski, Khoi D. Nguyen*, and Joseph M. Rosen* Stanford University Departments of Electrical Engineering and Surgery and the Palo Alto Veterans Administration Medical Center

A microelectrode array capable of recording from, and stimulating peripheral nerves at prolonged intervals after surgical implantation has been demonstrated. The microelectrode array, fabricated on a silicon substrate perforated by via holes, is implanted between the ends of a surgically severed nerve. Regenerating tissue fixes the array in place to provide a stable mapping between the microelectrodes and the axons in the nerve. Processes were developed for the fabrication of thin-film iridium microelectrodes, micromachined via holes, and silicon nitride passivation layers. All fabrication methods were designed to be compatible with standard active microelectronic circuit fabrication processes to allow for on-chip signal processing circuits in future designs. Devices with arrays of 64 via hole/microelectrode sites distributed over a 1mm^2 surface area were implanted bilaterally in the peroneal nerves of 9 male Sprague-Dawley rats. At post-operative durations of up to 13 months, the implants were surgically exposed and electrical probe needles used to make contact to probe points connected to the microelectrodes on the array. The arrays were used to record from and, in some cases, stimulate the nerve at up to 13 months post-operatively. While the initial design of these devices was not refined for optimal nerve regeneration, it allowed the demonstration of the basic principle and the durability of devices fabricated with the processes developed for this purpose. Optimization of the design in terms of minimal impact on nerve regeneration and electrical selectivity is currently underway.

227.17

BLOOD REPERFUSION FOLLOWING D.C. ELECTRICAL FIELD TREATMENT IN THE DAMAGED RODENT SPINAL CORD. M.J. Politis, M.F. Zanakis and B.H. Hallas. Dept. of Surgery, Univ. of Saskatchewan, Saskatoon, Saskatchewan, American BioInterface Corp., NY and New York College Osteopathic Medicine, NY.

Previous studies have demonstrated that DC electrical field application can result in increased axon populations in the damaged CNS. This process may involve axonal regeneration and/or a "sparing" of fibers. Since the changes occur rapidly, a vascular component is implicated. Thus, the following experiment was performed to determine the effects of DC electrical fields on reperfusion of damaged spinal cord. Adult rat spinal cords were lesioned using a 300 gm/cm weight drop. Animals were immediately implanted with TRAXON® CNS galvanic stimulators delivering 6μA at 3V DC throughout the length of the study. Control animals received inactive stimulators. The stimulating surfaces were positioned epidurally so that the cathode was located 5.0mm rostral to the lesion epicenter and the anode 5mm caudal. All animals were allowed to survive for 6 days and then sacrificed by transcardial perfusion of a Pelican ink solution. Spinal cord frozen sections were cut 1.0mm rostral or caudal to the lesion epicenter, and blood vessels were counted in a blinded fashion. The results showed that the animals treated with active stimulators had a statistically significantly greater number of vessels both rostral and caudal to the lesion than control animals. The vessels concentrated in the dorsal funiculi in all active stimulator animals. These data strongly implicate that reperfusion of damaged tissues is an early result of electric field treatment, and may be associated with the survivability or "sparing" of axons following CNS damage. Longer term evaluations are currently being performed.

227.18

RECONSTITUTION OF SYNAPSES FOLLOWING SURGICAL TREATMENT FOR INFANTILE HYDROCEPHALUS. J.P. McAllister, P.M. Hale*, R.A. Morano*, D.V. Shroff* and R.M. Kriebel. Depts. of Anatomy, Temple Univ. School of Medicine and Phila. Coll. Osteopathic Medicine, Philadelphia, PA 19140.

Controversy persists regarding the clinical decision of when to surgically decompress hydrocephalic children. To address this issue systematically, the present study was designed to analyze quantitatively the neuropil of hydrocephalic animals before and after ventriculoperitoneal (VP) shunting. Obstructive hydrocephalus was induced in 10 day old kittens by intracisternal injection of kaolin; VP shunts were placed in hydrocephalic animals at early (6-8 days post-kaolin) and late (10-15 days post-kaolin) stages of ventriculomegaly. Normal animals served as age-matched controls. Hydrocephalic animals were sacrificed at times corresponding to the immediate pre-shunt period, and about 7 days after shunt placement. Tissue from primary visual cortex was processed for electron microscopy and the number of synapses per unit area determined for superficial and deep laminae. To date, preliminary data indicate that in layers V and VI the number of synapses decreases 60% in hydrocephalic animals. While individual variations exist that appear to be related to the success of decompression, shunting largely restores the number of synapses in deep cortex (7% and less than 1% below control values for early and late shunts, respectively). Thus, early and late decompression seems equally effective in promoting recovery.

227.19

DISTRIBUTION AND CHARACTERIZATION OF RESPONSES TO LIGHT IN REINNERVATED HAMSTER SUPERIOR COLLICULUS. Y. Sauvé*, M. Rasminsky and D.A. Carter. Centre for Research in Neuroscience, McGill University, 1650 Cedar Ave., Montreal, Quebec, Canada H3G 1A4.

Axotomized rodent retinal ganglion cells (RGCs) can regrow axons for several cm into a peripheral nerve graft (PNG) apposed to the stump of the transected optic nerve and inserted into the CNS. The regenerated axon terminals form well differentiated, persistent and functional synapses with target neurons in the superior colliculus (SC) (Vidal-Sanz et al, J. Neurosci. 7:2894, 1987; Carter et al, J. Neurosci. 9:4042, 1989; Keirstead et al, Science 246:255, 1989). The extent and organization of SC reinnervation can be assessed physiologically by recording responses to light in the SC.

In adult hamsters examined 16-30 weeks after insertion of a PNG routed from the eye to the lateral aspect of the ipsilateral or contralateral SC, invasion of a substantial area of the SC by regenerated RGC axons was suggested by the finding of unitary responses to light within areas of the SC as large as 750 μm x 500 μm. Innervation of the SC by multiple regenerated RGC axons could be inferred from the observation of unique receptive fields for each of the visually responsive units. In some cases recordings were made from more than 20 different units, some of which could be identified as postsynaptic neurons rather than as RGC axon terminals by suppression of their responses to light by iontophoretic application of GABA. The visual fields of nearby SC units were frequently clustered, suggesting a tendency of axons regenerated from nearby RGCs to project together. However, the visual fields found for some closely spaced SC units identified as postsynaptic neurons were more widely separated than those found in recordings from the SC of intact animals in which a displacement of 20 μm represents a displacement of 1° in visual space.

227.20

PATTERNS OF THE ARBORIZATIONS MADE BY RETINAL GANGLION CELL AXONS REGENERATING INTO THE SUPERIOR COLLICULUS OF ADULT HAMSTERS. D.A. Carter*, G.M. Bray, and A.J. Aguayo. Centre for Research in Neuroscience, McGill University, 1650 Cedar Avenue., Montreal, Quebec, Canada, H3G 1A4.

Axotomized retinal ganglion cells (RGCs) in adult hamsters can regrow through peripheral nerve (PN) grafts and form persistent functional synapses in the superior colliculus (SC) (Carter et al., J. Neurosci. 9: 4042, 1989; Keirstead et al., Science 246: 255, 1989). We have now investigated the terminal arbors formed by RGC axons that penetrate the SC. To establish the retinal origin of the arbors studied, [^{3}H]-amino acids were injected into PN-grafted eyes. After 48 hours, HRP was applied into a small incision made in the graft near the SC; 12 hours later, the SCs were removed and incubated with DAB to visualize the fine details of the injury-filled terminal arbors. Forty-seven arbors labelled with both HRP and [^{3}H]-amino acids were studied from 6-9 months after the PN grafts were inserted into the SC. Double-labelled axons extended into the SC for up to 1 mm and remained confined to the retino-recipient layers of the SC. Their course was either directed towards the pial surface or followed a horizontal plane. These patterns resembled those of many normal RGC-SC arbors. However, many of the branches formed by the regenerated arbors were shorter than those of controls and their boutons were more densely clustered. In spite of these differences, many of the regenerated arbors had normal numbers of boutons.

227.21

FUNCTIONAL RECOVERY AND NEURONAL RECONNECTIVITY IN OLFACTORY TRANSPLANT AND OLFACTORY BULBECTOMIZED RATS. [1,2]K.R. Hendricks*, [2,3]L.E. Westrum, [2]J.N. Kott, [1]M.E. Hanson*, and [1]J.B. Simpson. Depts. of [1]Psychol., [2]Neurol. Surg., and [3]Biol. Struct., University of Washington, Seattle, WA 98195.

Recovery of function in rat and mouse after olfactory bulb (OB) transplants (OBT) and olfactory bulbectomies (OBE) is a controversial topic. We are examining the anatomical correlates involved in functional recovery. One day old Sprague-Dawley rats received unilateral OBE followed immediately by either an embryonic OBT or a gelfoam pack. At weaning, the rats were taught to find hidden pieces of cookie. When cookie-finding was reliable, the contralateral, normal OB was removed from all rats. Beginning one week after the second surgery, testing of cookie-finding was resumed. Some rats from both groups were immediately able to find the cookie. All rats in these two groups were able to find the cookie within 5 weeks, behaviorally demonstrating return of olfactory function. Histology was used to verify that the OBT had not been rejected (OBT material was labeled with tritiated thymidine) and that the lesions were complete. Olfactory Marker Protein (OMP)*, an olfactory nerve (ON) specific marker, combined with standard fiber and cell staining was used to show which brain regions were penetrated by ON fibers. Innervation of brain tissues by the ON was only achieved when the OBT or OBE occurred neonatally. The primary target of ON fibers in the lesion rats was the olfactory peduncle (OP; a target and relay area for OB and not ON efferents in the normal olfactory system). In one case, ON fibers innervated the nucleus accumbens as well. In the OBT rats, ON fibers innervated both the OBT tissue and the OP. The results suggest that in the absence of the secondary OB neurons in this pathway, the ON extends and connects directly to the "tertiary" neurons suggesting a mechanism for functional recovery. We are currently investigating the synaptic connectivity in OBT and OBE preparations. (Supported by NIH Grant NS 09678, and NS 07144. L.E.W. is an affliate of the CDMRC). *OMP was kindly provided by Dr. F. L. Margolis.

227.22

EVOKED SYNAPTIC POTENTIALS FROM REGENERATING DORSAL ROOT AXONS WITHIN INTRASPINAL FETAL SPINAL CORD TISSUE TRANSPLANTS. J.D. Houle, R.D. Skinner and K.L. Turner, Dept. of Anatomy, Univ. of Arkansas Med. Ctr., Little Rock, AR 72205

The apposition of injured dorsal roots (DR) into an intraspinal transplant of fetal spinal cord tissue results in the regeneration of sensory axons from the root, which can be promoted by co-grafting a nitrocellulose substratum treated with nerve growth factor (NGF). The present study tested whether these regenerating axons could form functional synaptic contacts with transplant and/or host neurons. Two to six months after transplantation, apposed DRs were cut and placed on hooked wires for electrical stimulation.

A ball tip electrode recorded cord dorsum potentials from the dorsal transplant surface and host spinal cord. A micropipette filled with 2 M NaCl was advanced through the transplant near the DR entry zone at 100 μm intervals. Negative intramedullary field potentials evoked by DR stimulation were maximal 400-700 μm below the dorsal surface, shifting to positive deeper into the transplant. Regions of maximum negativity indicated greatest density of active synapses. Single units were identified along the microelectrode tract by their negative spike potentials, sharp threshold, and temporal facilitation. Regenerated sensory axons were visualized by CGRP immunoreactivity. The results demonstrate structural and functional integration of regenerated nerve fibers with both transplanted and host spinal neurons.

Supported by NIH grant NS-26380.

TRANSPLANTATION: NEW TECHNOLOGY

228.1

FLUORESCENT LABELING OF FETAL CELLS FOR TISSUE CULTURE AND NEURAL TRANSPLANTATION. C. Paramore*, S. Meadows, R. D. Madison, and D. A. Turner. Neurosurgery and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710 and Durham VAMC.

One of the challenges in the field of neural transplantation is the absolute identification of grafted neurons in the host. A fluorescent marker to localize transplanted neurons in both physiological and anatomical preparations would be ideal. Potential benefits of labeling would include identification of cell type, identification of processes, and analysis of graft migration. We have used tissue culture as a model to test the feasibility of several fluorescent dyes for this application.

Fetal rat brain tissue (E12, E17) was harvested, mechanically dissociated, and incubated in the presence of the fluorescent labels rhodamine dextran amine and carboxy-fluorescein ester (CFSE). The cells were washed twice with medium, then plated on poly-L-lysine coated coverslips in 35 mm dishes at 37°C and 5%CO_2. Cultures were viewed and photographed live or after fixation with 4% paraformaldehyde at intervals up to one week. Cells labeled with rhodamine dextran were initially bright, but faded with time. Cells without processes appeared heavily labeled, while those with neurite-like processes were faint. At levels of 5μM, CFSE appeared toxic, as no processes arose from the cultured cells; however, at 0.05μM neurons were noted to put forth processes equal to controls, and the processes were well-labeled with dye.

We conclude that CFSE may be a suitable label for grafting of fetal rat brain cells, though longer periods in culture are being studied to determine the durability of the label. Parallel studies are being carried out on other labels, including DiI and latex nanosphere beads loaded with fluorescent dyes. Supported by B.S. Turner Foundation and the Alzheimer's Disease Association.

228.2

ANATOMICAL AND PHYSIOLOGICAL LOCALIZATION OF PRELABELED GRAFTS. G.K. Pyapali*, D.A.Turner and R.D.Madison. Neurosurgery and Neurobiology, Duke Univ. Med. Ctr. and Durham VAMC, Durham, N.C., 27710.

Neural grafting into CNS may restore function of the brain following pathologic insult. The anatomical and physiological analysis of grafted tissue could be facilitated with fluorescent labeling of grafted cells, ensuring that the graft is visible both at the time of physiological recording and during anatomical analysis. In this study we assess prelabeling dissociated fetal CNS cells with various fluorescent dyes and following the fate of these dyes after grafting, using both physiological and anatomical techniques.

Dissociated rat fetal cells (E12, E17-E20) were grafted into adult host hippocampus. The fetal cells were incubated with one of a number of fluorescent compounds (rhodamine beads, Cascade Blue beads, rhodamine-dextran-amine, DiI and carboxyfluorescein) at the time of the dissociation. The labeled cells were stereotaxically placed as a suspension into normal and lesioned host hippocampi. The prelabeled grafts were located using fluorescence optics 4-6 weeks later within live, *in vitro* hippocampal slices of the grafted host. Neurons within the graft were injected with Lucifer yellow. The rhodamine labels were the best in terms of intensity of labeling, percent of cells labeled, preference for labeling live cells, low toxicity and visibility in live slices. The processes of labeled cells were moderately integrated into the host hippocampus, as labeled by rhodamine-dextran-amine and Lucifer yellow.

The ability to prelabel the dissociated cell suspensions prior to grafting facilitates the identification of grafts for analysis of integration of grafted neurons. This technique will also improve our understanding of the survival, differentiation and migration of grafted neurons and their potential to restore function in the lesioned CNS. Supported by ADRDA and VAMC Research.

228.3

A CULTURED MULTIELECTRODE-CELL APPARATUS TO PROBE CNS INJURY. M.F. Zanakis, A. Feller*, B.H. Hallas, P.A. Femano*, D. Schwartz*, M. Sussman* and R. Cebelenski, H. Yoon* and M.R. Wells, American BioInterface Corp., NY, NY College of Osteopathic Medicine, NY, and Veterans Administration, Northport, NY.

Earlier experiments demonstrated that a multielectrode cable (MEC) could be constructed where fetal neurons (probes) could be cultured and monitored *in vitro*. These probes, along with the supporting MEC assembly, will be used for implantation into a damaged host CNS structure (such as the spinal cord). The synapse of damaged host neurites on the fetal probe cells will provide an opportunity to indirectly monitor host neuronal activity. With signal processing, monitoring host activity may allow us to more clearly understand the interconnections between these structures, and may ultimately allow us to stimulate musculature which was denervated by spinal cord injury. We have performed experiments using both rodent cardiac myocytes as well as dissociated fetal rat cortical cells as "test" and "actual" probe cells, respectively. The MEC surface is a group of 10μ diameter Pt/Ir lead wires encased in a polystyrene matrix. The MEC surface is polished and then prepared for cell adhesion by propane firing of the surface followed by layering of a protein mixture. The MEC assembly, varying from 30 to 200 electrodes, was tested on a support structure which includes the electrode lead wires, a channel scanner/selector and preamplifiers. Constant temperature is maintained and video imaging of the MEC surface (to identify cells) is provided. *In vitro* electrophysiological results through the MEC, verified by standard extracellular electrophysiological techniques, indicate that the MEC can provide good signal fidelity of cultured cells, while maintaining good cell viability. Stability testing indicates that probe cells on the MEC surface can survive implantation into the host CNS if the MEC surface is protected.

228.4

ENCAPSULATED PC12 CELLS AMELIORATE SPONTANEOUS AND DRUG-INDUCED BEHAVIORS IN THE 6-OHDA LESIONED RAT. D.F. Emerich, P.E. McDermott*, L. Christenson, M.A. Palmatier, F.A. Kaplan, T.R. Flanagan, M.P. Lavoie*, B.R. Frydel*, F.T. Gentile* and P.R. Sanberg, Cellular Transplants, Inc., Providence RI 02906.

Encapsulation of dopamine-secreting cells into a permselective polymer membrane has been proposed as a possible treatment for Parkinson's disease (PD). This technique provides an effective immunoprotective barrier for the encapsulated cells and permits the use of allogeneic or xenogeneic tissue without the need for immunosuppression of the recipient. Encapsulated PC12 cells ameliorated apomorphine-induced rotational behavior in 6-OHDA lesioned rats following implantation into the denervated striatum (Winn et. al., *Soc. Neurosci. Abstr.*, 16:39, 1990). In the following studies we further characterized the extent of behavioral recovery following implantation of encapsulated PC12 cells.

PC12 cells were coextruded into a polymer fiber, sectioned into 4 mm lengths and cultured in vitro to establish a basal level of dopamine metabolite release using HPLC. Following unilateral 6-OHDA lesions of the substantia nigra animals were tested for apomorphine-induced (0.1 mg/kg s.c.) rotational behavior and on a battery of sensorimotor and limb use tests. Lesioned rats exhibited a pronounced motor asymmetry in all tasks. Six weeks following surgery, animals received intrastriatal implants of encapsulated PC12 cells or empty, non PC12-containing capsules. At 2 and 4 weeks post-implantation those animals receiving PC12 implants exhibited a significant (50%) reduction in the number of apomorphine-induced rotations as well as a significant normalization of the aysmmetries observed using the sensorimotor and limb use tests. There were no beneficial or detrimental changes observed following implantation of empty capsules. This study further supports the previous evidence that implantation of encapsulated neurotransmitter-secreting cells may provide a useful treatment for PD and other human degenerative diseases characterized by secretory cell dysfunction.

228.5

AN ENCAPSULATED PC12 CELL LINE RELEASES LEVODOPA CONSTITUITIVELY *IN VITRO*. T.R. Flanagan, M.P. Lavoie*, F.A. Kaplan, W.J. Bell*, M.A. Palmatier, F.T. Gentile*, and P.R. Sanberg. Cellular Transplants, Inc., Providence, RI 02906.

Immunoisolation of xenogeneic cells allows a means of exploring their potential for cellular replacement therapies. Immunoisolation methods have been developed to produce polymer encapsulated PC12 cells for the treatment of experimental Parkinsonism. Suspensions of PC12 cells are co-extruded with a polymer solution to encapsulate cells within a hollow fiber. These fibers are designed with molecular weight perm-selectivities to restrict host immunoglobulin and complement access to encapsulated cells while permitting metabolite diffusion.

Encapsulated PC12 cells provide an interesting experimental system for characterizing changes in the aggregate properties of cell populations when compacted in a three dimensional array. It is well documented that PC12 cells synthesize, sequester, and also constitutively release dopamine. Previous studies have described surface-contact and density-dependent physiological responses among PC12 cells. Following encapsulation, PC12 cells exhibit evoked release of dopamine and also release of dopamine metabolites. Under basal release conditions, an encapsulated broadly available PC12 cell line primarily releases two dopamine derived metabolites; homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC). Under basal release conditions these encapsulated cells also release substantial amounts of the dopamine precursor levodopa. Thus, this cell line may provide a product for a constitutive local release of levodopa when implanted into Parkinsonian models.

While the basal release of metabolites from encapsulated cells cultured *in vitro* is a limited model for predicting the physiological performance of these encapsulated cells *in vivo*, we feel that this cell line offers an attractive system to study pharmacological manipulations which could optimize basal and evoked release of dopamine from this broadly available PC12 cell line after encapsulation.

228.6

IMMUNOCYTOCHEMICAL ANALYSIS OF ALLOGENEIC AND XENOGENEIC PC12 CELL IMPLANTS INTO STRIATUM. B.R. Frydel*, D.F. Emerich, P.E. McDermott*, F.A. Kaplan, M.A. Palmatier, L. Christenson, H. Duncan, and P.R. Sanberg. Cellular Transplants, Inc., Providence, RI 02906.

Transplantation of encapsulated dopamine-secreting cells has been proposed as a treatment for Parkinson's disease PD (Aebischer et al. *Exp. Neurol.* 111:269,1991). PC12 cells encapsulated within polymeric membranes survive for extended periods of time and reverse the behavioral deficits in both rodent and primate models of PD (Aebischer et al., *Soc. Neurosci. Abst.*16:393,1990). Because PC12 cells are tumor-derived, there is the potential for tumor formation if the cells escape from the capsule or the capsule is mechanically damaged during implantation. The following studies examined the survival and possible tumor formation of unencapsulated PC12 cells implanted into the striata of rats (allograft) and guinea pigs (xenograft). PC12 cells were suspended in RPMI at a concentration of either 10^4, 10^6, or 10^8 cells/4ul and were stereotaxically injected into rat and guinea pig striata. Animals were sacrificed at 1, 4, or 12 weeks following surgery and their brains were processed and stained for tyrosine hydroxylase (TH), dopamine beta hydroxylase (DBH), glial fibrillary acidic protein (GFAP), a cell surface marker for PC12 cells (Thy-1), Nissl substance, and hemosiderin. All rats injected with PC12 cells developed tumors within 1-2 weeks, with an associated 50% mortality. All surviving rats exhibited a massive growth of PC12 cells upon necropsy. In contrast, guinea pigs with PC12 cell implants survived at all time points with no observed mortality. Histological analysis showed that at one week after implantation 75% of animals had small clusters of cells, at 4 weeks 10% had some surviving cells, and at 12 weeks no surviving cells were observed. At 12 weeks post-implantation there remained a modest glial response. The survival of PC12 cells was not found to be dependent on the initial concentration implanted. These data suggest that PC 12 cells do not survive unencapsulated in a xenogeneic host. Furthermore, the use of encapsulated PC12 cells may be a viable and safe approach for treating human disease such as PD.

228.7

PHYSICOCHEMICAL CHARACTERIZATION AND MICROIMMUNO-CYTOTOXIC CHALLENGE OF MEMBRANE FOR CELL OR TISSUE TRANSPLANTATION. F.A. Kaplan, T.F. Hazlett*, M.P. Lavoie*, F.T. Gentile*, P.R. Sanberg, and L. Christenson. Cellular Transplants, Inc. Providence, RI 02906

Transplantation of endocrine or transmitter-secreting cells immunoisolated within a permselective polymeric membrane represents a novel therapeutic modality. In order to characterize the membrane transport properties, the rejection coefficients (R) of marker molecules of varying molecular weights (M) were measured with stirred filtration cells.

Marker Molecule	M	R (±10%)
Dextran	2,000,000	0.98
Bovine serum albumin	67,000	0.94
alpha-Chymotrypsinogen	24,000	0.25
Cytochrome C	12,500	0.12
Vitamin B12	1,400	0.05

Molecules the size of immunoglobulins do not cross the membrane, while molecules even larger than dopamine are freely permeable. Diffusive transport of dopamine in the membrane was determined in separate experiments: D_{mem}/D_{H2O} = 0.85±0.05. We then incubated capsules containing PC12 cells with an IgM antibody specific for the Thy-1.1 surface marker (clone HO-22-1) at titers 1:1, 1:4, and 1:8. Control wells contained unencapsulated PC12 cells, PC12 cells in capsules in which pin-holes had been deliberately created in the walls, and in capsules with incomplete seals. Samples were incubated with the antibody for periods of 2 hours, 3 days, and 8 days to allow the antibody to bind, at which time fresh rabbit complement (1:4, 1:20, 1:40) was added to the wells for 2 hours. Cell viability was assessed by uptake of propidium iodide (PI) using fluorescent microscopy. Controls exposed to anti-Thy-1.1 and complement took up PI at all time points measured, indicating that their cell membranes were damaged by the antibody and complement. In contrast, PC12 cells encapsulated in intact capsules showed the same viability as did capsules maintained for the same period of time. Cells encapsulated within this polymeric membrane are protected from complement-dependent, antibody-mediated cytolysis, while allowing passage of vital nutrients. NIH grant 1 R43 NS28992-01.

228.8

PC12 CELLS ENCAPSULATED IN AN IMMUNOISOLATORY POLYMER MEMBRANE SURVIVE FOR EXTENDED PERIODS *IN VITRO*. M.A. Palmatier, F.A. Kaplan, W.J. Bell*, F.T. Gentile*, T.R. Flanagan and P.R. Sanberg. Cellular Transplants, Inc., Providence RI 02906

Encapsulation of allogeneic or xenogeneic cells in an immunoisolatory synthetic thermoplastic polymer membrane provides a tool for the exploration of cellular replacement therapies without immunosuppression of the recipient. For example, implantation of PC12-containing capsules alleviates parkinsonian behaviors in animal models (Winn et al. Soc. Neurosci. Abst. 16:39, 1990).

Using a co-extrusion process PC12 cells can be encapsulated in a hollow polymer fiber with a perm-selective membrane. The fiber can be cut into segments and the ends sealed to form capsules which can be incubated *in vitro*. The present study was designed to determine the growth characteristics of encapsulated PC12 cells during extended *in vitro* culture.

PC12 capsules were incubated *in vitro* and MTT assays (Mosmann, J. Immuno. Methods 65:55, 1983; Uludag and Sefton, Biomaterials 11:708, 1990) were done at various times to determine the metabolic activity of the surviving cells. After 6 weeks *in vitro*, 4 mm long capsules loaded at 15 million cells per ml displayed the same metabolic activity as capsules assayed one week after fabrication (equivalent to approximately 15,000 unencapsulated cells). Two cm long capsules loaded at 5 million cells per ml showed gradually increasing metabolic activity over 6 weeks, approximately equivalent to 15,000 unencapsulated PC12 cells at 1 week and 40,000 unencapsulated PC12 cells at 6 weeks. Metabolic activity in the 2 cm long capsules, equivalent to approximately 40,000 unencapsulated PC12 cells, remained stable over the period from 6 to 15 weeks *in vitro*.

Encapsulation of PC12 cells in capsules of a synthetic polymer membrane provides a method for immunoisolating these cells, yet allows uncompromised metabolic activity of these encapsulated PC12 cells *in vitro*.

229.1

IMPLANTATION OF THE IMMORTALIZED HIPPOCAMPAL CELL LINE HIB5 INTO DEVELOPING AND MATURE BRAIN SITES: CHARACTERIZATION, EPIGENETIC INFLUENCE, AND IMPLICATIONS TOWARD FUNCTIONAL RECOVERY. M. G. Cunningham*, P. J. Renfranz, and R. D. G. McKay. Department of Brain and Cognitive Sciences, M.I.T., Cambridge, MA, 02139.

Immortalized cell lines were established from embryonic day 16 rat hippocampal primary cultures in order to address developmental issues and to assess the capacity of such cell lines to serve in brain cell replacement in disease states. The HiB5 cell line, one of a number of nestin-positive cell lines established, was reintroduced into the developing brain, specifically, the postnatal-day-2 hippocampus and cerebellum. A stereotaxic technique was used for implantation along with a combination of cell labeling techniques. HiB5 cells demonstrated a striking ability to integrate into both these sites and appeared to localize to neurogenically active regions within them. The morphologies assumed by the implanted cells were indistinguishable from those of endogenous neurons and glia that are being generated at the time of the implant. HiB5 cells were also implanted into the hippocampus and cerebellum of young and aged adults and showed much less integration and differentiation. Nevertheless, many of the implanted HiB5 cells survived beyond three months. Present studies are being conducted to determine whether these cells take on functional roles, and to investigate the potential of such cell lines in reestablishing specific cell populations in lesioned brain sites.

229.2

RAT ASTROCYTES CONTAINING A MOUSE NGF TRANSGENE ENHANCE THE SURVIVAL OF BOTH YOUNG POSTNATAL AND ADULT ADRENAL CHROMAFFIN CELLS GRAFTED INTO THE ADULT RAT STRIATUM. L.A. Cunningham, J.T. Hansen, M. P. Short[1], M.C. Bohn. Dept. of Neurobiology and Anatomy, University of Rochester Medical School, Rochester, NY 14642 and [1]Molecular Neurogenetics Unit, MGH, Harvard Medical School, Boston, MA 02114

Our previous studies demonstrated that primary rat astrocytes infected with a retroviral vector harboring a mouse ß-NGF transgene support the survival and neuronal differentiation of young postnatal adrenal chromaffin cells in intrastriatal grafts. The aim of the present study was to determine whether these effects were dependent upon adrenal age. Suspensions of partially purified chromaffin cells were prepared from postnatal day (PD) 12 or 90 rat adrenal medulla and grafted into the adult 6-OHDA-lesioned rat striatum alone (AC), or at a 1:1 ratio with either normal (AC + AS) or genetically altered astrocytes (AC + ASN.8). Surviving chromaffin cells were identified by tyrosine-hydroxylase immunoreactivity (TH-IR) two weeks after grafting.

Table 1.Number of surviving TH-IR chromaffin cells.*p<0.01

	AC	AC + AS	AC + ASN.8
PD12	1234 ± 197*	552 ± 97	3075 ± 77*
PD90	----	551 ± 325	2782 ± 750*

The genetically altered astrocytes enhanced the survival of both PD12 and PD90 chromaffin cells ~3-6 fold over control groups (Table 1). Histological evaluation revealed that PD12 chromaffin cells display extensive neurite-outgrowth when co-grafted with the genetically altered astrocytes, whereas PD90 chromaffin cells remain endocrine in morphological phenotype. Thus, the effect of the genetically altered astrocytes on chromaffin cell neurite-outgrowth, but not chromaffin cell survival, appears to be dependent on adrenal age.

(Supported by PEW Charitable Trust and NIH grants NS25778, NS08906).

229.3

TRANSPLANTATION OF POLYMER-ENCAPSULATED NGF-RELEASING CELLS PREVENTS LESION-INDUCED REDUCTION IN ChAT EXPRESSION BY SEPTAL CELLS. D. Hoffman[1], X. Breakefield[2], P. Short[2] and P. Aebischer[1]. [1]Division of Biology and Medicine, Brown University, Providence, Rhode Island; [2]Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts.

The delivery of nerve growth factor (NGF) to the lateral ventricle of a fimbria-fornix lesioned rat prevents the lesion-induced reduction in choline acetyltransferase (ChAT) expression by medial septal cells. The use of cell lines genetically engineered to produce NGF as a means of delivery poses the inherent threats of tumor formation and/or immune rejection. Transplantation of cells isolated within a permselective polymer capsule separates the cells from the host tissue and allows exchange of oxygen, nutrients, neurotransmitters, and other factors, while providing mechanical support and immunoprotection. Rat fibroblasts genetically engineered to produce NGF (R208N.8) were suspended at a density of $1x10^6$ cells/µl in a laminin-containing hydrogel, and surrounded by a permselective poly(acrilonitrile/vinyl chloride) membrane. Empty capsules and encapsulated control fibroblasts (R208F) were used as controls. The capsules were placed in culture with PC12 cells. Encapsulated fibroblasts remained viable after 2 weeks in vitro. R208N.8 capsules induced neurite extension from PC12 cells beginning at 4 days, while empty capsules and R208F capsules elicited no response. Unilateral aspirative fimbria-fornix lesions were performed in adult rats, and an empty or R208N.8 capsule was implanted into the ipsilateral lateral ventricle. After 2 weeks, ChAT(+) cells were evaluated in the medial septum. A significant prevention of ChAT(+) cell reduction was observed in rats receiving R208N.8 capsules, similar to that induced by NGF-releasing polymer rods. No undue reaction to the implants was noted, and viable fibroblasts were observed within the implanted capsules. These studies suggest that encapsulated genetically engineered cells may provide an efficient means for future applications involving delivery of neurotrophic factors.

229.4

TRANSPLANTATION OF AN IMMORTALIZED CELL LINE (A7) INTO THE RAT BRAIN. M. Isono*[1], H. M. Geller[2], M. Poltorak[1] and W. J. Freed[1]. [1]NIMH, Neuroscience Center at St. Elizabeths, Washington D. C., 20032, [2]Dep. of Pharmacology, UMDNJ-Rutgers Medical School, Piscatway, NJ 08854

The A7 cell line is an astrocyte-like cell from the rat optic nerve immortalized by retroviral transfer of the SV40 large T antigen (H. M. Geller and M. Dubois-Dalcq, J. Cell Biol. 107: 1977-1986, 1988). To investigate the properties of these cells as possible source of tissue for brain transplantation, A7 cells were transplanted into rat brains and the graft-host interaction was examined immunohistochemically. In 26 adult rats, dissociated A7 cells (25,000 cells in 5 ul) were transplanted stereotaxically into the hippocampus. After 2, 6 and 8 weeks, the brains were examined using indirect immunofluorescence staining. Survival of the transplanted cells was confirmed with Hematoxylin and eosin staining and with staining by an antibody against SV40 large T antigen. The cells grew focally and did not invade the surrounding brain tissue. In 3 of 26 animals, well encapsulated clumps of cells, which were probably displaced during the injection, were found in the subarachnoid space. After transplantation, the A7 cells retained immunocytochemical properties observed in culture. GAP-43 and N-cadherin, which were not expressed by A7 cells in culture, were strongly expressed throughout the graft and in surrounding brain tissue. This suggests integration of the A7 cells into the host brain structure and invasion of host neuronal elements into the graft area. No T lymphocyte infiltration was observed, although MHC class 1 immuno-reactivity was enhanced in the host tissue. These results suggest extensive interactions between transplanted A7 cells and host brain tissue, and suggest that this cell line may induce growth or anatomical alterations in surrounding regions of the host brain.

229.5

HSV-1 VECTOR-MEDIATED GENE TRANSFER: GENETICALLY MODIFIED POST-MITOTIC CELLS IMPLANTED INTO RAT STRIATUM EXPRESS A FOREIGN GENE.

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Herpes Simplex Virus (HSV-1) vectors (Science 241, 1667, 1988; PNAS, USA 87, 1149, 1990) are the only available method to deliver genes into postmitotic cells such as neurons; retrovirus vectors require a round of mitosis for integration. A potential gene therapy approach for Parkinson's Disease is to deliver genes into neurons using HSV-1 vectors and transplant these neurons into the adult brain. This strategy requires that the transfected neurons survive intracerebral implantation and stably express a foreign gene.

To develop such a system we used a HSV-1 vector (pHSVlac) expressing E.coli-ß-galactosidase (ß-gal); to optimize conditions for subsequent implantation, we examined infection parameters in culture using PC12 cells and cerebral cortex cells from rat embryos (E14). The highest infection rate was obtained by exposing cells to 10 infectious particles/cell at 37°C for 3 h. Infected cells were transplanted into the striatum of adult male rats (250-280g) by unilateral injection (5µl, 2 x 10^4 PC12 cells or 3.3 x 10^4 cortical cells). Expression of ß-gal was assayed 1-5 days after implantation; perfused brains were sectioned at 50 µm with a freezing microtome, and ß-gal was detected using X-gal or immunohistochemistry with rabbit anti-ß-gal-antibodies. Numerous ß-gal-positive cells were observed near the injection site using both assays. Thus, we have shown that pHSVlac infected cells survive implantation and express a foreign gene for least 5 days. The long-term survival of the transplanted cells and stable expression of ß-gal are currently under study.

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229.6

TRANSPLANTATION OF PRIMARY GLIAL CELLS THAT PRODUCE DOPA AFTER RETROVIRAL TH GENE TRANSFER IN THE RAT MODEL OF PARKINSON'S DISEASE.

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Dissociated striatal and mesencephalic cells from E15 rat fetuses were grown in the presence of 10% FCS, i.e. conditions allowing glial cell division. The cells were infected using recombinant retroviruses in which the human TH-I gene was placed under the control of either a CMV or a LTR promotor. The infected cells were cultivated for four weeks in the presence of 10% FCS. The production of DOPA was assayed by HPLC analysis of supernatants collected over an 24 hr incubation period. Most of the cells were found to be GFAP positive, suggestive of an astrocytic nature, and many showed TH immunoreactivity. Four dishes (each approx.5 million cells) exhibiting the highest DOPA production were selected for transplantation.

About 450 000 cells/ rat were implanted as a cell suspension into the striatum in rats with unilateral 6-OHDA lesions. Two weeks after grafting apomorphine-induced turning (but not amphetamine-induced turning) was reduced by an average of 45%. Microdialysis, through a probe placed in-between the three transplant deposits, was performed about three weeks after transplantation. From both types of cells dopamine overflow was detected in the perfusate, and it was increased after additon of depolarizing concentration of KCl. DOPA, by contrast, remained low during baseline conditions and after KCl, suggesting that DOPA released from the transplanted cells was rapidly decarboxylated by the host striatum.

The same gene transfer methods are now also being explored with the GABA synthesising enzyme. Glutamate decarboxylase cDNA has been expressed in various cell lines using the retroviral vector containing a CMV promotor. Cells showing high levels of GABA production are beeing characterized *in vitro* and transplanted to the ibotenic acid lesioned striatum of adult rats.

229.7

PRIMARY FIBROBLASTS GENETICALLY MODIFIED TO PRODUCE NERVE GROWTH FACTOR EMBEDDED WITHIN A COLLAGEN MATRIX ACT AS A PERMISSIVE GRAFT FOR AXONAL REGENERATION *IN VIVO*. M.D. Kawaja and F.H. Gage. The Department of Neurosciences, U.C.S.D., La Jolla, CA 92093-0624.

Grafts of a collagen matrix containing primary skin fibroblasts genetically modified to produce nerve growth factor (NGF) may serve as an unique graft environment for regenerating septal axons following fimbria-fornix (FF) ablation. Septal axons stained for acetylcholinesterase (AChE) were found within the deafferented hippocampus of female Fischer 344 rats with grafts of collagen/NGF-producing fibroblasts; the density of these fibers was variable among the different animals and was higher after eight weeks than at four weeks. In those rats that received grafts of collagen/control non-infected fibroblasts, the deafferented hippocampus was often devoid of AChE-positive fibers; a few axons were occasionally seen in the medial-most hippocampus. Data concerning the ultrastructural features of collagen/fibroblast grafts at eight weeks after unilateral FF lesion and implantation revealed that all grafts contained numerous fibroblasts with elongated processes passing along dense bundles of collagen. Capillaries, astrocytes and their processes, and blood-borne cells (e.g., plasma cells, lymphocytes) were also seen. In the NGF-producing grafts, variable numbers of small unmyelinated axons were surrounded by astrocytic processes. Axons were also closely associated with Schwann cells or the basal lamina of capillaries, while others were found within the graft extracellular matrix. In control grafts, small numbers of unmyelinated axons were associated predominantly with Schwann cells; few axons were found in the extracellular matrix of the grafts. These data reveal that this new grafting material - NGF-producing primary fibroblasts within a collagen matrix- can provide a conducive environment for regenerating cholinergic axons from the medial septum. Further, this unique grafting material allows for an ultrastructural assessment of the cellular and matrix substrates preferred by regrowing NGF-sensitive axons within the adult rat brain. Supported by AG08514 and NS28212.

229.8

Effect of Cytokines on Transgene Expression from the Moloney Murine Leukemia Virus LTR (MoMLV) and Collagen α2(I) Promoters in Fibroblasts. M. Schinstine, J. Ray, and F. H. Gage, Dept. of Neurosciences, UCSD, La Jolla, CA 92093.

Recently we have shown that MoMLV transgene expression is reduced in quiescent fibroblasts (Schinstine et al., submitted). In addition to becoming quiescent, fibroblasts transplanted into the CNS have mononuclear phagocytes associated with the core of the graft. These cells secrete many types of cytokines, including Il-1β, TGFβ1, and TNFα. The current study was undertaken to determine if these molecules can influence, and possibly be used to modulate, transgene expression.

Different reporter genes driven by the MoMLV or the collagen α2(I) promoter were transduced into primary dermal fibroblasts. The expression of the MoMLV-driven reporter gene was greatest in fibroblast cultures containing proliferating cells. Fibroblasts maintained in a postconfluent state for 7 days exhibited a ~50% decrease in reporter gene activity. The activity of the reporter gene driven by the collagen promoter was comparable in cultures containing growing or quiescent fibroblast. Thus, it appears that MoMLV-driven transgene expression decreases in quiescent cells, whereas collagen promoter-driven expression remains stable. Cytokines were used to test their influence on transgene expression in cultures containing quiescent cells. IL-1β (10 u/ml) and TNFα (120 ng/ml) did not affect transgene expression driven by MoMLV. TGFβ1 decreased transgene expression by ~50%. In comparison, IL-1β caused a 25% reduction in transgene expression driven by the collagen promoter. TNFα and TGFβ1 administration caused a ~200% and ~300% increase in transgene expression, respectively. These results indicate that cytokines differentially effect the MoMLV and collagen promoters. Data on the expression of cells that have been implanted into the brain will be presented.

Supported by AG08514 and an UC President's Fellowship to M.S.

229.9

IN VITRO AND IN VIVO CHARACTERIZATIONS OF PRIMARY FIBROBLASTS GENETICALLY MODIFIED TO PRODUCE ACETYLCHOLINE. L.J. Fisher, M. Schinstine, K.L. Eagle, K.S. Chen and F.H.Gage. Dept. Neurosci., Univ. of California, San Diego, La Jolla, CA 92093-0624.

In animal models of cognitive impairments, cholinergic-rich fetal neurons implanted within the brain have been found to reduce learning and memory deficits. Genetically modifying cells may be an alternative method for obtaining donor cells for intracerebral grafting which synthesize and release desired products. In the current work, primary skin fibroblasts from Fischer 344 rats were infected with a retroviral vector containing the cDNA for *Drosophila* choline acetyltransferase (dChAT), the enzyme which catalyzes the conversion of choline and acetyl-CoA to acetylcholine (ACh). Following selection, the ChAT activity of the primary fibroblasts was 2326.7 nmoles ACh/hr/mg protein. In situ hybridization and immunohistochemistry conducted on cells in vitro revealed that both dChAT message and protein was produced from the transgene. The ChAT enzyme was biologically active, catalyzing the formation of ACh within these cells. Approximately 16% of the ACh synthesized by the transduced cells was released into the extracellular medium. Administering the ACh precursor choline to the transduced cells in vitro was found to influence the production of ACh. Choline chloride levels ranging from 100 μm-500 μm increased extracellular ACh levels 75% to 288%. These ACh-producing fibroblasts have been implanted within the hippocampus of fimbria-fornix lesioned Fischer 344 rats. Some rats examined at 3 weeks post-grafting have been found to contain surviving grafts with fibroblasts which appeared to show positive immunoreactivity for dChAT. Currently, we are conducting experiments to assess whether ACh continues to be synthesized and released by the dChAT-expressing cells following implantation.
This work is supported by AG08514, UC President's Fellowship (M.S.). L.J.F. is a NINDS Javits Junior Clinical Investigator.

229.10

INTRAHYPOTHALAMIC GT1 CELLS DIFFERENTIATE AND STIMULATE REPRODUCTIVE DEVELOPMENT IN HYPOGONADAL MICE. A.-J. Silverman, J.L. Roberts, K.W. Dong*, G.M. Miller & M.J. Gibson, Dept. Anat.&Cell Biol., Columbia Univ., New York, NY, 10032; Dept.Med.and Fishberg Ctr. Neurobiol.,Mt.Sinai Sch Medicine, New York, NY,10029.

Bilateral injections of GT1-7 cells were made into the anterior medial hypothalamus of adult mutant hypogonadal (hpg) mice, which fail to produce GnRH. GT1 cell lines are immortalized neurons developed by genetically targeted tumorigenesis, that produce and secrete GnRH in culture. Neuronal-appearing cells with a plexus of fibers innervated the host brain at 1wk. Cells were immunoreactive to GnRH antibodies LR1, 1076 (detect precursor and product), and EL14 (which reacts with product only), suggesting complete processing of the GnRH prohormone in grafted cells. Numerous differentiated cells were seen in the hpg host brain at 5wk. Significant gonadal development in response to GT1-7 cells was evident in all 3 male hpgs studied 10 wk after grafting, with testes wts: 19.2, 20.4, and 33.8 mg (vs. untreated 7.6 mg). Two hpg female mice responded to GT1-7 implants with vaginal opening within 1wk, reflecting ovarian stimulation; at 10wk one hpg had significant uterine development (36.0 mg). We conclude that GT1 implants in hpg mice provide a powerful model to evaluate the utility of cell lines for neuronal transplants. Support: NS20335 (MJG) & HD19077 (MJG).

229.11

IN VITRO STRATEGIES FOR IDENTIFYING TRANSPLANTED PRIMARY CNS TISSUE AND NEURONAL CELL LINES. S. M. Onifer, L. A. White, S. R. Whittemore, and V. R. Holets. The Miami Project and Dept. of Neurological Surgery, University of Miami, Miami, FL 33136.

To address the fate of primary CNS tissues or genetically engineered cell lines after transplantation into the CNS, the transplanted cells must be labeled so as to be distinguished from the host neuropil. We reasoned that an optimal label could be chosen by first examining its fate in vitro. Using 11 different labels, we determined which labels were maintained in vitro by neurons and non-neuronal cells derived from fetal medullary raphe nuclei and by RN33B cells, a temperature-sensitive neuronal cell line derived from fetal medullary raphe (see White and Whittemore). Labelled RN33B cells were grown in clonal culture or in co-culture so as to model how the labeled cells might behave in vivo. The appropriate labels for fetal raphe neurons and RN33B cells were then examined in vivo after transplantation into the CNS.

In vitro, raphe neurons were most reproducibly labeled with the fluorescent dye DiI. Other labels were toxic or less effective. Labels in non-neuronal raphe cells were rapidly diluted. Retroviral infection with the E. coli β-galactosidase gene optimally labeled undifferentiated RN33B cells over multiple passages. The gene was expressed by RN33B cells at non-permissive temperatures and in co-culture. In vivo, DiI-labeled raphe neurons, many of which were 5-HT immunoreactive, were visualized up to 1 month after intra-spinal transplantation. β-galactosidase-positive RN33B cells were visualized up to 2 weeks after intra-hippocampal transplantation. These results suggest that to determine the most appropriate labeling approach for identifying transplanted cells, it is advisable to first examine its properties in vitro. Supported by The Miami Project and NS26887.

229.12

A NEURONAL CELL LINE DERIVED FROM MEDULLARY RAPHE, L.A. White & S.R. Whittemore, The Miami Project, Univ. of Miami School of Medicine, Miami, FL 33136.

Following infection of dissociated E13 rat medullary raphe cells with a retrovirus encoding the temperature-sensitive (ts)-mutant of SV40 large T antigen, a clonal cell line, RN33B, was isolated by serial dilution. At 33°C, RN33B cells divide with a doubling time of 36 hrs and show nuclear T antigen, vimentin, A2B5, and nestin immunoreactivities. RN33B cells are immortal, but not transformed as they will not grow in soft agar. At non-permissive temperature (39.5°C), T antigen immunoreactivity is markedly decreased and the cells cease mitotic activity. At 39.5°C, RN33B cells differentiate with phase bright cell bodies and "neuritic-like" processes. Immunohistochemical staining, northern blots, and Western blots showed that differentiated RN33B cells express vimentin, A2B5, nestin, and also neuron-specific enolase, neurofilament protein, and the NGF receptor, but not astrocytic or oligodendrocytic-specific cell antigens. Ultrastructurally, differentiated RN33B cells contain both microtubules and smaller caliber filaments. Differentiated RN33B cells returned to 33°Cre-express T antigen, but do not de-differentiate and begin dividing. Class I MHC expression was detected only on undifferentiated RN33B cells, although IFN-Γ could induce class I, but not class II MHC antigen expression on differentiated RN33B cells. Our results suggest that differentiated RN33B cells have many of the properties of mature CNS neurons. In addition, RN33B cells survive following transplantion into the CNS (see, Onifer et al.), suggesting that ts-neuronal cell lines may potentially be useful in replacing damaged neurons in vivo. Supported by The Miami Project to Cure Paralysis and NS26887.

229.13

ASTROCYTES AS CELLULAR VEHICLES FOR GENE THERAPY IN THE BRAIN. E.F. La Gamma, G. Weisinger, R.E. Strecker and N.J. Lenn. Depts. Pediatrics & Neurobiology, Psychiatry and Neurology, SUNY at Stony Brook, NY 11794-8111.

Genetically modified astrocytes can be transplanted into the central nervous system. Astrocytes may be the preferred cellular vehicle for gene replacement therapy via brain transplantation since they divide in culture, are region specific, possess a release mechanism, secrete growth factors, and migrate several milimeters from the transplant site. We have stably transfected neonatal rat astrocytes and selected for growth in G418 selective media for 3 weeks. RSV-driven and enkephalin promoter driven chloramphenicol acetyl transferase activity was demonstrated in vitro and again in vivo after 3 more weeks following transplantation into striatum. The transplanted astrocytes were also identified histologically by GFAP staining. In addition, we have demonstrated changes in transfected gene expression in vitro using dopaminergic agonists. This suggests another potential advantage of astrocytes over neuronal fibroblasts as transplant therapy in human disease entities such as Parkinson's Disease. Current work is in progress using the retroviral vector for the tyrosine hydroxylase gene LThRNL (provided to us by F. Gage and K. O'Malley) to further evaluate this approach in the Parkinsonian rat behavioral paradigm. We speculate that genetically modified astrocytes can be used for studying other expressed proteins as well.

229.14

EXPRESSION OF PLASMID DNA IN RAT BRAIN CELLS *IN VITRO AND IN VIVO*. S.S. Jiao, G. Acsadi*, A. Jani*, P.L. Felgner* and J.A. Wolff*. Dept. of Pediatrics & Medical Genetics, Waismain Center, University of Wisconsin, Madison, WI 53705 and Vical Inc., San Diego, CA 92121.

This study demonstrated that plasmid DNA was able to persist in rat brain cells *in vivo* for one to two months. A new cationic lipid formulation which contains equimolar concentrations of DOTMA {N[1-(2,3-dioleyloxy)propyl] -N, N, N-trimethyl-ammonium} and cholesterol was used to transfect reporter genes into fetal brain cells in culture with increased efficiency than the previously described cationic lipid formulation, Lipofectin (BRL). Suspended or clumped neocortical cells obtained from E17 fetuses had the greatest levels of luciferase activity after transfection of pRSVL, which contains the luciferase reporter gene. Using double immunohistochemical labelling, gene expression was localized both to glial and neuronal cells after transfection of fetal brain cells with pRSVLac-Z which contains the *E. coli* β-galactosidase reporter gene. Both glial and neurons were transfected with approximately equal efficiency. The transfected cells were also transplanted into the caudates of adult rats. One to two months after transplantation, the expression of the reporter genes were still found in the host brains. The ability for brain cells transfected with simple plasmids to have persistent expresssion of a foreign gene has implications for intracerebral viral infections and gene therapy of brain disorders.

MEMBRANE COMPOSITION: CELL SURFACE MACROMOLECULES I

230.1

THE AVIAN ALPHA$_6$ SUBUNIT OF INTEGRIN IS EXPRESSED IN THE DEVELOPING NERVOUS SYSTEM AND MUSCLE.
Michael Artinger*, M. Cullen and M. Bronner-Fraser, Developmental Biology Center, University of California, Irvine, CA 92717

The distribution of the alpha$_6$ subunit of integrin was examined in the trunk region in 2 to 8 day old avian embryos. Alpha$_6$ was first observed after neural tube closure and somite formation. As the somite differentiates into the dermomyotome and sclerotome, alpha$_6$ immunoreactivity was noted on prospective myotomal cells. At the same time, low levels of alpha$_6$ reactivity were observed in neuro-epithelial cells of the neural tube. Alpha$_6$ expression persisted in myotomal cells and later was present on myoblasts in the limb, as well as in the apical ectodermal ridge. In the developing neural tube, alpha$_6$ immunoreactivity became prominent near the lumen and in the ventrolateral portions of the neural tube, co-distributing with neurons and axons. Its expression was prominent in commissural neurons as they extended processes toward the floorplate via the basal lamina. Alpha$_6$ immunoreactivity was expressed on a subpopulation of neural crest cells around the dorsal aorta at later stages of neural crest cell migration; these were presumably sympathoadrenal cells. It was prominent in the ventral roots and first appeared in the dorsal root ganglia shortly after their formation. Expression was developmentally regulated and appeared to peak at embryonic days 5 to 6, decreasing thereafter. Alpha$_6$ co-localized with ß1 integrin, suggesting that they form alpha$_6$-beta$_1$ heterodimers. Our results show that the alpha$_6$ subunit of integrin is expressed throughout the developing nervous system and muscle. In the periphery, these regions are rich in laminin, which is thought to be its ligand. (supported by HD15527)

230.2

T-CADHERIN, A GPI-ANCHORED CADHERIN IN THE NERVOUS SYSTEM, IS COMPETENT TO MEDIATE CELL ADHESION. D. J. Vestal and B. Ranscht. La Jolla Cancer Research Foundation, La Jolla, CA 92037.

The cadherins are a family of calcium-dependent cell-cell adhesion molecules. The adhesive function of cadherins depends, in part, on the interaction of their highly conserved cytoplasmic domain with components of the cytoskeleton. This laboratory has identified a new member of the cadherin family, T-cadherin, that lacks the conserved cytoplasmic sequences (Ranscht and Dours-Zimmermann, in press). T-cadherin is a prominent component of many neuronal cell types and is detected in the paths of growing commissural and motor axons. This expression pattern is consistent with a possible role in nerve guidance.

In order to investigate if T-cadherin is competent to mediate adhesive interactions, T-cadherin cDNA was expressed in CHO cells. Transfected cells express both the 95 kda mature T-cadherin protein and an additional 110 kda polypeptide. The heterologously expressed T-cadherin is GPI-anchored, as determined by the incorporation of ^{3}H-ethanolamine and by the release of both polypeptides from the cell surface by treatment with PI-PLC. In cellular aggregation assays, T-cadherin induces calcium-dependent homophilic cell-cell adhesion. A variant T-cadherin cDNA has been identified (see Sacristan and Ranscht, this issue) which encodes T-cadherin polypeptides with similar biochemical and adhesive properties. The ability to promote cell-cell adhesion in the absence of the conserved cytoplasmic region, that links other cadherins with the cytoskeleton, implies that T-cadherin uses an alternative mechanism for generating adhesive interactions.

230.3

ASSOCIATION OF MULTIPLE FORMS OF HUMAN N-CADHERIN WITH BRAIN SYNAPTOSOMES. Duane D. Bronson*, Robert A. Reid*, and John J. Hemperly. Becton Dickinson Research Center, Research Triangle Park, NC 27709.

The establishment, maintenance, and modification of synaptic connections are critical to the proper functioning of the nervous system. The molecules involved in these processes, particularly cell adhesion molecules, are the subject of intensive study. We have prepared antibodies and DNA probes to the human calcium-dependent adhesion molecule N-cadherin and used them to identify the molecule in the synaptosomal fraction of human brain. Unlike in tissue culture cells, and in contrast to our studies of human L1 antigen and transmembrane forms of the neural cell adhesion molecule N-CAM, N-cadherin appears to be tightly associated with cytoskeletal elements. Moreover, antibodies prepared against a recombinant form of N-cadherin expressed in bacteria recognize two polypeptides (130 Kd and 100 Kd) in brain and in cell lines. The nature of these polypeptides and their relationship to each other and to other cadherins are being investigated.

230.4

CHARACTERIZATION AND DEVELOPMENTAL EXPRESSION OF A T-CADHERIN VARIANT IN THE CHICKEN NERVOUS SYSTEM. M.Sacristan* and B. Ranscht, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Cadherins are a multigene family of calcium-dependent cell-cell adhesion molecules that are suggested to play an important role in tissue morphogenesis. This laboratory has identified a new cadherin, T-cadherin, that lacks the conserved cytoplasmic region and, instead, is anchored to the cellular plasma membrane through a glycosylphosphatidylinositol glycan (Ranscht and Dours-Zimmermann, in press). We now report the characterization of a variant form of T-cadherin, Tcad$_v$, that is encoded by a cDNA differing in the 3' region from the originally reported clone, Tcad. In the open reading frame for Tcad$_v$ the carboxyterminal Leu of Tcad is changed into Lys and followed by five additional amino acids. To investigate if the expression of the two T-cadherin forms is regulated during chick neural development, mRNA from chick brain between embryonic day 2 and two days after hatching was analyzed by RNase protection with probes specific for Tcad and Tcad$_v$. mRNAs for both T-cadherin forms were expressed, at strikingly different levels, at all times examined. Tcad$_v$ was prevalent at embryonic day 2, while Tcad predominated, in a ratio of >10:1, at all later developmental stages. mRNA levels for both Tcad and Tcad$_v$ increased continually between embryonic day 4 and day 16 and appeared to be down regulated thereafter. The accumulation of Tcad over Tcad$_v$ mRNA appears to correlate with neuronal differentiation and was also observed in pure cultures of neurons. These observations indicate that the expression levels of the two T-cadherin forms are differentially regulated during neural development. The location of Tcad and Tcad$_v$ mRNAs at the single cell level will be examined by *in situ* hybridization.

230.5

NEURAL CELL ADHESION MOLECULE REGULATION IN CULTURED CHICK MYOTUBES. J.M. Lyles, W. Amin* and C.L. Weill. Department of Neurology, Louisiana State University Medical School, New Orleans, LA 70112.

The neural cell adhesion molecule, NCAM, is a cell surface glycoprotein that influences neuro-muscular adhesion in developing muscle. Since NCAM levels and molecular forms change during muscle development, the factors that affect NCAM regulation were investigated in primary, embryonic chick muscle cultures. Primary muscle cultures grew better and fibroblast proliferation was reduced in serum-free medium on reconstituted basement membrane, Matrigel. Serum factors enhanced the relative level of NCAM (per ug protein on myotube day 5) determined by ELISA, suggesting that growth factors regulate NCAM expression. Several purified factors which enhanced relative NCAM levels were: 1) nisoldipine (a calcium ion agonist), 2) insulin-like growth factor II (IGFII), 3) nerve growth factor (NGF), 4) veratridine (a sodium ion channel agonist) and 5) thyroxine (T4). Ineffective factors were vasoactive intestinal peptide, calcitonin gene-related peptide, acidic fibroblast growth factor, basic fibroblast growth factor, IGF I, dibutryl cyclic GMP and dibutryl cyclic AMP. Therefore, extracellular factors and intracellular ions appear to regulate NCAM expression in developing muscle.

230.6

DEVELOPMENTAL AND CELL-SPECIFIC CHANGES IN N-CAM EPITOPES Y. Yan*, W-W. Chung and C. Lagenaur. Dept. of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, Pittsburgh, PA. 15261.

N-CAM exists in three major isoforms in the CNS; varied glycosylation may account for further N-CAM heterogeneity. In this study, three monoclonal antibodies (12F11, 12F8 and 8A2) were used to study N-CAM expression in vivo and in vitro. Western blots of brain membranes from E14, P0 and adult mice were examined. Diffuse bands around 200 kD were observed with all antibodies at E14 although 12F11 stained weakly. In adult, polyclonal anti-N-CAM stained the three major isoforms of CNS N-CAM. The 12F8 antibody recognizes polysialic acid on N-CAM and showed little staining in adults. Interestingly, 12F11 stained the 180 and 140 kD forms of N-CAM but not the 120 kD form. The 8A2 antibody also failed to stain the 120 kD N-CAM, but did stain 140 and 180 kD forms. The anti-8A2 also weakly stained a number of unidentified proteins. Cultured mouse neocortex was stained with each antibody. Anti-12F8 stained neurons and their processes as well as some astroglia. Anti-12F11 stained some neurons but did not stain glia. Most strange was the anti-8A2 which stained the substrata of the cultures leaving an unstained "ghost image" in areas occupied by cells. These studies further define the developmental changes in N-CAM expression and the differing expression of particular forms of N-CAM by various neural cell types. Supported by NIH NS25543.

230.7

MOLECULAR STRUCTURE AND FUNCTIONAL TESTING OF HUMAN L1. M.L. Hlavin and V.P. Lemmon. Departments of Neurosurgery and Neuroscience. Case Western Reserve University School of Medicine. Cleveland, OH 44106.

The L1-like cell adhesion molecules are members if the immunoglobulin superfamily that have been implicated in axon growth and guidance. We have isolated the related molecule from human brain and found that, like its counterparts in rodents and chick, it also supports neurite growth *in vitro*. Additionally, we have cloned and sequenced the entire coding region for human L1. It has a very high degree of homology to mouse L1, 92% at the amino acid level. Although overall homology to chick L1 is substantially less, 40% at the amino acid level, certain regions are highly conserved. A domain by domain comparison of the mouse, chick, and *Drosophila* sequences to human L1 indicates that the L1 Ig domain 2 and Fn domain 2 are strongly conserved between species and thus are likely functionally important. The Ig 2 domain possesses a highly negatively charged region suggesting a significant functional region; similarly, a highly positively charged region was found in the Fn domain 3. We found 100% identity of the mouse cytoplasmic region to human L1, further supporting previous reports of the highly conserved nature of this region and suggesting an important function such as cytoskeleton interaction or cellular regulation.

DOMAIN	Ig 1	Ig 2	Ig 3	Ig 4	Ig 5	Ig 6	Fn 1	Fn 2	Fn 3	Fn 4	Fn 5	CP
M L1	83	93	91	87	90	93	89	89	86	82	75	100
NGCAM	44	66	55	57	39	43	38	56	33	30	18	63
NGCAM*	62	83	71	79	66	56	67	73	58	49	32	75
Neuroglian	26	32	27	24	29	32	32	36	31	29	18	26
Neuroglian*	41	54	47	49	49	48	51	68	50	52	40	40

*Analysis performed allowing conservative amino acid substitutions.

230.8

ISOLATION AND CHARACTERIZATION OF A 135 kDa MEMBRANE GLYCOPROTEIN FROM HUMAN BRAIN

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We have purified a 135 kDa membrane glycoprotein from human brain cortex using lentil lectin affinity chromatography and immunoaffinity chromatography with a specific monoclonal antibody, CF3 (Berglund, E. et al., (1987) J. Neurochem., 48(3),809-815, Berglund et al., (1991) Eur. J. Biochem., in press). The polypeptide core is estimated to be 115 kDa by N-glycanase digestion. The glycoprotein is anchored to the plasma membrane through glycosylphosphatidylinositol as shown by digestion with phospholipase C and liposome incorporation. Quantitative enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry techniques were used to determine the location of the 135 kDa protein in human brain. The 135 kDa glycoprotein is most abundant in the gray matter of frontal cortex and cerebellum, i.e. areas dense in dendritic and unmyelinated axonal processes. In contrast, the subcortical white matter, pons and medulla display only low levels of the 135 kDa protein. Amino acid sequence analysis of the amino terminus and of an internal peptide obtained by V8 protease digestion of the 135 kDa protein, revealed a strong similarity to three previously described glycoproteins from chicken (contactin and F11) and mouse (F3) brains. These glycoproteins belong to the immunoglobulin superfamily and are implicated in cell adhesion phenomena in the developing brain. Gp135 may be the human counterpart to one or several of these glycoproteins.

230.9

BRAVO, A NOVEL CELL-SURFACE MOLECULE FOUND IN A RESTRICTED PATTERN ON OPTIC FIBERS OF THE DEVELEOPING NERVOUS SYSTEM, IS CLOSELEY RELATED TO NgCAM AND L1.

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Cell-surface molecules play an essential role in guiding axons to their targets. We have developed a method to generate monoclonal antibodies (mAbs) which recognize cell-surface molecules of defined molecular weight that are expressed during the development of the chicken retinotectal system. The antigen distribution on optic fibers recognized by one of these mAbs, Bravo, is restricted to retinal ganglion cell axons in the retina, and absent from these same axons in the tectum. The complete derived sequence of Bravo has been obtained. It reveals a close relationship to the neural members of the immunoglobulin superfamily, with closest relationship to chicken NgCAM and mouse L1. Like NgCAM and L1, Bravo contains six immunoglobulin domains, five fibronectin type III repeats, a transmembane domain and a highly conserved but functionally uncharacterized cytoplasmic region. Like the other neural members of the Ig superfamily, Bravo carries the HNK-1 carbohydrate epitope, and specifically like L1 and NgCAM, Bravo is found predominantly in the form of a heterodimer, an intact chain cleaved in identical locations in all three molecules into two non-covalently associating parts. These data present an interesting view of retinotectal optic fiber outgrowth: As optic fibers grow in dense fascicles towards the optic nerve exit, they express both the known cell adhesion molecule NgCAM, and the closely related Bravo. As these fibers pass through the optic chiasm, Bravo is reduced on their surfaces, coincidentally in the same place and time that these fibers noticeably defasciculate, presumably to allow independent target seeking by individual axons. The possible role that Bravo's expression and topological restriction plays in the fasciculation and subsequent defasciculation of optic fibers is currently under investigation.

230.10

A RETINAL CELL SURFACE PROTEIN UNEQUALLY DISTRIBUTED ON OPTIC FIBERS

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Most scientists now agree that the proper establishment of axonal projections in the nervous system is achieved in part by an address system of cell surface recognition molecules. These address molecules are expected to be distributed unevenly both in space and in time during embryogenesis. One candidate for such a molecule was detected with the monoclonal antibody 10-22A8. In the chick retina, at embryonic stages E7 to E8, it stains the optic fibers of the nasal retina while staining the temporal fibers little if at all. Preliminary sequence information of a cDNA clone coding for a portion of this protein reveals that its closest homology is to the fibronectin Type III repeat of LAR (leukocyte antigen related, human) and DLAR (neuron specific, Drosophila), two members of the family of membrane bound tyrosine phosphatases. Determination of the entire coding sequence will reveal more information on protein domains with known functions and will establish whether this is in fact a novel member of the tyrosine phosphatase family of cell surface receptors. Additional histological studies, as well as in vitro and in vivo functional assays will be required in order to assess the significance of the striking difference between the staining of nasal vs. temporal axons in the developing retina at stages E7 to E8.

230.11

MONOCLONAL ANTIBODY BM88 DEFINES A NEURON SPECIFIC SURFACE ANTIGEN INVOLVED IN NEURITE OUTGROWTH. E. Patsavoudi*, V. Vlazakis* and R. Matsas. Lab. of Biochemistry, Hellenic Pasteur Institute, Athens 115 21, Greece.

BM88 antigen is a neuron specific surface protein present in the central and peripheral nervous system and composed of two 22KD polypeptide chains (E. Patsavoudi, C. Hurel and R. Matsas, 1989, Neuroscience 30 463-478). The immunoaffinity purified antigen has properties typical of an amphipathic membrane protein and is not glycosylated (E. Patsavoudi, C. Hurel and R. Matsas, 1991, J. of Neurochemistry 56 782-788).

The functional properties of this antigen were investigated in antibody mediated perturbation experiments using primary cultures. To this purpose an immunoaffinity and Fab fragment preparation of a polyclonal antibody raised against the BM88 antigen were prepared. When dissociated cells from brain of 19 day old rat embryos were cultured for 6h and 24h in a defined medium in the presence of the antibody, an inhibition of neurite outgrowth of these cultures was observed in a dose dependent manner. Our results strongly suggest that the BM88 antigen plays and important role in the promotion of neurite outgrowth.

230.12

DEVELOPMENTALLY AND SPATIALLY REGULATED EXPRESSION OF HNK-1 EPITOPE ON A NOVEL PI-ANCHORED GLYCOPROTEIN, PI-GP150 Y. Yoshihara, S. Oka*, Y. Watanabe* and K. Mori Dept. of Neuroscience, Osaka Bioscience Inst., Suita, Osaka 565 Japan

Cell surface adhesion and recognition molecules play critical roles in a variety of developmental events. HNK-1 carbohydrate antigen is a common epitope expressed on such molecules involved in cell-cell interactions in the nervous system. We purified and characterized from rat brain a novel phosphatidylinositol (PI)-anchored 150 kD glycoprotein with HNK-1 epitope. The molecule (PI-GP150) was detected by combination of PI-specific phospholipase C treatment of brain membranes and Western blot analysis with monoclonal antibody HNK-1. The expression of HNK-1 epitope on PI-GP150 was developmentally and spatially regulated. In newborn rats, HNK-1 epitope was expressed on PI-GP150 throughout the brain. The level of HNK-1-positive PI-GP150, however, decreased after postnatal day 7 only in hindbrain, and became completely absent in adult myelencephalon and metencephalon, while constitutive expression of HNK-1 epitope on PI-GP150 was observed in telencephalon. This developmental change results in formation of the rostro-caudal gradient of the HNK-1 epitope expression on PI-GP 150 in adult brain.

230.13

GALACTOSE-BINDING PROTEINS ISOLATED FROM THE LEECH CNS ARE COMPLEMENTARY, *IN VITRO*, TO A GALACTOSE-CONTAINING EPITOPE ON LEECH SENSORY AFFERENTS. R.N. Cole and B. Zipser. Department of Physiology, Michigan State University., East Lansing, MI 48824.

Carbohydrate recognition between cell surface glycoconjugates and carbohydrate-binding proteins (CBPs) is postulated to regulate neuronal pathfinding and target recognition. Previously, we demonstrated that a carbohydrate epitope on the surface of leech sensory afferents mediates the defasciculation of the sensory afferents into the synaptic neuropile of the CNS. This work predicted the presence of a CBP in the leech synaptic neuropile. We have now isolated three CBPs with apparent MWs of 37, 35, and 16 kD from crude membrane fractions of the leech CNS. They bind to an asialofetuin affigel column and are eluted by alpha-methyl-galactose or galactosamine, but not by xylose or alpha-methyl-mannose. These galactose-binding proteins have similar MWs and binding characteristics as the vertebrate galactose-binding proteins CBP-35 and CBP-16 (J. Biol. Chem. 258: 10657), which belong to the family of thiol dependent (S-type) lectins (J. Biol. Chem. 263: 9557).

In vitro, these galactose-binding proteins are complementary to a carbohydrate epitope on a subset of sensory afferents found on leech lips. The lip afferents, chemo- or heat detectors, express a galactose-containing epitope, as inferred from the sugar-binding specificity of the mAb Lan2-3a. Galactosamine inhibits the binding of mAb Lan2-3a to the lip afferents and elutes mAb Lan2-3a from an asialofetuin column. Molecular cloning and functional studies will determine whether the leech galactose-binding proteins are S-type lectins and whether they interact with the Lan2-3a epitope *in vivo*.

230.14

REGIONAL AND TEMPORAL VARIATIONS OF CARBOHYDRATE DISTRIBUTION IN THE EMBRYONIC CHICK BRAINSTEM. I. Lopez-Colberg and J.A. Wallace. Dept. of Anatomy, Univ. of New Mexico Sch. of Med. Albuquerque, New Mexico 87131.

Analysis of cell surface carbohydrates in central and peripheral neural tissue has revealed that certain neuronal subsets possess oligosaccharides that vary in composition or amount during development. The function of these oligosaccharides is unknown but it is postulated that they may serve as recognition signals during the processes of neuronal migration and synaptogenesis. In this study we examined the pattern of cell surface carbohydrates in the embryonic chick brainstem at ages related to active cell migration and pathway formation. To investigate the spatial and temporal distribution of sugar moieties in the chick brainstem, paraffin sections of the brainstem were screened with a battery of fluorescinated lectins. Lectins are glycoproteins of non-immune origin that bind to specific oligosaccharide residues. Our study demonstrated that only Peanut agglutinin (PNA) and Concanavalin A (Con A) exhibited regional and temporal variation in their binding during embryonic chick brainstem development. PNA staining was especially interesting in that it bound to areas known to contain differentiating and migrating serotonergic (5-HT) neurons. Work is in progress using an affinity isolation technique to enrich for 5-HT cells by their potential binding to peanut agglutinin. Supported by MBRS grant RR08139.

230.15

DIFFERENT DOMAINS OF J1/TENASCIN PROMOTE CEREBELLAR GRANULE CELL MIGRATION AND NEURITE OUTGROWTH. K. Husmann*, M. Schachner, and A. Faissner+ Dept. Neurobiology, Swiss Federal Institute of Technology, Zürich, Switzerland and +Dept. Neurobiology, University of Heidelberg, Heidelberg, Germany.

J1/tenascin is a hexameric extracellular matrix molecule which is involved in neuron-glia recognition. To study its role in astrocyte-mediated neuronal migration, five monoclonal antibodies were used in an in vitro assay to determine cerebellar granule cell migration (modified from Lindner et al., Nature, 305: 427, 1983). Rotary shadowing electron microscopy was used to determine the immunoreactive domains of J1/tenascin. The antibodies J1/tn1, 4 and 5 bound to the fourth or fifth fibronectin type III repeat, while J1/tn2 reacted with the tenth or ninth and tenth repeat and J1/tn3 with a proximal EGF-like repeat (for J1/tn1 and 2 see Lochter et al., J. Cell Biol., in press). Only antibodies 1,4 and 5 inhibited granule cell migration (by 55%). To study which of the domains are involved in extension of granule cell neurites, granule cells were cultured on a J1/tenascin-polyornithine substrate and the influence of J1/tenascin on neurite extension was measured in the presence of the antibodies. As for hippocampal neurons (Lochter et al., ibid.) only J1/tn2 inhibited the stimulatory effect. We conclude that J1/tenascin influences neurite outgrowth and neuronal migration by different domains in the fibronectin type III repeats.

230.16

VARIABLE MEMBRANE GLYCOPROTEINS IN DIFFERENT GROWTH CONE POPULATIONS. S. Quiroga*, H. Li*, K.H. Pfenninger. Cell. & Struct. Biol., Univ. Colo. Sch. Med., Denver, CO 80262

The question of whether growth cones generated by different neurons contain distinctive membrane glycoproteins was examined. Growth cones (GCPs) were isolated from defined regions of fetal or early postnatal rat brain and their membrane proteins analyzed by two-dimensional PAGE and Western blotting using wheat germ agglutinin (WGA) as a probe. The results indicate substantial pattern diversity for the different growth cone populations. Some WGA-binding proteins are uniformly present in the GCP fractions while others appear unique to a specific brain region. gp93 is a 90-97 kDa glycoprotein complex present in all GCPs analyzed. The complex covers a pI range from about 4.9 to about 6.4 and consists of at least twelve different species, probably isoelectric variants, in GCPs from whole fetal brain. Neuraminidase digestion simplifies the gp93 pattern only partially, indicating that variable sialic acid content explains the molecular diversity to some extent. In GCPs from different brain regions the sets of gp93 species are different and characteristic. This suggests that gp93 may be involved in selective cell-cell interactions. gp93 has been purified and experiments are being carried out to study the heterogeneity of the oligosaccharide and polypeptide chains included in the complex. (Supported by NIH grant NS24676.)

230.17

INTERACTIONS BETWEEN NEURONAL HEPARAN SULFATE PROTEOGLYCANS AND PROTEINS OF THE EXTRACELLULAR MATRIX. C. S. Stipp, M. E. Herndon*, and A. D. Lander, Dept. of Biology & Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Proteoglycans (PGs) are believed to play roles in complex neural cell behaviors such as cell attachment and neurite outgrowth. Biochemical analysis of PGs from cultured cells may provide clues about the roles of specific PGs in cell behavior. Therefore, membrane-associated PGs were isolated from PC12 cells that had been metabolically labelled with $^{35}SO_4$ and the core proteins of these PGs were biotin-labelled. SDS-PAGE analysis following heparitinase digestion confirmed the presence of a major heparan sulfate proteoglycan (HSPG) with a core protein M_r of ~60kd (Gowda, et al., JBC 264:11436). The effects of phosphatidylinositol-specific phospholipase C on Triton X-114 partitioning of this HSPG indicate that it is a lipid-anchored membrane component. The data suggest that this PG is identical to a rat brain PG whose expression is developmentally regulated (HSPG M12; Herndon and Lander, Neuron 4: 949). To investigate the possibility that this PG is a receptor for the extracellular matrix, PG binding to purified matrix proteins was measured by affinity coelectrophoresis (Lee and Lander, PNAS 88:2768) The PC12 PG preparation showed a single, heparitinase-sensitive affinity for each protein tested. The order of affinities was collagen I> laminin >> fibronectin (FN). Interestingly, this order correlates with the relative levels of adhesion displayed by PC12 cells toward substrata treated with these molecules. The low affinity observed for binding of PC12 HSPG to FN (K_d > 1μM) contrasts sharply with the behavior of HSPG M12 isolated from newborn rat brain, which contains a subfraction exhibiting high affinity (K_d ~10nM) for FN. These data are consistent with the idea that PGs can exhibit functional (e.g. binding) diversity sufficient to influence neuronal cell-matrix interactions. *Supported by NIH grant NS26862 (ADL) and NRSA predoctoral support (CSS and MEH).*

230.18

IMMUNOHISTOCHEMICAL LOCALIZATION OF THE GLYCOSAMINOGLYCAN (GAG) CHONDROITIN-4-SULFATE (CS-4) IN THE NORMAL ADULT RAT SPINAL CORD. F.J. LIUZZI. Dept. of Anatomy and Neurobiology, Eastern Virginia Med. Sch., Norfolk, VA 23501.

A chondroitin sulfate proteoglycan (PG) containing mainly CS-4 has been localized in adult rat brain by Aquino and colleagues (*J. Cell Bio. 187:211, 1984*). The PG has a widespread distribution and is present in neurons as well as astrocytes. More recently, an *in vitro* study showed that type-2 astrocytes express CS-4 but not CS-6 while type-1 astrocytes express neither form (Gallo and Bertolotto, *Exp. Cell Res. 99:1117, 1990*).

In light of the latter *in vitro* data, and the recent proposal that CS is inhibitory to axonal growth in the adult mammalian CNS by virtue of its localization in white matter and its purported absence in gray matter, it was decided to examine CS-4 localization in the adult rat spinal cord using a monospecific antibody to this GAG. The results of this work show that CS-4 is widely distributed not only in the spinal cord white matter but also in the gray matter. The GAG is ubiquitous in the gray matter and is found within neurons. In the white matter CS-4 is found in longitudinally oriented astrocytic processes and is particularly prominent at nodes of Ranvier, a finding that supports the idea that type-2 astrocytes form the nodal processes.

This work was supported by a grant from the NIH (NS24309).

230.19

A GENERAL COMPONENT OF ADULT BRAIN EXTRACELLULAR MATRIX, THE T1 CHONDROITIN SULFATE PROTEOGLYCAN. Mineo Iwata and Steven S. Carlson. Dept. of Physiology and Biophysics, University of Washington, Seattle, WA.

We have identified a large chondroitin sulfate proteoglycan (T1-PG) that has the characteristics of a general extracellular matrix component of rat brain (Iwata and Carlson,Abst. Soc.Neuro.,16:496,1990). This proteoglycan was identified with a mAb to a rare chondroitin sulfate epitope(T1). The T1-PG is immunocytochemically localized throughout the brain in white and gray matter. Like ECM components from other tissues, the T1-PG requires denaturing conditions to be solubilized from brain tissue. The T1 antigenicity appears to be secreted by cultured rat newborn cerebellar cells. Digestion of the T1-PG with chondroitin ABC lyase yields to core sizes of 210Kd and 250Kd.

The T1-PG appears to be distinct from the well-known spacefilling proteoglycans, versican and aggrecan. We generated an antibody against a common consensus sequence in the hyaluronic acid binding loop region(HABL) of these proteoglycans. This antibody crossreacts with monkey smooth muscle versican and calf nasal cartilage aggrecan with core sizes of 447Kd/528Kd and 447Kd, respectively. This anti-HABL antibody also recognizes two major core proteins of the putative rat brain versican with molecular weights of 361Kd and 447Kd. This putitive versican is extracted by non-denaturing detergents. Anti-HABL does not detectably bind to purified T1-PG. Furthermore, the following biochemical characteristics of T1-PG are found to differ from the putative brain versican:(1) T1-PG has a higher density on dissociative CsCl gradient. (2) It is tightly associated with the detergent-insoluble fraction of adult brain. (3) It has the T1 epitope; versican does not. (4) It is disulfide-linked to a 100Kd proteinz. (5)Its core size is smaller.

230.20

AN ANTIGEN EXPRESSED BY PRIMATE NEOCORTICAL NEURONS IN SPECIFIC LAMINAE IS A PROTEOGLYCAN M. Murtaugh*, A. F. Pimenta, P. L. Strick and P. Levitt, Department of Anatomy and Neurobiology, The Medical College of Pennsylvania, Philadelphia, PA 19129 and VA Med. Ctr./ Depts of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse, NY 13210.

The monoclonal antibody 8B3 recognizes an antigen present in the cortices of rhesus monkey, cat and rat. The antigen, which is expressed in mature and immature tissue, appears to be selectively associated with the cell surface of somata and dendrites in subpopulation(s) of neurons in all cerebral cortical areas.

Initial biochemical characterization of the antigen recognized by the 8B3 antibody has been performed using cat cerebral cortex. Western blot analysis reveals a large species macromolecule that migrates in gradient SDS-PAGE above 300,000 kiloDaltons. Subcellular fractionation shows that the 8B3 antigen is both membrane-associated and soluble. Enzyme digestion of these fractions with glycosaminoglycan lyases chondroitinase ABC and chondroiti-nase AC II at optimal pH results in a complete loss of immunoreactivity. Treatment with other lyases did not result in loss of immunoreactivity or molecular mass shift. Given the unique anatomical distribution of 8B3, the data suggest that this antigen is a novel chondroitin sulfate proteoglycan that adds to the growing family of membrane-associated, large macromolecules, that are anatomically specified in the brain. Supported by MH45507.

230.21

A neuronal surface, sub-population specific proteoglycan in mouse and Drosophila melanogaster nervous systems. C. Williams*, L. Reiter*, D. Hinton*, S. Benzer and C.A. Miller. Dept. of Pathology, U.S.C. School of Medicine, Los Angeles, CA 90033. Dept. of Biology, California Institute of Technology, Pasadena, CA 90025.

Monoclonal antibody (MAb) 6A2 labels an antigen localized to somal surfaces of neurons comprising the spinocerebellar system, and to a sub-population of non-pyramidal neurons in the cerebral cortex of human CNS, (Hinton et al, 1988, J. Comp. Neurol. 267:398). The immunoreactivity disappears in amyotrophic lateral sclerosis (Williams et al, 1990, Ann. Neurol. 27:215). We are now comparing the corresponding antigen (Ag) in the murine and Drosophila melanogaster nervous system. Ag 6A2 has a similar distribution in the murine and human CNS. Ultrastructurally, in mouse it lies in the extracellular space adjacent to neuronal surface membranes and in fly retina, in the intraretinular space. Affinity-purified mouse cerebellar and fly head homogenates reveal on immunoblots, 630kDa, and 400kDa proteins respectively. After incubation with chondroitinase ABC, both antigens showed increased migration on polyacrylamide gels, and the murine Ag 6A2 immunoreactivity with MAb 1B5, which labels chondroitin sulfate 'stubs'. The lectin, Jacalin, was immunoreactive with both antigens verifying the presence of O-linked oligosaccharides. The restricted distribution of Ag 6A2 to functionally distinct cellular subpopulations may be of importance in the formation and maintenance of these systems.

231.1

QUANTAL RELEASE AT INDIVIDUAL TERMINALS IS SPECIFIED BY POSTSYNAPTIC NEURONS OF *APLYSIA*.

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In *Aplysia* buccal ganglia, strengths of the same identified synapses vary from animal to animal. Additionally, the synaptic strengths of similar IPSCs vary in each ganglion, apparently specified by the postsynaptic neuron. Using quantal analyses as reported recently for hippocampal LTP, but in a preparation lacking questioned activation and saturation, I ascribe differences in synaptic amplitude to varying numbers of quanta released by presynaptic terminals, rather than to postsynaptic factors.

Recording from B4, B5, and two voltage-clamped postsynaptic cells allows analysis of four similar synapses sharing common presynaptic and postsynaptic cells. Mean and variance of g_{peak} yield quantal parameters consistent with a simple binomial, assuming that fluctuations of sequential PSCs represent differences in quantal release, rather than receptor number, desensitization, or noise. PSC ensemble variance time course is consistent with this view. The indirect analysis permits factoring g_{peak} into presynaptic $m/(1-p)$ and largely postsynaptic $\gamma(1-p)$ factors.

$\log_e [m/(1-p)]$ was correlated with $\log_e [g_{peak}]$ (r=0.87, slope = 1.1), consistent with different quantal release underlying different mean synaptic strength. In contrast, $\gamma(1-p)$ was independent of g_{peak} (r=0.19).

Pairing quantal values by common pre- or postsynaptic cells shows that $m/(1-p)$ is similar for presynaptic endings converging on a single follower (P=0.0001) but different for endings of the same presynaptic cell on different targets (P=0.34); no pairing specificity is seen for $\gamma(1-p)$.

As in hippocampus, varying postsynaptic specification of release from presynaptic terminals may involve dynamic retrograde modulation.

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231.2

CALCIUM-DEPENDENT NEUROSECRETION RECONSTITUTED IN *XENOPUS* OOCYTES: THE ROLE OF SYNAPTOPHYSIN.

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Total rat cerebellar mRNA was injected into stage VI *Xenopus* oocytes, and Ca-dependent neurosecretion was assayed 2 d later. Our aim is to reconstitute the molecular mechanism responsible for Ca-dependent secretion at the presynaptic nerve terminal in the *Xenopus* oocyte (Cavalli, et al., EMBO J., in press), which is more accessible to experimental manipulation than a neuron. We found oocytes injected with cerebellar mRNA expressed 5-fold higher uptake of radioactive glutamine over control oocytes, and a significantly higher fraction of radioactivity was secreted in the presence of 10 uM Ca ionophore (A23187) with 10 mM Ca than with 5 or 0 mM Ca. Background release of radioactivity from sham-injected oocytes showed no Ca-dependency. Co-injection of cerebellar mRNA with antisense oligonucleotides corresponding to the first 19 nucleotides of synaptophysin significantly inhibited the Ca-dependent secretion from the mRNA-injected oocytes (15.0 ± 5.2% of control secretion ± s.e., n=21). Co-injection of sense-oligos was without effect (96.0 ± 14.6% of control n=12). Western blot analysis indicated that mRNA-injected oocytes expressed synaptophysin, and that such expression was abolished in oocytes co-injected with antisense. The effect of antisense injection was on secretion rather than glutamine uptake. Finally, co-injection of anti-synaptophysin antibody also produced significant inhibition (9.4 ± 5.3% of control n=19) of Ca-dependent secretion, while only a small effect was observed for injection with antibody to GFAP (76.8 ± 7.4% of control n=10). These results suggest that the presence of synaptophysin in its functional form is required for Ca-dependent secretion of radioactivity from the oocyte. Whether the secreted substance is in the form of a neurotransmitter or neuropeptides derived from the precursor glutamine is currently under investigation.

231.3

TONIC TRANSMITTER RELEASE IS INHIBITED IN LIGHT ADAPTED PHOTORECEPTORS DURING POST-ADAPTATIONAL FACILITATION IN BLOWFLY VISUAL INTERNEURONS.

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The transmitter release of photoreceptors to second order neurons (LMCs) in dipteran insects has been proposed to be tonic even in complete darkness. We have verified this by observing a post-adaptational facilitation (PAF) in responses of the LMCs. After a strongly adapting light stimulation the subsaturating responses of the LMCs to test stimulus increase in size with longer latency. At the same time the conspicuous dark membrane noise typical of LMCs vanishes. This PAF effect in LMCs can be traced back to the post-adaptational hyperpolarization induced in pre-synaptic photoreceptors by the Na/K-pump (Jansonius, *J Comp Physiol A* **167**:461-467, 1990). Conceivably, the pump potential in photoreceptors hyperpolarizes the transmitter releasing axon terminals beyond the activation threshold of calcium channels thus stopping the transmitter release. The increase in response size of LMCs to test stimulus during the PAF can be interpreted in terms of calcium channels' inactivation properties.

231.4

FEEDBACK CONTROL OF TRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION OF THE RAT.

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The presynaptic regulation of transmitter release in the rat diaphragm was examined by testing the effects of nicotinic and muscarinic antagonists on evoked transmitter release. Nicotinic angatonists (α-bungarotoxin, d-tubocurarine, and hexamethonium) and muscarinic antagonists (atropine and scopolamine) were tested on evoked transmitter release in an attempt to determine if presynaptic nicotinic or muscarinic receptors are present on the nerve terminal and whether they serve a role in regulating transmitter release. Intracellular recording techniques were used to monitor end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) in the isolated cut-muscle rat diaphragm-phrenic nerve preparation. Quantal release in the presence and absence of the antagonists were examined. In the presence of each of the nicotinic antagonists a significant increase in quantal release was observed. The muscarinic antagonists, atropine and scopolamine failed to exhibit presynaptic effects in a concentration range that did not affect the nicotinic postsynaptic receptors. In higher concentrations atropine enhanced release but this action was associated with nicotinic blockade and inhibition of acetylcholinesterase. These results suggest that the nerve terminal lacks muscarinic receptors but contains nicotinic receptors that serve a negative feedback role

(Supported by NIH grant NS-27260).

231.5

HYPERTONIC SOLUTIONS MAY INCREASE QUANTAL SIZE AT FROG NEUROMUSCULAR JUNCTIONS BY ACTIVATING PROTEIN KINASE A.

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Permeable cAMP derivatives roughly double quantal size at frog neuromuscular junctions. So does pretreatment in a hypertonic solution containing 200 mM NaCl; if 200 mM Na gluconate is used quantal size is increased fourfold. H8 (N-[2-methylamino)ethyl]-5-isoquinolonesulfonamide) inhibits protein kinases. When hypertonic gluconate solution contained 100μM H8 the quantal size increase was significantly less. The (Rp)- diastereomer of cAMPS (cyclic adenosine 3',5'- phosphothoate) blocks PKA activation by cAMP and 100μM lessened the increase in quantal size produced by hypertonic solution. The (Sp)-diastereomer activates PKA; (Sp)-cAMPS added to Ringer doubled quantal size.

The phosphodiesterase inhibitor IBMX in Ringer has no effect on quantal size. Quantal size was increased significantly in experiments in which preparations were exposed for 10 min to hypertonic solution containing IBMX and then kept in IBMX solution for 40 min (controls were in IBMX only after hypertonic exposure).

231.6

ANTISENSE GAP-43 mRNA BLOCKS EVOKED RELEASE OF DOPAMINE FROM PC12 CELLS.

K.J. Ivins, S.A. Fidel, K.A. Neve, and R.L. Neve, Dept. of Psychobiol, UCI, Irvine, CA.

The neuronal growth associated protein GAP-43 has been implicated in the release of neurotransmitters from permeabilized synaptosomes. We previously reported the generation of PC12 cells stably transfected with human sense or antisense GAP-43. The spontaneous and evoked release of dopamine from these cells has been measured using HPLC. Spontaneous release of dopamine from cells overexpressing GAP-43 (i.e. transfected with sense GAP-43) was greater than the release from control cells. Depolarization of cells with 56 mM K^+ caused a 5-10 fold stimulation in dopamine release from control cells and from cells transfected with GAP-43, but evoked release of dopamine from cells transfected with antisense GAP-43 was greatly reduced or absent. Similarly, the ability of the calcium ionophore A23187 to stimulate dopamine release from cells transfected with antisense GAP-43 was decreased or absent. These results indicate that GAP-43 is involved in the release of dopamine from PC12 cells and suggest that GAP-43 may function in neurotransmitter release at a step beyond an increase in intracellular calcium.

231.7

SYNCHRONOUS SPONTANEOUS SYNAPTIC RELEASE OF GABA IN CULTURED RAT HIPPOCAMPAL NEURONS.

J. Vautrin, Anne Schaffner and J.L. Barker. Laboratory of Neurophysiology, NINDS, NIH Bethesda, MD 20892.

Edwards et al. (*J.Physiol.* 430:213, 1990) reported that release of γ-aminobutyric acid (GABA) by hippocampal neurons is quantal. A quantal process varies by integer multiples of a defined amplitude and one expects amplitudes of unitary postsynaptic responses (PRs) to form a bell-shaped distribution. In fact, the amplitude distribution of single events due to release of GABA by hippocampal neurons recorded in the slice or in culture (reported here) are skewed and not bell-shaped. GABAergic PRs were analyzed in rat hippocampal neurons cultured for 1-3 weeks and recorded using patch electrodes containing CsCl. The PRs analyzed decayed with a time constant longer than 20ms at 22-25°C, were bicuculline-sensitive and reversed at E_{Cl}. The positive correlation between amplitude and rise time of individual PRs occurring spontaneously in tetrodotoxin indicates that electrotonic factors cannot account for smaller amplitudes and/or longer rise times. Careful observation of time courses strongly suggests that most PRs are composed of subunits that vary in number and synchronization. This suggests that the actual quantal size may be even smaller than the mode (or the mean) of the PR amplitude distribution (Ropert et al. *J.Physiol.* 428:707, 1990; Otis et al. *Brain Res.* in press, 1991).

231.8

CHARACTERIZATION OF VESICULAR RELEASE FROM INDIVIDUAL BOVINE ADRENAL MEDULLARY CELLS.

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Release of catecholamines from individual bovine adrenal medullary cells that have been physically stimulated has been measured using carbon-fiber microelectrodes placed adjacent to the cell. The calcium-dependent release was observed as sharp spikes, or changes in current, corresponding to the oxidation of catecholamines which has been confirmed by cyclic voltammetry. With temporal resolution on the order of microseconds per point, individual spikes were determined to be the result of single secretory events. The average spike area was 1.03 + 0.08 pC (avg + s.e.m.) corresponding to the average adrenal vesicle content of 5 attomoles of catecholamine. A probability density function derived for the distribution of spike areas confirmed the observation of single secretory events based on a gaussian distribution of vesicle radii and a constant vesicular concentration. Both the direct chemical measurements and mathematical modeling are consistent with exocytotic release from the individual cells.

231.9

TETRODOTOXIN-SENSITIVE CALCIUM-DEPENDENT RELEASE OF 3H-GLUTAMATE FROM BATRACHOTOXIN-TREATED RAT BRAIN SYNAPTOSOMES.

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We are using ^{3}H-glutamate release from synaptosomes as a model system to study regulation, especially that mediated by presynaptic receptors, of Ca-dependent neurosecretion at central synapses. An effective means of depolarizing synaptosomes is to elevate external [K+], since the membrane potential of these structures approximates E_K. However, any presynaptic modulation mediated by alterations of K channel activity will be occluded by a "High K" paradigm. An alternative approach was developed, taking advantage of the toxinology of action-potential Na channels found in nerve endings. Synaptosomes, preloaded with ^{3}H-glutamate in Na-containing buffer were pelleted and resuspended in Na-free, N-Methyl Glucamine (NMG) based saline. Batrachotoxin (BTX, 5μM), a persistent activator of Na channels, was added and the synaptosomes were incubated 30 min. on ice. A superfusion device designed to measure ^{3}H-glutamate release on a subsecond time scale was used to evoke release by switching the superfusion buffer from NMG to a Na-containing saline with 2.4 mM Ca^{+2}. The kinetics of evoked ^{3}H-glutamate release were biphasic, similar to those obtained using High K depolarization, although the amplitude was reduced. The release was Ca-dependent and was blocked by tetrodotoxin, whereas release evoked in a similar manner in Gramicidin-treated synaptosomes was not altered by tetrodotoxin. These results are consistent with depolarization mediated by Na influx through BTX-modified Na channels, enabling us to study the roles of K and Ca channels in presynaptic modulation of neurosecretion.

231.10

CALCIUM AND SODIUM EFFECTS ON VASOPRESSIN SECRETION FROM NEUROHYPOPHYSIAL NERVE ENDINGS.

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Neurohormone/neurotransmitter release is highly dependent on the free $[Ca^{2+}]_i$ at release sites in nerve endings and suggests that the kinetics of release should be related to the kinetics of Ca^{2+} entry, rise in $[Ca^{2+}]_i$ and to processes which restore $[Ca^{2+}]_i$ to prestimulatory levels. We have examined in isolated rat neurohypophysial nerve endings the kinetic relationship between averaged $[Ca^{2+}]_i$, $[Na^+]_i$, and vasopressin (VP) release to depolarizing stimuli. VP release was quantified with high temporal resolution from isolated endings while $[Ca^{2+}]_i$ and $[Na^+]_i$ were monitored in single endings using fura2 and SBFI. Elevation of $[K^+]_o$ leads to a dose-dependent, dihydropyridine- (DHP) sensitive, increase in $[Ca^{2+}]_i$ that is sustained throughout the depolarizing stimulus. Under voltage clamp, depolarizing voltage steps evoke changes in $[Ca^{2+}]_i$ reflecting the amplitude of the Ca^{2+} current and suggest an absence of voltage- or Ca^{2+}-induced Ca^{2+}_i release. Succeptibilty of the sustained Ca^{2+} increase to DHP or removal of Ca^{2+}_o demonstrates the sustained increase results from continued influx via "L-type" Ca^{2+} channels. VP release to a sustained depolarizing stimulus results in a transient release response (t 1/2 = 7 sec). Subsequent to secretory decline an additional depolarizing step produces a further increase in $[Ca^{2+}]_i$ and a resurgent, transient, increase in release. In contrast, treatments which evoke substantial increases in $[Na^+]_i$ but little change in $[Ca^{2+}]_i$ show dramatic release responses which show little secretory decline. Recovery and restimulation to elevated $[Na^+]_i$ shows little decrement in subsequent secretory responses. The kinetic difference in the Na^+- and Ca^{2+}- evoked secretory responses suggests that the Ca^{2+}-evoked secretory decline does not result from depletion of releasable granules, saturation of available release sites or complete inactivation of Ca^{2+} influx.

231.11

RESIDUAL CALCIUM AND ENHANCEMENT OF TRANSMITTER RELEASE: EVIDENCE FROM INVERTEBRATE AND VERTEBRATE SYNAPSES FOR CAUSALITY AND UNIVERSALITY.

K.R. Delaney, W.G. Regehr and D.W. Tank. Biophysics Res. Dept., AT&T Bell Labs, Murray Hill, NJ 07974.

We have directly demonstrated that elevated presynaptic residual calcium, measured with fura-2, shows a linear correspondence with short-term synaptic enhancement at the crayfish neuromuscular junction (nmj) (Delaney et al. 1989, J. Neurosci. 9(10): 3558). We have now addressed two related questions: (1) does the raised presynaptic calcium cause the enhancement, or is the observed correlation simply an epiphenomenon, and, (2) do synapses in other species show the same linear relationship?

To address the issue of causality, we have modified the calcium handling capabilities of presynaptic terminals in the crayfish nmj using microinjected calcium buffers and changes in temperature. Both synaptic enhancement and calcium decay kinetics following brief trains of action potentials are similarly affected by these manipulations which change calcium accumulation and removal rates. This strongly suggests that the residual calcium is a causal link in the expression of the synaptic enhancement.

To address the issue of universality, we have performed experiments at the guinea pig hippocampal mossy fiber synapse similar to our earlier experiments on crayfish. At this mammalian synapse we also observe a linear relationship between presynaptic residual calcium levels and synaptic enhancement similar to that seen at crayfish nmj. This suggests that some of the mechanisms of calcium-induced short-term enhancement of synaptic transmission may be phylogenetically widespread and perhaps universal.

231.12

THE ROLE OF RESIDUAL CALCIUM IN SYNAPTIC PLASTICITY AT THE HIPPOCAMPAL MOSSY FIBER SYNAPSE.

W.G. Regehr and D.W. Tank. Biophysics Res. Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974.

We have examined the role of presynaptic residual calcium ($[Ca^{++}]_{res}$) in use-dependent changes in synaptic strength at mossy-fiber synapses between hippocampal dentate granule cells and CA3 pyramidal cells. Mossy fiber terminals in guinea-pig hippocampal slices were labeled with fura-2 using our localized-perfusion loading technique and $[Ca^{++}]_{res}$ levels in individual terminals were measured while stimulating the mossy-fiber pathway and recording field excitatory post synaptic potentials. Action potential trains that did not induce LTP produced a transient buildup of $[Ca^{++}]_{res}$ that correlated with short-term increases in synaptic connection strength. The properties of this accumulation and its relationship to synaptic enhancement are similar to what is observed at other synapses (see Delaney et al. accompanying abstract) and suggest that the rise in $[Ca^{++}]_{res}$ *causes* the enhanced release. The enhancement is very calcium sensitive: a two-fold increase in transmitter release was accompanied by a 10-30 nM increase in $[Ca^{++}]_{res}$.

Despite the strong sensitivity of synaptic enhancement to $[Ca^{++}]_{res}$, sustained increases in $[Ca^{++}]_{res}$ are not responsible for the maintained synaptic enhancement observed during mossy-fiber LTP. Following induction of mossy-fiber LTP, $[Ca^{++}]_{res}$ decayed to prestimulus levels while enhancement of synaptic transmission persisted.

Our methods presently enable us to measure transient 20-50 nM increases in $[Ca^{++}]_{res}$ in single nerve terminals produced by a single action potential. We are attempting to use such measurements of $[Ca^{++}]_{res}$ to indirectly measure changes in presynaptic calcium current, another possible mechanism for the maintenance of LTP.

231.13

MODELING A SYNAPTIC CHEMICAL COMPUTATION: THE BUILDUP AND DECAY OF PRESYNAPTIC CALCIUM. D.W. Tank, W.G. Regehr & K.R. Delaney, Biophys. Res. Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974.

Calcium influx produced by action potentials invading a presynaptic nerve terminal produces a buildup of intracellular free calcium ions that modulates the strength of synaptic transmission produced by subsequent action potentials. Like the buildup and decay of charge on nerve cell membranes, this buildup and decay of intracellular calcium ions implements two computationally important dynamic functions: integration, and short-term (decaying) memory. To study how cellular properties alter this simple form of chemical computation and more accurately interpret our experimental results (see accompanying abstracts), we have developed a theoretical model and computer simulation for the presynaptic terminals we study based on solving calcium and buffer reaction-diffusion equations for a set of spherical shells. The model predicts that the decay of elevated calcium concentration produced by short trains of action potentials should be approximated by an exponential decay with a time constant proportional to calcium buffer concentration, consistent with the changes in calcium kinetics we have observed when exogenous buffers are introduced into the nerve terminal. Computer simulations also demonstrate that the sodium ion buildup we have measured during long action potential trains, could, by reversal of the sodium/calcium exchange, lead to a second, longer, time constant in the calcium decay, corresponding to post-tetanic potentiation, as experimentally observed. To test the model, we are also attempting to predict the calcium accumulations produced by long trains from measurements of accumulations produced by single action potentials. We also examined to what extent spatial and temporal averaging affect our fluorometric ion concentration measurements.

231.14

INHIBITION OF QUANTAL GLUTAMATE RELEASE AT HIPPOCAMPAL SYNAPSES:CONTRIBUTION OF MECHANISMS OCCURING DOWNSTREAM FROM Ca^{2+} INFLUX. K.P. Scholz and R. J. Miller, Dept. of Pharm. and Physiol. Univ. of Chicago, 60637.

The mechanisms responsible for the inhibition of quantal glutamate release during presynaptic inhibition were studied using whole-cell voltage-clamp techniques in cultured rat hippocampal pyramidal neurons. Small spontaneous inward current transients were identified as miniature excitatory post-synaptic currents (mEPSCs) by several criteria, including reversal potential, insensitivity to TTX and sensitivity to glutamate receptor antagonists. In the presence of high divalent cations (6mM Mg2+ and 3 mM Ca2+) spontaneous action potentials were suppressed. Addition of the adenosine receptor agonist cyclopentyladenosine (CPA, 100-200 nM) reduced the frequency of mEPSCs to 62±9% of control(n=6). This result was not accompanied by a shift in the cumulative histogram of mEPSC amplitudes, indicating a presynaptic origin for the reduction in frequency. The effects of CPA on mEPSC frequency were blocked by pretreatment with pertussis toxin. Divalent cation Ca2+ channel blockers were used to characterize the response further. Co2+ (1 mM) or Cd (100 uM) had variable effects on the frequency of mEPSCs, ranging from no effect to as much as 80% inhibition. In the presence of these agents, CPA induced a further reduction in the frequency of mEPSCs (to 70±7% of control;n=5), with no consistent change in amplitude. These results suggest that adenosine receptors may act through a G-protein to inhibit some component of the quantal release apparatus.The purified component of black-widow spider venom alpha-LTX (gift of J. Meldolesi), induced a dramatic increase in the frequency of mEPSCs, even in the presence of Cd2+. We are currently testing whether CPA can reduce this action in an attempt to assess whether CPA alters the sensitivity of the release apparatus to Ca2+.

231.15

RELEASE OF ACETYLCHOLINE FROM THE PREGANGLIONIC CHOLINERGIC FIBERS OF THE RAT MAJOR PELVIC GANGLION (MPG). G.T. Somogyi and W.C. de Groat. Departments of Pharmacology and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh PA, 15261.

Cholinergic modulation of ^{3}H-acetylcholine (ACh) release was studied in the rat MPG, which was prelabelled with ^{3}H-choline. In a superfused system two consecutive electrical field stimulations (S_1 and S_2) noticeably increased ^{3}H-ACh outflow in the presence of 10 uM hemicholinium-3 (S_2/S_1 ratio 0.85±0.04). ACh release was not dependent on the frequency of stimulation, 0.3 Hz producing the same volley output as 10 Hz. TTX (1 uM) or omission of Ca^{2+} completely abolished ACh release. Oxotremorine (1 uM) a muscarinic agonist decreased (0.69), whereas the antagonist atropine (1 uM) slightly increased the release of ACh (0.94). The cholinesterase inhibitor, physostigmine (1-5 uM), the nicotinic agonist, DMPP (10 uM) as well as the nicotinic antagonist, d-tubocurarine (50 uM) did not change ACh release. 4-aminopyridine a K^+ channels blocker significantly increased the release of ACh (1.85). 7 days after decentralization of the MPG the release of ACh was completely abolished. It is concluded that measurable amounts of ACh can be released by electrical stimulation in a single parasympathetic ganglion and that release occurs from the preganglionic nerve terminals rather than from the cholinergic cell bodies. The release is subject to modulation via muscarinic receptors, but not by nicotinic receptors. Supported by NSF Grant BNS-890-8934; NIH Grants DK 37241, DK 42369 and Clinical Research Center Grant MH 30915.

231.16

BIFEMELANE ENHANCES GLUTAMATE RELEASE FROM MOSSY FIBER SYNAPTOSOME OF GUINEA-PIG HIPPOCAMPUS: INVOLVEMENT OF PROTEIN KINASE C. Y. Kuraishi, M. Ueda*, T. Fujii* and M. Satoh, Dept. of Pharmacol., Fac. of Pharm. Sci., Kyoto Univ., Kyoto 606, Japan.

Bifemelane (BIF), which has an anti-amnesic action, enhances the long-term potentiation of the mossy fiber-CA3 pyramidal, but not the Schaffer collateral-CA1 pyramidal, system in the hippocampus. As a step to elucidate the mechanism of its selective action on the CA3, the effects of BIF on the mossy fiber terminals were examined. Mossy fiber synaptosomal fraction (P3) and conventional synaptosomal fraction (P2) were prepared from guinea-pig hippocampus. P3 fraction was richer in dynorphin A(1-8), dynorphin B and zinc than P2 fraction. BIF (0.01-1 μM) increased dose-dependently the 30 mM K+-evoked release of glutamate (Glu) from P3 fraction *in vitro*, without effect on Glu release from P2 fraction at 1 μM. Such increasing effect of 1 μM BIF on K+-evoked Glu release from P3 fraction was abolished by H-7 (100 μM), which also suppressed the increase of K+-evoked Glu release by phorbol 12,13-dibutyrate (1 μM). BIF (1 μM) translocated protein kinase C (determined by binding assay using [3H]phorbol 12,13-dibutyrate) from cytosol to membrane in P3, but not P2, fraction. The present results suggest that BIF directly acts on mossy fiber terminals to increase depolarization-induced Glu release, which may at least partly mediated by translocation of protein kinase C.

231.17

THE VARIABILITY OF TRANSMITTER RELEASE PROBABILITY AT CENTRAL SYNAPSES BETWEEN LOCAL INTERNEURONS IN THE LOCUST. G. Laurent. California Institute of Technology, Biology Div., CNS Program, 139-74, Pasadena CA 91125.

Quantal analysis was applied to inhibitory synapses between spiking and nonspiking local interneurons in the locust central nervous system. Paired intracellular recordings were made from pre- and postsynaptic neurons *in vivo*, and IPSP amplitude distribution histograms were constructed from large samples. Clear and regularly interspersed peaks in the histograms were found and taken to indicate quantal release. A simple binomial model (implying uniformity of release probability) was then used to fit the probability distribution of peak amplitudes. The average values obtained for n and p were 13.1±2.8 and 0.452±.16 (n=10) respectively. When several targets to a single spiking interneuron were found, the binomial n and p describing each synapse were compared. Whilst each distribution could be described by a simple binomial model, both n and p (and also their product m), however, differed between the different synapses made by the same presynaptic neurone. This suggests that, for a given interneurone, though the probability of release at all release sites of one synapse may be equal, this probability may differ from one synapse to the next.

231.18

INFLUENCE OF CALMODULIN INHIBITORS ON STRIATAL NEUROTRANSMITTER SYNTHESIS AND RELEASE. S.L. Garber, C. Ndubuka, R. McCormick* and M.H. Makman*, Depts. of Biochemistry, Molecular Pharmacology and Neuroscience, Albert Einstein College of Medicine, NY, NY 10461.

These studies concern the role of calmodulin and second messenger systems in synthesis and release of transmitters in rat striatal synaptosomes. Dopamine (DA) and serotonin (5HT) formation were measured by (1) decarboxylation of ^{14}COOH-labeled amino acid (AA) precursor, (2) conversion of ^{3}H-AA precursor to DA and 5HT and (3) net in vitro increase in endogenous synaptosomal DA and 5HT, purified by HPLC. Release of endogenous DA and 5HT and labeled DA, 5HT, glutamate (GLU) and γ-amino-butyric acid (GABA) were also measured. The calmodulin inhibitor calmidazolium (CMZ) at 10 μM inhibited basal as well as forskolin-stimulated synthesis of both DA and 5HT, while DA and 5HT release were stimulated by CMZ. DA and 5HT synthesis were also inhibited by another calmodulin inhibitor, W7 (N-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide), and by inhibitors of cyclic AMP-dependent protein kinase. CMZ stimulated release of GABA and GLU. W7, however, inhibited depolarization-induced release of GABA and GLU, indicating differential effects of CMZ and W7 on calmodulin and related processes. These results for transmitter release correspond to and may be mediated by effects on Ca^{2+} metabolism (see Ndubuka and Makman, this vol.). Also, there are several different sites at which calmodulin-dependent processes influence synthesis and release of neurotransmitters.

231.19

STRIATAL INTRASYNAPTOSOMAL CALCIUM CONCENTRATION AND CALCIUM FLUX: INFLUEUNCE OF CALMODULIN INHIBITORS.
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This study evaluates the influences of the calmodulin inhibitors, calmidazolium (CMZ) and N-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide (W7) on calcium ion fluxes and concentration in striatal synaptosomes. $^{45}Ca^{2+}$ fluxes were measured at various times (1s to 15 min) in normal and in high potassium (K^+) medium. Intrasynaptosomal [Ca^{2+}] was measured with the Ca^{2+}-sensitive fluorescent dye, Fura-2. CMZ and W7 affected Ca++ fluxes differently. High K^+ alone increased $^{45}Ca^{2+}$ influx. CMZ alone caused a smaller but significant increase in Ca^{2+} influx. The effects of CMZ plus high K^+ on Ca^{2+} influx were additive. W7 did not significantly alter basal Ca^{2+} influx. However, W7 inhibited the high K^+-induced increase in $^{45}Ca^{2+}$ influx. Qualitatively, these results agreed with the effects of W7 and CMZ on intrasynaptosomal [Ca^{2+}]. Quantitatively, however, the effects were appreciably different in the two Ca^{2+} assays, possibly due to the proportion or compartment of synaptosomes measured by each assay. $^{45}Ca^{2+}$ efflux was not influenced by either W7 or CMZ. We conclude that the calmodulin inhibitors influence striatal intrasynaptosomal Ca^{2+} by at least two mechanisms: a) inhibition of calmodulin-dependent Ca++ channel activation; and b) inhibition of calmodulin-dependent intracellular Ca^{2+} sequestration.

231.20

Ca STIMULATES SECRETION IN RAT PITUITARY CELLS WITHIN MILLISECONDS
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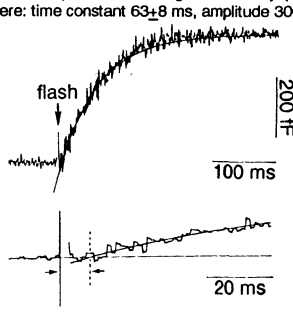
Neurons release transmitter in <1ms after cytosolic [Ca^{++}] (Ca_i) rises. To see how fast peptidergic endocrine cells secrete, we measured the membrane capacitance (C, proportional to cell surface) after flash photolysis of caged Ca. We voltage-clamped rat melanotrophs in whole-cell (-60mV, 32°C) and dialyzed them with 0.1mM fura-2, 10mM "cage" (DM-nitrophen) with 5mM $CaCl_2$, 4mM $MgCl_2$, 2 mM ATP plus buffer (pH 7.2) and Cs-glutamate to isotonicity. A <1mS UV flash transiently raised Ca_i to >1 uM (fura-2) and increased C (shown at slow and fast speed). C was well fitted with an exponential starting after a delay (arrows). Means (± S.E.) in 7 cells were: time constant 63±8 ms, amplitude 300±50 fF (750 vesicles), maximal dC/dt 5000±1000 fF/s or 12,500 vesicles/s. Conclusions: Ca_i stimulates secretion 60,000 fold over biochemically-measured basal rates in <20 ms. It does so with a delay (10±1 ms) that is more than 10 fold longer than at fast synapses, but the delay is too short for vesicles to move significant distances along microtubules. Hence most of the vesicles released here are probably already docked at the cell membrane. Supported by AR-17803.



231.21

COCAINE IN UTERO ENHANCES STRIATAL DOPAMINE RELEASE IN ADULT RATS.
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Although considerable work has focused on the effects of adult exposure to cocaine on dopamine (DA) neurotransmission and behavior, less has been written on the effects of cocaine treatment in utero on DA release or behavior. We measured the effect of repeated cocaine exposure of impregnated mothers on [^{3}H]-DA release from the brains of their offspring. Mother rats were divided into groups of saline in utero vs cocaine in utero (40 & 80 mg/kg; sc) and treated during the 20 day gestation period. At birth, offspring were taken from the mother and cross-fostered with non-cocaine treated nursing mothers. At three months of age, pups were given either a cocaine (10 mg/kg; ip) or saline injection and were sacrificed 24 hours later. [^{3}H]-DA release was measured from superfused 400 μm striatal slices under basal conditions and after exposure to amphetamine (2 μmM, 2.5 min) or electrical stimulation (5 Hz, 60 sec). In all saline offspring, one injection of cocaine produced a 47% increase in amphetamine-stimulated [^{3}H]-DA release, ($F(1,9)=6.00$; $p<.05$), as seen before (Peris & Zahniser; 1987). Amphetamine-stimulated release was also significantly increased by 44% in rats treated in utero with cocaine compared to animals from saline-treated mothers ($F(1,17)=6.55$; $p<.05$), regardless of whether rats were injected 24 hrs earlier with saline or cocaine. Neither basal nor electrically-stimulated release was affected by the cocaine in vivo or in utero treatments. Total tissue tritium levels were significantly decreased 35% in animals treated cocaine in utero, ($F(1,17)=7.21$; $p<.05$). Behavior testing must be performed to see if this increased release is correlated with any behavioral changes.

231.22

EFFECTS OF Ca^{2+}- AND Na^+-DEFICIENT CONDITIONS ON GABA RELEASE AND CORTICAL SYNAPTIC EXCITATION OF CELLS IN STRIATUM.
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We investigated the role of extracellular Na^+ in Ca^{2+}-independent release of GABA by measuring [^{3}H]GABA efflux from slices of rat striatum. In a parallel series of experiments, electrophysiological activity of single striatal cells was recorded extracellularly using a corticostriatal slice preparation. In the presence of low [Na^+] (27.25 mM, substituting choline Cl for NaCl) we observed that: (a) both spontaneous and electrically evoked GABA efflux increased, (b) only evoked GABA release was sensitive to TTX. In addition, in the corticostriatal slice preparation, synaptically-evoked action potentials of striatal cells gradually disappeared, but recovered upon returning to normal media. Total omission of Na^+ increased spontaneous GABA efflux more than 17-fold and blocked electrically evoked GABA release. In Ca^{2+}-free conditions (+1 mM EGTA): (a) electrically-evoked GABA efflux was enhanced in the presence of normal [Na^+], but decreased in low [Na^+]; (b) in normal [Na^+] an outburst of action potentials lasting 10-20 sec was induced, after which no spontaneous or synaptically-evoked activity was observed. This effect was prevented by Mg^{2+} (10 mM) or TTX (5 µM). Together, these results suggest that depolarization-evoked release of GABA in Ca^{2+}-free conditions can occur as a result of Na^+ influx in the absence of action potential events. Supported by NINDS (NS19608) and NIMH (MH00343).

231.23

CHARACTERIZATION OF GLUTAMATE RELEASE FROM PREOPTIC AREA SYNAPTOSOMES OF OVARIAN STEROID-PRIMED FEMALE RATS.
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Progesterone (P) treatment of estradiol (E_2)-primed, ovariectomized (OVX) rats in vivo was previously found to enhance the glutamate (GLU)-induced release of GABA, but not of GLU itself, from preoptic area (POA) synaptosomes incubated in vitro. Further studies have indicated that E_2 + P enhances GABA release via a Ca^{2+}-independent, carrier-mediated process that is inhibited by 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), a Cl^--channel antagonist. Release of ^{3}H-GLU from POA synaptosomes, derived from OVX rats treated with E_2 + P, was induced by GLU and by the GLU carrier agonist D-aspartate (D-ASP). The excitatory amino acid (EAA) receptor agonists N-methyl-D-ASP and kainate were without effect while quisqualate exerted a small stimulatory effect on ^{3}H-GLU release. Release of ^{3}H-GLU induced by GLU itself or by D-ASP was not blocked by the EAA receptor antagonists, D-2-amino-5-phosphonovaleric acid, 6,7-dinitroquinoxaline-2,3-dione or kynurenate. GLU-induced release of ^{3}H-GLU was Ca^{2+}-independent and blocked by DIDS. GLU altered neither basal nor K^+-induced increase in intrasynaptosomal [Ca^{2+}], as measured by the fura-2 method. It is suggested that GLU-induced release of ^{3}H-GLU in POA synaptosomes involves an atypical Cl^--dependent GLU carrier that may play a role in steroid-modulated amino acid release.

231.24

IN VITRO PATCH CLAMP ANALYSIS OF BACLOFEN EFFECTS IN THE RAT NUCLEUS TRACTUS SOLITARIUS (NTS) - COMPARISON OF SENSITIVITY OF SYNAPTIC AND DIRECT ACTIONS.
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Baclofen, acting at $GABA_B$ receptors in the NTS, causes increases in blood pressure and heart rate (Florentino et al, Brain Res. 535:264, 1990). We have used a brainstem slice preparation to examine responsiveness of NTS neurones and spontaneous or evoked synaptic potentials/currents (PSPs/PSCs). Current or voltage clamp recordings were made in whole cell patch clamp mode with intracellular solutions containing K-gluconate and ATP. All neurones (n=75) displayed spontaneous excitatory PSPs/PSCs and in some neurones (n=43), inhibitory PSPs/PSCs were also observed. Evoked PSPs/PSCs were obtained by electrical stimulation in the region of the tractus, and had both excitatory and inhibitory components. These could be studied in isolation by perfusing bicuculline or CNQX containing solutions respectively.

Concentrations of baclofen above 2µM caused membrane hyperpolarisation or outward currents which were insensitive to TTX and Co^{2+} and were accompanied by decreased resistance. At lower concentrations the baclofen was effective at blocking both spontaneous and evoked EPSPs and IPSPs at concentrations down to 250nM. IPSPs were more sensitive than EPSPs (ED_{50} 300vs. 750nM).

The pre- and postsynaptic effects of baclofen could be antagonised by 2-OH-Saclofen (400µM) confirming action at the $GABA_B$ receptor subtype.

Supported by NIH XXXXX , MRC Programme Grant & Wellcome Travelling Fellowship to PAB.

231.25

C-FOS ACTIVATION REFLECTS EXTRACELLULAR DOPAMINE LEVELS IN THE RAT CAUDATE-PUTAMEN AS MEASURED BY BRAIN DIALYSIS

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A combined study on the extracellular concentration of dopamine (DA) measured in freely moving rats and the activation of the nuclear proto-oncogene c-fos, was performed in the rat caudate-putamen (CPu) of the same animal in order to evaluate pre and post synaptic effects of drugs influencing DA activity. MAO inhibitors, pargyline (75 mg/kg) or clorgyline (10 mg/kg) increased DA extracellular concentration by 100% and 80% over basal values, respectively. The DA uptake inhibitor GBR 12909, increased DA extracellular concentration by 150% and 400% depending on the dose used (5 and 20 mg/kg). Histochemical visualization of c-fos like-immunoreactivity in the CPu, revealed significant activation of c-fos in pargyline and clorgyline treated rats; after GBR 12909 (5 mg/kg) stronger activation was observed. After 20 mg/kg of GBR 12909, dense and numerous c-fos granuli were found all over the CPu. Blockade of D-1 receptors by SCH 23390 (0.1 mg/kg) prevented GBR 12909-induced c-fos activation although it increased extracellular levels of DA by 20 folds; the D-2 antagonist (-)sulpiride (50 mg/kg), which increased extracellular DA levels by 50 folds, decreased but not abolished c-fos activation. The results show a good correlation between extracellular DA concentration and c-fos activation. Moreover the differential effect on c-fos activation of D-1 and D-2 receptor blockade indicates that D-1 receptor stimulation is crucial for c-fos activation while D-2 receptors play a permissive role.

POSTSYNAPTIC MECHANISMS: SIGNALLING PATHWAYS

232.1

WHOLE CELL RECORDING OF SEROTONIN RESPONSES IN RAT HIPPOCAMPUS.

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Recent implementations of the patch clamp technique have allowed for recordings in adult rats brain slices. However little is known regarding how results obtained using this technique compare with those obtained using microelectrodes. To address this issue whole cell recordings were used to examine the effects of serotonin(5-HT) in the CA1 region and to compare them to results previously obtained using microelectrodes.

Whole cell recordings were obtained from slices prepared using conventional methods. Patch pipettes contained 140 mM $KMeSO_4$; 5mM KCl; 2mM $MgCl_2$; 0.5mM EGTA; 10mM HEPES; 2mM ATP and 0.5 mM GTP and had resistances of 1.5 to 7 MΩ. Cell membrane potential and input resistance were comparable to those seen with microelectrodes.

Two distinct responses to serotonin are present in the CA1 region, a 5-HT_{1A}induced membrane hyperpolarization and a slower 5-HT_4 receptor mediated depolarization and decrease in the AHP. The 5-HT_{1A}response was readily observed in all cells tested and was stable for over 1 hr. Using this technique it could be readily demonstrated that the hyperpolarization was mediated by an inwardly rectifying potassium conductance and signalled by a G protein. In contrast the 5-HT_4 depolarizing responses were seen infrequently and the rapid wash-out of the AHP precluded repeated testing of the effects of serotonin on this afterpotential. Thus both techniques offer different advantages for studying the effects of serotonin in the hippocampus.

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232.2

ALKALINE EXTRACELLULAR pH SHIFTS EVOKED BY GLUTAMATE AND GABA IN RAT HIPPOCAMPAL SLICE.

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Activity dependent alkaline shifts (AS) have been described in many brain regions (1) and are particularly large in hippocampus (2). We studied the pharmacologic basis of this response using pH microelectrodes in the CA1 layer of hippocampal slices. Baseline extracellular pH was 7.20±0.13 (±SD, n=54 slices). Schaffer collateral stim (20 Hz, 10s) evoked an AS of 0.052±0.031 pH units, (n=138) followed by a variable, small acidification. Local pressure ejection of glutamate (GLU) or GABA elicited a similar AS, which averaged 0.052±0.032 (n=110) and 0.073±0.035 (n=137) pH units respectively. Picrotoxin (100 μM) did not affect the stimulus or GLU evoked AS, but abolished the GABA evoked response, indicating that the latter was mediated by GABA-A receptors.

Since bicarbonate generated pH shifts depend on the dehydration of carbonic acid, we tested the sensitivity of the GABA response to inhibitors of carbonic anhydrase. Acetazolamide (1 μM) or benzolamide (0.1 μM) completely blocked the GABA evoked AS. By contrast, similar concentrations of these agents enhanced the stimulus and GLU evoked AS up to 8.5-fold. These results indicate that the GABA evoked AS shift is most likely generated via bicarbonate efflux across GABA-A anion channels, as noted in cerebellum (3). By contrast, the excitatory synaptic and GLU evoked AS are generated by a distinct, bicarbonate independent mechanism.

Transmitter dependent shifts in extracellular pH are particularly relevant in that the activity of the NMDA receptor is sensitive to pH changes in the physiological range (4). Our results suggest that both excitatory and inhibitory activity can give rise to pH changes which may modulate NMDA receptor dependent processes.

(1) Chesler, Prog. Neurobiol. 34:401 (2) Carlini & Ransom Soc. Neurosci Ab. 12:452. (3) Chen & Chesler Neurosci. Lett. 116:130. (4) Tang et al. PNAS. 87(16):6445. Supported by NINDS Grant NS 27011.

232.3

GABA$_A$ AND GABA$_B$ RECEPTORS ARE LOCALIZED AT SEPARATE SYNAPSES ON CA1 HIPPOCAMPAL PYRAMIDAL CELLS.

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Application of GABA evokes three responses in neurons of the hippocampal slice, hyperpolarizing GABA$_A$ and GABA$_B$ responses and depolarizing GABA$_A$ responses. We have used two procedures to address the synaptic localization of these effects: 1. focal electrical stimulation in the presence of CNQX and APV and 2. nipecotic acid (NIP), an uptake inhibitor that can release cytoplasmic GABA by heteroexchange (Johnston et al., J. Neurochem. 26:83, 1976).

Focal stimulation could evoke pure monosynaptic hyperpolarizing GABA$_A$ IPSPs from all regions of the cell. Depolarizing IPSPs were not observed. Sites were found in the dendritic region, especially s. lacunosum-moleculare, which evoked pure GABA$_B$ IPSPs. Local application of NIP evoked responses with characteristics compatible with the activation of GABA$_A$ and GABA$_B$ receptors. These actions of NIP were reduced by SKF 89976A, a GABA uptake inhibitor that is not taken up, or by low Na^+ solutions. In addition, outside-out patches responded to GABA but not to NIP. These results suggest that the responses to NIP occur indirectly from release of GABA. We have used the GABA releasing properties of NIP to examine the distribution of synaptic GABA receptors facing GABAergic terminals. Hyperpolarizing GABA$_A$ mediated responses could be evoked from all regions of the cell. Depolarizing GABA$_A$ mediated responses were not observed. GABA$_B$ responses could be induced in the dendritic region, with pure responses occurring mainly in s. lacunosum-moleculare. The fact that focal stimulation and NIP can evoke pure GABA$_A$ and GABA$_B$ responses indicates that the receptors for these responses are segregated at some synapses. The failure to evoke depolarizing responses suggests that they are mediated by extrasynaptic GABA$_A$ receptors.

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232.4

GABA$_A$ RECEPTOR-MEDIATED SHUNTING INHIBITION OF PERFORANT PATH EXCITATORY INPUT TO DENTATE GYRUS GRANULE CELLS.

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The depolarizing postsynaptic potential elicited in granule cells by stimulation of perforant path fibres in the outer molecular layer of the dentate gyrus recorded in 400 μm hippocampal slices with the whole-cell method was increased by 55% following blockade of GABA$_A$ receptors, despite a Cl^- reversal potential 20 mV positive to RMP (-85 mV). Effective shunting by GABA$_A$ conductances of more depolarizing conductances mediated by dendritic glutamate receptors requires that GABA$_A$ conductances have a similar time course and relatively larger amplitude than the excitatory conductances.

The compound postsynaptic conductance (PSC) was analyzed at -35 mV with Cs-gluconate electrode solutions and specific blockers of GABA$_A$, NMDA and nonNMDA receptors. The time course of the PSCs mediated by NMDA and GABA$_A$ receptors were identical. The nonNMDA receptor-mediated conductance had more rapid kinetics. The ratios of peak PSCs were 8:1.5:1 for GABA$_A$:nonNMDA:NMDA receptors. This relationship was constant over a wide range of compound postsynaptic current amplitudes (90-400 pA). Even at the Cl^- reversal potential the GABA$_A$ shunt produced a 50-100% decrease in the amplitude of excitatory currents measured at the soma.

Thus, the GABA$_A$ receptor-mediated conductance had the characteristics necessary to shunt the glutamate-mediated PSC. The synaptically driven firing of granule cells at high stimulus intensities is due to the faster kinetics of nonNMDA *vs* GABA$_A$ conductances rather than differences in input-output relationships for inhibitory and excitatory conductances. Supported by a Dana Postdoctoral Fellowship (K.J.S.) and NIH grant NS 12151 (I.M.).

232.5

GLYCINERGIC IPSC KINETICS IN THE GOLDFISH MAUTHNER CELL ARE INDEPENDENT OF GLYCINE UPTAKE. M.J.Titmus, W. Young, H.Korn, and D.S. Faber. *Dept. Physiol. SUNY, Buffalo, NY, and Pasteur Inst.,Paris.*

Earlier we reported that blocking Na^+-dependent glycine uptake by replacing the extracellular Na^+ with Li^+, choline, or n-methylglucamine did not alter the time course of inhibitory responses evoked by the collateral network in the goldfish Mauthner (M-) cell. But it did enhance conductance changes produced by glycine iontophoresis. We have extended these studies by i) using two additional uptake blockers, p-chloromercuriphenyl sulphonate and sarcosine, and ii) examining the effect of all types of blockers on inhibitory responses evoked by single and repetitive VIIIn stimulations to produce small, graded conductances involving few inhibitory interneurons. The falling phase of the collateral IPSC could generally be fit accurately with a single exponential function and was not significantly altered by any of the uptake blockers (at -75mV, $\tau \sim 10$ms). IPSCs generated by VIIIn stimulation decayed in two phases, with a larger rapid component followed by a slower one ($\tau_{fast} = 5.7 \pm 0.9$ms, $\tau_{slow} = 132 \pm 43$ms, current ratio $= 2.8 \pm 1.4$, n=5), neither being altered by any of the uptake blockers. Repetitive stimulation of the VIIIn (5-7 stimuli, 500Hz) produced a more complex response with a larger and longer slow decaying phase that was not significantly changed by the blockers. We conclude that high affinity uptake of glycine does not play a role in determining the time course of the inhibitory synaptic currents even after repetitive stimulations, which may cause a buildup of transmitter in the synaptic cleft. Monte Carlo simulations of quantal glycinergic synaptic responses, using a range of binding and conformational rate constants, are consistent with rapid clearance of transmitter by diffusion alone.

232.6

ADENOSINE MAY LIMIT ACTIVATING BRAINSTEM INFLUENCES IN GENICULOCORTICAL NEURONS THROUGH TWO IONIC MECHANISMS H.C. Pape. Abt.Neurophysiologie, Ruhr-Univ., D-4630 Bochum, Germany

Adenosine (Ado) is considered as part of a negative feedback system which modulates neuronal activity depending upon the available energy. Local application of Ado (2-5 mM) to relay neurons in the guinea pig dorsal lateral geniculate nucleus (LGNd), maintained in vitro as slices, elicited a membrane hyperpolarization associated with an increase in potassium (K^+) conductance. The K^+ currents induced by activation of Ado and $GABA_B$ receptors were non-additive. Blockage of K^+ currents through barium unmasked an Ado evoked decrease in input conductance associated with a 1-2 mV hyperpolarization from -60 mV. The response was highly voltage dependent, appeared as an outward current negative to -66 mV, and was due to a reduction of the hyperpolarization activated cation current, I_h, whose amplitude and rate of rise were decreased by Ado. The adenylyl cyclase (AC) inhibitor 2',3'-dideoxyAdo (100 µM) imitated the decrease in I_h, while the AC stimulant forskolin (25 µM) increased I_h. The increase in K^+ conductance and the decrease in I_h, were mimicked by agonists (CPA, CHA; 50-300 µM) and blocked by an antagonist (DPCPX; 1-10 µM) selective for the A_1 receptor subtype.

While activity of the brainstem system is capable in the thalamus to create a state which is conducive of wakefulness (Steriade & Llinás, Physiol.Rev., 68: 649, 1988), the metabolic state of LGNd relay neurons may limit their responsiveness to brainstem inputs. An increased level of Ado inhibits single spike firing and promotes burst activity through a membrane hyperpolarization due to an increased K^+ conductance. The additional decrease in I_h directly inhibits the dampening influence of monoaminergic receptor activation on rebound burst oscillations.

232.7

EXCITATION OF GABAERGIC INTERNEURONS BY ACETYLCHOLINE IN RAT HIPPOCAMPUS. T.A. Pitler & B.E. Alger, Dept. of Physiol., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

Cholinergic agonists modulate GABAergic responses. Some reports show inhibition of GABAergic IPSPs with application of cholinergic agonists, while others suggest ACh enhances GABA release. We used intracellular recording techniques in the rat hippocampal slice to examine these apparent discrepancies.

Carbachol inhibited the evoked GABAergic IPSP, but increased picrotoxin-sensitive spontaneous membrane potential fluctuations. Using Cl^--containing electrodes to invert $GABA_A$-mediated events, we observed an atropine-sensitive increase in frequency of spontaneous IPSPs (dependent on interneuron firing) with carbachol application or stimulation of cholinergic afferents. Spontaneous IPSPs were blocked by picrotoxin or TTX. The increase in spontaneous IPSPs was not secondary to postsynaptic muscarinic actions since similar results were obtained under whole-cell voltage-clamp or when postsynaptic responses were blocked. Enhancement of spontaneous IPSPs was not blocked by CNQX and APV, ruling out indirect cholinergic excitation of interneurons by glutamatergic synapses.

We conclude that GABAergic interneurons possess muscarinic receptors, that activation of these receptors increases the excitability of interneurons and that synaptically released ACh increases interneuronal activity. Depression of the evoked IPSP by application of cholinergic agonists is presumably through the inhibition of excitatory synapses which drive interneurons.

232.8

ACTIVITY-DEPENDENT DEPRESSION OF GABAERGIC IPSCS IN RAT HIPPOCAMPUS. D.S.F. Ling and L.S. Benardo. Dept. of Pharmacology, SUNY-HSCB, Brooklyn, NY 11203.

Synaptic inhibition in hippocampal neurons consists of two distinct components: a fast inhibitory postsynaptic potential (IPSP) mediated by $GABA_A$ receptors and a slow IPSP mediated by $GABA_B$ receptors. Both of these inhibitory events appear vulnerable to activity-dependent depression since repetitive stimulation significantly reduces their amplitudes. To evaluate this in more detail, synaptic currents were recorded in CA1 pyramidal cells using whole-cell techniques in thick (400 µM) rat hippocampal slices (Blanton *et al., J. Neurosci. Meth.*, 30: 203, 1989). These cells had resting membrane potentials greater than -55 mV and input resistances >150 MΩ. Orthodromic stimulation of stratum radiatum evoked a fast EPSC followed by a fast and a slow IPSC. Stimulus frequencies >0.1 Hz seemed to reduce slow IPSCs, while fast IPSCs appeared depressed at frequencies >1-3 Hz. Precise determination of the degree of depression was limited due to temporal overlap of currents. Application of excitatory amino acid antagonists CPP and CNQX (10 µM) allowed IPSCs to be viewed in isolation, but was insufficient to quantitatively define the activity-dependent reduction of each component of inhibition. Subsequent blockade of $GABA_B$ currents with intracellular cesium allowed examination of the remaining fast IPSC, while bath application of picrotoxin blocked $GABA_B$ events, isolating slow IPSCs, thereby permitting unambiguous assessment of depression of the respective IPSC components.

232.9

LARGE INCREASES IN INTRACELLULAR Ca^{2+} REQUIRE POSTSYNAPTIC ACTION POTENTIALS IN HIPPOCAMPAL PYRAMIDAL NEURONS *W.N. Ross, D. Jaffe, N. Lasser-Ross, J. Lisman and D. Johnston*, Dept. of Physiology, New York Medical College, Valhalla, NY 10595, Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030, and Dept. of Biology, Brandeis Univ., Waltham, MA 02254.

A high speed CCD camera was used to measure the spatial and temporal characteristics of intracellular Ca^{2+} transients in individual hippocampal neurons injected with Fura-2. Subthreshold depolarizations produced only a small rise in Ca^{2+}. Suprathreshold depolarizations, which evoked trains of action potentials (APs), produced large (10-fold higher) changes in $[Ca^{2+}]_i$. In the same neurons, we studied the Ca^{2+} elevation produced by tetanic synaptic stimulation (50-100Hz). This produced large Ca^{2+} transients only when the postsynaptic depolarization was sufficient to evoke APs. The increase in Ca^{2+} concentration was correlated with APs rather than with the period of the tetanus. If APs were abolished by reducing the stimulus strength or hyperpolarizing the cell, the Ca^{2+} changes were about 10-fold smaller. Since Ca^{2+} entry produced by tetanic stimulation in the absence of APs was small, we studied a larger synaptic event, the paroxysmal depolarizing shift (PDS), after addition of picrotoxin. Even during these large synaptic events, Ca^{2+} changes were about 4-fold smaller when the PDS did not elicit APs. Generally, results in CA3 and CA1 cells were similar. These results suggest that Ca^{2+} transients resulting from activation of voltage-gated Ca^{2+} channels are driven primarily by Na^+ spikes (see also Jaffe et al., this volume). Furthermore, these results demonstrate that suprathreshold synaptic stimulation is required to produce significant Ca^{2+} changes, consistent with a literal Hebbian mechanism of synaptic potentiation. (NS16295, MH09996, and MH44754)

232.10

THE DISTRIBUTION OF DENDRITIC CALCIUM ENTRY PRODUCED BY ACTION POTENTIALS IN A MODEL HIPPOCAMPAL CA3 NEURON. *D.B. Jaffe, W.N. Ross, and D. Johnston*, Div. of Neurosci., Baylor College of Medicine, Houston, TX 77030 and Dept. of Physiology, New York Medical College, Valhalla, NY 10595.

Action potentials (APs) produce large transient increases in $[Ca^{2+}]_i$ in the proximal dendrites of hippocampal pyramidal neurons. Smaller Ca^{2+} transients are observed in the soma and distal processes (Regehr et al., 1989 and Ross et al., this volume). The distribution of the Ca^{2+} transients described above may be accounted for by a low density of Ca^{2+} channels in distal processes or by attenuation of Na^+-dependent APs propagating into distal dendrites. A computational model of a CA3 pyramidal neuron constructed using CABLE (Hines, 1989) was used to model Ca^{2+} transients produced by activation of voltage-gated Ca^{2+} channels in response to EPSPs or trains of APs. Free parameters were adjusted to produce repetitive, overshooting APs in response to a depolarizing current step. Two distributions of Na^+ and Ca^{2+} channels were considered. The first was a uniform Na^+ channel density in all compartments and a Ca^{2+} channel density reduced by 90% in distal dendrites. In the second, the Ca^{2+} channel density was uniform while the Na^+ channel density was restricted to the soma and proximal dendrites. Both models qualitatively reproduced the dendritic distribution of Ca^{2+} transients. In addition, subthreshold depolarizations, evoked intrasomatically or synaptically produced small Ca^{2+} transients in all dendritic compartments. The model suggests that the distribution of Ca^{2+} transients measured in the imaging experiments could be explained not only by a gradient of Ca^{2+} channel density, but also by attenuation of APs propagating to distal dendrites. (NS16295, MH09996, and MH44754).

232.11

CALCIUM-DEPENDENT SLOW AFTER-DEPOLARIZATION IN RAT LOCUS COERULEUS NEURONS. S.S. Osmanović and S.A. Shefner. Dept. Patho-Physiology, Med. Faculty Belgrade, Yugoslavia and Dept. Physiology and Biophysics, University of Illinois, College of Medicine. Chicago, IL 60612.

Intracellular recordings were made from rat locus coeruleus (LC) neurons in a totally submerged brain slice preparation. We have previously reported that trains of action potentials in LC neurons elicit a long-lasting, biphasic, post-stimulus hyperpolarization (PSH) mediated by two different Ca^{2+}-activated K^+ conductances. In the present experiments, reduction of PSH with externally applied Ba^{2+} or TEA, unmasked a large, slow depolarization following a train of action potentials in all cells tested (n=20). In order to elicit this after-depolarizing potential (ADP), the current stimulus had to exceed a certain threshold level. The ADP lasted for up to several minutes, during which time neuronal excitability was increased, resulting in prolonged high frequency firing. The ADP was associated with an increase in conductance. A single-electrode voltage clamp circuit was used to record the current underlying the ADP. The reversal potential of this current was -48 mV (n=4) and did not change when the intracellular Cl^- concentration was altered. The ADP was not affected by 1 μM tetrodotoxin, but was inhibited by the Ca^{2+} channel blocker Cd^{2+} (200 μM). Intracellular injection of the Ca^{2+} chelating agent EGTA depressed ADP. These data suggest that in LC neurons, Ca^{2+} influx during an action potential train can activate a slow inward current which may be similar to the Ca^{2+}-activated nonspecific cation current which has been described in other preparations. Grant Support: AA-5846.

232.12

EXTRACELLULAR CALCIUM IS NEEDED FOR POSTSYNAPTIC INDUCTION OF LONG-TERM DEPRESSION (LTD) AT CA1 SYNAPSES IN RAT HIPPOCAMPAL SLICES. G. Christofi*, A. Nowicky*, S. Bolsover* & L. Bindman. Dept. of Physiology, University College London, London WC1E 6BT, U.K.

Long-term depression of EPSPs evoked by stratum radiatum stimulation at 0.1Hz can be induced postsynaptically, by antidromic conditioning trains (6 at 100Hz for 0.5s every 10s) applied to CA1 axons in slices *in vitro*, either in normal bathing medium or in one containing high [Mg^{++}] to reversibly block all synaptic activity during conditioning (Pockett et al., 1990, Exp. Brain Res. 80, 196-200).

To test whether Ca^{++} entry was required for postsynaptic induction of LTD, we repeated the experiments in a bathing medium with high [Mg^{++}] and either 2mM Ca^{++} or 0 Ca^{++}. LTD of the depolarizing slope of the EPSPs was obtained in the presence of extracellular Ca^{++} (mean EPSP slope 52.6% ± 11.4% SEM of its control at 30 min post-conditioning, n=7) but **not in 0 Ca^{++}** (mean EPSP slope 102.7% ± 2.8% of its control at 30 min postconditioning, n=6) establishing that extracellular Ca^{++} is needed for induction of LTD. Postsynaptic Ca^{++} entry during depolarization persisted in high Mg^{++} solutions, as shown by FURA2 measurements in organotypic slices.

232.13

EXPERIENCE-DEPENDENT REGULATION OF PROTEIN KINASE C ACTIVITY IN THE OLFACTORY BULB AND STRIATE CORTEX OF DEVELOPING RATS. S. Elkabes*, J. A. Cherry, A. A. Schoups and I. B. Black . Dept. Neuroscience and Cell Biology , UMDNJ / Robert Wood Johnson Medical School, Piscataway, NJ, 08854.

Transduction of environmental signals into neuronal information involves complex processes mediated by regulatory molecules. Protein Kinase C (PKC) is one such molecule that has been implicated in mechanisms underlying synaptic function.

As a first approach in elucidating its role in synaptic plasticity during development, we have studied experience-induced alterations in PKC activity in the rat olfactory bulb by neonatal, unilateral odor deprivation and in the developing striate cortex by visual deprivation.

PKC associated with soluble and particulate fractions of olfactory bulb or striate cortex homogenates was partially purified by DEAE-cellulose chromatography. Activity was measured by incorporation of ^{32}P from [γ-^{32}P]ATP into Histone H1 (type III-S) in the presence or absence of Ca^{2+}, phosphatidylserine and diolein. Our studies indicate that soluble and membrane-bound PKC activity is gradually decreased 21 and 50 days following neonatal closure of one naris by cauterization. In addition, preliminary results suggest that PKC activity in striate cortex of rats reared in complete darkness for four weeks following birth is also decreased. These findings raise the possibility that experience-dependent regulation of PKC activity is similar in different brain regions during development.

232.14

BIOCHEMICAL PROBES THAT REVEAL RECEPTOR-G PROTEIN COUPLING M. Diversé-Pierluissi*, M.F. Goy†, and K. Dunlap. Physiology Departments, Tufts Medical School, Boston, MA 02111 and †Univ. of North Carolina, Chapel Hill, NC 27599-7545.

Norepinephrine (NE) and GABA inhibit voltage-dependent calcium channels in dorsal root ganglion (DRG) neurons. Distinct receptors mediate the inhibition and activate pertussis toxin (PTX)-sensitive G proteins. To determine whether the two receptors share a common G protein, we have developed methods for specifically labeling receptor-coupled pools of G protein. In one approach, DRG neurons are preincubated with PTX, with or without the transmitter(s). When present, the transmitter activates (dissociates) those G proteins that transduce the stimulus, thereby protecting them from ADP-ribosylation by the toxin. Following preincubation, transmitter is removed and membranes prepared and exposed to activated toxin with a radioactive ADP-ribosylation substrate. We find: 1) In the absence of transmitter, a 2 hour preincubation with PTX reduces the substrate available for labeling by >80%. 2) Inclusion of either NE or GABA increases the available substrate by >7 fold; we believe that those G proteins protected by the transmitter during the preincubation period are now available to incorporate radioactivity. 3) Specific receptor agonists and antagonists demonstrate that the protection displays the appropriate NE and GABA receptor pharmacology. 4) NE and GABA protect more than a single G protein substrate, suggesting that a given receptor interacts with more than one G protein. 5) The two transmitters appear to protect the same substrates, implying that individual (or highly similar) G protein subtypes can interact with more than one receptor *in vivo*.

232.15

G-PROTEINS IN DIABETIC ENCEPHALOPATHY: MOLECULAR MECHANISMS UNDERLYING THE FUNCTIONAL ALTERATIONS. A.M. Di Giulio, C. Finco*, M.L. Malosio*, M.P. Abbracchio, B. Paternieri*, F. Cattabeni, P. Mantegazza and A. Gorio. Dept. of Medical Pharmacology, Univ. of Milano, v. Vanvitelli 32, 20129 Milano, Italy.

We have previously shown that 5 and 14 weeks after diabetes induction by a single s.c. injection of alloxan (100 mg/kg), there is a progressive functional reduction of the Gi/o system in the rat striatum. ADP-ribosylation experiments of striatal pertussis toxin sensitive Gi/o subunits have indicated that 14 weeks following diabetes induction there is a 50% reduction of 32P incorporation. To test whether these data could be explained by a reduced Gi/o protein synthesis, we performed Western and Northern blotting experiments. The Western blotting analysis indicates that the amounts of Gi, Go and Gs proteins are apparently unchanged in diabetic animals. The mRNA analysis indicates that the expression of Gi and Gs is apparently normal in diabetic animals, whereas the 5.7 kb transcript of Go shows a 30% reduction in diabetic animals 14 weeks after the induction of diabetes. These data would suggest a specific alteration of the Go system in diabetic encephalopathy.

232.16

SOME CHARACTERISTICS OF G-PROTEIN COUPLING BETWEEN RECEPTORS AND POTASSIUM CONDUCTANCE OF THE LATE IPSP. R.H. Thalmann and M.I. Al-Dahan* Baylor College of Medicine, Houston, TX 77030.

In some dorsal root ganglion neurons, pertussis toxin (PTX) blocks the ability of GTPγS to promote functional coupling between G-proteins and their effector channels. We examined the generality of this phenomenon to potassium (K) channels of the late IPSC in hippocampal CA3 pyramidal neurons. After gigohm seals were established in slices from one month old rats, whole cell current underlying the late IPSP, the late IPSC, was recorded via pipettes containing gluconate as the major anion, and calcium buffered to 100 nM. Artificial CSF at 32°C was drawn over both sides of the slices via nylon meshes. Following a pulse (50V, 50μsec) delivered to the mossy fiber pathway, the late IPSC peaked at 200 mSec and 75±25 pA (holding potential=-60mV). GTPγS (0.5-1.0mm) in the recording pipette activated an outward current of 50-100 pA, after which the late IPSC could no longer be elicited. Intra-hippocampal injection of 2 μg PTX 3 days prior to recording blocked the late IPSC. Surprisingly, specific ^3H-baclofen binding was reduced by only 18%, and Gα01, Gα02, and Gαi1 were only partially ADP-ribosylated (ADP-ribosylation assay performed by Juan Codina). These PTX injections did not block the activation of outward current by GTPγS. Thus, PTX did not block the ability of GTPγS to promote G-protein coupling with the K channels of the late IPSC. Supported by NIH grant NS-21713.

232.17

DOPAMINE ENHANCES BOTH ELECTROTONIC COUPLING AND CHEMICAL EPSP AT MIXED SYNAPSES ON THE MAUTHNER (M) CELL.

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Previous work has shown that intracellular injections of cAMP and the catalytic sub-unit of cAMP-dependent protein kinase into the M-cell lateral dendrite enhanced both the electrotonic coupling potential and the chemical glutamatergic component of the mixed EPSP evoked by stimulating the posterior eighth nerve. Dopamine is generally believed to act through mechanisms that involve cAMP as a second messenger. Dopaminergic innervation of the M-cell was demonstrated by using a polyclonal anti-dopamine revealed with an avidine biotine complex (ABC) immunoperoxidase method and confirmed with an anti-tyrosine hydroxylase. Near the distal part of the lateral dendrite, immunoreactive fibers meander between the large myelinated club endings of the eighth nerve. As determined with electron microscopy, they contain some small vesicles, and they never establish synaptic contact with the M-cell, remaining in its synaptic bed, at a distance from its plasma membrane. Extracellular pressure-application of dopamine (10 mM in a vehicle solution of 130 mM NaCl, 10 mM Hepes and 1mM Ascorbic Acid) produced enhancements of both components of the mixed EPSP, which averaged 28.7% (range: 7.6 to 44.2) for coupling and 36.6% (range: 13.1 to 85.7) for the chemical component (n=10). Resting potential remained unchanged and antidromic spike height (a measure of M-cell input resistance) decreased, the average change being -6.3%. Following post-synaptic injections of the protein kinase inhibitor (Walsh), dopamine applications did not produce any enhancement of either component of the synaptic response, implicating a post-synaptic site of action. These results suggest that dopamine modulates this synaptic input via the cAMP second messenger system of the M-cell.

ACETYLCHOLINE RECEPTORS: MUSCARINIC II

233.1

MUSCARINE INCREASES CATION CONDUCTANCE AND DECREASES POTASSIUM CONDUCTANCE OF RAT LOCUS COERULEUS NEURONS IN VITRO. K-Z. Shen and R.A. North. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Whole-cell patch clamp recordings were made from locus coeruleus neurons in rat brain slices. Muscarine (30 μM) evoked an inward current at -60 mV, which was usually associated with a decrease in membrane conductance of some cells. This current persisted in TTX (1 μM) and cadmium (200 - 300 μM), and in solutions that were magnesium-free, low (14 mM) in chloride, calcium-free and high (10 mM) in magnesium; it was reduced by 78.4 $\pm$ 2.7 % in solution containing low (20 mM) sodium concentration. In control conditions, the muscarine induced current often showed two reversals, at about 0 mV and at a potential tens of millivolts negative to E_K (about -100 mV). When sodium was substituted by Tris, the reversal potential was close to E_K, and the current was blocked by barium (100 μM) and cesium (3 mM). When cesium replaced potassium in pipets and external solution, the muscarine current became linearly dependent on potential and reversed at 10 mV. The results indicate that muscarine produces an inward current by decreasing potassium conductance and by increasing cation conductance.

233.2

Heterogeneity of Muscarinic Depolarizations in Guinea Pig Celiac Neurons in Primary Culture. J.S. Coggan, S.L. Purnyn* and D.L. Kreulen. Department of Pharmacology, University of Arizona Coll. of Med., Tucson, AZ, 85724.

Voltage and current responses induced by muscarinic agonists were examined in celiac ganglion neurons in primary culture. Muscarinic agonists induced a depolarization which in 80% of cells was associated with only an increase (30%) in input resistance and in 20% of the cells was associated with an initial increase followed by a decrease (36%) in input resistance. The order of potency of agonists (900 ms application) used was ACh > oxotremorine-M > muscarine. During current clamp, muscarine (100 μM) increased the tendency of cells to fire repetitively and this effect was blocked by atropine (1 μM). Under whole cell voltage clamp, muscarine (1.5 sec application) induced inward currents with latency of less than 100 ms and durations of 20 - 30 s. Amplitudes of these currents diminished with hyperpolarization and disappeared near -65 Mv, without evidence of reversal. Maximal responses occurred at 500 μM with an EC50 of 60 μM. In 27% of cells examined, muscarine also induced a longer latency, slower inward current of a time-course similar to the secondary changes in input resistance observed with current clamp. Support:HL27781.

233.3

ACETYLCHOLINE-ACTIVATED SINGLE CHANNEL CURRENTS IN CULTURED APLYSIA NEURONS. M. Fejtl and D. O. Carpenter. Wadsworth Laboratories and School of Public Health, Albany, NY 12201.

Acetylcholine (ACh) activates Na-, K- and Cl- currents, all of which show desensitization. To reveal the underlying ion channel behavior responsible for that decay, we employed the single channel patch clamp technique on cultured Aplysia neurons. Ganglia from juvenile Aplysia were dissected and the isolated neurons plated on poly-l-lysine coated cover slips. Cell-attached patches were obtained with electrodes pulled from borosilicate glass and filled with artificial sea water containing either 0.5 μM ACh or 5 μM ACh.

We successfully recorded outward currents while holding the pipette potential between -10 and -150 mV. We never observed single channel events by hyperpolarizing the cell. Some patches showed openings at 0 mV, whereas the remainder showed openings at -40 mV or even lower. This may reflect the difference between K- and Cl- currents, but the reason for the lack of inward currents caused by positive pipette potentials is unclear. Outward currents were in the range from 1 pA to 5 pA and the slope conductance was about 60 pS. With increasing the hyperpolarization 2 or 3 channels opened simultaneously, independent of the agonist concentration used. We obtained 2 patches from the same cell with 0.5 μM ACh and 5 μM ACh, respectively. The 5 μM patch showed higher unitary currents than the 0.5 μM patch, but only in the range from -40 mV to -60 mV. Open time and closed time histograms could be best fitted with a double and single exponential probability density function, respectively. Since we recorded in steady state (the patches lasted between 5 an 10 minutes), non-desensitizing concentration of ACh must be used to provide sufficient channel openings. We observed bursts and long closed periods even with 0.5 μM ACh. Thus, the long closed periods reflect a desensitized state and lower agonist concentration is necessary to study non-desensitizing channel behavior.

233.4

OOCYTE EXPRESSION OF ACETYLCHOLINE-INDUCED INWARD CURRENTS FROM PHYLOGENETICALLY PRIMITIVE METAZOANS. Ian G. Welsford. Dept of Biology Bradley Univ., Peoria, IL 61625.

As a prelude to the molecular characterization of AChRs from divergent animal phyla, studies were undertaken to begin to characterize the physiological and pharmacological profiles of ACh-induced currents expressed in *Xenopus laevis* oocytes injected with total cellular RNA isolated from CNS tissue of the coelenterate, *Cyanea*, the annelid, *Nereis*, and the echinoderm, *Asteris*. The ACh-induced inward currents from each organism ranged from 50 to 250 nA and had linear I-V curves over the range of holding voltages between -100 and +10 mV. The sensitivity of each current to curare and alpha bungarotoxin was tested at holding potentials ranging from -100 to -20 mV. The currents from both *Asteris* and *Nereis* exhibited very similar sensitivities to alpha bungarotoxin (20 $\pm$ 5% decrease over control current levels for*Nereis* and 35 $\pm$ 8 % decrease in *Asteris*; mean $\pm$ SEM, n=5 oocytes) at all holding voltages tested. In contrast, *Cyanea* demonstrated marked voltage-dependence of blockade with alpha bungarotoxin, showing significant blockade at -80 mV (60 $\pm$ 7 % decrease over controls), no blockade at -60 mV (8 $\pm$ 5% decrease) and mild augmentation of inward current (20 $\pm$ 10% increase) at +20 mV. *Cyanea* currents were relatively insensitive to curare at all holding potentials tested (range 27 -19 % decrease over controls) while currents from *Nereis* and *Asteris* demonstrated marked voltage-dependence to curare blockade. Currents from both organisms were insensitive to curare at -80 mV (0-5 % decrease) but blockade steadily increased at more positive holding potentials, reaching a maximal value at -20 mV (48 $\pm$ 10 % decrease in *Asteris* and 53 $\pm$ 6 % decrease in *Nereis*). Supported in part by the Grass Foundation, The AMOCO Foundation and OTEFD at Bradley Univ.

233.5

ROLE OF RECEPTOR RESERVE IN THE DETERMINATION OF AGONIST/ ANTAGONIST SUBTYPE SELECTIVITY IN CHO CELLS EXPRESSING HUMAN MUSCARINIC RECEPTORS (ml-m5). R.D. Schwarz, D.K. Boyd, C.J. Spencer*, and G.L. Woodward*, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI, 48105.

Molecular characterization of muscarinic receptors based upon cloning, sequencing, and expression studies has shown that there are five subtypes of receptors (ml-m5). Recently, each subtype of human receptor has been stably expressed in CHO cells (Bonner and Brann, NIH). Using whole cell [^{3}H]-N-methyl-scopolamine (NMS) binding in these cells, the five subtypes were found to have K_d values of 0.11 - 0.63nM and B_{max} values ranging from 210 - 2450 fmoles/mg protein. Displacement of [^{3}H]-NMS by known muscarinic agonists showed that none were truly subtype selective. For antagonists, pirenzepine, methoctramine, and hexahydrosiladifenidol showed selectivity for m1, m2, and m3 respectively. To correlate the binding with functional activity, phosphatidylinositol (PI) turnover (m1, m3 m5) and adenylate cyclase activity (m2, m4) were measured. However, it was found that agonist potency and efficacy were markedly affected by differences in total receptor number among the subtypes, while antagonist results were not. For example, in PI turnover, alkylation of 90% of m1 receptors with propylbenzyl choline mustard rightshifted the EC_{50} for carbachol from 1.6uM to 109.6uM and decreased production of total [^{3}H]-inositol phosphates by almost 80%. Thus, differential receptor reserve must be taken into account when using functional measures to determine subtype selectivity.

233.6

IDENTIFICATION OF THE MUSCARINIC RECEPTOR SUBTYPE STIMULATING ADENYLATE CYCLASE ACTIVITY IN RAT OLFACTORY BULB. M.C. Olianas and P. Onali, Dept. of Neurosciences, University of Cagliari, Italy.

The pharmacological profile of the muscarinic stimulation of adenylate cyclase (a.c.) activity in rat olfactory bulb (o.b.) was investigated by using antagonists selective for the different muscarinic receptor subtypes. The potency of the compounds in antagonizing the acetylcholine (ACh) inhibition of a.c. in rat striatum and heart were also determined under similar conditions. Schild analysis showed that the ACh stimulation of a.c. was counteracted with high affinity by both the M2 antagonists methoctramine (pA2 = 8.08) and AF-DX 116 (6.84) and the M3 antagonists 4-DAMP (8.30), hexahydro-sila-difenidol (7.21) and p-fluoro-hexahydro-sila-difenidol (7.13). The M1 antagonist pirenzepine exibited the lowest potency (6.45). In striatum, ACh inhibition of a.c. was blocked by the M3 antagonists more potently than the M2 antagonists, whereas in the heart the opposite was observed. These data indicate that, whereas the striatal and cardiac inhibitory receptors can be classified as M3 and M2, respectively, the stimulatory receptors of o.b. show a pharmacological profile different from that typical of either the M1, M2 or M3 subtype.

233.7

MUSCARINIC RECEPTORS IN LOCUST GANGLIA ARE COUPLED TO PHOSPHATIDYL INOSITOL TURNOVER AND TO ADENYLATE CYCLASE. S. Qazi* and G. G. Lunt. Department of Biochemistry, University of Bath, Bath, BA2 7AY, U.K.

We have previously obtained preliminary evidence that muscarinic receptors in the supraoesophageal ganglion of the desert locust (Schistocerca gregaria) are coupled to phosphatidyl inositol turnover and to adenylate cyclase. We have extended these studies and have shown the incorporation of [^{3}H]inositol into inositol phosphates, particularly IP_3. The incorporation is enhanced by carbachol and a further enhancement is seen in the presence of $Li^+(10m^M)$. The stimulated incorporation is blocked by atropine. Pilocarpine is more effective than carbachol in the stimulation experiments. In the same tissue a carbachol inhibition of adenylate cyclase activity is seen that is reversed by atropine. Both pilocarpine and oxotremorine are more effective inhibitors than carbachol. Examination of the effects of a wide range of muscarinic agonists and antagonists suggests that the pharmacological profile of the locust cyclase-coupled muscarinic receptor is different from the well-characterised mammalian receptor classes.

We are grateful to ICI Plant Protection Division and the SERC for support.

233.8

ACTIVATION OF MUSCARINIC RECEPTORS INDUCES BOTH INOSITOL PHOSPHATE FORMATION AND Ca^{2+} UPTAKE IN PORCINE ADRENAL CHROMAFFIN CELLS. Y. Xu and E. J. Forsberg. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Activation of muscarinic receptors on porcine adrenal chromaffin cells evokes the secretion of catecholamines and ATP, and also evokes an increase in intracellular free Ca^{2+} ($[Ca^{2+}]_{in}$), in a manner largely dependent on extracellular Ca^{2+}. In contrast to secretion induced by nicotine and high K^+, which is inhibited by nifedipine in a does dependent fashion in the range of 0.1 to 10 μM, secretion induced by a muscarinic agonist, methacholine, can not be blocked by nifedipine in the same concentration range. These results indicate that muscarinic responses, unlike responses induced by nicotine and high K^+, are not mediated by L-type voltage-gated Ca^{2+} channels. Moreover, unlike nicotine-induced responses, methacholine-induced responses are not blocked by prior depolarization of the cells, again suggesting that voltage-gated Ca^{2+} channels are not involved (Xu, Duarte and Forsberg, J. Neurochem., in press). Methacholine (0.5 mM) induced an increase in $^{45}Ca^{2+}$ uptake by 2 fold and also activated Mn^{2+} uptake as measured in fura-2 loaded cells. The effect of methacholine on inositol phosphate formation was also examined. Methacholine at 0.5 mM induced a rapid increase in inositol 1,4,5-trisphosphate ($I(1,4,5)P_3$) by a factor of about 2. It also caused an increased production of IP, IP_2 and $I(1,3,4)P_3$. These findings strongly support the hypothesis that predominantly Ca^{2+} influx but also the release of Ca^{2+} from internal Ca^{2+} stores contribute to the secretion and $[Ca^{2+}]_{in}$ signals induced by activation of muscarinic receptors.

233.9

MUSCARINIC RECEPTORS COUPLED TO IP_3 IN ACUTELY DISSOCIATED HIPPOCAMPUS CA1 NEURONS OF RAT. *T. Nakaye*, M. Wakamori*, N. Harata*, H. Uneyama*, Y. Kataoka# and N. Akaike. Dept. of Neurophysiology, Tohoku Univ. Sch. of Med., Sendai 980. #Dept. of Pharmacology, Nagasaki Univ. Fac. of Med., Nagasaki 852, Japan.*

There are a few reports that ACh hyperpolarized CA1 neurons followed by the depolarization in mammalian hippocampus slice preparation. In this study, we investigated intracellular mechanisms of the controversial ACh responses in pyramidal neurons dissociated from rat hippocampus CA1 region. ACh evoked hyperpolarization under current-clamp condition and outward current (I_{KACh}) at a holding potential of -60 mV under "perforated" patch-clamp. Muscarine played as ACh agonist, and I_{KACh} was inhibited more preferentially by pirenzepine than by AF-DX-116. Fura-2 fluorometric measurement showed that ACh increased the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) even in Ca^{2+} free extracellular solution. Cholera toxin and pertussis toxin had no effect on I_{KACh}. Calmodulin antagonists (W-7, chlorpromazine, trifluoperazine) inhibited I_{KACh} in a concentration-dependent manner, whereas a protein kinase inhibitor (H-7) had no effect on it.

These observations suggested us following intracellular pathway of ACh response mediated by M1 receptor. Phospholipase C is activated by G_p, and inositol trisphosphate (IP_3) releases Ca^{2+} from intracellular stores. This free Ca^{2+} activates calmodulin kinase followed by protein phosphorylation and K channel opening.

233.10

USE OF M_1/M_2 CHIMERIC MUSCARINIC RECEPTORS FOR THE DELINEATION OF STRUCTURAL DOMAINS INVOLVED IN LIGAND SELECTIVITY AND FUNCTIONAL REGULATION. J. Lai, S.W. Ma*, S. Waite*, L. Nunan*, J.W. Bloom*, H.I. Yamamura and W.R. Roeske. Departments of Pharmacology and Internal Medicine, University of Arizona, Tucson, AZ 85724.

Several chimeric M_1/M_2 muscarinic receptors were expressed in murine fibroblasts (B82) transfected with various recombinant murine m1/m2 genes. These chimeric receptors and the native M_1 and M_2 receptors all have similar affinities for non-selective ligands such as [^{3}H](-)MQNB (K_d=358-519 pM) and atropine (K_i=0.51-0.99 nM). The binding properties of a number of agonists and selective antagonists to these receptors were analyzed. Preliminary data based on the binding studies of pirenzepine and AF-DX 116 suggest that the high affinity, selective binding of AF-DX 116 to the M_2 receptors may reside between the 1st and 4th transmembrane domains whereas that for pirenzepine to the M_1 receptors may reside between the 4th and 6th transmembrane domains. The latter region also modulates the binding of the agonist carbachol (CCh). When this region is derived from the M_1 receptors, the agonist exhibits multiple affinity states (K_H=2.5 μM; K_L=53.6 μM). A single affinity state for the agonist is observed, however, when the same region is derived from the M_2 receptors (K_i=6 μM). Substitution of the N terminal to 4th transmembrane domain of the M_1 receptors with that of the M_2 receptors reduces the potency (7 fold) and efficacy (3 fold) of CCh in inducing inositol lipid (PI) hydrolysis but enhances CCh induced cAMP formation (7 times that of the M_1 receptors at 1 mM CCh). This may suggest that the M_1 receptor mediated cAMP production is not entirely a secondary effect of PI hydrolysis mediated by the receptor. Supported in part by AHA, NHLBI and NIMH.

233.11

ASYMMETRY IN THE ALLOSTERIC INTERACTIONS BETWEEN AGONISTS AND GUANYL NUCLEOTIDES AT CARDIAC MUSCARINIC RECEPTORS P. Chidiac* and J.W. Wells Department of Pharmacology and Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 1A1

The muscarinic agonist carbachol and the guanyl nucleotides GDP, guanosine-5'-O-(3-thiotriphosphate) (GTPγS), and guanylyl imidodiphosphate (GNP-PNP) were studied for their effects on the binding of N-[^{3}H]methylscopolamine ([^{3}H]NMS) and [^{35}S]GTPγS to highly washed left ventricular membranes from Syrian hamsters. The specific binding of 1 nM [^{3}H]NMS was inhibited by carbachol to yield Hill coefficients less than one. An overall decrease in the affinity of the agonist was observed in response to each nucleotide. The specific binding of 160 pM [^{35}S]GTPγS similarly was inhibited by the unlabeled nucleotides to yield Hill coefficients less than one. An overall decrease in the affinity of GDP was observed in response to carbachol, which had no effect on the binding of either GMP-PNP or GTPγS. The allosteric interaction between agonist and guanyl nucleotide at receptors linked to G proteins commonly is rationalized in terms of an equilibrium between free receptors (R) and free G proteins (G) and an RG complex. The model predicts that the effect of an agonist on the binding of a nucleotide to the relevant G protein will mirror the effect of the nucleotide on the binding of the agonist to its receptor. The asymmetry inherent in the failure of carbachol to perturb the binding of GTPγS and GMP-PNP suggests that the dispersion of affinities revealed in binding of carbachol and guanyl nucleotides does not reflect the coexistence of coupled and uncoupled receptors and G proteins.

233.12

DIFFERENT ALLOSTERIC MODULATORS INTERACT AT A COMMON SITE ON CARDIAC MUSCARINIC RECEPTORS. John Ellis and Margaret Seidenberg*. Neuroscience Research Unit, Department of Psychiatry, University of Vermont, Burlington VT 05405.

A number of compounds from diverse pharmacological groups interact allosterically with muscarinic receptors. However, it is not known whether the allosteric effects of these different modulators are mediated by a common site, or indeed whether any modulator interacts at a well-defined site. Many of the allosteric ligands act at concentrations where nonspecific effects might occur. We have noted that different allosteric modulators affect rat cardiac muscarinic receptors to different extents. That is, gallamine, TMB-8, and THA slow the dissociation of [^{3}H]NMS (reduce the apparent k_{off}) by more than 90%. On the other hand, obidoxime slows the dissociation by only about 50%, even at maximal concentrations. If these allosteric modulators interact in a competitive manner at the allosteric site, one would expect obidoxime to be able to partially reverse the allosteric effects of the other ligands (the situation is mathematically analogous to the case of a partial agonist interacting with a full agonist). We have observed that obidoxime does partially reverse the allosteric effects of gallamine, TMB-8, and THA, in a concentration-dependent manner that suggests a common site of action. This finding is encouraging for studies aiming to manipulate receptor function via the allosteric site. *(Supported by R01AG05214)*

233.13

ALLOSTERIC MODULATION OF MUSCARINIC RECEPTORS BY PROTAMINE. J. Hu*, S-Z. Wang*, C. Forray and E.E. El-Fakahany. Department of pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

A large number of diverse pharmacological agents bind to a secondary domain on the muscarinic receptor to allosterically influence the interaction of ligands at the primary binding site. Based on common structural features of these antagonists we examined the interaction of protamine, an endogenous polycationic peptide and polyamines with muscarinic receptors in rat heart. Our results provide several lines of evidence that protamine allosterically modulates the conformation of muscarinic receptors in a marked negative cooperative manner. It decelerated the dissociation of [^{3}H]NMS in a concentration-dependent manner, with a 50% inhibition of ligand dissociation at 20.2±5.3 μg/ml. Inhibition of [^{3}H]NMS binding by protamine at equilibrium showed a distinct plateau which increased in magnitude at higher ligand concentrations. Scatchard analysis of saturation isotherms of [^{3}H]NMS binding indicated that protamine did not alter maximal ligand binding. However, it decreased the affinity of [^{3}H]NMS in a concentration-dependent manner, but with a ceiling effect. This allosteric effect of protamine is selective for muscarinic m2 receptors as compared in Chinese hamster ovary cells transfected with the muscarinic m1, m2, m3 and m4 genes. Arginine residues play an important role in this allosteric interaction. No effects of polyamines were observed on [^{3}H]NMS binding. This is the first report on the allosteric modulation of a G-protein-coupled receptor by an endogenous peptide.

233.14

ACRIDINE ARAPHANES: A NEW CLASS OF DRUGS USED TO EVALUATE PHARMACOPHORE INTERACTIONS WITH CHOLINERGIC RECEPTORS. K.P. Shaw[1,5], S. Pou[4*], R.S. Aronstam[2] & E.X. Albuquerque[1,3]. Depts. [1]Pharmacol. Exp. Ther. Sch. Med. & [4]Pharmacol., Sch. Pharm., Univ. MD, Baltimore, MD 21201; [2]Pharmacol. Toxicol., Med. Coll. Georgia, Augusta, GA 30912; [3]Lab. Mol. Pharmacol.II, IBCCF, RJ, Brazil 21941; [5]NDMC, Taipei, Taiwan, ROC.

Structure-activity relationship studies have shown that agonists of muscarinic receptors bind simultaneously to three sites. In addition, hydrophobicity is important to muscarinic antagonists. A novel series of bis-ammonium acridine derivatives with a variable number of carbons (n) in the alkyl chain was synthesized and characterized by hydrophobicity, electrostatic interaction and steric rigidity. Biochemical studies showed that as n increases from 2 to 4 the affinity of the compound to the muscarinic receptor enhances by ≈ 10-fold such that 1,4-butane acridine araphane (1,4-BAA) is the most potent in this series ($IC_{50} \approx 10^{-9}$ M). Increasing n to 7 decreases the affinity by ≈ 300-fold. When n = 9 (1,9-nonane acridine araphane: 1,9-NAA) the affinity suddenly increases to values similar to 1,4-BAA. The length of the alkyl chain also affects the binding of these compounds to nicotinic receptors. Acridine araphanes have at least two nitrogens protonated, with pk_a values between 11.6 and 8.2. According to quantum mechanics calculation by computer-aided molecular-modeling, protonation occurs at N_{ar} (nitrogen in acridine ring). By geometry optimization, the acridine rings are relatively parallel when n=2-4, the inter-N_cs distince being from 3.6 to 5.8 Å. They tend to become coplanar as n increases to 7 because of double bond formation between N_c (nitrogen in alkyl chain) and C_9 and extention of the distance inter-N_cs to 7.7 Å. After n=9, the rings fold together, decreasing the inter-N_c distance (6.2 Å). In conclusion, hydrophobic regions and a distance from 5.8 to 6.8 Å between inter-cationic (N_c) sites and hydrophobic groups are critical to determine the antagonist potency of the acridine araphanes which appear as a new class of cholinergic probes. Support: U.S. Army Med. Res. & Devel. Comm. Contr. DAMD17-88-C-8119.

233.15

SINGLE CHANNEL CURRENTS ACTIVATED BY PHYSOSTIGMINE (PHY) IN HIPPOCAMPAL NEURONS ARE BLOCKED BY BENZOQUINONIUM (BZQ) BUT NOT BY METHYLLYCACONITINE (MLA). E.X. Albuquerque[1,2], A. Maelicke[3] and E.F.R. Pereira[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD, USA, 21201; [2]Lab. Mol. Pharmacol. II, IBCCF, RJ, 21944, Brazil; [3]Inst. Physiol. Chem., Johannes-Gutenberg Univ. of Med. Sch.; Duesbergweg, D-6500 Mainz, Germany.

It has been shown that PHY activates nicotinic acetylcholine receptors (AChR) on muscles (*Mol. Pharmacol.*, **28**:527, 1985). However, studies performed on *Torpedo* demonstrated that the agonistic action of PHY could not be blocked by α-BGT, d-TC or by a monoclonal antibody to the well-established agonist binding sites of the AChR. Thus, PHY might be activating the AChR by a second pathway (Okonjo *et al.*, *in press*). Here, we evaluated single channel currents activated by PHY (10 μM) in outside-out patches excised from fetal rat hippocampal neurons grown in culture for 20-30 days. The effects of BZQ (3-30 μM) and MLA (2 nM) on these currents were also analyzed. All solutions contained TTX (0.1 μM), atropine (1 μM) and APV (50 μM), and were delivered via a glass mini-pipe connected to a perfusion system. PHY activated most 30-pS single channel currents which appeared as both brief and long events. Kinetic analysis revealed that channel open, closed and burst times could be fit by double exponential functions. Open times (τ_o) were voltage-dependent and increased with membrane hyperpolarization. BZQ (3-30 μM) significantly reduced the τ_o of these channels in a concentration- and voltage-dependent manner. The higher the membrane hyperpolarization, the greater the blockade, such an effect being reversed at positive potentials, which indicates that BZQ is an open channel blocker. The agonist effect of PHY, in contrast to that of (+)anatoxin-a, was not blocked by MLA (2 nM), reinforcing the notion that PHY binding sites on the AChR may be different from those of ACh. Support: U.S. Army Med. Res. & Devel. Comm. DAMD 17-88-C-8119 & USPHS NS25296. CAPES Fellow (EFRP).

233.16

INTERACTION OF 4-DAMP MUSTARD WITH MUSCARINIC RECEPTORS IN THE BRAIN, HEART AND SALIVARY GLANDS. E.J. Ehlert, H.H. Hsu*, A.L. Hunter* and E.A. Thomas*. Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

A 2-chloroethylamine derivative (4-DAMP mustard; N-(2-chloroethyl)-4-piperidinyl diphenylacetate) of the selective muscarinic antagonist 4-DAMP (N,N-dimethyl-4-piperidinyl diphenylacetate) was synthesized, and its conversion to an aziridinium ion and interaction with muscarinic receptors was investigated. When dissolved in aqueous solution at pH 7.4 and 37°C, 4-DAMP mustard released an equivalent amount of chloride. The release of chloride was consistent with a first order process having a half-time of approximately 15 min. Under the same conditions, the peak concentration of the aziridinium ion was reached at approximately 45 min and corresponded to about 60% of the initial concentration of 4-DAMP mustard. The concentration of the aziridinium ion decayed to half of its peak concentration at approximately 4 hr after dissolution. Treatment of the isolated rat ileum with 4-DAMP mustard caused an irreversible blockade of contractions elicited by the muscarinic agonist oxotremorine-M, and this blockade persisted after extensive washing. When homogenates of the brain, heart and submaxillary gland were incubated with 4-DAMP mustard (10 nM) for 1 hr, washed extensively, and then assayed for muscarinic receptor binding properties, a decrease in the binding capacity of the muscarinic antagonist [^{3}H]N-methylscopolamine was observed. We conclude that 4-DAMP mustard forms an aziridinium ion that binds irreversibly to muscarinic receptors.

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233.17

CHOLINERGIC PARTICIPATION IN MASCULINE SEXUAL BEHAVIOR REGULATION IN RATS. S. Retana*, E. Dominguez* and J. Velazquez-Moctezuma. Dept. Biol. Reprod. Universidad Autonoma Metropolitana-Iztapalapa. C.P. 09340. México City. MEXICO.

The role of the cholinergic system in the regulation of masculine sexual behavior (MSB) remains controversial. Contradictory reports in the literature suggest that both muscarinic an nicotinic receptors systems could be involved. In this study, we analyzed the acute effect of several doses of a muscarinic antagonist, scopolamine (Sco), as well as, the effect of its chronic administration. Additionally, the acute effects of several doses of nicotine were also assessed. Acute administration of Sco induced a dose-dependent decrease of the percentage of rats displaying MSB, as well as an increase of mounts an intromissions latencies. MSB after finishing the chronic administration of Sco showed an increase of intromission frequency as well as an enlargement of ejaculation latency. The main effect of acute nicotine administration was a trend towards the inhibition of ejaculation frequency.

ACETYLCHOLINE RECEPTORS: MUSCARINIC III

234.1

DISTINCT KINETIC PROPERTIES OF *N*-[^{3}H]-METHYLSCOPOLAMINE BINDING AFFORD DIFFERENTIAL LABELING AND LOCALIZATION OF M1, M2, AND M3 MUSCARINIC RECEPTOR SUBTYPES IN THE PRIMATE BRAIN. D. D. Flynn, G. Maxwell*, A.A. Vaishnav, M. Basile, and D. C. Mash. Depts. of Pharmacology & Neurology, University of Miami School of Medicine, Miami, FL., 33101.

Three classes of muscarinic receptors in mammalian brain have been postulated on the basis of equilibrium and kinetic binding data (Giraldo et al., 1987; Ehlert et al., 1989). However, equilibrium binding assays alone have not permitted a clear demonstration of M1, M2, and M3 receptor subtype localization in the brain because of the overlapping selectivities of muscarinic antagonists. In this study, we demonstrate that the distinct kinetics of *N*-[3H]-methylscopolamine (NMS) binding permit receptor subtype-selective labeling in the primate brain. Equilibrium and kinetic binding conditions were established using cells (AL9) transfected with the genes for the m1, m2, m3 and m4 receptor subtypes. For autoradiographic studies, the m1 receptor was directly labeled with 3 nM [^{3}H]-pirenzepine. The m2 receptor was labeled with short pulses of 0.5 nM [^{3}H]-NMS after preincubation with 0.3 μM pirenzepine to occlude m1 and m3 sites. Selective labeling of the m3 receptor was obtained by incubating cell membranes or sections with 0.25 nM [^{3}H]-NMS followed by a 60 minute dissociation in the presence of 1 μM atropine. The distribution of M1 and M2 receptor subtypes corresponded well to previous autoradiographic studies (Mash et al., 1988). The distribution of the M3 receptor subtype was largely coincident with the pattern of the M1 sites labelled by [^{3}H]-pirenzepine with some notable exceptions. The M3 receptor subtype was elevated over the dorsolateral prefrontal cortex and the CA2 sector of the primate hippocampus. Peak densities of M3 sites were apparent throughout the dorsal sector of the caudate, while M1 receptors were most prevalent in the ventromedial sector. These results provide pharmacological and anatomic evidence for M1, M2 and M3 subclasses of muscarinic receptors in the primate brain. (Funded by NS19605 and NS25785.)

234.2

Autoradiographic Characterization of m_3 Muscarinic Cholinergic Receptors in Rat Brain

J.K. Zubieta, MD and K.A. Frey, MD, PhD, *Neuroscience Laboratory, University of Michigan. Ann Arbor, MI 48109*

The binding characteristics of [3H]4-DAMP, a muscarinic antagonist with high affinity for m_1 and m_3 and intermediate affinity for m_2 receptor subtypes, were studied in an attempt to visualize the distribution of the m_3 site in rat brain. Equilibrium binding conditions and optimal post-incubation wash times were determined for 20 μm-thick cryostat sections. Autoradiographic saturation analysis demonstrated apparently homogeneous populations of binding sites with K_D varying between 4 nM in striatum and 12 nM in the hypoglossal nucleus. Displacement of 2 nM [3H]4-DAMP with unlabeled pirenzepine (PZ), an m_1-selective muscarinic antagonist, revealed evidence of high and low affinity sites in striatum (K_H 20 nM, K_L 500 nM; attributable to m_1 and m_3 sites) while only the low affinity site (m_2 or m_3) was observed in the hypoglossal nucleus. Displacement of [3H]4-DAMP with AF-DX 116, a selective m_2 antagonist, revealed single site competition with K_I of 6 to 7 nM in all regions studied. On the basis of these findings, selective m_3 autoradiographic images were generated by incubation in 5 nM [3H]4-DAMP in the presence of both 1 μM PZ and 3.5 μM AF-DX 116 to block binding to the m_1 and m_2 subtypes, respectively. The distribution of m_3 receptors defined in this manner is diffuse throughout the nervous system, but is quantitatively minor in comparison to levels of other subtypes. These results indicate that the binding of [3H]4-DAMP in the absence of unlabeled selective inhibitors is predominantly to non-m_3 receptors.

234.3

Effects of DFP Treatment on m3 Receptors: An Autoradiographic Analysis

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Prior studies from our laboratory have demonstrated significant reductions in muscarinic receptor binding in rat brain after 7 days of treatment with the acetylcholinesterase inhibitor DFP. The changes appear to involve both high and low affinity sites defined by pirenzepine (PZ) and AF-DX 116 competition of [3H]scopolamine binding. We have now extended these results by studying the effects of DFP treatment on muscarinic m_3 receptors defined by the regional distribution of [3H]4-DAMP binding in the presence of unlabeled PZ and AF-DX 116. The specific binding of [3H]4-DAMP (5 nM), a muscarinic receptor antagonist with affinity for m_3, m_1, and m_2 receptors, was significantly decreased (20-30%) in the striatum and cerebral cortex following cholinesterase inhibition. When unlabeled AF-DX 116 (3.5 μM) was added to eliminate m_2 subtype binding, detectable down-regulation was present but of reduced magnitude in cerebral cortex. After addition of cold PZ (1 μM) to eliminate m_1 binding, relative down-regulation was enhanced in both striatum and cortex. The selective m_3 autoradiograms obtained after the addition of both unlabeled PZ and AF-DX 116 did not reveal significant down-regulation after DFP treatment in any of the forebrain areas studied. The present results suggest differences in the regulation of muscarinic cholinergic receptor subtypes following acetylcholinesterase inhibition. Down-regulation of muscarinic receptors by DFP treatment appears to involve m_1 and m_2 subtypes to differing extent, but does not affect m_3 muscarinic cholinergic receptors.

234.4

AUTORADIOGRAPHIC ANALYSES OF THE BINDING OF NIPECOTIC ACID ESTERS TO MUSCARINIC RECEPTOR SUBTYPES IN MOUSE BRAIN. C.N. Hinko, A. El-Assadi*, A.M. Crider* and W.S. Messer. College of Pharmacy, Univ. of Toledo, Toledo,OH 43606 and College of Pharmacy, Northeast Louisiana Univ., Monroe, LA 71209.

Prior studies have shown that the ethyl ester of (-)nipecotic acid (NA), (+)ethyl nipecotate hydrogen tartrate ((+)ENT), possesses anticonvulsant activity but also elicits signs of cholinergic stimulation such as tremors, salivation, lacrimation and diarrhea in mice. The ethyl ester of (+)NA, (-)ethyl nipecotate hydrogen tartrate ((-)ENT), has minimal anticonvulsant effect and no obvious cholinergic action. The *m*-nitrophenyl ester of (-)NA, (-)*m*-nitrophenyl-3-piperidine carboxylate HCl ((-)MNPC-HCl), and the *m*-nitrophenyl ester of (+)NA, (+)MNPC-HCl, are both active as anticonvulsants with no obvious cholinergic effect. In the present study the binding of the ethyl and *m*-nitrophenyl esters of (+)NA and (-)NA to muscarinic receptors in the mouse brain was examined through quantitative autoradiography. The overall order of potency for inhibition of [3H]-l-quinuclidinyl benzilate ([3H]-l-QNB) binding to mouse brain sections was (+)ENT>(-)ENT>(+)MNPC-HCl>(-)MNPC-HCl. Regional quantification of the autoradiograms indicated that (+)ENT bound with highest affinity to midbrain nuclei (eg. lateral hypothalamus, IC_{50} = 5.0 μM) and with lowest affinity to forebrain nuclei (eg. dentate gyrus, IC_{50} = 25 μM). (-)ENT displayed slightly less selectivity and a lower affinity for muscarinic receptors with IC_{50} values for the lateral hypothalamus and dentate gyrus of 91 and 170 μM, respectively. Both (+)MNPC-HCl and (-)MNPC-HCl bound with even lower affinity and a relative lack of selectivity. Analysis of the data yielded a Hill coefficient of less than 1 for (+)ENT, and of slightly less than 1 for (-)ENT. The Hill coefficients for both (+)MNPC-HCl and (-)MNPC-HCl were close to unity. These data provide support for (+)ENT as an M_2-selective agonist. (-)ENT appears to be M_2 selective but has lower affinity than (+)ENT, while (+) and (-)MNPC-HCl bind weakly to muscarinic receptors and in a nonselective manner. The observed order of affinity for binding to muscarinic receptors is in agreement with their previously observed tendency to produce cholinergic side effects.

234.5

THE DISTRIBUTION OF M3 MUSCARINIC RECEPTOR SUBTYPE AND m3 MESSENGER RNA IN HUMAN OCULAR STRUCTURES

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We localized M3 muscarinic receptor subtype and its messenger RNA in human eye sections by using *in vitro* autoradiography and *in situ* hybridization respectively. M3 binding sites were detected with [^{3}H]4-diphenylacetoxy-N-methyl-piperidine methiodide ([^{3}H]4DAMP) (M1 and M3 antagonist)) in the presence of unlabelled pirenzipine (M1 antagonist). M3 binding sites were highly concentrated in the ciliary body and iris, and were also present in the corneal epithelium, anterior lens epithelium, retina, retinal pigment epithelium, and choroid. m3 messenger RNA was localized with a human m3 specific [^{35}S]-labelled oligonucleotide probe. The specificity of this probe was verified by Northern blotting of total RNA extracted from the human anterior segment. m3 transcript was localized in each of the structures found to have M3 binding sites, but in addition, m3 transcript was noted in the corneal endothelium and the trabecular meshwork. These two complementary techniques show the presence of M3 muscarinic receptor subtype and its messenger RNA in several defined human ocular structures. Comparison of these results with the known distributions of M1 and M2 subtypes suggests the potential for selective targeting of these muscarinic receptor subtypes in ocular drug therapy.

234.6

MUSCARINIC RECEPTOR SUBTYPES IN HUMAN EYE.

Alicia M. Bogardus*, Barbara J. Feldmann*, Elizabeth WoldeMussie*, and Daniel W. Gil. Allergan, Inc., Irvine, CA 92715.

Muscarinic receptor agonists act at several sites in the eye to regulate intraocular pressure. They contract the ciliary muscle, enhancing outflow of aqueous humor (AH). They also mobilize calcium in non-pigmented epithelial (NPE) cells, which secrete AH into the eye. The subtypes involved in these actions have a M3 pharmacology. To better understand muscarinic actions in the eye, we are identifying ocular muscarinic receptors at the molecular biological level, using the polymerase chain reaction (PCR). Primer pairs corresponding to regions of unique sequence encoding the amino terminal and third cytoplasmic loop of each of the five muscarinic receptors were used. These were incubated with cDNAs generated from 1) ciliary muscle obtained from donor eyes, 2) early passage cultures of human ciliary smooth muscle cells, and 3) a transformed NPE cell line. In each cDNA, a fragment encoding the Hm3 muscarinic receptor was amplified. A low level of the Hm2 subtype was also detected in the ciliary muscle sample. Fragment identities were confirmed by size and digestion with restriction enzymes. The PCR data is consistent with pharmacological evidence that the Hm3 receptor regulates intraocular pressure.

234.7

BINDING OF VESAMICOL TO RAT BRAIN PREPARATIONS.

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Vesamicol (AH5183) is an potent inhibitor of acetylcholine transport into cholinergic vesicles that may be useful for in situ quantification of cholinergic innervation. However, while its binding to purified cholinergic synaptic vesicle preparations is well characterized by the work of Parsons and co-workers, its binding to more complex preparations such as mammalian brain is less well understood. We found that the (-)vesamicol bound to rat brain preparations in a high affinity (KD = 30 nM), stereospecific manner and that binding was most concentrated in a vesicle-enriched fraction. Displacement with several vesamicol analogs (kindly provided by Dr. Stanley Parsons) paralleled that seen in electroplax-derived vesicles. However, there was also a low affinity binding component (KD = about 500 nM) that was neither stereospecific nor concentrated in vesicle fractions. High affinity binding was most concentrated in the cerebellum, a region with sparse cholinergic innervation, followed by striatum, hippocampus and cerebral cortex, in that order. However, the ratio of high/low affinity binding was highest in striatum, followed by cerebrum and cerebellum. These results suggest that (-)vesamicol may be useful for the antemortem diagnosis of Alzheimer's disease, but only if performed under the appropriate conditions to control for non-specific binding.

EXCITATORY AMINO ACIDS: PHARMACOLOGY IV

235.1

DECREASE IN TOLERANCE DEVELOPMENT TO CHRONIC MORPHINE WITH COMPETITIVE AND NON-COMPETITIVE NMDA RECEPTOR ANTAGONISTS.

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Recent reports have demonstrated that pretreatment with the NMDA receptor antagonist MK-801 can decrease the rate of tolerance development and physical dependence observed with chronic morphine administration (Trujillo and Akil, *Science*, **251**, 1991). As the psychomimetic effects produced by MK-801 (MK) and the other non-competetive NMDA antagonists may limit their clinical usefulness, the current focus is on the development of competetive antagonists with fewer limiting side effects. The purpose of this study was to determine if the competetive NMDA antagonist LY-274614 (LY), (Eli Lilly and Co.), could alter the development of morphine tolerance and to compare MK and LY with respect to behavioral profiles and tolerance development when administered prior to morphine on a repetetive dose schedule. Adult male SD rats were administered saline (SAL) (1 ml/kg), MK (0.2 mg/kg i.p.), or LY (1, 3 mg/kg, i.p.) 30 min prior to morphine administration (10 mg/kg, s.c, b.i.d.) for 10 days. Behavioral and analgesic responses (hot-plate, 52.5° C) were assessed at 60 min post-morphine on alternate mornings for the duration of the study. Tolerance development was quantitated by measuring the AUC (%MPE vs time). SAL animals rapidly developed tolerance to the analgesic effects of morphine and reliably returned to baseline HP latencies (%MPE=0) by day 6. Animals pretreated with MK or LY demonstrated significantly less tolerance with the LY effects being-dose related, and with neither the MK nor the high dose LY animals returning to baseline during the study. Analysis of tolerance using AUC shows that SAL animals have an AUC that is 22% of the maximum possible area, while the LY-1 mg, the LY-3 mg, and the MK animals have AUC's of 40%, 85% and 75% of the maximum area respectively. Thus, although MK and LY bind to different sites on the NMDA receptor, both are able to decrease the development of tolerance to morphine. However, the more favorable side effect profile of LY may provide a clinical advantage in analgesic studies. (Supported in part by DA-01457)

235.2

PHARMACOLOGICAL SPECIFICITY OF DRUG DISCRIMINATION BASED ON THE N-METHYL-D-ASPARTATE ANTAGONIST NPC 12626.

D.J. Bobelis* and R.L. Balster. Depts. of Pharmacology & Toxicology and Psychology, Virginia Commonwealth Univ., Richmond, VA 23298-0613.

A drug discrimination based upon the competitive N-methyl-D-aspartate (NMDA) antagonist 2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid (NPC 12626) was assessed for pharmacological specificity by stimulus generalization testing of a number of representative drugs from a variety of pharmacological classes. Adult male Sprague-Dawley rats were trained to discriminate 20 mg/kg i.p. NPC 12626 from saline on a standard two-lever fixed ratio 32 schedule of food reinforcement as described previously (Willetts, Bobelis and Balster, Psychopharmacology 99:458-462, 1989). During test sessions the competitive NMDA antagonists NPC 12626 (3-100 mg/kg), cis-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755, 1-10 mg/kg i.p.) and D,L-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849, 0.3-5.6 mg/kg i.p.) all completely substituted for the training dose of NPC 12626 with ED50 values of 16.4, 2.30 and 0.90 mg/kg, respectively. In contrast, drugs that failed to substitute for NPC 12626 when tested at two or more doses included MK-801, dextromethorphan, amphetamine, ketocyclazocine, morphine, muscimol, diazepam, valproate, phenytoin, chlorpromazine, physostigmine, baclofen, methocarbamol, L-phenylisopropyladenosine and imipramine. These results provide evidence that the discriminative stimulus produced by NPC 12626 is unique and specific, shared fully only by other competitive NMDA antagonists. (Supported by NIDA Grants DA-01442 and DA-07027).

235.3

PHARMACOLOGICAL SPECIFICITY OF N-METHYL-D-ASPARTATE DISCRIMINATION IN RATS. D. M. GRECH, J. WILLETTS AND R. L. BALSTER. Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

The pharmacological specificity of N-methyl-D-aspartate (NMDA) discrimination was examined in male Sprague-Dawley rats trained to discriminate 30 mg/kg NMDA from saline under a 2-lever FR-32 reinforcement schedule. Drugs with diverse central actions were evaluated in substitution tests. Morphine, (+)amphetamine, caffeine, picrotoxin and pentylenetetrazol failed to substitute for NMDA, each producing an average of less than 40% NMDA-lever responding. The cholinergic antagonist mecamylamine and the cholinomimetic agents, (-)nicotine and physostigmine, produced 40-60% NMDA-lever responding, however, arecoline failed to substitute for NMDA. Pentobarbital and the benzodiazepine inverse agonist, methyl-6,7-dimethyoxy-4-ethyl-beta-carboline-3-carboxylate, (DMCM) produced greater than 97% NMDA-lever responding but only at doses which markedly disrupted behavior. These results provide evidence for considerable pharmacological specificity of the NMDA discriminative stimulus in rats. (Supported by NIDA Grants DA-01642 & DA-07027).

235.4

SELECTIVE NMDA ANTAGONISTS AFFECT THE URINARY BLADDER FUNCTION THROUGH THE CENTRAL NERVOUS SYSTEM IN THE RAT. Y. Gotoh*, M. Ishida and H. Shinozaki. The Tokyo Metro. Inst. Med. Sci. Tokyo 113, Japan.

Systemic administration of selective NMDA antagonists reduced the spontaneous contraction of the rat urinary bladder in a dose dependent manner, while it increased the gastric motility. Specific NMDA antagonists and broad-type glutamate blockers such as kynurenate and CNQX showed reverse actions on the stomach and bladder in the rat, respectively. Recently peripheral distribution of NMDA receptors has been reported, therefore, we examined the site of inhibitory actions of these glutamate blockers on the bladder micturition. Under urethane anethesia (1.5 g/kg, s.c.), a catheter was introduced into the bladder through the apex of the bladder in order to record to the intravesical pressure. The bladder contraction was elicited by stimulation of the distal end of the pelvic nerve, which was preganglionically cut near the pelvic plexus. CPP, kynurenate and MK-801 did not affect the urinary bladder contraction elicited by electrical stimulation of the distal end of the pelvic nerve. On the other hand, MK-801 and CPP reduced the urinary bladder contraction elicited by the proximal stimulation of the pelvic nerve. These results suggest the possibility that selective NMDA antagonists affect the micturition function through the central nervous system.

235.5

NMDA CONVULSIONS PRODUCED BY ICV ADMINISTRATION IN RATS: DIFFERENTIAL SENSITIVITY TO VARIOUS ANTAGONISTS. L. Robles, E. Echevarria and F.C. Tortella. Neuropharmacol. Br., Walter Reed Army Inst. Res., Washington, DC 20307.

Parenteral administration of NMDA produces severe clonic (popcorn) convulsions and lethality in rodents. We previously reported the effect of centrally (icv) administered NMDA to produce similar convulsions in rats (Robles et al., Neurosci. Abst., 1990). This study further characterizes the icv NMDA convulsion. In rats icv NMDA (0.78-12.5 nM) dose-dependently elicits popcorn convulsions (CD50=2.8 nM; 15-20 sec latency). Unlike sc or iv NMDA, maximally effective icv doses (6.25-12.5 nM) are not lethal and exhibit a unique sensitivity to various NMDA antagonists. Namely, while the competitive antagonist APV (1-25 ug, icv) or the glycine antagonists HA966 (75-150 ug, icv) and 7-chlorokynurenic acid (1-25 ug, icv) provide 100% protection against the icv NMDA convulsion, the noncompetitive antagonists MK801 (0.5 mg/kg, sc) or PCP (5 mg/kg, sc), given at 5x their respective anticonvulsant ED50 doses, are ineffective. Thus, these results suggest the possible existence of a NMDA recognition site insensitive to non-competitive NMDA antagonists.

235.6

DEXTROROTATORY OPIOIDS AND PHENCYCLIDINE EXERT ANTICONVULSANT ACTION IN RAT PREPIRIFORM CORTEX. J.E.Ishmael-Roth, P.H.Franklin and T.F.Murray. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Although the anticonvulsant action of the dexrorotatory opioids, dextrorphan (DX) and dextromethorphan (DXM), is comparable to that of other putative noncompetitive NMDA antagonists, such as phencyclidine (PCP) and MK-801, the underlying mechanism is less certain (Tortella et al., Life Sci., 42:2509,1988). We have recently described a specific binding site for [^{3}H]dextrorphan in rat forebrain membranes, that exhibited a pharmacological profile consistent with the labelling of NMDA receptors (Franklin and Murray, Eur.J.Pharmacol. Mol.Pharmacol.Sect., 189:89,1990). In the present study we investigated the ability of DX, DXM and PCP to suppress seizures induced by a unilateral, focal injection of bicuculline methiodide (118 pmol) into the rat prepiriform cortex.

All compounds protected against bicuculline-induced seizures in a dose dependent manner. DX and PCP potently suppressed seizures with complete efficacy; ED_{50} values ± S.E. were 16.5 ± 2.9 nmol and 19.3 ± 4.5 nmol respectively. DXM was approximately 4-fold less potent than DX, with an ED_{50} value of 65.0 ± 3.2 nmol. These anticonvulsant potencies corresond to the respective affinities of DX, DXM and PCP for the [^{3}H]dextrorphan binding site in forebrain membranes. The anticonvulsant action of these compounds therefore appears to be a consequence of noncompetitive antagonism of NMDA receptors. This observation is consistent with a proposed NMDA receptor modulated output pathway thought to be essential for the generation of seizures from this brain area.

235.7

CIRCLING INDUCED BY AN INTRACEREBRAL INJECTION OF THE RECEPTOR NMDA ANTAGONIST MK801: IMPLICATION OF DOPAMINERGIC SYSTEM? J.A. St-Pierre*, D. Gaudin, L. Grégoire*, and P.J. Bédard, Centre de Recherche en Neurobiologie, Hôpital de l'Enfant-Jésus, 1401, 18ième Rue, Québec, Canada G1J 1Z4, Département de Pharmacologie, Université Laval.

It is admitted that systemic injection of MK801 induces an ipsilateral rotation in the rats with an unilateral nigrostriatal lesion produced by 6-OHDA (Clineschmidt B.V. *et al.*, Drug Dev. Res. 2:135-145, 1982; Kashihara K. *et al.*, Brain Res. 528:80-82, 1990.)

The present investigation is aimed at elucidating the site of action of this non-competitive NMDA antagonist on the nigrostriatal dopaminergic pathway. Female rats were lesioned with 6-OHDA 8µg/2µl in the left substantia nigra. At least one month later they were tested with apomorphine (0.25mg/kg s.c.). Microinjection of MK801 was performed by four guide-cannulae implanted stereotaxically into the striatum (5.0µg/1.0µl) and into the substantia nigra pars reticulata (5.0µg/0.5µl). Both sites were chosen for their implication in the dopamine-induced circling. Analysis of results show that intrastriatal injection of MK801 on either side (intact or lesioned) induced practically no circling response. On the other hand, a similar injection in the substantia nigra reticulata caused a robust response to the side contralateral to the injection. There was no difference between the intact and the lesioned side. However, the systemic administration of MK801 (100µg/kg i.p.) in the lesioned rats induced a remarkable ipsilateral rotation (referred to the lesion) response. The same type of injection in non-denervated rats increased locomotion without direction predominance.

Taken together, our results suggest that MK801 administered systemically may interact with the dopaminergic pathway on the intact side to induce circling. However, results of the intracerebral injections suggest that output neurons of the substantia nigra reticulata which receive an important glutamatergic input from the subthalamic nucleus may play an important role in the response to this antagonist. Supported by MRC of Canada.

235.8

NMDA RECEPTOR ANTAGONISTS ARE ANTINOCICEPTIVE IN THE MOUSE FORMALIN MODEL DURING ACUTE AND CHRONIC ADMINISTRATION. K.J. Elliott*†, D.J. Cerbone*, K.M. Foley*† and C.E. Inturrisi, Dept. of Pharmacology, Cornell U. Med. Coll. and Memorial Sloan-Kettering Cancer Center†, New York, NY.

The formalin test results in excitatory amino acid (EAA) release. The EAA-NMDA receptor antagonist, AP5 given IT, is antinociceptive in the formalin test (Murray, C.M. et al. Pain 44:179, 1991). We compared the effect of acute and chronic ip MK-801 (MK, non-competitive NMDA antagonist) and LY274614 (LY, competitive) on formalin-induced licking and flinching in mice. Acute MK and LY blocked flinching behavior in a dose-dependent manner. The ED50 (95% CI) for acute MK is .74 mg/kg (.31 - 1.74) and the ED50 for LY is 7.07 mg/kg (5.19 - 9.64). Response to licking behavior was much more variable but both MK and LY suppressed licking behavior at all doses after acute and chronic administration. Chronic ip MK blocked flinching behavior in a dose-dependent manner with an ED50 of .91 mg/kg (.62 - 1.33) while LY did not. With chronic dosing MK (2.5 - 5.0 mg/kg) resulted in persistent agitation and ataxia while LY (10-20 mg/kg) resulted in initial somnolence which resolved prior to the formalin challenge. Further analgesic evaluation of competitive NMDA antagonists such as LY is required as MK has limiting behavioral side effects. (Supported in part by DA01457 (CEI) and CA09461 (KJE.)

235.9

PHENCYCLIDINE-INDUCED ACTIVATION OF A_{10} DOPAMINE NEURONS IS BLOCKED BY COMPETITIVE NMDA-RECEPTOR ANTAGONISTS. E.D. French, Department of Pharmacology, University of Arizona, College of Medicine, Tucson, AZ 85724

Phencyclidine (PCP) is a non-competitive antagonist of glutamate activation of NMDA receptor-ion channel function. In vitro studies have shown that PCP binding within the channel can be positively or negatively affected by NMDA receptor agonists and antagonists, respectively. Thus, the present study sought to examine the consequences of competitive NMDA receptor blockade in vivo on PCP-induced alterations of A_{10} neuronal activity.

Male rats anesthetized with chloral hydrate were prepared for extracellular recording from A_{10} neurons identified by well-characterized electrophysiological criteria. All drugs were injected I.V. and dose-response comparisons were constructed from cumulative dosing regimens. The high affinity and selective competitive NMDA antagonists, CGS 19755 and (+)CPP were administered in incremental doses, with 45 min from the last CGS or CPP dose to the first dose of PCP.

PCP alone produced a bimodal change in A_{10} firing characterized by a low dose excitation followed by an attenuation of this effect with increasing doses. After 40 mg/kg of CGS or CPP the stimulatory effects of PCP were completely attenuated but the inhibitory component remained. 10 mg/kg of CGS produced only a partial blockade of PCP. CGS and CPP alone failed to alter A_{10} activity at either dose, and at the highest dose did not antagonize morphine-induced activation of dopamine cell firing.

These findings provide functional evidence to suggest that competitive NMDA antagonists can block the effects of PCP on NMDA-ion channel function. This finding along with previously published observations strongly suggest that the activation of the mesolimbic-mesocortical dopaminergic system elicited by PCP is mediated through an NMDA receptor mechanism.

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235.10

EFFECT OF CHRONIC MK801 TREATMENT ON MK801-INDUCED BEHAVIORS AND QUINOLINIC ACID-INDUCED NEUROTOXICITY IN RAT BRAIN. A.B. Norman, L.M. Wyatt, M.J. Gerwe* and L.M. Ford. Dept. of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Acute treatments with the noncompetitive NMDA glutamate receptor antagonist MK801 can prevent neurodegeneration mediated via the NMDA receptor. However, some disease processes are ongoing and clinical use of such drugs may require chronic treatments. Chronic treatments with antagonists of other receptors can produce tolerance to the effects of antagonists and supersensitivity to agonists. We therefore investigated the behavioral and neurochemical effects of chronic treatments with MK801.

Male Sprague-Dawley rats received 28 daily injections of MK801 (1 mg/kg/day i.p.) or vehicle. The behavioral effects of MK801 were significantly modified following chronic MK801 treatments with a tolerance to the locomotor stimulating effects. Furthermore, MK801 produced a marked stimulation rather than inhibition of rearing behavior following chronic MK801 treatments. However, there were no significant changes in the B_{max} or K_D of [^{3}H]MK801 binding sites in rat brain homogenates. Two days following the last treatment with MK801 or vehicle rats received bilateral intrastriatal quinolinic acid (QA: 75, 100 or 150 nmol) lesions or vehicle. 21 days postlesion striata were removed and the binding of [^{3}H]SCH23390 to D_1 dopamine receptors was determined as a quantitative index of neurotoxicity. QA produced a significantly greater decrease in [^{3}H]SCH23390 binding in striata of rats withdrawn from chronic MK801 treatment than in controls. Therefore, chronic MK801 treatment produces tolerance to some of the behavioral effects and increases the sensitivity to QA-induced neurotoxicity.

Supported by Huntington's Disease Society of America.

235.11

COMPARISON OF COMPETITIVE AND PCP-LIKE, NON-COMPETITIVE NMDA ANTAGONISTS ON TWO SHORT-TERM MEMORY TASKS IN PIGEONS IN RELATIONSHIP TO THE NMDA ANTAGONIST DOSE. J.D. Leander, N.A. Moore* and P.L. Ornstein. Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285 and Lilly Research Laboratories, Windlesham, Surrey, U.K.

NMDA antagonists show promise as novel therapeutic agents for the treatment of a variety of neurodegenerative conditions. Since NMDA antagonists have been reported to impair the development of long-term potentiation and various learning and memory tasks, the purpose of the present studies was to compare competitive and PCP-like, non-competitive NMDA antagonists on two different memory tasks. The two were a spatial alternation task and a variable delay matching task. The data from these two memory tasks were compared to the doses of antagonists necessary to antagonize the behavioral suppressant effects of NMDA in birds responding under a multiple schedule of grain presentation. On both memory tasks, the non-competitive NMDA antagonists (PCP and MK-801) increased errors and decreased rates of responding at the same doses that antagonized the behavioral effects of NMDA. In marked contrast, the competitive NMDA antagonists (LY233053 and LY274614) antagonized the behavioral suppressant effects of NMDA at doses >20-fold lower than the doses that impaired memory performance. These data show that competitive NMDA antagonists may be far less disruptive of memory processes than non-competitive antagonists and thus suggest that they may have wider therapeutic indexes in man than has been seen with the PCP-like compounds.

235.12

PHENCYCLIDINE AND SIGMA LIGANDS HAVE OPPOSING EFFECTS ON NMDA ACTIVITY. C.S.Hornfeldt and A.A.Larson. Department of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

Phencyclidine (PCP) and related compounds bind to a high affinity site associated with the NMDA channel, inhibiting NMDA activity, and to a low affinity site thought to be the σ-receptor. σ-ligands also bind to a high and low affinity site, corresponding to the σ-receptor and the PCP binding site, respectively. Electrophysiologic evidence indicates σ-receptors modulate NMDA activity which may be potentiated or inhibited by haloperidol, a σ-antagonist. These data suggest that, because of different binding affinities to high and low affinity sites, the effect of a specific compound on NMDA activity may be highly dose-dependant (*Eur J Pharmacol* 179; 441, 1990). Previous behavioral studies, however, have found σ-ligands to be without effect on NMDA-induced behaviors (*Eur J Pharmacol* 159; 149, 1989). We wished to test the hypothesis that PCP and 1,3-di-o-tolylguanidine (DTG), a σ-ligand may, at different doses, have opposing effects on NMDA activity in a behavioral model. Mice were pretreated with an intrathecal injection of PCP or DTG. After 5 min, each animal was reinjected intrathecally with NMDA (0.25 nmol) and the number of bites and scratches counted during the next 1 min. Pretreatment with a low dose of DTG (5 nmol) caused a potentiation of NMDA behaviors which was reversed by simultaneous pretreatment with haloperidol (2.5 nmol). In contrast, pretreatment with a high dose of either DTG (25 nmol) or PCP (3 nmol) inhibited NMDA behaviors. This inhibition was potentiated by simultaneous pretreatment with haloperidol. PCP (0.2 pmol) also potentiated NMDA behaviors but only when coadministered with NMDA. This potentiation was inhibited by pretreatment with haloperidol. These results support the hypothesis that PCP and σ-receptor ligands have opposing effects on NMDA activity that can be monitored in the same behavioral assay system (NIDA 04090, 04190, 00124).

235.13

THE NMDA RECEPTOR DOES NOT MEDIATE THE BEHAVIORAL EFFECTS OF PCP. L.M. Wyatt and A.B. Norman. Departments of Psychiatry and of Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267

PCP acts as a non-competitive antagonist of the NMDA receptor at the associated ion channel. The psychotomimetic actions and behavioral effects in animals are assumed to be mediated through this site. Metaphit, an irreversible antagonist of PCP receptors, should therefore be expected to bind to the ion channel of the NMDA receptor and to block its actions. Male Sprague-Dawley rats were given 2 μmoles metaphit i.c.v., sacrificed 24 hours later, the appropriate brain regions dissected, and [^{3}H]MK801 and [^{3}H]TCP binding assessed. There was no significant decrease in [^{3}H]MK801 binding in any brain region used. [^{3}H]TCP binding was decreased 66% in the striatum, 39% in the hippocampus, and 16% in the cortex. K_D was not changed in any region. Competition of PCP for [^{3}H]MK801 binding in rat cortical homogenates demonstrated a single population of binding sites consistent with the NMDA receptor. In contrast, competition of PCP for [^{3}H]TCP demonstrated 2 sites, K_H=2.5 nM, K_L=756 nM, %R_H=23%. Metaphit treatment eliminated the [^{3}H]TCP binding site with high affinity for PCP. The remaining site had characteristics consistent with the NMDA receptor. Furthermore, metaphit treatment antagonized the locomotor effects of PCP (9 mg/kg, i.p.) but did not antagonize the behavioral effects of MK801 (0.5 mg/kg, i.p.). Therefore, the non-NMDA, high affinity PCP, metaphit sensitive [^{3}H]TCP binding site may mediate PCP behaviors. [supported by The Huntington's Disease Society of America]

235.14

NEUROPROTECTION BY MK801 IN TEMPERATURE MAINTAINED GERBILS. Boast, C.A. and Hoffman, C.* Wyeth-Ayerst Research, Princeton, NJ 08543-8000.

Hypothermia reduces ischemic brain damage (Welsh et al, JCBF 1990, 10:557), confounding interpretation of the neuroprotective effects of novel drugs. Specifically, the neuroprotectant MK801 has been shown to cause hypothermia. Some have claimed that when body temperature (BT) is maintained, MK801 is not a neuroprotectant, (Buchan & Pulsinelli, J. Neurosci. 1990 10:311; Corbett et al, Brain Res. 1990 514:300), while others have claimed it still is (Gill & Woodruff, Eur. J. Pharmacol., 1990 176:143; Neill et al., SN Abstr. 1990 16:278).

Gerbils received bilateral carotid occlusion (BCO) for 5 or 10 min (halothane), while on a heating pad at 37 C. Immediately after surgery the animals were restrained, a rectal temperature probe inserted, and BT maintained using a thermistor controlled heat lamp and cooling fan. When more cooling was required, an animal was placed in a refrigerator with the heat lamp and fan. In free regulating animals (10 min BCO), 3 mg/kg i.p. MK801 at the time of occlusion significantly reduced brain damage. When BT was maintained (5 min BCO) at 38.5 C, no reduction in brain damage was seen up to 10 mg/kg i.p. When BT was maintained (5 min BCO) at 36.5 C, 10 mg/kg i.p. significantly reduced brain damage. Thus, although a higher dose of the drug is required, MK801 can reduce ischemic brain damage in the absence of hypothermia. .

235.15

EVIDENCE THAT IN PHYSIOLOGICAL CONDITIONS NMDA RECEPTORS PLAY FUNDAMENTALLY DIFFERENT ROLES IN THE HIPPOCAMPUS AND IN THE CEREBRAL CORTEX. N.Ludvig, P.K.Mishra, R.L.Burger and P.C.Jobe. Dept. of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, IL 61656.

Combined EEG and intracerebral microdialysis techniques were used to compare the effects of NMDA in the hippocampus and in the cerebral cortex, in freely behaving rats. The recording electrodes were implanted chronically in either structure. At the beginning of the experiments, a loop-type microdialysis-probe was inserted into the recording site. For a 2 hour period, artificial cerebrospinal fluid was perfused (10 ul/min). After this period, a selected dose of NMDA (concentration range: 0.01 - 10.0 mM) was included into the perfusion fluid for 5 min, then washed out.

In hippocampus the drug provoked epileptiform electrical activity in a dose-dependent manner; 1.0 mM concentration was the lowest dose to induce such EEG changes. In contrast, in the sensorimotor cortex 1.0 mM or higher concentrations of NMDA induced marked reductions in both EEG amplitude and frequency. These electrographic changes were indistinguishable from those characteristic of K^+-induced spreading depression. In this area, NMDA did not produce epileptiform spike activity. Subsequent analyses of the microdialysates and complementary electron microscopic studies indicated that the depressant effect of NMDA in cortex was neither due to an increased release of GABA nor to a neurotoxic action.

Interestingly, dibutyryl cyclic AMP appeared to potentiate the effects of NMDA, at least in hippocampus, indicating that NMDA receptor functions may be under the regulation of a cyclic AMP-dependent mechanism. A differential operation of such regulatory mechanisms in the cerebral cortex and in the hippocampus may underlie in the apparently different roles of NMDA receptors in these structures.

235.16

αKETOGLUTARATE UPTAKE INTO GLUTAMATERGIC NERVE TERMINALS: AUTOREGULATION OF EXCITATORY NEUROTRANSMISSION. J.Lehmann, E. Oei*, and C. Tsai. Division of Behavioral Neurobiology, Dept of Mental Health Sciences, Hahnemann University, Philadelphia PA 19102, and Research Department, Ciba-Geigy Corp., Summit NJ.

[^{14}C]αKetoglutarate ([^{14}C]αKG) uptake by crude synaptosomes (P_2 fraction) was sodium-dependent but insensitive to malate and phthalonate, unlike mitochondrial [^{14}C]αKG uptake. The excitatory amino acids L-glutamate, L-aspartate, and kainate inhibited [^{14}C]αKG uptake into synaptosomes. Following cortical lesions, uptake of [^{14}C]αKG into striatal synaptosomes was decreased in parallel nwith [^{3}H]L-glutamate uptake. Thus, this well-documented glutamatergic projection would appear to contain a high-affinity αKG uptake system.

Taken together, these data are suggestive of a receptor-mediated interaction of excitatory amino acids with nerve terminals, resulting in inhibition of αKG uptake. Since αKG may serve as a precursor for neurotransmitter glutamate and/or aspartate, the inhibition by excitatory amino acid of αKG uptake may represent a homeostatic negative feedback loop, i.e., an "autoreceptor".

235.17

BINDING OF A NOVEL, SELECTIVE SIGMA SITE LIGAND, [^{3}H] 3-PHENYL-1-(1,2,5,6-TETRAHYDRO-1-PROPYL-3-PYRIDINYL)-1-PROPANONE OXIME (PD 128298) TO RAT BRAIN MEMBRANES. **C.M. Hanchin*, L.L. Coughenour*, H. Tecle*, W.H. Moos and F.M. Hershenson. Departments of Pharmacology and Chemistry, Parke-Davis Pharmaceutical Research Division, Warner Lambert Co., Ann Arbor, MI 48106-1047.**

A series of tetrahydropyridine oximes recently has been described as potent and selective inhibitors of [^{3}H]1,3-di-o-tolylguanidine (DTG) and [^{3}H]-(R)-3-(1-propyl-3-piperidinyl)phenol ((+)3PPP) binding (Pei et al., Ab# MEDI 124, 201st ACS National Meeting, 1991). PD 128298, a high affinity member of this series, was tritium labeled, and its binding to rat whole brain homogenates was characterized using methods described for [^{3}H](+)3PPP binding (Largent et al., J Pharmacol Exp Ther. 238: 739, 1986). Binding of [^{3}H]PD 128298 was saturable and reversible. Scatchard analysis revealed a single, high affinity binding site with a K_d = 2 nM and a B_{max} = 0.1 pmoles/mg wet wt. tissue. The rank order of potency (IC_{50}) of agents to inhibit the binding was haloperidol (0.35 nM) > pentazocine (68 nM) > (+)3PPP (145 nM) ≈ DTG (180 nM) > dextromethorphan (584 nM) > phencyclidine (3660 nM). Binding was 500 to 60,000 fold selective for other receptors including phencyclidine, serotonin-2, dopamine D_2, alpha-1 and alpha-2 adrenergic, muscarinic, nicotinic and sodium channel site 2. [^{3}H]PD 128298 should provide a useful tool for studying sigma site pharmacology and mechanisms.

235.18

NEOMYCIN IS AN AGONIST AT THE POLYAMINE SITE OF THE NMDA RECEPTOR.

L.M. Pullan, R.J. Stumpo, R.J. Powel*, K.A. Paschetto*, M. Britt*. Dept. of Pharmacology, ICI Americas, Inc., Wilmington, DE 19897

The aminoglycoside antibiotic neomycin, enhances the binding of the non-competitive antagonist [3H]TCP (1-[1-(2-thienyl)cyclohexyl]piperidine) to the NMDA (N-methyl-D-aspartate) receptor. The maximal enhancement is greater than that seen with the polyamine spermidine, and the enhancement by neomycin of [3H]TCP binding is competitively inhibited by spermidine. These results suggest that neomycin may be a full agonist and spermidine may be a partial agonist at the site where polyamines enhance [3H]TCP binding. Other aminoglycosides also enhance [3H]TCP binding with efficacies (neomycin>gentamycin=kanamycin) roughly proportional to the number of primary amine groups. The polyamine antagonist arcaine inhibits the enhancement by spermidine or neomycin. The inhibition of [3H]TCP binding by arcaine is competitively reduced by neomycin and spermidine. Neomycin, in contrast to spermine and spermidine, does not enhance the binding of the NMDA receptor competitive antagonist [3H]CPP (3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid). However, neomycin does inhibit the ascending arm of the biphasic spermidine enhancement of [3H]CPP binding, while not affecting the descending arm. Thus it appears that neomycin binds to the site where polyamines modulate [3H]TCP and [3H]CPP binding at the NMDA receptor, but neomycin may not cause the conformational change through which spermidine enhances [3H]CPP binding.

235.19

ISCHEMIA INDUCED EXCITATORY AMINO ACID RELEASE FROM THE GERBIL HIPPOCAMPUS: EFFECTS OF ANESTHESIA AND GM1 GANGLIOSIDE. G. Lombardi, G. Cherici* and F. Moroni. Dept. of Pharmacology, University of Florence, 50100 Florence, Italy.

The ischemia-induced release of aspartate (ASP) and glutamate (GLU) was studied both in freely moving and in chloral hydrate anesthetized gerbils having a transverse microdialysis tubing in their hippocampi. The amino acids were measured in the dialysis fluid (Ringer) using HPLC and fluorimetric detection. Transient forebrain ischemia was obtained by occluding both common carotid arteries for different periods (3-8 min). This occlusion was performed 2 h after the implantation of the dialysis tube in anesthetized and 24 h later in freely moving (locally anesthetized) animals. Two hours after cannula implantation the apperance of ASP and GLU in the perfusion fluid was 1.8±0.2 and 8.9±0.9 uM respectively, while at 24 h it was 0.87±0.1 and 4.4±1.7 uM. When the animals were pretreated with GM1 ganglioside (30 mg/Kg/day i.p. for 2 days) the output of ASP and GLU 2 h after cannula implantation was approximately 50% lower than in controls. Common carotid occlusion for 8 minutes in anesthetized animals increased EAA appearance in perfusates 3 to 4-fold. This increase (measured as the area under the curve) was significantly reduced by GM1 ganglioside pretreatments. In freely moving animals 5 min of common carotid occlusion caused a 3 to 4-fold increase in EAA output lasting approximately 20 min. However in most of the animals a further peak of EAA appeared 2-3 h later. The meaning of this late EAA output in unanesthetized animals is currently under investigation.

235.20

EFFECTS OF OXIRACETAM AND PIRACETAM ON THE RELEASE OF DIFFERENT NEUROTRANSMITTERS FROM HIPPOCAMPAL SLICES AND SYNAPTOSOMES. M. Raiteri, M. Marchi, G. Lunardi*, and A. Augliera*. Istituto di Farmacologia e Farmacognosia, Viale Cembrano 4, 16148 Genova, Italy.

The in vitro effects of some nootropic drugs on the release of both ^{3}H-D-aspartate (^{3}H-D-ASP) and endogenous glutamate (GLU) from hippocampal slices has been previously reported. Oxiracetam increased in a concentration-dependent way (0.01 - 1 μM) the depolarization-evoked release, but not the spontaneous release, of ^{3}H-D-ASP and endogenous GLU. Piracetam also increased the K^+-evoked release of ^{3}H-D-ASP but its effect was consistently less pronounced (Marchi et al. EJP 185, 247, 1990). We have extended this study on synaptosomes and found that oxiracetam and piracetam did not increase the ^{3}H-D-ASP release in this preparation. We report here also the data on the effects of these two drugs on the release of ^{3}H-ACh, ^{3}H-GABA and ^{3}H-NE from synaptosomes and slices of rat hippocampus.

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236.1

MODIFICATION OF RECEPTOR-G PROTEIN FUNCTION IN NG108-15 CELL MEMBRANES BY LOW pH TREATMENT. D. E. Selley and S.R. Childers. Dept. Physiology/Pharmacology, Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

Pretreatment of rat brain membranes at pH 4.5 increases GTP regulation of opioid agonist binding and blocks G_s-stimulated adenylyl cyclase (AC). To determine the effect of low pH treatment in a cell culture system, membranes from the δ opioid receptor-containing NG108-15 cell line were treated at pH 4.5 and assayed for [^{3}H][D-Ser^2-Leu^5-Thr^6]-enkephalin ([^{3}H]DSLET) binding and AC activity. Although low pH treatment did not affect the binding of the δ-selective agonist in the absence of sodium or guanine nucleotides, this treatment increased sodium inhibition of [^{3}H]DSLET binding between 3-100 mM NaCl. Inhibition of [^{3}H]DSLET binding by guanine nucleotides was not observed in the absence of sodium in either control or low pH-treated NG108-15 cell membranes. In the presence of NaCl, however, inhibition of [^{3}H]DSLET binding by GTP was slightly increased by low pH treatment. Assays of AC activity in low pH-treated NG108-15 membranes indicated that this treatment did not alter basal or forskolin-stimulated AC, but markedly reduced AC stimulated by G_s, using NaF or PGE_2. Inhibition of forskolin- and PGE_2-stimulated AC by DSLET was relatively unchanged by pH 4.5 treatment of membranes. These results indicate that effects of low pH treatment on NaCl/GTP regulation of agonist binding and on G_s function in NG108-15 cell membranes are similar to the effects of this treatment on rat brain membranes.

Supported by PHS grant DA-02904 from NIDA.

236.2

ALTERATIONS IN G-PROTEIN EXPRESSION IN NEONATAL RAT BRAIN FOLLOWING MORPHINE TREATMENT R. Basheer*, K. Espinoza* and A. Tempel. Lab. of Molecular Pharmacology, Hillside Hosp., Glen Oaks, NY 11004.

G-Proteins have been identified as membrane transducing components in receptor-mediated events. Opiate receptors are representatives of a class of neurotransmitter-binding proteins that are coupled to G-proteins. Previous work has suggested that opiate receptor density is mediated through the cAMP system via G-binding proteins. We have shown that chronic morphine treatment of rat pups produces a significant decrease in brain μ opioid receptor density with no change in receptor affinity. This downregulation is in direct contrast to what is observed in the adult brain where no significant change in opiate receptors can be demonstrated. Our hypothesis is that the factors that influence this difference in neonates are related to the G-protein/cAMP system. In order to understand the interaction between opioid receptors and G-proteins, our preliminary approach was to quantitate mRNA of G-proteins in the developing rat brain before and after morphine treatment. Changes in the levels of G-protein mRNA will be correlated with receptor density changes. Data from these studies may provide insight into the interaction between the opiate receptor and the membrane associated signal transducing systems during addiction. (Supported by NIDA Grant DA-05440).

236.3

M_1 MUSCARINIC ANTAGONISTS INTERACT WITH σ RECOGNITION SITES. Diane L. DeHaven-Hudkins[1] and Robert L. Hudkins[2]. [1]Department of Enzymology and Receptor Biochemistry, Sterling Research Group, Malvern, PA 19355-1314 and [2]Department of Medicinal Chemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0540.

The receptor binding profile of representative compounds for σ and muscarinic M_1 and M_2 sites was studied in an attempt to determine if a correlation exists between muscarinic and σ binding affinity, and if a relationship exists between σ binding affinity and a particular muscarinic receptor subtype. A variety of M_1-selective muscarinic antagonists potently inhibited binding to σ sites in brain. Both basic ester and non-ester type M_1-selective antimuscarinics exhibit affinity for the σ site, while the classical antimuscarinic antagonists atropine and QNB, and the tricyclic pirenzepine, were ineffective in binding to this site. The only agonist which displaced σ binding was the selective M_1 agonist McN-A-343. We also observed a significant correlation between the IC_{50} values for σ compounds to inhibit [^{3}H]pirenzepine binding and their IC_{50} values to inhibit carbachol-stimulated phosphoinositide turnover. These observations may aid in elucidating the relationship of σ binding to inhibition of phosphoinositide turnover stimulated by cholinergic agonists.

236.4

IN VIVO MOUSE BRAIN LOCALIZATION OF [^{3}H]DPDPE TO DELTA OPIOID RECEPTORS. S.J. Weber, D.L. Green*, V.J. Hruby*, H.I. Yamamura and T.P. Davis. Depts. Pharmacology and Chemistry, University of Arizona College of Medicine, Tucson, AZ 85724.

The opioid receptors μ, δ, and κ have been shown to have distinct patterns of distribution within the brain. In particular, the highest concentrations of the δ receptors are found within the striatum (ie., caudate nucleus and putamen) and frontal cortex (layers I-II and V-VI). The intent of this study was to examine regional brain distribution of [^{3}H]DPDPE following i.v. administration and compare results to δ receptor localization. Three groups of anesthetized CD1 male mice were studied. Group 1 was administered (i.v.) a known amount of [^{3}H]DPDPE (20 Ci/mmol; 0.4 nmol/100 g). Groups II and III were pretreated with naloxone (NLX, 10 mg/kg, i.v.) or the specific δ receptor antagonist naltrindole (NTI, 10 mg/kg, i.v.) before [^{3}H]DPDPE. Mice were sacrificed, a sample of blood taken, the brain perfused with saline, removed and rapidly dissected. Brainstem, cerebellum, frontal cortex, hippocampus, striatum and remaining brain were solubilized and counted. No significant difference in the levels of [^{3}H]DPDPE between regions was seen (0.070-0.083%/g tissue) at 10 min, but the striatum was highest. NLX pretreatment resulted in significant ($p<0.01$) reductions of [^{3}H]DPDPE in all regions with the largest percent reduction in striatum (73.5%). NTI pretreatment had little effect on detectable levels of [^{3}H]DPDPE in the cerebellum and brainstem but significant ($p<0.05$) reductions occurred in the frontal cortex and striatum (24.0 and 37.4%, respectively). These results suggest that the pattern of i.v. [^{3}H]DPDPE distribution to the brain is similar to δ opioid receptor autoradiographic localization. (Supported by NIDA-DA06284)

236.5

CHARACTERIZATION AND AUTORADIOGRAPHIC ANALYSIS OF ^{3}H-NALTRINDOLE IN THE RAT, E.J. Drower*, M.S. Dappen*, C. Dorn, C. Markos, M.F. Rafferty* and P.C. Contreras*, Central Nervous Systems Disease Research* and Radiochemistry Division, G.D. Searle & Co. Skokie, Il, 60077.

Naltrindole has been characterized as a select δ-opioid receptor antagonist in several isolated smooth muscle systems. We now report the biochemical characterization of ^{3}H-naltrindole (^{3}H-NTI) as conducted in a crude rat brain homogenate and slices. Binding of ^{3}H-NTI was temperature dependent with increased affinity at higher temperatures. Scatchard analysis revealed a single population of binding sites displaying a K_d of 0.06 nM and Bmax of 100 fmole/mg protein. Delta selectivity was confirmed by the rank order of IC_{50} values of select δ and μ ligands; DSLET (0.3 nM), DPDPE (6.2 nM), levorphanol (39 nM) and DAMPGO (685 nM). Saturation isotherms of ^{3}H-NTI was also conducted on both thaw-mounted brain slices and brain paste yielding an approximate K_d of 0.3 nM for each. Autoradiographic distribution of ^{3}H-NTI was performed in the presence and absence of levorphanol to determine specific binding. Binding sites labeled by ^{3}H-NTI were localized in the caudate putamen, nucleus accumbens and outer layers of the cortex.

236.6

Differential Antagonism Of [D-Ala^2, Glu^4]deltorphin- and [D-Pen^2, D-Pen^5]enkephalin Mediated Antinociception by [D-Ala^2, Cys^4]Deltorphin in the Mouse. P. Horan, J. Taylor, A. Misicka*, A.W. Lipkowski*, V.J. Hruby* and F. Porreca Departments of Pharmacology and Chemistry*, University of Arizona, Tucson, AZ 85724.

Supraspinally in mice, two opioid δ receptor subtypes can be demonstrated on the basis of sensitivity to the irreversible antagonists, [D-Ala^2, Leu^5, Cys^6]enkephalin (DALCE) and 5'-naltrindole isothiocyanate (5'-NTII). These δ receptors are selectively activated by [D-Pen^2, D-Pen^5]enkephalin (DPDPE, DALCE-sensitive site) and [D-Ala^2, Glu^4]deltorphin (DELT II, 5'-NTII-sensitive site); no antinociceptive cross-tolerance occurs between DPDPE and DELT II. It is not known if a sulfhydryl group, perhaps critical for ligand binding, is located in or around the binding site of the 5'-NTII sensitive δ receptor. To test this hypothesis, cysteine has been substituted for glutamic acid in the 4 position of DELT II, and the agonist and potential antagonist properties of this compound ([D-Ala^2, Cys^4]deltorphin)(Cys^4-DELT) were tested against intracerebroventricular (*i.c.v.*) DPDPE and DELT II. *I.c.v.* Cys^4-DELT produced a dose-related antinociceptive effect (55°C water tail-flick test) in male, ICR mice; the A_{50} (95% C.L.) was 0.78 (0.24-2.54) nmol/mouse, and peak effect was at +10 min with the agonist effect dissipating by 30 min. Pretreatment with an A_{90} dose of Cys^4-DELT (3 nmol) blocked the antinociceptive effect of DELT II tested 4, 8, 12 and 24 hrs later, but not that of an equiantinociceptive dose of DPDPE up to pretreatment times of 120 hrs. The differential antagonism of DPDPE and DELT II by Cys^4-DELT suggests the presence of a sulfhydryl group in the 5'-NTII-sensitive δ opioid receptor and supports a structural basis for opioid δ subtypes.

236.7

SWIM-STRESS INDUCED ANTINOCICEPTION IN MICE IS MEDIATED BY A SUBTYPE OF OPIOID δ RECEPTOR. Kenneth D. Wild, Todd W. Vanderah* and Frank Porreca. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Supraspinal antinociception produced by opioid δ agonists has recently been shown to be associated with subtypes of δ receptors which can be distinguished on the basis of sensitivity to the novel, irreversible δ antagonists, [D-Ala^2, Leu^5, Cys^6]enkephalin (DALCE) and naltrindole-5'-isothiocyanate (5'-NTII). These δ receptor subtypes are selectively activated by [D-Pen^2, D-Pen^5]enkephalin (DALCE-sensitive δ receptor) and [D-Ala^2]deltorphin II (5'-NTII-sensitive δ receptor); no antinociceptive cross-tolerance occurs between these agonists. Cold-water swim-stress (CWSS) has been previously shown to produce antinociception in mice, apparently resulting from endogenous activation of opioid δ receptors. The present experiments attempted to identify the opioid δ receptor subtype associated with CWSS. Male, ICR mice (20-30 g) were exposed to water (5°C) for 3 min and tested after a further 10 min in the 55° C water tail-flick test. CWSS produced a time-related antinociceptive effect which was maximum at approximately 10 min and which dissipated by 15 min after swim. The antinociception was antagonized by naloxone (1 mg/kg, *s.c.*, -13 min) or the δ antagonist, ICI 174,864 (4.4 nmol, *i.c.v.*, -18 min) but not by the μ antagonist β-funaltrexamine (18.8 nmol, *i.c.v.*, -24 hr, a time and dose which blocks μ agonist effects). Additionally, CWSS-induced antinociception was antagonized by 5'-NTII (17.5 nmol, *i.c.v.*, -24 hr), but not by DALCE (4.46 nmol, *i.c.v.*, -24 hr). Under these conditions, CWSS-induced antinociception is mediated by activation of the 5'-NTII-sensitive δ receptor in mice.

236.8

σ RECOGNITION SITES IN BRAIN AND PERIPHERAL TISSUES: CHARACTERIZATION AND EFFECTS OF CYTOCHROME P450 INHIBITORS. L.C. Fleissner*, F.Y. Ford-Rice, M.A. Ator* and D.L. DeHaven-Hudkins. Dept. Enzymology & Receptor Biochemistry, Sterling Research Group, Malvern, PA 19355.

Binding to σ sites in guinea pig and rat was characterized with the [^{3}H]ligands (+)pentazocine and DTG. The affinities of selected σ reference compounds and cytochrome P450IID1 inhibitors, and the effects of cytochrome P450 induction were also determined. The K_D values for [^{3}H](+)pentazocine-labelled σ sites were similar in all tissues, but the number of sites varied, with an order of liver > testes > brain > heart. The B_{max} values for [^{3}H]DTG-defined σ sites were greatest in testes, followed by liver, brain and heart, while the K_D values in liver and testes were two-fold lower than those in brain and heart. The rank order of potency for reference compounds was similar in brain, liver and testes. Following induction with phenobarbital or β-naphthoflavone, the number of σ sites was unchanged in brain but increased in liver. The potency of representative compounds to displace σ binding in guinea pig liver failed to correlate with the affinities of these compounds to inhibit cytochrome P450IID1 activity in human liver. These results suggest that σ sites in the periphery are similar to those in brain, and that the σ binding site is not identical with cytochrome P450IID1.

236.9

INTERACTION OF [^{3}H]-DTG WITH σ_2 RECEPTORS IN RAT HEART MEMBRANE PREPARATIONS. M. Dumont and S. Lemaire. Department of Pharmacology, University of Ottawa, Ottawa, Ontario Canada K1H 8M5.

Using membrane binding techniques, we have characterized the binding properties of [^{3}H]-(1,3 di(2-tolyl) guanidine ([^{3}H]-DTG) in membrane preparations of rat heart. Binding assays were performed with 2 ml-aliquots of membrane (1 mg protein) in 5 mM Tris-HCl (pH 7.4) at 37°C for 30 min followed by filtration through polyethyleneimine treated glass microfiber filters. Scatchard plot analysis of [^{3}H]DTG binding revealed the presence of one high affinity saturable binding site with a Kd of 7.1 nM and a Bmax of 86 pmol/g protein. The drug specificity profile of the receptor correlated with that of the σ receptor with the following order of potency: DTG > haloperidol > (-)butaclamol > (+)butaclamol > (-)SKF-10047 > PCP > TCP > MK-801 > (+)SKF-10047. [^{3}H]DTG binding was sensitive to the Ca^{2+} channel blocker, verapamil but not to the K^+ channel blocker, 4-aminopyridine. The reverse stereoselectivity of [^{3}H]DTG binding for (-)SKF-10047 indicated that the cardiac σ receptor belongs to the σ_2 subtype. The presence of verapamil-sensitive σ_2 receptor in the heart indicates that it may be involved in some of the cardiovascular effects of the non-selective drug of abuse, PCP. Supported by the HSFO.

236.10

CHARACTERIZATION OF THE DOSE-DEPENDENT ANTAGONISM OF SPINAL OPIOID RECEPTORS BY NALOXONE AND NALTRINDOLE. FURTHER EVIDENCE FOR DELTA RECEPTOR SUBTYPES ?
Tony L. Yaksh, Donna L. Hammond and Paul J. Tiseo Dept. of Anesthesiology, U. C. San Diego and the University of Chicago.

The possibility that *delta*-opioid receptor subtypes or coupled *mu*/*delta* receptor complexes were mediating antinociception in the rat spinal cord was investigated using the intrathecal (i.t.) administration of highly and partially selective *mu* and *delta* opioid receptor agonists and antagonists. The *mu* agonist DAGO and the *delta* ligand DPDPE, as well as the partially selective agonists morphine (μ/δ) and DADLE (δ/μ) were examined using the hot-plate test (52.5° C). The i.t. doses producing a maximal response (DAGO, 0.3 μg; DPDPE, 60 μg; morphine, 10 μg; and DADLE, 1 μg) were then systematically challenged with either naloxone (NAL), or the more selective *delta* opioid antagonist naltrindole (NTL). The time course of drug administration was designed such that the times of both agonist and antagonist peak effects coincided. NAL antagonized the analgesic response of *all* the agonists tested with the antagonist dose-response curves of all agonists virtually overlapping. In contrast, the degree of antagonism produced by NTL varied greatly. The MPE produced by DPDPE was significantly attenuated in a dose-related manner whereas the response produced by DAGO was unaffected. However, when DADLE was challenged by NTL, the response was *also* completely unaffected. Interestingly, morphine demonstrated a suprising sensitivity to NTL with the high dose (30 μg) reducing the MPE of morphine to 46%. The NAL results suggest that a strong *mu* component is involved in the analgesic response produced by *all* the agonists, including the δ-preferring agonists DADLE and DPDPE. In a similar fashion, the NTL results suggest that a *delta* component is involved in the analgesia produced by the μ-preferring morphine. Such results may support the existence of a *mu*/*delta* receptor complex in the spinal cord of the rat whose mediation of analgesic effects can be antagonized by both NAL and NTL. Finally, the fact that DADLE is unaffected by NTL in this study suggests that although DADLE shows a strong *delta* selectivity *in vitro*, it does not appear to be binding to the same *delta* receptor that NTL does in the rat spinal cord.

236.11

ALTERATIONS IN LOCAL CEREBRAL GLUCOSE UTILIZATION FOLLOWING UNILATERAL MICROINJECTION OF δ OPIATE LIGAND IN THE SUBSTANTIA NIGRA. R.W. Clement, A.G. Hohmann, and J.M. Walker, Schrier Research Laboratory, Department of Psychology, Brown University, Providence, RI, 02912.

Previous studies demonstrate that unilateral microinjection of opioid ligands in the substantia nigra (SN) result in contralateral circling behavior in the rat; however, the mechanisms for this behavior differs with the particular class of receptors involved. Whereas μ and δ opiates exert effects through dopaminergic neurons of the pars compacta, kappa opiates increase movement by their actions on nondopaminergic neurons in the pars reticulata. The current study investigates regional activity following unilateral intranigral application of δ opioid ligands using the quantitative autoradiographic 2-deoxy-D-[1-^{14}C]glucose method as adapted for use in freely moving rats. Subjects were microinjected with 10nmol of the delta ligand [D-penicillamine^{2,5}]enkephalin (DPDPE) in a volume of 0.5 μl normal saline just prior to infusion of 125μCi/Kg of 2-deoxy-D-[1-^{14}C]glucose. Behavioral data was concurrently obtained via microcomputer and confirms the potent locomotor stimulation induced by DPDPE. Regional cerebral glucose utilization was determined. Following application of DPDPE in SN, bilateral effects were observed in the nucleus accumbens, habenula, superior colliculus, ventral tegmental area (VTA), and the cingulate cortex. Unilateral differences in glucose utilization were not apparent. Since there are few δ receptors in the VTA (Mansour, et al. TINS, 11:308 '88). it seems unlikely that δ opiates had direct effect on this structure by spreading from the microinjection site in the adjacent substantia nigra.

236.12

EFFECTS OF 1,3-DI-O-TOLYLGUANIDINE, A SIGMA LIGAND, ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT BRAIN. A.G. Hohmann[1], R.R. Matsumoto[1], M.K. Hemstreet[1], S.L. Patrick[1], J.E. Margulies[2], R.P. Hammer[2], and J.M. Walker[1]. [1]Schrier Res. Lab., Dept. of Psych., Brown Univ., Providence, RI 02912. [2]Dept. of Anat. and Reprod. Biol., Univ. Hawaii Sch. Med., Honolulu, HI 96822.

The 2-deoxy-D-[1-^{14}C]glucose ([^{14}C]DG) method was used to examine the effects of the relatively selective sigma (σ) ligand 1,3-di-o-tolylguanidine (DTG) on cerebral metabolism in freely moving rats. Each animal received an i.p. injection of DTG (0.2 [n = 8], 1 [n = 5], or 5 [n = 5] mg/kg) or normal saline (n = 6) 20 min prior to the infusion of [^{14}C]DG. DTG induced dose-dependent changes in local cerebral glucose utilization (LCGU) in certain motor and limbic structures. For most structures, the maximum effect occurred at 1mg/kg. The most profound increases in LCGU were found in regions that are rich in σ receptors. These include cerebellar and related nuclei (interpositus, lateral and medial cerebellar n., vestibular n., olivary n., red n.), ambiguus n., facial n., motor trigeminal n., hippocampus (CA2, CA1, CA3, DG), n. basalis of Meynert, interpeduncular n., substantia nigra pars compacta and pars reticulata. It is unlikely that the observed alterations in LCGU resulted from artifacts of the procedure, since the pattern of changes induced by DTG is not typical of alterations produced by changes in blood pressure, pCO_2 or body temperature. The areas affected by DTG are similar to those previously reported for other σ ligands (Puppa & London, *Br. Res.* 505: 283, '89). The increased functional activity in the substantia nigra pars compacta is consistent with data from microdialysis experiments which suggest a dopamine-releasing effect of DTG and (+)-pentazocine following parenteral administration (Walker et al. this meeting). Future studies employing additional selective σ ligands at different doses must be carried out in order to confirm whether these changes in LCGU are σ-mediated.

236.13

FUNCTIONAL ANTAGONISM OF SIGMA RECEPTORS BY A NOVEL ISOTHIOCYANATE LIGAND. MK Hemstreet[1], L Bluth[2]*, BR DeCosta[3], WD Bowen[2], and JM Walker[1]. [1]Program in Neural Science and [2]Division of Biology and Medicine, Brown University, Providence, RI 02912, and [3]National Institutes of Health, Bethesda, MD 20892.

Both the in vivo and in vitro characteristics of a novel isothiocyanate (ITC) ligand for sigma receptors (BD713) were examined in male Sprague-Dawley rats. In radioligand binding assays, brain membranes were pretreated for 10 min. with either BD713 or a structurally-related sigma compound lacking the ITC moiety; membranes were then repeatedly washed. Under these conditions there was no remaining sigma binding of the non-ITC compound, but wash-resistant blockade of sigma sites was evident following BD713. This reduction in sigma binding following BD713 pretreatment may be due to covalent attachment of the ITC compound to sigma receptors following interaction with the ligand binding region. The specificity of BD713 does not overlap with phencyclidine receptors, as seen by minimal attenuation of [^{3}H]TCP binding following pretreatment with the ITC compound.

In behavioral experiments, animals were pretreated with BD713 by unilateral intracerebral administration either into the red nucleus or the substantia nigra. These sites were chosen because of the predictable and quantifiable nature of the behaviors elicited by microinjection of sigma ligands into these structures. Control injections consisted of vehicle or the reversible sigma ligand, (+)pentazocine. Following a 5hr. pretreatment interval, animals received a second microinjection into the same structure, with all rats receiving (+)pentazocine as the second injection. Only BD713 pretreatment produced a functional antagonism of the in vivo actions of (+)pentazocine, with significant attenuation of cervical dystonia following intrarubral administration ($p < 0.05$) and of contralateral rotatory behavior following intranigral injection of the reversible sigma ligand ($p < 0.01$). These results imply that the behavioral actions of sigma ligands may be due to occupation of sigma receptors, rather than nonspecific actions of these compounds; experiments are currently underway to address this possibility.

236.14

IONTOPHORETIC EFFECTS OF PUTATIVE SIGMA LIGANDS ON RAT CEREBELLAR PURKINJE CELLS. W.J.Martin, B.R. deCosta* and J.M. Walker. Schrier Research Laboratory, Dept. of Psychology, Brown University, Providence, RI 02912. *Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD, 20892.

The early ligands for the σ receptor were tainted by a lack of selectivity, leading to attempts to develop more selective ligands for the site. The purpose of this study was to quantify the electrophysiological responsiveness of rat cerebellar Purkinje cells to iontophoretic application of more selective σ ligands. Male Sprague-Dawley rats were anesthetized with Urethane (1.25g/kg, i.p.). 1,3-di-o-tolulguanidine (DTG), dextrallorphan (DEX), (+)-pentazocine (PENT), (+)-3-(3-Hydroxyphenyl)-N-propylpiperidine ((+)-3-PPP), and a novel diamine, BD1008, were ejected from a multi-barrel pipette onto individual Purkinje cells. Significant drug effects were determined according to a 12% change in the frequency of baseline firing. Inhibitions were seen following ejections of DTG (68%, n = 19), BD1008 (60%, n = 15), DEX (52%, n = 21) and PENT (20%, n = 15). These inhibitions were dose-dependent and occurred with changes in the frequency of cell firing, but without changes in spike amplitude or duration, indicating that local anesthesia effects were not responsible for observed inhibitions. The effects of (+)-3-PPP were atypical, inducing excitations in 29% of cells studied (n = 21). Vehicle controls for pH, as well as controls for current were employed. The results of this study indicate that σ ligands are predominantly inhibitory on Purkinje cells in the rat cerebellum, with the exception of (+)-3-PPP, which is excitatory. However, it cannot be concluded that the effects of (+)-3-PPP were σ-mediated or that this drug is acting on the same σ receptor as the other ligands. These data are consistent with the possibility that BD1008 is a potent σ ligand.

236.15

CHARACTERIZATION OF [^{3}H]DTG AND [^{3}H](+)-3-PPP BINDING SITES OF NB41A3 NEUROBLASTOMA, C6 GLIOMA, AND NG108-15 HYBRID CELLS. B.J. Vilner and W.D. Bowen. Section of Biochemistry, Division of Biology and Medicine, Brown University, Providence, RI 02912.

We have characterized the binding of sigma ligands to crude membrane homogenates from NB41A3 neuroblastoma, C6 glioma, and NG108-15 hybrid cells (50 mM Tris-HCl, pH 8.0). [^{3}H]DTG bound with K_d (nM) and B_{max} (fmol/mg protein) of 62±6 and 7324±670 (NB41A3), 101±7 and 5507±537 (C6), and 75±1 and 3134±229 (NG108-15), respectively. The corresponding values for [^{3}H](+)-3-PPP were 137±11 and 2256±642, 500±31 and 6268±682, and 183±25 and 1673±344, respectively. Competition studies with various sigma ligands *vs.* 5 nM [^{3}H]DTG gave the following rank order of potency: DTG > haloperidol > (-)-pentazocine > (+)-3-PPP = fluphenazine > (+)-pentazocine > (-)-SKF 10,047 > (+)-SKF 10,047. Ligands for opiate (DAGO, DSLET, morphine), PCP (MK-801), dopamine (apomorphine) and amino acid (GABA, glutamate) receptors failed to displace [^{3}H]DTG binding at 10 uM. Low affinity for (+)-pentazocine (K_i= 1.7-3.3 uM) and (+)-SKF 10,047 (K_i= 19.3-91.8 uM), and much higher affinity for the corresponding (-)-enantiomers suggest that these cells express sigma-2 receptors similar to those found in PC12 cells (*Brain Res.* 527:244-253, 1990). However, the affinity of (+)-3-PPP (K_i= 286-493 nM) is significantly lower than reported for the PC12 cell site. This may suggest a sub-form of sigma-2 site and deserves further investigation. Finally, binding of [^{3}H]DTG to intact cells under physiological conditions suggests that these are cell surface receptors. The presence of sigma-like binding sites on cell lines of both glial and neuronal origin will facilitate studies of the biochemical and physiological processes mediated by this receptor. (PHS Grant NS-26746)

236.16

RELATIONSHIP OF KAPPA OPIOID RECEPTOR BINDING AND INHIBITION OF ADENYLYL CYCLASE IN GUINEA PIG BRAIN. C.S. Konkoy and S.R. Childers, Dept. Physiology/Pharmacology, Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

Previously, we showed that κ-selective ligands inhibit adenylyl cyclase (AC) in guinea pig cerebellar membranes. The present studies explore the relationship between κ binding sites and inhibition of AC. Various κ opioids displaced [^{3}H]U-69,593 (U-69) binding at a single site with sub-nM affinities. These agonists were several hundred fold weaker in inhibiting AC, but the rank order of AC inhibition paralleled the displacement of [^{3}H]U-69 binding except for α-neo endorphin. The correlation of AC and binding IC_{50} values was significant except for α-neo endorphin, which was relatively weak at inhibiting AC despite a K_i value of .08 nM vs. [^{3}H]U-69 binding. A correlation between [^{3}H]U-69 binding and U-50,488H (U-50)-inhibited AC was determined across 11 regions of the guinea pig brain. κ-inhibited AC was highest in the cerebellum, absent in thalamus and sup. colliculus, and moderate in other regions. In most regions, [^{3}H]U-69 binding correlated with the efficacy of U-50-inhibited AC. However, hippocampus had high levels of U-50-inhibited AC despite low levels of [^{3}H]U-69 binding, while cortex exhibited a high density of [^{3}H]U-69 sites but a relatively low level of U-50-inhibited AC. Irreversible reaction of cerebellar κ receptors with ß-CNA blocked both [^{3}H]U-69 binding and U-50-inhibited AC, with no effect on agonist potency, thus suggesting a relatively small κ receptor reserve for this effector system.

Supported by PHS grants DA-02904 and DA-05400 from NIDA.

236.17

KAPPA AND DELTA OPIOID RECEPTORS: RELATIONSHIP TO RAT MIDBRAIN DOPAMINERGIC NEURONS. S.G. Speciale, M. Sadeq, K.F. Manaye and D.C. German. Dept. of Psychiatry, UT Southwestern Med. Cntr., Dallas, TX.

We have described the relationship between mu opioid receptors and midbrain dopaminergic (DA) neurons (Speciale et al., Neurosci. Abstr. 15: 230, 1989). Receptor densities (fmol/mg tissue) ranged from 40-150 within specific DA nuclei. The present study extends this investigation. Brain sections were incubated in 5 nM ^{3}H-U69593 for kappa (k) binding, or 6 nM ^{3}H-DPDPE for delta (δ) binding, and standard in vitro autoradiographic methods used. The k receptors were specifically localized within the midbrain DA regions: throughout the rostral-caudal extent of nucleus A9, and within several nucleus A10 subnuclei (e.g., paranigral and interfascicular nuclei) at densities of 6.6 ± 0.6; within nucleus A8, however, the density was 4.1 ± 0.4. There were low densities of δ receptors uniformly throughout the midbrain DA regions. Within nuclei A8, A9 and A10 the binding was ≈ 7 , and slightly higher within the caudal nucleus A9. These results demonstrate a heterogeneous distribution of opioid receptor subtypes within the midbrain DA nuclear regions. Supported by DA-05314.

236.18

CHARACTERIZATION OF KAPPA OPIOID RECEPTOR SUBTYPES IN GUINEA PIG BRAIN AND ILEUM. W.E. Polgar*, S.R. Brandt*, J.L. Webster*, I. Berzetei-Gurske and L. Toll. Neuroscience Dept. SRI International, Menlo Park, CA 94025.

Recent studies have clearly demonstrated the presence of κ_1 and κ_2 opioid binding sites. κ_2 sites, have been labeled with [^{3}H]bremazocine, or [^{3}H]EKC, in the presence of reversible or irreversible blockers of μ, δ and κ_1. We have used [^{3}H]bremazocine binding to guinea pig brain membranes and directly compared reversible and irreversible blockade of μ, δ and κ_1 for analysis of κ_2 receptors. We have also used selective antagonists to look for κ_2 receptors in guinea pig ileum. Our results showed similarity between the two methods of incubation conditions. Using either method of tissue incubation, many ligands tested displayed shallow inhibition curves, suggesting the presence of additional sites. This was most dramatically demonstrated by the dynorphin gene products tested. Each dynorphin analog inhibited a maximum of 60-70% of the [^{3}H]bremazocine binding, even after μ, δ and κ_1 are blocked. These results suggest that even κ_2 binding is heterogeneous. Because dynorphin does not inhibit a portion of "κ_2" binding, this portion should not be considered a κ subtype.

236.19

KAPPA OPIOID RECEPTOR SUBTYPE IN RAT SPINAL CORD: COINCIDENCE WITH AUTONOMIC REGIONS. M.A. Romagnano, E.K. Richfield, L.S. Brady, and R.W. Hamill. Neurology Dept. Monroe Community Hospital/Univ. of Rochester, N.Y. 14620 and Unit on Functional Neuroanatomy, NIMH, Bethesda, MD. 20892

The distribution of the kappa opioid receptor subtype was examined throughout spinal cord levels C8-S2-4 in the rat using the *in vitro* receptor autoradiography technique. Fresh frozen horizontal serial sections were incubated with 10nM [^{3}H]bremazocine in the presence of either unlabeled DAGO and DADLE or BIT and FIT (mu and delta opioid receptor subtype blockers). Adjacent sections were coincubated with naloxone (10μM) or levorphanol (1μM) to determine nonspecific binding. Following incubation, slides were apposed to ^{3}H-Hyperfilm (Amersham) and allowed to expose for 3-4 months.

In upper and lower thoracic spinal cord, moderate-dense binding was found in lamina VII in nucleus intercalatus and nucleus intercalatus, pars paraependymalis.Moderate-dense kappa binding was present in the dorsal commissural nucleus while moderate binding found throughout the gray matter. Specific dense kappa binding was found in laminae I and II in upper and lower thoracic and lumbosacral spinal cord. Moderate-dense kappa binding was found in the sacral parasympathetic nucleus, dorsal gray commissure, and within bands traversing the intermediate zone.The rest of lumbosacral spinal cord gray matter contained moderate kappa binding. The dorsal root ganglia at all spinal cord levels contained moderate to moderate-dense kappa binding. These data establishing the presence of the kappa opioid receptor subtype in spinal cord regions involved in autonomic functions support the involvement of opioid peptides in autonomic regulation.

236.20

EFFECT OF KAPPA AGONISTS, PD 117302 AND CI-977, ON GLUTAMATE-STIMULATED PHOSPHATIDYLINOSITOL (PI) METABOLISM IN NEURONAL CULTURES. D. L. Yourick, M. A. DeCoster and F. C. Tortella. Dept. Med. Neurosci., Walter Reed Army Inst. Res., Washington, D. C. 20307-5100.

Neuroprotective and anticonvulsant effects of the novel kappa opioid agonists, PD 117302 and CI-977, have been described. We have also shown that a reduction in intracellular calcium may be involved in this neuroprotective action. The effects of these kappa opioids on glutamate-induced inositol phosphate (IP) accumulation were evaluated in primary rat cortical neuronal cultures. Glutamate (40 μM) increased IP accumulation to 1523% of basal; addition of 50 nM PD 117302 or CI-977 with glutamate decreased this stimulation by 57 and 48% respectively. In control experiments, both drugs were seen to stimulate IP accumulation alone, indicating that kappa receptors may be linked to PI metabolism. These results 1) suggest that kappa opioid-induced reductions in glutamate-stimulated IP accumulation may represent a mechanism by which intracellular calcium is decreased and 2) provide a molecular basis for the role of second messengers in the neuroprotective actions of these novel kappa opioid drugs.

236.21

NALOXONE INDUCED ANALGESIA:INVOLVEMENT OF KAPPA OPIATE RECEPTORS. M.Bianchi* and A.E.Panerai. Dept. Pharmacology, Univ. of Milano, 20129, Milano, Italy.

The analgesic effect of naloxone has been consistently demonstrated in post-stressful experimental or physiological conditions. Several hints suggest the involvement of opiate receptors of the kappa subtype in this effect. We show now in rats that acute naloxone, at the dose of 30mg\kg, exerts an analgesic effect as measured on the hot plate, that peaks thirty minutes after administration, and is reversed by the kappa opiate receptor antagonist MR 1452. Consistently with this observation, the analgesic effect of naloxone was observed also after the subcutaneous administration of the drug for five days, and this effect too was reversed by the kappa opiate receptor antagonist. The analgesia observed is not limited to naloxone, but another opiate receptor antagonist, naltrexone, exhibit an analgesic effect, that is reversed by the kappa opiate receptor antagonist. We conclude that naloxone induced analgesia is part of the activation of a complex pattern of mechanisms that might be not strictly related to stress, and involves kappa opiate receptors.

236.22

PHARMACOLOGY AND BINDING OF KAPPA$_3$ OPIATE RECEPTORS IN MICE. J. Cheng, C.G. Pick and G.W. Pasternak, The Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neuroscience and Pharmacology, Cornell Univ. Medical College, New York, NY 10021.

Naloxone benzoylhydrazone (NalBzoH) labels both μ and κ3 receptors in calf brain membranes. In the present study we demonstrate the presence of κ3 receptors in mouse brain and examine κ3 analgesia in genetically distinct strains of mice. Like the calf, the density of κ3 sites in mouse brain was quite high, typically twice that of μ receptors. The selectivity of κ3 binding also was similar to that in calf. The poor affinity of a number of selective drugs (morphine, U50,488H, DPDPE) supported the existence of a distinct κ3 site. NalBzoH (50 mg/kg, s.c.) elicited a potent κ3 analgesia in CD-1 mice (55%), as previously reported. However, its analgesic potency in additional strains varied considerably. In fact, no tailflick analgesia was observed in C57 or CXBK mice. The sensitivity of Swiss (30%) and Balb/C (15%) were intermediate. Whole brain binding studies did not demonstrate significant differences in either the affinity or B_{max} of the various strains. The fact that the behavioral differences were not accompanied by the changes in the binding values may have a number of reasons. The receptors responsible for analgesia may represent a small percentage of the total or the effectiveness of the κ3 system may be modulated by changes downstream from the receptor.

236.23

CHARACTERIZATION OF KAPPA OPIOID BINDING SITES ON MURINE LYMPHOCYTES. L. D. Saripalli and J. M. Bidlack, Dept. of Pharmacology, Univ. of Rochester, Rochester, NY 14642.

Membranes from the R1.1 murine lymphoma cell line, prepared in 50 mM Tris-HCl, pH 7.5, bound [^{3}H]naloxone in a saturable and specific manner. Scatchard analysis of [^{3}H]naloxone binding at 25°C to R1.1 cell membranes showed a curved plot, suggesting multiple binding sites. Competition experiments with opioids, specific for μ, δ, and κ brain opioid binding sites, indicated that some of the [^{3}H]naloxone binding was to κ opioid binding sites, while the remaining [^{3}H]naloxone binding was only inhibited by naloxone. The κ opioid binding site was further characterized using [^{3}H]U69,593, a selective κ opioid agonist. Specific [^{3}H]U69,593 binding increased linearly with protein concentration. At 25°C, binding equilibrium was attained within 20 min, and was stable for up to 120 min. Scatchard analysis of [^{3}H]U69,593 binding to R1.1 cell membranes showed a linear plot with a K_d value of 0.23 ± 0.018 nM and B_{max} value of 30.3 ± 47 fmol/mg protein. The δ-selective opioid peptides, [D-Pen^2,D-Pen^5]enkephalin (DPDPE) and ICI 174,864, at concentrations up to 10 μM did not inhibit 0.4 nM [^{3}H]U69,593 binding. Micromolar concentrations of the μ-selective opioid peptide [D-Ala^2,(Me)Phe^4,Gly(ol)^5]enkephalin (DAGO) were needed to inhibit [^{3}H]U69,593 binding. The IC_{50} values for the κ-selective opioids U50,488H, nor-binaltorphimine (nor-BNI), and (-) pentazocine were in the low nM range, while the IC_{50} value of (+) pentazocine was 26 times greater than the (-) isomer. Protease-resistant analogues of dynorphin also inhibited [^{3}H]U69,593 binding to R1.1 cell membranes. These results indicate that R1.1 lymphoma cells exhibit κ opioid binding sites that have similar characteristics to brain κ opioid receptors. (Supported by USPHS grant DA04355).

237.1

DIFFERENTIAL EFFECTS OF MU AND DELTA OPIATES IN THE RAT HIPPOCAMPAL SLICE. G.B. Watson and T.H. Lanthorn. CNS Diseases Research, Searle R & D, Skokie, IL 60077.

Various opiate agonists and antagonists were examined for their ability to alter extracellular CA1 evoked population spike amplitude. DPDPE, DSLET, DTLET, DAMGO and Leu- and Met-enkephalin all increased the amplitude of the evoked primary population spike. In addition, those compounds with delta receptor selectivity of < 100 induced secondary population spike(s). In contrast to DPDPE, (D-ala^2)-deltorphin II (10-1000 nM), another compound with high affinity and selectivity for delta receptors, had no agonist-like effect on primary population spike amplitude. The effects of DPDPE were antagonized by the delta antagonist naltrindole (NTI), while the agonist actions of DSLET were relatively insensitive to NTI. The actions of DSLET were antagonized by the mu antagonist naltrexone. Taken together, these results suggest that the actions of delta selective compounds like DPDPE can be differentiated from mu receptor-mediated actions. In addition, the lack of activity of deltorphin II and the apparent lack of delta activity of DSLET suggest that one subtype of delta receptor mediates this activity. This appears to be most like the proposed $delta_{noncomplexed}$ site.

237.2

8-BROMO-cAMP BLOCKS OPIOID ACTIVATION OF A VOLTAGE-GATED POTASSIUM CURRENT IN ISOLATED HIPPOCAMPAL NEURONS T.L.Wimpey and C. Chavkin. Dept. of Pharmacology, University of Washington, Seattle, WA 98195

We have previously shown that mu-selective opioid agonists activate both an inward rectifying and a voltage-gated potassium conductance in acutely dissociated hippocampal neurons. We now report that the opioid-activated voltage-gated potassium conductance is blocked by the membrane permeable cAMP analogue 8-Bromo-cAMP. Hippocampal neurons acutely dissociated from adult rats were voltage-clamped using the whole cell patch clamp technique. The neurons were continuously superfused with oxygenated buffer containing TTX (1 μM) and cadmium (500 μM) to block sodium and calcium currents respectively; drug responses were determined by pressure application through drug pipettes. Only dissociated neurons that were not pyramidal in appearance were chosen for recordings. As previously reported the opioid-responsive neurons make up only a small fraction of the total neuronal population in this brain region. Only about one in six of the selected neurons responded to opioid application. PL017 or another mu selective agonist DAMGO increased outward potassium currents recorded during depolarizing voltage steps in these neurons. This effect was blocked by simultaneous application of the antagonist naloxone. Application of 8-Bromo-cAMP from a second drug pipette reversed the opioid action and blocked the effect of subsequent opioid application. In contrast, 8-Bromo-cGMP failed to inhibit opioid activation of the voltage-gated potassium current. This is the first demonstration of an opioid-regulated membrane conductance that is sensitive to cAMP levels. Supported by DA04123.

237.3

INTERACTIONS BETWEEN OPIATE AND ACETYLCHOLINE RESPONSES ON MEDIAL VESTIBULAR NEURONS. D.O. Carpenter, D.G. Tieman, and N. Hori, NYS Dept. Health and School of Public Health, Albany, NY 12201.

We have recorded electrical activity and responses to transmitters and peptides on rat brain slices of medial vestibular nucleus. These neurons exhibit an endogenous pacemaker discharge which varies with membrane potential but is independent of manipulations which block synaptic transmission. With ionophoretic application of neurotransmitter agonists the neurons are excited by the excitatory amino acids and acetylcholine and inhibited by GABA. Morphine and leucine enkephalin altered the spontaneous activity of every neuron tested and these effects were blocked by naloxone. Most (>90%) were excited by morphine, but some cells showed either pure inhibition or a biphasic excitation/inhibition. When morphine was bath perfused a few neurons showed a significant and maintained increase in spontaneous discharge frequency. In the presence of morphine most showed a clear increase in amplitude of response to acetylcholine but not to quisqualate. This effect on acetylcholine response was dose dependent. Naloxone, which blocked all opiate effects, also reduced the response to acetylcholine in the presence or the absence of morphine. These observations show that medial vestibular neurons exhibit excitatory opiate receptors on almost all neurons and that there is an interaction between opiate and acetylcholine responses which has not been described elsewhere.

237.4

EFFECTS OF PHOSPHATASE INHIBITION ON THE TRANSIENT DESENSITIZATION OF μ- OPIOID RECEPTORS IN RAT LOCUS COERULEUS. P.B. Osborne and J.T. Williams. Vollum Institute, Oregon Hlth. Sci. Univ, Portland, OR 97201.

The locus coeruleus (LC) is a noradrenergic nucleus in the brainstem that is thought to play a role in mediating opiate withdrawal responses. In slice preparations agonists to both μ opioid receptors and α_2 adrenoceptors hyperpolarize LC neurons by increasing a potassium conductance. In a previous microelectrode study it was shown that exposure of neurons to supermaximal concentrations of μ receptor agonists resulted in a transient desensitization of opioid receptors but had little or no effect on hyperpolarizations caused by adrenoceptor agonists.

To study this effect further, experiments were conducted using whole cell patch clamp recording in rat brain slices. Outward currents induced by test doses of Met-enkephalin (ME) (300nM) were compared before and after a 5 min exposure to a desensitizing dose of ME (30 μM). 3 min after washout of the high dose, the current induced by the low dose was reduced by 77%. Recovery occured over 15min but was always incomplete. Addition of the phosphatase inhibitor microcystin (100nM) to the electrode solution prolonged the time taken to recover from desensitization. Microcystin at this concentration fully inhibits type 1 and type 2a phosphatases but not Ca/calmodulin -dependent type 2b phosphatases. With both microcystin and the Ca chelator BAPTA (10mM) in the electrode solution the amount of desensitization was increased and further slowing of the recovery from desensitization was observed. These results indicate that dephosphorylation of an as yet unidentified substrate appears to be necessary for the reversal of μ opioid receptor desensitization. Supported by USDHHS DA 04523 and MH45003.

237.5

PRE- AND POSTSYNATPIC EFFECTS OF OPIOIDS MEASURED IN PATCH/SLICE RECORDINGS OF THE RAT NUCLEUS TRACTUS SOLITARIUS (NTS). H.W. Rhim, M.McCarren, S.R. Glaum and R.J. Miller, Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago IL 60637.

The NTS mediates central respiratory, cardiovascular and gastrointestinal homeostasis. Opioid-containing nerve terminals and opioid receptors are widely distributed in the NTS (Atweh et al.,Brain Res. 124:53,1977). Exogenous opioid agonists produce potent respiratory and cardiovascular effects by unknown mechanisms within the NTS (Hassen et.al.,Peptides 4:621,1983). We therefore examined the effects of morphine (MOR, 10uM), the selective mu agonist D-Ala-MePhe-Gly-ol-enkephalin (DAGO, 0.1-10uM), the selective delta agonist D-Pen-D-Pen-enkephalin (DPDP, 4uM) and the selective kappa agonist dynorphin (DYN, 4uM) on synaptic transmission in the NTS.

Whole cell patch recordings were made with K-gluconate filled electrodes (3-5 Mohm) containing (mM): K-gluconate 145; $MgCl_2$ 2; HEPES 5; K_2ATP 5; $CaCl_2$ 0.1; EGTA 1.1.

All neurons (n=34) displayed spontaneous (sp) EPSPs. In addition, approximately half of the cells exhibited spIPSPs at their resting membrane potential (-65 to -50mV). Application of 10uM MOR (n=3) reduced the amplitude of both spPSPs and PSPs evoked by electrical stimulation of the tractus solitarius. The effect of MOR was not mimicked by DYN (4uM, 5/6 cells) or DPDP (4uM, 23/24 cells). In contrast, DAGO (0.1-10uM) reduced both evoked and spPSPs in 32/34 cells. Accompanying this response was a dose-dependent hyperpolarization (23/32 cells) with a reversal potential of -97mV and a decrease in R_{in}. Naloxone (10-30uM) reversibly inhibited the effects of MOR and DAGO. These data suggest that mu opioid receptor activation can depress synaptic transmission in the NTS.

237.6

THE INFLUENCE OF OPIATE ANTAGONISTS ON DYNORPHIN-INDUCED ELECTROPHYSIOLOGIC EFFECTS. **H. Ristic* and L. Isaac, Department of Pharmacology, Univ. Ill. Col. of Medicine, Chicago, IL 60612**

Dynorphin A is thought to be the endogenous ligand for the kappa opiate receptor. When applied directly onto the spinal cord it results in a reversible depression of the dorsal root potential (DRP). Our goal was to determine whether this effect is opiate receptor mediated.

A laminectomy was performed on urethane anesthetized rats. Lumbar spinal roots were isolated, cut and placed on platinum wire electrodes.The DRP was recorded before and following application of saline, DYN (1-13), DYN (2-13), U-50488H (U50), morphine (MOR), naloxone (NAL), nor-binaltorphimine (NOR-BNI) or a combination of these drugs.

Saline, NAL and NOR-BNI had no influence on the DRP. The non-opiate peptide DYN (2-13) did not influence the DRP. Naloxone, at doses which blocked MOR, also did not influence the response to DYN. On the other hand, the kappa antagonist NOR-BNI blocked both DYN- and U50-induced depression of the DRP and itself in high doses depressed the DRP.

These data suggest that a kappa opiate receptor component is involved in DYN-induced depression of the DRP.

237.7

NALOXONE PARADOXICALLY PROLONGS THE ACTION POTENTIAL AFTER ACUTE GM1 GANGLIOSIDE TREATMENT OF SENSORY NEURONS BUT CONTINUES TO ANTAGONIZE OPIOID-INDUCED SHORTENING. K.-F. Shen and S.M. Crain. Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, N.Y. 10461.

Low (nM) concentrations of opioid agonists prolong the calcium-dependent component of the action potential duration (APD) of many mouse dorsal-root ganglion (DRG) neurons in culture (Shen & Crain, Br. Res.491, 1989), whereas higher (μM) levels shorten the APD. Both effects are blocked by naloxone (1-10nM). Opioid-induced APD prolongation appears to be mediated by excitatory opioid receptors that are positively coupled via a cholera toxin-A-sensitive G_s protein to cAMP-dependent ion conductances (S.& C., Br. Res.525, 1990); opioid-induced APD shortening, by inhibitory receptors linked to pertussis toxin-sensitive G_i/G_o. Cholera toxin B-subunit, which binds to GM1 ganglioside, also selectively blocks opioid-induced APD prolongation (S.& C., Br. Res.531, 1990). After brief treatment with GM1 ganglioside (>10nM for a few min), the opioid agonists, dynorphin (1-13) or morphine prolonged the APD at fM vs. the usual nM concentrations (S. et al,Br.Res.,in press). Furthermore, the opioid antagonists, (-) naloxone or diprenorphine (1-30nM), but not (+) naloxone, unexpectedly prolonged the APD of the GM1-treated cells, yet still continued to antagonize opioid-induced APD shortening. These results suggest that the supersensitivity of GM1-treated DRG neurons to the excitatory effects of opioid agonists and antagonists is due to a remarkably increased efficacy of excitatory opioid receptor activation of G_s, rather than simply to increased binding affinity. Similar supersensitivity develops after chronic opioids (C.&S., SN abstr., 1991). (Supported by NIDA grant DA 02031.)

237.8

CHRONIC OPIOID-TREATED SENSORY NEURONS BECOME SUPERSENSITIVE TO THE EXCITATORY EFFECTS OF OPIOIDS AS OCCURS AFTER ACUTE ELEVATION OF GM1 GANGLIOSIDE LEVEL. S.M. Crain and K.-F. Shen. Dept. of Neuroscience, Albert Einstein Coll. Med., Bronx, N.Y. 10461.

Chronic DADLE- or morphine-treated DRG neurons (1uM for 1 wk in culture) become tolerant to opioid inhibitory effects, i.e., shortening of the action potential duration (APD) (Crain et al, Br. Res., 1988), and supersensitive to opioid excitatory effects, i.e., prolongation of the APD. Dynorphin (1-13) or morphine prolong the APD of treated DRG neurons at fM levels, whereas nM levels are required in naive cells (C.&S. TiPS, 1990), and the opioid antagonists, naloxone or diprenorphine (1-10 nM) unexpectedly prolong the APD of most of the treated cells. Similar opioid supersensitivity occurs after acute treatment of naive DRG neurons with GM1 ganglioside (Shen et al,Br. Res. in press; S.& C., SN abstr, 1991). Furthermore, whereas cholera toxin-B subunit (1-10 nM) blocks opioid-induced APD prolongation in naive DRG neurons (presumably by interfering with endogenous GM1 modulation of excitatory opioid receptor functions: S.& C., Br. Res. 531, 1990), even higher concentrations of CTX-B were ineffective in chronic opioid-treated neurons. These and related data suggest that excitatory opioid supersensitivity of chronic opioid-treated DRG neurons may be due to a cAMP-dependent increase in GM1 ganglioside levels that greatly enhances the efficacy of excitatory opioid receptor activation of G_s. Our results may clarify mechanisms of opioid dependence and the paradoxical supersensitivity to naloxone which triggers withdrawal symptoms after opiate addiction. (Supported by NIDA grant DA 02031.)

237.9

ACTIVATION OF MU AND DELTA OPIOID RECEPTORS ON F11 NEUROBLASTOMA CELLS INCREASES VOLTAGE-DEPENDENT K^+ CURRENTS. S.F. Fan[1], K.-F. Shen[1], M.A. Scheideler[2*] and S.M. Crain[1]; Dept. of[1] Neuroscience, Albert Einstein Coll. Med., Bronx, NY, 10461 and[2] CNS Div., NovoNordisk AIS, 2860 Soeborg, Denmark

The F11 cell line is a fusion product of embryonic rat dorsal root ganglion (DRG) cells with the mouse neuroblastoma cell line N18TG-2 (Platika et al., PNAS '85). This cell line is derived from sensory neurons (Boland & Dingledine, Dev. Br. Res. '90), and expresses μ & δ opioid receptors that are negatively coupled to adenylate cyclase (Francel et al., J. Neurochem. '87; Dawson & Scheideler, in Biochem. & Physiol. of Substance Abuse, 2,R.R. Watson, ed., CRC Press, '90). The present study presents direct binding measurements & electrophysiologic evidence of μ & δ opioid receptor expression. Tight-seal patch-clamp recordings of F11 cells after several days in a differentiating culture medium (low serum, cAMP and NGF) showed that: (1) the outward K^+ current during pulsed depolarization in most of these cells was increased by either the δ agonist, DPDPE (1uM) or the μ agonist, DAGO (1uM), but none were responsive to both opioids; (2) cells without any processes responded neither to DPDPE nor to DAGO. The opioid-induced increase in voltage-dependent membrane K^+ current in F11 cells resembles the inhibitory effect elicited by μ and δ opioid agonists in primary cultures of mouse DRG neurons (Werz and Macdonald, JPET '83; Shen & Crain, Br. Res. '89). The F11 cell line may therefore provide a valuable model system for correlative pharmacologic, electrophysiologic and biochemical analyses of opioid functions in sensory neurons. (Supported by NIDA research grants DA05203 and DA 02031 to S.M.C.)

237.10

OPPOSITE MODULATION OF SUBSTANCE P RELEASE FROM RAT TRIGEMINAL NUCLEUS CAUDALIS SLICES BY KAPPA AND DELTA OPIOID-RECEPTORS. H. Suarez-Roca and W. Maixner. Dept. of Pharmacology and Dental Research Center, University of North Carolina, Chapel Hill, N.C. 27514.

We previously reported that the release of substance P (SP) from trigeminal nucleus caudalis (TNC) slices is inhibited and facilitated by activation of distinct populations of μ-opioid receptors with nM concentrations of morphine. In addition, SP release is respectively inhibited and facilitated by activation of δ- and k-opioid receptors with μM concentrations of morphine (Suarez-Roca et al., Neurosci.Abs.16:367,1990). In the present study, we have examined the effect of nM concentrations of U50488H (k-opioid receptor agonist) and D-Pen^2,D-Pen^5,enkephalin (DPDPE, δ-opioid receptor agonist) on K^+-evoked SP release from rat TNC slices. Immunoreactive SP was measured in perfusates. U50488H produced facilitation of SP release with a maximal effect at 30 nM. Naloxone (30 nM; non-selective) and nor-binaltorphimine (3 nM n-BNI; k-selective) blocked U50488H facilitatory effect whereas β-funaltrexamine (20 nM β-FNA; μ-selective), naloxonazine (1 nM; μ_1-selective), and ICI174,864 (0.3 μM; δ-selective) were inactive. DPDPE inhibited SP release with a maximal inhibition at 3 nM. DPDPE inhibition of SP release was blocked by naloxone and ICI174,864 but not by n-BNI, naloxonazine, or β-FNA. Both U50488H and DPDPE produced U-shaped concentration-response curves that were completely autoinhibited at 100 nM. These findings support our previous observations that activation of k- and δ-opioid receptors respectively enhances and reduces the release of SP in TNC and that opioids produce bi-directional modulatory effects that are concentration- and receptor-subtype-dependent. (Supported by DE08013 & OAS Fellowship).

237.11

ONTOGENY OF THE ZETA (ζ) RECEPTOR IN RAT CEREBELLUM. P.J. McLaughlin, D.M. Gibo* and I.S. Zagon. Dept. of Neuroscience and Anatomy, Penn State Univ. College of Medicine, Hershey, PA 17033.

An opioid growth factor (OGF), [Met^5]-enkephalin (MET), serves as an important regulatory peptide of nervous system development. OGF interacts with the zeta (ζ) receptor to inhibit growth processes. The ontogeny of the ζ receptor during cerebellar development has been examined. Tissues from rat pups were collected at embryonic (E) days 15, 18, and 20, as well as postnatal days 1, 3, 6, 10, 15, 18, 21, 35, and adult; whole brains were assayed at E15 and E18, whereas cerebella were studied from day E20 onwards. Specific and saturable binding of ^{3}H-MET was detected in the whole brain at E15 and E18 (6.2 fmol/mg protein). The embryonic cerebellum (E20) contained 6.0 fmol/mg protein. Binding capacity markedly increased from birth to day 10, with 29.8 fmol/mg protein measured on day 10. Binding decreased substantially by day 15 (7.0 fmol/mg protein), and was not detected by weaning. These data are consistent with the timetable of cerebellar neurogenesis defined in earlier studies, and correlates closely with the extent of the germinative pool of cells (i.e., external germinal (granule) layer).

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237.12

HYPOTHALAMIC LOCALIZATION OF KAPPA, DELTA AND MU OPIOID RECEPTORS IN THE SONGBIRD BRAIN. P. Deviche and P. Cotter*. Inst. Arctic Biology, Univ. Alaska Fairbanks, Fairbanks, AK 99775.

To determine the distribution of opioid binding sites in the songbird hypothalamus, whole brain sections obtained from dark-eyed juncos (*Junco hyemalis*) were incubated with tritiated ligands that bind either to kappa (ethylketocyclazocine), mu (DAGO) or delta (DPDPE) opioid receptors. Nonspecific binding was defined as the difference between the total binding and the binding measured in the presence of high concentrations of appropriate unlabelled ligands. Preliminary studies using brain homogenate sections showed that the binding of the 3 labelled ligands is specific, time-dependent, and saturable (Km $\leq$ 5 nM; Bmax $\leq$ 120 fmol/mg protein). Within the hypothalamus, specific kappa, delta, and mu binding sites are discretely and differently distributed. In particular, there is a high density of delta sites in the n. paraventricularis (PVN). Immunocytochemical studies demonstrate that the junco PVN contains opioid peptides including methionine-enkephalin and dynorphin (1-13), suggesting that these peptides influence PVN activity. The PVN, which contains steroid receptors, may be a region where steroids and opioids interact functionally.

237.13

CHARACTERIZATION OF OPIOID RECEPTORS IN PHEOCHROMOCYTOMA CELLS M.E. Abood and J.S. Eubanks*. Dept. of Pharm., Med. Coll. of Virginia, Richmond, VA 23298.

PC12 rat pheochromocytoma cells are useful as a model system for neuronal development. In one subclone of PC12 cells, PC12h, low levels of δ-type opioid receptors markedly increase in response to nerve growth factor (NGF) (Inoue, N. and Hatanaka, H. *J. Biol. Chem.* 257: 9238 1981). We are currently investigating the consequences of the appearance of opioid receptors in PC12h cells and examining concurrent changes in the expression of opioid-regulated genes. After 10 days of treatment with NGF, the number of opioid receptors (as measured by ^{3}H-diprenorphine binding), increases from a B_{max} of 40 to 220 fmols/mg protein, with concentrations up to 10 nM. Above 10 nM, a second site appears. The NGF-induced increase in receptor binding was reproducible in several experiments. The induction of binding does not necessarily mean these sites are functional receptors. In order to estimate whether these receptors are coupled to a potential second messenger, we took advantage of two known properties of δ receptors in cell lines, desensitization and down-regulation in response to opioid agonists. In preliminary experiments, the receptors in the cells show a time-dependent reduction in affinity for agonist, followed by a loss of receptor binding on the cell surface upon exposure to etorphine. Such results indicate that the receptors on NGF-treated PC12h cells are capable of responding to opioid agonists. Along with the increase of opioid binding following NGF treatment we find changes in the message levels of G proteins and other opioid-related molecules.

237.14

OPIATE RECEPTORS IN NEURONAL AND ASTROGLIAL PRIMARY CULTURES FROM DIFFERENT BRAIN REGIONS - association with dopamine (D1) receptor adenylate cyclase. Peter S. Eriksson*[1], Elisabeth Hansson[1] and Lars Rönnbäck[1,2] Institute of Neurobiology[1] and Department of Neurology[2], University of Göteborg, 400 33 Göteborg, Sweden.

Primary cultures enriched in neurons or astroglial cells from three phylogenetically different regions of the rat brain, the cerebral cortex, the striatum and the brain stem, were used to investigate the presence of opiate receptors coupled to adenylate cyclase. Morphine (MO) was used as a μ receptor agonist and D-ala2-D-leu-enkephalin (DADLE) was used as a δ receptor agonist. In the neuronal cultures both ligands inhibited the prostaglandin (PG)E1 stimulated intracellular cAMP accumulation dose dependently with the most prominent effects seen in the striatum cultures and with DADLE being more potent than MO. The opiate receptor antagonist naloxone reversed the effects. MO and DADLE added together inhibited the PGE1 stimulated cAMP accumulation less than the sum of each drug. Therefore, it might be that these opioid receptors are co-localized on the same neuron. Striatal neurons contained DA receptors coupled to cAMP as second messenger. The DA D1 receptor-stimulated adenylate cyclase activity was inhibited by the μ and δ opioid receptor ligands. Thus, interactions at the adenylate cyclase level seem to exist between D1, μ and δ opiate receptors. In the astroglial enriched cultures DADLE inhibited the PGE1 induced cAMP accumulation, however with a less prominent effect in the brain stem cultures. MO did not influence the basal or the PGE1 stimulated intracellular cAMP accumulation in the cultures used. DADLE was less effective to inhibit the PGE1 induced cAMP accumulation in the astroglial cultures than in the neuronal enriched cultures. Our results thus suggest the presence of δ receptors on astroglial cells in culture while no μ receptors with cAMP as second messenger were identified.

237.15

VISUALIZATION OF ^{125}I-ß-ENDORPHIN OPIOID RECEPTOR SITES IN NG108-15 AND SK-N-SH NEUROBLASTOMA CELLS BY MICROSCOPIC AUTORADIOGRAPHY. **Ric I. Cone, Jelveh Lameh*, Stuart A. Rosenfeld* and Wolfgang Sadée*. Departments of Pharmacy and Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446 (USA).**

Detection of opioid receptors by microscopic autoradiography on intact SK-N-SH and NG108-15 neuroblastoma cells was obtained with the use of ^{125}I-ß-endorphin (ßE) as tracer, together with ßE(6-31) to block high affinity non-opioid binding. Labeling was blocked by 10 μM diprenorphine.

Previously, we have measured μ and δ opioid receptor sites in intact SK-N-SH and NG108-15, using ^{125}I-ßE (K_d = 3 & 7 nM, respectively), and demonstrated rapid loss of δ receptor sites from the cell surface of NG108-15 following pretreatment with DADLE (Cone & Sadee, submitted). Using microscopic autoradiography, opioid receptor binding will be examined following differentiation with retinoic acid, and pre-treatment with opioid agonists, and cDNA libraries will be screened for opioid receptor expression in pCDM8-transfected COS -7 cells.

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237.16

MORPHINE REGULATION OF PROTO-ONCOGENE C-FOS AND PRO-OPIO-MELANOCORTIN EXPRESSION IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS. S.L. Chang, J.E. Zadina[1], L. Spriggs*[2]. Dept. of Physiology, LSU Sch. of Dentistry, New Orleans, LA. 70119, [1]VAMC, Dept. of Med. and Neuroscience Program, Tulane Univ. Sch. of Med., New Orleans, LA. 70146, and Dept. of Pharmacology, Tulane Univ. Sch. of Med., New Orleans, LA. 70112.

Morphine has been previously demonstrated to increase expression of the proto-oncogene c-fos in rat brain. The FOS nucleoprotein complex was then proposed to couple morphine binding to alteration of expression of proenkephalin (PE) and pro-opionmelanocortin (POMC), whose promoter contains an AP-1 sequence. (BBRC 157:698, 1988). The SH-SY5Y human neuroblastoma cells express predominantly μ-subtype opiate receptors (JBC 261:1065, 1986) with receptor density increased by retinoic acid and decreased by PMA (Excerpta Medica 914:151, 1990). In this study, we examined the time course of change in c-fos and POMC mRNA concentrations upon morphine treatment in SH-SY5Y cells. For undifferentiated cells, the c-fos mRNA levels were elevated by morphine (10 μM) rapidly and transiently during the initial period. By 2-6 hours, c-fos mRNA levels were reactivated and remained elevated for 7 days, the length of the testing period. The POMC mRNA levels were also activated upon chronic morphine treatment. These results indicated that chronic exposure to morphine can result in prolonged activation of c-fos and POMC mRNA in SH-SY5Y cells.

237.17

OPIOID REGULATION OF PRO-ENKEPHALIN MRNA LEVELS IN RAT PRIMARY FOREBRAIN NEURONAL CULTURES. S.R. Childers, T. Sexton and D.R. Marckel, Dept. Physiology/Pharmacology, Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

The role of opioid-inhibited adenylyl cyclase (AC) in the regulation of pro-enkephalin (PE) mRNA levels was examined in primary neuronal cultures from neonatal rat forebrain. The opioid peptide agonist dynorphin A_{1-13} (DYN) inhibited cAMP accumulation in intact cells by 40%-60%, and directly inhibited AC in isolated membranes by 15%. In these cultures, PE mRNA levels were induced 2-5-fold by either dibutyryl cAMP or forskolin. Addition of 1 μM DYN decreased forskolin-stimulated PE mRNA by 20%-60%, producing maximal effect between 1-4 hr. This effect was specific for neuronal cultures, since no effect of opioids was observed in glial cultures. Blockade of protein synthesis by cycloheximide increased PE mRNA levels but the inhibitory actions of DYN were still evident. The inhibitory action of DYN was blocked by naloxone and by pertussis toxin treatment of the cultures. Although DYN was effective in reducing forskolin-stimulated PE mRNA levels, the opioid agonist had no effect on dibutyryl cAMP-stimulated message. These results demonstrate that opioid receptor inhibition of AC may be the mechanism by which opioids regulate PE mRNA levels, providing a negative feedback mechanism to control their own synthesis. In addition, these results demonstrate a function for AC in brain which could regulate neurotransmitter expression and play an important role in long-term neuronal homeostasis.

Supported by PHS grants DA-02904 and DA-04534 from NIDA.

237.18

MORPHINE EFFECTS ON PROTEIN PHOSPHORYLATION AND ADENYLYL CYCLASE ACTIVITY IN RAT THALAMIC MEMBRANES. L. M. Fleming and S. R. Childers, Dept. Physiol./Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103, Dept. Pharmacology, Univ. Florida Coll. Med., Gainesville, FL 32610.

We previously reported that opioid-inhibited adenylyl cyclase (AC) decreased protein phosphorylation of two proteins (MW 63 and 85 kDa) by cAMP-dependent protein kinase. Chronic (but not acute) administration of morphine increased phosphorylation of these two protein bands. This chronic effect of morphine occurred in a short time period, with maximal effects 1-2 days after morphine pellet implantation. The increase in protein phosphorylation was not caused by an increase in cAMP-dependent protein kinase. However, preliminary results showed an increase in AC basal activity in thalamic membranes from chronically morphine-treated rats. This effect occurred within 9-24 hours, using periodic morphine injections. Maximum stimulation of AC by forskolin was not changed by morphine treatment, but when expressed as % of basal AC, forskolin-stimulated AC was blunted. Inhibition of AC by D-ala enk was not changed by morphine treatment. Lineweaver-Burke analysis indicated a change in the kinetics of AC in thalamus from these animals. These results suggest that subchronic administration of morphine may alter the parameters of opioid-inhibited AC in thalamic membranes, and may explain the observed increase in phosphorylation of membrane phosphoproteins.

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237.19

EFFECTS OF LHRH AND ESTRADIOL ON cAMP IN OPIOID-RESPONSIVE NEUROBLASTOMA CELLS. A. Ratka and J.W. Simpkins, Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, FL 32610.

SK-N-SH neuroblastoma cells, which express opioid receptors were evaluated for the inhibitory effects of opiates on cyclic AMP (cAMP) accumulation. Prostaglandin E_1 (PGE_1, 1μM) caused dramatic increase in cAMP level. Treatment with morphine (MOR, 10 μM) significantly inhibited the stimulatory effect of PGE_1 (from 2066.9±244.8 to 1179.2±196.3 pmol/mg protein). Estradiol (E_2) dose of 5 nM given for 1 hr., 8 hrs. and 6 days and the dose of 0.5 nM for 1 hr. significantly potentiated PGE_1 effect. In all cells pretreated with E_2 the inhibitory effect of MOR was not changed. Treatment with LHRH (1 ng/ml for 10 min.) increased PGE_1 stimulation of cAMP from 1879.6±118.9 to 2475.9±146.0 pmol/mg protein. The same LHRH treatment resulted in significant reduction of the inhibitory effect of MOR on cAMP level. Ten times higher dose of LHRH or longer duration of LHRH treatment had no effect on cell responses to either PGE_1 or MOR. When LHRH was added to E_2-treated cells, MOR-induced inhibition of cAMP accumulation decreased from 43-50% to 14-26%. LHRH antagonist (10 ng/ml, 10 min.) attenuated the stimulatory effect of PGE_1 on cAMP (2247.2±126.4 vs 3292.5±156.3 pmol/mg protein). The antagonist did not change either E_2 or MOR effects on cAMP accumulation. We conclude that in cells which express opioid receptors (1) the stimulatory effects of PGE_1 on cAMP accumulation is potentiated by E_2, (2) activation of LHRH receptors resulted in significant potentiation of PGE_1 effect and attenuation of the inhibitory effect of MOR on cAMP level, while antagonism of LHRH receptors reduced the effect PGE_1, (3) E_2 may modify the function of opioid receptors via LHRH, the neurons which are present in SK-N-SH cultures.

(Supported by AG 0202).

CATECHOLAMINE RECEPTORS: DOPAMINE II

238.1

ALTERNATIVE DOPAMINE D3 RECEPTOR mRNA SPLICE FORMS IN RAT AND HUMAN. L.A. Snyder, J.L. Roberts, S.C. Sealfon. Fishberg Research Center for Neurobiology, Mt. Sinai Medical School, New York, NY 10029

The molecular cloning of the rat dopamine D3 receptor (D3R) was recently reported (Nature 347:146,1990). Based on the published amino acid sequence, degenerate oligonucleotides for polymerase chain reaction (PCR) were designed to the N-terminal region and to the putative second cytoplasmic loop. In PCR reactions using rat cDNA as a template, two reaction products were observed; one of the expected size of the D3R, and another approximately 100 bp smaller. Multiple subcloned PCR products were sequenced and a cDNA with a 113 bp deletion, that was otherwise identical to the published D3R, was found The 113 bp is flanked by the nucleotide sequence AGGT, a consensus sequence for an RNA splice donor/acceptor site. The segment deleted, encompassing the first extracellular loop and third transmembrane domain, alters the reading frame, introducing 19 amino acids not found in the full length D3R, followed by a premature stop codon. Partial length clones of the human D3R were also isolated by PCR. Sequence analysis revealed that these D3R splice variants have been evolutionarily conserved

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238.2

EXPRESSION OF AN ALTERNATELY SPLICED D3 DOPAMINE RECEPTOR. M.W. McLane*, T.E. Norris, and M.M.S. Lo. ICI Americas, Inc., Wilmington, DE 19897.

Using RT-PCR methods, we have isolated a rat cDNA clone, pD3b, that is an alternately spliced form of the D3 dopamine receptor that has been recently reported. The D3b cDNA's coding region is 1284 bp long and is identical to the original D3 sequence except for an in-frame deletion that removes an internal eighteen amino acids. Nuclease protection assays will be done to confirm that the D3b transcript is authentic and coexists with the D3 form in the rat brain region containing olfactory tubercle. A broader tissue survey by RT-PCR will also determine if the D3b transcript is CNS specific.

238.3

ISOLATION AND CHARACTERIZATION OF THE RAT DOPAMINE D4 RECEPTOR. K.L. O'Malley, S. Harmon*, L. Tang*, S. Han*, and R.D. Todd. Departments of Anatomy and Neurobiology, Genetics and Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

Ongoing molecular studies have shown an unexpected variety of dopamine receptors generated from multiple genes and/or alternative splicing of RNA transcripts. Here we describe the isolation and characterization of a new D2 family gene which is expressed both in the CNS and the periphery. A rat genomic library was screened at low stringency using a DNA fragment corresponding to transmembrane domains VI and VII of $D2_A$ (Bunzow et al., 1988) and D3 (Sokoloff et al., 1990). Three types of clones were isolated, two corresponding to the previously isolated genes, $D2_A$ and D3, and the third representing a novel D2-family receptor. DNA sequence analysis revealed the greatest identity with D2 dopamine receptors followed by the alpha adrenergic and muscarinic receptors. The gene is composed of several exons some of whose splice junctions have been conserved within this family. Subsequent to this finding Van Tol et al. (1991) reported the cloning of a human D4 dopamine receptor. Sequence comparison indicates the novel rat receptor reported here is analogous to that of D4. In contrast to the human studies, the rat D4 is expressed primarily in the heart.

238.4

CLONING AND FUNCTIONAL CHARACTERIZATION OF THE DOPAMINE D_4 RECEPTOR. C. Bouvier*, H.H.M. Van Tol*, J. Bunzow*, R.A. Johnson*, H. Niznik* and O. Civelli. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201, Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8

A new dopamine receptor has been cloned from a human neuroepithelioma SK-N-MC cDNA library on the basis of its homology to the D_2 receptor. This new receptor, the D_4 receptor, has a higher degree of sequence similarity with the D_2 and D_3 than with the D_1 or D_5 receptors. It is encoded by a gene which organization is similar to that of the D_2 and D_3 receptors. The D_4 receptor, when expressed through DNA transfection in COS-7 cells, has a pharmacological profile resembling that of the D_2 receptor. Most importantly, the D_4 receptor binds the antagonist clozapine with a ten fold higher affinity than does the D_2 receptor. This suggests that the D_4 receptor may serve as the primary target for clozapine, an antipsychotic devoid of extrapyramidal side effects. The ability of the D_4 receptor to induce second messengers has been studied in SK-N-MC cells and in transfected GH_4C_1 cells. In GH_4C_1 cells, stimulation of the D_4 receptor by dopamine results in an inhibition of the forskolin-stimulated cAMP levels. In SK-N-MC which harbor endogenous D_1 and D_4 receptors, the D_1 dependent dopamine-stimulated cAMP levels are inhibited by the co-stimulation of the D_4 receptor. These results show that the D_4 receptor is able to modulate adenylyl activity in a way similar to that of the D_2 receptor, studies of the D_4 activity on other second messenger systems will be discussed.

238.5

TWO HUMAN PSEUDOGENES HOMOLOGOUS TO THE D5-DOPAMINE RECEPTOR B.F. O'Dowd*, T. Nguyen*, H. Jin*, L. Grupp and P. Seeman
Addiction Research Foundation (ARF) and the Department of Pharmacology, University of Toronto, Toronto, Canada M5S1A8.

Receptors for the neurotransmitter dopamine belong to the family of receptors characterized by having seven transmembrane (TM) regions and by acting through G-proteins. Molecular cloning studies have now identified five structurally homologous genes for the biosynthesis of human dopamine receptors, namely D1, D2, D3, D4 and D5. Two of these dopamine receptors (D1 and D5) are encoded by genes intronless in their coding regions. In an attempt to ascertain whether there are other intronless genes with homology to the gene encoding the D5 receptor we used a cloning method based on the polymerase chain reaction (PCR). Human genomic DNA was subjected to amplification by PCR with oligonucleotides based on the D5 receptor nucleotide sequence. Amplification of nucleotide sequences between these oligonucleotides, from TM1 to the carboxy tail, has allowed for the isolation of two independent intronless genes with close homology to the D5 receptor, namely PG-1 and PG-2. The deduced amino acid sequences of each of these genes, PG-1 and PG-2, shows an overall homology to the D5 receptor of 90%. However, the genes PG-1 and PG-2 each contains a stop codon in their coding regions that would render these genes incapable of encoding functional receptors. Thus, in common with the family of human genes encoding the phosphoglyccrate kinases, human dopaminergic genes are represented by intron-containing genes (D2, D3 and D4), by intronless genes (D1 and D5), and the pseudogenes PG-1 (ΨD5A) and PG-2 (ΨD5B). Southern blot analysis, using PG-1 as a probe, has also detected hybridizing bands in rat and mouse genomic DNA. Funded by ARF and MRC (Canada).

238.6

Localization of D_4 and D_5 Dopamine Receptor mRNAs in the Human Brain. J. Meador-Woodruff, A. Mansour, C. Work*, H.H.M. Van Tol*, D. Grandy, O. Civelli, and S.J. Watson. Department of Psychiatry and Mental Health Research Institute, University of Michigan, Ann Arbor MI, 48109-0720; Oregon Health Sciences University, Portland OR, 97201.

The dopamine receptor, pharmacologically resolved into D_1 and D_2 subtypes, has been more recently shown to consist of a family of 7-transmembrane domain, G-protein-coupled receptors. To date, five distinct genes encoding different dopamine receptors have been cloned. We have previously reported on the distribution of the mRNAs encoding D_1 and D_2 receptor mRNAs in brain. In this preliminary investigation, we compare the distributions of the mRNAs encoding the D_4 and D_5 receptors in the human nigrostriatal system. While both D_4 and D_5 receptor mRNAs were identifiable in the caudate and the putamen, only D_4 receptor mRNA could be visualized in the pars compacta of the substantia nigra. These results suggest that both D_4 and D_5 receptors exist as postsynaptic receptors in the striatum, while the D_4 receptor may also function as an autoreceptor in the midbrain. Based on transfection studies, the D_4 receptor appears to be pharmacologically more D_2 -like, and the D_5 more D_1 -like. The present results are in agreement with these findings: anatomically, D_4 receptor mRNA is similarly distributed to that corresponding to the D_2 receptor, while D_5 mRNA has a similar tlocalization as D_1 receptor mRNA. More detailed mapping studies of the distributions of these mRNAs are underway in both the human and the monkey brain.

238.7

Differential Distribution of D_2 and D_4 Dopamine Receptor mRNAs in the Rat Brain: An *in situ* Hybridization Study. A. Mansour, J. Meador-Woodruff, S. Burke*, J. Bunzow*, H. Akil, H.H.M. Van Tol*, O. Civelli, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI, 48109-0720; The Oregon Health Sciences University, Portland, OR, 97201.

Several dopamine receptors (D_1 - D_5) have recently been cloned and appear to be part of a larger family of G-protein coupled seven transmembrane (TM) receptors found in the CNS. The present *in situ* hybridization study compares the distribution of the D_2 and D_4 receptors which are highly homologous to one another, but differ pharmacologically, with the D_4 having a 10 fold greater affinity for clozapine (Van Tol et al, 1991, Nature, 350; 610-614). Using adjacent rat brain sections and standard *in situ* hybridization procedures (Mansour et al, 1990, J. Neurosci., 10; 2587-2600), the D_2 receptor mRNA was localized with a 495 bp cRNA generated to the third cytoplasmic loop and TM VI and VII of the rat D_2 receptor, while the D_4 mRNA was visualized with a riboprobe generated to a 420 bp fragment of the human D_4 receptor that corresponds to TM VI and VII and the entire 3' untranslated region. *In situ* hybridization studies demonstrate that the two mRNAs are differentially distributed with the D_2 receptors being particularly abundant in the striatum and substantia nigra, while the D_4 receptors are more widely distributed with relatively high levels in the cortex, hypothalamus, amygdala, hippocampus and pineal gland. The relative lack of D_4 receptors in the nigrostriatal system and its distribution within cortical and limbic areas is of particular interest given clozapine's therapeutic effects and its relative lack of extrapyramidal side effects.

238.8

Structural Determinants of Dopamine Receptor Binding: Site-Directed Mutagenesis of the Human D_2 Receptor. F. Meng*,A. Mansour, J. Meador-Woodruff, L.P. Taylor*, and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI, 48109-0720; The Oregon Health Sciences University, Portland, OR, 97201.

Site-directed mutagenesis studies with the β_2-adrenergic receptor have identified several amino acid residues that are critical for agonist binding (Strader et al 1989, J. Biol. Chem. 264; 13572-8 and 16470-7). These include an aspartate in transmembrane (TM) three that interacts with the cationic anion and two serines and in TM5 that interact with hydroxyl moieties located on the aromatic ring of catecholamine agonists. In the present study we have mutated the homologous residues in the human D_2 receptor by site-directed mutagenesis to determine if dopamine agonists bind in a similar manner in the D_2 receptor. In addition Met (116 and 117) were mutated to evaluate whether residues near Asp (114) of the D_2 receptor are critical in differentiating dopaminergic from adrenergic agonists. The results suggested the following - 1. Removal of the charge with mutation of Asp (114) to either Asn or Gly led to a total loss of both agonist and antagonist binding. 2. Methionine mutations Met 116 to Leu or Met 117 to either Cys or Gly to make the D_2 binding pocket more closely resemble the β_2-adrenergic receptor did not result in a change in selectivity toward noradrenergic agonists or antagonists. 3. Mutations of Ser (194) and Ser (197) to Ala produced a greater loss of affinity for agonists as compared to antagonists with Ser (197) being particularly important for dopamine receptor binding. The affects of these mutations varied with agonist or antagonist tested and will be discussed in relation to ligand structure and their interaction with the putative binding pocket.

SEROTONIN RECEPTORS: $5HT_3$

239.1

CHARACTERIZATION OF A SOLUBILIZED SEROTONIN $5HT_3$ RECEPTOR FROM BRAIN. K.J. Miller and M. Teitler. Dept. of Pharmacology and Toxicology, Albany Medical College, 47 New Scotland Avenue, Albany, N.Y. 12208.

Bovine area postrema has been found to contain a high density of $5HT_3$ receptors (1). Thus this may be a useful source of brain $5HT_3$ receptors for molecular characterization of this ligand-gated ion channel. $5HT_3$ receptors were solubilized from bovine area postrema using CHAPS, according to the method of Miquel et al., 1990 (2). Recovery of the $5HT_3$ receptor from the soluble preparation was approximately 50% (B_{max}=50±6 fmol/mg). Scatchard analysis revealed an equilibrium dissociation constant (K_D) for [^{3}H]-GR65630 of 1.0±0.3nM. Kinetic studies determining association and dissociation rate constants revealed a K_D of 0.5nM. The affinities of prototypical $5HT_3$ receptor agonists and antagonists for the solubilized receptor were essentially identical to the affinities for the membrane-bound receptor. Gel filtration chromatography of the soluble extract indicated a major peak of specific [^{3}H]-GR65630 binding corresponding to a molecular mass of about 600kDa, as well as several smaller peaks. WGA-agarose affinity chromatography of the soluble extract resulted in a significant enrichment of the specific binding activity. These results are qualitatively similar to those reported by Miquel et al., using a neuroblastoma-glioma cell line as their $5HT_3$ receptor source, and indicate that purification and detailed molecular neurobiology of brain $5HT_3$ receptors should be feasible using the bovine area postrema $5HT_3$ receptor preparation. Supported by MH-40716

1. M. Teitler and E. Weisberg, Biotech Update, January, p.1 (1991)
2. Miquel, et al., J. Neurochem., 55, 1526-1536 (1990).

239.2

THE EFFECT OF WAY-100,289, A NOVEL 5-HT3 RECEPTOR ANTAGONIST, ON A9 AND A10 DOPAMINE (DA) NEURONAL ACTIVITY: ACUTE AND CHRONIC STUDIES. C. W. Uzzle and J. T. Haskins.
Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000

WAY-100,289 (WAY; Endo-N-[[8-methyl-8-azabicyclo[3.2.1] octan-3-yl)amino]carbonyl]2-cyclopropylmethoxy)benzamide) binds with high affinity and selectivity to the 5-HT_3 receptor subtype and is a functional antagonist of the 5-HT_3 site in both *in vitro* and *in vivo* models. 5-HT_3 receptors have been identified in brain areas receiving DA input and 5-HT_3 antagonists can alter DA metabolism. It was therefore of interest to compare the effects of WAY with those of the standard 5-HT_3 antagonist BRL-43694 (BRL) and GR-38032 (GR) on central DA neuronal activity following acute or chronic administration. Following acute i.v. administration neither BRL, GR nor WAY had any significant effect on A10 neuronal activity. When administered prior to the DA agonist apomorphine (APO), however, all compounds potentiated the inhibitory effects of APO, although this potentiation did not reach statistical significance. The effects of WAY, GR and haloperidol (HAL) on the number spontaneously active A9 and A10 DA neurons were also determined following chronic (21 days) administration at doses of 5.0, 5.0 and 0.5 mg/kg i.p., respectively. HAL produced a significant reduction in the number of spontaneously active DA neurons in both the A9 and A10 areas. No other significant effects were noted. Additional doses of WAY are being examined to determine their effects, if any, following chronic administration.

239.3

5-HYDROXYTRYPTAMINE (5HT) RECEPTORS AND HYPERLOCOMOTION IN RODENTS. D.M. Jackson✚■, B. Chieng*■, D.M. Gillies*■ and E.J. Mylecharane*■✚ Astra Research Centre AB, Södertälje, Sweden and ■ Pharmacology Department, Sydney University, NSW, Australia.

5HT plays a role in regulating locomotor function in rodents. In this study we report some studies utilizing intracerebroventricular injections of 5HT agonists and antagonists into various parts of the rat brain. We have earlier shown that the 5HT1$_{A/B}$ agonist RU24969 stimulates activity in mice via a mechanism sensitive to dopamine (DA) D1 and D2, α1 and 5HT1$_B$ receptor antagonists and is dependent on intact stores of DA and to some extent noradrenaline. RU24969 (0.1 to 10 μg/side) injected into the accumbens (Acb) of rats had little or no effect on locomotion, but it produced marked and prolonged hyperlocomotion when injected into the ventral tegmental area (VTA, 1 and 5 μg/side). The simultaneous injection of (-)-propranolol into the VTA (5 μg/side) blocked RU24969's effect, suggesting that 5HT1$_B$ receptors modulating locomotion are located on the cell bodies rather than in the terminal regions of the mesolimbic pathways. To investigate the role of 5HT$_3$ receptors, 2-methyl-5HT (0.001 to 0.1 μg/side), odansetron (0.001 to 0.1 μg/side) or granisetron (0.001 to 1 μg/side) were injected into the Acb, but had no significant effect on either basal motor activity or on hyperlocomotion induced by subsequent injection of d-amphetamine (20 μg/side). However, injection of 2-methyl-5HT into the VTA (0.01 or 0.1 μg/side) produced locomotion. These findings suggest that 5HT1$_B$ and 5HT$_3$ receptors within the VTA play a role in modulating locomotor function in rats, probably by regulating DA release within, *inter alia*, the Acb. Supported in part by a grant from the ARC, Australia.

239.4

THE ACTION OF SEROTONIN IN THE RAT MEDIAL PREFRONTAL CORTEX: MEDIATION BY SEROTONIN$_3$-LIKE RECEPTORS. C.R. Ashby, Jr., E. Edwards and R.Y. Wang. Dept. of Psychiatry and Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794-8790.

The aim of the present study was to test the hypothesis that 5-HT$_3$-like receptors play a major role in mediating the inhibitory action of 5-HT in the rat medial prefrontal cortex (mPFc). Experiments were carried out in chloral hydrate anesthetized male Sprague-Dawley rats, using the techniques of single unit recording and microiontophoresis. The iontophoresis of 5-HT inhibited current-dependently mPFc cell firing. This effect was significantly attenuated by concurrent iontophoresis of selective 5-HT$_3$ receptor antagonists such as granisetron, ICS 205930 and zacopride, but not by 5-HT$_{1,2}$ receptor antagonists such as metergoline, pindolol, ritanserin and spiperone. The blockade of 5-HT's action by the 5-HT$_3$ receptor antagonists reached 60-80%. Furthermore, the i.v. administration of zacopride (5-50 μg/kg), but not metergoline (4-2400 μg/kg) or ritanserin (5-2000 μg/kg), markedly attenuated the suppressive action of 5-HT on mPFc cell firing. In addition, the suppression of mPFc firing produced by electrical stimulation of the caudal linear raphe nucleus (CLi), where ascending 5-HT fibers pass, was significantly blocked by the iontophoresis of granisetron and by i.v. administration of S-zacopride (0.1 mg/kg). Interestingly, i.v. injection of ritanserin (0.5-1.5 mg/kg) also significantly attenuated CLi-induced suppression of mPFc cell activity, whereas the iontophoresis of ritanserin or another 5-HT$_2$ receptor antagonist (+)MDL 11939 failed to alter CLi-induced effect. Overall, our results suggest that 5-HT's action in the mPFc may be primarily mediated by 5-HT$_3$-like receptors. Moreover, the blockade of CLi-induced effect by systemic ritanserin may be an indirect effect.
(Supported by USPHS grants MH-41440 and MH-00378).

239.5

EFFECTS PRODUCED BY CHRONIC TREATMENT WITH GRANISETRON ALONE OR IN COMBINATION WITH HALOPERIDOL ON MIDBRAIN DOPAMINE CELLS R.Y. Wang, Y. Minabe and C.R. Ashby, Jr. Dept. of Psychiatry and Behav. Sci. SUNY at Stony Brook, Putnam Hall-South Campus, Stony Brook, NY 11794-8790.

Evidence obtained from recent studies suggests that 5-HT$_3$ receptor antagonists may represent a new class of antipsychotic drugs (APDs). The aim of the present study was to evaluate the antipsychotic potential of granisetron (BRL) using an electrophysiological model. Rats were randomly allocated to one of the following treatment groups: control (1 ml/kg of 0.9% saline), haloperidol (HAL, 0.5 mg/kg, i.p.), BRL (0.1, 1.0 or 10 mg/kg, i.p.) or BRL + HAL. The experimenter was "blind" as to the drug treatment of the animal. Standard electrophysiological techniques were used to record A9 and A10 DA cells in chloral hydrate anesthetized rats. Chronic administration of 0.1 or 1 (but not 10) mg/kg BRL selectively decreased the number of spontaneously active A10 DA cells compared to controls, mimicking the effect produced by chronic treatment with the atypical APD clozapine. However, unlike the effect produced by APDs, this BRL-induced effect was not reversed by apomorphine (APO, 50 μg/kg, i.v.). This suggests that the chronic BRL-induced effect on DA cells is not the result of depolarization inactivation. Acute BRL (0.01, 0.1 or 1 mg/kg, i.v.) significantly potentiates the inhibitory action of APO on A10 DA cells. The chronic co-administration of HAL + BRL either attenuated or prevented completely the HAL-induced decrease of the number of spontaneously active DA cells. Our results are consistent with the report showing that 5-HT$_3$ receptor antagonists markedly attenuate or reverse the DA hyperactivity induced by various drugs including HAL. In conclusion, the results of this study suggest that whereas chronic BRL at low doses may possess atypical antipsychotic potential, the administration of BRL and HAL diminishes the antipsychotic effect of either drug.

239.6

AS-5370 ANTAGONISES THE 5-HT$_3$ RESPONSE OF THE RAT VAGUS. N. R. Newberry*, C. J. Watkins*, R.A.Leslie and D.G. Grahame-Smith*. (SPON: Brain Research Association) Oxford University-SmithKline Beecham Centre for Applied Neuropsychobiology, Oxford, OX2 6HE, England.

AS-5370, N-(1-methyl-4-(3-methyl-benzyl)hexahydro-1H-1,4-diazepin-6-yl)-1H-indazole-3-carboxamide 2HCl, is a potent and long lasting 5-HT3 antagonist (Ito T. et al, 1990, Eur. J. Pharmac., 183, 1214-1215). We have assessed its potency to antagonise the 5-HT$_3$ receptor mediated depolarisation of the rat vagus nerve in vitro.

The methods for grease-gap recording from the desheathed vagus nerve (taken from male Sprague-Dawley rats) were similar to those previously described (Newberry et al, 1991, Brit. J. Pharmac, 102, 615-620). Using 1 minute superfusions of 5-HT, we studied its dose-response curve before and after a 1 hour incubation of the preparation with or without an antagonist.

The mean EC$_{50}$ for 5-HT was 0.7 μM (range 0.2-1.9, n=32). Repeating the dose-response curve in the absence of antagonist gave a mean dose-ratio of 1.5 (n=6). AS-5370 (0.1-3.0 nM) (a kind gift from Dainippon Pharmaceutical Co.) produced a dose dependent depression of the maximum response to 5-HT (n=16) with an IC$_{50}$ of 0.3-1.0 nM. This contrasted with another 5-HT3 antagonist d-tubocurarine (0.3-3 μM) which produced parallel shifts of the dose-response curve to 5-HT (pK$_b$ =7.1, IC$_{50}$ = 0.3-1.0 μM, n=13).

The data suggest that AS-5370 is a potent, noncompetitive 5-HT$_3$ antagonist on the rat vagus nerve.

239.7

EVIDENCE FOR MODULATION OF DOPAMINE RELEASE BY 5-HT$_3$ RECEPTORS WITHIN THE NUCLEUS ACCUMBENS

G.A. Kennett* and T.P. Blackburn* (Spon: D.C. Rogers), SmithKline Beecham Pharmaceuticals, The Pinnacles, Harlow, Essex, CM19 5AD, UK.

The possibility that 5-HT$_3$ receptors within the nucleus accumbens modulate dopamine (DA) release was studied.

Male SD rats were implanted with dialysis probes in the right nucleus accumbens. On the next day these were perfused with artifical CSF (0.5 μl/min). Dialysates were collected every 20 min and DA, DOPAC and HVA determined by HPLC-ECD. Injection of the 5-HT$_3$ receptor antagonists BRL 46470 (Thomas et al., 1990) or ondansetron (0.01 and 0.1 mg/kg s.c.) did not alter DA release over the next 40 mins. Dose-dependent increases in DA release, however, were observed following infusion of the putative 5-HT$_3$ agonists 2-methyl-5-HT and phenylbiguanide (PBG) into the nucleus accumbens for 40 min, although the effect of PBG perfusion was much larger. The response to 1 mM 2-methyl-5-HT was significantly attenuated by BRL 46470 0.01 and 0.1 mg/kg s.c. given 40 min pretest. Ondansetron was also effective but only at the higher dose of 0.1 mg/kg. However BRL 46470 0.1 mg/kg s.c. did not affect the response to PBG (0.1 mM). 2-Methyl-5-HT had little effect on DOPAC or HVA levels but PBG reduced both markedly. These results suggest that PBG may have an amphetamine-like or re-uptake inhibitor action as suggested by Schmidt and Black (1989). They also support evidence of presynaptic modulation of mesolimbic dopamine release by 5-HT$_3$ receptors and imply that these may be located within the nucleus accumbens.

Schmidt, C.J. and Black, C.K. (1989) Eur.J.Pharmacol., 167, 309-310
Thomas, D.R. et al. (1990). J.Psychopharmacol., 4,7

239.8

Expression of Tryptophan Hydroxylase in the NG108-15 cell line by 5-HT$_3$ Receptor Stimulation. J.C. Poblete[1], X. Zhang[2], J. Rubinstein[2], E.C. Azmitia[1], and P.M. Whitaker-Azmitia[2]. 1.Dept. of Biology, New York University, NY, NY 10003, and 2.Dept. of Psychiatry, SUNY-Stonybrook, Stonybrook, NY 11794.

The neuroblastoma x glioma hybrid NG108-15 cell line has been shown to possess serotonergic, cholinergic and peptidergic properties (Ghahary et al.,Cell. Mol. Neurobio. 9(3),1989). In addition, undifferentiated NG108-15 cells have detectable levels of 5-HT. The purpose of this study is to investigate the serotonergic identity of this cell line after treatment with dibutyryl-cAMP (dBcAMP), which induces NG108-15 cellular differentiation, and with phenylbiguanide (PG), a 5-HT$_3$ agonist. The serotonergic property of the NG108-15 cell line was determined by utilizing a new antibody (WOH-66) developed against tryptophan hydroxylase, an enzyme localized specifically in serotonergic neurons. Glial properties of this cell line were determined using antibodies against S100 protein and glial fibrillary acid protein (GFAP). The neuronal characteristic of this cell line was determined using an antibody against neuron specific enolase. Undifferentiated cells were incubated for 2,4,6,8 days with PG (100nM) and dBcAMP (10mM), and then fixed in 4% paraformaldehyde (0.1M PBS, pH 7.4) and processed for immunocytochemical staining using the HRP-ABC method. Staining for S100, GFAP and neuron-specific enolase was observed in all cells in control and both treatment groups (PG and dBcAMP). However, staining for tryptophan hydroxylase was more intense in PG-treated cells than in control and dBcAMP-treated cells. This observation indicates that the NG108-15 cell line possesses 5-HT$_3$ receptors, which when stimulated, increase the expression of tryptophan hydroxylase. Support: NICHD and NSF 88-12892.

239.9

EFFECTS OF PRENATAL TREATMENT WITH A 5-HT3 AGONIST AND ANTAGONIST ON THE DEVELOPMENT OF NOCICEPTIVE PATHWAYS. *J.BELL, X. ZHANG, AND P.M. WHITAKER AZMITIA.* Dept. Psychiatry and Behavioral Science, SUNY at Stony Brook, Stony Brook NY 11794.

In previous studies we have found that the development of ascending serotonergic pathways are regulated through the balance of two high affinity receptors-one the 5-HT_{1A} receptor which releases a growth factor, and a receptor which regulates the release of 5-HT and is inhibitory to growth. In the current study, we examined the types of 5-HT receptors regulating the development of descending 5-HT pathways. Using two tests, tail flick and the binding of ^{3}H-paroxetine (a 5-HT uptake inhibitor) as a measure of 5-HT terminal density, we tested the effects of chronic prenatal exposure to both phenylbiguanide (PG), a 5-HT_3 agonist and MDL-72222 (MDL), a 5-HT antagonist, on both nociception and receptor development in the spinal cords of neonatal rat pups. Pregnant Sprague Dawley rats were either given 5mg/kg PG, 5mg/kg MDL or a vehicle control (s.c.) starting on gestation day 12 and continuing to birth. Pups were assessed for tail flick latency on postnatal D10 ,D18 and D30 or their spinal cords were removed on D18 and D30 for paroxetine binding. PG animals showed significant increases in analgesia on D10 [$p<.03$] and D30 [$p<.003$] while MDL animals showed significant decreases in latency on D10 [$p<.001$] and D18 [$p<.008$]. ^{3}H-paroxetine binding for both groups revealed significant elevations in 5-HT terminal density in the spinal cords of D18 animals when compared to controls (PG [$p<.009$] and MDL [$p<.03$] respectively). These findings show that the development of this system can be altered by prenatal treatment with agents acting on the 5-HT_3 receptor. Support by NICHD.

239.10

NG108-15 CELLS AS A MODEL SYSTEM FOR STUDYING THE EFFECTS OF 5-HT_3 RECEPTORS ON BRAIN DEVELOPMENT P.M. Whitaker-Azmitia, X. Zhang and J.E. Rubinstein. Dept. of Psychiatry, State University of New York, Stony Brook, NY,11794.

We have recently found a role for 5-HT_3 receptors in regulating development of serotonergic neurons (Bell and Whitaker-Azmitia, abs. this meeting). NG 108-15 cells, a hybridoma cell line, have previously been shown to express high amounts of 5-HT_3 receptors. We have therefore tested these as a system to further study the role of these receptors in development.

NG-108-15 cells were grown in culture for 1 to 4 days, in the presence of varying concentrations of phenylbiguanide (PG), a 5-HT_3 agonist. The number of cells was assayed using a colorimetric assay. A significant decrease in cell growth (73% of control, $p<.005$) was observed. The maximum decrease was observed by three days, and the maximum response induced by 100 nM PG with 50% of maximum observed at 10 nM. These effects were reversed by 10 nM MDL 7222 - a 5-HT_3 antagonist. PG did not stimulate phosphlipase C or adenylate cyclase in these cells. We are currently studying the effects of ion channel antagonists, such as nimodipine, to block the effect.

239.11

PHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF 5-HT_3 RECEPTORS OF RABBIT, MOUSE AND GUINEA-PIG NODOSE GANGLION NEURONES. H.M. Malone*, J.A. Peters* and J.J. Lambert. Department of Pharmacology, University of Dundee, Ninewells Hospital, Dundee, DD1 9SY, Scotland, U.K.

Mammalian primary visceral afferents express 5-HT_3 receptors which mediate membrane depolarization via an integral cation channel. We have utilized whole-cell recordings to investigate and compare the properties of 5-HT_3 receptors of cultured adult rabbit, mouse and guinea-pig nodose ganglion neurones (NGN's) voltage-clamped at -60mV. In rabbit, mouse and guinea-pig NGN's, 5-HT (10μM) induced an inward current associated with a conductance increase which was abolished by 1nM, 3nM and 300nM ondansetron respectively. A marked differential sensitivity in the 5-HT_3 antagonist potency of (+)-tubocurarine [(+)-Tc] and cocaine was observed between the three species, as the IC_{50} values for (+)-Tc in mouse, rabbit and guinea-pig NGN's were 1.6nM, 170nM and 9μM, and for cocaine, 4.6μM, 80nM and 2μM respectively. Ion substitution experiments indicate the 5-HT_3 activated ion channel in rabbit NGN's to be permeable to small monovalent cations with permeability ratios relative to sodium (Px/PNa) of 1.17, 1.13, 1.11 and 0.94 for Cs^+, K^+, Rb^+ and Li^+ respectively, assuming Cl^- to be impermeant. The organic cation derivatives, ammonium (1.73) and methylamine (1.26) were more permeant than sodium, whereas ethylamine (1.07) and dimethylamine (0.95) were approximately equipermeant. Supported by the Wellcome Trust.

239.12

5-HT_3 RECEPTORS IN GUINEA-PIG COELIAC NEURONS. A. Surprenant, S. Matsumoto and V. Gerzanich. Vollum Institute and Dept. of Anatomy, School of Dentistry, Oregon Hlth. Sci. Univ., Portland, OR 97201

Whole cell and outside out single-channel recordings were made from adult coeliac ganglion neurons maintained in culture for 12 - 72 h. Using internal Cs-gluconate solution without ATP or GTP, 5-HT evoked desensitizing inward currents at the holding potential of -70 mV in all neurons; EC_{50} was 2 μM for steady-state and 6 μM for peak current. Time constant for recovery from desensitization by 100 μM 5-HT was 10 s. IV relationship showed inward rectification and a reversal potential of -1 mV. 5-HT (3 μM) induced channel openings of at least three unitary amplitudes; unitary conductance of the predominant channel type was 10 pS. Macroscopic and single-channel currents were blocked by ICS 205-930 (100 nM) and GR 38032F (1 μM). Thus, adult coeliac ganglion cells possess the 5-HT_3 ligand-gated ion channel whose properties are similar to 5-HT_3 receptors in enteric neurons.

239.13

5-HT3 SEROTONIN AGONISTS AS DISCRIMINATIVE STIMULI R.A. Glennon,* R. Young, M. Dukat* Department of Medicinal Chemistry, MCV/VCU; Richmond, VA 23298

The drug discrimination paradigm has proven of considerable utility in neuropharmacology. Specifically, in the realm of 5-HT research, various 5-HT1 and 5-HT2 agonists have been used as training drugs. To date, there are no reports of 5-HT3 agonists being used in a similar manner. Male SD rats were trained to discriminate the 5-HT3 agonist 2-methyl 5-HT (5 mg/kg) from saline using a VI 15s schedule of reinforcement employing standard two-lever operant techniques. The 2-methyl 5-HT stimulus (ED50 = 2.6 mg/kg) generalized to the new 5-HT3-selective agonist *meta* chlorophenylbiguanide (*m*CPBG; ED50 = 1.7 mg/kg). The stimulus was potently antagonized by zacopride and ICS 205-930 (ID50 = 6.3 and 11 μg/kg, respectively). Even at doses 10,000 times the ID50 dose of ICS 205-930, its quaternary salt (which does not readily penetrate the blood-brain barrier) never fully (max 38% inhibition) attenuated the 2-methyl 5-HT stimulus, suggesting that the stimulus is likely centrally mediated. Animals have also been trained to discriminate *m*CPBG from saline. These studies are the first to report the use of 5-HT3 agonists as training drugs; this procedure should be useful in the future study and development of 5-HT3 agonists and antagonists. [Supported in part by NS 23520.]

240.1

IN VITRO PHOSPHORYLATION OF PROTEIN KINASE C SUBSTRATES BY ISOZYMES TYPE I, II AND III. F.M.J. Heemskerk, P.N.E. de Graan and F.L. Huang. NICHD, NIH, Bethesda, MD 20892.

In brain two protein kinase C (PKC) substrates, B-50 (GAP-43) and MARCKS, have been extensively characterized. Both are acidic proteins, with apparent Mr (by SDS-PAGE) of 48 and 87 kDa, respectively. Previously B-50 has been shown to be phosphorylated in vitro at one site, bovine MARCKS can be phosphorylated at 4 sites by PKC. We have investigated whether there might be differences in substrate specificity and preference for phosphorylation site using highly purified PKC isozymes. We isolated synaptosomal plasma membranes (SPM) to study the phosphorylation of these proteins in the membrane-associated state and compared their kinetics of phosphorylation with those of histone IIIS. PKC type II and III were found to have 2-3 times higher activity than type I, using either histone or SPM proteins as substrates. Tryptic peptides of phosphorylated SPM-proteins were analyzed by two dimensional peptide mapping. Phosphorylation of B-50 by each of the isozymes showed only one phosphopeptide. MARCKS gave multiple phosphopeptides, but no differences were detected among samples phosphorylated by each PKC isozyme. Using highly purified B-50 or a 25 amino acid synthetic peptide, containing the 4 reported phosphorylation sites in MARCKS, we found a similar lack of substrate specificity for the PKC isozymes. Although there was an apparent preference for a particular site over the others, all three PKC isozymes phosphorylated these sites in the same order of preference. These results suggest that there is little evidence for a difference in preference of any PKC isozyme for one of these substrates, or even a phosphorylation site. It seems likely that the functional specificities of the various PKC isozymes are determined by their unique localization and sensitivity to second messengers, rather than their substrate specificity.

240.2

INCREASED [^{3}H]-FORSKOLIN AND [^{3}H]-PHORBOL 12,13 DIBUTYRATE BINDING IN THE HIPPOCAMPUS FOLLOWING EXCITOTOXIC LESION OF THE RAT MEDIAL SEPTUM. K. Horsburgh[a*], F. M. Inglis[b*], and J. McCulloch[b], [a]University of California, San Diego, Dept. of Neurosciences, 0624, School of Medicine, La Jolla, CA 92093 and [b]Wellcome Surgical Institute, University of Glasgow, U.K. G61 1QH

The rat septo-hippocampal pathway provided a model system in which to examine possible alterations of ligand binding to second messenger systems following denervation. Quantitative autoradiography of [^{3}H]-forskolin binding to G_S-adenylate cyclase and [^{3}H]-phorbol 12,13 dibutyrate (PDBu) binding to protein kinase C was examined 21days following stereotaxic injection of ibotenate (n=7) or phosphate buffer (n=7) into the rat medial septum. Ibotenate lesion of the rat medial septum was confirmed histologically in cresyl violet stained brain sections adjacent to those used in ligand binding studies. [^{3}H]-Forskolin binding was significantly decreased at the lesion site (-19%) whilst a significant increase in [^{3}H]-forskolin binding was observed in the polymorph layer of the dentate gyrus (19%) in the ibotenate-lesioned group compared to shams. A significant increase in [^{3}H]-PDBu binding was demonstrated in the superficial layers of entorhinal cortex (29%) following lesion of the medial septum. There were no alterations in [^{3}H]-forskolin or [^{3}H]-PDBu binding in any other brain region post-lesion. The heterogeneous alterations in second messenger ligand binding sites and the direction of the responses may be supportive of plastic modifications of second messenger systems in the hippocampus following denervation.

240.3

PROTEIN KINASE C DELTA AND EPSILON ARE INCREASED IN PC12 CELLS EXPOSED CHRONICALLY TO ETHANOL. P. J. Petersen*, C. J. Henrich*, and R. O. Messing. E. Gallo Clinic & Research Ctr., UCSF, San Francisco, CA 94110.

Chronic ethanol exposure increases expression of L-type Ca^{2+} channels in neural tissues. In PC12 cells, this is prevented by inhibitors of protein kinase C (PKC). Therefore we examined whether ethanol regulates PKC. Exposure to 100 mM ethanol for 6 days increased phosphorylation of KRTLRR, a selective PKC substrate, by 32 ± 4% in digitonin-permeabilized cells, whereas ethanol exposure for 10 min had no effect. Ethanol could increase PKC-mediated phosphorylation by increasing PKC activation or PKC levels. Ethanol did not alter enzyme affinity for Ca^{2+}, phosphatidylserine, diacylglycerol, or histone, and did not change cellular levels of diacylglycerol . However, ethanol treatment for 6 days caused an increase in PKC activity in cell homogenates, with a significant increase of 21 ± 2% with 50 mM ethanol, and a maximal increase of 32 ± 2% with 100 mM ethanol. This effect was time-dependent and was significant 2 days after exposure to 200 mM ethanol. Ethanol (200 mM, 6 days) also increased the number of binding sites for [^{3}H]phorbol 12,13-dibutyrate by 82% without changing binding affinity, suggesting that increases in PKC activity reflected increased PKC levels. Using isozyme specific antibodies we found that PC12 cells contained PKCα, δ, ε, and ζ immunoreactivity. Treatment with 100 mM ethanol for 6 days increased immunoreactivity only to PKC δ and PKCε, by 39 ± 9 and 54 ± 5 %, respectively. Therefore, chronic exposure to ethanol appears to increase levels of PKCδ and ε, and thereby enhance PKC-mediated phosphorylation in PC12 cells.

240.4

CYCLIC AMP-DEPENDENT PROTEIN KINASE: AN INTERMEDIARY IN THE MODULATION OF $GABA_A$ RECEPTOR FUNCTION IN CEREBELLAR PURKINJE CELLS. J.E. Cheun and H.H. Yeh Dept. Neurobiology and Anatomy, Univ. Rochester Med. Ctr., Rochester, NY 14642.

Work from our laboratory has demonstrated a synergistic interaction between beta-adrenoceptor activation and $GABA_A$ receptor-mediated chloride current (I_{GABA}) in Purkinje cells acutely dissociated from the rat cerebellum. Because beta-adrenoceptors are coupled positively to adenylate cyclase and because cyclic AMP has the potential to initiate many intracellular events through phosphorylation, ongoing experiments are focusing on testing the participation of the cAMP cascade in modulating the functional efficacy of the $GABA_A$ receptor as part of an effort to elucidate the underlying cellular and molecular mechanisms.

Isolated Purkinje cells were recorded under voltage-clamp in the whole cell configuration and I_{GABA} examined under conditions which activated the cAMP cascade. Both forskolin (≤200 uM) and 8-Br cAMP (≤1 mM) reversibly potentiated I_{GABA}, suggesting that an elevated level of intracellular cAMP affected either through triggering the adenylate cyclase or through transmembrane delivery can lead to an enhancement of $GABA_A$ receptor function.

To test the hypothesis that cAMP-dependent phosphorylation is involved in the potentiation of I_{GABA}, the catalytic subunit of the cAMP-dependent protein kinase (PKA) was included in the pipet solution and allowed to dialyze into cells. The amplitude of I_{GABA} increased after 4-5 minutes following rupture of the membrane to achieve the whole-cell mode. The time course of the change in I_{GABA} amplitude is consistent with that of intracellular dialysis having occurred since it correlated well with the results of a separate study in which lucifer yellow was included in the recording solution. In the absence of PKA, I_{GABA} remained at a relatively constant level throughout the duration of recording. These data suggest that phosphorylation may be involved in the modulation of I_{GABA} by cAMP. *Supported by PHS grants NS24830 and NS 01340.*

240.5

PHOSPHATIDYLCHOLINE POTENTIATES THE SYNERGISTIC ACTIVATION OF PROTEIN KINASE C. S.G. Chen, D. Kulju*, S. Halt*, and K. Murakami Dept. of Biochem. Pharmacol. State Univ. New York, Buffalo, NY 14260.

Cis-fatty acid has been recently shown to activate protein kinase C (PKC) synergistically with diacylglycerol. This synergistic activation does not require Ca^{2+} but micromolar concentrations of Ca^{2+} strongly enhance the PKC activity. Among the PKC subtypes, types III (α) PKC is particularly sensitive to the synergistic activation mode; micromolar range of cis-fatty acid is sufficient to activate this subtype of PKC in the presence of diacylglycerol. This activation is dependent on the presence of phosphatidylserine (PS) and diacylglycerol but significantly reduces the PS requirement. These observations suggest that the generation of three second messengers, i.e., the increase in the intracellular Ca^{2+} concentrations, and the formation of both cis-fatty acid and diacylglycerol in the cell are necessary for the full activation of this PKC subtype.

We have further characterized the synergistic activation of PKC subtypes by cis-fatty acid and diacylglycerol with respect to the requirement of phospholipids. Phosphatidylcholine (PC), a neutral phospholipid, was found to support the synergic activation of PKC. This PC potentiation effect is totally dependent on the presence of cis-fatty acid; PC does not activate PKC at all in the absence of cis-fatty acid, but it significantly stimulates the cis-fatty acid-induced PKC activity in concert with Ca^{2+}. Diacylglycerol plays a modulatory role; it further stimulates the PC potentiated cis-fatty acid activation of PKC. The synergistic activation in the presence of PC is equivalent to that observed in the presence of PS. There are several differences, however, between PC- and PS- supported synergism including Ca^{2+} sensitivity and autophosphorylation of the PKC subtypes. In vitro mechanism of the synergy is thus quite different from the previously reported PKC activation mechanisms. These unexpected observations of PC effect on the synergism suggest that neutral phospholipids may also participate in the activation of PKC.

240.6

Visualization of calcium/calmodulin-dependent protein kinase type II mRNA using a sensitive non-radioactive *in situ* hybridization technique. E. M. McGowan, P.C. Emson and M. Rigby[1]*. MRC Group, Dept. of Neuroendocrinology. AFRC, Babraham, Cambridge, U.K. [1] Merck, Sharpe and Dohme. Harlow, Essex, U.K.

Calcium/calmodulin-dependent protein kinase type II (CAM II kinase) is a multimeric enzyme consisting of two principle subunits, an alpha (α) subunit of 58kDa and a beta (β) subunit of 58/60kDa. The two subunits are differentially expressed throughout the central nervous system and also in peripheral tissues. A sensitive non-radioactive *in situ* hybridization technique, using alkaline phosphatase coupled oligonucleotides, was used to visualize the respective α and β CAM II kinase mRNAs in the gerbil forebrain. The strongest hybridization signal for both the α and β CAM II kinase antisense oligos was seen in the hippocampal formation. The alkaline phosphatase signal was concentrated in the cell bodies of the pyramidal cells of the CA1–CA4 field and the granule cells of the dentate gyrus, in contrast to, observations made using radio-labelled probes where CAM II Kinase α mRNA was readily identified in the dendrites. Elsewhere in the forebrain strong staining for both enzyme subunit mRNAs was detected in the cerebral cortex and amygdala, with lower levels of staining in the thalamus. In addition only mRNA for the β subunit was detected in the granule and Purkinje cells of the cerebellum. Currently, observed changes in the levels of α and β CAM II kinase mRNAs are being studied in the gerbil hippocampus following an ischemic insult.

240.7

AGE-RELATED CHANGES IN [^{3}H]PHORBOL BINDING AND PROTEIN KINASE C IMMUNOREACTIVITY IN FISHER-344 RAT BRAIN. K.A. Yurko, M.R. Pisano*, E. Yadin and E. Friedman. Departments of Pharmacology and Psychiatry, Medical College of PA, Phil., PA 12129. Protein kinase C (PKC), a major neuronal kinase, plays a key role in many neurochemical processes that have shown age-related changes. [^{3}H]PDBu binding in 6- and 24-month-old Fischer-344 rat brains (10 μm coronal slices) was examined. The effects of Carbachol stimulation (CARB, 10 μM) and of calorie restricted diet across these age groups was also investigated. Significant age-related increases in [^{3}H]PDBu binding were observed in the dentate gyrus of rats fed an unrestricted or a calorie-restricted diet. In the amygdala, significant age-related increased binding was seen in the unrestricted diet condition. Stimulation with CARB significantly increased [^{3}H]PDBu binding, regardless of age, in the unrestricted diet condition, in both these brain regions. In the restricted diet condition, the effect of CARB stimulation on binding was reduced across both age groups in these brain areas. No significant differences in binding across age and diet conditions, with or without CARB stimulation, were seen in caudate, ventral thalamus or corpus callosum. Immunoreactivity of PKC isozymes (α,ß,γ) from 6- and 24-month-old rats, under either diet condition, is currently being studied. These age-related changes in [^{3}H]PDBu binding may reflect associated changes in membrane phospholipids or diacylglycerol production necessary for functional PKC.

240.8

QUANTITATIVE RECEPTOR AUTORADIOGRAPHY OF PROTEIN KINASE C / [3H]-PDBu BINDING SITES FOLLOWING UNILATERAL FIMBRIECTOMY IN RATS BRAIN. A.R. Parent and R. Quirion. Douglas Hospital Research Centre and Dept. of Psychiatry, McGill University, 6875 Lasalle Blvd., Verdun, Quebec, Canada, H4H 1R3.

Cellular responses to neurotransmitter receptor activation depend upon the integrity of receptor coupling with their associated signal transduction mechanisms. Protein kinase C (PKC) is an important second messenger directly involved in receptor transduction mechanisms of various neurotransmitters. On the basis our interest in the neurochemical features of Alzheimer disease (AD) in which deficits in muscarinic receptors possibly coupled to PKC were reported, we determine the activity of PKC in fimbriectomized rats, a model of the hippocampal cholinergic deafferentation observed in AD. Ten days after lesion, PKC was evaluated using membrane binding assays and quantitative receptor autoradiography, using the selective ligand [3H]-phorbol 12,13-dibutyrate (PDBu). While binding affinities were similar in most brain regions (6 nM), [3H]-PDBu binding density was greatest in the hippocampus (30 pmol/mg protein) followed by frontal cortex (15 pmol), temporo-occipital cortex (8 pmol), thalamus-hypothalamus (6 pmol), cerebellum (6 pmol) and brainstem (3 pmol). On the ipsilateral side of the fimbriectomized animals, the apparent density and affinity of [3H]-PDBu binding were not modified in the hippocampus. However, the distribution of [3H]-PDBu binding was enlarged in the ipsilateral septal areas. Thus, deafferentation of the hippocampus does not affect PKC activities in this region measured by [3H]-PDBu binding. However, the distribution of cell expressing [3H]-PDBu / PKC binding may be enlarged in the septal area suggesting the possible sprouting of transected septo-hippocampal neurons in response to lesion.

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240.9

PROTEIN KINASE C-RELATED SIGNAL TRANSDUCTION PATHWAY IS TRIGERED BY SUBNANOMOLAR CONCENTRATION OF VIP IN ASTROCYTES.

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LCO, NCI[1]; LENP, NINDS[2] and LDN, NICHD[3], NIH, Bethesda, MD 20892

Neurotransmitters and neuropeptides can trigger the release of glia-derived factors that are important to neuronal growth and survival. Vasoactive intestinal peptide (VIP) has been shown to release a complex array of substances that can influence the survival of spinal cord neurons grown in dissociated cell cultures. This secretagogue action of VIP, observed at a concentration of 0.1 nM, is 1000-fold less than that required to produce a significant elevation in cAMP, the recognized second messenger for the VIP receptor. Previous studies have shown that astroglia exhibit two subtypes of VIP-receptors, a low affinity site with a Kd of 0.8 µM and a high affinity site with a Kd value of 70 pM. We have investigated the possibility that a protein-kinase C (PKC)-related signal-transduction mechanism may mediate the actions at the high affinity VIP receptor in rat cortical astrocytes. Western blotting with a pan-PKC specific antibody has shown that the subcellular localization of this enzyme is cytosolic in control astrocytes. Stimulation of cells with 0.1 nM VIP resulted in a partial translocation of the PKC immunoreactivity from the soluble to a nuclear fraction, which can be mimicked by treatment with low concentration of TPA (1 nM). Endogenous substrates of PKC were also studied by incubating cytosolic and nuclear extracts from VIP-treated and control astrocytes in the presence of [γ-^{32}P]ATP. Specific phosphoprotein bands were detected at 40, 30, and 24 kD in the absence of Ca^{2+} (but in the presence of phosphatidylserine and diacylglycerol), suggesting the presence of Ca^{2+}-independent PKC forms. No additional substrates (i.e. the 80 kD phosphoprotein) were seen in the presence of both Ca^{2+} and phospholipid, although presence of this PKC form could be detected by externally added histone H1 phosphorylation. All PKC forms could be down-regulated by a short pre-treatment with 250 nM TPA. These data suggest that a PKC-related signal transduction mechanism mediates the action of VIP at the high affinity receptor in astrocytes.

240.10

REGULATION OF THE NEUROTRANSMITTER RELEASE MACHINERY BY SIGNAL TRANSDUCTION PATHWAYS: EXPRESSING UNREGULATED SIGNAL TRANSDUCTION ENZYMES AND PARVALBUMIN IN AXON TERMINALS, FROM HSV-1 VECTORS. J. Bryan*, A.I. Geller, O. Ashe*, C.L. Wilcox, R.L. Neve, and M.J. During. Dana Farber Can. Inst., Boston MA. 02115; Dept. Microbiol. U. CO., Denver CO. 80262; Dept. Psychobiol., U. CA., Irvine CA. 92717; Dept. Neurosurg., Yale Univ. Sch. of Med., New Haven CT. 06510.

Signal transduction pathways may regulate the neurotransmitter release machinery in the axon terminal. We have expressed, from HSV-1 vectors, catalytic domains of signal tranduction enzymes. These unregulated enzymes activate specific signal transduction pathways, but they are localized to neuronal cell bodies. To deliver the catalytic domains to axon terminals, the catalytic domains of adenylate cyclase, calcium/calmodulin dependent protein kinase II, and protein kinase C were fused to the process targetting domain of GAP-43 (the 10 N terminal aa), which we have shown targets foreign proteins to processes. Activating each of these three signal transduction pathways in axon terminals may change the phosphorylation state of specific components of the neurotransmitter release machinery, and thus alter neurotransmitter release. To study the role of calcium in regulating release, we expressed parvalbumin, a calcium binding protein localized to GABAergic neurons. Parvalbumin was expressed, for at least 1 week, in both cell bodies and processes of cultured sympathetic and occipital cortex neurons. Parvalbumin caused a long term increase in monoamine or excitatory amino acid neurotransmitter release from sympathetic or cortical neurons, respectively, in both the basal state, and following depolarization (high K or veratridine). Cultures infected with pHSVpUC (pUC19 polylinker), or mock infected did not alter release; the increased release was dependent on calcium. These studies may provide insights into the roles of specific signal transduction pathways in the long term regulation of the neurotransmitter release machinery, in the axon terminal.

240.11

EXPRESSION OF AN UNREGULATED PROTEIN KINASE C IN CORTICAL NEURONS, FROM A HSV-1 VECTOR, CAUSES A LONG TERM, ACTIVITY DEPENDENT INCREASE IN NEUROTRANSMITTER RELEASE. A.I. Geller, J. Bryan*, O. Ashe*, M.J. During, and R.L. Neve. Div. Cell Growth and Regulation, Dana Farber Can. Inst., Boston MA. 02115; Dept. Neurosurg., Yale Univ. Sch. of Med., New Haven CT. 06510; Dept. Psychobiol., U. CA., Irvine CA. 92717

We are investigating the role of signal transduction pathways in mediating long term changes in mammalian neuronal physiology. The strategy is to express catalytic domains of signal transduction enzymes in neurons using our HSV-1 vector system (Science 241, 1667, 1988; Proc. Natl. Acad. Sci, 87, 1149, 1990; Biochem. Pharm. 40, 2189, 1990). The catalytic domains encode unregulated enzymes that are always active, thereby stably activating a signal transduction pathway; the effect of a particular pathway on neuronal physiology is then studied. Following this strategy, the rat protein kinase C ß-II catalytic domain (pHSVpkcΔ) was fused to a 10 aa peptide recognized by an antibody. pkcΔ protein was expressed, for at least 1 week, in cultured sympathetic and occipital cortex neurons, and was predominantly localized to cell bodies. Depolarization (high K or veratridine) revealed a long term increase in monoamine or excitatory amino acid neurotransmitter release from sympathetic or cortical neurons, respectively, infected with pHSVpkcΔ, but not from cultures infected with pHSVpkc (full length pkc), pHSVpUC (pUC19 polylinker), or mock infected. The increase in release required calcium. The catalytic domain of calcium/calmodulin protein kinase II (pHSVCaCKΔ) also caused a long term, activity dependent increase in release, but of smaller magnitude. In the basal state, pHSVpkcΔ or pHSVCaCKΔ did not alter release; in contrast, an adenylate cyclase catalytic domain increased release in the basal state, but not following depolarization. These results suggest that the cAMP pathway and the calcium activated protein kinases cause specific and distinct long term changes in neuronal physiology. The underlying biochemical mechanisms are under investigation.

240.12

A MECHANISTIC MODEL FOR THE ARACHIDONIC ACID-DIACYLGLYCEROL SYNERGISTIC ACTIVATION OF PROTEIN KINASE C IN NEURONAL SYSTEMS. David S.Lester , Thomas J.Nelson*, and Daniel L.Alkon*Neural Systems Section, NIH, Bethesda, MD, 20892.

Diacylglycerol (DAG) and arachidonic acid (AA) are established activators of the Ca^{2+}/phospholipid-dependent protein kinase, protein kinase C (PKC). Using a model membrane bilayer system, we have found that in the presence of suboptimal activating concentrations of DAG (1 mol% of total lipid, similar to what is reported for physiological systems), addition of AA (1.1-22 µM) activates PKC phosphorylation of exogenous and endogenous substrates in a synergistic manner. This synergistic activation is not related to phosphoprotein substrate requirements, nor does the AA compete with phorbol ester at the DAG-phorbol ester binding site of PKC. Based on enzymatic assays, the AA seems to make the lipid activator, DAG, more available such that more PKC can be bound and activated. This study supports the notion that there are more than one pathway to activate PKC all of which involve phospholipase(s) A_2 and/or C. We have used the associative learning cellular system of the Type B photoreceptor of the sea snail, Hermissenda, to propose a model for how two external stimuli (light and rotation) can have convergence of their effects (depolarization and GABA) and these phospholipase pathways to activate the enzyme, PKC. Physiological evidence from our laboratory and others support the notion that this synergistic interaction between AA and DAG may be an important mechanism in neuronal systems.

240.13

CELLULAR ACTIONS OF PHARMACOLOGICALLY DISTINCT FORMS OF PROTEIN KINASE C. F.J. Thomson*, M.S. Johnson*, D.J. MacEwan*, G. Fink and R. Mitchell* MRC Brain Metabolism Unit, 1 George Square, Edinburgh EH8 9JZ, U.K.

We have developed various cellular models of protein kinase C (PKC) action which have allowed us to explore, in a physiological context, the selective pharmacology of different species of PKC. Depolarisation-induced $^{45}Ca^{2+}$ influx through dihydropyridine-sensitive Ca^{2+} channels into anterior rat pituitary tissue and into GH_3 cells were differently influenced by phorbol esters and putative PKC inhibitors. In GH_3 cells, 4β-, but not 4α-phorbol 12,13-didecanoate (PDD) inhibited K^+-induced $^{45}Ca^{2+}$ influx in a staurosporine- and H7-sensitive manner. In pituitary tissue, by contrast, PDD enhanced K^+-induced $^{45}Ca^{2+}$ influx in a staurosporine-sensitive, but H7-insensitive manner. We have also found certain PKC actions in other models to show differential sensitivity to H7. For example, H7 blocked phorbol 12,13-dibutyrate (PDBu)-induced release of luteinizing hormone (LH) but not growth hormone (GH), whereas staurosporine inhibited both. These results were supported by the fact that both Ca^{2+}-independent and Ca^{2+}-dependent PDBu-stimulated kinase activities were blocked with similar potency by staurosporine, whereas Ca^{2+}-independent kinase activity was found to be much more resistant to H7 relative to Ca^{2+}-dependent activity. These pharmacologically different PKCs in anterior pituitary also differ in their cellular targets. Phospholipase A_2 (PLA_2) inhibitors (eg quinacrine) blocked PDBu-induced LH but not GH release suggesting that in gonadotrophes, but not somatotrophes, an H7-sensitive PKC(s) can act to modulate PLA_2 activity. These data suggest that PKC species may differ in their sensitivity to PKC inhibitors, and that they may have distinct cellular targets.

240.14

POSSIBLE INVOLVEMENT OF PROTEIN KINASE C (PKC) AND cAMP IN THE CONTRACTILE RHYTHMIC ACTIVITY OF THE CNIDARIAN, RENILLA KOELLIKERI. E.W.Awad*, M. Anctil and T. Cabana. Dept.Sci.Biol., Univ. de Montreal, Qc., Canada, H3C 3J7.

Previous studies have reported modulatory effects of epinephrine,norepinephrine,serotonin and the neuropeptide Antho-RFamide on the colonial muscular activity of the sea pansy,Renilla koellikeri. In an effort to establish a relationship between these neuroactive substances and specific receptors, the possible involvement of the second messengers diacylglycerol and cAMP was examined. A force transducer and a chart recorder were used to monitor colonial contractile rhythmic activity. Phorbol 12,13-dibutyrate and 1,2-dioctanoyl-sn-glycerol induced tonic contractions and an increase in the amplitude of rhythmic contractions. These responses were reduced by the absence of Ca^{++} and the presence of PKC inhibitors. Basic cAMP levels measured in colonial tissues by competitive protein binding assay were sensitive to several serotonergic and adrenergic agents. These results suggest the presence of PKC- and cAMP-mediated mechanisms involved in the modulation of rhythmic contractile activity of the sea pansy.

240.15

ACUTE AND CHRONIC EFFECTS OF CALCIUM ANTAGONISTS ON PROTEIN PHOSPHORYLATION IN THE RAT HIPPOCAMPUS. F.J. Hoffman Jr.* and R.A. Janis. Miles Institute for Preclinical Pharmacology, West Haven, CT 06516 USA.

Nimodipine has neuroprotective and cognition enhancing effects, but the mechanisms by which it exerts these effects are not known. Protein kinase C (PKC) and calcium are implicated in both ischemic cell damage and neuroplasticity, and therefore, the effects of acute and chronic nimodipine treatment were studied. Chronic nimodipine treatment (40 mg implants for 2 and 4 weeks) was found not to affect the phosphorylation of major substrates by PKC in the rat hippocampus as studied by two-dimensional electrophoresis and direct counting of the gels. The acute effects of nimodipine and other calcium antagonists were also studied. K^+-induced depolarization (50 mM) of rat hippocampal slices resulted in significant changes (33 to 38%) in the phosphorylation state of the major PKC substrates MARCKS (myristoylated, alanine-rich C-kinase substrate) and neuromodulin. Pretreatment of the slices with 500 μM Cd^{2+}, but not 300 nM nimodipine, 10 μM ω-conotoxin GVIA, or MK-801, blocked the K^+-induced change in phosphorylation. The results suggest that K^+-induced changes in phosphorylation of MARCKS and neuromodulin are mediated by calcium influx mechanisms other than, or in addition to, those sensitive to the organic antagonists employed.

240.16

IBOTENATE STIMULATES PROTEIN PHOSPHORYLATION IN CULTURED HIPPOCAMPAL PYRAMIDAL NEURONS. W.K. Scholz. Dept. Pharmacol. and Pysiol. Sci., Univ. Chicago, 947 E. 58th St., Chicago, IL 60637.

Previously we showed that glutamate stimulation of cultured pyramidal neurons resulted in the elevation of calcium and diacylglycerol (DAG), and the phosphorylation of 3 proteins: MARCKS, a 120- and a 48-kDa protein. When pyramidal neurons were stimulated in an extracellular solution containing 50 nM calcium (approximately equal to the resting level in these cells), generation of DAG and phosphorylation still occurred, although the response was reduced (Scholz and Palfrey, J.Neurosci., in press). This suggests that a metabotropic type glutamate receptor, one which is associated with the hydrolysis of inositol phospholipids, might be partially responsible for the phosphorylation observed. The ability of glutamate agonists to activate phospholipases in 1.26 mM and 50 nM calcium was investigated. NMDA and quisqualate did not stimulate the generation of inositol phosphates in normal or low calcium in pyramidal neurons. Kainate stimulated inositol phosphates production in the presence of 1.26 mM Ca but not in 50 nM Ca. Ibotenate was able to stimulate the generation of inositol phosphates with similar maximal responses in normal and low extracellular Ca. The maximal response to ibotenate was approximately equal to that of glutamate, when pyramidal neurons were stimulated in 50 nM Ca. Glutamate and ibotenate stimulated generation of inositol phosphates could be partially blocked (approximately 40% reduction) by prior treatment of neurons with pertussis toxin (250 ng/ml) suggesting that a G-protein might be involved. The second product of inositol phospholipid hydrolysis, DAG, directly binds and activates protein kinase C. Indeed we have shown that 1) ibotenate stimulates the phosphorylation of the same three PKC substrates as glutamate, 2) kainate stimulates phosphorylation only in the presence of 1.26 mM Ca and 3) NMDA and quisqualate were ineffective in stimulating phosphorylation. Thus stimulation of pyramidal neurons with glutamate leads to the phosphorylation of specific proteins. This occurs through the activation of ionotropic (kainate) receptors, which depolarize the neuron resulting in Ca-activation of phospholipases and metabotropic (ibotenate) receptors, which directly activate phospholipases possibly through a G-protein.

240.17

THE DISTRIBUTIONS OF INHIBITOR-1 AND DARPP-32 IN BRAIN AND PERIPHERAL TISSUES OF VARIOUS SPECIES H.C. Hemmings, Jr., J.-A. Girault, A.C. Nairn and P. Greengard. Lab. of Mol. and Cell. Neuroscience, The Rockefeller Univ., New York, NY 10021

The distribution of inhibitor-1 (I-1) and of DARPP-32, two homologous, cAMP-regulated inhibitors of protein phosphatase-1 (PP-1), was analyzed in various brain regions and peripheral tissues of several species by immunoblotting using specific antibodies. In rat CNS, a single I-1 immunoreactive protein of M_r=30,000, was widely distributed. In contrast, DARPP-32 was highly concentrated in the basal ganglia. I-1-immunoreactive proteins were detected in brain tissue from frog (M_r=27,000), turtle (M_r=29,000/33,000), canary (M_r=26,000), pigeon (M_r=28,000), mouse (M_r=31,000), rabbit (M_r=26,500), cow (M_r=27,000) and monkey (M_r=27,500), but not from goldfish. In peripheral tissues of the rabbit, I-1 was identified in skeletal muscle, liver, fat, kidney, heart, spleen and pancreas. In other species, I-1 was detected in the same peripheral tissues with the exception of rat liver, cow liver and spleen, and pigeon and turtle fat, spleen and pancreas. DARPP-32 was detected in brain tissue of all the species tested except frog, but was absent from most peripheral tissues. Both I-1 and DARPP-32 were concentrated in the cytosol and synaptosomal cytosol of rat caudate-putamen. The developmental expression of I-1 and DARPP-32 in rat caudate-putamen differed. I-1 peaked in the first postnatal week at twice the level found 1 week before birth, and then declined and stabilized by the third postnatal week. In contrast, DARPP-32 increased to a peak level by the third postnatal week, and remained elevated thereafter. Since I-1 and DARPP-32 have distinct but partially overlapping distributions and developmental expression in rat CNS, and have distinct distributions in various peripheral tissues, it appears that their functions are not fully interchangeable.

240.18

D1 AGONIST 38393 INCREASES THE STATE OF PHOSPHORYLATION OF ARPP-21 IN NIGRAL SLICES. K. Tsou and P. Greengard. Lab. of Molecular & Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

ARPP-21 is a cAMP-regulated phosphoprotein (Mr=21,000 in SDS-PAGE) that has a distribution in brain similar to that of DARPP-32. It is enriched in the medium spiny neurons in the striatum and in the striato-nigral projection nerve terminals in the pars reticulata of the substantia nigra. ARPP-21 is phosphorylated by cAMP-dependent protein kinase on a seryl residue located at position 55 of its 88 amino acid sequence. Both forskolin and 8Br-cAMP increase its state of phosphorylation in striatal and nigral slice preparations.

In the present study, rat nigral slices were first preincubated in warm, oxygenated RPMI 1640 culture medium containing 0.5 mM IBMX for 45 min. Drugs were added to the medium for 10 min. The tissue was frozen in liquid nitrogen and homogenized by sonication in 5 mM Zn acetate. Proteins were extracted in acid, and back phosphorylated by exogenous catalytic subunit of cAMP dependent protein kinase and γ^{32}P-ATP. The phosphorylated ARPP-21 was immunoprecipitated by a polyclonal anti-ARPP-21 antibody. The immunoprecipitate was subjected to SDS-PAGE and autoradiography. D1 agonist 38393 (10^{-4}M) increased the state of phosphorylation of ARPP-21 by 25% on average (range 11-50%). This effect could be blocked by pretreatment of the nigral slices for 5 min with the D1 antagonist SCH 23390 (10^{-5} M). This result indicates that ARPP-21 is a dopamine regulated phosphoprotein.

240.19

EFFECTS OF CALRETININ ON BRAIN PHOSPHOPROTEINS: COMBINED INHIBITORY AND STIMULATORY ACTIONS. L. Winsky, T. Yamaguchi* and D.M. Jacobowitz. Lab. of Clinical Science, NIMH, Bethesda, MD 20892

Calretinin is a neuronal calcium binding protein of the EF hand type. As with calbindin-D28k, its closest homolog, the function of calretinin remains to be elucidated. The present study investigated whether calretinin, like some other calcium binding proteins (calmodulin, calcineurin), might produce effects on protein phosphorylation. Synaptic membranes were prepared by sucrose density centrifugation and samples were incubated in buffer containing combinations of 5 mM EGTA or 200 μM calcium with calretinin alone or in combination with other effectors. Results indicated a dose dependent inhibitory effect of calretinin on the appearance of a 39 kDa synaptic membrane phosphoprotein with 30-50 % inhibition produced by 100 nM calretinin. Phosphorylation of the 39 kDa band was stimulated by calcium but was not affected by calmodulin or a protein kinase C inhibitor at doses producing clear effects on other proteins. These compounds also did not affect the inhibitory action of calretinin on the phosphorylation of the 39 kDa band. Calretinin in combination with either calmodulin or phosphatidyl serine produced additive stimulatory effects on several phosphoprotein bands. These results suggest that calretinin could produce some physiological action through effects on protein phosphorylation.

240.20

Ca^{2+}/PHOSPHOLIPID-DEPENDENT PROTEIN PHOSPHORYLATION IN RAT BRAIN AFTER LONG-TERM TREATMENT WITH ANTIDEPRESSANTS M. Asakura*, H. Asou, J. Nakanishi*, T. Tsukamoto*, H. Matsui*, M. Ino*, J. Shimada* and K. Hasagawa*
Department of Neuropsychiatry and Division of Clinical Pharmacology of Medical Sciences, St.Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa, 216 Japan

The Ca^{2+}/phospholipid-dependent phosphorylation plays an important role for the regulation of transmembrane signal transduction systems in the brain. In the present study, we examined the influence of long-term treatment with antidepressants on the Ca^{2+}/phospholipid-dependent protein phosphorylation in the cerebral cortex and hippocampus of the rat. The proteins were separated by the reverse phase HPLC procedure of the ammonium sulfate precipitate fraction from the membrane and soluble fractions of the brain regions. Long-term treatment with imipramine or chlorimipramine increased the Ca^{2+}/phospholipid-dependent phosphorylation of the 47 kDa and 26 kDa protein bands on the SDS-PAGE- autoradiogram from the membrane fraction of cerebral cortex. The effect appeared to be associated with the long-term treatment with antidepressant agents since we did not observed the increase in the photoactivated incorporation of 47 kDa and 26 kDa proteins after acute treatment with the agents.

240.21

INSULIN AND IGF-1 STIMULATE TYROSINE PHOSPHORYLATION OF PHOSPHATIDYL INOSITOL 3-KINASE IN NEONATAL RAT NEURONS. R.A. Patel*, P. Kurian*, M. K. Raizada, and F. T. Crews. Departments of Pharmacology and Physiology, College of Medicine, University of Florida, Gainesville, FL 32610.

In the central nervous system insulin and insulin-like growth factor 1 (IGF-1) are known peptide growth factors which can stimulate neurite outgrowth and induce specific neuronal proteins. Both insulin and IGF-1 seem to require the tyrosine kinase activity of their respective receptors to influence physiological processes. However, the identification of the cellular targets involved in the signal transduction pathways for these growth factor receptor tyrosine kinases have not been elucidated. Recently, phosphatidylinositol 3-kinase (PtdIns 3-kinase), a novel enzyme which specifically phosphorylates the *myo*-inositol ring of phosphatidylinositol (PtdIns), phosphatidylinositol 4-phosphate [PtdIns(4)*P*], and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] on the three position has been implicated in the transduction of mitogenic and oncogenic signals. To determine if PtdIns 3-kinase was involved in neuronal growth factor responses, we investigated the effects of insulin and IGF-1 on phosphatidylinositol 3-kinase activity. Treatment of neonatal rat neurons with various concentrations of insulin and IGF-1 resulted in increased immunoprecipitable PtdIns 3-kinase by anti-phosphotyrosine monoclonal antibodies in a concentration-dependent manner. These studies indicate that PtdIns 3-kinase activity is present in non-mitogenic neonatal rat neurons and that it is tyrosine phosphorylated by the tyrosine kinase growth factor receptors for insulin and IGF-1. To determine if the increased tyrosine phosphorylation of PtdIns 3-kinase increased activity *in vivo*, intact neurons were stimulated with insulin in the presence of ^{32}P-orthophosphate. Insulin receptor stimulation increased the formation of 3-polyphosphoinositides. Specifically, insulin stimulation increased the levels of both phosphatidylinositol 3-phosphate [PtdIns(3)*P*] and phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2] as determined by SAX-HPLC. These results indicate that insulin and IGF-1 hormonal stimulation of neonatal rat neurons increases the formation of the unique 3-phosphorylated phosphoinositides and that growth factor signals in neonatal rat brain which do not involve mitogenic responses do stimulate PtdIns 3-kinase activity.

SECOND MESSENGERS VII

241.1

EFFECT OF UCB 11056, A NEW ANTIDEMENTIA DRUG, ON NOREPINEPHRINE (NE) AND FORSKOLIN (FSK) - INDUCED FORMATION OF CYCLIC AMP (cAMP) IN HIPPOCAMPAL TISSUE. A. El Tamer[a,b], J. C. Corey[a], E. Wulfert[a,b], and I. Hanin[a]. [a]Loyola Univ. Chicago Stritch Sch. Med., Maywood, IL 60153 and [b]UCB s.a. Pharmaceutical R&D, Brussels, Belgium.

We observed a significant increase in cAMP levels (+25%) in rat hippocampus within 10 min post-intraperitoneal injection of ucb 11056 (5.4 and 10.0 mg/kg). To determine whether this effect is mediated via adrenergic β-receptor activation, hippocampal slices (0.5 mm), prepared from naive rats, were first preequilibrated in Krebs Ringer buffer at 37°C for 75 min. They were then challenged for 20 min with ucb 11056 (5, 10 and 50 μM), NE (10 μM), FSK (1 μM), or a combination of ucb 11056 with either of the two other drugs. cAMP levels were measured using radioimmunoassay. Increases in cAMP levels, expressed as pmol/mg protein, were as follows:

ucb11056(μM)	Δincrease vs baseline (12 ± 1.5)	Δincrease vs NE (10μM) (19.8 ± 2.34)	Δ increase vs FSK (1μM) (45.8 ± 6.4)
5	0	5.0 ± 0.25	17.4 ± 3.2
10	0	9.0 ± 0.75	25.0 ± 2.2
50	5.6 ± 0.1	17.0 ± 1.2	37.6 ± 3.4

Number of samples were 5-25 per group. These combined data show that ucb 11056: 1) increases rapidly cAMP levels in the rat hippocampus in-vivo; and 2) potentiates both NE and FSK-activated cAMP formation in vitro. These data suggest that ucb 11056 might be acting as a permissive agent favoring cAMP generation in the brain.

241.2

HIGH AFFINITY LIGAND BINDING BY THE DETERGENT-SOLUBILIZED CANNABINOID RECEPTOR. D.B. Houston and A.C. Howlett, Department of Pharmacological and Physiological Science, Saint Louis University Medical Center, 1402 S. Grand Blvd., St. Louis, MO 63104.

A receptor which specifically binds the active component of marijuana, delta-9-tetrahydrocannabinol (THC) and several more potent synthetic analogs has been previously characterized in rat brain by our laboratory. The cannabinoid receptor is a member of the family of receptors linked to GTP-binding proteins, as it is coupled to G_i to inhibit adenylate cyclase activity in brain and neuronal cells. Binding with the cannabinoid agonist [^{3}H]CP-55,940 reveals a receptor density of 1-2 pmoles/mg of protein in rat brain membranes. Scatchard analysis indicates a K_d for [^{3}H]CP-55,940 of 0.8 nM. Affinity for agonist ligands is decreased by non-hydrolyzable GTP analogs, confirming the regulation of the cannabinoid receptor by a G-protein. We were able to solubilize the receptor from rat brain membranes using the zwitterionic detergent CHAPS. The solubilized receptor was assayed by binding to [^{3}H]CP-55,940 with bound and free ligand separated on small gel filtration columns. A detergent:protein ratio of 0.5:1 resulted in almost 50% of membrane receptor appearing in solubilized form. The solubilized receptor exhibits high affinity agonist binding in the presence of Mg^{2+}, which is decreased in the presence of guanine nucleotides, suggesting that the associated G-protein is also solubilized with the receptor. The agonist binding characteristics of the solubilized receptor are similar to those seen in membranes. The K_i for binding of desacetyllevonantradol (DALN), another cannabinoid agonist, is 0.8 nM, comparable to that seen for receptor in membranes. The apparently successful solubilization of the cannabinoid receptor will enable us to develop a purification scheme for the receptor and associated G-protein(s). Supported by NIDA grants DA03690 and DA06312.

241.3

THYROID HORMONE REGULATION OF G-PROTEIN AND ADENYLATE CYCLASE GENE EXPRESSION IN RAT BRAIN. S. F. Colin and E. J. Nestler, Laboratory of Molecular Psychiatry, Yale University School of Medicine, New Haven, CT 06508

Past studies have established that hypothyroidism induces an approximate two-fold increase in levels of $G_{i\alpha 2}$, $G_{i\alpha 3}$, and $G\beta$ mRNA and protein in peripheral tissues without affecting levels of $G_{s\alpha}$. In the current study, we have examined the effects of hypothyroidism on G-proteins in rat brain. After three weeks of treatment with the anti-thyroid drug 6-n-propyl-2-thiouracil (resulting in serum T4 < 0.7ug/dl), we found a 30-40% increase in cerebral cortical mRNA levels of $G_{i\alpha 1}$, $G_{i\alpha 2}$, and $G\beta$ (in comparison to euthyroid controls) as determined by Northern blotting. As with past studies in adipose and heart tissue, hypothyroidism did not affect $G_{s\alpha}$ mRNA. Similar changes in G-protein mRNA levels were noted in hippocampus. Hypothyroid regulation of adenylate cyclase and protein kinase A subunits is under current investigation.

In parallel studies we have shown that chronic lithium (1 mM for 4 weeks), but not acute (1 mM for 6 days) or chronic low dose (0.5 mM for 4 weeks) lithium, induces a 20% decrease in $G_{i\alpha 1}$ and $G_{i\alpha 2}$, and a 50% increase in adenylate cyclase types 1 and 2, mRNA and protein (*PNAS* submitted). Together, these results indicate that regulation of G-protein and adenylate cyclase gene expression may underlie clinical phenomena such as hypothyroid-induced depression and the efficacy of lithium therapy and thyroid supplement in the treatment of affective disorders.

241.4

ESTROGEN UNCOUPLES β-ADRENERGIC RECEPTORS FROM ADENYLYL CYCLASE IN FEMALE RAT HYPOTHALAMUS. S. Ungar and A.M. Etgen. Depts. Psychiat. & Neurosci., Albert Einstein Coll. Med., Bronx, NY 10461.

Previous work from this laboratory demonstrated that estrogen desensitizes β-adrenergic receptor stimulation of cAMP accumulation in female rat hypothalamic and preoptic area slices without modifying either receptor number or antagonist binding affinity. The current experiments compared β-adrenoceptor activation of adenylyl cyclase (AC) in membranes prepared from combined hypothalamus-preoptic area of ovariectomized (OVX) and OVX, estrogen-primed rats. OVX rats received oil vehicle or 2 μg of estradiol benzoate (EB) 48 and 24 hr prior to sacrifice. Stimulation of AC by the β agonist isoproterenol (10^{-8}-10^{-4}M) and by forskolin (10^{-8}-10^{-6}M), a receptor-independent activator of AC, were assayed. Membranes from OVX animals displayed a concentration-dependent stimulation of AC when incubated with isoproterenol, with enzyme activity maximally increased by approximately 60% above basal levels at 10^{-6}M. In contrast, membranes from EB-treated animals showed no isoproterenol-dependent elevation in AC activity. EB administration had no measurable effect on basal or forskolin-stimulated AC activity. These data suggest that estrogen promotes a stable uncoupling of β-adrenergic receptors from AC in the hypothalamus and preoptic area either at the level of receptor coupling to G protein or G protein coupling to AC.

241.5

S-ADENOSYL-METHIONINE (SAM) MODULATION OF ß-ADRENOCEPTOR MEDIATED CYCLIC AMP PRODUCTION: POSSIBLE RELATIONSHIP TO METHYLATION OF THE CARBOXYL TERMINUS OF A SMALL G-PROTEIN. P. Zhong and K.J. Kellar. Department of Pharmacology, Georgetown University School of Medicine, Washington DC 20007.

We demonstrated previously that SAM potentiates ß-adrenoceptor-mediated cyclic AMP production in rat brain slices without affecting the basal cyclic AMP level (Zhong et al., Soc. Neurosci. Abst. 16:1302, 1990). SAM does not alter the affinity, nor the Bmax of ß-adrenoceptor binding sites in rat brain; nor is cyclic AMP production by forskolin affected by SAM. Therefore, the possibility of modulation of G-protein functions by SAM has been explored. In solubilized extracts from cerebral cortex slices incubated with [^{3}H]methyl-SAM, immunoprecipitation under non-denaturing conditions with antisera raised against the different α and the common ß subunits of Gs, Gi and Go did not reveal significant methylation of these subunits. Next, the incorporation of base-volatile [^{3}H]-methyl groups into brain slices was examined by a diffusion assay (Clarke et al., PNAS 85:4643, 1988). A major methylation peak was found in a 25 kD fraction of the labeled membrane protein. Binding of $^{32}P\alpha$GTP in a 25 kD fraction of protein from solubilized brain membranes also was detected on nitrocellulose blots by the method of Bhullar and Haslam (Biochem J. 245:617, 1987). Acetyl-farnesyl-cysteine (AFC), a selective competitive inhibitor of "-CAAX" box methylation (Volker, et al., Methods, 1:283, 1990), inhibited [^{3}H]methyl group incorporation in the 25 kD protein from brain in a concentration dependent manner. AFC also blocked potentiation of NE-stimulated cyclic AMP production by SAM in a similar concentration-dependent manner. These data suggest that a small G-protein containing a "-CAAX" box in the carboxyl terminus might be involved in modulation of cyclic AMP production by SAM.

Supported by NIMH grant #MH41819.

241.6

FUNCTIONAL INTERACTIONS OF STRIATAL D_1 AND D_2 DOPAMINE RECEPTORS: EFFECTS OF TISSUE PREPARATION AND LESIONS. L.L. Cook, D.M. Mottola, J.M. Petitto*, M.H. Lewis, R.B. Mailman. Brain and Development Research Center, Univ. of North Carolina, Chapel Hill, NC 27599.

These studies examined functional D_1/D_2 interactions of dopamine receptors in rat striatum. Molecular or biochemical studies have shown that D_1 (and "D_5") receptors can be positively coupled to adenylate cyclase (AC), whereas D_2 ("D_3" and "D_4") receptors are negatively coupled. As reported previously, dopamine (100 μM) increased cAMP efflux in slices of rat striatum, an effect blocked by SCH23390. The relative potency and efficacy of D_1 agonists to increase cAMP synthesis in slices was similar to that seen in striatal homogenates. As expected, the addition of a selective D_2 antagonist (sulpiride, domperidone or spiperone) resulted in an enhanced efflux of cAMP in slices, presumably due to blockade of inhibitory D_2 receptors. Conversely, in homogenates, D_2 antagonists (domperidone, remoxipride, and sulpiride) did not increase cAMP synthesis. Since this functional interaction between D_1 and D_2 receptors is eliminated following gentle homogenization, two possibilities are suggested. One is that the biochemical coupling of D_2 receptors to G proteins is more sensitive to disruption compared to D_1 receptors in this tissue preparation. Alternately, this biochemical interaction may require intact cellular architecture, consistent with the hypothesis that these receptor classes are located on different cells.

The D_2 interaction with D_1-coupled AC was assessed in slices from rats with unilateral 6-OHDA substantia nigra lesions. Such lesions are known to increase the density of D_2 receptors, to increase (in homogenates) the potency of dopamine in the AC assay, and to abolish behavioral D_1/D_2 interactions. Striatal slices were prepared from sham controls, and from each striatum of the 6-OHDA-lesioned rats. In slices from the lesioned side, there was a 58% increase in cAMP efflux compared to sham controls and a 47% increase compared to the unlesioned side. Sulpiride (30 μM) produced a 32% increase in cAMP efflux in control slices, a 73% increase on the lesioned side and a 77% increase on the contralateral side. These results demonstrate that after deafferentation this functional interaction between D_1 and D_2 receptors remained intact, and appears to be potentiated, consistent with up-regulation of D_2 receptors. (Supported by Grants MH40537 and ES01104, and Foundation of Hope)

241.7

CANNABINOID RECEPTOR CHARACTERISTICS IN RAT CEREBELLAR GRANULE CELLS AND NG108-15 CELLS. M.A. PACHECO, S.R. CHILDERS, AND S.J. WARD. Dept. Physiol./Pharm., Bowman Gray Sch. Med., Winston-Salem, NC 27103; Dept. Neuroscience, Univ. of Florida, Gainesville, FL.; Sterling Drug Inc., Rensselaer, NY.

Both cannabinoid analogs (Cn) and aminoalkylindoles (AAI) bind to G-protein coupled Cn receptors. Cn receptors were assayed in membranes by binding of the AAI agonist ^{3}H-WIN 55212 (^{3}H-212) and, in intact cells by inhibition of forskolin-stimulated cAMP formation by Cn and AAI analogs. ^{3}H-212 binding sites and Cn-inhibited cAMP were detected in cerebellar granule (CG) cells after 12 days in culture. ^{3}H-212 binding was inhibited by GTP in CG membranes. In CG cells levonantradol (LN) and WIN 212 inhibited cAMP in a dose-dependent manner. An AAI antagonist blocked both LN and WIN 212 inhibited cAMP in CG cells. The AAI antagonist alone had no effect on cAMP. Pertussis toxin blocked LN and WIN 212 inhibited cAMP in CG cells. These data suggest that Cns and AAIs are working through the same inhibitory G-protein coupled receptor in CG cells.

The levels of Cn receptors were similar in NG108-15 and CG cell membranes but only 20% the level in adult rat cerebellar membranes. Although LN and WIN 212 were equipotent in displacing the ^{3}H-212 binding to cerebellar membranes, LN was much less potent than WIN 212 in displacing binding to NG108-15 cell membranes. These data suggest that CG and NG108-15 cells may differ in their sensitivity to various Cn analogs.

Supported by PHS grant DA-06784 from NIDA.

241.8

IN VIVO ASSESSMENT OF D1 RECEPTOR ACTIVATION IN RAT PREFRONTAL CORTEX. W. A. Clark and R.H. Roth. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

The medial prefrontal cortex (PFC) receives dopaminergic input from cells originating in the ventral tegmental area. This mesoprefrontal dopamine system exhibits exquisite sensitivity to mild stressors and to a class of benzodiazepine receptor inverse agonists, the anxiogenic β-carbolines. Both D1 and D2 receptors are found in this dopaminergic terminal field. In order to evaluate postsynaptic responses to changes in presynaptic dopamine turnover, we have evaluated extracellular (EC) cAMP, recovered by in vivo microdialysis, as a biochemical index of postsynaptic D1 receptor activity (c.f. Egawa et al., *Brain Research 458:303-308, 1988*).

We utilize commercially available RIA materials to quantify cAMP to a lower limit of 0.5fmol. This high sensitivity assay eliminates the requirement for IBMX or other phosphodiesterase inhibitors in the perfusion buffer and allows measurement of basal EC cAMP.

All drugs are administered through a microdialysis probe located in the PFC of the anesthetized rat. Basal EC cAMP levels are insensitive to GTPγS (1μM to 250μM) suggesting that cAMP is generated within and transported from intact cells. While perfusion of the D1 selective agonist SKF38393 (up to 100μM) results in a dose-dependent increase in EC cAMP, this effect is TTX- and EGTA-insensitive (2μM and 4mM, respectively) indicating that the cAMP is formed from direct D1 receptor-mediated stimulation of adenylate cyclase.

Our data suggest that EC cAMP is a useful index of in vivo activation of D1 receptors in the PFC (c.f. Hutson & Suman-Chauhan, *Neuropharmacology 29:11: 1011 -1016, 1990*).

Supported by MH-14092 & GM-07324-14

241.9

DEVELOPMENTAL CHANGES IN CYCLIC AMP-DEPENDENT PROTEIN KINASE ASSOCIATED WITH RESPONSE TO PROTHORACICOTROPIC HORMONE. A.H. Varghese*, K.J. Lou*, and W.A. Smith. Dept. of Biology, Northeastern University, Boston, MA 02115.

Prothoracicotropic hormone (PTTH) is an insect cerebral neuropeptide which stimulates ecdysteroid secretion by the prothoracic glands. PTTH has previously been shown to activate glandular cAMP-dependent protein kinase in Manduca sexta, particularly during the mid-portion of the final larval stage. At this time, glandular response to PTTH is maximal with regard to steroidogenic output. We have further characterized glandular kinase, and have noted a predominance of the type II (54-55 kD) regulatory subunit. Further, a protein co-migrating with this subunit on 2-dimensional gels is a prominent substrate for thiophosphorylation in the presence of [^{35}S]ATP. Exposure to PTTH enhanced regulatory subunit occupancy by cAMP, primarily in the cytosol at this stage of development, though substrate phosphorylation occurred in plasma-membrane and microsomal cell fractions. Thiophosphorylation permitted us to examine regulatory subunit content independent of subunit occupancy by endogenous cAMP. Putative RII examined in this manner increased dramatically during the period of ecdysteroid secretion occurring between days 3 and 5 of the last larval stage. Increased RII content was also suggested during the prepupal ecdysteroid peak on day 8. Thus a strong correlation exists between PTTH responsiveness, secretory capacity, and RII subunit content, which we hypothesize may all be regulated by enhanced glandular levels of cAMP. (Supported by NIH DK37435 to WAS)

241.10

MECHANISMS UNDERLYING THE DIFFERENTIAL MODULATION OF GLYCINE CURRENT BY CYCLIC AMP DEPENDENT PROTEIN KINASE IN ACUTELY DISSOCIATED VENTROMEDIAL HYPOTHALAMIC NEURONS. N. Agopyan, N. Tokutomi* and N. Akaike. Dept. of Neurophysiology, Tohoku Univ. Sch. of Med., Sendai 980, Japan.

We recently reported that in acutely dissociated ventromedial hypothalamic (VMH) neurons cyclic AMP (cAMP) reduces the amplitude of 100 μM glycine (Gly)-induced chloride current (I_{Cl}) without affecting that induced by Gly concentrations up to 10 μM (Proc. of third IBRO World Congress of Neuroscience 1991, Montreal). Such a specific action of cAMP on >10 μM Gly-induced I_{Cl} may reflect the presence of either two different Gly receptor subunits or different conductance states of a single Cl channel (Hamill et al. 1983, Nature 305:805-808) with different sensitivities to cAMP dependent phosphorylation. To differentiate between these possibilities we studied the effect of cAMP (20 μM - 1 mM) on the inactivation kinetics of I_{Cl} induced by Gly (3 - 100 μM). Whole cell currents from acutely dissociated VMH neurons, held at -20 mV in symmetrical [Cl] (120 mM), were recorded at room temperature by patch- pipettes filled with internal solution containing 4 mM ATP and 1 mM GTP.

Under control conditions the amplitudes of I_{Cl} evoked by <10 μM Gly do not desensitize while that evoked by >10 μM Gly decrease to a steady-state level upon prolonged Gly application. Internal perfusion with cAMP induced a transient enhancement and the desensitization of 10 μM Gly-evoked I_{Cl} without significantly affecting the inactivation kinetics of that evoked by 30 and 100 μM Gly. H-8 (10 μM), a cyclic nucleotide -dependent kinase inhibitor (Hidaka et al. 1984, Biochem. 23:5036-5041), blocked the effect of cAMP, indicating the involvement of protein kinase A. Presently we are investigating the effects of cAMP on single channels to further elucidate the mechanism underlying Gly receptor modulation. Our preliminary data favours the presence of two different Gly receptor subunits in the VMH neurons.

This study was supported by the JSPS fellowship awarded to N. Agopyan.

241.11

SUBCELLULAR DISTRIBUTION OF THE CALMODULIN-DEPENDENT CYCLIC NUCLEOTIDE PHOSPHODIESTERASE IN THE RAT BRAIN. V.Burmeister*, N.Ludvig, P.C.Jobe and R.L.Kincaid. Depts. of Basic Sciences and Pathology, University of Illinois College of Medicine at Peoria, Peoria, IL 61656 and Section on Immunology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

In this study the subcellular localization of the calmodulin-dependent cyclic nucleotide phosphodiesterase (CaM-dependent PDE) in cerebral cortex, hippocampus and inferior colliculus of rat brain was analyzed by electron microscopic immunocytochemical methods. Affinity purified antibodies were used. The immunoreactivity was found exclusively within neurons whereas glial cells were unstained. Preabsorption of antibody with phosphodiesterase eliminated this reactivity demonstrating the specificity of immunostaining. In the neuronal perikarya, deposits of immunoreaction product occurred as sparse patches, and were often associated with organelles such as mitochondria and endoplasmic reticulum. Nuclei, however, were free from immunoreaction product. In the neuronal processes, immunoreactivity was found within dendrites and dendritic spines, whereas the myelinated axons and axon terminals were immunonegative. The postsynaptic densities of asymmetric synapses were associated with especially high concentrations of immunoreaction product. The immunopositive profiles appeared to be quite selective; they comprised only a small percentage of the total number of synapses in the examined areas.

These data indicate that the CaM-dependent PDE is localized predominantly at postsynaptic sites in the rat brain. This supports other morphological evidence indicating a principal involvement of the cyclic nucleotide second messenger system in postsynaptic and not presynaptic functions in brain. Furthermore, CaM-dependent PDE may play an important role in the postsynaptic integration of Ca^{2+}-mediated neuronal inputs.

241.12

ISOLATION AND CHARACTERIZATION OF A cDNA WHICH IDENTIFIES BOTH NEURAL-SPECIFIC AND UBIQUITOUSLY EXPRESSED NOVEL Gsα mRNAs. B.A. Habecker, J.M. Martin, and N.M. Nathanson. Dept. of Pharmacology, Univ. of Washington Sch. of Med., Seattle, WA 98195.

A clone has been isolated from a 1321N1 human astrocytoma cDNA library which is identical to Gsα-1 except for a novel 460 bp of 5' sequence, thus implying that the mRNA results from the use of an alternative promoter and alternative splicing of the Gsα gene. Polymerase chain reaction confirmed the presence of an mRNA corresponding to this cDNA in astrocytoma cells. Northern blot analyses indicate that two mRNAs containing this novel sequence are present in rat, a 2.0 Kb RNA found primarily in neural and neuroendocrine tissues, and a 1.8 Kb mRNA expressed in many tissues.

The novel sequence does not contribute an in-frame ATG, which may be due to the presence of 160 bp of alphoid satellite DNA at the 3' portion of this novel sequence. Insertion of this alphoid DNA (which ordinarily is centromeric and not transcribed) into the transcription unit of this Gsα variant results in an mRNA encoding a truncated form of Gsα, probably translated from the first in-frame ATG in exon 2 as seen in Gsα-5 (Ishikawa et. al., 1990). Studies are currently underway to determine whether this alphoid insertion is only found in astrocytoma cells, or if it is also present in Gsα related mRNA found in other neural cell lines and in normal brain tissue.

241.13

LOCALIZATION OF PUTATIVE PI LINKED G PROTEINS BY *IN SITU* HYBRIDIZATION. C.E. Glatt and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University, Baltimore, MD 21205.

PI turnover is a major second messenger system. Several G proteins have recently been identified which can stimulate PI turnover (Moriarty, (1990) *Nature* 343,79 and Smrcka, (1991) *Science* 251,804). These G proteins include G_0 and the G_q/G_{11} family. It remains unclear, however, which neurotransmitter systems stimulate PI turnover through which G proteins in the CNS. Detailed mapping of these putative PI linked G proteins by *in situ* hybridization may help determine which receptors couple to which G proteins by co-localization.

Complementary cDNA oligonucleotide probes have been synthesized against G_0, G_q, and G_{11}. These probes recognize unique sequences from each respective G protein. Northern analysis shows that each probe recognizes a single band of the appropriate size for each message. *In situ* hybridization histochemistry shows high levels of expression for G_0 in the Purkinje cell layer of the cerebellum, cortex and hippocampus, particularly the dentate gyrus and CA4 regions. G_{11} labels the cortex, granule cell layer of cerebellum,superior and inferior colliculi, olfactory bulb and all regions of the hippocampus. G_q shows similar distribution but lower levels in all regions particularly the olfactory bulb and colliculi.

241.14

CHARACTERIZATION OF G-PROTEINS IN XENOPUS MELANOCYTES. S. Karne*, C. K. Jayawickreme*, T.P. Nguyen*, M.L. Anderson and M. R. Lerner. Howard Hughes Medical Inst., Depts. of Internal Medicine, Pharm. & Neuroscience, Yale University School of Medicine, New Haven, CT. 06510

Xenopus Melanocytes translocate their pigment granules in response to a variety of stimuli, including light, melatonin, melanocyte stimulating hormone, norepinephrine, and serotonin. These ligands are believed to stimulate G-protein coupled receptors. Using the polymerase chain reaction and degenerate oligonucleotides to the conserved regions of known G-proteins, thirteen different PCR generated fragments for the G-proteins in these cells have been isolated and sequenced. Comparison of these sequences to the known G-proteins reveals that these cells contain all known members of the G-protein superfamily, save for transducin which is coupled to rhodospin in rods and cones. There are six different Gs-like, one Gi-1, one Gi-2, one Go, one Gz, and three Gp-like proteins in these cells.

241.15

MOLECULAR CLONING AND SEQUENCING OF A G-PROTEIN α_i-like SUBUNIT FROM THE LOBSTER OLFACTORY ORGAN. A.P. Byrnes*, T.S. McClintock and M.R. Lerner. Section of Molecular Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510.

The polymerase chain reaction (PCR) was used to amplify DNA sequences encoding G-protein α subunits from lobster olfactory organ cDNA. Sequencing of two cloned PCR fragments revealed homology with α_i and α_s subunits of G-proteins. One cloned PCR fragment was used to probe an olfactory organ cDNA library. A clone with a 1.4 kb insert containing the entire coding region was isolated and sequenced. This clone's predicted amino acid sequence has 77% - 82% identity to α_i subunits, 66% - 68% to α_o subunits, 62% - 65% to transducins, and 44% - 45% to α_s subunits according to a search of the Genbank and EMBL databases. A Northern blot probed with this clone revealed a single 8 kb message in lobster olfactory organ, brain, eyestalk, leg, and tail muscle.

241.16

EFFECT OF MASTOPARAN, A WASP VENOM TOXIN, ON GTP-BINDING PROTEINS THAT REGULATE RAT CORTICAL ADENYLATE CYCLASE. S. Hatta, H. Ozawa*, and H. Ohshika. Departments of Pharmacology and *Neuropsychiatry, Sapporo Medical College, Sapporo 060, Japan.

Mastoparan, a wasp venom toxin, has been shown to be a potent stimulator of exocytosis for several mammalian cells. Mastoparan (MP) has recently been suggested to interact with Go and/or Gi protein directly in the reconstituted system, and to inhibit an IAP-insensitive putative G-protein which activates phospholipase C in 1321N1 human astrocytoma cells. In this study, we examined the effect of MP on the function of G-proteins which regulate adenylate cyclase (AC) in rat cerebral cortex (CCX). MP(10^{-4} M) potentiated GppNHp-stimulated AC activity and lowered the EC_{50} value of AC activation for GppNHp in CCX membrane. Furthermore, GppNHp-stimulated AC activity in the membranes that preincubated with GppNHp was augmented by MP. On the other hand, there was no significant effect of MP either on isoproterenol-stimulated AC activity or on GppNHp inhibition of forskolin (100 μM)-stimulated AC activity. AC activation with $MnCl_2$ (20 mM) was not different in the membranes incubated with or without MP. From these results, it appears that MP potentiates the Gs protein function in the rat cortical AC system. However, no significant effect of MP on the Gi protein function was observed in rat CCX membranes.

241.17

CA++ / CALMODULIN AUGMENTS CHRONIC ANTIDEPRESSANTS' EFFECT ON THE COUPLING OF G PROTEIN (Gs) TO ADENYLATE CYCLASE H. Ozawa, N. Katamura, H. Kamada, H. Ikeda, N. Takahata, and T. Saito Dept. of Neuropsychiatry, Sapporo Medical College, Sapporo 060 Japan.

Our previous studies have shown that enhanced adenylate cyclase (AC) activity after chronic treatment with antidepressants is attributable to changes in the nature of the stimulatory GTP-binding protein (Gs) or increased coupling between Gs and the catalytic unit of AC. Thus, the molecular locus of action of antidepressants may reside at Gs. Recently it has been reported that although AC appears to bind calmodulin, the regulation of enzyme activity by that compound is complicated and likely to involved G proteins. The present study examines the modulation of antidepressants' effect on the coupling of G protein to adenylate cyclase by Ca++/calmodulin. Male Sprague-Dawley rats (150-200g) were injected (intraperitoneal) once daily for 21 days (chronic) with amitriptyline, iprindole (10mg/kg) or saline (as control) and cerebral cortex membranes were prepared and assayed for AC. When membranes were assayed under conditions in the absence of 1 mM EGTA, chronic treatment with antidepressant increased basal AC activity but not in the presence of EGTA. Enhancement of GppNHp stimulated AC activity by antidepressants, however it was not affected by EGTA. Ca++ dependent AC activity was enhanced in membranes from chronic antidepressant treatment by the addition of 1uM calmodulin. In the absence of calmodulin, however, Ca++ dependent AC activity was not altered by chronic antidepressant treatment. Ca++dependent AC activity stimulated by GppNHp was increased in membranes from rats treated by chronic antidepressant with or without calmodulin. These results suggest that increased sensitivity of Ca++/calmodulin by chronic antidepressant treatment on the AC system may be attributed to enhanced coupling between Gs and the catalytic unit of AC.

241.18

G_o ACTIVATES A CONDUCTANCE IN RAT HIPPOCAMPAL NEURONS SIMILAR TO THAT INDUCED BY SEROTONIN. R. D. Blitzer, O. D. Gil, D. Carty*, M. DeVivo*, R. Iyengar* and E. M. Landau. Depts. of Psychiatry and Pharmacology, Bronx VAMC and Mt. Sinai Med. Center, New York, NY 10029.

Serotonin hyperpolarizes hippocampal neurons by activating a K+ conductance. This effect is sensitive to pertussis toxin, suggesting that either G_o or G_i transduces the 5HT response. We are investigating the properties of activated G-proteins on these neurons, and report that activated G_o (G_o*) induces a membrane conductance which is similar, and possibly identical, to the 5HT-activated conductance.

CA1 pyramidal cells were voltage clamped in slices (500 μm thick) obtained from either immature or adult rats, using whole-cell patch methods. In control cells, 5HT (10 μM) evoked an outward current of 140 ± 23 pA at V_H=-60 mV, with a reversal potential of -80 ± 1 mV (n=12). In other cells, the pipette solution included 30 nM G_o*. 4/6 of these cells exhibited a slowly-developing steady outward current, peaking at 105 ± 28 pA and with a reversal potential of -78 ± 5 mV. The G_o*-induced current did not occlude the response to 5HT, indicating that G_o* and 5HT may activate different types of K+ channel. An alternative explanation is that the receptor-mediated response may be transduced by G_o, but that either (1) the maximal agonist response is not limited by the number of available channels, or (2) the channels activated by the introduced G_o* are spatially distinct from those activated by 5HT. We are presently exploring these possibilities. (Supported by the VA Merit Program and NIH-NIA AG02219).

241.19

EXPRESSION OF MAMMALIAN MUSCARINIC RECEPTORS AND TRIMERIC G-PROTEINS IN THE YEAST SACCHAROMYCES CEREVISIAE. P. Payette, M. Caron*, E. Leberer* and M. Dennis*
National Research Council of Canada, Biotechnology Research Institute, Montreal, CANADA, H4P 2R2

It is now well known that yeast cells possess a G-protein signal transduction pathway similar to mammalian cells. This signalling pathway is used by the pheromone receptor (STE2 or STE3) in haploid cells to activate the mating response. In the resulting diploid cell the production of the signalling pathway components is repressed. We have expressed in diploid cells, trimeric mammalian G-proteins ($\alpha_i\beta\gamma$ or $\alpha_o\beta\gamma$) and either the human muscarinic receptor subtype 1 (Hm1) or subtype 2 (Hm2) using different expression vectors. We have assesed the production of the G-protein subunits by western blot and have tested the ability of pertussis toxin to ADP ribosylate the associated trimer. Binding displacement experiments with agonists were also performed to verify if a high affinity state is conferred to the receptor by the active trimer.

It appears that $G\alpha_i$ is produced in greater quantity than $G\alpha_o$ and pertussis toxin labels only the complete trimer and not alpha alone. Agonist binding profiles are now being investigated, and preliminary experiments demonstrate no change for Hm1 binding whereas Hm2 shows a slight difference (i.e. High affinity state in low proportion) when in the presence of the $G\alpha_i$-protein trimer.

241.20

EFFECTS OF COCAINE SENSITIZATION ON G-PROTEIN RIBOSYLATION. C.D. Striplin and P.W. Kalivas. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Sensitization is a behavioral and a neurochemical process that results from repeated administration of psychostimulants. Neurochemically sensitization has been correlated with an increase in dopamine (DA) transmission in nuclei of the mesolimbic system. Biochemically, the reason for this augmented DA release is not well understood; however, it is known that the G-protein second messenger system is involved. Recently, Nestler et al. (J. Neurochem., 55:1079-1083, 1990) demonstrated that chronic cocaine (30 mg/kg) causes a decrease in pertussis toxin (PTX) labelling of G_i and G_o proteins in several mesolimbic nuclei. However, since both high and low doses of cocaine cause sensitization, it is important to determine if changes are a general phenomena of sensitization or a toxic effect of the dosage regimen. To investigate this question, rats were sensitized for five days with different doses of cocaine (0, 15, and 30 mg/kg, ip). On the fifth day, animals were sacrificed at 1, 6, or 24 h after their last injection. The ventral tegmental area (VTA) and nucleus accumbens (NA) were dissected over ice, and *in vitro* PTX activated ^{32}P-ADP ribosylations were performed. Ribosylated proteins were separated by SDS-PAGE and analyzed by densitometry following autoradiography. After the last cocaine injection in both dosage regimes, a slight decrease in the ribosylation of G-proteins was observed in the VTA at 1 h but not at 24 h. No change was apparent in the NA. The short term decrease in G-protein ribosylation produced by cocaine argues that G-proteins are not involved in the longer expression of behavioral sensitization to cocaine.

241.21

MODULATION OF SIGNAL TRANSDUCTION PATHWAYS BY ELECTROCONVULSIVE SHOCK (ECS).H.Manji, G.Chen*, J.Bitran*, M.Masana, and W.Z.Potter*Section on Clinical Pharmacology, ETB,NIMH,Bethesda, MD 20982

Electroconvulsive therapy (ECT) is an effective treatment for depression, catatonic states, and even Parkinson's disease. Despite extensive research, the molecular mechanisms underlying its efficacy in these clinical conditions remains to be elucidated.We have recently investigated signal transduction pathways as putative targets for electroconvulsive shock (ECS). 10 male sprague-dawley rats were administered a series of 10 electroconvulsive shocks (alternate day) via ear-clip electrodes. Sham-treated animals were handled similarly without electric current being administered. 24 hours following the last shock, the animals were decapitated, and frontal cortex (CORT), hippocampus (HIPP), accumbens (ACC), and striatum (STR) were dissected.

Chronic ECS did not have any effecst on the amount of α_s,α_{i1-3},α_o, as assessed by western blotting. In contrast, ECS produced a significant increase in the cholera toxin [^{32}P] ADP-ribosylation of Gs in striatum, in the absence of any changes in the pertussis toxin [^{32}P]ADP-ribosylation of Gi/Go. ECS also produced a selective decrease in the levels of PKC γ in the HIPP, without altering PKC α or PKC β. These findings may have relevance for both the therapeutic (mood-elevating and motoric) and deleterious (memory-impairing) effects of ECT.

REGULATION OF DOPAMINE RECEPTORS

242.1

FURTHER STUDIES OF AGONIST- AND ANTAGONIST-INDUCED REGULATION OF mRNA OF MUSCARINIC CHOLINERGIC RECEPTORS IN CEREBELLAR GRANULE CELLS. F.Fukamauchi, C.Hough and D.-M.Chuang, Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

We have previously demonstrated that cerebellar granule cells express mRNA of m2- and m3-muscarinic acetylcholine receptors (mAChRs) and that their mRNA contents are up- and down-regulated by exposure to the receptor antagonist atropine and agonist carbachol, respectively. In this study, we investigated some pharmacological details involved in m2- and m3-mAChR mRNA regulation. Exposure of cells to 1μM atropine induced a differential up-regulation of m2- and m3-mAChR mRNA with a maximal increase at 24 and 8 hr for m2- and m3-mAChR mRNA respectively. Treatment of cells for 2 hr with 10μM AF-DX 116, a selective m2-mAChR antagonist, significantly increased m2-mAChR mRNA. The copresence of AF-DX 116 with 100μM carbachol blocked carbachol-induced m2-mAChR mRNA down-regulation. On the other hand, treatment for 8 hr with 10μM 4-DAMP, a selective m3-mAChR antagonist, resulted in a marked increase in m3-mAChR mRNA content. Moreover, 4-DAMP completely blocked m3-mAChR mRNA down-regulation induced by 8 hr stimulation with 100μM carbachol. The level of m3-mAChR mRNA was decreased by 20, 40 and 43% after 8 hr treatment with 10, 100 and 1000μM carbachol. Under these conditions, m2-mAChR mRNA was decreased by less than 20%. Exposure of cells to 500nM PDBu and 50μM H7 for 2 hr did not affect m2- and m3-mAChR mRNA contents. These results provide further evidence for differential regulation of levels of m2- and m3-mAChR mRNA by the receptor agonists and antagonists. Moreover, agonist-induced mAChR mRNA down-regulation appears to be independent of a change in protein kinase C activity.

242.2

CHRONIC NICOTINE ADMINISTRATION DOES NOT AFFECT MESOLIMBIC NICOTINIC RECEPTOR BINDING OR FUNCTION. Peter P. Rowell and Laurence A. Carr, Dept. of Pharmacology, Univ. of Louisville Sch. of Med., Louisville, KY 40292.

It has generally been found that chronic nicotine administration to rodents leads to an increase in the number of nicotine binding sites in most brain areas which have been investigated. The explanation for this effect is that chronic nicotine causes receptor desensitization resulting in a consequent upregulation. Many of nicotine's effects are thought to result from the nicotine-induced stimulation of mesolimbic dopaminergic neurons, particularly those terminating in the nucleus accumbens; however, the effects of chronic nicotine on the density of nicotine binding sites in this tissue have not been investigated. In this study, rats were administered nicotine (3 mg/kg/day) for two weeks via osmotic minipumps, after which [^{3}H]-methylcarbamylcholine binding in the nucleus accumbens and amygdala was determined. The ability of 10 μM nicotine to release [^{3}H]-dopamine from these brain areas was also investigated using *in vitro* superfusion. It was found that there were no differences between control and nicotine-treated animals with respect to the Bmax for [^{3}H]-MCC binding in either tissue, nor were there differences in nicotine-evoked dopamine release. These results indicate that chronic nicotine treatment does not induce receptor upregulation nor does it alter dopamine release in the mesolimbic system as it does in some other brain areas. This supports evidence that tolerance to the reinforcing properties of nicotine, thought to be mediated in part via mesolimbic neurons, may not develop with chronic nicotine use. (Supported by a grant from the Tobacco and Health Research Institute.)

242.3

CHRONIC SUBSTANCE P INCREASES MUSCARINIC RECEPTOR BINDING IN RAT CORPUS STRIATUM BUT NOT IN FRONTAL CORTEX. NW Pedigo, JS Fu*, MA Rice*, and JK Rowlett*. Depts Pharmacology, Anesthesiology and Psychology, Univ. of Kentucky Med. Ctr., Lexington, KY 40536.

The neuropeptide substance P (SP) is widely distributed in mammalian brain, including corpus striatum (CS), where it provides a major excitatory output pathway, and frontal cortex (FC), where it may coexist with acetylcholine. The purpose of this study was to evaluate possible neuromodulation by SP of postsynaptic muscarinic receptors or function. In vitro SP (final concentration = 0.1 μM) did not directly alter binding of ^{3}H-quinuclidinyl benzilate (^{3}H=QNB) or carbachol-stimulated phosphoinositide (PI) hydrolysis in rat FC. In vivo treatment of rats with repeated intracerebroventricular (icv) injections of SP (50 ng/2 μl, 2X/day for 10 days) did not alter muscarinic receptor binding or function in FC. However, chronic icv SP did elevate ^{3}H-QNB binding density (B_{max}) in CS (2095 ± 54 vs 1730 ± 55 fmol/mg protein for SP- and vehicle-treated rats, respectively; $p<.001$). No change in receptor affinity was noted (K_d's = 46 vs 50 pM). This effect may be due to SP actions within CS or due to SP activation of dopaminergic nigrostriatal neurons which, in turn, inhibit cholinergic neurons in CS. Subsequent decreased cholinergic activity could lead to muscarinic receptor up-regulation.

242.4

Effect of chronic neuroleptic treatment on central nervous system muscarinic receptors. **T.A. Cawley, Jr. and G.R. Luthin, Dept. of Physiology and Biophysics and Institute for Neuroscience, Hahnemann University, Philadelphia PA 19102.**

Clozapine is an atypical neuroleptic agent that has a much-decreased frequency of motor side effects when compared to typical neuroleptics. Clozapine has a high potency to inhibit both muscarinic and dopamine receptor activity in binding studies. This is consistent with clinical observations that motor side effects, due to chronic treatment with typical neuroleptics, may be reduced by co-administration of anticholinergic drugs. Administration of anticholinergic drugs generally produces increases in muscarinic receptor levels in brain. Therefore, we hypothesize that the decrease in motor side effects during chronic clozapine treatment is due to its ability to act as an antagonist to modulate muscarinic receptor levels. It was the general purpose of this study to measure levels and distribution of muscarinic receptor subtypes following chronic administration of typical and atypical neuroleptics and anti-cholinergic agents in rats. Animal treatment groups consist of the following: clozapine (20 mg/kg), atropine (20 mg/kg), fluphenazine (12.5 mg/kg), fluphenazine/atropine or saline (0.5 ml). All animals were chronically treated for 14 days. Striata were dissected from the brains and immediately frozen in liquid nitrogen and stored at -80°C. Receptors were solubilized using a digitonin/cholate method. Antibodies to muscarinic receptor subtypes were used in immunoprecipitation assays to assess the effect of the treatments to modulate the levels of the different subtypes. Binding assays demonstrated the expected increase in the total muscarinic receptor density in the atropine and fluphenazine/atropine groups but no increase in the clozapine group. The increases due to atropine treatment could be accounted for by increases in the m1 receptor subtype. No alterations in subtype levels were seen with clozapine. This study suggests that the clinical profile of clozapine activity may not be related to its anti-cholinergic activity.

Supported by Scottish Rite Schizophrenia Research Program Grant to G.R.L

242.5

CHRONIC ESTRADIOL TREATMENT INCREASES ANTERIOR PITUITARY BUT NOT STRIATAL D-2 DOPAMINE RECEPTOR mRNA LEVELS IN RATS. D. Lévesque[1], B .Gagné*[2], N. Barden[2], and T.Di Paolo[1], [1]Dept. Mol. Endocrinol., CHUL Research Centre and School of Pharmacy, Laval University, Québec, G1K 7P4, and [2]Dept. Molecular Psychogenetics, CHUL Research Centre, Laval University, Québec, Canada, G1V 4G2.

Much evidence suggests that estrogens modulate central dopaminergic system activity and the most interesting aspect of this interaction is the effect of estrogens on dopamine (DA) receptors. However, the mechanism underlying this interaction is yet to be fully understood. In this report, we investigated the effect of chronic 17β-estradiol (E2) treatment (10 µg, b.i.d., for 2 weeks) on D-2 DA receptor mRNA levels in striatum and anterior pituitary tissues. We used ^{32}P-labeled probes specific for D-2 receptor and β-actin mRNAs in Northern blot analysis. We observed that the ratio of D-2 DA receptor mRNA/β-actin mRNA level was increased in anterior pituitary tissue of E2-treated rats compared to vehicle-treated animals (70% increase, $p<0.01$). Anterior pituitary weight and protein content were also increased in E2-treated animals, but β-actin mRNA levels remained constant. The D-2 DA receptor mRNA/β-actin mRNA ratio in the striatum was not affected by E2 treatment. However, the medial striatum showed a significantly lower ratio compared to the lateral striatum ($p<0.01$) in both vehicle- and E2-treated rats. Thus, E2 effects on anterior pituitary D-2 receptors may implicate transcriptional regulation, while these results support the hypothesis of an alternative mechanism of action for E2 in the striatum, and which may implicate synaptic membrane components. This work was supported by the MRC of Canada.

242.6

DOPAMINE D1 AND D2 RECEPTORS IN ADULT RAT BRAIN AFTER NEONATAL 6-OHDA TREATMENT: AN AUTORADIOGRAPHIC STUDY. F. Radja*, K.M. Dewar T.A. Reader and L. Descarries. Centre de recherche en sciences neurologiques (Départements de physiologie et de psychiatrie), Université de Montréal, Montréal (Québec), CANADA H3C 3J7.

A membrane binding study has already demonstrated an increased number of dopamine (DA) D2 but not D1 receptors in the neostriatum of adult rats subjected to neonatal destruction of the nigrostriatal DA system by intraventricular 6-OHDA (Dewar et al., *Brain Res.*, 536:287, 1990). In the present study, the regional distribution of D1 and D2 receptor binding was investigated by quantitative autoradiography, 3 and 6 months after the neonatal 6-OHDA lesion. The selective antagonists [^{3}H]SCH23390 and [^{3}H]raclopride were used as radioligands for the D1 and the D2 receptors, respectively. In all lesioned rats, the DA content of the neostriatum was reduced by more than 90% of control values. Three months after the lesion, the density of [^{3}H]raclopride binding was increased by approximately 30% over controls in both the rostral and the caudal neostriatum. A similar increase in D2 receptors was found at 6 months post lesion. In the substantia nigra examined at 6 months, there was an obvious reduction of D2 receptor density. In contrast, there were no apparent changes in D1 receptor density in either the neostriatum nor the substantia nigra, as observed after 3 months. These results confirm the up-regulation of D2 receptor binding previously described in the rostral neostriatum, 3 months after the neonatal, nigrostriatal DA lesion. They also indicate that this up-regulation takes place in the caudal as well as the rostral neostriatum and suggest that it is a permanent phenomenon. The reduction in nigral [^{3}H]raclopride binding is consistent with a somato-dendritic localization of the D2 receptors on the DA neurons in this part of the brain. (Supported by MRC grants MT-3544 and MT-6967).

242.7

D1 AND D2 RECEPTORS DO NOT UP-REGULATE IN RESPONSE TO NEONATAL INTRASTRIATAL 6-HYDROXYDOPAMINE (6-OHDA) LESIONS. B.S. Neal and J.N. Joyce. Departments of Psychiatry and Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

In adult rats, extensive lesions of DA neurons produce profound behavioral impairments which mimic changes observed in Parkinson's disease. This syndrome is not seen following neonatal lesions. Adult-lesioned rats are supersensitive to D2 agonists and antagonists, whereas neonatally-lesioned rats are supersensitive to D1 agonists and subsensitive to antagonists (Neal and Joyce, Devl. Brain Res., in press). Therefore, differential receptor regulation following adult vs. neonatal lesions would be expected. Adult lesions cause an increase in D2 receptor density, with either no change or a decrease in D1 density (Joyce, Expt. Neurol., in press). No clear-cut results have been forthcoming with neonatal lesions. Conflicting findings have been reported for both D1 and D2 binding depending upon the degree of DA depletion and the age at lesioning. To determine what effect the extent of DA depletion had on receptor regulation, we administered a range of 6-OHDA doses (0-20 µg/striatum) bilaterally into the striatum on day of birth or postnatal day 1. Quantitative receptor autoradiography revealed a dose-dependent loss of DA uptake sites (^{3}H-mazindol) and µ-opioid receptor patches (^{3}H-naloxone) in adult striatum, as a consequence of neonatal 6-OHDA lesions. Doses of 2-16 µg of 6-OHDA caused an approximate 10% loss of D1 binding (^{3}H-SCH23390) in the central and medial portions of the striatum; 20 µg caused a greater loss (up to 24% in the central striatum). D2 binding (^{125}I-epidepride) did not increase; in fact, there was a slight decrease (5-10%) in the central and medial striatum, which was not dose-dependent. These results suggest that D1 and D2 receptors are affected differently by DA depletion during development or in adulthood. Supported by grants from the Tourette Syndrome Association and USPHS (MH09888 and HD26979).

242.8

MECHANISMS UNDERLYING THE DEVELOPMENT OF DA RECEPTORS SUPERSENSITIVITY: ROLE OF G-PROTEINS. C. Ventra, T. Florio, S. Talia*, E. Cocozza*, A. Avallone*, G. Schettini. Dept. of Pharmacology, II School of Med., Naples, Italy.

We have shown an enhanced response of striatal adenylate cyclase activity (AC) to GTP in up-regulated D1 and D2 receptors, suggesting the involvement of G-proteins in the development of DA receptors supersensitivity. Here, we used NaF, an agent activating AC via Gs, that, unlike GTP, stimulated striatal AC by the same extent in saline and 21 days neuroleptic-treated rats. To evaluate whether the increased response to GTP is due to an enhanced expression or functioning of G-proteins, we tested the effect of 21 days Haloperidol and SCH 23390 on striatal mRNA encoding Gs and Gi alpha subunits. Unexpectedly, the only difference observed, so far, was in the levels of Gi1 mRNA following SCH treatment, while no changes in Gs mRNA levels were observed. [3H]-GTP binding studies are in progress to detect possible modifications in the GTP-binding activity of supersensitive DA receptors. Finally, no changes in AC-stimulated by MnCl2 or forskolin were detected, thus excluding the involvement of the catalyst in the development of DA receptors supersensitivity.

242.9

EFFECT OF LONG-TERM HALOPERIDOL TREATMENT ON DOPAMINE-INDUCED INOSITOL PHOSPHATE FORMATION IN RAT BRAIN SLICES. R.Li,* L.L.Wing, R.J.Wyatt, D.G.Kirch and D.-M.Chuang. Neuropsychiatry Branch, NIMH, Neuroscience Center at St. Elizabeths, Washington, D.C. 20032 and Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

Although it is firmly established that haloperidol blocks dopamine receptors, the actual mechanism of its antipsychotic action remains unclear. Previous studies have shown that in vitro stimulation of dopamine D1 receptors can enhance phosphoinositide (PI) turnover in the brain. We examined dopamine receptor-mediated PI turnover in rats treated with saline or haloperidol (1.5mg/kg/day, IM) for 6 weeks. Basal and dopamine stimulated PI hydrolyses were assayed by lithium-dependent accumulation of ^{3}H-inositol monophosphate (IP_1) in rat brain slices pre-labeled with ^{3}H-myo-inositol. Our results showed that: (1) basal PI turnover was unchanged after haloperidol treatment: (2) dopamine stimulation in vitro significantly increased PI hydrolysis in striatum, hippocampus and frontal cortex; (3) long-term haloperiodol treatment significantly attenuated dopamine-induced ^{3}H-IP_1 accumulation in striatum and frontal cortex ($P<0.05$) and to a lesser extent in hippocampus. The relationship between dopaminergic receptors and PI turnover will be discussed.

242.10

D_2 DOPAMINE RECEPTOR REGULATION IN THE INTERMEDIATE LOBE OF THE RAT PITUITARY. Daniel S. Dickerson*, Belinda S. Pratt*, William R. Millington and Bibie M. Chronwall. University of Missouri-Kansas City, School of Basic Life Sciences, Kansas City, MO 64108.

Peptide secretion from the rat intermediate lobe is regulated by D_2 dopamine receptors. This study demonstrates the change in D_2 dopamine receptor kinetics in conjunction with changes in D_2 mRNA level in the intermediate lobe of the rat pituitary following chronic treatment (2mg/kg/d, 12d) with the D_2 receptor antagonist haloperidol and agonist bromocriptine. Receptor autoradiography using emulsion coated coverslips apposed to slide-mounted tissue sections was employed to assay the effects of haloperidol and bromocriptine on the D_2 receptor population. ^{3}H-Spiperone (0.04 nM to 0.4 nM) was used as the D_2 binding ligand and butaclamol (3.0 nM) as the D_2 competitive ligand. Analysis of the D_2 receptor kinetics indicates that haloperidol treatment leads to a statistically significant increase in B_{max} (from 5.63 ± 0.68 fmol/µm^2 to 10.12 ± 1.12 fmol/µm^2) and an increase in K_d (from 525 ± 134 to 839 ± 140) as compared to control values. Bromocriptine treated animals show no change in B_{max} (6.16 ± 0.46 fmol/µm^2) or K_d (591 ± 71).

Radioactive and non-radioactive *in situ* hybridization of D_2 receptor mRNA levels indicate that D_2 mRNA levels increase 50% following chronic haloperidol treatment with no change following chronic bromocriptine treatment. This parallel change in receptor concentration and affinity as well as mRNA levels follows the classic paradigm by which a cell, in response to an antagonist, up-regulates the receptor population.

242.11

AMYGDALA KINDLING ALTERS STRIATAL AND EXTRA-STRIATAL DOPAMINE D2 RECEPTOR REGULATION UNILATERALLY. A. Janowsky, K.A. Neve, L.A. O'Toole*, J.K. Belknap, and C.D. Applegate. VAMC and Oregon Health Sci. Univ., Portland, OR., and Univ. of Rochester Sch. of Med., NY

Previous reports indicate that hippocampal and (or) amygdala kindling alters dopamine D2 receptor density in the nucleus accumbens. We now report that amygdala kindling produces long-term unilateral changes in dopamine D2 receptor regulation in striatum. Extrastriatal brain regions that contain a very low density of neurotransmitter binding sites were also examined. Electrodes were implanted into the left amygdala and rats were kindled to 6 consecutive stage 5 seizures. Animals were sacrificed 30 days after the last seizure, and 16μm sections from frozen brain were incubated with [^{125}I]epidepride (40 to 76 pM) in the presence and absence of spiperone (1 μM) for autoradiographic determination of dopamine D2 receptor density. Analysis of tissue sections indicates that there is a 20% to 50% increase in specific radioligand binding on the side ipsilateral to the kindled site, as compared to the contralateral side. Changes were most obvious in lateral neostriatum. Binding to nucleus accumbens, hippocampus, cortex, hypothalamus, amygdala, and other extrastriatal regions is being analyzed. The long term effects of kindling suggest the involvement of altered genetic expression, and experiments concerning the temporal aspects of, and the molecular mechanisms involved in the receptor changes are now in progress. *Supported by NIMH, NIAAA, and VA Merit Reviews.*

242.12

NEUROCHEMICAL BASIS FOR THE ABSENCE OF OVERT STEREOTYPED BEHAVIORS IN RATS WITH UP-REGULATED STRIATAL D2 DOPAMINE RECEPTORS (DAr). JZ Fields, GE Drucker, L Wichlinski & JH Gordon. Res Svce, VA Hosp, Hines IL 60141; Dept Pharm, Loyola U Med Sch.

Because of substantial evidence for the hyperdopaminergic hypothesis of tardive dyskinesia (TD), animal models, especially rats, treated chronically with neuroleptics are used to study this disorder. The rat model has been criticized because, unlike TD, there is an apparent lack of spontaneous abnormal movements even when striatal D2 DAr density is increased. Our data suggest a mechanism by which rats suppress these abnormal movements normally associated with elevated DAr. We correlated neurochemical with behavioral changes using several animal models, including non-neuroleptic ones, which elicit varied levels of DAr upregulation. There was (as expected) a significant robust, *positive* correlation between striatal DAr density and apomorphine-induced stereotypy. In contrast, there was a significant *negative* correlation between increased DAr and synthesis capacity for striatal DA (Vmax for tyrosine hydroxylase). We conclude that this decrease in Vmax is compensation by the nigrostriatal DA tract for the increased DAr density induced in our animal models. Our data suggest that an observed increase in receptor density does not necessarily predict a functional change (spontaneous behavior, neuropathology) because compensatory mechanisms exist. In TD this compensation may fail, leading to spontaneous abnormal behaviors. (Supported by VA Med Res & Ro1NS26449).

242.13

PREVENTION AND REVERSAL OF DOPAMINE RECEPTOR (DAr) SUPERSENSITIVITY (SS) BY CYCLO(LEU-GLY) (CLG). GE Drucker, RF Ritzmann[a], LJ Wichlinski, JH Gordon & JZ Fields. Res Svce 151, VA Hospital, Hines IL 60141; Dept Pharm, Loyola U Med Sch, Maywood IL; [a]Psych Svce, VA Med Ctr, Sepulveda CA

Chronic administration (21 d) of haloperidol (HAL) (IP, 1.0 mg/kg/d) induced a behavioral SS to dopamine (DA) agonists (stereotypic sniffing) and up-regulation of striatal and limbic D2 DAr. Co-administration of CLG (8 mg/kg, SC, every third day, every other day, or every day) with HAL attenuated the behavioral SS except for CLG every day. D2 DAr binding assays showed CLG-induced changes parallel to these behavioral changes, suggesting that CLG's ability to prevent behavioral SS to DA agonists involves a decrease in the total number (Bmax) of D2 binding sites. DAr binding changes also suggest that the biphasic behavioral dose response curve involves a failure of CLG at the highest cumulative doses to reverse the elevated density of D2 DAr. CLG was also able to reverse an already established DAr up-regulation, since a similar down-regulating effect of CLG on behavior and binding was observed when CLG was injected daily (8 mg/kg, SC) for 4 days during the period of withdrawal from HAL. Chronic CLG by itself had no significant effects on behavior or binding. These data suggest that CLG may be useful, within a therapeutic window, in disorders that are thought to involve up-regulated striatal or limbic DAr including tardive dyskinesia, L-DOPA induced dyskinesias, and schizophrenia. (Supported in part by VA Med Res & Ro1NS26449)

242.14

CROSS-SENSITIZATION BETWEEN D1 AND D2 DOPAMINE AGONISTS: EFFECT OF A SINGLE INTRA-ACCUMBENS INJECTION ON SUBSEQUENT LOCOMOTOR ACTIVITY IN MICE. C. Messier, O. Mrabet*, and C. Destrade. Laboratoire de Psychophysiologie, URA CNRS n°339, Université de Bordeaux I, 33405 Talence Cédex, France. *Département de Biologie, Faculté des Sciences, BP 20, ElJadida, Morocco

We tested the effect of a single unilateral injection of a specific D1 agonist into the nucleus accumbens on the behavioral response to a subsequent unilateral intra-accumbens injection of a selective D2 agonist ten days later. The effect of the inverse order of presentation (D2 agonist followed ten days later by a D1 agonist) was also tested. No significant differences between the locomotor effects of the intra-accumbens injection of either SKF-38393 (3.5 µg) or LY-171555 (10 µg) were observed during the first test. Ten days later, during the second test, intra-accumbens injection of both the LY-171555 and SKF-38393 increased the percentage of contralateral rotations relative to the first test while LY-171555 also increased the total number of rotations. Control injections showed that these effects of LY-171555 and SKF-38393 were not due to a conditioning process. Rather, the results suggested that the locomotor changes observed during the second test were the result of behavioral sensitization due to the initial acute injection of the agonists.

CARDIOVASCULAR REGULATION: BRAINSTEM MECHANISMS I

243.1

PROJECTIONS FROM THE PERIAQUEDUCTAL GRAY (PAG) TO THE PERIAMBIGUAL AREA: RELATION TO VAGAL OUTPUT NEURONS. M. Ennis, T.A. Rizvi, M.T. Shipley, M.M. Behbehani, D. Smith, E.J. Van Bockstaele, P. Luppi and G. Aston-Jones. Univ. Cinti. Coll. Med.; Hahnemann Univ.

Activation of PAG elicits selective pressor and depressor responses. PAG projections to the rostral ventrolateral medulla sympathoexcitatory zone is a likely substrate for PAG-evoked pressor responses. Circuits mediating PAG-evoked depressor responses are less clear. Here, we report PAG projections to nucleus ambiguus (NA) and the periambigual area (pNA) in rat.

WGA-HRP and PHA-L injections into lateral and ventrolateral PAG labeled a rostrocaudally oriented, longitudinal fiber plexus surrounding, and occasionally within, NA. Labeled fibers increase along the rostral to caudal axis of pNA. These injections also labeled neurons in pNA and, less frequently, in NA. Injections into dorsomedial, dorsolateral, or ventromedial PAG produced only sparse fiber labeling in NA and pNA. WGA-HRP injections into the vagus nerve retrogradely labeled longitudinally organized columns of neurons in NA and pNA. The distribution of pNA cells labeled after vagus nerve injections appeared to substantially overlap with the fibers anterogradely labeled from PAG.

These results indicate that PAG has reciprocal connections with NA and pNA. pNA contains preganglionic parasympathetic neurons that project to the heart (Bieger and Hopkins, 1987). Experiments in progress will determine if PAG innervates identified vagocardiac neurons and if this pathway mediates PAG-evoked depressor responses. (Support: PHS Grants HL08097, NS20463 and NS24698).

243.2

HEMODYNAMIC RESPONSES PRODUCED BY MICROINJECTION OF [D-ala^2-met^5]ENKEPHALINAMIDE (DAME) IN ROSTRAL MEDULLA OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR). L. R. Portis and R. L. Tackett. Department of Pharmacology and Toxicology, Cardiovascular Pharmacodynamics Laboratory, College of Pharmacy, University of Georgia, Athens, GA 30602.

Previous studies have shown that stimulation of opiate receptors in rostral ventrolateral medulla (RVLM) by an enkephalin analogue produces hypotension and bradycardia in normotensive rats. The present study was designed to determine what, if any, hemodynamic changes are produced by microinjection of the enkephalin analogue [D-ala^2-met^5]enkephalinamide (DAME) into RVLM of genetically hypertensive animals. Experiments were performed on pentobarbital anesthetized spontaneously hypertensive (SH) rats instrumented for measuring arterial pressure and heart rate and ventilated artificially. A pulsed doppler flowprobe was placed on the abdominal aorta for measurement of hindlimb blood flow. The RVLM was identified by bilateral microinjection of L-glutamate (300 ng/side). Bilateral injection of DAME (100 ng/side) in RVLM produced a decrease in arterial pressure (-41 ± 9 mmHg). No significant change in heart rate was observed. Hindlimb vascular resistance was decreased 19% from baseline. These data suggest that in SH rats bilateral microinjection of the enkephalin analogue DAME produces a decrease in arterial pressure and hindlimb vascular resistance.

243.3

EVIDENCE THAT STIMULATION OF OXYGEN SENSORS IN VENTRAL MEDULLA INITIATES THE CEREBRAL ISCHEMIC RESPONSE. M.-K. Sun and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Brief (<5 sec) ischemia of the brainstem elevates arterial pressure (AP) secondary to excitation of sympathoexcitatory reticulo-spinal neurons of the rostral ventrolateral medulla (RVL-SE neurons), and elicits bradycardia and apnea: the cerebral ischemic response (CIR). The adequate stimulus for the CIR is unknown. Here we investigated whether it could be hypoxia. In anesthetized paralyzed rats the activity of RVL-SE neurons was recorded extracellularly. In sino-denervated rats, systemic hypoxia (pO_2=27.3±1.8 mmHg) excited RVL-SE neurons (by 122.5±18.4% increase in firing rate from 19.3±3.1 spikes/s, n=7). In contrast, hypercarbia (pCO_2=54 mmHg, pH=7.11) or extracellular iontophoresis of hydrogen ions did not produce a comparable change of RVL-SE neuron activity nor AP. H^+ however attenuated the GABA-mediated baroreceptor inhibition of these neurons. Iontophoresis of cyanide (CN) or sulfide onto RVL-SE neurons elicited a dose-dependent, site specific, rapid (<2 sec), reversible increase in neuronal activity (2-fold increase, n=29). Microinjections of cyanide increased AP and reduced phrenic nerve discharge. Adjacent respiratory neurons were inhibited by CN. We conclude that hypoxia, but not acidosis or hypercarbia, may be the main stimulus for the sympathoexcitatory component of the CIR. RVL-SE neurons themselves may be O_2 sensors.

243.4

CENTRAL RESETTING OF THE ARTERIAL BAROREFLEX IN RENAL HYPERTENSIVE RATS. M.Hay, L. F. Hayward, J. A. Smith and R. B. Felder. Cardiovascular Center, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Previous studies have shown impairment of baroreflex control of arterial pressure (MAP), heart rate (HR), and sympathetic nerve activity in animals with some forms of experimentally induced hypertension. The present study was designed to determine the effect of Grollman hypertension on the central regulation of baroreflex function. MAP and HR responses to aortic depressor nerve (ADN) stimulation were compared in anesthetized, age-matched control and 21 day post figure-8 Grollman hypertensive Sprague Dawley rats. Renal hypertension resulted in a shift in the baroreflex mediated MAP and HR responses upward and to the right. In control rats (n=4), resting MAP and HR were 106.2 ± 9 mmHg and 372.3 ± 27 beats/min, respectively. Stimulation of the ADN (8.0 V, 2.0 msec, 1.0 min) at 2, 5, and 25 Hz resulted in a 30, 44 and 82 beat/min. decrease in HR and a 24, 39 and 49% decrease in MAP. Resting MAP and HR in the renal hypertensive rats (n=3) were 121.7 ± 6 mmHg and 332 ± 49 beats/min, respectively. The ADN stimulation resulted in a upward parallel shift of the MAP response as well as a significant attenuation of the HR response as compared to the control animals. ADN stimulation at 2, 5, and 25 Hz resulted in only a 3, 10 and 28 beat/min decrease in HR and a 4, 29 and 44% decrease in MAP. These results suggest that the central regulation of baroreflex function is impaired in chronic renal hypertensive rats.(Supported by HL29302)

243.5

ARTERIAL BARORECEPTORS AND CNS HISTAMINE CONTRIBUTE TO THE BRADYCARDIA DURING PERIPHERAL HYPEROSMOLALITY. M. J. Kenney and S.L.Bealer. Rhodes College and University of Tennessee, Memphis, TN 38112.

Peripheral hyperosmolality produced by infusion of 2.5M NaCl (10µl/100g/min) hypertonic saline (HTS) increases mean arterial pressure (MAP) and reduces heart rate (HR). The current study tested the hypothesis that decreases in HR observed during HTS infusion in conscious rats are dependent on afferent baroreceptor mechanisms and involve central histamine. HTS infusion increased MAP (26±3 mmHg) and reduced HR (54±10 mmHg) in rats (n=5) with intact arterial baroreceptors whereas in sino-aortic denervated rats (n=5), HR remained unchanged despite a similar increase in MAP. After the central (icv) administration of cimetidine (specific H_2 antagonist) in intact rats, HTS infusion increased MAP (20±3 mmHg), however, the reduction in HR (18±6 mmHg) was significantly attenuated. In contrast, central cimetidine did not alter the HR responses to peripheral phenylephrine and nitroprusside. These results suggest that the bradycardia accompanying HTS infusion is mediated through the arterial baroreceptor reflex and involves a selective histaminergic pathway.

243.6

THE ROLE OF REFLEX HYPOTENSION IN ACUTE RESETTING OF THE CAROTID BAROREFLX IN RABBITS. L. Hayward, M. Hay and R.B. Felder Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Previous work has shown that carotid sinus baroreflex control of arterial pressure is acutely reset by electrical stimulation of the aortic baroreceptors (*FASEB J.* 5,1033, 1991). The present study examined the role of reflex hypotension associated with this resetting protocol. Reflex changes in mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) were recorded during unilateral ramp increases (0-200 mmHg) in carotid sinus pressure (CSP) in anesthetized, vagotomized rabbits with bilaterally isolated carotid sinuses. Acute resetting was induced by 5 minutes of electrical stimulation of both aortic depressor nerves (ADN) cut distal to the stimulating electrodes (25 Hz, 10 V, 0.2 ms). In the same animal, reflex characteristics were compared: 1) before and after acute resetting alone and; 2) before and after acute resetting with resting MAP maintained during ADN stimulation with phenylephrine (PE) infusion (15 µg/ml over the first 4 min. of ADN stimulation). Resetting protocols were separated by 40-60 minutes. In 4 animals, 1 minute following ADN stimulation alone, the curve relating MAP to CSP was shifted upward and to the right. This shift was characterized by significant increases ($p<0.05$) in the resting MAP (79±7 to 91±8 mmHg; mean±SE; control vs reset), mid-range CSP (116±7 to 124±9 mmHg CSP), and minimum MAP (39±6 to 47±10 mmHg); there was also a decrease in the resting RSNA (92±14 to 63±5 imp/sec). All values returned to control in 10 minutes. There were no significant differences between these "reset" reflex characteristics and those induced by ADN stimulation with PE maintained pressure, demonstrating that reflex hypotension does not significantly influence acute resetting of the carotid sinus baroreflex. These findings also provide further support for the concept of a central change in the processing of baroreflex information during acute resetting. (Support: HL29302, HL44546).

243.7

UNEQUAL EFFECT OF CHEMORECEPTOR ACTIVATION ON THE CAROTID SINUS BAROREFLEX IN RABBITS. Long Qu and S.L. Stuesse. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

Carotid sinus baroreceptors influence various components of the carotid sinus baroreflex unequally when carotid sinus pressure (CSP) is increased. We examined the effect of chemoreceptor activation on the unequal control. In rabbits anesthetized with urethane, the aortic depressor nerves and vagus nerves were cut, and isolated carotid sinuses were bilaterally perfused with physiological saline. CSP was increased in steps from 50 to 200 mmHg while heart rate (HR), cardiac contractile force, renal nerve activity (RNA), and hindlimb perfusion pressure (HPP) were recorded. Stimulus-response curves were constructed for control (95%O_2 - 5%CO_2) and 6%CO_2 perfusates, and the slopes of the curves were calculated. In controls, the sum of the gains of the reflex changes is maximal (1.62) when CSP is 90 mmHg. HR and HPP were very sensitive to changes in CSP. In high CO_2, the total baroreflex activity was attenuated at middle and high CSP levels (> 80 mmHg) as indicated from the sum of the reflex gains (1.3). However, the gains of HR and RNA were increased. This suggests that hypercapnia influences the regulatory mechanism of baroreceptors on cardiovascular components in a nonlinear fashion. Supported by a grant from the American Heart Association.

243.8

PARAVENTRICULAR EFFERENTS INFLUENCE AREA POSTREMA NEURONS. P. Smith* and A.V. Ferguson. Dept. of Physiology, Queen's University, Kingston ONT. Canada K7L 3N6.

The rat area postrema (AP) is a midline circumventricular organ (CVO) situated at the level of the obex on the floor of the fourth ventricle. Previous electrophysiological studies from our laboratory have demonstrated AP neurons to be influenced by changes in blood pressure, circulating peptides such as angiotensin, endothelin, and vasopressin, and parabrachial nucleus stimulation. Anatomical tracing studies have demonstrated that a major afferent input to the AP is provided by the paraventricular nucleus (PVN).

In order to investigate the functional nature of such neural connections sodium pentobarbitol anaesthetized male Sprague Dawley rats were fitted with femoral arterial and venous catheters and endotracheal tubes. A bipolar stimulating electrode was positioned in PVN using stereotaxic coordinates and fixed into position using dental cement. The AP was exposed by a midline incision dorsal to the medulla and a glass recording electrode (tip diameter <1µm, resistance 10 - 30 Mω) was placed on the surface using a micromanipulator. Extracellular single unit recordings were obtained from AP cells and peristimulus histograms were used to determine the effects of PVN stimulation on these neurons. Of the 13 AP cells tested 38.5% were excited by PVN stimulation while the remainder were unaffected. In animals that had stimulation sites outside of PVN 12.5 % of AP cells tested were inhibited by such stimulation while the remaining 87.5% were unaffected.

These data demonstrate that activation of PVN neurons elicit excitatory effects on cells in the AP. They thus further emphasise the potential significance of descending hypothalamic inputs in controlling neuronal activity in this CVO.

Acknowledgements: Supported by the Medical Research Council of Canada

243.9

RENAL AFFERENT INPUT TO THE VENTROLATERAL MEDULLA OF THE CAT
M. A. Vizzard, A. Standish and W. S. Ammons, Department of Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

Experiments were performed to determine the effect of renal nerve stimulation on neurons within the rostral ventrolateral medulla. Extracellular action potentials were recorded from 91 cells within the rostral ventrolateral medulla of alpha-chloralose anesthetized cats. Cells were tested for responses to electrical stimulation of both left and right renal nerves. Renal nerve stimulation excited eighty-nine cells (97.8%) and inhibited two (2.2%). Forty cells (43.9%) responded to both left and right renal nerve stimulation. Forty-six cells (50.5%) responded to electrical stimulation of renal nerves on the opposite side and five cells (5.5%) responded to electrical stimulation of renal nerves on the same side. For cells which received bilateral excitatory renal nerve input, a conditioning-test paradigm revealed that a stimulus to renal nerves on one side decreased the response elicited by a stimulus applied to the renal nerves on the opposite side for at least 300 msec. All cells had somatic receptive fields that included the forelimbs, hindlimbs, abdomen and flank. Twenty-four cells with short latency responses to renal nerve stimulation required stimulus intensities greater than the A-delta volley threshold but less than the C-fiber volley threshold of the compound action potential recorded from the least splanchnic nerve. Fourteen cells exhibited both early and late responses to electrical renal nerve stimulation. The threshold of the late response was always greater than the threshold for the C-fiber volley in the compound action potential. Thus it was concluded that C-fibers mediate the late responses to renal nerve stimulation. Twenty-one cells (26.7%) responded to activation of renal mechanoreceptors or chemoreceptors. Occlusion of the renal vein or ureter increased the activity of nine cells from 21.4±7.8 imp/s to 47.6±12.8 imp/s and decreased the activity of eight cells from 14.8±2.6 imp/s to 4.9±1.4 imp/s. Renal artery occlusion increased the activity of twelve cells from 17.8±7.8 imp/s to 40.8±11.6 imp/s. These studies demonstrate that the rostral ventrolateral medulla is a supraspinal site to which information from both kidneys projects and that cells receiving renal input also receive convergent input from somatic structures. Furthermore, the demonstration that mechanical stimulation of renal receptors elicits responses from cells within the rostral ventrolateral medulla suggests a functional significance to this renal afferent input. The existence of a functional afferent connection between the kidneys and the ventrolateral medulla suggests that the rostral ventrolateral medulla may play a role in supraspinal reflexes of renal origin. [Supported by AHA, national affiliate and PA affiliate]

243.10

MYELINATED AND NONMYELINATED VAGAL AFFERENTS PROJECTION TO THE INSULAR CORTEX IN RATS.
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I have shown the projection of vagal C-afferent to the rostral insular cortex. Further analysis on A-afferent are presented here. SD rats anesthetized with sodium amobarbital or alpha-chloralose were used. Microelectrodes were placed at the solitary tract nucleus (NTS) and the cortex to record field potentials (FPs) evoked by cervical vagus nerve stimulation. Under chloralose anesthesia, A-afferent activation resulted in surface-recorded positive-negative FPs with the latency around 40ms in the granular insular cortex (GI). Barbital diminished this response. Irrespective of the anesthetics, A-afferent activation produced FPs with 4-8ms latency at NTS, and stimulation of NTS produced positive-negative FPs in GI with 10-20ms latency. The latency of vagally evoked cortical FPs was longer than a simple sum of those of vagus-NTS response and NTS-GI response. These results indicate that vagal myelinated afferent projects to the cerebral cortex probably by way of thalamic relay nucleus and that this ascending system has certain structure at NTS extremely susceptible to barbital which is absent from C-afferent projection system. Supported by Uehara Foundation.

243.11

GLUTAMATE - IMMUNOREACTIVE TERMINALS IN THE NUCLEUS TRACTUS SOLITARIUS: ULTRASTRUCTURAL IMMUNOGOLD STUDY.
T.F.C. Batten* and S. Saha*. (SPON: European Neuroscience Association). Cardiovascular Studies and Physiology, The University, Leeds LS2 9JT, U.K.

Glutamate has been implicated as a neurotransmitter at the site of synaptic termination of vagal afferent fibres in the nucleus tractus solitarius (NTS), but little morphological evidence is available to support this hypothesis. Vibratome or cryo-sections of rat and cat brain stem and nodose ganglia, perfused fixed with 2% formaldehyde/ 2% glutaraldehyde, were incubated with anti-glutamate antibodies (Arnel and Incstar), and bound antibody was visualized by a biotin-avidin-peroxidase method for light microscopy (LM). Tissue was also processed for electron microscopy (EM), and ultrathin resin sections were incubated in anti-glutamate, followed by colloidal gold labelled IgG conjugate. Immunoreactive (IR) cell bodies of vagal afferent neurons were found in the nodose ganglion. At EM level the gold particles were at highest density over the smaller ganglion cells. Intense glutamate-IR cells were found in many brain stem nuclei, but not the NTS, which had a moderately dense matrix of fibre varicosities. Under EM, gold particles were associated with terminals forming mainly asymmetrical synapses on NTS neurons, and containing round synaptic vesicles. A combined neuronal tracing and EM immunogold study is underway to confirm the identity of glutamate-IR terminals in the NTS as vagal afferents.

243.12

HEMODYNAMIC EFFECTS OF NMDA ± MK-801 INFUSED INTO EITHER THE AREA POSTREMA OR THE NTS IN RATS.
D.K. Hartle and B. Tian, Department of Pharmacology and Toxicology, College of Pharmacy, University of Georgia, Athens, GA 30602.

The hemodynamic effects of microinfusing NMDA (10 ng/min x 3 min) into either the area postrema (AP) or unilaterally into the medial nucleus of the solitary tract (NTS) was tested in 8 Pb-anesthetized SD rats. NMDA infusion reduced mean blood pressure (MBP) 28% at area postrema and 31% at NTS. Analysis of regional hemodynamics in renal, mesenteric and iliac vascular beds revealed that while no significant changes in vascular resistance occurred during NMDA infusion into either AP or NTS, blood flow decreased significantly in the renal bed, but not in the mesenteric and iliac beds. MK-801 (1 nmol/50 nl/min) did not alter MBP, HR or regional hemodynamics by itself. This dose of MK-801 effectively blocked all the hemodynamic effects of NMDA at AP/NTS. In separate experiments, this dose and site of administration of MK-801 was shown to also significantly attenuate reflex bradycardia during pressor events elicited by intravenous phenylephrine. Conclusion: MK-801 infused into the AP/NTS of Pb-anesthetized rats produced no blood pressure or heart rate changes alone, but effectively blocked the cardiovascular effects of subsequent NMDA administration at this site. Supported by HLBI 37706 to DKH.

243.13

CHRONIC SINO-AORITC DENERVATION ELIMINATES INCREASES IN VASOPRESSIN AND MEAN ARTERIAL PRESSURE INDUCED BY NUCLEUS TRACTUS SOLITARIUS LESIONS IN RAT
A.M. McDonald and A.F. Sved, *Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

We previously reported (Neurosci. Abst. 98:12, 1990) that the pressor response elicited by bilateral microinjection of the $GABA_A$ agonist muscimol into the nucleus tractus solitarius (NTS) in chloralose-anesthetized, ventilated rats was absent following chronic sino-aortic denervation (SAD). Those results suggested that, upon removal of arterial baroreceptor afferents by SAD, NTS neurons involved in cardiovascular control are no longer tonically activated. To further investigate this phenonmenon the present study examined the effects of prior SAD on the typical responses of increased plasma vasopressin (VP) and hypertension that occur following bilateral electrolytic NTS lesion in conscious rats. One week following SAD or sham-denervation, baroreceptor reflexes (phenylephrine-induced bradycardia and nitroprusside-induced tachycardia) were tested in conscious, cannulated rats and were totally absent in SAD rats. One hour following reflex testing a baseline blood sample was collected to assess plasma levels of VP. Rats were then anesthetized with halothane and bilateral electrolytic lesions (1mA, 10sec) were placed in the NTS. Mean arterial pressure (MAP) and heart rate were recorded one hour later while rats were totally conscious. A second blood sample was then collected and completeness of lesion was confirmed by the absence of hypotension in response to bolus i.v. injections of phenylbiguanide (50µg/kg). In sham-denervated rats NTS lesions markedly increased MAP and VP levels($p<0.01$), but NTS lesions did not significantly alter MAP or VP levels in SAD rats.

	MAP (mmHg)		VP (pg/ml)	
	Pre	Post	Pre	Post
Control (n=7)	117±3	165±6	9±2	100±27
SAD (n=8)	123±3	108±6	13±5	27±7

These results are consistent with our previous data and lend additional support to the hypothesis that in chronic SAD rats NTS neurons modulating cardiovascular function are no longer tonically activated. Further, these data are inconsistent with the notion that remaining cardiopulmonary baroreceptor afferents are responsible for the restoration of normal average resting MAP in SAD rats.

243.14

THE HYPOTENSIVE EFFECT OF PURINES IN THE NUCLEUS OF THE SOLITARY TRACT IS ASSOCIATED WITH SELECTIVE GLUTAMATE RELEASE.
R. Mosqueda-Garcia, M. Appalsamy* and D. Robertson. Department of Pharmacology, Vanderbilt University, Nashville TN 37232.

We have previously reported that microinjection of purinergic substances into the nucleus of the solitary tract (NTS) evokes excitatory cardiovascular effects. These effects were blocked only by specific adenosine and/or excitatory amino acid receptor antagonists. In the present study we addressed whether perfusion of adenosine in the NTS affects cardiovascular function and the release of glutamate (GLU), glycine (GLY) or taurine (TAU) in the NTS.

New Zealand white rabbits (2.5-3.0 kg) were anesthetized with urethane and cannulated for direct recording of blood pressure (BP) and heart rate (HR). A microdialysis probe (1 mm membrane tip, 240 um o.d.) was implanted in the right NTS. Artificial cerebrospinal fluid (CSF) was perfused at a rate of 1 ul/min. After implantation of the probe, a stabilization period was followed by perfusion over 60 min with CSF containing either no adenosine or adenosine in concentrations of 10^{-6} or 10^{-3}M. Samples were analyzed for their GLU, GLY and TAU content using a HPLC system. After placement of the microdialysis probe in the NTS, a period of 120 min was allowed for stabilization.

Basal BP, HR and glutamate concentrations during CSF perfusion were 86 ± 3 mmHg, 258 ± 26 bpm, and 16 ± 3 umol/L, respectively (n=6). Perfusion of 10^{-6}M of ADO through the probe only slightly increased GLU levels by 31%. In contrast, 10^{-3}M adenosine increased GLU levels by 694%, decreased BP by 21 ± 10 mmHg and HR by 15 ± 8 bpm. No significant changes were observed in GLY or TAU levels. These results indicate that local release of glutamate is one mechanism by which purinergic substances in the NTS exert cardiovascular control.

243.15

INTRINSIC PROPERTIES OF MEDIAL NTS NEURONS IN SPONTANEOUSLY HYPERTENSIVE RATS. S.M. Johnson and R.B. Felder. Cardiovascular Center, Univ. of Iowa Coll. of Med., Iowa City, IA 52242.

Previous work has shown that spontaneously hypertensive (SH) rats have a central abnormality in the baroreflex control of arterial pressure. We tested the hypothesis that the intrinsic properties of neurons in the medial portion of the nucleus tractus solitarius (NTS) might be altered in hypertensive SH rats. An *in vitro* brainstem slice preparation was used to record intracellularly from 15 neurons in 5 month old SH rats and 25 neurons in 3-5 month old, non-hypertensive Fischer 344 (F344) rats. Comparison of SH and F344 medial NTS cells showed that there were no differences in resting membrane potential (-55 ± 4.0, -58 ± 7.5 mV [mean ± std.dev.]), action potential amplitude (85 ± 7.5, 79 ± 7.9 mV), action potential undershoot (7.0 ± 2.1, 9.8 ± 3.4 mV), and action potential duration (.93 ± .25, .78 ± .13 ms). The input resistance, however, was significantly greater for SH cells than for F344 cells (125 ± 70, 81 ± 38 MΩ). SH cells with a high input resistance had a nearly flat steady-state current-voltage relationship (-40 to -100 mV). In addition, more action potentials were expressed for the same amount of positive current injection. Spike frequency adaptation did not differ in SH and F344 cells, suggesting that the defect reported in superior cervical ganglion cells is not present at the medial NTS level. SH and F344 NTS cells similarly expressed post-tetanic hyperpolarization (>90% of the cells), delayed excitation (≈50% of the cells), and post-inhibitory rebound (<10% of the cells).

We conclude that SH rats have a larger proportion of medial NTS neurons with a high input resistance, which may affect the post-synaptic responsiveness of these neurons to central and peripheral inputs. (Supported by AHA 89-1017, AHA 85-235)

243.16

TIME COURSE OF HEMORRHAGE-INDUCED C-FOS EXPRESSION IN BRAIN STEM CARDIOVASCULAR NUCLEI. R.K.W. Chan and P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037.

In situ histochemical localization of c-fos immunoreactivity (IR) and mRNA was carried out in brain stems harvested from rats sacrificed at varying intervals following an hemorrhagic stress, in order to clarify the sequence and extent of functional activation in cell groups targeted by, and presumably involved in adaptive responses to, reduced blood volume. Hemorrhage was carried out in conscious animals by withdrawing 10 ml/kg blood via indwelling jugular catheters at a rate of 0.5 ml/min, and resulted in a transient 50% reduction in mean arterial pressure. A progressive increase in the number of cells displaying nuclear c-fos-IR was seen predominantly in tyrosine hydroxylase (TH)-IR neurons in the caudal nucleus of the solitary tract (NTS), and in the A1 and C1 cell groups of the ventrolateral medulla from 0.5-1.5 hr after hemorrhage. Using concurrent retrograde tracing methods, some fos-expressing C1 and NTS cells were found to project to the spinal cord. Recruitment of cells in the locus coeruleus and the A5 cell group became evident at about 1.5 hr, and in the parabrachial nucleus at 2 hr after hemorrhage. A massive activation of cells in the area postrema occurred at 2 hr after the challenge. Expression of c-fos-IR at all of these loci diminished beginning at about 2.5 hr, and was indistinguishable from control levels at 4 hr after hemorrhage. Analyses of c-fos mRNA in this paradigm revealed a similar pattern of results, which preceded alterations in c-fos-IR. These data emphasize the importance of medullary catecholamine neurons in orchestrating integrated neuroendocrine and autonomic responses to cardiovascular challenge, and are compatible with an hierarchical organization in which aminergic neurons in the caudal NTS and the ventrolateral medulla play important roles.

243.17

THE CARDIOVASCULAR EFFECTS OF 5-HT IN THE NUCLEUS TRACTUS SOLITARIUS. M.E. Hall*, R.A. Greer* & J.M. Stewart, Dept. of Biochem. & Neuroscience Training Program, University of Colorado School of Medicine, Denver, CO 80262

The role of 5-HT in regulating baroreceptor reflex function in the Nucleus Tractus Solitarius (NTS) is unclear. Previous studies have shown that intra-NTS injections of 5-HT can alter blood pressure and heart rate. However, some studies report a depressor effect while others describe a pressor effect.

We now report that the effects of 5-HT are biphasic and dose-dependent. Following intra-NTS injection, 5-HT has a depressor effect at picomole doses but a pressor effect at nanomole doses. Similar effects are seen when p-chloroamphetamine is injected into the NTS to release 5-HT from endogenous stores. We also observed that, while the pressor effect of 5-HT is often of short duration, there is a prolonged inhibition of responses to subsequent injections of 5-HT.

The pressor and depressor effects of 5-HT appear to be mediated by different receptor subtypes. 5-HT_2 antagonists effectively block the depressor response to picomole amounts of 5-HT, but not the pressor response to nanomole amounts. In contrast, the 5-HT_{1A} agonist buspirone can reproduce the pressor effects of nanomole amounts of 5-HT.

These and other findings indicate that within the NTS, 5-HT may function as an important modulator of baroreceptor reflex function.

243.18

DIFFERENTIAL AUTONOMIC AND CARDIOVASCULAR EFFECTS OF SEROTONIN AND A SEROTONIN 5-HT_{1A} RECEPTOR AGONIST. A. Dedeoğlu* and L.A. Fisher. Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Different subtypes of serotonin (5-HT) receptors within the central nervous system (CNS) mediate varying effects on autonomic outflow, arterial pressure (AP) and heart rate (HR). The present studies compared the mechanisms underlying the cardiovascular effects of 5-HT and the selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) in conscious rats. Intracerebroventricular (icv) administration of 5-HT (100 nmol) elevated AP (15 mm Hg), plasma epinephrine (E) levels (10-fold), plasma norepinephrine (NE) levels (2-fold), and reduced HR (50 beats/min) at 5 min post-injection. Prior bilateral adrenalectomy (ADX) abolished the AP and plasma E responses but did not modify the HR and plasma NE responses. Prior intravenous (iv) injection of propranolol (PROP) doubled the AP increase without altering the HR response. Prior iv injection of methylatropine (ATR) blunted the HR decrease by 40% but did not alter the AP response. Icv injection of 8-OH-DPAT (100 nmol) did not change AP at 5 min post-injection but elevated plasma E (30-fold) and NE (3-fold) levels and reduced HR (60 beats/min). ADX blocked the E response, did not alter the NE response, unmasked a small decrease in AP (5 mm Hg), and slightly enhanced the HR response (75 beats/min). Iv injection of PROP unmasked a large increase in AP (22 mm Hg) but did not change the HR response. Iv injection of ATR abolished the acute HR response but did not modify the acute AP response. The depressor response (13 mm Hg) to 8-OH-DPAT at 30 min post-injection was not modified by ADX, but was enhanced and attenuated by ATR and PROP, respectively. These results suggest that 5-HT and 8-OH-DPAT stimulate adrenal E release but have differential effects on sympathetic and vagal activity.

243.19

Central 5-HT-induced pattern of cardiovascular regulation. R.L. Davisson & A.K. Johnson. Dept. of Psychology and Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

Evidence exists to support either a facilitative or inhibitory influence of centrally administered serotonin (5-HT) on sympathetic outflow and cardiovascular functions. Recent results from our laboratory indicate that central 5-HT induces a bradycardia, hypotension, and natriuresis which is dependent on renal nerves. The present study examined the central 5-HT-induced pattern of sympathetic outflow mediating hemodynamic regulation.

We examined the effect of ICV injection of vehicle or 5-HT (20 µg) on blood pressure, heart rate, and regional vascular resistances in anesthetized Sprague Dawley rats. Central 5-HT produced a brief pressor response and sustained bradycardia. Hindquarter resistance was elevated above baseline, and reached maximum levels by 10 min post-injection. Renal vascular resistance was significantly reduced below baseline by 10 min post-injection. Mesenteric resistance remained unchanged. These results indicate a pattern of sympathetic outflow that is under differential control by central 5-HT mechanisms.

243.20

EFFECTS OF ANESTHESIA ON BLOOD PRESSURE CHANGES MEDIATED BY KAINIC ACID STIMULATION OF THE FASTIGIAL NUCLEUS. T.J. Parry and J. G. McElligott. Dept. of Pharmacology, Temple Univ. Sch. of Med., Phila., PA 19140

Microdialysis administration of kainic acid (KA) into the rostral fastigial nucleus (RFN) of awake rats produces tonic and phasic increases (pressor response) in mean arterial pressure (MAP) [Parry and McElligott, Soc. Neurosci. Abst. #233.17, 1990]. In contrast, other studies (Chida et al., Brain Res. 370: 378-382, 1986) have shown that local injection of KA into the RFN of α-chloralose anesthetized rats produces a tonic decrease in MAP (depressor response). In order to determine if this difference was due to the method of KA administration (microdialysis *vs.* injection) or due to anesthesia, we injected KA (5 nmole) into the RFN of either awake (n = 4) or anesthetized (α-chloralose, 55 mg/kg i.p., n=4) rats. Initially, the blood pressure modulatory site within the RFN was localized by electrical stimulation in head restrained awake animals. Probe placement was also confirmed by histological verification following the experiment. Half of the animals were anesthetized and half were kept unanesthetized. After approximately 40 minutes, a vehicle injection into the RFN was made producing no change in MAP in either awake or anesthetized rats. KA was then injected into the same site producing within 2 minutes a tonic depressor response (Δ = 23 ± 2 mmHg) in anesthetized rats and a tonic pressor response (Δ = 36 ± 2 mmHg) in awake animals. In awake rats, phasic increases in MAP also appeared within 1 hour of injection which are similar to those in our previous microdialysis studies. Phasic increases also appeared in the α-chloralose treated group only upon recovery from anesthesia. KA treated rats (including those which had recovered from anesthesia) also exhibited severe disturbances in postural tonus seen in previous studies. These results show that injection of KA into the RFN of awake rats produces a pressor response by stimulation of local RFN neurons. The use of α-chloralose anesthesia not only inhibits this increase in MAP but converts it to a depressor response. (Supported by a grant from the American Heart Assoc., Southeastern PA Chapter)

243.21

EXCITATORY AMINO ACIDS MAY MEDIATE NUCLEUS TRACTUS SOLITARIUS EXCITATORY INPUT TO RAT PARABRACHIAL NEURONS. J.H. Jhamandas & K.H. Harris, Dept. of Medicine (Neurology) & Div. of Neuroscience, University of Alberta, Edmonton, Alberta, Canada T6G 2B7.

The pontine parabrachial nucleus (PBN) is a recipient of predominantly excitatory input from the nucleus of the solitary tract (NTS). Recently the presence of glutamate-like immunoreactivity in these brainstem nuclei suggests a role for excitatory amino acids (EAAs) in neurotransmission within this projection. We characterized electrophysiologically in vivo the NTS-evoked excitation of PBN neurons by blocking the synaptic input with locally applied EAA antagonists. Extracellular recordings in urethane-anaesthetized rats were obtained from 64 PBN neurons following electrical stimulation (50-300 μA, 200 μsec current pulses at 0.5 Hz) applied through bipolar electrodes implanted in the NTS. Non-selective EAA antagonist kyneurinic acid (KYN), the selective N-methyl-D-aspartate (NMDA) antagonist DL-2-amino-5 phosphonovalerate (APV) and non-NMDA blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were applied by iontophoresis and pressure ejection from multi-barreled micropipette attached to the recording electrode. Post-stimulus histogram data revealed that NTS-evoked excitation could be reversibly blocked by KYN, APV, and CNQX in 15 of 36,7 of 15 and 6 of 13 cells respectively. These data suggests a role for both NMDA and non-NMDA receptors in mediating the excitatory input from NTS to PBN.

Supported by the MRC of Canada.

243.22

DORSAL RAPHE NUCLEUS (DRN) NEURONS PROJECT SIMULTANEOUSLY TO THE RAT PARAVENTRICULAR NUCLEUS (PVN) AND THE LATERAL PARABRACHIAL NUCLEUS (LPB). T.Petrov, T.L.Krukoff and J.H.Jhamandas. Depts. of Anatomy & Cell Biol. and Medicine (Neurology), Univ. of Alberta, Edmonton, Alberta T6G 2H7.

The DRN is known to project to LPB and PVN, sites involved in autonomic and neuroendocrine regulation. The chemical anatomy of these pathways is unclear, but serotonin (5-HT) is the most abundant transmitter in DRN perikarya. This retrograde tracer investigation was designed to study the presence of 5-HT DRN neurons, projecting both to the LPB and PVN. Rhodamine and fluorescein labelled latex microspheres were stereotaxically injected in the LPB and the PVN areas, respectively. After 4-5 days, fixed and sectioned tissues were processed for immunocytochemical detection of 5-HT.

Thirty five percent of all the labelled neurons within the DRN contained both tracers indicating the presence of collateral branching to the PVN and LPB. Double labelled cells were predominantly observed close to the aqueductal wall within the caudal portion of the DRN, but were located more ventrally at rostral levels of the nucleus. Less than 5% of the double labelled cells demonstrated 5-HT immunoreactivity.

These data suggest that mainly non-5-HT DRN neurons are capable of influencing simultaneously, through collateral input, the activity of LPB and PVN for the control of autonomic functions.

Supported by the MRC of Canada and AHFMR.

243.23

LATERAL PARABRACHIAL AREA (LPB) UNITS WHICH RESPOND TO CARDIOVASCULAR STIMULI DO NOT REACT TO GUSTATORY STIMULI J.A. Lovell and S. L. Stuesse, Neurobiology Dept., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272

The parabrachial region (PB) receives visceral sensory information from the nucleus tractus solitarius (NTS). Subnuclei within the NTS project to discrete subregions of the PB. In general, the medial PB receives gustatory information while cardiovascular information is believed to terminate more laterally. However, previous studies have indicated electrical stimulation of widespread areas of the PB elicits cardiovascular responses. We tested units in the lateral PB (LPB) for convergence of inputs from these two modalities. Multiple unit activity (MUA) and arterial pressure were monitored in the LPB of anesthetized male Sprague-Dawley rats (200-250 gm) before, during, and after the presentation of the following stimuli: 1) phenylephrine-induced hypertension (0.2 mg,i.v.), 2) nitroprusside-induced hypotension (0.75 mg,i.v.), 3) saline washing of oral cavity (0.5 M), 4) sucrose washing of oral cavity (0.1 M), 5) acid washing of oral cavity (0.01 N HCl), 6) distilled water washing of oral cavity. Units were first tested for their responsiveness to blood pressure changes. LPB units responded in a vigorous excitatory fashion to hypotension; they displayed no change or a decrease in spontaneous activity in response to hypertension. When the blood pressure-responsive units were then tested with the gustatory stimuli, none were found which responded to gustatory stimuli. These results show that the segregation of visceral information in the NTS is maintained in the LPB.

243.24

Fos IMMUNOREACTIVITY IN RAT BRAIN ELICITED BY ELECTRICAL STIMULATION OF THE PARABRACHIAL COMPLEX. K.H. Harris, J.H. Jhamandas, and T.L. Krukoff. Depts. of Medicine (Neurology), and Anatomy & Cell Biology, Univ. of Alberta, Edmonton, Canada T6G 2E1.

To further study the role of the pontine parabrachial complex (PBN) in autonomic regulation, Fos-immunoreactivity (Fos-ir) was examined as a marker of neuronal activity after electrical stimulation of the PBN. In urethane-anesthetized rats, the PBN was unilaterally stimulated (7-25 V, 10 sec pulsed trains, 20 Hz, 100 usec duration) for 1-2 h so that arterial pressure (AP) was elevated 20-35 mm Hg. Controls included rats with (1) stimulation of PBN below threshold for AP changes, (2) placement of electrode but no current passed, or (3) stimulation of non-PBN area (cerebellum) with equal current. In controls, Fos-ir was found in the n. tractus solitarius, ventrolateral medulla, lateral PBN, ventral supraoptic nucleus (SON), and central n. of the amygdala. PBN stimulation also led to induction of Fos-ir in the ipsilateral SON, piriform cortex, and insular cortex. No changes in Fos-ir could be attributed to elevation of AP. Results support a role for PBN modulation of autonomic function in the forebrain and show that increases in Fos-ir observed are due to stimulation-evoked activation of PBN efferents and not to changes in AP.

Supported by the Medical Research Council of Canada.

CARDIOVASCULAR REGULATION: HYPERTENSION AND ENDOTHELINS

244.1

ATROPINE BLOCKADE OF CENTRAL ENDOTHELIN'S PRESSOR EFFECT. T. LaHann and G. Daniell*, College of Pharmacy, Idaho State University, Pocatello, ID 83209

Endothelin-1 (ET-1) binding sites exist in brain and ET-1 has been found in CNS neurons. We report here that ET-1 applied to the ventral surface of rat medulla oblongata elicits dose-dependent increases in systemic blood pressure and that this effect can be antagonized by i.v. pretreatment with atropine sulfate, but not by i.v. pretreatment with atropine methyl nitrate. Although ET-1 is a potent vasoconstrictor, the pressor effect of topically applied ET-1 was not duplicated by application of the vasoconstrictor drug clonidine. Bilateral vagotomy not only did not eliminate the pressor effect of ET-1, it seemed to enhance it. Sinoaortic denervation reduced ET-1's pressor effect by about half. Our results suggest that 1) ET-1 acts within the central nervous system to increase systemic blood pressure, 2) this action is, at least in part, the result of a direct, specific effect on neurons lying on or near the ventral surface of the medulla oblongata and 3) this pressor action is mediated through a central, atropine-sensitive mechanism.

244.2

ENDOTHELIN-1 MICROINJECTED INTO NUCLEUS TRACTUS SOLITARII OF RAT BLOCKS ARTERIAL BAROREFLEXES. C.C. Lee, H. Ohta, and W.T. Talman. Dept. of Neurology and CV Center, VAMC and University of Iowa, Iowa City, IA, USA 52242.

Injection of endothelin-3 into the cisterna magna attenuates baroreflexes and increases arterial pressure (AP). We have sought to determine whether endothelin acts at the nucleus tractus solitarii (NTS) to modulate AP, heart rate (HR), and arterial baroreflexes. Endothelin-1 (Et-1) was microinjected bilaterally into NTS of rats anesthetized with chloralose. The baroreflex was assessed by means of a sigmoidal curve fitting analysis applied to reflex changes in HR when AP was increased or decreased by i.v. injection of phenylephrine or nitroprusside respectively. A high dose (10 pmol) of Et-1, but not low doses (1 and 2 pmol), elicited prolonged increases in AP and HR (42.5±4.2 mmHg and 38.8±7.1 bpm respectively) and decreases in respiratory rate. There was a dose-related decrease of the slope of the baroreflex curve (slope=-1.5 before and -0.1 after ET-1 after a 10 pmol dose, $p<.05$). Unilateral microinjection of Et-1 completely blocked decreases in AP and HR elicited by microinjection of N-methyl-D-aspartate (1 pmol), glutamate (250 pmol) or acetylcholine (300 pmol) at the same site. This study suggests that Et-1 abolishes baroreflexes by nonselectively blocking neurotransmission in NTS. Support: VA Merit Review and NIH HL-32205, HL-14388, and NS-24621. CCL is a Merck & AHA Fellow.

244.3

CENTRAL REGULATION OF BLOOD PRESSURE INDUCED BY VASOPRESSIN AND ENDOTHELIN IN RATS. N. Ono, M. Kaneko*, S. Etoh* and H. Kamiya*. Dept. of Pharmacol., Fac. of Pharmaceut. Sci., Fukuoka Univ., Fukuoka, 814-01, Japan.

Comparative study on central regulation of blood pressure induced by vasopressin and endothelin (ET-3) found recently the presence in the CNS carried out using urethane-anesthetized rats. Vasopressin (1-10 nmol) and ET-3 (30-60 pmol) administered intracerebroventricularly (i.c.v.) elicited dose-relatedly the pressor and positive chronotropic effects with long duration. These pressor actions of vasopressin and ET-3 were inhibited by the intravenous pretreatment with pentolinium and phentolamine. The pressor action of vasopressin was inhibited by the i.c.v. pretreatment with α-agonists, norepinephrine, phenylephrine, methoxamine and V1-antagonist, but not clonidine, α-blocker, β-blocker and aspirin. On the other hand, ET-3 was inhibited by the i.c.v. pretreatment with aspirin, indomethacin, and propranolol and ICI 118,551, but not metprolol and naloxone. Also prostaglandin F2α, which elicited a pressor action by i.c.v. treatment, produced similar manner to the mode of ET-3 action. These results suggest that these peptides may have a different mechanism in cardiovascular regulation through the activation of sympathetic nervous system , all involving in the CNS. It appears that vasopressin participates in the α-1 subsystem and ET-3 mediates prostaglandin sytem of the CNS.

244.4

CENTRALLY MEDIATED HEMODYNAMIC RESPONSES OF PROENDOTHELIN-1: EVIDENCE FOR THE PRESENCE OF AN ENDOTHELIN-CONVERTING ENZYME IN RAT BRAIN. A. S. Tadepalli and M. A. Hashim. Division of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Previously, we reported that central application of endothelin-1 (ET) produces long-lasting decreases in mean arterial pressure (MAP), renal blood flow (RBF) and heart rate (HR). ET is produced from its precursor, big endothelin-1 or proendothelin-1 (PET) by an endothelin-converting enzyme (ECE). The aim of the present study was to determine the presence of an ECE in rat brain. Application of ET (1-10 pmol) or PET (30-100 pmol) to the IV ventricle of anesthetized, ventilated rats, evoked similar dose-dependent, falls in MAP, RBF and HR lasting 90-120 min. The latency of the responses was longer with PET (3 ± 0.8 min) than with ET (1 ± 0.2 min). Compared to ET, approximately 10 times higher doses of PET were required to produce similar effects, and doses of PET lower than 30 pmol were ineffective. Thus, falls in cardiovascular parameters evoked by 10 pmol of ET were: MAP=-48 ± 4%; RBF=-46 ± 7%; HR=-19 ± 3%, while those elicited by 100 pmol of PET were: MAP=-43 ± 3%; RBF=-37 ± 10%; and HR=-10 ± 2%. Application of phosphoramidon (PRD; 90 nmol), a metalloproteinase inhibitor, to the IV ventricle, 15 min prior to PET, inhibited the hemodynamic responses to PET. Thus, after PRD pretreatment, peak falls produced by PET (100 pmol) were: MAP=-9 ± 2%; RBF=-6 ± 2%; HR=-6 ± 1%. PRD pretreatment failed to significantly affect the ET-induced responses from the IV ventricle (MAP=-43 ± 3%; RBF=-32 ± 5%; HR=-15 ± 2%). Intravenous administration of the same low doses of PET or ET did not produce any cardiovascular effects. Saline (vehicle) applied to the IV ventricle was also without effect. These data indicate the presence of a phosphoramidon-sensitive ECE in rat brain and suggest a physiological role for endothelin in the central nervous system.

244.5

EFFECT OF EXCITATORY AMINO ACID (EAA) RECEPTOR BLOCKADE ON CENTRALLY MEDIATED CARDIOVASCULAR RESPONSES OF ENDOTHELIN-1 (ET). M. A. Hashim and A. S. Tadepalli. Division of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

We have previously shown that ET (1-3 pmol) applied to the IV ventricle of anesthetized, ventilated rats decreases mean arterial pressure (MAP) and renal blood flow (RBF). These responses occur in two phases, with immediate *phase I* decreases of short duration (5 min) followed by long-lasting (90 min) *phase II* decreases. ET or glutamate elicited similar depressor responses when microinjected into the same, discrete cardiovascular sites (dorsal strip, commissural subnucleus) within the solitary complex. Microinjections of NMDA or quisqualate at these sites, or application to the IV ventricle, also evoked depressor responses (blocked by aminophosphonovaleric acid (APV) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), respectively), while kainate produced a pressor effect (blocked by kyneuranic acid). Therefore, in the present study, we examined the role of EAAs in the central ET-induced cardiovascular effects. Prior application of a combination of APV (2-5 μmol) and CNQX (100-300 nmol) to the IV ventricle significantly attenuated phase I decreases in MAP and RBF by ET (3 pmol). Thus, peak phase I decreases to ET were (% change, pretreated *vs.* unpretreated rats) : MAP=-8 ± 7 *vs.* -26 ± 4; RBF=1 ± 6 *vs.* -16 ± 4. Peak phase II decreases due to ET (3 pmol) were also reduced, but not significantly: MAP=-22 ± 6 *vs.* -34 ± 4; RBF=-13 ± 6 *vs.* -29 ± 7. Pretreatment with a combination of MK-801 and CNQX produced similar results. These data suggest that the centrally induced hemodynamic responses of ET are mediated, in part, indirecly via release of an endogenous EAA which probably activates NMDA and/or AMPA receptors.

244.6

L-N^G-NITRO ARGININE (LNA) MARKEDLY REDUCES ARTERIAL PRESSURE LABILITY (APL) IN CONSCIOUS SINOAORTIC BARORECEPTOR DENERVATED (SAD) RATS. R.A. Shaffer*, A.K. Johnson, W.W. Kaelber and S.J. Lewis. Depts. Pharmacol., Psychol. and Anatomy, & Cardiovasc. Ctr., Univ. Iowa, Iowa City, IA 52242

Our laboratory has shown that (1) the marked APL in conscious SAD rats is dependent upon increased sympathetic neurogenic drive to the vasculature and (2) the generation of the vascular endothelium-derived relaxing factor nitric oxide (NO) is also dependent upon sympathetic drive. This study examined whether the generation of vascular NO is involved in the APL in conscious SAD. In these experiments, the effects of the NO-synthesis inhibitor LNA (0.02 mmol/kg, i.v.) and equipressor infusions of the α_1-adrenoceptor agonist phenylephrine (≈4 μg/kg/min) on the mean arterial pressure (MAP) and APL of conscious SAD rats (n=10) were examined. The injection of LNA increased MAP (pre vs post, 108±6 vs 162±7 mmHg, +50±7%, $p<0.05$) and decreased APL (pre vs post, 13.4±1.7 vs 2.4±0.8 mmHg, -82±7%, $p<0.05$). The infusion of phenylephrine produced a hypertension (pre vs post, 104±3 vs 159±8, +49±6%, $p<0.05$) of equal magnitude to that produced by LNA but in contrast potentiated APL (pre vs post, 12.1±2.3 vs 19.6±3.8 mmHg, +62±17%, $p<0.05$). These findings strongly suggest that vascular NO is involved in the generation of APL in SAD rats. (Supported by HL-14388)

244.7

L-N^G-NITRO ARGININE (LNA) PRODUCES AN EXAGGERATED HYPERTENSION IN ANESTHETIZED SPONTANEOUSLY HYPERTENSIVE RATS (SHR). A.K. Johnson, P.J. Lacolley, M.J. Brody and S.J. Lewis. Depts. Psychol. and Pharmacol., & Cardiovasc. Ctr., Univ. Iowa, Iowa City, IA 52242.

In vitro studies suggest that endothelium-dependent vascular relaxation is impaired in various hypertensive rat models including the SHR. The aim of these studies was to examine the effects of i.v. injection of the nitric oxide (NO)-synthesis inhibitor LNA on mean arterial pressure (MAP) and heart rate (HR) of urethane-anesthetized 16 week old Sprague-Dawley (SD), Wistar-Kyoto (WKY) and SHR rats. The resting MAPs (mmHg) were SD (116±4) > SHR (103±3) > WKY (92±3). The resting HRs (bpm) were SD (389±12) = WKY (354±8) = SHR (351±16). There were no differences in the baroreceptor reflex activity (bpm/mmHg) of the SD (-1.8±0.2), SHR (-1.4±0.3) and WKY (-2.0±0.2). The two cumulative i.v. doses of LNA (0.005 mmol/kg) given 30 min apart produced dose-dependent hypertension in the three strains. The LNA-induced hypertension was most marked in the SHR (+89±8%, post-2nd dose of LNA) whereas the increases in MAP were equal in the two normotensive strains, i.e., SD (+40±7%) = WKY (+52±4%). These data suggest that the synthesis/release or biological activity of NO may actually be augmented in the SHR and that this vasodilator may modulate the expression of the hypertension in this strain. (Supported by HL-14388)

244.8

ARGININE-VASOPRESSIN (AVP) IMMUNOREACTIVITY IN CENTRAL AND PERIPHERAL NEURONAL TISSUES OF DOCA-SALT HYPERTENSIVE RATS. J.S. Simon, S.J. Lewis, B.G. Kasson* and A.K. Johnson. Depts. Pharmacol. & Psychol. & Cardiovasc. Ctr., Univ. Iowa, Iowa City, IA 52242

We have shown previously that AVP is present and may be synthesized within peripheral blood vessels of both rat and cow. However, AVP synthesis and storage within peripheral afferents has not been studied. Since the development of DOCA-salt hypertension (DOCA) is an AVP-dependent process, the aims of this study were to examine whether (1) AVP is stored within vagal afferent perikarya in the nodose ganglion (NG) and (2) DOCA alters AVP levels in pituitary (PIT), hypothalamus, hippocampus, medulla/pons, NG and efferent perikarya of the superior cervical ganglion (SCG). Neural tissues were removed from DOCA and sham rats, homogenized in 1M HCl and centrifuged at 25,000 x g. Supernatants were collected and assayed in radioimmunoassay for AVP using a specific antiserum. Both the NG and SCG contained significant amounts of AVP immunoreactivity (≈25 ng/gm tissue) which were unaltered in DOCA rats. AVP levels in the PIT (≈20 μg/g) of DOCA rats were markedly reduced in comparison to levels in sham rats (≈135 μg/g). AVP levels in other brain tissues were unchanged. These studies indicate that peripheral afferent and efferent neuronal tissue contain AVP immunoreactivity and PIT stores of AVP are markedly depleted in DOCA rats. (Supported by HL-44546)

244.9

Hypertension and Longevity in Rapp-Dahl Rats: Influence of Repeated Stress and Food Intake. R.J. Servatius, J.E. Ottenweller*, S.D. Drastal*, M.T. Bergen, and B.H. Natelson. University of Medicine and Dentistry of New Jersey; Neurobehavioral Unit (127A), V.A. Medical Center, E. Orange, NJ. 07019

We have studied the effects of repeated stress on the development, course, and outcome of hypertension in salt-sensitive Rapp-Dahl spontaneously hypertensive rats. In Experiment 1, rats were habituated for 3 weeks to the blood pressure apparatus, matched for blood pressure and body weight, and then randomly assigned to the stress group or to the non-stressed control group. Stress consisted of supine 4-limb restraint (2 hr sessions, 5 days a week, every other week, for 4 weeks). Blood pressure was measured on weeks between stress, then monthly thereafter. Stressed animals exhibited a delayed rise in blood pressure and lived significantly longer than non-stressed controls. The stressed rats lost weight during stress weeks, gained weight during non-stress weeks, but never attained the weight of the nonstressed group. Because reduced food intake with its consequent reduction in salt ingestion might have explained these results, we undertook Experiment 2. All procedures were identical to Experiment 1, except that stressed and non-stressed rats were food-yoked. The development of hypertension and life-span were equivalent in the two groups in this experiment. The results closely resembled those from the stressed rats in Experiment 1. These data do not support the hypothesis that repeated stress exacerbates the hypertensive process and indicate that control of food intake is a critical variable for further research into the effects of stress on blood pressure. Supported by V.A. Medical Research Funds.

244.10

ACUTE AUTONOMIC RESPONSES IN A HYPERTENSIVE RAT STROKE MODEL. K.S. Butcher, V.C. Hachinski and D.F. Cechetto. Robarts Research Institute, University of Western Ontario, London, Ontario, Canada N6A 5K8.

Acute increases in sympathetic activity, and frequency of myocardial damage, occur following middle cerebral artery occlusion (MCAO) in Wistar rats (Cechetto, et. al., 1989). Hypertension is a major risk factor for stroke. The autonomic responses to MCAO in the spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats were therefore investigated. Arterial pressure (AP), heart rate (HR), renal sympathetic nerve discharge (SND), and ECG were measured in 16 SHR and 16 WKY male rats, which were subjected to either MCAO or sham MCAO. Infarct size did not differ between SHR and WKY rats, as shown by tetrazolium staining. Initial AP and SND were significantly ($p \leq 0.05$) higher in SHR (96.2 ± 4.4 mmHg; 18.3 ± 4.1 $\mu v \cdot s$) than in WKY (70.9 ± 1.3 mmHg; 9.1 ± 1.3 $\mu v \cdot s$). No significant differences in initial HR were observed between SHR and WKY. By 6 hours after MCAO, AP and SND in SHR significantly decreased (69.4 ± 9.9 mmHg; 8.0 ± 2.3 $\mu v \cdot s$), while HR showed a significant increase (352.8 ± 11.1 bpm). The decrease in AP and SND may be associated with damage to the insular cortex. AP, SND and HR in the WKY were not significantly affected by MCAO. These responses contrast with previous findings in Wistar rats. Differences may arise from the relative amounts of baseline sympathetic activity in the three strains.
Supported by the Heart and Stroke Foundation of Canada.

244.11

BRAINSTEM AND PERIPHERAL ENDOTHELIN RECEPTORS IN THE CAT: A ROLE FOR ENDOTHELIN IN BARORECEPTOR AND CHEMORECEPTOR REFLEXES.
R.M. Sykes*, M.R. Dashwood*, D.S. McQueen*[1], M.de B. Daly* and K.M. Spyer. Dept. of Physiology, Royal Free Hospital School of Medicine, London and Dept. of Pharmacology, University of Edinburgh Medical School*[1], United Kingdom.

Endothelin (ET), a powerful vasoconstrictor peptide, is present within certain regions of the central nervous system. Using autoradiography [^{125}I] ET binding sites have been identified in the medulla oblongata involving the nucleus tractus solitarius, dorsal vagal nucleus and rostro-ventrolateral medulla. Dense binding was also observed in the carotid body and at the level of the carotid bifurcation. Injection of ET into the common carotid artery caused a dose-related fall in baroreceptor discharge but had little effect on chemoreceptor activity. Chemoexcitation evoked by acetylcholine or sodium cyanide injected during ventilation with 11% O_2 was markedly potentiated following ET. These changes together with its localised CNS binding suggest that ET has a potent influence on reflex control of circulation and respiration.

CARDIOVASCULAR REGULATION: SPINAL MECHANISMS

245.1

SYNAPSES ON AXONS OF SYMPATHETIC PREGANGLIONIC NEURONS IN RAT AND RABBIT SPINAL CORD. I.J. Llewellyn-Smith, P.M. Pilowsky, J.B. Minson* and J.P. Chalmers*. Centre for Neuroscience and Department of Medicine, Flinders University, Bedford Park, SA 5042 Australia.

In searching for a retrograde tracer that would allow us to visualize distal dendrites of sympathetic preganglionic neurons (SPN), we have managed to reveal their axons and have examined them for the presence of synapses. Cholera toxin B subunit (CTB) was injected into the superior cervical ganglion of rats and rabbits, into the stellate ganglion of rabbits or into the adrenal medulla of rats. After perfusion with fixatives suitable for both light and electron microscopy, CTB-immunoreactivity was found in the axons of sympathetic preganglionic neurons as well as in their cell bodies and dendrites. At the light microscope level, most SPN axons ran through the ventral horn near its lateral border with the white matter, although some ran more medially. A small proportion of axons were branched. At the ultrastructural level, synapses were found on the proximal parts of the SPN axons and in one case on an axonal branch immediately after it diverged from the main axon. These axoaxonic synapses are likely to be an important part of the neuronal circuitry that regulates the activity of SPN. This work was supported by grants from the National Health and Medical Research Council of Australia and the National Heart Foundation of Australia.

245.2

NEURONS IN CERVICAL SPINAL CORD PROJECT TO THE INTERMEDIOLATERAL CELL COLUMN (IML) IN THE RAT. M.A. Haxhiu and C.J. Helke*. Case Western Reserve Univ., Cleveland, OH and Uniformed Services University, Bethesda, MD 20814.

Neurons in the dorsolateral funiculus (DLF) of cervical spinal cord can affect sympathetic outflow (Schramm and Livingstone, 1987). To provide direct anatomic evidence for this effect, we evaluated cervical cord for the retrograde transport of rhodamine-labeled microspheres (50-60 nl) or 4% Fluoro-Gold from the T_3 IML. Control injections were made into surrounding areas. Five to 10 days later the rats were perfusion fixed and cryostat-cut sections (20μm) of cervical and thoracic spinal cords were prepared. The site and spread of IML injections were determined. Following restricted IML injections, retrogradely-labeled cells were found in the nuclei of the DLF (Giesler and Elde, 1985) of C_2-C_3 both ipsilateral [7$\pm$3 (mean$\pm$SD) cells/section] and contralateral [3$\pm$2 cells/section] to the IML injection. The cells were oval, triangular or spindle-shaped. Numerous tracer-labeled cells were also found in laminae IV, V, and VII, whereas only a few labeled cells were observed in laminae X. In addition, retrogradely-labeled neurons were found in the ventral horn of upper thoracic segments. This study provides anatomic evidence for intraspinal projections to the IML from neurons in the cervical DLF, and from regions of the cervical gray and thoracic ventral horn. (USUHS grant R075A2; NIH grants NS24876 and HL38701).

245.3

EFFECT OF RENAL NERVES ON RENAL AND CARDIOVASCULAR FUNCTION IN CHRONIC SPINAL RATS. K.A. Trostel* and J.W. Osborn. Dept. of Vet Biol., U. of MN, St. Paul, MN 55108.

Previous studies have demonstrated that renal nerve activity has acute effects on renal function in rats with cervical spinal cord transection (CST). The present study addressed the question: **Do renal nerves have chronic effects on renal and cardiovascular function in CST rats?** Three groups of conscious Sprague-Dawley rats were studied: renal denervated + CST (RDNX+CST), sham-RDNX + CST (SHAM+CST), and sham-RDNX + sham-CST (INTACT). CST or sham-CST surgeries were performed 8 days after RDNX or sham-RDNX. Sodium and water intake were fixed by i.v. infusion. Mean arterial pressure (MAP) and plasma renin activity (PRA) were measured before and for 9 days after CST/sham-CST. In addition, urine flow, urinary sodium excretion, and urine pH were measured in the two groups of CST rats. One day after CST, MAP had fallen 25 mm Hg in both RDNX+CST and SHAM+CST groups. PRA was lower in RDNX rats compared with sham-RDNX rats before CST/sham-CST (1.2 $\pm$ 0.3 vs 2.1 $\pm$ 0.2 ng AI/ml*hr). One day after CST, PRA had fallen to 0.7 $\pm$ 0.1 ng AI/ml*hr and was not different between CST groups. MAP and PRA remained depressed throughout the study. There was no difference between SHAM+CST and RDNX+CST rats in any of the renal or cardiovascular variables measured after CST. In summary, we found no evidence for a chronic effect of renal nerves on renal or cardiovascular function in CST rats. NIH grant HL 39619

245.4

SOURCES OF SYMPATHETIC PREGANGLIONIC CONTROL OF RENAL, SPLENIC AND MESENTERIC NERVES IN RATS. R. B. Taylor*, M.M. Robinson and L.C. Weaver. John P. Robarts Research Inst. and Dept. of Physiol., Univ. of Western Ontario, London, Canada.

The locations of sympathetic preganglionic neurons (SPNs) controlling renal, splenic, and mesenteric nerves were studied in anesthetized rats. SPNs were excited by injecting D,L-homocysteic acid (DLH, 3 nL,.16 M) into the thoracolumbar gray matter (T4-L4) while recording sympathetic firing. Responses of the three nerves could be elicited from overlapping spinal segments extending from T4 to T13, demonstrating no discrete topography of SPNs controlling these organs. Increases in renal nerve discharge were greatest after ipsilateral injections into T8-T12 (12 rats); the largest change (+58±10%) occurred at T10. Increases in splenic (8 rats) and mesenteric (12 rats) nerve discharge were produced from either side of the cord uniformly in segments T4-T12 and T4-L3, respectively. Their largest increases of 20±5% (T5, splenic) and 33±13% (T13, mesenteric) were smaller than those of renal nerves. The larger renal nerve responses demonstrate that sympathetic output can be differentiated at the level of the spinal cord. Further experiments were done to determine if tonic afferent input to the cord contributed to the excitation of renal nerves. In 6 rats, dorsal root rhizotomy from T7-L2 caused no significant change in renal nerve firing (+4±12%) and did not alter responses to DLH injections. In contrast, in 10 acute spinal rats, this rhizotomy decreased firing by 29±10%. No predominant contribution of rootlets from any segment or side of the cord was detected. This suggests that afferent excitation of renal nerves may become important after loss of bulbospinal drive. (Support:MRC Canada)

245.5

Renal and adrenal sympathetic preganglionic neurons in rabbit spinal cord and presumed premotor neurons in brain: tracing with Herpes Simplex Virus. Y. -W. Li, Z. -Q. Ding*, S. L. Wesslingh* and W. W. Blessing. Centre for Neuroscience, Flinders University of S.A., Bedford Park, SA 5042.

We studied the location of renal and adrenal sympathetic preganglionic neurons in the spinal cord, and premotor neurons in the brain, using Herpes Simplex Virus type 1 (HSV1) as a transneuronal retrograde tracer. NZW rabbits (n = 28) were anesthetized and a clinical isolate of HSV1 was injected into the left renal nerve or the left adrenal gland, or DiI was injected into the adrenal gland and HSV1 into the renal nerve, or both tracers were injected into the adrenal gland. After 3 - 8 days rabbits werereanesthetized, the spinal cord and brain perfused and sectioned, and HSV1 was localized using the immunofluorescence or the avidin-biotin-peroxidase procedure. Five days after renal injection, virus labeled neurons were observed in spinal segments T_7 - L_2, with 95% in the ipsilateral intermediolateral column (IML). Three days after adrenal injection, virus labeled neurons were seen ipsilaterally in segments T_4 - T_{12}, with 91% in the IML. In rabbits with renal injection of virus and adrenal injection of DiI, surviving 4 - 7 days after viral injection, 5 - 12% of DiI labeled neurons were HSV1-positive. In rabbits with both DiI and HSV1 injection into the adrenal gland, 50% of DiI labeled neurons were HSV1-positive. In rabbits surviving 7 days after renal injection and 5 days after adrenal injection, virus labeled neurons appeared in restricted brain regions, including the rostral ventrolateral medulla, parapyramidal area, A5 area and paraventricular nucleus. In the rostral ventrolateral medulla a proportion of labeled neurons (61% after adrenal and 40% after renal injection) contained tyrosine hydroxylase, identifying them as C_1 catecholamine neurons. Results suggest that most renal and adrenal sympathetic preganglionic neurons belong to separate neuronal populations. The presumed premotor neurons in the brain were located in similar regions after injection of virus into either the adrenal gland or the renal nerve.

245.6

CNS NEURONS INFECTED BY RENAL INJECTION OF PSEUDORABIES VIRUS. L.P. Schramm, A.M. Strack, K.B. Platt*, and A.D. Loewy. The Johns Hopkins School of Medicine, Baltimore, MD 21205, The Washington University School of Medicine, St. Louis, MO 63110, and Iowa State University, Ames, IA 50011.

The purpose of these experiments was to delineate the CNS neurons regulating renal circulation and function. Others have located the renal postganglionic neurons (RPN) using conventional retrograde tracers. However, these tracers did not cross synapses and, therefore, did not demonstrate the synaptic antecedents of the RPN. Studies indicate that pseudorabies virus (PRV) is transported centrally across synapses (Br. Res. *491*:274, 1989; J. Neurosci. *10*:2139, 1990). Under anesthesia, we injected kidneys of Sprague-Dawley rats with PRV. Rats were sacrificed 48 to 96 hours later. Brains, spinal cords, and most sympathetic ganglia were removed, sectioned, and reacted immunohistochemically for the presence of virus. Rats exhibited a wide range of severity of CNS infection. In lightly infected rats, most infected spinal neurons were in the intermediolateral column between T7 and T13, and their morphology suggested that they were sympathetic preganglionic neurons (SPN). Infected neurons were also observed in the rostral ventrolateral medulla (RVLM), A5 cell group, and the paraventricular nucleus of the hypothalamus (PVN). However, none were detected in rostral thoracic or cervical spinal cord. Rats in which the infection involved more medial, thoracic, interneurons exhibited infected neurons in the dorsolateral funiculus of the rostral cervical spinal cord. We conclude that several brain stem regions, including the RVLM, A5 group and PVN, may affect renal function *via* direct projections to SPN. Cervical spinal neurons also may be involved in regulating renal circulatory or tubular function. Their effects, however, are mediated *via* spinal interneurons rather than by direct synapses on SPN. Supported by NIH grants HL16315 and HL25449.

245.7

CARDIAC RESPONSES TO INJECTIONS OF ADRENERGIC RECEPTOR AGONISTS INTO THE INTERMEDIOLATERAL COLUMN (IML) OF THE SPINAL CORD IN THE RAT.
V. Malhotra, A. Kachroo, A.J. Krieger & H.N. Sapru, Section of Neurosurgery & Pharmacology, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

In pentobarbital-anesthetized, artificially ventilated Wistar rats, injections (20 nl) of L-glutamate (1.77 nmole) into the right IML at T1-T3 increased (30%) heart rate (HR). Different doses (0.001-0.1 pmoles (pm) in 20 nl) of agonists [phenylephrine (alpha-1), dobutamine (beta-1), clonidine (alpha-2) & terbutaline (beta-2)] and antagonists [prazosin (alpha-1), metoprolol (beta-1), idazoxan (alpha-2) & butoxamine (beta-2)] were injected into the IML. The following changes in HR were observed: activation of alpha-1 and beta-1 receptors increased (10-20%) HR, alpha-2 decreased (15-20%) it and beta-2 produced no change. The response to each agonist was blocked by its specific antagonist. Microinjections (20 nl) of Epi in lower doses (0.001-0.8 pm) increased (10-20%) while higher doses (2000-3200 pm) decreased (5-10%) HR. Excitatory actions of Epi were blocked by injections of prazosin & metoprolol (0.1 pm/20 nl each) while the inhibitory action was blocked by idazoxan. These results suggest that in the IML at T1-T3 the receptors mediating the actions of Epi are: (1) alpha-1 and beta-1 for excitatory action and (2) alpha-2 receptors for inhibitory action.
Support: NIH (HL24347) and Am. Heart Assoc. (NJ).

245.8

DIFFERENTIAL RELATIONSHIPS AMONG DISCHARGES OF POSTGANGLIONIC SYMPATHETIC NERVES. S. Zhong, M.J. Kenney, S.M. Barman, and G.L. Gebber. Dept. of Pharmacol. & Toxicol., Mich. State Univ., E. Lansing, MI 48824.

The relationships among the simultaneously recorded discharges of as many as four postganglionic sympathetic nerves were studied with coherence and phase spectral analyses in baroreceptor-denervated cats. Activity was recorded from the inferior cardiac (CN), vertebral (VN), and renal (RN) nerves. Most of the power in sympathetic nerve discharge (SND) was below 6 Hz, and the discharges of any two nerves cohered at frequencies between 0 and 15 Hz. Our most significant findings are as follows: 1) Coherence values were significantly higher in chloralose-anesthetized than in unanesthetized-decerebrate cats. 2) Coherence values were higher for near ipsilateral nerves (e.g., CN and VN) than for widely-separated ipsilateral nerves (e.g., CN and RN). 3) Coherence values for most pairs were higher when the nerves were located on the same side (ipsilateral nerves) rather than opposite sides (contralateral nerves) of the body. 4) Coherence values were higher for some functionally complementary nerves (e.g., CN and RN) than for noncomplementary nerves (e.g., VN and RN). Functionally complementary nerves are those whose discharges change in the same direction (increase or decrease) during affective behaviors, whereas the discharges of noncomplementary nerves usually change in opposite directions. 5) As revealed by phase spectral analysis, the interval between the discharges of two nerves was constant within the coherent frequency band at a time when the interval was frequency-dependent for other nerve pairs. These results indicate that both the anatomy of spinal sympathetic outflow and the functional diversity of postganglionic nerves are reflected in the coupling of central circuits responsible for SND. (Supported by NIH grants HL-13187 and HL-33266.)

245.9

CARDIOVASCULAR EFFECTS OF SEROTONIN$_{1C/2}$ (5-HT$_{1C/2}$) AGONISTS IN THE THORACIC SPINAL CORD OF THE RAT. E.T. Phillips and C.J. Helke. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Recent receptor autoradiographic studies demonstrated the discrete and preferential localization of 5-HT$_{1C/2}$ binding sites to the intermediolateral cell column of the thoracic spinal cord (Thor et al., this meeting). To determine the mean arterial pressure (MAP) and heart rate (HR) effects of the activation of 5-HT$_{1C/2}$ receptors in spinal cord, the 5-HT$_{1C/2}$ agonists dimethoxy-iodophenyl-aminopropane (DOI) and α methyl serotonin (αM-5HT) were intrathecally (i.t.) administered to anesthetized, artificially-respired rats. DOI (1-100 μg) caused initial decreases followed by increases in MAP and HR whereas αM-5HT (1-30 μg) only decreased MAP and HR. Studies with i.t. administration of ^{125}I-DOI, and studies with peripheral administration of a 5-HT$_2$ antagonist (LY53857) prior to i.t. DOI, showed that the pressor effects of i.t. DOI were due to peripheral leakage and that the depressor effects were due to a spinal cord site of action. The depressor effects of DOI were prevented by peripheral administration of phentolamine. Pretreatment with i.t. administration of 5-HT$_2$ antagonists (LY53857 and ketanserin) was inconsistent in blocking the depressor effects of i.t. administration of DOI or αM-5HT. These data suggest that activation of spinal 5-HT$_{1C/2}$ receptors decreases MAP by reducing sympathetic outflow to the vasculature. Whether the receptor activated is an atypical 5-HT$_2$ site or a 5-HT$_{1C}$ site remains to be determined. [Supported by NIH grant NS24876]

245.10

RESPONSES OF RAT LOWER THORACIC AND LUMBAR SYMPATHETIC PREGANGLIONIC NEURONES TO IONTOPHORESED MET-ENKEPHALIN. M.P. Gilbey and S.Y. Zhou* Department of Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, England.

Recent studies have shown enkephalin immunoreactive neuronal projections from the medulla to the inter-mediolateral cell column (Sasek C.A. and Helke, C.J., J. Comp. Neurol., 287: 484, 1989). Furthermore, endogenous opioid systems appear to decrease the cardio-vascular response to stress (Morris, M. et al., Psycho-neuroendocrinology 15:185, 1990). Consequently, in this investigation the responses of lower thoracic and upper lumbar sympathetic preganglionic neurones (SPNs) to iontophoresed metenkephalin were examined.

Twenty eight SPNs were studied in chloralose anaesthe-tized rats. Twenty one SPNs had their activity decreased by met-enkephalin, 2 had their activity increased and the remainder were unaffected. Naloxone given either intra-venously or iontophoretically was observed to antagonize the decrease in sympathetic preganglionic neuronal discharge (n=2).

These results indicate that endogenous opioids may act directly on SPNs to modulate cardiovascular responses to stress.

245.11

PHYSIOLOGICAL AND ANATOMICAL EVIDENCE FOR A MONOSYNAPTIC PATHWAY BETWEEN A CARDIOVASCULAR REGULATORY SITE IN THE ANTERIOR CINGULATE CORTEX AND THE THORACIC SPINAL CORD IN THE RAT S. J. Bacon and A. D. Smith Department of Pharmacology, South Parks Road, Oxford OX1 3QT UK

In 42 anaesthetised rats, blood pressure was recorded from the femoral artery and a double-barrelled glass micropipette positioned stereotaxically in the cingulate cortex. One barrel contained a solution to stimulate neurons (0.1 M glutamate or 25 mM KCl), and the other contained the anterogradely transported tracer *Phaseolus vulgaris* leucoagglutinin (PHAL). Extracellular injections of 100-150 nl of stimulant were made by pressure until a change in blood pressure was recorded. PHAL was then injected iontophoretically at the same site. Immunohistochemistry was used to reveal the PHAL containing cells, and light and electron microscopy (LM and EM) used to study their targets. In all 42 rats injections of stimulant elicited a depressor response. Blood pressure fell by 15.0 +/- 8.3 mm Hg (mean +/- SD) from a resting value of 92.4 +/- 14.6. Injections of artificial cerebrospinal fluid or saline had no effect. PHAL injection sites consisted of a few hundred labelled somata and their associated dendrites and axons. The vast majority of somata were confined to the Cg3 region of the anterior cingulate cortex. LM showed PHAL labelled axons and varicosities in the cervical and thoracic spinal cord. 10 varicosities from the central autonomic region of the thoracic spinal cord were serially sectioned and examined in the EM. All varicosities formed asymmetric synaptic specialisations with small dendritic profiles or spine-like processes. These results demonstrate the existence of a monosynaptic pathway between cells from a region of the anterior cingulate cortex shown to be involved in cardiovascular regulation and regions of the spinal cord with known autonomic function.

RESPIRATORY REGULATION II

246.1

LABELED BULBOSPINAL NEURONS FROM NEONATAL RAT IN CULTURE. P.G. Wagner and M.S. Dekin. School of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506-0225.

Intrinsic membrane properties of bulbospinal neurons from the dorsal respiratory group have been well characterized using an *in vitro* brainstem slice preparation (Dekin et al., *J.Neurophysiol.*, 58: 195, 1987). These studies, however, were limited to macroscopic current measurements. Analysis of single channel properties would allow a more quanitative description of these currents and provide direct observation of how putative neurotransmitters/modulators affect channel kinetics. Although the whole-cell patch clamp technique has been applied to the slice, the quality of the patch seals is often not sufficent for resolution of single channel currents. We have, therefore, combined the technique of primary cell culture which allows direct visualization and access to the cell surface with the use of fluorescent retrograde tracers. Tracers were used to circumvent the loss of functional/anatomical identity usually associated with cell culture. Discrete pressure-injections of rhodamine filled latex beads (Lumofluor, Inc.) were made in the ventrolateral regions of cervical spinal cord (C4-C6) in 3-4 day old rats. After 24 hrs, brainstems were removed and transverse slices just caudal to the obex extending 1 mm rostral were made. The slices were subdivided into dorsal and ventral portions and cultured separately. Cells extended both dendrites and axons. Labeled neurons were observed in both ventral and dorsal cultures. The numbers of labeled neurons in both cultures were relatively low. This is consistent with reports on the numbers of respiratory bulbospinal neurons (Berger et al., *J. Comp. Neurol.*, 224:60, 1984). Neurons had resting membrane potentials in the range of -70 to -55 mV and conducted action potentials in the whole-cell current clamp mode. Calcium channels were observed in both cell-attached and inside-out patches in which 110 mM $BaCl_2$ (pipette) was used as the charge carrier. (Supported by NIH Grants HL40369, HL02314 and RR07114).

246.2

IN VIVO AND IN VITRO RESPONSES OF VENTROLATERAL MEDULLARY NEURONS TO HYPOXIA. P.C. Nolan* and T.G. Waldrop. Dept. of Physiology & Biophysics, College of Medicine, Univ. of Illinois, Urbana, IL 61801.

Previous studies from this laboratory have shown that ventrolateral medullary (VLM) neurons modulate the respiratory response to hypoxia and that hypoxia increases the discharge rate of VLM neurons. The purposes of the present study were to determine if hypoxia can stimulate VLM neurons in the absence of peripheral chemoreceptors and if the stimulation is dependent upon synaptic input from other brain areas. In a first set of experiments, the extracellular responses to hypoxia of VLM neurons were examined in anesthetized rats after transection of peripheral chemoreceptor and baroreceptor afferents. Inhalation of a hypoxic gas (10% O_2/90% N_2) elicited increases in the discharge frequency of 67% of the neurons studied in these peripherally-chemodenervated rats. Most of these neurons had a basal discharge related to the cardiac and/or respiratory cycle. Additional experiments were performed *in vitro* with 400 μm brain slices containing the medulla from 3-6 week old rats. The slices were maintained in an interface chamber perfused with nutrient media and bubbled with 95% O_2/5% CO_2. VLM neurons increased their discharge frequency during perfusion of the chamber with anoxic (5% CO_2/95% N_2) and hypoxic (10% O_2/5% CO_2/85% N_2) gas; cells increased their discharge in a dose-related manner. These results suggest that neurons in an area of the medulla known to be involved in cardiorespiratory regulation are excited by hypoxic stimulation (Supported by NIH 38726; American Heart Association).

246.3

PHRENIC AND SYMPATHETIC NERVE RESPONSES TO INTRAMEDULLARY NaCN MICROINJECTION IN CAT J. Mitra, N.B. Dev*, J.R. Romaniuk*, R. Trivedi* and N.S. Cherniack. Case Western Reserve University, Dept. of Medicine, Cleveland, Ohio 44106.

We showed that intravertebral artery injection of either sodium cyanide (NaCN) or hypoxic saline solution in normoxic cats depresses breathing and stimulates sympathetic nerve activity. To investigate the site(s) of action of NaCN in the brainstem, we microinjected NaCN (100nl of 100 μg/ml sol.) into the ventral medulla 1-12mm caudal to foramen caecum (FC) and 2-5mm lateral, to a depth of 1mm from the surface, in anesthetized, paralyzed and ventilated cats and recorded from phrenic and cervical sympathetic nerves. Following observations were made: (a) phrenic excitation (8.0 ± 2.0%) without changing sympathetic activity at 1-4mm caudal (Area1). (b) Phrenic depression (37.5 ± 9.4%) and simultaneous excitation of sympathetic (26.0 ± 9.2%) at 5-8mm caudal (Area2) (P<0.05). (c) Variable effect on sympathetic nerve response without significant change in phrenic amplitude at 9-12mm caudal (Area3). NaCN did not alter breathing frequency at any injection site.

Our results suggest that both respiratory excitation and depression can be elicited by NaCN. However, significant respiratory depression along with sympathetic excitation obtained only from Area2. We conclude that the neural substrates underlying Area2 may be involved in intravertebral NaCN responses.

246.4

EFFECTS OF SMALL VOLUME INJECTION OF GABA AGONISTS AND ANTAGONISTS ON MEDULLARY RESPIRATORY NEURONAL DISCHARGE DURING OPOSSUM DEVELOPMENT. J. P. Farber and S. R. Barker*. Dept. Physiol.; Univ. Okla. HSC; OKC, OK; 73190

Changes in breathing pattern between immature and adult animals could be due in part to changing post-synaptic sensitivity to particular neurotransmitters and/or to the fate of these neurotransmitters after release. We examined inhibition of extracellularly recorded action potentials from medullary respiratory neurons after localized pressure injection of GABA (~25 pl) using multibarrel pipettes in Inactin-anesthetized adult and suckling opossums down to about 20 days of age (1.8 g). Injection volumes were estimated by droplet diameter of injections both before and after the pipette was inserted into the medulla. GABA concentrations were 0.5 and 10 mM/L (balance NaCl; total conc. = 150 mM/L; pH=7.4). Effects were assessed as the interval between time of injection and resumption of action potentials. Results showed longer intervals of inhibition (approx. 3 fold on average) with the higher concentration of GABA. Using the higher concentration of GABA, where the results would be less influenced by breathing pattern because of the relatively longer duration of inhibition, average time of inhibition was 9.8±0.7 sec SE (n=33) among adult animals. For animals approx. 60 days of age to weaning, 40-60 days, and 20-30 days, the times of inhibition were, 6.4±0.4 (n=36), 5.1±0.4 (n=38), and 7.4±0.80 (n=8) sec, respectively. Similarly, the GABAa receptor antagonist, bicuculline (2 mM/L; 25 pl), increased the discharge of some respiratory units at all ages. Some aspects of GABAergic systems appear to be functional in young opossums; clearance of exogenous GABA is effective despite incomplete glia proliferation in the young animal. (Supported by HL-37318 and Presbyterian Health Foundation).

246.5

SIMULATION OF RESPIRATORY NEURAL NETWORKS. U. J. Balis*, K. F. Morris, J. Koleski* and B. G. Lindsey. Dept. Physiol. & Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

We tested whether synaptic relationships among respiratory neurons of the ventrolateral medulla inferred from spike train cross-correlation (J. Neurophysiol. 57:1101; 61:1185) were sufficient to generate discharge patterns similar to those of bulbospinal I neurons. Neurons with accommodation, but no "bursting" properties, were simulated with SYSTM11 (MacGregor, Academic Press, 1987). Five populations were connected using a symbolic network editor running under X Windows. Terminals from each population were randomly distributed on each specified target population. Firing patterns were similar to those previously described for I/EI, I-AUG, I-DEC, E-AUG, and E-DEC neurons. For example, I/EI neurons became active during the second half of the E-phase as E-DEC inhibition declined; some E-AUG neurons discharged transiently prior to their ramp activity coincident with the onset of E-DEC neuron firing. Inhibition of E-DEC and I-DEC neurons decreased the duration of their respective phases and increased respiratory rate; reduced I-DEC activity also increased the slope of the inspiratory ramp. "Apneustic" patterns were generated by varying the balance of tonic excitatory and inhibitory inputs to the network. Inhibition of some E-AUG neurons by other E-AUG cells increased respiratory rate. The results: (a) show that our network schemes are sufficient to generate normal and abnormal respiratory phase durations and discharge patterns similar to those observed in anesthetized vagotomized adult *in vivo* preparations, (b) predict consequences of modulating specific synapses, and (c) provide a foundation for addressing the scaling of network parameters and roles of various membrane conductances. Supported by NS19814.

246.6

ALTERATION OF BREATHING BY NON-NMDA RECEPTOR BLOCKADE IN PRE-BÖTZINGER REGION OF ADULT CATS AND RATS. C.A. Connelly, J.D. Hernandez*, E.G. Finlon, M.R. Otto-Smith*, & J.L. Feldman. Systems Neurobiology Lab., Dept. Kinesiology, UCLA, Los Angeles, CA 90024-1527.

The pre-Bötzinger complex (pre-BötC), a region of the ventral respiratory group (VRG) located immediately caudal to expiratory Bötzinger (BötC) neurons, is hypothesized to contain neurons responsible for the rhythmic drive underlying breathing in mammals (Feldman et al., Eur J. Neurosci.Suppl. 3: 171, '90). The discharge patterns and distribution of cell types found in the pre-BötC were examined in adult rats and cats. Results in both spontaneously breathing (vagi intact) rats and anesthetized, paralyzed, artificially ventilated cats and rats indicate a heterogeneous mixture of inspiratory (I), expiratory (E) and phase-spanning respiratory neurons. Pre-I neurons (discharge in late E and continue into mid to late I) in the pre-BötC of the adult rat were excited by iontophoresis of L-glutamate and DL-homocysteic acid. To determine if blockade of non-NMDA receptors in pre-BötC alters breathing, CNQX (1 mM in saline, 20-100 pmoles) was microinjected in subregions of the pre-BötC; decreased breathing frequency was observed in both species following unilateral microinjections. Control injections of CNQX in adjacent respiratory regions, including VRG, BötC, and subregions of the pre-BötC did not alter respiratory frequency or amplitude. Research supported by NIH grant HL 37941. CAC is a Parker B. Francis Foundation Fellow.

246.7

MICROINJECTION OF MORPHINE INTO THE MEDIAL PONTINE RETICULAR FORMATION (MPRF) CAUSES INCREASED NUMBER OF APNEIC EPISODES. J. Keifer*, H.A. Baghdoyan, R. Lydic (Spon: J.F. Biebuyck) Dept. of Anesthesia, The Penn State University, College of Medicine, Hershey, Pa 17033

Morphine, a potent analgesic and respiratory depressant, decreases rapid eye movement (REM) sleep. The mPRF is a cholinoceptive area which is involved in REM sleep generation and is believed to mediate sleep-dependent changes in ventilation (*Neurosci. Lett.* 102:211,1989). Since several lines of evidence point to an interaction between cholinergic systems and narcotic receptors, we are testing the hypothesis that the mPRF is a site which mediates morphine's effect on states of consciousness and ventilation. To date we have injected normal saline (0.25μl) and morphine sulphate (15.2μg/ 0.25μl) into the mPRF in 4 intact cats chronically instrumented for measuring sleep and ventilation (saline control = 12 trials, morphine = 8 trials). The mean (±SD) number of apneic episodes in a two hour period following microinjection was significantly greater ($p<.05$) after morphine (9.3 ± 5.9) than after saline (3.8 ± 2.4). These preliminary results suggest that the mPRF, a cholinoceptive region containing no known clusters of respiratory neurons, can mediate some of the respiratory effects of morphine.

Supported by an Eric A. Walker Fellowship (JCK), MH-45361 (HAB) and HL-40881(RL).

246.8

CHOLINERGIC RETICULAR MECHANISMS PRODUCE STATE-DEPENDENT DECREASES IN PARABRACHIAL RESPIRATORY NEURON DISCHARGE. K.A. Gilbert and R. Lydic. Department of Anesthesia, Pennsylvania State University, College of Medicine, Hershey, PA 17033.

Pontine respiratory group (PRG) discharge decreases during sleep, but the mechanisms which cause these state-dependent changes in firing rate are poorly understood. Since microinjection of carbachol into the medial pontine reticular formation (mPRF) of intact, unanesthetized cats produces a rapid eye movement (REM) sleep-like state (DCarb) and respiratory depression (*Neurosci. Letts. 102*:211, 1989), the current study is using the DCarb model to test the hypothesis that cholinergic reticular mechanisms can also cause state-dependent decreases in PRG neuron discharge. These studies recently reported that parabrachial REM-on and REM-off cells recorded during REM sleep had similar state-related discharge during DCarb (Gilbert & Lydic, *Neurosci. Letts, 120*:241, 1990). PRG neurons have now been recorded in the cat and these cells decrease discharge during DCarb similar to decreases observed during REM. Average discharge during DCarb compared to waking (W) revealed a 42% decrease in inspiration (I) and a 76% decrease in expiration (E). This carbachol-induced decrease was blocked by mPRF pretreatment with the muscarinic antagonist pirenzipine. During the REM sleep-like state produced by mPRF microinjection of neostigmine, PRG neuron discharge also decreased during E (-26.7%). These results demonstrate that cholinergic reticular mechanisms can causally mediate state-dependent decreases in PRG neuron discharge.

Support: National SIDS Foundation (KAG) and HL 40881 (RL).

246.9

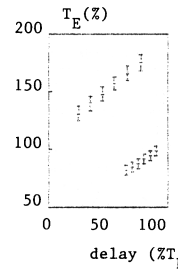
MEDIUM FREQUENCY OSCILLATIONS (MFO) OF PHRENIC UNIT DISCHARGES IN THE COURSE OF INSPIRATION (I). W-X. Huang*, S. Lahiri*, W.R. See, R. Barnhardt* and M.I. Cohen. Univ. of Penna. School. of Med., Phila., PA 19104 and Albert Einstein Col. of Med., Bronx, NY 10461.

In 11 pentobarbital-anesthetized, paralyzed and vagotomized cats, we studied the activities of single phrenic fibers using spectral analysis. Of 34 units recorded, 20 were early-I (onset delay ≤ 80 ms) and 14 were late-I. Out of a total of 43 pairs of phrenic fibers, 12 pairs were early-I. The autospectra of unit discharges, based on activity in the whole inspiratory phase, always showed a prominent frequency peak between 10 and 50 Hz (MFO), followed by several harmonic peaks, indicating the rhythmicity of the discharge. For all units, the frequency of the main peak was lower during the first half of I than during the second half (19.7 ± 1.2 Hz vs 32.0 ± 1.9 Hz). This is a characteristic of MFO rhythms (Christakos et al., FASEB J. 2: A510, 1988). A statistically significant coherence between activities of members of a pair near their autospectral MFO frequencies was found for 16/43 pairs (range of coherence values 0.04 to 0.35). This incidence was predominantly due to the activity of early-I pairs in the first half of I; 12/12 pairs showed such coherences in the first half whereas only 2 had a significant MFO coherence during the second half. This contrasts with previously reported results (Christakos et al., ibid.) that unit-(whole phrenic) MFO coherences were rare and weak in unanesthetized decerebrate cats. (Supported by NIH Grants NS-21068 and HL-27300.)

246.10

SHORTENING OF THE EXPIRATORY PHASE BY LUNG INFLATION (LI) IN DOGS. J.R. Romaniuk*, T.E. Dick, E. Bruce*, G. Supinski*, A.F. DiMarco*. MetroHealth Medical Center, Case Western Reserve University, Cleveland, OH

It has been reported that LI during the last 30% of expiration (E) does not change the duration of expiration (T_E). However, we noted that expiratory motor activity could be terminated by LI (50% of V_T, 1 sec. duration) when applied in late E. In the present study, we reassessed the effect of pulse LI applied in E on T_E. We recorded airway pressure (P) and EMG activities of triangularis sterni (TS) and parasternal intercostal muscles in spontaneously breathing, anesthetized dogs (n = 11). T_E was correlated with the delay (normalized as percent of E, 100% control) at which pulse LI was applied in expiration (See Fig. 1). When LI is applied late in E, vagal input can randomly either prolong or shorten T_E according to two separate linear functions. This bifurcation of the response of T_E to LI suggest the existance of long-lasting central after-effects of LI, becoming evident during repeated stimulation. Supported by HL34143.



246.11

POST-INHIBITORY EFFECTS OF LUNG INFLATION (LI) ON EXPIRATORY PHASE (E) IN DOGS. T.E. Dick, J.R. Romaniuk*, M.R. Bachoo*, N.S. Cherniack, G. Supinski*, A. DiMarco*. MetroHealth Med. Ctr., Case West. Res. Univ., Cleve., OH.

We have previously shown that LI during E inhibits chest wall expiratory muscle EMG, inhibits cervical sympathetic activity (CS) and prolongs E. Release of LI is followed by a return of expiratory EMG prior to the next inspiration. In anesthetized, spontaneously breathing dogs (n=11), we further characterized the return of expiratory activity (EA) by varying the duration and magnitude of LI. We also studied the effects of electrical vagal stimulation on EA in vagotomized, artificially ventilated dogs with open chest. We recorded triangularis sterni (TS) EMG and phrenic (Phr) and CS nerve activity as activities modulated by the central pattern generator for respiration (CPG). The duration of EA after release of LI was inversely proportional to the duration of LI and to the train duration of vagal stimulation and unrelated to blood gas tensions. Increasing amplitude of LI decreased TS activity during LI and increased the duration of EA after release of LI. Release of LI caused excitation of CS which correlated with the degree of decrease in Phr and T_E prolongation of the next breath. These results suggest that LI applied during expiration resets the CPG rather than promoting the expiratory phase. Supported by PHS 25830 and HL34143.

246.12

DYNAMICAL ANALYSIS OF NONLINEAR RESPIRATORY SYSTEMS BY RECURRENCE PLOTS AND ENTROPY CALCULATIONS. Charles L. Webber, Jr. and Joseph P. Zbilut*. Department of Physiology, Loyola University of Chicago, Maywood, IL 60153 and Departments of OR/Surgical Nursing and Physiology, Rush Medical College, Chicago, IL 60612.

Recurrence plots are becoming an important graphical tool for the analysis of nonlinear dynamical systems. Careful examination of contour maps reveals subtle time (or spacial) correlation information as distinguished from stochastic behaviors (noise) and system transients (drifts). Deterministic structures appear as linear segments in a field of rarified or clustered points. To quantify the amount of determinism present, recurrent linear segments are distributed into length histograms. From these binned segments, Shannon entropy values (information content) are computed as complexity measures. To obtain a composite profile of any dynamic ranging from local to global scales, recurrence plot calculations are repeated for step increases in a normalized cutoff parameter. For low cutoff values (local dynamic), entropy values differentiate between aperiodic random and aperiodic deterministic time series. Applying this analysis to respiratory data from spontaneously breathing, unanesthetized rats shows deterministic (rule-obeying), non-random structuring of the local dynamic both in terms of breathing periods and pressure amplitudes. As experience is gained in their interpretation, it is envisaged that recurrence plots will accrue respect for utility in analyzing nonlinear dynamical systems.

246.13

SDH ACTIVITY OF FIBER SUBTYPES ACROSS RESPIRATORY MUSCLES. J.F. Watchko*, W.Z. Zhan, Y.H. Fang*, M.J. Daood*, B.S. Brozanski*, R.D. Guthrie* and G.C. Sieck, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213, and Mayo Clinic, Rochester, MN 55905.

Fibers classified histochemically as type IIB in the rat diaphragm (DIA) express primarily a 2X myosin heavy chain (MHC) isoform, whereas IIB fibers in the external oblique (EO) muscle express a 2B MHC isoform. To indirectly compare the oxidative capacity of different respiratory muscle fiber subtypes, we quantified SDH activity of histochemically classified fibers in the DIA and EO muscles in adult rats using a microdensitometric procedure. SDH activity was expressed as mmol fumarate/l/min.

	Type I	Type IIA	Type IIB
DIA % fibers	35±2	24±8	41±8
SDH	5.95±1.25	6.72±1.31	4.82±1.32
EO % fibers	16±2	14±3	70±2
SDH	3.67±0.63	3.94±0.53	1.58±0.49

SDH activity was lower in all EO fiber types compared to the DIA. The SDH activity of IIB fibers in the EO was disproportionately lower than IIB fibers in the DIA. We conclude that the oxidative capacity of a specific fiber type can vary markedly between respiratory muscles and that this reflects differences in activation history between the DIA and EO muscles. We also conclude that the SDH activity of fibers expressing MHC 2X is intermediate to those expressing MHC 2B and MHC 2A isoforms.

Supported by HL 02491, HL 34817, and HL 37680.

246.14

MATURATION OF THE VAGAL NERVE IN FETAL LAMB. S.U. Hasan, H.B. Sarnat*, R. Auer and A. Rigaux*. University of Calgary, Departments of Paediatrics and Pathology, Reproductive Medicine Research Group, Calgary, Alberta, CANADA T2N 4NI.

Lung distension and oxygenation induce arousal and continuous fetal breathing movements (FBM) in sheep. These breathing and behavioral responses are critically dependent on intact vagal nerves and fetal maturity; almost always occurring after 136 days of gestation (term = 147 days). The objective of this study was to delineate the vagal myelination and axonal maturation at various gestational ages, ranging from 79 to 147 days (n=18). Also, comparison was made with neonatal (n=3) and adult (n=6) vagal nerves. Methods included transmission electron microscopy and computer-assisted analyses of ratios of small, medium sized and large myelinated nerve fibres and axonal diameters in semi-thin plastic embedded sections, stained with toluidine blue. Scattered myelinated fibres are already evident by 92 days gestation. Unmyelinated fibres and lightly myelinated fibres show numerous microtubules in their axoplasm, but more heavily myelinated nerves have relatively fewer microtubules and more neurofilaments. The axoplasm in even the youngest animals examined showed mature neurofilaments with short perpendicular branches; elongated mitochondria with well formed criste also were observed. Degenerative and abnormal proliferative changes were not observed at any age. During the phase of myelination, chromatin of Schwann cells becomes finely dispersed, while completely myelinated fibres are enclosed by Schwann cells. There is a progressive increase in the number of myelinated nerve fibres in the last third of gestation and adult level of vagal maturation is achieved by 138 days of gestation. We speculate that the lack of fetal breathing and behavioral responses prior to 136 days might be due to vagal immaturity.

247.1

ENRICHMENT OF GLUTAMATE-LIKE IMMUNOREACTIVITY IN CERVICOTHALAMIC AND ROUND LARGE TYPE TERMINALS IN THE VENTRAL POSTEROLATERAL NUCLEUS OF THE CAT THALAMUS. J. Broman and O.P. Ottersen. Dept. Cell Biol., Fac. Health Sci., Univ. Linköping, Sweden (JB) and Dept. Anatomy, Inst. Basic Med. Sci., Univ. Oslo, Norway (OPO).

The distribution of glutamate-like immunoreactivity (Glu-LI) in the ventral posterolateral nucleus (VPL) was quantitatively evaluated with the electron microscopic immunogold technique. Cervicothalamic tract (CTT) terminals were identified through the presence of peroxidase activity following injections of WGA-HRP into the lateral cervical nucleus (LCN). Three sections from each of 3 cats were analyzed.

Figure 1 shows the results expressed in percent of the mean gold particle density for all structures analyzed in each of the sections. Enrichment of Glu-LI was evident in RS (round small) terminals of probable cortical origin and also in CTT terminals and RL (round large) terminals, the latter probably originating from the dorsal column nuclei and/or the LCN.

The present findings suggest that glutamate is a neurotransmitter in ascending somatosensory afferents to the VPL.

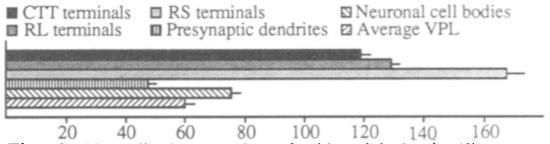


Fig. 1. Normalized mean values of gold particle density (%)

247.2

AN ULTRASTRUCTURAL ANALYSIS OF CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVE (CGRP-IR) SYNAPTIC CONTACTS IN THE THALAMUS OF THE RAT. A.M.Williamson and H.J.Ralston,III. Dept of Anatomy, UCSF, San Francisco, CA 94143. USA.

Several recent studies have shown a large CGRP-ir projection from the medial and ventral lateral parabrachial nuclei to the ventroposterior nucleus (VPpc) of the thalamus and to the cortex in the rat. The function of CGRP in this system is not known, but may be linked to somatic or visceral central pathways including those mediating nociception.

We used standard immunohistochemical and electron microscopic techniques to examine the nature of the CGRP-ir synaptic contacts in VPpc.

Our initial analysis of 300 synaptic contacts in VPpc (3 rats) showed both symmetric and asymmetric types in roughly equal numbers. We saw only 4 contacts on cell bodies. We did not observe synaptic contacts onto presynaptic elements, or onto CGRP-ir dendritic elements. CGRP-ir fibers were primarily small and unmyelinated, but several myelinated fibers were also seen. The postsynaptic element varied in size sufficiently to conclude that CGRP-ir input occurs on all calibers of dendrites.

We suggest that the dual forms of synaptic contacts, symmetric and asymmetric, made by CGRP-ir axons may represent different types of synaptic function.

This work was supported by NS23347 and NS21445.

247.3

POST-SYNAPTIC EFFECTS FROM THE DORSAL COLUMN NUCLEI IN THALAMIC VL NEURONS. R. Mackel and E. Miyashita*. The Rockefeller University, New York, NY 10021.

To reinvestigate of whether dorsal column (DC) information may reach motor cortex via the ventrolateral (VL) nucleus of the thalamus, we recorded intracellularly from VL neurons while stimulating the DC nuclei. Experiments were performed in cats anaesthetized with chloralose. Recordings were obtained with glass micropipettes filled with 2 M K-acetate saturated with fast green. VL neurons were identified by their direct input from the cerebellum and, if possible, antidromic activation from motor cortex. Recording sites were marked by dye injection, stimulation sites by electrolytic lesions.

80/105 (76%) neurons (20 fully identified as projection neurons), located throughout VL, including the border area with VPL, responded with excitation or inhibition to DC stimulation. One EPSP occurred at a latency of 2 ms, did not display temporal facilitation and may have been monosynaptic. The remaining EPSPs had latencies of 3-20 ms (median 10 ms) and showed temporal facilitation, consistent with polysynaptic transmission. Increasing the stimulus intensity did not shorten the EPSPs, mitigating the possibility of restricting stimulation sites. Results show that DC input can reach motor cortex polysynaptically via VL. However, no evidence was obtained for a direct, short latency route via VL to motor cortex.

Supported by NS 26288.

247.4

DIFFERENTIAL SYNAPTIC ORGANIZATION OF THE TRIGEMINAL PRINCIPALIS AND INTERPOLARIS PROJECTIONS TO THALAMIC BARRELOIDS. M.N. Williams, D.S. Zahm, & M.F. Jacquin. Anatomy & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Most thalamic VPM cells in rodents have single vibrissa receptive fields (RFs) that establish similar RFs in layer IV of barrel cortex. However, it is not clear how VPM RFs are synthesized. Multiple lines of evidence indicate that extensive inputs from trigeminal nucleus principalis (PrV), where single whisker RFs are prevalent, play a major role. A less robust projection to VPM arises from subnucleus interpolaris (SpVi), yet these multi-whisker inputs are only expressed after PrV lesions. To account for the differential effectiveness of these 2 parallel pathways in activating VPM cells, Rhoades et al. (J. Neurophysiol. 57, '87) hypothesized that SpVi fibers end on more distal portions of VPM dendrites than those from PrV. As a first step in testing this hypothesis in adult rats, HRP transport to VPM from left (L) SpVi and right (R) PrV in the same cases was processed for TMB reaction product and electron microscopy. Analysis of all labeled terminals in matching L and R barreloids (N=2), as well as the majority of labeled terminals in all of the L and R VPM (N=3), indicated that PrV terminals contacted VPM dendrites with a mean (±SD) diameter of 1.51±.10 um. SpVi contacts were on VPM dendrites with a mean diameter of 1.27±.07 um. This difference was statistically reliable ($F=11.6$, $df=1,4$, $p<.05$). PrV endings were also more likely to contact VPM somata (11.0±4.2%) than those from SpVi (3.0±1.0%, $p<.05$). Insofar as primary dendrites are thicker than distal dendrites in VPM (e.g. Harris, JCN 251, '86), these data suggest a differential distribution of V inputs onto VPM cells that may account for the different roles of PrV and SpVi in VPM RF synthesis. NIH DE07662, DE07734, NS23805.

247.5

EFFECTS OF NEONATAL INFRAORBITAL NERVE DAMAGE UPON THE MORPHOLOGY AND RESPONSE PROPERTIES OF CELLS IN VENTRAL POSTEROMEDIAL NUCLEUS OF THE RAT. N.L. Chiaia, W.H. Bauer, S. Zhang* and T.D. King*. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Infraorbital nerve (ION) damage in neonatal rodents alters the somatotopic organization of the ventral posteromedial thalamic nucleus (VPM). This investigation utilized intracellular recording and HRP injection techniques to assay the effects of such lesions upon the morphology and response properties of individual VPM neurons. In normal adult rats (N=148 cells), 82% of VPM cells respond only to vibrissa deflection and the average number of vibrissae in a receptive field is 1.4. Only 3% of the VPM neurons recorded in normal rats are unresponsive. In nerve damaged rats (N=409 cells), only 23% of the neurons responded to vibrissa deflection and the average receptive field "size" was 2.0 vibrissae; 21% of the vibrissa-sensitive cells also had other tactile inputs. Thirty five percent of the VPM cells in the nerve damaged rats were unresponsive. VPM cells recovered from normal rats (N=34) had an average total dendritic length of 4,476 μm and an average cross-sectional area of 34,764 μm^2. The average values for the 30 VPM cells recovered from nerve damaged rats were 4,799 μm and 48,011 μm^2, respectively. There were no significant correlations between receptive field size or type and measures of dendritic morphology for the recovered neurons. These data thus indicate that neonatal ION lesions render many VPM cells unresponsive to peripheral stimulation and increase the receptive field sizes of many other neurons. Both of these functional changes are associated with an increase in the extent, but a reduction in the density, of the dendritic arbors of these cells. DE 07734, DE 08971

247.6

FRACTAL DIMENSIONS OF NEURONS LOCATED IN THE CAT'S THALAMIC VENTROBASAL COMPLEX (VB) AND ITS VENTRAL PERIPHERY (VB_{vp}). K.-D. Kniffki(1), D. Chialvo(2), C. Vahle-Hinz(1) and A.V. Apkarian(2). (1)Physiologisches Institut, Universität Würzburg, D-8700 Würzburg, Germany; (2)Depts. of Neurosurg. and Pharmacol., SUNY Health Science Center, Syracuse, N.Y. 13210, U.S.A.

The complex dendritic branching pattern of neurons is thought to result from local environmental as well as from genetic influences. Which mechanisms underlie the formation of these branching patterns during development is an unresolved question. Recently, the shapes of retinal neurons were found to have a fractal dimension of D=1.68±0.15 (Caserta et al., Physical Rev. Lett. 64: 95-98, 1991). The authors proposed an explanation of certain stages of neuronal shape development in terms of a diffusion-limited-aggregation model which predicts D=1.70±0.10.

In the present study we examined the fractal dimension of Golgi-stained neurons located in VB and VB_{vp} using 2-dimensional drawings. Two different protocols were employed to determine D. Hausdorff (D_h) dimensions were determined by the box-counting protocol. For a new protocol (D_s) a Sholl analysis (Sholl, J. Anat. 87: 387-406, 1953) with a grid of concentric circles was used. D_s was determined by the slope of the log-log plots of the cumulative number of intersections of the dendrites with the grid versus the radius of the circles. Corrections were made for differences in the thickness of the dendrites.

In general, D_s (range 1.39-1.81) was larger than D_h (range 1.27-1.49). There seems to be a tendency, however, for both D_s and D_h to be larger for neurons within the core of both nuclei as compared to those located near the ventral border of the VB_{vp}. The fractal dimension might be a useful parameter to characterize the morphology of neurons.

247.7

MAPS WITHIN THE CAT'S SOMATOSENSORY THALAMUS.
J.W. Crabtree. Department of Human Anatomy, University of Oxford, U.K.

The rabbit's visual thalamus contains precise maps of the visual field both within the main thalamic relay nucleus and the thalamic reticular nucleus (TRN). In the present study of the cat's somatosensory thalamus, injections of HRP and/or [3H]proline were made into somatosensory cortical areas S1 and S2. The cortical representation of the chorda tympani nerve (CT) was also injected. The resultant anterograde labelling in the thalamus was analyzed. The thalamus was also processed for immunoreactivity to monoclonal antibody Cat-301.

The cat's main thalamic somatosensory relay, the ventrobasal complex (VB), is immunoreactive to Cat-301. Three major reactive regions are distinct, which laterally to medially appear to correspond to the electrophysiologically defined hindlimb, forelimb, and head representations, respectively. The patterns of labelling within VB following tracer injections into focal regions of S1 confirm this correspondence. The somatosensory sector of the TRN is also immunoreactive to Cat-301, revealing distinct regions of strong and weak reactivity that correspond to the sector's outer and inner aspects, respectively.

Single injections of a tracer into S1 result in single zones of terminal label within the TRN. Each zone lies within the plane of the nucleus, occupies only a fraction of its thickness, and is broadly distributed in the dorsoventral and oblique rostrocaudal dimensions. Injections into the three major body representations of S1 (hindlimb, forelimb, and head, respectively) progressively produce labelling in lateral to medial zones within the region of the TRN that is strongly Cat-301 reactive. Injections of S2 produce labelling that overlaps that resulting from S1 injections. Injections of CT (cortical taste representation) produce labelling that lies adjacent to and inside the zone of the head representation, demarcating the innermost aspect of the strong Cat-301 reactive region.

The present results indicate that there is a precise map within the somatosensory sector of the cat's TRN, the spatial organization of which mimics the map within VB. Precise maps within the TRN provide evidence for a specific functional role for the nucleus. (Supported by the MRC, Grant No. G8720770N)

247.8

THE ROLE OF THE RETICULAR THALAMIC NUCLEUS IN THE PROCESSING OF SOMATOSENSORY INFORMATION IN THE VENTROPOSTERIOR LATERAL THALAMIC NUCLEUS OF THE CAT. R.A. Warren and E.G. Jones. Dept. of Anatomy and Neurobiol., University of California, Irvine, CA 92717.

In the cat ventroposterior lateral thalamic nucleus (VPL), GABAergic inhibition originates both from local acting interneurons and from the GABAergic projections from the reticular thalamic nucleus (RTN). We used an inactivating paradigm in order to evaluate the contribution of the RTN to the inhibition observed in the VPL. Pressure ejected cobaltous chloride was used to inactivate the somatosensory part of RTN while recording single VPL neurons responding to mechanical stimulation of their receptive field.

Fourteen VPL neurons from 6 cats were studied. The neuronal activity of certain neurons was enhanced during and/or just after the ejection of the cobalt solution in RTN. In a few cases the inactivation of RTN appeared to produce a transient inhibition of neuronal activity while in the remaining cases no change in neuronal activity could be detected.

While the excitation is consistent with the hypothesis that the cell under study is under the direct influence of RTN inhibition, the inhibitory effects resulting from the inactivation also suggest an indirect projection via inhibitory interneurons. These two hypothesis are currently under investigation. Supported by NIH grant NS22317 and FRSQ of Canada.

247.9

PRIMARY PROJECTION TARGETS OF THERMORECEPTIVE LAMINA I TRIGEMINOTHALAMIC AND SPINOTHALAMIC CELLS IN THE CAT. J.O. Dostrovsky and A.D. Craig. Dept. of Physiology, Univ. of Toronto, Toronto, Canada M5S-1A8 and Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Preceding anatomical (PHA-L) studies indicated that lamina I trigeminothalamic (TrT) and spinothalamic (STT) cells terminate in n. submedius (Sm), the dorsomedial aspect of the ventral posterior medial n. (dmVPM), the ventral aspect of the basal part of the ventral medial n. (vVMb), and other sites. We are using antidromic activation from a roving array of 8 electrodes to identify the projections of single thermoreceptive-specific (cold sensitive) lamina I TrT and STT cells. We have previously demonstrated that all "cold" lamina I cells that project to Sm also project to vVMb and dmVPM. In the present experiments, the array was initially positioned to select for lamina I cells that projected to dmVPM rather than Sm. Antidromic maps with 0.25 mm vertical and 0.5-1.0 mm horizontal resolution were made for 9 lamina I TrT and 7 lamina I STT (L7) "cold" cells recorded with tungsten microelectrodes in 16 barbiturate-anesthetized cats. The preliminary histological analyses indicate that 6 of the 16 cells did not project to Sm, although all projected to vVMb. Thus, dmVPM and vVMb, but not Sm, appear to be the primary projection targets for thermoreceptive lamina I TrT and STT cells. These data support the possibility that dmVPM, where Landgren ('60) first identified cold-specific activity, may be an important thalamic site for thermoreception.
(supported by NIH grants to J.O. Dostrovsky and A.D. Craig)

247.10

ANALYSIS OF INTRACELLULARLY FILLED THALAMO-CORTICAL PROJECTION (TCP) NEURONS IN THE SOMATOSENSORY THALAMUS OF THE PRIMATE. P.T. Ohara and L.A. Havton. Dept of Anatomy, UCSF, San Francisco, CA 94143. USA.

Standard techniques were used to record receptive fields and label TCP neurons in sodium pentobarbitol anaesthetized primates using glass micropipettes containing 4% HRP or 2% Neurobiotin. The animals were perfused with aldehyde fixatives and 50μm coronal sections through the thalamus were cut, processed and mounted for light microscopy. The filled cells were located in VPM or VPL and responded to the normal range of non-nociceptive stimuli.

Somata and all dendrites of 13 cells were fully reconstructed using a light microscope with camera lucida attachment. The reconstructed neurons have between 7 and 10 dendrites (mean=7.5; SD=1.2) and range in total surface area from 33,000μm^2 to 73,600μm^2. Most dendritic branching occurs in close proximity to the soma resulting in short pre-terminal segments while terminal segments are long, comprising 88.5% (SD=2.2) of the total dendritic length. Analysis reveals that dendritic nodes are unevenly distributed in the perisomatic space and the majority of neurons have a different but preferred orientation of dendrites.

Analysis of all the measured parameters suggests that non-nociceptive somatosensory TCP neurons form a homogeneous population and have a stereotyped morphology.

Support: NS23347, NS21445 and NS26488

247.11

ANALYSIS OF DENDRITES FROM INTRACELLULARLY FILLED THALAMO-CORTICAL PROJECTION (TCP) NEURONS IN THE SOMATOSENSORY THALAMUS OF THE PRIMATE. L.A. Havton and P.T. Ohara. Dept of Anatomy, UCSF, San Francisco, CA 94143. USA.

Quantitative analysis of physiologically characterized, intracellularly filled TCP neurons in the primate somatosensory thalamus reveals the neurons to form a homogenous population with a stereotyped morphology. In order to determine whether individual dendrites of the labelled neurons also have a stereotyped morphology we have examined the dendrites from all labelled neurons as a single group.

98 dendrites from 13 neurons were fully reconstructed and quantatively analysed at the light microscopic level. Dendrites range in total surface area from 1418μm^2 to 6848μm^2 with a mean of 2393μm^2 (S.D. 1372).

There is a positive correlation between primary dendritic size and total dendrite size. The mean segment length increases with increasing segment order for preterminal segments, whereas the mean segment length of end segments decreases with increasing segment order. Analysis of dendritic branching reveals a consistant pattern of fairly asymmetrical distribution of dendritic end segments at branch points. (PSAD=0.60, S.D. 0.19).

There is considerable variation in the morphology of individual dendrites indicating that the more stereotyped structure of individual TCP neurons results from a combination of dendrites with different features.

Support: NS23347, NS21445 and NS26488

248.1

THE ORGANIZATION OF CHOLINERGIC PROJECTIONS FROM THE NUCLEUS BASALIS OF MEYNERT TO SENSORY-MOTOR CORTICES. K. A. Baskerville*, P. Herron, and H. T. Chang, Dept. of Anatomy and Neurobiology, The Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163

The purpose of this study was to determine in rats 1) the topography of cholinergic neurons in nucleus basalis of Meynert (NBM) of the basal forebrain that project to the somatosensory and motor cortices, and 2) the percentage of retrogradely-labeled neurons that are cholinergic.

Double-labeling experiments were done using fluorescent retrograde tracers and choline acetyltransferase (ChAT) immunocytochemistry. Tracers were injected in electrophysiologically-identified sites of sensory-motor cortices. Following survival periods of 24 to 48 hrs, animals were perfused with 4% paraformaldehyde in phosphate buffer, the brains were sectioned, and the tissue reacted for ChAT immunocytochemistry.

The results show that a medial-to-lateral distribution of injections in the somatosensory cortex labeled a rostral to caudal sequence of neurons in the NBM. Injections in homotopic regions in somatosensory and motor cortices labeled different groups of NBM neurons. Seventy to 96% of retrogradely-labeled NBM neurons were also labeled by ChAT immunofluorescence.

These results suggest that most afferents from NBM to the sensory-motor cortices are ChAT-positive and that these afferents are topographically organized. Moreover, cholinergic input to homotopic regions of sensory-motor cortices arise from separate populations of NBM neurons. (Supported by NIH grant AG05944, Alzheimer's Association, and The Center for Neuroscience of The Univ. of Tennessee, Memphis).

248.2

THALAMOCORTICAL CONNECTIVITY PATTERNS IN AN OLD WORLD PRIMATE, Erythrocebus patas. **S. Warren and J. E. Taylor*. Univ. of Mississippi Medical Center, Dept of Anatomy, 2500 N. State St., Jackson, MS. 39216-4505.**

The topographic organization of cutaneous inputs within the primary somatosensory (SI) cortex of the Old World primate, Erythrocebus patas (Warren and Carlson, '87) has been examined electrophysiologically. However, patterns of thalamocortical connectivity between the ventral posterior (VP) nuclei and SI cortex in E. patas have not been described. Consequently, these connections were studied in five animals by placement of separate, discrete injections of different retrograde tracers into physiologically identified, SI body, hand and/or face recipient areas. Similar to other Old World anthropoids, neurons retrogradely labelled from injections confined to areas 3b and 1 form somatotopically arranged lamellae with lower extremity laterally placed in ventral posterior lateral (VPL) nucleus, the hand medial, and the face in ventral posterior medial (VPM) nucleus. There is a tendancy for lamellae resulting from digit injections to be located in the rostral half of the nucleus, while lamellae resulting from injections of palm and hand dorsum appear restricted to more caudal portions of nucleus. Injections into SI digit areas responding to both cutaneous and joint stimulation of the same digit produce an intermingling of labelled neurons of differing submodalities within individual digit lamellae. In addition, labelled neurons are seen within the posterior (Po) group. To contrast with other monkeys, some injections confined to SI cortex, also label an additional separate population of neurons confined to a location medial and dorsal to the labelled lamellae within the confines of VP. This particular pattern suggests a possible connection between intralaminar nuclei and/or ventral lateral nucleus and SI cortex in this species. Thus, while displaying patterns of thalamocortical connectivity similar to other species, fine details of these patterns in E. patas suggest additional species specific connections. Supported in part by BRSG #2SORR5386 and NIH #NS29776.

248.3

MORPHOLOGY OF INDIVIDUAL THALAMOCORTICAL AFFERENTS IN THE PRENATAL RAT NEOCORTEX S. Catalano*, R.T. Robertson and H.P. Killackey, Depts of Psychobiology and of Anatomy and Neurobiology, University of California, Irvine, CA 92717

Dawson and Killackey ('85, Dev. Brain Res. 17:309) reported that on the day of birth, topography within the thalamocortical projection to rat somatosensory cortex is similar to that of the adult. On the other hand, Naegele et al ('88, J.Comp.Neurol. 277:593) reported that geniculocortical topography within the neonatal hamster visual cortex is progressively refined during postnatal development; these authors attribute this refinement to loss of multiple short collaterals from individual afferents within the intermediate zone. In this context we examined the morphology of thalamic afferents in the somatosensory cortex of rats aged embryonic day 16 (E 16) through 21 (the day of birth). Afferents were labeled with DiI, photoconverted and drawn using a camera lucida. On E 17, fibers run parallel to the pial surface within the intermediate zone without branching, and turn at the lower border of layer VIb. These fibers course obliquely through layer VIb to reach the bottom of the cortical plate where they terminate in a complex growth cone. At later ages fibers begin to form obliquely oriented branches within the lower cortical layers. At any age examined, only a very small proportion of fibers within the intermediate zone give rise to radially oriented collaterals. These observations suggest that topographic cortical maps may develop differently in different areas of cortex or different species of rodent. Supported by NSF grant BNS90-22168.

248.4

Volume Rendering of Mouse Barrel Components Labeled in Fixed Tissue with Lipophilic Dyes

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Elements of the developing mouse trigeminal pathway were labeled in fixed tissue using crystals and solutions of a variety of lipophilic dyes, including DiA, DiI, and DiQ (Molecular Probes). Serial confocal optical sections were acquired using a Laser Scan Microscope (LSM, Carl Zeiss), and transferred across an IEEE interface into an imaging workstation (Silicon Graphics). Three-dimensional views of thalamocortical afferents and intrinsic cortical cells were obtained, as volume renderings of the scanned data, using commercially available software (Vital Images). The spatial interrelationships of labeled ingrowing axons and cortical dendrites, with each other and with the developing barrel septa, are clearly evident in stereo pairs and film loops of the volume reconstructions. They support the view that these neurites are selectively remodeled to match (and form) emerging barrel boundaries. Attempts are currently being made to analytically extract the three-dimensional boundaries of the labeled material for structural analysis, electrical modeling and simulation of interacting neurite outgrowth in a synthetic population of cells. Supported by NIH grants NS 17763 and NS 07057.

248.5

DIFFERENTIAL LAMINAR DISTRIBUTION OF CORTICOTHALAMIC PROJECTION NEURONS IN RAT PRIMARY SOMATOSENSORY CORTEX. K. E. Good* and H. P. Killackey, Department of Psychobiology, University of California, Irvine, CA 92717.

Several previous studies have reported that corticothalamic projection neurons arise from layers V and VI of rat primary somatosensory cortex (Wise & Jones 1977; Chmielowska et al., 1989). These studies, however, did not differentiate between two potential targets of such projections in the rat thalamus, namely, the ventral posterior nucleus (VP) and the medial division of the posterior complex of nuclei (POM). The present study was undertaken to assay the differences, if any, between the cortical projection to these two thalamic cell groups.

Stereotaxic pressure injections of rhodamine or flouroscein conjugated microspheres were made into either VP or POM. In most cases, an individual injection of a tracer was confined to one of the two targets on a given side of the thalamus although in some cases dual injections were made into the same side of the thalamus. Forty eight hours after the injections the rats were euthanized, perfused and their brains removed. The brains were sectioned in the coronal plane and processed for examination by epiflourescent microscopy.

The dual injections revealed that few, if any, cortical neurons projected to both thalamic targets. Further, both the dual and individual injections revealed that projection neurons which project to VP and POM have a differential laminar distribution. Cortico-VP projection neurons were distributed in a single dense band which was largely confined to the upper portion of layer VI. Cortico-POM projection neurons, on the other hand, were distributed in a bilaminar fashion consisting of a dense band of labeled neurons in the lower portion of layer VI, and a more superficial and sparse band in layer V. This differential distribution of corticothalamic projection neurons is presumably related to differences in the functional properties of the two thalamic targets.

248.6

COMBINED INJECTIONS OF IBOTENIC ACID AND HORSERADISH PEROXIDASE (HRP) FOR STUDYING RECIPROCAL CONNECTIONS. E.L. WHITE and D. CZEIGER, DEPT OF MORPHLOGY, BEN-GURION UNIV. BEER SHEVA, ISRAEL.

Reciprocal pathways are particularly prominent in the cerebral cortex where they link cortical areas with each other and with specific thalamic nuclei. Previously, we have examined reciprocal thalamocortical (TC) relationships by combining injections of HRP into mouse thalamus to label corticothalamic neurons, with electrolytic lesioning of the injection site, one day later, to label synapses made in cortex by TC afferents. Here we describe a simplified approach: thalamic lesions and HRP labeling are effected by a single injection of a 40% solution of HRP containing 1.0% ibotenic acid.

The axon terminals of chemically lesioned TC afferents to layer IV of the SI barrel field form approximately 20% of the asymmetrical synapses within barrel hollows. At 4 days post-injection, all affected terminals are in medium/late stages of degeneration; about 80% synapse onto spines. The retrograde labeling of corticothalamic cells by HRP is unaffected by the presence of ibotenic acid; the local axon collaterals of these cells form asymmetrical synapses mainly onto dendritic shafts. These are precisely the same results obtained when HRP injections are followed by electrolytic lesions. We conclude that combined HRP/ibotenic acid injections represent an efficient alternative to HRP injection/electrolytic lesioning, since with the former, the lesion is placed easily and accurately at the site of the HRP injection. NIH 20149 and BSF 89000-52.

248.7

DISTRIBUTION OF GABA-IMMUNOREACTIVE NEURONS IN CAT SI CORTEX. J. Li* and H.D. Schwark. Department of Biological Sciences, University of North Texas, Denton, TX 76201.

Rapidly- (RA) and slowly-adapting (SA) submodality-specific regions have been described in area 3b of the cat primary somatosensory (SI) cortex (Sretavan & Dykes '83). Compared to SA regions, a higher proportion of neurons located in RA regions are sensitive to locally applied bicuculline (Dykes et al. '84), suggesting differences in the organization of GABAergic systems between these two regions.

In the present study, we have plotted the distribution of GABA-immunoreactive cells in 16 um parasagittal sections through SI in order to determine if differences in GABA cell densities might correlate with the segregation of submodalities. In each section, all GABA-positive profiles throughout SI were plotted in a Cartesian plane. Heterogeneities in GABA-immunoreactive cell densities were assessed by visual inspecting cell plots and by counting the number of GABA-immunoreactive cells in a grid of 100 um x 100 um bins. Reliability of detected patterns was determined by analyzing series containing 4-6 alternate sections.

We have analyzed three cortical hemispheres. The distribution of GABA neurons in area 3b was somewhat patchy, but the patterns were not uniform across a series of sections, suggesting that the differences in densities are not related to the interdigitating band pattern described by Sretavan and Dykes ('83). GABA-immunoreactive cells are more densely distributed in superficial than in deep cortical layers in all areas. The middle layers of area 3b contain higher densities of GABA neurons than the same layers in area 2. We are presently quantifying total cell numbers to determine the percentages of GABA-immunoreactive cells in SI.
Supported by NIH grant NS25729.

248.8

ONTOGENY OF EXCITATORY AMINO ACID RECEPTORS IN BARREL FIELD CORTEX OF IMMATURE RAT. M.E. Blue, J.W. McDonald and M.V. Johnston. Kennedy Research Institute, Dept. of Neurology, Johns Hopkins Univ. Sch. of Med. Baltimore MD, 21205.

The ontogeny of quisqualate (QUIS) and *N*-methyl-D-aspartate (NMDA) receptors in barrel field cortex was studied in rats between postnatal days 1 and 21 (P1-P21). The pattern of QUIS and NMDA sites was examined in flattened sections of cortex using QUIS-sensitive and NMDA-sensitive [^{3}H]-glutamate binding. No distinct pattern of QUIS labeling is observed at birth (P1), but by P5, dense patches of QUIS sites are localized in discrete barrel centers, forming a vibrissa-related map. After P14, the pattern becomes less distinct and by P21, QUIS sites no longer form a vibrissa-related map. In contrast, NMDA binding shows a complementary pattern with fewer sites in barrel centers at ages P7-P10. With time (P14-P21), the density of NMDA sites increases in barrel centers, yet the number of NMDA sites remains low in barrel septa. These results show a differential ontogeny of NMDA and QUIS sites in the barrel field of the immature rat. The developmental expression of QUIS sites follows that of thalamocortical and serotonergic axons, which are distributed over barrel centers by P3 (Blue et al., 1990). Unlike the thalamocortical axons, the expression of QUIS sites in a vibrissa-like pattern is transient. The initial appearance of QUIS labeling in barrels most closely coincides with the onset of barrel formation, suggesting that the ontogenetic pattern of these postsynaptic receptors may reflect the cytologic differentiation of barrels. The period of rise in the density of NMDA sites occurs after that of QUIS sites suggesting that NMDA sites may influence later stages in barrel development. Supported by NIH grant NS28208.

248.9

THE DISTRIBUTION OF LOCAL CIRCUIT NEURONS AND GABAergic BOUTONS WITHIN THE GUINEA PIG THALAMUS. C. Asanuma. Laboratory of Neurophysiology, NIMH, NIH Animal Center, Poolesville, MD 20837.

There is considerable variation in the relative proportions of GABAergic local circuit neurons within thalamic relay nuclei among mammals. For example, GABAergic neurons may account for as much as 30% of the relay nuclei neuronal population in cats, while local circuit neurons are essentially absent from many nuclei in rats and mice, as well as in opossums (LGN, MGN, and LP excepted).

To determine if the striking paucity of local circuit neurons is seen as well in guinea pig thalamic nuclei, frontal or horizontal sections of guinea pig brains were stained with GABA or GAD. As in all species examined thus far, GABA$^{(+)}$ neurons occur within the LGN, MGN and LP of guinea pigs. In addition to these nuclei, GABA$^{(+)}$ neurons are prevalent also within the guinea pig VB. This contrasts with the virtual absence of interneurons well documented in the VB of other phylogenetically primitive mammals. The GABA$^{(+)}$ VB neurons in the present material are, in general, smaller (ca. 10-15 µm in diameter) than nearby GABA$^{(-)}$ neurons (ca. 20-25 µm in diameter). GABA$^{(+)}$ neurons account for ~10 % of the neurons in this nucleus; this is somewhat less than the proportion seen in LGN, MGN, and LP. The rest of the guinea pig dorsal thalamus contains only occasional GABA$^{(+)}$ neurons.

While GABA$^{(+)}$ neurons are found only in a few select relay nuclei within the guinea pig thalamus, small GABA$^{(+)}$ or GAD$^{(+)}$ puncta, possibly corresponding to GABAergic axonal boutons, occur virtually throughout. The distribution of the labeled puncta, however, is not uniform, and their densities are significantly higher within those nuclei containing appreciable numbers of local circuit neurons.

Thus, there is considerable variation in the presence and distribution of local circuit neurons and GABAergic puncta among thalami in different species – such variation can be marked even within a single order of mammals. The differential distribution of the GABAergic elements within the guinea pig thalamus described here may be of relevance for studies of thalamic function in this species.

248.10

SIMULTANEOUS EM-IMMUNOCYTOCHEMICAL IDENTIFICATION OF SEROTONIN TERMINALS AND GABA PROCESSES IN CAT VPL THALAMIC NUCLEUS. X.-B. Liu, J. DeFelipe and E.G. Jones. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Only 7-10% of monoaminergic terminals make conventional synaptic contacts in the VPL thalamic nucleus. The majority however form close membrane appositions that may represent atypical synaptic sites. (X.-B. Liu and E.G. Jones, Exp. Brain Res., in press). In the present study, preembedding immunoperoxidase (for serotonin) and postembedding immunogold labeling (for GABA) were combined to demonstrate light and electron microscopically the neuronal targets of serotonergic afferents.

Adult cats were perfused with 4% paraformaldehyde or 2% paraformaldehyde plus 0.2-0.5% glutaraldehyde in 0.1M phosphate buffer. Vibratome sections through VPL were processed for serotonin immunocytochemistry by ABC peroxidase and embedded in Araldite. Thin sections were collected on Formvar-coated single slot nickel grids and postembedding immunogold staining for GABA was carried out on the grids.

5-HT immunoreactive fibers and terminal varicosities surround both relay cells and GABA positive interneurons. Ultrastructurally, the majority of serotonin terminals make close contact without membrane specializations with GABAergic axon terminals (F1 type) or GABAergic presynaptic dendrites (PSD) and some contact GABAergic somata, but few conventional synapses are detectable. A small number of 5-HT terminals contact GABA negative dendritic shafts of relay cells and form typical asymmetric synaptic contacts. These findings suggest a dual innervation pattern in VPL. Supported by USPHS grant no. NS 22317.

248.11

CHEMICAL COMPARTMENTS IN VPL NUCLEUS OF MONKEY THALAMUS. E. Rausell[1], C.S. Bae[1], C.G. Cusick[2], E.G. Jones[1]. [1]Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717 and [2]Dept. of Anatomy, Tulane University Medical Center, New Orleans, LA 70112.

The ventral posterior lateral nucleus (VPL) of the monkey thalamus is the relay for medial lemniscal and spinothalamic pathways to somatosensory cortex (SI). Two different classes of thalamo-cortical relay cells in VPL can be distinguished by differential immunoreactivity (IR) for the calcium binding proteins parvalbumin and 28Kd-calbindin (Jones and Hendry, Eur.J.Neurosci., 1:222, 1989). In the associated ventral posterior medial nucleus (VPM) parvalbumin and calbindin-IR neurons form separate compartments, which relay principal and spinal inputs respectively to different layers of SI (Rausell and Jones, J.Neurosci., 11:226, 1991). In this study, we investigated the distribution, and connectional relationships of parvalbumin and calbindin neurons in VPL by combining immunohistochemical, histochemical, and fluorescent tracing techniques in normal monkeys and in monkeys subjected to chronic dorsal rhizotomy. a) Parvalbumin-IR neurons are concentrated in cytochrome-oxidase (CO) rich patches that form discontinuous lamellae oriented anteroposteriorly in VPL. These neurons project to deep layers of SI. b) Calbindin-IR neurons are distributed in CO poor spaces that intervene between the parvalbumin patches, form a shell around the nucleus, and project to superficial layers of SI. c) The calbindin domain of VPL is the recipient of spinothalamic terminals and the parvalbumin domain of lemniscal terminals. d) Chronic dorsal rhizotomy results in enhanced calbindin immunostaining, and decreased parvalbumin immunostaining in the regions of the VPL deprived of afferent activity. These results indicate that lemniscal and spinothalamic inputs are relayed to different layers of SI by separate VPL neuronal populations. The two populations show differential responses when the balance of afferent activity is perturbed. Supported by USPHS grants NS 21377 and NS 22317.

248.12

FUNCTIONAL ASYMMETRIES IN THE RODENT BARREL CORTEX. J.S. McCasland, G.E. Carvell, D.J. Simons and T.A. Woolsey. Dept. Neurosurgery, Washington University, St. Louis MO 63110, and Dept. Physical Therapy and Dept of Physiology, University of Pittsburgh, Pittsburgh PA 15261.

Asymmetries in the functional organization of rodent whisker-barrel cortex have been demonstrated by previous 2-deoxyglucose (2DG) and neurophysiological studies. We examined the possibility that the activity gradients observed in metabolic studies can be attributed to subtle rostral/caudal and dorsal/ventral asymmetries in physiologically measured surround or cross-whisker inhibition by comparing 2DG results with predictions generated from quantitative single-cell receptive field data. Despite differences in the two experimental approaches there is remarkable agreement between them. 1) The density of 2DG labeling declines across the barrel cortex of the behaving animal from anteromedial to posterolateral barrels in a manner qualitatively and quantitatively similar to the values predicted from neurophysiology. 2) The strength of surround inhibition in barrel neurons predicts the two-fold increase in activation of the C3 barrel following acute trimming of adjacent whiskers. And, 3) within a cortical column the decrease in metabolic activity associated with adjacent whisker stimulation is greatest in layer IV and least in the infragranular layers, a finding qualitatively similar to the laminar distribution of inhibitory interactions observed physiologically.

Supported by NIH Grants NS 19970 (D. J. S.) and NS 17763 (J. S. M. and T. A. W.), the McDonnell Center for Studies of Higher Brain Function (T. A. W.) and the Spastic Paralysis Foundation of the Illinois-Eastern Iowa District of the Kiwanis International (T. A. W.).

248.13

SUPPLEMENTARY SENSORY AREA VERSUS THE PARIETAL OPERCULUM: WHICH IS THE REAL SECOND SOMATOSENSORY AREA? Richard J. Caselli, M.D., Department of Neurology, Mayo Clinic, Scottsdale, Arizona 85259

Isolated lesions of the parietal operculum, currently regarded as the second somatosensory area (SII) and supplementary sensory area (SSA) are rare, but two patients are presented to contrast the striking differences resulting from selective damage to each somatosensory area. Damage to SII causes a subtle, non-disabling disturbance of somesthetically mediated object recognition (tactile agnosia), which cognitively may represent dysfunction of the "first" cortical somatosensory system at a very distal point of processing, analogous to other agnosic disturbances. Damage to SSA, in contrast, causes much greater somesthetic impairment. Despite normal elemental somesthetic sensibility (touch, vibration, proprioception, superficial pain), other basic (2-point discrimination), intermediate (barognosis), and tactile object recognition functions were significantly impaired. This patient also had profound apraxia, probably due to concurrent, but less extensive damage to the supplementary motor area (SMA). Following SSA and SMA infarction, both somesthetic and motor deficits resolved within a year, in contrast to tactile agnosia following SII infarction which was found four years following infarction. Initial severity followed by dramatic resolution is consistent with the notion of a "second" system that can function in parallel to the "first", and such is known to be the pattern following damage to the second motor area (MII), or SMA. Historical considerations aside, the greater known anatomical and physiological parallels of SSA than parietal operculum with SMA, and the pattern of deficits following SSA infarction suggest that SSA, and not SII should be considered the true second somatosensory area.

248.14

STIMULUS-EVOKED 2DG LABELLING IN SI: IN VIVO AND IN VITRO STUDIES. M. Tommerdahl, C.-J. Lee, and B. Whitsel. Physiol, UNC, Chapel Hill, NC 27599.

C14-2-deoxyglucose (2DG) labelling in somatosensory cortex (SI) of monkeys and cats was quantitatively characterized. Repetitive stimuli were used to evoke the labelling patterns. In regions of SI corresponding to the stimulated skin field the first-order distribution of C14-2DG concentration values was multimodal, and most frequently, trimodal. For regions outside the SI representational field, and for all of SI in animals receiving no skin stimuli, the distribution was unimodal. A trimodal distribution is predicted by a recently proposed model of stimulus-driven pericolumnar lateral inhibitory interactions (Whitsel, et al, 1990). Additional evidence was obtained in 2DG studies which employed pharmacological or neurosurgical manipulations. These studies showed that the the first-order distribution of C14 concentration values in SI is modified in a predictable manner in subjects in which the cortical (1) GABAergic inhibitory neurotransmission was blocked by bicuculline, (2) NMDA receptor system was antagonized by either PCP or APV, and (3) input was interrupted by spinal dorsal column lesion. The 2DG method was adapted for use with the cortical slice. The advantages it offers are considerable: (1) input drive to a cortical network can be precisely controlled and applied at a known locus, and (2) agents (2DG and/or neuroactive drugs) can be administered easily at known concentration and for precisely defined time periods. The characteristics of stimulus-evoked 2DG labelling in cortical slices are consistent with the idea that a dynamic process involving NMDA receptor supported GABAergic pericolumnar lateral inhibitory interactions is triggered into activity by repetitive extrinsic excitatory drive. Collectively, these observations suggest that SI should be regarded as a dynamic network possessing short- and long- term mechanisms enabling it to respond to input in a manner that reflects the prior history of sensory experience. Supported by grants DE07509 and MH09942.

248.15

MINICOLUMNAR ORGANIZATION OF SI SEGREGATES. O.V. Favorov and D.G. Kelly*. Depts of Physiology, Endodontics, Mathematics, Univ. of N. Carolina, Chapel Hill, NC 27599.

Recent evidence suggests that SI is organized as a mosaic of discrete column-shaped topographic entities - "segregates" - 0.3-0.5 mm in dia. A segregate consists of 40-80 minicolumns (MCs) - radial cords of cells ca. 45 μm in dia. Whereas receptive fields (RFs) of neurons in the same MC are very similar, RFs of neighboring MCs have much less overlap. Moving across a segregate from one MC to the next, RFs jump back and forth on the skin, but with no appreciable drift (Favorov & Diamond 1990 J Comp Neurol 298:97-112). To study how RFs of a segregate might be arranged on the skin, we implemented a single-segregate model with 61 MCs. Each MC was represented by an input "spiny-stellate" cell (distributor of thalamic input to all other cells of the same MC and, to some degree, to other MCs); an intrinsic "double-bouquet" cell (inhibitor of neighboring MCs); and an output "pyramidal" cell. Connections from thalamus to spiny-stellates were made Hebbian. The network was stimulated with a random series of point "skin" stimuli until its connections settled into a dynamic equilibrium. In this "matured" state, MC RFs formed a regularly permuted arrangement, consistent with experimental observations. A majority of MCs showed a degree of orientation selectivity in response to elongated stimuli and directional selectivity in response to moving stimuli. As in the visual cortex, 1) preferred orientation changed gradually across the segregate with all angles represented, and 2) MCs in the center of the segregate lacked orientation tuning. These results demonstrate the power of Mountcastle's concept of the minicolumn as the basic functional unit of cortical organization. Supported by NIDR DE07509.

248.16

INTRACORTICAL ORGANIZATION OF PROJECTIONS TO INDIVIDUAL MODULES IN SI CORTEX. R.J. Weinberg and O.V. Favorov, Dept. of Cell Biology & Anatomy, Dept. of Physiology, and Dept. of Endodontics, U. of North Carolina, Chapel Hill, NC 27599.

Receptive field mapping studies of primary somatosensory cortex (SI) have demonstrated that the body surface is represented as a mosaic of discrete place-defined cortical columns, or "segregates," approximately 0.3-0.5 mm in diameter. The intracortical organization of these modules has been studied in cats and rats. Small injections of retrograde tracers, especially gold-labeled wheat germ agglutinin conjugated to apo-horseradish peroxidase were made in individual physiologically-identified SI segregates in anesthetized rats and cats. After 12-48h survival, animals were reanesthetized and perfused with mixed aldehyde fixative. Vibratome and frozen sections processed with silver intensifier (Janssen IntenSE BL) were mounted on slides and examined under darkfield and polarization optics.

Large numbers of labeled neurons were found in the ipsilateral cortex, even after very small and confined injections. Labeled neurons tended to cluster into vertical patches whose dimensions corresponded to typical segregates. Labeling was also observed in diffusely scattered neurons, whose frequency declined with distance from the injection center. Work is now in progress to test whether the patches of retrograde labeling coincide with electrophysiologically-defined segregates functionally related to the injected area. Portions of this work were supported by NIH awards #NS-16264 and DE-07509.

248.17

MULTINEURONAL RESPONSES IN THE SI AND MI CORTEX DURING LEARNING OF A STIMULUS CUED MOTOR TASK. J.K. Chapin and R.T. Mariano*, Dept. Physiology, Hahnemann Univ., Phila, PA 19102

Our aim is to define the neuronal network mechanisms by which the cortex processes sensory stimuli to trigger motor responses. For this we trained rats in a task in which they were required to hold their forepaw on a bar and to move the bar immediately after a vibratory cue. Ensembles of neurons were recorded simultaneously through microwire electrodes chronically implanted in the MI cortical forelimb area, and in the SI cortical forepaw- forelimb area, including the granular zone (GZ, homologous with area 3b) and the caudally adjacent perigranular zone (PGZ, homologous with areas 1-2). The GZ neurons had cutaneous receptive fields (RFs) on the forepaw, and PGZ neurons had deep/proprioceptive RFs in the forelimb. In one rat, 22 microwires were implanted, of which 16 recorded discriminable neurons. 100Hz vibratory stimuli produced responses in both GZ and PGZ neurons during rest, but only in GZ neurons during and just before forelimb movement. 50Hz stimuli produced strong excitatory responses in the GZ at 10 msec latency, and weak excitatory or inhibitory responses in the PGZ at 15-20 msec latency. Initially, the MI neurons had no RFs, and did not respond to the 50Hz cue (though one did respond to 100Hz). However, after the animal learned to move the bar immediately after the cue, the MI neurons did respond to the cue, at 20-30 msec latency. Cross-correlating the activity of the simultaneously recorded neurons revealed serial functional connections between neurons within the GZ and PGZ, but not between the PGZ and MI, which nevertheless are known to be neuroanatomically connected. However, after learning the task such serial connections between the PGZ and the MI began to be observed, suggesting a learning related enhancement of the pathway for transmission of cue related sensory information to the MI cortex. *Supported by grants AFOSR-90-0266, NS23722, and AA06965 to JKC.*

248.18

EFFECTS OF CIRCUIT OSCILLATIONS AND NOREPINEPHRINE-LIKE MODULATION IN A NEURONAL NETWORK MODEL OF THE SOMATOSENSORY SYSTEM. J.P. Utz, B.D. Waterhouse and J.K. Chapin. Department of Physiology, Hahnemann University School of Medicine, Philadelphia, PA, 19102-1192.

In order to investigate the effects of oscillations and norepinephrine (NE) in simple neuronal networks within the somatosensory system, a computer simulation was used. The simulation consisted of layers of "neurons" (30x30 2-D arrays) representing the ventral basal thalamus (VB), cerebral cortex (CTX) and nucleus reticularis thalami (nRT). Each neuron incorporates a membrane potential (Vm) calculated from membrane conductances and Nernst potentials for Na^+, K^+, and Cl^-. State variables (eg., conductances and Vm) are re-calculated every ms of simulation time. The VB is driven by a 30x30 array of randomly-timed input fibres, with a bar of increased activity serving as a focal stimulus. VB excites nRT and CTX, CTX excites VB and nRT, and nRT inhibits VB. Each connection fans out in a 5x5 array. In this model, spatiotemporal oscillations are observed throughout the network under certain conditions. During increased oscillatory behavior stimulus discrimination by the network decreases. Effects of NE were modeled by increasing synaptic efficacies. When NE is set to increase all synaptic efficacies by up to 30%, the observed spatiotemporal oscillations and discrimination by the network decreases. When NE increases all synaptic efficacies by 30%, except the CTX to VB feedback, the ability of the network to discriminate a stimulus increases without increasing the oscillatory behavior of the network. This simulation demonstrates that NE's cellular modulatory actions can increase stimulus discrimination by sensory neuronal network, and that control of oscillations is important for discrimination by the somatosensory system. *Supported by grants NS23722, AA06965 & AFOSR-90-0266 to JKC.*

248.19

MODELING THE CIRCUIT CHARACTERISTICS WHICH MAY UNDERLIE OSCILLATORY PROPERTIES OF THALAMOCORTICAL NETWORKS. T. Fisher, A. Gupta; M.A.L. Nicolelis, and J.K. Chapin. Dept. Physiology/Biophysics, Hahnemann Univ., Phila, PA 19102

In simultaneous recordings of single neurons in mulitple regions of the somatosensory thalamus and cortex in chronically implanted rats we have observed strong oscillatory firing under conditions of drowsiness, slow wave sleep, and pentobarbital anesthesia. Several findings suggest that purely circuit characteristics of this system may be extremely important for such oscillations: 1) Cross-correlation studies showed that the neurons oscillated synchronously, but at different phase angles. 2) The oscillations exhibited well known characteristics of nonlinear dynamical systems, such as sudden frequency bifurcations, and low-dimensional chaos. We have used computer modeling techniques to investigate the characteristics of neuronal circuits which may support such oscillatory functions. Several types of simple and complex circuits have been modeled, culminating in models which incorporate the major circuits of the somatosensory system. From these we have determined that, to support oscillations, a circuit must incorporate both positive and negative feedback of sufficient strength and non-zero delay, nonlinear (e.g. sigmoid) neuronal activation functions, and some initial activation of at least one of the neurons. If the synaptic strength of the excitatory feedback, or slope of the sigmoid activation curve are initially set to low levels, oscillation will develop suddenly as one of these parameters is incrementally increased above a certain threshold. The frequency of oscillation is determined complexly by the synaptic delays, time constants of decay of neuronal activation, and the relative strength of the inhibitory feedback. If multiple feedback systems exist within the network, complex frequencies can be produced. Long loop feedbacks can support chaotic properties. Strong afferent inputs to such networks can abolish the oscillations. After cessation of the afferent input, the oscillations resume in a phase reset fashion. Conversely, strong continuous oscillations tended to suppress network responses to weak afferent inputs. Thus, in situations where two dynamic processes were competing for control of the network (e.g. afferents vs. endogenous circuit oscillation) the stronger process tended to dominate, and suppress the weaker process. *Supported by grants AFOSR-90-0266, NS23722, and AA06965.*

248.20

APPLYING MULTI-SINGLE UNIT RECORDING TECHNIQUES TO THE STUDY OF PLASTICITY AT MULTIPLE LEVELS OF THE RAT TRIGEMINAL PATHWAY. M. A. L. Nicolelis, R. C.S. Lin, and J. K. Chapin. Department of Physiology and Biophysics. Hahnemann University, Philadelphia, PA 19102-1192, USA.

Peripheral sensory deprivation induces a reorganization of cortical receptive fields (RFs). Whether this cortical phenomenon is influenced or not by a reorganization of subcortical structures is still unclear. Here, we approached this issue by mapping the cutaneous RFs of neurons in the ventral posterior complex (VP) of the thalamus in anesthetized rats which were subject to an unilateral removal of their facial whiskers at the day of birth. These experiments revealed that thalamic neurons undergo extensive reorganization of RFs. Typically, larger facial RFs and the coexistence of continuous face-body RFs were observed in the VP of these rats. Although these results point to the existence of thalamic rearrangements which may parallel the cortical reorganization, they do not provide a definitive link between the processes and endow little insight in what happen in the awake rat. To approach these issues we have developed new methods for chronic multi-single unit recordings in awake, freely behaving rats. Thus, up to 48 50 μm teflon-coated stainless steel microwires are simultaneously implanted in the trigeminal nuclei, dorsal thalamus and somatosensory cortex of adult animals, as well as wires for recording facial muscle activity (EMGf). Single units are studied in behaving rats in terms of their waveforms, receptive fields, tuning curves and correlation to the EMGf. Principal components analysis is used for a quantitative spike classification, providing criteria for following single spikes in chronic recordings. Single trigeminal, thalamic, and cortical units are then chronically recorded during different whisking behavior paradigms in order to study networks of neurons located at different levels of the trigeminal pathway. ***Sponsored by grants NS26722 and FAPESP 88/4044-9.***

SUBCORTICAL VISUAL PATHWAYS: LGN

249.1

REORGANIZATION OF RETINO-GENICULATE PROJECTIONS FOLLOWING EXCISION OF AREAS 17 AND 18 IN NEWBORN AND ADULT CATS. S.G. Lomber, B.R. Payne, P. Cornwell* and H.E. Pearson. Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118.

The purpose of the present study was to evaluate the pattern of retinal projections to the dorsal lateral geniculate nucleus (dLGN) at least six months after ablation of primary visual areas 17 & 18 in the newborn kitten and adult cat. The terminations of retinal projections to the degenerated dLGNs in both groups of cats were studied following the injection of tritiated amino acids into one eye. In the dLGN of adult lesioned animals there is substantial neuron death in both laminae A & A1 and moderate neuron loss in the C-laminae. Quantification of the retinal projections show their density and distribution to be very similar to those in the intact cats. However, in cats which had areas 17 & 18 removed on the day of birth both the configuration and cytoarchitecture of dLGN and retinal projections to the nucleus are abnormal. The entire nucleus failed to rotate so that its orientation is close to its initial vertical position. Laminae A & A1 have collapsed completely and virtually all surviving neurons are in the C-laminae. Retinal projections to dLGN terminate almost exclusively in the C-laminae with only a hint of projections to laminae A & A1. These results show substantial retraction of retinal projections from laminae A & A1 following ablation of areas 17 & 18 on the day of birth and provide little evidence for retraction of retinal projections from the degenerated layers of dLGN following equivalent ablation in adulthood. These data show that immature retinal projections are plastic and can change in response to ablation of areas 17 & 18. (Supported by MH44647 and Whitaker Health Sciences Fund.)

249.2

DEGENERATION OF MEDIUM-SIZED RETINAL GANGLION CELLS IN RESPONSE TO LOSS OF POSTSYNAPTIC NEURONS IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE ADULT CAT . H.E. Pearson and D.J. Stoffler*. Dept. of Anat. and Cell Biol., Temple Univ. Sch. of Med., Philadelphia, PA 19140.

We have demonstrated previously that postsynaptic targets are essential for the survival of mature retinal ganglion cells. In the absence of normal targets, ganglion cells will first retract their axon terminals and then degenerate. We have now expanded this study to determine whether this degeneration is confined to a specific ganglion cell population. Kainic acid (3 nmol/ul) was injected at multiple sites within the dLGN of adult cats. Following survivals of 2,4 and 6 months, the cats received multiple injections of HRP into the dLGN. After a further 72 hr, the retinae were reacted for the presence of HRP and counterstained with cresyl violet. Adjacent brain sections were processed for thionin staining and HRP histochemistry. At each survival, counts of retinal ganglion cells were made at comparable locations in peripheral nasal retina, at a site corresponding retinotopically to regions of degeneration within the dLGN. Cell densities were determined separately for cells labelled retrogradely with HRP and for unlabelled cells stained for Nissl. Separate analyses of small, medium and large ganglion cells revealed that only medium sized cells were lost at 2 months, whereas both medium and large cells were lost at 4 and 6 months. By 6 months, 92% of medium cells and 65% of large cells had degenerated. These results show that the dependence of mature retinal ganglion cells on target integrity varies with ganglion cell type, being greatest among those with medium-sized cell bodies and least among the small cells. Supported by NS25196.

249.3

THE PROJECTION OF PRETECTAL AXONS TO THE LATERAL GENICULATE NUCLEUS (LGN). D.J. Uhlrich and K.A. Manning. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

The midbrain pretectal region contains distinct cell groups that are involved in vision. Within this region is a column of cells, mostly in the nucleus of the optic tract (NOT), that projects to the LGN. Because the NOT is involved with optokinetic nystagmus, this projection may coordinate the sensory and motor aspects of eye movements.

Little is known about the projection from the NOT to the LGN at the single cell level. Therefore, we labeled pretectal axons with iontophoretic injections of the anterograde tracer, *Phaseolus vulgaris* leucoagglutinin, targeting the NOT. We find labeled fibers in all laminae of the LGN as well as in adjacent visual nuclei. The terminal fibers contain boutons, mostly *en passant*, that are larger (1.8μm ±0.6 μm) than the boutons of other extraretinal fibers that innervate the LGN. Serial reconstructions of portions of labeled pretectal axons indicate that they freely cross laminar borders in the LGN. Preliminary results show that pretectal terminal arbors are asymmetrical. They are relatively flat in the rostral-caudal dimension (i.e., visual field elevation in the LGN retinotopic map), but they can encompass one third of the medial-lateral extent of the LGN (i.e., visual field azimuth). These terminal fields are consistant with observations that the pretectum is well organized retinotopically for visual field elevation, but not for azimuth. It is also consistent with observations that NOT cells respond best to large visual stimuli that are drifting along the horizontal plane.

Supported by NIH grant EY-06610.

249.4

QUANTITATIVE IMMUNOGOLD EVIDENCE FOR HIGH LEVELS OF GLUTAMATE BUT NOT ASPARTATE IN SYNAPTIC TERMINALS OF RETINO-GENICULATE, CORTICO-GENICULATE AND GENICULO-CORTICAL AXONS IN THE CAT. V.M. Montero and R.J. Wenthold. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53705, and Lab. Molecular Otology, NIDCD, NIH, Bethesda, MD 20892

In a previous study, high Glu immunogold reactivity (IGR) was found in synaptic terminals of retinal and cortical afferents to LGN, and of geniculo-cortical axons in the cat (Montero, Visual Neurosc. 4:437-443,1990). However, several pharmacological and uptake-release studies in cats that indicate excitatory amino acids (EAA) as neurotransmitters in these retino-geniculo-cortical synapses leave still open the possibility that Asp may be a neurotransmitter in these pathways: Asp and Glu act on the same type of EAA receptors (although with slight different preferences) and are taken up by the same mechanisms; NMDA and non-NMDA receptors are known to be involved in retino-geniculate transmission. It was of interest, therefore, to compare the IGR intensity to an Asp antibody in the retino-geniculo-cortical synapses with that in GABAergic terminals in LGN, in order to detect any relative enrichment of Asp in these synapses (and to compare these results with those obtained with a Glu antibody). The results showed that Glu-IGR in ultrastructurally identifiable synaptic terminals from retinal (R) and cortical (C) axons in LGN, and from geniculo-cortical (G-C) axons in the perigeniculate nucleus was 1.6 - 2.1-fold that seen in GABAergic F terminals, confirming the previous results. By contrast, the intensity of Asp-IGR in C, R and G-C terminals was similar or less than that seen in F terminals. These immunocytochemical results support the notion that Glu but not Asp is the neurotransmitter in the retino-geniculo-cortical pathways. Supported by NIH grants EY02877 and HD03352.

249.5

CALBINDIN ANTIBODIES LABEL SPECIFIC CELL CLASSES IN THE CAT LATERAL GENICULATE NUCLEUS. Francis Banfro and Ranney Mize, Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN 38163.

Calbindin 28Kd, a calcium binding protein, is important in the regulation of calcium and may be associated with calcium spiking neurons in some regions of the CNS. We have localized calbindin (CaBP) in the cat dorsal lateral geniculate nucleus (dLGN) using antibody immunocytochemistry to determine if it labels specific cell classes in this nucleus. Anti-CaBP labeled neurons were found in all layers of the dLGN. Anti-CaBP cells in layers A, A1, and the magnocellular and parvicellular C layers were mostly small to medium sized cells with a mean area of 139.3 μm^2. Labeled cells in the interlaminar zones were larger (range 250-677 μm^2). By contrast, most unlabeled neurons in the main layers were large neurons (mean area 270.9 μm^2). Labeled neurons in the A layers had round or ovoid soma morphologies. Over 75% of these neurons had vertically distributed dendrites whose orientation was 45^0 or more from the longitudinal axis of the lamina. The labeled neurons constituted 60-75% of neurons in the A layers. Labeled neurons in the C layers had similar soma morphologies, but over 50% of these neurons had horizontally distributed dendrites whose orientation was within 45^0 of the longitudinal axis of the laminae. Labeled neurons were more than 75% of the total neuron population in the C layers, and included virtually all neurons in the parvicellular C laminae. At the electron microscope level, we found label in F2 presynaptic dendrites within the retinal glomeruli, and in conventional dendrites and myelinated axons. Some neurons with cytoplasmic laminated bodies were also labeled by CaBP antibodies. Double labeling studies showed that CaBP was colocalized with GABA in a subset of neurons in both the A and C layers. We conclude on the basis of cell size and ratio data that all X and W cells, and GABAergic interneurons are labeled while Y neurons are not labeled by CaBP in the cat LGN. Supported by NEI EY-02973.

249.6

THE DISTRIBUTION OF SOME PROTEINS IN CLASS 3 CELLS IN THE CAT LATERAL GENICULATE NUCLEUS A.J. Scheetz and Mark Wm. Dubin, Department of MCD Biology, University of Colorado, Boulder CO 80309.

The cytoarchitecture of the cat lateral geniculate nucleus (LGN) has been characterized to the point that the origin and connectivity of a given synaptic profile can be determined based on synaptic relationships. Because of this, we have been able to study the distribution of proteins within specific cell classes in the LGN using elctron microscopic immunocytochemistry. We examined the distribution of some proteins normally associated with either dendrites or axons in LGN class 3 cells. These cells had normal axons but their dendrites were capable of interacting as pre- and postsynaptic partners. We examined whether some proteins that are normally associated with dendrites are present in these atypical dendrites, and if some proteins that are normally associated with axons are included in class 3 cell dendrites. We examined both adult and neonatal (3 week) LGN tissue that had been stained with various antisera. We were unable to detect any form of spectrin or fodrin in class 3 cell dendrites and their terminals (F2 terminals). This is probably due to the activation of calcium dependent proteases specific to spectrins during the tissue preparation. Calcium and calmodulin dependent protein kinase II (CAM II) staining was seen in class 3 cell dendrites and their terminals where it was associated with postsynaptic densities and microtubules. Synapsin 1 was found in F2 terminals. Map-2 staining was absent from class 3 cell dendrites and F2 terminals. Tau was seen in all axons but was excluded from F2 terminals . We found that class 3 cell dendrites have cytochemical compositions that differ from other axons and dendrites. This confers special functional capabilities to these synapses. CAM II is incapable of phosphorylating Map-2 in class 3 cell dendrites and F2 terminals. These terminals are the only place in the cat LGN where CAM II can phosphorylate synapsin 1. We conclude that class 3 cell dendrites have atypical distributions of some proteins which may reflect certain functional differences between class 3 cell synapses and other synapses in the LGN. Such differences may be a ubiquitous feature of presynaptic dendrites in general.

Supported by EY04629 to MWD

249.7

NAAG DEPOLARIZES NEURONS IN SLICES OF RAT LGN. D.G. Tieman, N. Hori*, D.O. Carpenter and S.B. Tieman. Neurobiology Research Ctr and School of Public Health, SUNY, Albany NY 12222 and Wadsworth Labs, NYS Health Dept, Albany NY 12201.

N-Acetylaspartylglutamate (NAAG) is an endogenous brain dipeptide satisfying many of the criteria for a neurotransmitter. In particular, it is found in retinal ganglion cells and their terminals in rat and cat, and is released from those terminals in a calcium-dependent manner following electrical stimulation of the optic nerve. Further, NAAG activates glutamate receptors, and glutamate receptors have been implicated in retinogeniculate transmission. We have therefore investigated the effects of NAAG on the target cells of the retinal pathway using intracellular recordings from slices of rat lateral geniculate nucleus (LGN), iontophoretic application of agonists, and bath application of antagonists. Synthetic NAAG (10 mM in 150mM NaCl, pH 7.0-7.5) was iontophoresed by passing negative current for 1 sec intervals with 30 sec in between. During the inter-stimulus intervals a small retention current was applied. Under these conditions NAAG depolarized cells in LGN. This depolarization was largely, but not completely, blocked by bath application of 50 μM APV or CPP, and could be enhanced by perfusion with Mg^{++}-free Ringer's, suggesting that NAAG activates NMDA channels. The response could also sometimes be decreased by bath application of 10 μM CNQX, suggesting a kainate or quisqualate component. The pharmacology of these responses is similar to those for electrical stimulation of the optic tract, but not identical in that they imply a greater dependence on the NMDA channel. These results are consistent with a role for NAAG in retinogeniculate transmission. (Supported by NSF grant BNS 811039 to SBT and PHS grant NS 2380705 to DOC.)

249.8

THE MODULATION OF TRANSMITTER RELEASE BY PRESYNAPTIC $GABA_B$ RECEPTORS IN THE DORSAL LATERAL GENICULATE NUCLEUS (dLGN). Vincenzo Crunelli, Katalin Toth*, Zsuzsa Emri* and Ivan Soltesz. Dept. of Visual Science, Inst. of Ophthalmology, London, U.K.

A wealth of experimental evidence suggests that GABA can decrease its own release as well as that of glutamate via presynaptic $GABA_B$ receptors (Bowery et al, Nature, 1980; Dolphin & Scott, J.Physiol., 1987; Deisz & Prince, J.Physiol., 1989). In the dLGN similar evidence has been lacking, though it is well established that both $GABA_A$ and $GABA_B$ IPSPs evoked by electrical stimulation of the optic tract (OT) show a decline in amplitude with increasing frequencies of stimulation (Crunelli et al, J.Physiol., 1988). Here we tested the hypotheses that this frequency-dependent decrease in the amplitude of GABA IPSPs in the dLGN might be due to presynaptic $GABA_B$ receptors.

2-OH-Saclofen (0.4mM), which abolished the postsynaptic $GABA_B$ responses, greatly reduced the frequency-dependent decrease in the amplitude of $GABA_A$ IPSPs. Phaclofen (1mM) had no effect on the frequency-dependent decrease of $GABA_A$ IPSPs, suggesting that this antagonist is less potent than 2-OH-saclofen on presynaptic $GABA_B$ receptors, at least in the dLGN. The $GABA_B$ agonist baclofen (10μM), in the presence of Ba^{2+} (1-2mM) which blocks the postsynaptic, K^+ dependent $GABA_B$ receptors, decreased the amplitude of the OT evoked EPSPs. Thus in addition to the regulation taking place through interneurone-interneurone and perigeniculate neuron-interneuron synapses, dLGN interneurons are capable of controlling GABA release by negative feedback mechanisms through presynaptic $GABA_B$ receptors. Moreover, $GABA_B$ receptors on the OT terminals might regulate glutamate release. This negative control of glutamate and GABA release by presynaptic $GABA_B$ receptors will be of major importance during visual processing in vivo owing to the high firing rate of retinal afferents.

249.9

SYNAPTIC RESPONSES TO STIMULATION OF THE VISUAL CORTEX PROJECTION TO THE FERRET LATERAL GENICULATE NUCLEUS IN VITRO. M. Esguerra and M. Sur. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

Corticofugal afferents were activated by electrical stimulation of the optic radiations in isolated slices of adult ferret lateral geniculate nucleus (LGN). Optic radiation stimulation elicited both excitatory and inhibitory postsynaptic responses in the LGN. The excitatory responses (EPSPs; n = 17) were monosynaptic and appeared to be similar to optic tract-evoked postsynaptic potentials in amplitude and duration. In contrast to retinogeniculate EPSPs, optic radiation-evoked responses could be divided into "fast" and "slow" types based on the EPSP latency-to-peak and EPSP rise time. Most corticogeniculate EPSPs were attenuated by blockade of NMDA receptors; one EPSP was observed only in low-magnesium medium, and was blocked completely by normal levels of extracellular magnesium.

Optic radiation stimulation also led to purely inhibitory synaptic events (n = 2) which did not include any sign of depolarizing or excitatory components at resting potentials. This pure inhibition may represent direct stimulation of axons arising in the perigeniculate nucleus of the thalamic reticular complex. In 2 cells optic radiation stimulation elicited mixed excitatory and inhibitory responses characterized by a short excitatory component followed by a longer duration inhibitory component (IPSP). These most likely represent direct excitatory input from corticofugal afferents paired with disynaptic feed-forward inhibition by intrinsic geniculate interneurons.

We propose that a possible role of the corticofugal projection is to produce local dendritic depolarizations that can modulate nearby sites of retinogeniculate input.

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249.10

VISUALLY EVOKED BURST DISCHARGES IN CAT LGN CELLS: SPATIAL AND TEMPORAL TUNING. S.M. Sherman, S.-M. Lu, and W. Guido, Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

LGN cells display voltage-dependent, low threshold (LT) spikes, which are large depolarizations due to an increased Ca^{2+} conductance. Typically riding the crest of each is a burst of 2-7 action potentials. *In vitro* studies show that, once initiated, LT spikes may continue spontaneously at roughly 6 Hz, signaling a *burst discharge mode* that may interfere with retinogeniculate transmission. This differs from the *tonic discharge mode*, during which LT spikes are inactivated. We now extend our preliminary *in vivo* observations that LT spikes do not necessarily interrupt retinogeniculate transmission. From intracellular recording, we found that bursts of action potentials with interspike intervals $\leq$4 msec occur only during LT spikes, so we could recognize these events extracellularly. We thus recorded extracellular responses from cat LGN cells to drifting, sinewave gratings that varied in spatial frequency, temporal frequency, and contrast. We separately resolved the total responses into those due to more tonic responses and those limited to bursts. Among our sample, burst responses represented a variable proportion of the total response, from practically zero for some cells to the majority of the response for others. Y cells tended to display more visually-evoked bursts than did X cells. The spatial and temporal tuning functions were indistinguishable between tonic and burst responses, indicating that bursts clearly can be stimulus-evoked. No differential temporal tuning existed between the burst and tonic responses, suggesting that any rhythmic firing due to LT spiking does not contribute noticeably to these responses. We also found no difference between tonic and burst discharges in contrast response functions. We conclude that LT spikes are visually-evoked and may be a component of useful retinogeniculate transmission that helps to amplify signals while a cell is relatively hyperpolarized. (Supported by USPHS grants EY03038 and EY06082.)

249.11

FEEDBACK FROM V1 IMPROVES THE ABILITY OF MONKEY LGN NEURONS TO DISCRIMINATE AMONG PICTURES. J. W. McClurkin, L. M. Optican, B. J. Richmond. Laboratory of Sensorimotor Research, National Eye Institute, and Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, Maryland 20892.

Cooling the striate cortex (V1) altered the magnitude and temporal properties of the responses of lateral geniculate (LGN) neurons. Cooling V1 also reduced the average information transmitted by LGN neurons about 16 black-and-white pictures presented at each of 9 luminance pairs. We related the change in the responses to the change in transmitted information to elucidate the influence of cortical feedback on the LGN.

The responses of LGN neurons are multivariate and can be represented as points in a 3-D space. Neuronal codes for the picture parameters of pattern and luminance can be represented by 2-D planes fit to sets of points lying in this space. The variance of the points from the planes represents the noise in the code.

Transmitted information would be reduced if either the noise in the codes increased or the difference between the codes decreased. If cooling V1 increased the noise in LGN codes, we would expect the planes representing heavily affected parameters to fit the data less well than planes representing unaffected parameters. However, there was no relationship between the fits of the planes and changes in transmitted information. Rather, cooling changed the orientations of the planes representing the affected parameters.

We hypothesize that the role feedback from V1 is to improve the ability of LGN neurons to discriminate among pictures by increasing the difference between their temporal codes. The basis for this increased differentiation may be visual information from neighboring retinal locations that is fed back to LGN neurons from V1.

249.12

CONTRIBUTION OF GENICULATE MECHANISMS TO THE GENERATION OF LENGTH TUNING IN THE VISUAL CORTEX. H.E. Jones & A.M. Sillito, Dept. of Visual Science, Inst. of Ophthalmology, Judd St, London WC1H 9QS, UK.

Although length tuning is conventionally regarded as a higher order cortical property, some reports have indicated that dLGN cells also exhibit length tuning. To explore this issue, we examined the responses of cat dLGN cells to stimuli routinely used to assess cortical length tuning. The majority of dLGN cells were highly length tuned (mean 71%, n = 186) with many cells showing a degree of length tuning that matched or surpassed that seen in tightly tuned cortical hypercomplex cells. These data suggest that cortical length tuning may merely reflect the dLGN input, and that interactions in the dLGN play a major role in generating length tuning in the visual cortex.

A component of dLGN length tuning arises from centre-surround interactions in the retina and from the enhanced surround generated in the dLGN through GABAergic inhibitory mechanisms. However, our data indicate that strength of surround antagonism and length tuning are not always correlated. It seems that a significant component of dLGN length tuning derives from a corticofugal drive to geniculate inhibitory interneurons, which could be mediated via intrinsic dLGN or PGN interneurons. The interneurons might be expected to show rapid spatial summation for lengths over which the decline in relay cell responses occur, to be non-endstopped and to be strongly driven by their corticofugal input. To dissect out whether PGN cells are involved, we have compared their length tuning curves in the presence and absence of corticofugal feedback. While the tuning profiles of many PGN cells appear suited to generating dLGN length tuning, removal of the cortex did not provoke any obvious change in these profiles. Thus while PGN cells may well play a role in the subcortical generation of dLGN length tuning, they are unlikely to mediate the corticofugal component which is therefore most likely to be mediated via intrinsic inhibitory interneurons.

249.13

SPATIAL FREQUENCY AND ORIENTATION DOMAIN SUBSTRUCTURE TO THE FEEDBACK FROM THE VISUAL CORTEX TO THE LATERAL GENICULATE NUCLEUS. J. Cudeiro*, A. M. Sillito & P.C. Murphy, Dept. of Visual Science, Institute of Ophthalmology, London.

The dorsal lateral geniculate nucleus (dLGN) in the cat receives a major projection from layer VI cells in the visual cortex. This projection can enhance inhibitory interactions in dLGN cell fields (Murphy and Sillito 1987, Nature 329: 727-729) and hence provides the potential for an orientation sensitive influence on mechanisms in the dLGN. Utilising a visual stimulus (drifting grating) subdivided into an inner window located over the receptive field centre and an outer window, we have examined the effect of varying the orientation alignment and spatial frequency of the two components on dLGN cell responses. The orientation and spatial frequency of the inner and outer stimuli were varied independently and when in alignment they appeared as a grating moving over the field.

For non-endstopped cortical cells tested with this stimulus, the orientation aligned condition produced the largest responses. In dLGN cells, the converse applied, when the stimulus was in alignment the smallest responses were observed. For any given dLGN cell the sensitivity to the aligned condition appeared to apply for any given orientation of the whole stimulus. Effects of orientation alignment were greatest when spatial frequencies in the inner and outer windows matched and were in the range 0.1 - 0.5 cycles/degree. These data match the effects that would be predicted from the action of the stimuli used on layer VI cell responses. They suggest the feedback may serve to enhance the sensitivity of the dLGN field to discontinuity between stimulus components moving over centre and surround mechanisms.

249.14

INHIBITORY AND EXCITATORY COMPONENTS TO THE SUBCORTICAL AND CORTICAL INFLUENCE OF LAYER VI CELLS IN THE CAT VISUAL CORTEX. K.L. Grieve*, P.C. Murphy & A.M. Sillito. Dept. of Visual Science, Inst. of Ophthalmology, Judd St, London WC1H 9QS. U.K.

Layer VI cells in the visual cortex provide a major corticofugal contribution to the synaptic input to the dLGN and send collaterals up to layer IV. Recent anatomical evidence suggests that the distribution of the corticofugal terminals from layer VI is biased towards the processes of inhibitory interneurones whilst the cortical terminals are biased to those of excitatory interneurones. We have explored the functional influence of layer VI cells at these two locations.

Pharmacological blockade of layer VI (iontophoretic application of GABA or muscimol) produced shifts in the properties of cells recorded in the overlying layer IV. These were commensurate with a reduction in an excitatory drive. In particular we noticed a selective decrease of responses to short stimuli in end-stopped layer IV cells. We were unable to detect any reduction in inhibitory influences underlying layer IV cell fields. Conversely, in the dLGN, elimination of the corticofugal influence reduced the response attenuation normally elicited when the length of a moving bar was increased so that it encroached on the receptive field surround. In the presence of corticofugal feedback attenuation of responses to a bipartite drifting bar were sensitive to the orientation alignment of the components crossing centre and surround, reinforcing the view that a component of the inhibitory drive derives from effects mediated via the cortical influence. Thus layer VI cells seem to exert a spatially organised influence on the translation of visual information into the cortex via a facilitatory mechanism in layer IV and an enhancement of inhibitory mechanisms in the dLGN.

249.15

EFFECTS OF STIMULUS ORIENTATION ON SIGNAL TRANSMISSION THROUGH THE LGN. Y. Chino, E.L. Smith III, H. Cheng*, and A. Langston*. College of Optometry, University of Houston, Houston, TX 77204-6054.

The mammalian lateral geniculate nucleus (LGN) regulates signal transmission from the retina to the visual cortex in a highly complex manner. Besides the non-specific effects associated with an animal's arousal level, local mechanisms appear to gate visual information in a stimulus-dependent manner. Because the majority of LGN neurons exhibit a substantial orientation bias, we investigated the effects of stimulus orientation on LGN signal transmission. We recorded extracellular action potentials from single LGN neurons and their S-potentials (their retinal inputs) in anesthetized and paralyzed cats. Responses were measured for drifting sinusoidal gratings that varied in spatial frequency and/or orientation. We found that the transmission ratio, defined as the firing rate of an LGN relay cell divided by that of its S-potential, was highest at the optimal stimulus orientation. Furthermore, the transmission ratio at all orientations was directly related to the firing rate of the LGN neuron. Therefore, our results provide additional evidence for the hypothesis that LGN relay cells can act as non-linear spatio-temporal filters. Supported by NIH R01-EY08128, R01-EY03611 and P30-EY07551.

249.16

THE INFLUENCES OF EYE POSITION ON THE RESPONSES OF X AND Y CELLS IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE AWAKE, BEHAVING CAT. S. Lehmkuhle and J.A. Baro. School of Optometry, University of Missouri–St. Louis, St. Louis, MO 63121

Recent work on the synaptic circuitry of cells in the dorsal lateral geniculate nucleus (dLGN) clearly demonstrates that information from retinal ganglion cells can be modulated in several ways. To begin to elucidate the modulatory functions of these neural circuits, we have measured the responses of cells in dLGN as a function of eye position in awake, behaving cats.

Cats were trained to fixate LED targets situated in front of a CRT display on which grating stimuli were presented. Scleral search coils recorded eye position. The eye position signal from the coil, via a sample and hold circuit, was used to displace the entire display horizontally and vertically on the CRT in concert with the movement of the eyes. Stimulus position was updated according to eye position at the 200-Hz frame rate of the display. Because the eyes were relatively stable due to fixation, and the 200-msec duration stimulus was stabilized on the retina, the phase of the grating within a receptive field could be precisely controlled. From these data, we classified cells as X or Y and quantified the effects of eye position on a variety of response measures. Response amplitudes and latencies of both X and Y cells varied with eye position.

(Supported by NSF BNS-8819706)

249.17

LATERAL GENICULATE NUCLEUS (LGN) RESPONSE TO FLASH: CURRENT SOURCE DENSITY (CSD) AND PRINCIPAL COMPONENTS (PCA) ANALYSES. C.E.Tenke and C.E.Schroeder, Dept. Biopsych./ Psychophysiol., NYS. Psych.Inst.,NY,NY and Dept. Neurosci., A.Einstein Coll.Med., Bronx, NY

The surface flash-VEP of the awake monkey arises largely from the activation of cortical regions. However, An early negativity (N25) may be traced from the frontolateral surface to the LGN, where it abruptly inverts in polarity within lamina 6. This inversion occurs in association with a sink/source configuration in the derived CSD profile. Although the superficial laminae of LGN appear to be crucial for the production of N25, multielectrode recordings through LGN indicate considerable concurrent activity in deeper laminae, much of which is not discernable above the LGN. Cancellation of this activity may occur locally, from closed field generators which are detectable only within a lamina. Alternatively, each LGN lamina may act as an open field generator whose contribution is largely cancelled by activity of opposite sign in superficial laminae. We explored these alternatives using two CSD methods: 1) CSD estimates computed directly from field potential profiles using a range of differentiation grids; 2) quasistationary CSD estimates computed from waveforms extracted by a PCA. Although considerable cancellation was seen within laminae using high resolution CSD profiles, the use of wider differentiation grids revealed regions of source and sink which summate across laminae without evidence of closed field artifacts. At the latency of the N25 peak, an effective sink extends throughout laminae 3-5, in association with a source in magnocellular laminae; a corresponding PCA factor showed the same pattern. Additional peaks, including oscillatory activity, also build within the parvocellular laminae. These findings support the second alternative, and extend the applicability of VEP techniques to the study of LGN physiology. (Supported by MH36295 and MH06723)

AUDITORY AND VESTIBULAR HAIR CELLS: ULTRASTRUCTURE, REGENERATION AND TUNING

250.1

SIMILAR FREQUENCY RESPONSES FROM FIBERS INNERVATING MORPHOLOGICALLY DIFFERENT PARTS OF THE LATERAL LINE SYSTEM IN AN ANTARCTIC FISH. S. Coombs and J.C. Montgomery*. Parmly Hearing Institute, Loyola Univ., Chicago, Il. 60626 and Dept. of Zoology, University of Aukland, Aukland, NZ.

Extracellularly-recorded responses to a sinusoidally vibrating sphere (6 mm in diameter) were obtained from both anterior and posterior lateral line nerve fibers innervating different canals on the head and trunk of the antarctic notothenioid fish, *Trematomus bernacchii*. Despite large regional variations in anatomical dimensions known to affect the biomechanics of the system, fibers innervating different regions showed remarkably similar frequency responses. In response to stimuli of equal pk-pk acceleration levels, all fibers had (1) maximum responsiveness in the range between 10 and 45 Hz that was nearly flat and independent of frequency and (2) a decline in responsiveness that began around 45 Hz and continued at the rate of around 18 dB/octave. Attempts to model the filtering effects of the different sized canals and cupulae indicate that all morphologies predict the flat, low frequency portion of the neural response function, but quite a wide range of high frequency cut-offs that are well above those measured from single fibers. These results suggest that (1) there may be considerable variability in the morphology with little consequence for function and (2) there are additional filters between the cupula and primary afferent fibers.

250.2

ULTRASTRUCTURAL COMPARISON OF STRIOLAR AND EXTRASTRIOLAR HAIR CELLS OF FISH UTRICLE, J. Chang* and A. N. Popper, Dept. of Zoology, Univ. of Md., Collage Park, MD 20742.

Different types of vestibular hair cells have been characterized from striolar region and extrastriolar region of fish utricle using EM 3D computer reconstruction technique.

The results showed that fish inner ears have type I like and type II like hair cells. Type I hair cells (striolar cells) restrict to the striolar region while type II hair cell (extrastriolar cells) locate in extrastriolar regions. Its ultrastructural difference correlates to our recent finding on calcium binding protein immunoreactivity, trigger zone like membrane activity, and gentamycin toxicity. This study suggests that the two types of hair cells as transducers may produce different types of information by releasing presynaptical vesicles at different frequencies. They may have different intracellular mechanisms involved in intracellular calcium activity as well.

In addition, the ultrastructural characteristics of the two types of cells resemble the type I and type II hair cells in mammals. These findings suggest that the two types of hair cells evolved as early as in piscine species.

250.3

IMMUNOHISTOCHEMICAL LOCALIZATION OF CALCIUM BINDING PROTEINS IN AUDITORY HAIR CELLS OF THE BARN OWL AND THE CHICKEN. C. Hue and C. E. Carr, Univ. Maryland. Dept. Zoology, College Park, MD 20742

Calbindin-28kD has been found in the chick cochlea (Oberholtzer et al,1988) and calbindin-28kD and calmodulin have been localized to bull frog hair cells (Shepherd et al,1989). These studies have characterized the calcium binding proteins from isolated hair cells and stereocilia. We describe the distribution of calcium binding proteins over the basilar papilla. The avian basilar papilla has two hair cell types, tall hair cells and short hair cells (Smith et al, 1985). Short hair cells are thought to receive primarily efferent innervation, while tall hair cells are innervated mainly by afferent fibers.

Immunohistochemical techniques were used on tissue sections as well as whole basilar papillae to determine if calcium binding proteins were differentially distributed in the different hair cell types. Both monoclonal and polyclonal antibodies against calbindin and calretinin, parvalbumin and S-100 were used.

Calbindin-like immunoreactivity labels both short and tall hair cell bundles and cuticular plates. The antibodies also labelled some ganglion cells, nerve terminals and axons, but did not stain supporting cells.

Parvalbumin-like immunoreactivity reveals a gradient of label across the basilar papilla. The hair cell bundles and cuticular plates of the short hair cells were more darkly stained than those of the tall hair cells. Some nerve terminals, ganglion cells and axons were also immunoreactive.

Thus short hair cells may be differentiated from tall hair cells on the basis of calcium binding protein immunoreactivity.

250.4

COMPARTMENTAL MODELS OF CURRENT FLOW IN RAT VESTIBULAR CALYCEAL AND NERVE BRANCH PROCESSES USING 3-DIMENSIONAL RECONSTRUCTIONS FROM ELECTRON MICROGRAPHS OF SERIAL SECTIONS. T.C. Chimento, D.G. Doshay*, and M.D. Ross*, NASA, Ames Research Center, Mountian View, CA 94035

As the first stage in the development of computational models of the rat vestibular macula, current flow within single primary afferents is being simulated. This work is focused on the flow of current within collateral-like processes of calyces and nerve fiber branches that are either pre- or postsynaptic to type II hair cells. Detailed morphometrics and synaptic areas of processes are obtained from electron micrographs of serial sections cut at 20 nm. The models simulate current flow with both passive and active membranes using NEURON, generously provided by Michael Hines, Duke U. Med. Center.. We are testing the hypothesis that small changes in morphology produce significant changes in the current at the base of afferent processes, with corresponding changes in the total current reaching the spike initiation zone. Similarly, the hypothesis is that morphological variations in efferent-type processes determine whether transmitter release will occur for a given depolarization of the calyx. For example, resistance is so high in some small diameter stems that the heads are effectively decoupled from the calyx or nerve branch, indicating that local processing could occur in the network. These results demonstrate that morphometric details that can be resolved only at the EM level have large effects on the current at the output end of the process and thus upon simulations of the functioning network.This work was supported by NASA and the National Research Council.

250.5

WITHDRAWN

250.6

REGENERATIVE CELL PROLIFERATION IN ORGAN CULTURES OF THE MATURE AVIAN COCHLEA. M.E. Warchol, C. Laverack*, and J.T. Corwin. Dept. of Otolaryngology-HNS and Dept. of Neuroscience. University of Virginia School of Medicine, Charlottesville VA 22908 USA.

Sensory hair cells in the avian cochlea are regenerated after acoustic trauma or aminoglycoside toxicity *in vivo*. Regeneration appears to result from renewed proliferation in an otherwise mitotically quiescent cell population within or near the sensory epithelium. We are attempting to conclusively identify the progenitor cells and the signals that lead to this renewed mitosis by experimentation in an organ culture preparation of the avian cochlea.

The chick cochlea is anatomically mature at hatching. Cochleae from 7- to 14-day-old chicks are removed to Hank's balanced saline under sterile conditions. The tegmentum vasculosum and lagena are dissected away and the remaining sensory epithelium is transferred to a collagen coated culture well containing 50 µl of medium (80% Medium-199, 20% fetal bovine serum). The well is sealed in a Rose chamber to allow microscopic visualization of the sensory epithelium. Normal hair cell morphology can be maintained under these conditions at 37° C for at least five days.

Approximately 50 adjoining hair cells near the distal tip of the cochlea are individually lesioned using a UV laser microbeam. Immediately following lesioning, ^{3}H-thymidine (1 µCi/ml) is added to the culture medium. After 24 hr of incubation with ^{3}H-thymidine, specimens are fixed for autoradiography. Near the lesion sites labeled supporting cells are found within the sensory epithelium. A lesser number of labeled supporting cells are found at locations away from the lesions. Labeling is also common among cells located just outside the inferior edge of the sensory epithelium. The onset time of the proliferative response following lesioning is currently being determined.

(Supported by an NRSA to M.E.W. and NIDCD and DRF grants to J.T.C.)

250.7

HAIR CELL REGENERATION IN THE LATERAL LINE: IDENTIFICATION OF PROGENITOR CELLS AND LINEAGE RELATIONSHIPS. J.E. Jones and J.T. Corwin. Dept. of Neuroscience and Dept. of Otolaryngology-HNS, University of Virginia Sch. of Medicine, Charlottesville, VA 22908 USA.

As a step toward understanding mechanisms that underlie the regeneration of sensory hair cells in the ear, we have used lateral line hair cell epithelia as models for determining the lineage relationships of the cells that give rise to replacement hair cells. Two types of injury to the system were used in anesthetized salamanders, *Ambystoma mexicanum*. The responses evoked by each were observed during nearly continuous time-lapse video microscopy until hair cells had been regenerated, usually over the course of one week.

In the first experiments, amputation of a segment of the tail that contained lateral line neuromasts stimulated macrophage activity and a dramatic increase in proliferation of mantle-type supporting cells at the posteroventral edge of the posterior-most neuromast of the tail stump. The new cells continued to divide and migrated posteriorly as the regenerative placode. Their progeny ultimately differentiated to form replacement neuromasts.

In a second series of experiments, a UV laser microbeam was used to individually kill all pre-existing hair cells in one neuromast, leaving a sensory epithelium that contained only supporting cells. Replacement hair cells were regenenerated during time-lapse recording in the week following the laser treatment. In some cases replacement cells differentiated directly from cells that were present in the epithelia immediately after the laser treatment. In other cases, cells went through one or two divisions prior to the differentiation of one daughter as a hair cell. These results suggest that cell differentiation during replacement of sensory hair cells in the lateral line depends both on cell lineage and on cellular response to environmental or positional cues.

(Supported by grants from NIDCD and the DRF to J.T.C.)

250.8

RECOVERY OF THE MAMMALIAN ORGAN OF CORTI AFTER LASER MICROBEAM ABLATION OF EMBRYONIC HAIR CELLS. M.W. Kelley, C.M. Laverack*, and J.T. Corwin. Dept. of Otolaryngology - HNS and Dept. of Neuroscience, Univ. of Virginia School of Medicine, Charlottesville, VA 22908.

Loss of hair cells from the adult mammalian cochlea results in the formation of epithelial scars that are believed to be permanent. It has been suggested that recovery of the organ of Corti following hair cell loss may be constrained by its complex cytoarchitecture. In light of this, we felt that at earlier stages in development more complete recovery might occur.

To test this hypothesis, cochleae were dissected from embryonic (E15 - E20) or neonatal (P0 - P3) mice and maintained *in vitro* using a modification of the Sobkowicz technique. Individual hair cells were ablated using an ultraviolet laser microbeam. The response to ablation of different numbers of hair cells was assessed through DIC microscopy, phalloidin staining of filamentous actin, and tritiated thymidine labelling of proliferative cells.

After lesioning of hair cells in neonatal (P0 - P3) cochleae, cells migrated into the lesion sites from the greater epithelial ridge (GER). Proliferative cells were observed adjacent to some lesion sites and in several cases were located in or near the sensory epithelium, but recovery was also observed without proliferation. In control regions, no proliferative cells were observed in either the GER or the sensory epithelium. Preliminary data from E16 cochleae indicate that hair cell lesions made at this stage are completely repaired in 24 to 48 hours. No proliferation was observed during these repairs.

Prior to maturation of cytoarchitecture, the organ of Corti is capable of self-repair. In addition, the observed replacement of ablated hair cells suggests that differentiation of cells as hair cells is a function of the cell's response to environmental cues rather than an autonomous determination of cell fate.

(Supported by funds from NIDCD and DRF to JTC.)

250.9

CURRENT-CLAMP RESPONSES OF VESTIBULAR HAIR CELLS. A.J. Ricci*, C.H. Norris, P.S. Guth Departments of Otolaryngology, Pharmacology and the Neuroscience Training Program, Tulane University, New Orleans, LA 70112

The whole-cell patch configuration was used to record from frog semicircular canal hair cells. A current-clamp protocol was used that stepped in both positive and negative directions from the zero-current level, in an attempt to measure the cell's physiologic voltage responses to a variety of stimuli. Three types of hair cell voltage responses were observed. The first type responded in a relatively ohmic fashion to both depolarizing and hyperpolarizing steps. The second type showed large voltage shifts in the hyperpolarizing direction that tended to relax with time. The depolarizing direction was ohmic with small voltage changes. The third type of cell produced major non-linear voltage changes in both depolarizing and hyperpolarizing directions. Both responses tended to relax with time. These results suggest that vestibular hair cells have different proportions of channel types. They also suggest that an active conductance is present at rest. A comparison and characterization of voltage-clamp responses will be given. This work was supported by PHS grant DC303.

250.10

FREQUENCY SELECTIVITY AND ITS DEPENDENCE ON SOURCE LOCATION IN THE FROG. T.D. White[1], B. Schmitz[2]*, and P.M. Narins[1]. [1]Dept. of Biology, Univ. of California, Los Angeles, CA 90024 and [2]Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz 1, FRG.

The directional characteristics of hearing in the leopard frog (Rana pipiens) were investigated by obtaining frequency threshold curves (FTCs) from nerve fibers of cranial nerve VIII. Three populations of neurons were identified: low-frequency sensitive (LFS, 63-500 Hz), mid-frequency sensitive (MFS, 720-1199 Hz), and high-frequency sensitve (HFS, 1404-2177 Hz). In LFS neurons, the characteristic frequency (CF) did not vary significantly with speaker azimuth but thresholds at the CF were least sensitive when obtained from the anterior field and most sensitive from the posterior field. For MFS neurons, the posterior field produced FTCs with CFs significantly higher (0.16 octave) than the anterior field. CFs obtained from HFS neurons with ipsilateral stimulation were significantly lower (-0.29 octave) than those from the anterior field. Differences in frequency selectivity were attributed to acoustic transmission through extratympanic pathways. NIH #DC00222 to PMN.

250.11

DOWN-REGULATION OF CELL PROLIFERATION IN THE AVIAN VESTIBULAR EPITHELIUM. P. Weisleder and E.W Rubel. Hearing Devel. Labs., RL-30, U. of Washington, Seattle, WA 98195.

A low, ongoing level of cell proliferation and hair cell differentiation has been described in the intact vestibular epithelium of postnatal chicks and adult budgerigars. Recent studies in our laboratory have demonstrated that the rate of proliferation increases following streptomycin toxicity. In the present study we sought to determine if VIIIth-nerve activity blockade influences the amount of cell proliferation following aminoglycoside damage.

Ten, two-week old chicks received daily injections of streptomycin for 5 days. On the sixth day, five animals had a tetrodotoxin-impregnated (TTX) Elvax (a plastic polymer) -bead placed in the oval window of one ear. The other five animals were implanted with beads not containing TTX. Beginning two hours later, the animals received four bi-hourly injections of the cell proliferation marker, bromodeoxyuridine (BrdU). Following the last BrdU injection, the animals were sacrificed; the cristae ampullaris were dissected, embedded in plastic, and every fourth section was processed for BrdU-immunocytochemistry.

A ratio, derived by dividing the number of labeled cells by the number of sections analyzed, was obtained for each organ. The tissue obtained from ears that were not exposed to TTX consistently had 30% more BrdU-positive nuclei than tissue exposed to TTX. These results suggest that silencing the VIIIth-nerve reduces the pace at which the damaged avian vestibular epithelium replaces lost elements. Supported by DC00395 and Cora M. Poncin Foundation.

250.12

ORGANIC Ca^{++} CHANNEL BLOCKERS HAVE HIGHER POTENCY THAN K^+ CHANNEL BLOCKERS IN BLOCKING K^+ CONDUCTANCE IN THE ISOLATED OHCs OF GUINEA PIG. X. Lin*[1], R.I. Hume[2], A.L. Nuttall[1], Kresge Hearing Research Institute[1] and Department of Biology[2], University of Michigan, Ann Arbor, MI, 48109.

Many types of voltage-gated membrane conductances have been studied in the isolated outer hair cells (OHCs) of guinea pig. Under whole cell voltage-clamp, the dominant ionic current at depolarizing voltages is an outward K^+ current. In this work, the potency of a variety of K^+ and Ca^{++} channel blockers in blocking this K^+ conductance was investigated.

The whole cell voltage-clamp technique was used to study the voltage-dependent currents in isolated outer hair cells (OHCs) maintained in short-term culture. The pipette internal solution contained (in mM): KCl 134, $MgCl_2$ 2, NaCl 4, BAPTA 10, Hepes 10, Glucose 10, pH 7.4. The external solution had: NaCl 134, $MgCl_2$ 2, KCl 4, EGTA 1, Hepes 12.5, Glucose 10, pH 7.4. Ion channel blockers were applied by pressure ejection from a fine glass pipette (tip size 3μm) located near the OHC. The typical whole-cell current to clamped depolarizing voltages was an outward current which showed little inactivation at the end of a 200ms test pulse. The reversal potential and ion substitution tests both indicated that most of the current was carried by K^+ ions. Pharmacological tests showed this current was reversibly suppressed by a variety of conventional K^+ channel blockers. Surprisingly, organic Ca^{++} channel blockers also blocked the K^+ current, with even higher potency. The order of potency of these channel blockers was (estimated half maximum blocking concentration listed in the parentheses): nimodipine(10μM)> verapamil= D600(20μM) > nifedipine(100μM)> 4-AP(150μM)> quinine(700μM)> TEA(5mM).

The ionic composition and strong Ca^{++} buffers used in this experiment suggested that it is unlikely the effect of these organic Ca^{++} channel blockers on the K^+ current was mediated by a Ca^{++} dependent K^+ current. (supported by NIH DC00141)

250.13

ELECTRON MICROSCOPIC LOCALIZATION OF ACETYLCHOLINESTERASE ACTIVITY IN THE RAINBOW TROUT INNER EAR. K.M. Khan[1], J.S. Hatfield[2*], M.J. Drescher[1*], and D.G. Drescher[1]. [1]Laboratory of Bio-otology, Wayne State University School of Medicine, Detroit, MI 48201, and [2]V.A. Medical Center, Allen Park, MI 48101.

The hair cells of the trout inner ear are morphologically similar to type II hair cells of the avian and mammalian vestibule. These cells are innervated by two kinds of nerve terminals. The nonvesiculated terminals are considered to be afferent, while the efferent endings contain numerous clear, round vesicles and a few that are dense-cored. Morphological features of the vesiculated terminals are similar to those of cholinergic endings present in the avian and mammalian inner ear. The aim of the present study was to characterize the efferent terminals in the rainbow trout inner ear sensory epithelia at the electron microscopic level, by histochemical localization of acetylcholinesterase (AChE) activity. Tissues were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 10^{-1} M phosphate buffer, pH 7.2 for 2 h. All procedures were carried out at 2-4 °C. After a brief rinse in buffer, specimens were incubated according to the method of Karnovsky and Roots (J. Histochem. Cytochem. 12: 219, 1964). Eserine sulfate (an inhibitor of AChE and pseudocholinesterase) was added to the medium as control for nonspecific staining. Vesiculated endings in the cristae ampullares, and saccular and utricular maculae exhibted AChE activity. The reaction product was present on or adjacent to the plasma membranes of the efferent terminals. Nonvesiculated endings did not show any reaction product, except at presumed axodendritic synapses. No staining was observed in control specimens. In some instances the reaction product was not intense, perhaps caused by limited penetration of the incubation medium through the dense connective tissue associated with the basal surface of the sensory epithelia. These results suggest that the vesiculated nerve endings in the trout inner ear contain cholinergic elements. Supported by NIH grant DC 00156.

250.14

THE DEVELOPMENT OF THE INNER EAR EFFERENTS IN MICE: A DiI AND AChE STUDY. B. Fritzsch and D.H.Nichols, Creighton Univ., Div. of Anat., Omaha, NE 68178

Three populations of cells in the brainstem provide efferent innervation to the vestibule, the outer, and the inner hair cells of the cochlea, respectively. We employed DiI in fixed tissue and AChE-histochemistry to analyze the time course of arrival of efferents at the ear, the segregation of fibers at the target and of the cells in the brainstem during development. At E11% we could label efferents retrogradely from the ear and we anterogradely labelled their fibers from the brainstem to the ear. At E 12% we could fill efferent fibers from one ear to the other ear where they reached all future sensory epithelia. AChE-histochemistry showed a continuous band of cells from the ventricle to the meninges between E 11% and E12% suggesting migration at that time. At E 14 the distribution of olivocochlear efferents as revealed with DiI gave no indication of a segregation into the two adult subsystems but the numbers of retrogradely labelled cells were already approaching known adult values and distribution of all olivocochlear efferents (Campbell, J.P. & Henson, M.M., Her. Res. 35 (1988) 271). These data suggest that (1) the efferents reach the future sensory epithelia at the same time as afferents and around the time hair cells are born, i.e much earlier then suggested using other techniques, (2) peripheral segregation may precede onset of migration of olivocochlear neurons, and (3) the sorting of the two subpopulations of olivocochlear neurons (LSO and MSO) is a late ontogenetic event.

250.15

INFLUENCE OF ATP ON THE SEMICIRCULAR CANAL (SCC) OF THE FROG. A. Aubert*[1], C.H. Norris[1,2] and P. S. Guth[1,2]. Depts. of Pharmacology[1] and Otolaryngology[2]. Tulane University, New Orleans, LA 70112

Ashmore and Ohmori (1990) and Shigemoto and Ohmori (1990) have shown that ATP induced an alteration of calcium processing in the cochlear outer hair cells. In an attempt to determine the possible role of ATP as a neuromodulator in the SCC, we have investigated the dose-responsive influence of disodium-ATP on the bioelectrical activities of the isolated SCC of the frog. At a concentration of 10^{-12} M, disodium-ATP induced an increase of the spontaneous afferent activity while the evoked afferent activity was decreased. ATP caused a depolarization followed by a hyperpolarization of the transepithelial D.C. potential. The nerve direct current at rest and its variation during mechanical stimulation were decreased. These data imply the existence of ATP receptors in the SCC. The possible role of ATP and its receptors in the physiology of the SCC will be discussed. (Supported by USPHS grant # DC - 00303)

250.16

GLUTAMATE DECARBOXYLASE AND GABA-TRANSAMINASE LOCALIZATION IN THE GUINEA PIG VESTIBULE. IMMUNOCYTOCHEMICAL SUPPORT FOR AN AFFERENT GABAERGIC NEUROTRANSMISSION.

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In a recent report a GABA-like immunoreaction in the guinea pig vestibular hair cell and adjacent fibers was demostrated(1). In order to investigate the source and fate of this GABA pool, pre and postembedding techniques were used to study the localization and distribution of glutamate decarboxylase(GAD) and GABA-Transaminase(GABA-T) (enzyme of synthesis and degradation of GABA respectively) in the guinea pig vestibule. Animals were perfused with a mixture of aldehydes, the vestibular organs were dissected dehydrated and embedded in paraffin. Vestibular cristae sections were incubated with either GAD or GABA-T antisera and processed for pre and postembedding immunoperoxidase staining. GAD-like immunoreactivity was found in both type I and II hair cell cytoplasm, whereas GABA-T-like immunore activityclosely followed GABA distribution being found in the calyx, some nerve fibers and ganglionar cells. These results show that GABA is synthesized in the hair cell cytoplasm and after its release and action upon its receptor isdegraded by the afferent fiber,thus strongly supporting its afferent neurotransmitter role in the vestibule.

1.- López,I.et al (1990) Brain Res. 170-175.

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250.17

CHOLINERGIC AND PURINERGIC RECEPTORS ARE NOT CO-LOCALIZED ON OUTER HAIR CELLS ISOLATED FROM THE GUINEA-PIG ORGAN OF CORTI. G.D. Housley[1] and J.F. Ashmore[2] Depts. of Physiology, [1]University of Auckland, New Zealand & [2]University of Bristol, England.

It has been suggested that adenosine-5'-triphosphate (ATP) may act as a neuromodulator of the cholinergic olivo-cochlear innervation to the outer hair cells (OHCs) of the mammalian cochlea (Nakagawa et al., J. Neurophysiol. 63:1068-1074, 1990). Using the whole-cell patch-clamp technique we have shown that acetylcholine (ACh) elicits a rapidly inactivating outward potassium current when applied by pressure injection from a fine glass pipette to a region around the basal pole of isolated OHCs (Housley & Ashmore, Proc. R. Soc. Lond. B. 1991, *in press*). These responses were highly variable (<+10pA to +678pA at a holding potential of -50mV, using 100μM ACh) and limited to cells shorter than 65μm. We have now used this procedure to study the effect of ATP and ACh on the same cell.

Our evidence suggests that purinergic receptors (P2) which produce an inward cationic current in OHCs are not co-localized with the nicotinic-like cholinergic receptors around the basal pole of OHCs. ATP (0.1 - 100μM) produced the largest inward currents with minimum onset latencies when applied to the apical region of all OHCs tested (n =60), irrespective of cell length (19μm - 87μm). Using double-barrelled ejection pipettes, application (0.5 - 2s) of ACh (50μM) + ATP (10μM) to the base of the cells voltage-clamped to -60mV yielded outward current responses no different from using ACh (50μM) alone. This contrasted with the application of ACh + ATP to the apical region of these cells where an inward current (ATP response) was produced, ACh alone had no effect when applied here (n=4). There was no difference between the minimum onset latencies of ATP and ACh responses (approximately 30-60ms). (Supported by: Health Research Council (N.Z.), Deafness Research Foundation (N.Z.), New Zealand Lottery Grants Board).

250.18

VESTIBULAR OTOCONIAL MATRIX OF YOUNG CHICKS (*Gallus domesticus*). C.D. Fermin and D. Martin. Dept. of Pathology. Tulane University Medical School. New Orleans, La 70112-2699.

Otoconia or statoconia are microscopy calcite crystals (1-10 μm) located over the sensory epithelia of the inner ear organs that respond to gravitational forces. High resolution transmission electron microscopy, histochemical and biochemical assays demonstrated that the hexagonal crystals of the chick inner ear vestibular organs could be made up of intercalating fibrillar arrays that conform to mature crystal shape. Usually the crystals of hatchlings have angle of 115º and short face sides of >1 μm. Crystals treated with sodium hypochlorite and ultrasonic vibrations yielded submicroscopy subunits with similar angle value but <100 nm short face sides, indicating that each large crystal could be made up of interconnecting subunits. Previous work from this lab has shown that early in development, these crystals may be made up mostly of organic substances, including glycosaminoglycans. Potassium pyroantimonate staining, indicated that calcium is probably gradually added between the fibrils of the organic matrix. It is possible that after a certain critical period of development the organic components of the matrix are aligned in register with the calcic components in such a way that perfect hexagonal subunit crystals can be formed. Whether otoconia is recycled or not is still a mystery despite numerous works in several species. A permanent organic template (e.g. glycosaminoglycans and glycoproteins) could be selectively inactivated and also reactivated if necessary providing sufficient flexibility for crystal recycling and/or maintenance. Because of the exquisite homeostatic control of the endoymphatic environment where crystals reside this idea deserves further analysis. (Supported by NASA grant NAGW1515 to CDF and Departmental funds).

CHEMICAL SENSES: PERIPHERAL MECHANISMS II

251.1

Regulation of Differentiation of Cultured Human Olfactory Neuroblasts. Trey Sunderland, B. B. Zheng*, H. Coon*, B. L. Wolozin. Lab. of Clinical Science, NIMH and Lab. of Genetics, NCI, Bethesda, MD 20892.

We are studying the regulation of differentiation of human olfactory neuroblasts grown in cell culture. We now report that TPA stimulation at 200 ng/ml for 24 hrs or 33 ng/ml for 48 hrs results in the extension of multiple long narrow processes. Immunocytochemical and immunoblotting experiments indicate a corresponding increase in the density of neurofilament and olfactory marker protein OMP staining. Immunoblots of tau protein however, shows no TPA induced increase. db-cAMP and IBMX (0.5 mM each) also promote process outgrowth though over a somewhat longer time course, requiring 24-48 hrs. This paradigm, however, did not elicit any increase in neurofilament or OMP immunoreactivity seen by immunocytochemistry. Immunoblots indicate that db-cAMP + IBMX elicits a small decrease in neurofilament and larger decrease in OMP reactivity. Because cAMP is implicated in the odorant transduction pathway, this reduction in neuronal immunoreactivity may represent a general down-regulation event. Growth factors, such as EGF and NGF, however, act via a cascade that includes protein kinase C. Hence, the TPA induced upregulation of neuroreactivity may mimic the stimulation of neuronal differentiation or axonal outgrowth by endogenous factors *in vivo* resulting in an upregulation of these elements.

251.2

FINE-STRUCTURAL LOCALIZATION OF THE OLFACTORY TRANSDUCTION APPARATUS. B. Ph. M. Menco. Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208.

We localized important components of the olfactory transduction apparatus in chemosensory receptor cells and of some mucus regulating factors in olfactory supporting cells in the rat's olfactory epithelium. For most of the work we used rapid-freeze, freeze-substitution and post-embedding (in Lowicryl K11M) gold-label electron microscopic immunocytochemistry. G-proteins and adenylate cyclase act, together with among others cyclic nucleotide-gated channels, to transduce the olfactory signal. We used antibodies to $G_{olf\alpha}$ (*Jones & Reed, Science, 244:790 (1989)*), $G_{s\alpha}$ (*Mania-Farnell & Farbman, Devl. Brain Res., 51:103 (1990)*) and Type III olfactory adenylate cyclase (*Bakalyar & Reed, Science, 250:1403 (1990)*). All three antibodies co-localized in majority in the same cellular compartment, i.e., antigens were mainly found in the long distal parts of rat olfactory cilia, suggesting that these regions are specifically modified to transduce, and most likely also receive, the olfactory message. Apical regions of olfactory supporting cells share several properties with apical regions of cells of transporting epithelia, in particular those of kidney collecting tubules (*Brown, Am. J. Physiol., 25:F1 (1989); Brown et al., Am. J. Physiol., 25:F366 (1989); Tousson et al., J. Cell Sci., 93:349 (1989)*). Supporting cell microvilli labeled with antibodies to amiloride-sensitive sodium channels and, with light microscopy, their apices seemed also to bind antibodies to a proton pump. This suggests that these cells play a role in the maintenance of the mucous pH and sodium concentration around receptor cell cilia. *Drs. Heather Bakalyar, Dale Benos, Steve Gluck, David Jones, and Randy Reed and their colleagues are thanked for sharing their antibodies. Supported by NSF grant BNS-809839.*

251.3

VOMEROMODULIN, A NOVEL GLYCOPROTEIN OF LATERAL NASAL GLAND: A PUTATIVE PHEROMONE TRANSPORTER. M. Grillo[1], Y.S. Khew-Goodall*[2], M. Getchell[3], W. Danho*[4], T. Getchell[3], F.L. Margolis[1]. [1]Dept. of Neurosciences, Roche Inst. of Molec. Biol., Nutley, NJ 07110. [2]The Hansen Ctr. for Cancer Res., Adelaide, Australia. [3]Dept. of Surgery & Sanders-Brown Ctr. on Aging, Univ. of Kentucky, Lexington, KY 40536. [4]Peptide Res. Dept., Hoffmann-La Roche, Inc., Nutley, NJ 07110. [5]Dept. of Physiol. & Biophys., Univ. of Kentucky, Lexington, KY 40536

Vomeromodulin, a novel glycoprotein of the lateral nasal gland, has been cloned by differential hybridization of a cDNA library from nasal/olfactory tissue. The 2.2kb mRNA directs the in vitro synthesis of a 60 kD primary translation product in reticulocyte lysates. Differential sensitivity to endoglycosidases indicates that vomeromodulin is post-translationally modified in vivo by N-glycosylation to form a 70 kD glycoprotein of the complex type. Immunocytochemical localization with two different antisera demonstrated that vomeromodulin is abundant in the lateral nasal glands and is also present in the posterior septal and vomeronasal glands. Most striking is the observation that it is highly concentrated in the mucus of the vomeronasal organ but is not detectable in the mucus of the main olfactory neuroepithelium. Evaluation of mRNA and protein distribution by Northern and Western analyses indicated that vomeromodulin is absent from 15 other tissues. The glandular and mucosal distribution of this glycoprotein implies a transport function that may be related to the mechanism by which pheromone molecules of low volatility gain access to their receptors in the vomeronasal organ. We propose that vomeromodulin participates in perireceptor events.

251.4

DIFFUSION AND TRANSPORT OF ODORANTS AND TRACERS IN THE OLFACTORY MUCUS LAYERS OF THREE SPECIES OF SALAMANDERS

P.A. Moore, M.N. Friedemann, T.E. Finger+, G.A. Gerhardt. Departments of Pharmacology and Psychiatry, +Dept. of Cellular and Structural Biology, University of Colorado Health Science Center, Denver, CO 80262

Perireceptor events are important aspects of chemoreception. These include the movement of chemicals by turbulence and diffusion, the binding of odorants to transporter proteins and diffusion to receptor sites. In terrestrial olfaction, odor molecules must diffuse through a mucus layer before binding with receptor proteins and subsequently back through the mucus layer to be removed from the olfactory epithelium. Thus, the diffusion properties of molecules in mucus have remained relatively unknown. Using high speed *in vivo* electrochemical recordings coupled with local chemical application techniques, we have determined the apparent diffusion coefficients of dopamine (tracer) and several odorants, including vanillin, eugenol, and safrole, in the mucus layer covering the olfactory epithelium of the tiger salamander (*Abystoma tigrinum*). In addition, diffusion coefficients were determined for both the marbled (*Abystoma opacum*) and spotted salamanders (*Abystoma maculatum*). Diffusion coefficients in the mucus (0.2×10^{-6} cm/s) were significantly slower than in solution (0.6×10^{-6} cm/s). In addition, these values were at least an order of magnitude slower than those previously used in theories and models. These studies are needed to understand the role that diffusion and transporter proteins play in determining the structure of chemical signals as they arrive at receptor cells.

Support by USPHS (AG00441) and NSF 2534920 to GAG.

251.5

EXPRESSION OF RAT OLFACTORY RECEPTORS IN XENOPUS OOCYTES. N. Dahmen*[1], H. Wang[1], T.V. Getchell[2], F.L. Margolis[1]. [1]Dept. of Neurosciences, Roche Inst. of Molec. Biol., Nutley, NJ 07110. [2]Dept. of Physiol. & Biophys., Univ. of Kentucky, Lexington, KY 40536

We demonstrate that ligand specific olfactory receptors are expressed by Xenopus oocytes following the injection of mRNA isolated from rat olfactory epithelium. Application of appropriate ligands activates stimulus-dependent transmembrane currents in the oocytes, measured under two-electrode voltage clamp recording, that reverse direction at the chloride equilibrium potential. The currents show characteristic secondary oscillations, that are assumed to reflect the underlying Ca^{2+} oscillations. Similar ligand activated membrane currents induced in oocytes after injection of other mRNAs have been shown to be due to Ca^{2+} dependent chloride channels. These are dependent on an intracellular rise of free Ca^{2+} that is released from intracellular stores by inositoltriphosphate. Mammalian olfactory signals therefore appear to be capable of being coupled not only through the well-established cAMP pathway, but also through the Ca^{2+}/IP_3 pathway. Currently, we are using the Xenopus oocyte in vitro expression system as a tool for the identification of ligand specificity profiles of olfactory receptor molecules and for the expression cloning of these receptors.

251.6

EXPRESSION OF CATFISH L-AMINO ACID OLFACTORY RECEPTORS IN XENOPUS OOCYTES INJECTED WITH IN VITRO TRANSCRIBED RNA. H. Wang[1], N. Dahmen*[1], K. Kodukula*[1], N. Yan*[1], J. Teeter[2] and F.L. Margolis[1]. [1]Dept. of Neurosciences, Roche Inst. of Molec. Biol., Nutley, NJ 07110. [2]Monell Chemical Senses Center, Philadelphia, PA 19104

We report the functional expression of catfish olfactory receptors in Xenopus oocytes injected with either isolated mRNA or with in vitro transcribed RNA. Catfish olfactory tissue contains high-affinity receptors for L-alanine (A) and L-arginine (R), which are odor stimuli for catfish. These L-amino acid olfactory receptors are coupled to phosphotidylinositol (PI) turnover through G proteins. Two electrode voltage-clamp recording of RNA-injected oocytes is being used to clone olfactory L-amino acid receptors. Oocytes injected with mRNA purified from catfish olfactory tissue were monitored by two electrode voltage clamp recordings. Superfusion of mRNA injected oocytes with A or R resulted in oscillating and outward rectifying membrane currents. A and R activated currents reversed direction at about the Cl^- equilibrium potential. This result is consistent with the hypothesis that A and R evoked Ca^{++}-dependent Cl^- currents of the oocytes by activating the endogenous IP3-Ca^{++} second messenger system. A cDNA library was constructed using the mRNA preparation responsible for expression of A and R receptors in the oocytes. RNA transcribed from a subpool of the cDNA library was injected into the oocytes. A and R stereospecifically evoked Ca^{++}-dependent Cl^- currents in these oocytes. Stepwise fractionation of this cDNA library subpool is in progress to isolate individual cDNA clones encoding the L-amino acid olfactory receptors.

251.7

Molecular Modelling of the Receptor-like Binding Sites in Monoclonal Antibodies to Intense Sweet Taste Ligands. Jerry M. Anchin and D. Scott Linthicum, Dept. Vet. Pathobiol., College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4467,USA

Knowledge-based computer-assisted molecular modelling of monoclonal antibodies is now feasible using a modelling protocol involving "canonical structures" from known crystallographic coordinates of emperically solved myeloma proteins and monoclonal antibodies. Modelling and energy minimization techniques permits the construction of interactive residues involved in ligand binding. In our study we examined the binding sites of monoclonal antibodies directed against intense sweet taste compounds derived from guanidines. The high potency of these sweetners is useful for the identification of sweet taste epitopes which bind the antibody. These epitopes are probably important in sweetner-receptor interactions and molecular modelling of the antibody binding sites can reveal structural features and chemical interactions which may be similar to those postulated for the receptor site model. The use of antibody binding sites as a paradigm for taste receptor binding sites is an important concept in our understanding of ligand-receptor interactions. Detailed structure-activity relationships and molecular modelling studies of sweet taste ligands and receptors are important tools if valid comparisons are to be made. Supported by the Nutrasweet Co. Gig'em Aggies!

251.8

ANATOMIC AND SPECIES DISTRIBUTION OF THE N-CAM GLYCOFORM RECOGNIZED BY MONOCLONAL ANTIBODY 1D9. M.J. Crowe & S.K. Pixley. Dept. of Anatomy & Cell Biology, Univ. of Cincinnati, Cincinnati, OH 45267.

We report here on the anatomic distribution of the antigen recognized by the monoclonal antibody (MAb) 1D9 in the olfactory epithelia (OE) of several species. The preparation and initial characterization of MAb 1D9 has been described (Neurosci. Abst. #466.13, 1988 & #364.10, 1990). The N-CAM character of the antigen was reported by Key & Akeson (Neuron, 6:381-396, 1991). Using coronal sections of E19 and P7 rat heads, we see a varied antigen distribution. In E19 tisssues, 1D9 immunoreactivity (IR) is strongest in neuron-like cells in the vomeronasal organ and in taste buds. Both of these tissues have receptors sensitive to non-volatile compounds borne primarily within a fluid medium. No 1D9 IR is seen in the E19 main OE. In P7 rat OE, a subset of neurons within the main olfactory region is labeled. We examined an amphibian, *Ambystoma tigrinum*, to see if any differences between OE located at a tissue/air interface versus a tissue/fluid interface could be observed. The flat sheet of OE comprising the main part of the olfactory sac in the post-metamorphosed land phase (air-breathing) animal is labeled with MAb 1D9 specifically in a subset of cells with processes. The remnants of water phase OE, which are present in land phase animals, are completely labeled by 1D9. We then tested OE from the pre-metamorphosed water phase (gill breathing) *A. tigrinum*. In this animal, the main part of the olfactory sac is composed of folds of water phase OE with a small portion of the OE being flat and sheet-like. Again, a subset of olfactory cells is immunolabeled with MAb 1D9 in the flat OE, while all cells in the folds of water phase OE are strongly 1D9-reactive. In rat and *A. tigrinum*, MAb 1D9 appears to differentially label those sensory epitheliums dependent upon a tissue/air interface and those dependent upon a tissue/fluid interface. Supported by NIH DC00342 and DC00347.

251.9

LOCALIZATION OF N-CAM AND N-CADHERIN IN THE FETAL HUMAN PRIMARY OLFACTORY PATHWAY. M.I. Chuah and C. Au*. Dept. of Anatomy, Chinese University of Hong Kong, Shatin, Hong Kong.

The cell adhesion molecule N-CAM has been shown to be present in the adult mouse primary olfactory pathway. Results from recent in vitro studies indicate that N-CAM, in addition to N-cadherin, mediates neurite extension from olfactory neurons cultured on astrocyte monolayers (Chuah et al. Developmental Brain Research, In press, 1991). In the light of this functional role, we have examined human olfactory epithelium and bulb for the presence of N-CAM and N-cadherin during a stage when there is extensive growth of olfactory axons into the bulb. Immunofluorescence staining was performed on olfactory tissues from fetuses of 17 1/2 to 21 weeks of gestation. Moderate staining for N-CAM on olfactory neurons and basal cells was observed while the most intense staining was present in the nerve bundles in the lamina propria. Immunoreactivity for N-CAM was slightly reduced as the nerve bundles approached the olfactory bulb. All layers of the olfactory bulb showed labeling for N-CAM although increased staining was generally found in the olfactory nerve and glomerular layers. For N-cadherin, the most intense labeling was found in the olfactory epithelium. The olfactory nerve axons also showed immunopositivity but the labeling decreased progressively as they reached their termination in the glomerular layer of the bulb. Some staining for N-cadherin was also found in the other layers of the bulb.

251.10

THE GROWTH OF OLFACTORY NERVES IN EXPLANT CULTURES IS NOT INHIBITED BY CNS MYELIN. Albert I. Farbman and Judith A. Buchholz, Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208.

Transplantation experiments have shown that the olfactory nerve is unique in its ability to grow in all kinds of environments within the CNS (Morrison & Graziadei, 1983, Brain Res., 279:241). The CNS is not permissive for the growth of several other types of neuron, both central and peripheral, apparently because of the presence in CNS myelin of 2 proteins not present in PNS myelin (Caroni & Schwab, 1988, J. Cell Biol., 106:1281). We have made fresh frozen sections of adult rat cerebrum and cerebellum and placed them on polyornithine (PORN)-coated glass cover slips. Explant cultures of fetal rat olfactory sensory epithelium were grown on these cover slips, both on and off the sections. After 2-5 days *in vitro*, there was little difference in the amount of axonal outgrowth from explants in contact with the white matter, the grey matter, or the PORN substrate. The results suggest that olfactory neurons do not have the receptors that respond to the growth inhibitory proteins of CNS myelin.

Supported by NIH Grant # DC 00347.

251.11

GLOSSOPHARYNGEAL NERVE SECTION DOES NOT IMPAIR SALT TASTE DISCRIMINATION IN RATS. A. C. Spector and H. J. Grill[1]. Dept. of Psychology, University of Florida, Gainesville, FL 32611 and [1]Dept. of Psychology, University of Pennsylvania, Philadelphia, PA 19104.

Recent behavioral and electrophysiological findings suggest that the peripheral coding of taste qualities may, to some extent, be topographically organized. We have previously shown that rats trained to discriminate potassium chloride (KCl) from sodium chloride (NaCl) across a concentration range show severe performance deficits at this task following deafferentation of the anterior tongue by section of the chorda tympani nerve (removes 15% of the total taste buds). The present study demonstrates unequivocally that the glossopharyngeal nerve (GLn), which innervates the posterior tongue (65% of the total oral taste buds), is not necessary to maintain performance in the KCl vs NaCl discrimination task. Eight water-deprived Sprague-Dawley rats were trained in a specially-designed gustometer to maintain licking during brief presentations (5 sec) of one taste stimulus (S-) and to avoid licking a second tastant (S+). Rats were trained (counterbalanced) to respond one way to KCl (0.05, 0.10, 0.20 M) and another way to NaCl (0.05, 0.10, 0.20 M). On S+ trials, if the rat did not suppress licking during the latter 3 sec of the 5 sec trial it received a brief footshock. On S- trials, if the rat suppressed licking during the latter 3 sec of the trial, the rat received a 30 sec time-out further delaying the next fluid trial. Stimuli were presented randomly during 60 min sessions. Rats easily learned this discrimination. Next, half of the rats received bilateral sections of the GLn and the other half served as full surgical controls. Postsurgical assessment of their discrimination performance revealed no deficit. These findings suggest that different oral receptive fields provide differential contributions to the peripheral signals characterizing various chemical stimuli. Supported by PHS grants DC-00161 and MH-43787.

251.12

RECONSTITUTION OF THE OLFACTORY EPITHELIUM AND RE-INNERVATION OF THE OLFACTORY BULB AFTER METHYL BROMIDE LESIONS. J.E. Schwob and S.L. Youngentob. Depts. of Anatomy and Cell Biology, Physiology, and the Clinical Olfactory Research Center, SUNY Health Sci. Ctr., Syracuse, NY 13210.

In adult animals, the olfactory epithelium has some capacity to recover from direct injury by generating a new population of sensory neurons. However, the timing and degree of neuronal recovery is poorly characterized. We have been examining the reconstitution of the epithelium of adult rats following lesions induced by a single inhalation exposure to methyl bromide gas (330 ppm X 6 hr). A variety of immunohistochemical markers for immature (e.g., anti-GAP-43) and mature (e.g., anti-OMP) olfactory neurons and their axons have been used. In addition, the status of the axonal projection of the newly born neurons onto the bulb was assessed (1) by anterograde transport of WGA-HRP, to identify when the bulb had been re-innervated, and (2) by immunohistochemical staining with the monoclonal antibody RB-8, to selectively mark axons that derive from the ventrolateral olfactory epithelium. We find that the epithelium recovers in thickness by 2 weeks after lesion, but at this time it contains mostly immature neurons. The normal balance between immature and mature olfactory neurons is not struck until 6 weeks post exposure. By comparison, reinnervation of the bulb begins as soon as 1 week after MeBr, and is largely complete by 4 weeks. The newly formed axonal projection recapitulates the restricted spatial distribution revealed by the RB-8 antibody in normal rats. That is, after recovery, axons from the ventrolateral epithelium reinnervate the ventrolateral bulb. In conclusion, the adult primary olfactory projection has the capacity for virtually complete anatomical recovery after MeBr lesions. Supported by DC 00467 and DC 00220.

251.13

OLFACTORY FUNCTION FOLLOWING PERIPHERAL OLFACTORY LESIONS. S.L. Youngentob and J.E. Schwob. Departments of Physiology, Anatomy and Cell Biology, and the Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse NY 13210.

Several investigators have demonstrated that the olfactory epithelium has a remarkable capacity to recover anatomically following experimentally induced lesions. However, there is a paucity of information regarding the degree of functional recovery. Therefore, the present study was undertaken to assess functional recovery, as defined by psychophysical techniques, following lesions that induce the cycle of neuronal degeneration, epithelial reconstitution and axonal reconnection with the bulb. Long-Evans rats were trained to criterion (>90% correct) on a five odorant identification task and then lesioned by intranasal infusion with $ZnSO_4$ or by exposing them to 330 ppm MeBr gas for 6 hrs. Animals were randomly allocated into either the 3 day survival or full behavioral recovery group. For each animal the anatomical state of the olfactory epithelium was evaluated relative to behavioral performance on the odorant identification task (see also Schwob and Youngentob, this meeting). The results of this study demonstrated for the first time that the reconstituted olfactory epithelium preserves the coding of odorant quality. Furthermore, restoration of behavioral function is supported by less than complete anatomical recovery. Supported by NIH DC00220.

251.14

CONVERGENCE IN THE VOMERONASAL AND MAIN OLFACTORY SYSTEM OF NEONATAL AND MATURE MAMMALS AND ITS RELATION TO SENSITIVITY. Essie Meisami, Physiol. Dept., Univ. of Illinois, Urbana, IL 61801.

The high convergence of receptor neurons to central relay cells in the olfactory system is believed to be a basis for high olfactory sensitivity. To compare sensitivity between the main olfactory and vomeronasal systems and to predict postnatal changes in sensitivity in the two systems, we determined the number of receptor neurons in the olfactory epithelium and vomeronasal organ and the number ofmitral cells in main and accessory olfactory bulbs of newborns and neurologically mature weanlings of rat, hamster and rabbit. From these numbers we obtained the convergene ratios at each age and for the various animals. In the main olfactory system of all three species, the convergence ratio was found to increase postnatally by 5 to 10 folds, mainly due to increase in the number of receptor neurons, reaching maximum values of 1000:1. Compared to the main olfactory system,the adult's vomeronasal convergence ratios were markedly lower. However, like the main olfactory system, the vomeronasal convergence ratio also increased postnatally, although less markedly. The results suggest two general conclusions: one, the main olfactory system is much more sensitive than the vomeronasal system; two, sensitivity is likely to increase postnatally in both the main and vomeronasal system. Supp: Univ. Illinois Research Funds

251.15

GNRH IMMUNOREACTIVE SOMATA IN THE PERIPHERAL AND CENTRAL OLFACTORY SYSTEM OF ATLANTIC SALMON (*SALMO SALAR*): A LIFE HISTORY ANALYSIS. G.A. Nevitt, M.S. Grober and A. Bass. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York, 14853.

Salmon are known for their ability to imprint and home to the stream in which they were spawned in order to reproduce. Behavioral studies have demonstrated an olfactory basis for home stream recognition (Scholz, *et al*, 1976). Changes in olfactory receptor cell sensitivity have also been linked to behavioral imprinting (Nevitt and Moody, 1989). However, the potential role that hormones play in establishing these changes is unknown. The present study is the first in a series designed to identify possible sites of hormone modulation in the peripheral olfactory system of salmonids. Because of its established localization to the terminal nerve ganglion and putative involvement with olfaction, initial studies have focused on gonadotropin releasing hormone (GnRH).

Using a monoclonal antibody to salmon GnRH, GnRH-like immunoreactive somata were identified within four regions of the olfactory system of early life stage animals (fingerlings, parr and smolts): 1) the medial component of the olfactory nerve as it immediately arises from the olfactory epithelium, 2) the entire rostro-caudal extent of the olfactory nerve, 3) the peripheral layers of the olfactory bulb, and 4) the lateral preoptic area. Prior to this study, GnRH positive somata have not been reported anterior to the olfactory bulb in salmonids. Preliminary analysis suggests a similar distribution of these cells between all three life stages, and studies will continue as fish mature into adults. These data provide new morphological evidence for the potential role of GnRH in modulating olfactory receptor cell function at multiple life history stages. References cited: Scholz, A.T., *et al.* (1976). *Science* 192, 1247-1249; Nevitt, G.A. and Wm.J. Moody (1989). *Soc. Neurosci. Abst.* 15:748.

251.16

NEURAL GUSTATORY RESPONSES IN TWO INBRED STRAINS OF MICE DIFFERING IN NACL PREFERENCE. K. S. Gannon and R. J. Contreras. Florida State University, Department of Psychology, Tallahassee, FL 32306-1051.

When allowed to self-select between water and a taste solution, inbred strains of mice display unique fluid preferences. For example, 129/J mice exhibit remarkably strong preferences for NaCl compared to the inbred strain, C57BL/6J (Beauchamp, AChemS Abstr., 1990). No data exist on the underlying mechanism(s) of this strain difference in NaCl preference. It is generally accepted that taste plays an important role in the control of NaCl ingestion. The present study examined possible underlying neural gustatory mechanisms of NaCl preference in 129/J and C57BL/6J mice. We found that 129/J mice exhibited a significantly greater preference for 0.08 and 0.3 M NaCl compared to C57BL/6J mice. Peak and tonic response amplitudes of the whole chorda tympani nerve to NaCl and KCl were similar in the two strains. However, neural studies of salt-sensitive neurons may reveal strain differences not evident at a whole nerve level of analysis. Furthermore, behavioral, membrane-transport, and electrophysiological studies indicate that the initial step of NaCl taste transduction involves specific sodium channels on the plasma membrane of taste receptor cells. Use of the reversible sodium transport blocker, amiloride, may produce differential neural response inhibition to NaCl stimulation among 129/J and C57BL/6J mice. (Supported by NIH grant HL38630).

251.17

THE DEVELOPMENT OF SEXUAL BEHAVIOR AND PARTNER PREFERENCE IN MALE RATS AFTER REMOVAL OF THE VOMERONASAL ORGAN. C. Tardivel, A. N. Clancy and D. A. Edwards. Department of Psychology, Emory University, Atlanta, Georgia 30322.

Recent reports suggest the importance of the vomeronasal organ (VNO) for rodent social behavior. We studied the effect of removing the VNO on the development of copulation and partner-preference in sexually naive male rats. Sexually inexperienced Long-Evans male rats about 70 days old were randomly divided into two groups. We removed the VNO from individuals in one group (VNX); individuals in the other group were sham-operated. Beginning about two weeks after surgery, males were tested in an arena where the male could choose to spend time with (and mate with) a sexually receptive female, a nonreceptive female, or be in a neutral compartment. Males were tested a total of 6 times over a period of about 1 month.

Seven of 8 control males mated to ejaculation. Five of 9 VNX males mated and the sexual performance of these males was comparable, and in some respects superior to, that of controls. Four VNX males never ejaculated, but 3 of these did mount receptive females. Non-ejaculating VNX males showed many episodes of anogenital investigation, the majority of which were directed at the receptive female. While the VNO may be important for sexual arousal in some males, the development of sexual competence in many others is not compromised by the absence of this chemosensory organ. Control and VNX males (maters and nonmaters) developed a strong preference for receptive females. Since all VNX males who mounted showed a strong preference for a receptive female over a nonreceptive female the VNO is apparently not crucial to the process by which males recognize, and become attracted to, sexually receptive females. Supported by NSF Grant BNS 8718797.

CHEMICAL SENSES: CENTRAL PATHWAYS I

252.1

TASTE RESPONSES TO PERITHRESHOLD STIMULI IN THE PARABRACHIAL PONS OF THE RAT. P.M. Di Lorenzo, G.S.Hecht, and S. Monroe. Dept. of Psychology, SUNY at Binghamton, Binghamton, NY 13902.

In the study of the neural code for gustation, it has been argued that there are 4 basic taste qualities and that these qualities are encoded by separate subsets of units. If this were true, then it can be predicted that, although taste-responsive neural elements are generally broadly tuned across taste qualities, at concentrations that are just identifiable, i.e. at threshold, there should be 4 separate groups of units that each respond to only one quality. To test this prediction, electrophysiological responses to prototypes of the 4 basic qualities were recorded in the parabrachial nucleus of the pons (PbN). Single units were tested initially with gustatory stimuli presented at concentrations that evoked generally robust responses. Next, progressively lower concentrations of each stimulus were presented until no response was apparent. Test stimuli were sapid solutions of NaCl (.1 M, .01 M, .001 M, .0001 M), HCl (.01 M, .005 M, .001 M, .0001 M), quinine-HCl (.01 M, .005 M, .001 M, .0001 M) and sucrose (.5 M, .1 M, .01 M, .001 M, .0001 M). Threshold concentrations of all stimuli were considered to be .001 M based on previous reports of the threshold of the chorda tympani whole-nerve response. At present, taste responses from 19 PbN units have been recorded. At the highest concentration of each stimulus tested, 9 units (47%) responded to all 4 taste stimuli and 9 units (47%) responded to 3 of the 4 taste stimuli. Only one unit (5%) was stimulus-specific. At threshold concentrations, 12 units (63%) responded to at least one tastant; 8 of these 12 units (67%) responded to at least 2 of the 4 prototypical taste stimuli. Because it appears that the majority of taste-responsive units are multisensitive at threshold concentrations of taste stimuli, it is possible to suggest that the neural code for gustation in the PbN may rely on the across unit pattern of responses to encode quality, rather than on the responses from a subset of taste-responsive elements.

This work was supported by a grant from the Whitehall Foundation to P.D.

252.2

WHOLE CELL PATCH AND OPTICAL RECORDINGS OF SYNAPTIC RESPONSES IN SALAMANDER OLFACTORY BULB. D.P. Wellis and J.S. Kauer Neurosci. Program, Tufts-New England Med. Center, Boston, MA 02111.

To further understand the synaptic mechanisms underlying odor coding in the olfactory bulb (OB), we have applied patch clamp and pharmacologic techniques to an *in vitro* tiger salamander hemibrain preparation. Here we describe both spontaneous and olfactory nerve-driven synaptic currents in a population of 37 OB neurons, many identified as mitral/tufted cells with biocytin filling. Input impedances of OB output cells averaged about 700MΩ and resting potentials averaged -60 mV. Under symmetric Cl^- conditions and V_h=-70 mV, spontaneous inward currents ranged in size from <10 pA to >100 pA, peaked in a few ms, and decayed over periods up to 100 ms. These currents were evoked at a greater frequency for up to 3 s following olfactory nerve stimulation. The sensitivities of these currents to changes in external and internal Cl^-, to bicuculline and picrotoxin, and to transmembrane voltage suggest that this activity is associated with activation of $GABA_a$ receptors. These results thus suggest that output neurons are under a low level of tonic inhibition which is further increased by nerve stimulation. The response to olfactory nerve stimulation also included an early bicuculline-insensitive inward current. Latencies to the onset of this component (23 ms) and to the onset of the bicuculline-sensitive component (70 ms) were similar to the onsets of depolarization and hyperpolarization, respectively, in OB neurons *in vivo* (Hamilton and Kauer 1988). We are further investigating the pharmacologic nature of the early current, which underlies the direct depolarization by olfactory receptor axons. We have also begun to obtain simultaneous optical recordings from the same preparation. Preliminary results show that changes in RH414 (kindly provided by A. Grinvald) fluorescence correlate well with the single cell physiology and that the dye does not appear to have a detrimental effect on neuronal function.

Supported by grants from the NIH, the ONR, the Pew Charitable Trust, and by the Department of Neurosurgery, NEMC.

252.3

MULTIPLE SINGLE UNIT RECORDING FROM OLFACTORY BULB OF AWAKE BEHAVING RATS. U.S.Bhalla and J.M.Bower. Division of Biology, Caltech, Pasadena, CA 91125

The coding of information in the olfactory system has become a major focus of our laboratory's efforts. Here we describe the results of experiments in which activity from multiple single olfactory bulbar neurons has been recorded simultaneously in behaving rats using chronic recording techniques. This data is crucial to our continuing efforts to develop realistic network models of the system.

The current experiments are based on a microdrive we have developed that weighs less than 6 gm and allows 8 stereo electrodes to be independently positioned. This device is also used to monitor respiration and EEG rhythms. Implanted rats have been exposed to odors in both passive and behavioral contexts while activity from multiple bulbar neurons, presumably mitral cells, is recorded. In the passive situation the implanted rat is placed in an air stream with odorants added at defined times. The behavioral paradigm requires the rat to identify an odor and select one of two paddles for a water reward.

Using these procedures we have investigated temporal and spatial patterns in bulbar responses, and their relation to the behavioral context. We have found that most neurons do not show a simple change in firing rate in response to the presence of odors, although many neurons show phasic responses correlated to the respiratory and EEG cycles. In some cells we have detected a modulation of these phase relationships in the presence of specific odors.

Supported by ONR contract N00014-88-K-0513.

252.4

TASTE RESPONSES IN THE NUCLEUS OF THE SOLITARY TRACT OF AWAKE RATS: AN EXTENDED STIMULUS ARRAY K. Nakamura and R. Norgren Dept. of Behavioral Science, Col. of Medicine, Pennsylvania State University, Hershey, PA 17033.

Forty taste neurons were isolated in the nucleus of the solitary tract (NST) and tested with 15 sapid chemicals delivered via an intraoral cannula. Each trial consisted of an infusion of 50 µl of water, followed by a similar amount of sapid solution, and then a water rinse. Although the range was large, on average, NST neurons responded well to NaCl, sucrose, monosodium L-glutamate (MSG), $NaNO_3$, and glycine ($\bar{X}$ = 8.9-12.0 spikes/sec). Mean responses to KCl, NH_4Cl, HCl, malic acid, and quinine HCl (QHCl) were low ($\bar{X}$ = 0.4-2.3). The average responses to the other stimuli (citric acid, $MgCl_2$, fructose, maltose, and polycose) fell between these extremes ($\bar{X}$ = 4.3-5.6). Based on the largest response to the 4 standard stimuli, the neurons were classified as follows: 11 NaCl-best, 15 sucrose-best, 12 citric acid-best, and 2 QHCl-best. Sodium-best neurons responded robustly and nearly equally to the 3 sodium salts ($\bar{X}$ = 19.5-24.9) and much less to non-sodium, Cl salts ($\bar{X}$ = 0.5-4.7). Sucrose-best neurons also responded strongly to glycine and MSG ($\bar{X}$ = 18.2 & 14.4, respectively), but only moderately to other, normally preferred chemicals (fructose, maltose, and polycose; $\bar{X}$ = 8.7, 9.5, & 9.6). Citric acid-best neurons responded moderately to citric and malic acid ($\bar{X}$ = 9.4 & 5.1), but only weakly to HCl ($\bar{X}$ = 1.5). The 2 QHCl-best neurons responded moderately to QHCl and $MgCl_2$ ($\bar{X}$ = 12.0 & 9.5), but weakly or not at all to the other stimuli. Supported by PHS grants DC 00240, MH 43787, and MH 00653.

252.5

THE INFLUENCE OF SUBSTANCE P ON CELLS IN THE GUSTATORY PORTION OF THE HAMSTER SOLITARY NUCLEUS: AN *IN VITRO* STUDY. H. Liu*, M. M. Behbehani and D. V. Smith. Univ. Cincinnati Coll. Med., Cincinnati, OH 45267.

Gustatory afferent fibers from the VIIth and IXth nerves project into the rostral portion of the nucleus of the solitary tract (NST), ending primarily within the rostral central (RC) and rostral lateral (RL) subdivisions. Fibers containing substance P (SP) are closely associated with taste buds in the periphery and project centrally into all subdivisions of the NST. We recorded extracellularly from cells in the rostral NST from an *in vitro* slice preparation of the hamster medulla. Sixty neurons were isolated within the NST at the level of the dorsal cochlear nucleus, their activity recorded with 6-8 MΩ micropipettes, and their locations marked with pontamine. Of these 60 neurons, 41 were located within the gustatory projection zone (RL and RC) and the remaining 19 were within the medial or ventral subdivisions. Cells were classified according to characteristics of their spontaneous discharge: 1) 70% slow firing (0.03 - 5 Hz), 2) 20% fast (6 - 40 Hz), 3) 3% bursting, or 4) 7% irregular. Of the 41 cells within the RL and RC, 27 were slow firing, 9 fast, 2 bursting and 3 irregular. SP excited 20 of these cells, inhibited 9, and had no effect on 12 cells. Of the 19 cells within the medial and ventral subdivisions, areas that do not receive gustatory afferent input, 15 were slow, 3 were fast, and 1 was irregular. Within these subdivisions, SP excited 17 of the 19 cells and had no effect on 2. Thirty of the excited cells were tested with SP + the SP antagonist, [D-Pro^2,D-Trp^{7,9}]-Substance P (DPDT); the response to SP was effectively blocked in 27 cells. Seven of the inhibited cells were similarly tested with DPDT; responses were blocked in 6 cells. These results suggest that SP, which is associated peripherally with taste receptors, may play some role in the central processing of gustatory information.

Supported in part by NIDCD Grant DC-00353 to D.V.S.

252.6

CO-LOCALIZATION OF SUBSTANCE P, TYROSINE HYDROXYLASE AND GABA IMMUNOREACTIVITIES IN THE HAMSTER OLFACTORY BULBS. T.Jang, R.M.Kream*, and F.Macrides. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, Tufts University Schools of Medicine, Boston, MA 02111.

Postnatal development of substance P (SP), tyrosine hydroxylase (TH) and GABA expression were studied with single and double immunofluorescence techniques. TH- and GABA-positive neurons were present in the main olfactory bulb (MOB) on the 1st postnatal day. Their dendrites arborized in the glomerular layer (GL). SP-positive perikarya were not seen in the olfactory bulbs until the 2nd week; however, prominent SP-positive centrifugal fibers entered the granule cell layer (GRL) of both the MOB and accessory bulb (AOB) in 1-day old pups. By the 2nd week, the distributions on SP-, TH-, and GABA-positive perikarya were like those in adult hamsters.

Neurons co-expressing GABA and TH immunoreactivites were evident in the GL on the 1st posnatal day. Their somal sizes and location indicated that they were peri-glomerular (PG) cells. During the 2nd week, virtually all the SP-positive neurons in the MOB were also TH-positive, and had the morphological features of external tufted cells. The proportion of such double-labeled cells then declined with age. Some SP and GABA double-labeled cells were seen in the GL by the second week, but these were relatively rare at all ages. These observations suggest multi-subclasses of PG and external tufted cells.

252.7

IMMUNOCYTOCHEMICAL EVIDENCE FOR DOPAMINERGIC CELLS IN THE SALAMANDER OLFACTORY BULB. K.A. Hamilton and S.S. Foster. Department of Cellular Biology and Anatomy, LSU Medical Center, Shreveport, LA 71130.

In the salamander olfactory bulb, cells located in the granule cell and external plexiform layers exhibit intense tyrosine hydroxylase immunoreactivity (K.A. Hamilton, Neurosci. Abstr., 16: 128, 1990). The cell bodies give rise to smooth dendrites that branch in the external plexiform and glomerular layers, producing varicose processes. Although cells exhibiting both tyrosine hydroxylase and dopamine-like immunoreactivity exist elsewhere in the salamander brain, olfactory bulb cells that are tyrosine-hydroxylase positive do not stain intensely with dopamine antiserum.

To enhance dopamine-like immunoreactivity in the salamander olfactory bulb, in the present study land phase animals were injected with monoamine oxidase inhibitor either alone or prior to injecting methyl L-DOPA (Wallace *et al.*, Neurosci. Abstr., 16:646, 1990). Intense staining was then observed in a large number of granule-layer cells and a small number of external plexiform-layer cells. Both smooth dendrites and varicose processes were stained in the external plexiform and glomerular layers. Staining was also observed within the glomeruli. The cells resembled granule cells and tufted cells described in previous studies.

The results suggest that a heterogeneous population of dopaminergic cells may exist in the salamander olfactory bulb. The cell bodies occur in the granule cell and external plexiform layers, but not in the glomerular layer. The presence of dopamine-like immunoreactivity within the glomeruli, however, suggests that, regardless of soma location, the putative dopaminergic cells might modulate afferent input to the olfactory bulb at an early synaptic level.

Supported by NIH Grant DC00300.

252.8

SPATIAL CODING AND CELLULAR TUNING OF FATTY ACID ODORS IN RABBIT OLFACTORY BULB K.Mori, N.Mataga*& K.Imamura. Dept. of Neuroscience, Osaka Bioscience Institute, Furuedai, Suita, Osaka 565 Japan.

To examine spatial representation of odors in the brain, odor-evoked oscillatory(40Hz)local field potential(OLFP) was recorded in the external plexiform layer of extensive regions of the main olfactory bulb in urethane anesthetized rabbits. Stimulation of olfactory epithelium with normal(n)-fatty acids elicited large OLFPs within a region at the rostro-dorso-medial part of the bulb.

Single unit recordings from bulbar neurons revealed that in the region of the bulb, about 80% of mitral cells showed excitatory spike responses to fatty acids. Each mitral cell showed tuning to one to four n-fatty acids with hydrocarbon chain of similar length. In addition to n-fatty acids, a group of mitral cells responded to iso-fatty acids. These results indicate that mitral cells responsive to fatty acid odors are grouped together in the region at the rostro-dorso-medial part of the bulb. Above results also suggests that chemical structures of odorants are spatially coded in the main olfactory bulb and that each mitral cell in the bulb is tuned to specific structural features of odorants.

252.9

OMP mRNA IS TRANSPORTED TO THE AXON TERMINALS OF OLFACTORY RECEPTOR CELLS. C.H. Wensley*, J.S. Kauer, F.L. Margolis[1] and D.M. Chikaraishi (SPON: D.M. Chikaraishi) Neuroscience Program, Tufts Univ. School of Med., Boston, MA 02111 and [1]Roche Institute of Mol. Bio., Nutley, NJ 07110

Until recently, neuronal mRNA has been thought to be restricted to the cell body and dendritic portions of the cell. By *in situ* hybridization and RNAase protection, we have studied the distribution of olfactory marker protein (OMP) mRNA in the receptor cells of the rat olfactory epithelium. We found that this mRNA is located not only in the perikaryon but also in the axons as they approach and synapse in the olfactory bulb. To verify that this signal was in the receptor cell axons, we performed unilateral axotomies on the olfactory nerve of one month old rats. The axotomized animals were sacrificed 10-14 days after surgery. *In situ* hybridization on this tissue showed that the OMP mRNA disappeared from the axotomized side while remaining intact on the control side. Similiar results were obtained using mice which had undergone intranasal irrigation with 0.17M $ZnSO_4$ to destroy inputs to the olfactory bulb. Tissue from these animals sacrificed 7 days after lavage revealed no OMP message in either the perinuclear region in the olfactory epithelium or the terminal axon field in the olfactory bulb.

252.10

DIFFERENTIAL DISTRIBUTION OF SYNAPSIN ISOFORMS IN THE MAIN OLFACTORY BULB OF THE RAT. M.R.Dino, E. Mugnaini, A. Czernick[1], and P. Greengard[1] Neuromorphology Lab, U-154, Univ. of Conn., Storrs, CT. 06269 and [1]Lab. of Molecular and Cellular Neuroscience, Rockefeller University, 1230 York Ave. Box 296, New York, N.Y. 10021

Synapsins, of which four isoforms have been uncovered, are a class of molecules involved in the mechanism of neurotransmitter exocytosis.By immunocytochemistry with antisera specific for the four isoforms, we show that synapsins are unequally distributed in the main olfactory bulb of albino rats.Synapsin-like immunoreactivity is mostly situated in punctate structures interpreted as axonal or dendritic presynaptic profiles. Immunoreactivites to synapsins Ia and Ib are present throughout the bulb and are particularly enriched in the external plexiform layer (EPL); in the granular layer (GRL) immunoreactivity for synapsin Ia is relatively lower than that for synapsin Ib. Immunoreactivity to synapsin IIa is strikingly enriched in the glomeruli; in the remaining layers, its distribution resembles that of synapsin Ia and Ib. Synapsin IIb immunoreactivity is localized mostly in the GRL and in a band of immunoreactive puncta at the border between the glomerular layer (GL) and EPL, and it is nearly absent within the glomeruli; it is found only in scattered puncta in the interglomerular neuropil and in the EPL.Severance of the lateral olfactory tract leads to conspicuous reduction of synapsins Ia, Ib, and IIa in the GRL, but produces little change of the respective immunoreactivities in the GL and EPL. This indicates that these isoforms are situated in presynaptic elements belonging to centrifugal afferents as well as to olfactory nerve terminals and intrinsic profiles. The same lesion removes some immunoreactivity to synapsin IIb, indicating that this isoform is localized mostly in centrifugal afferent terminals and in some short axon cells.

252.11

VALPROATE DISTRIBUTION IN THE MOUSE BRAIN AFTER INTRANASAL ZINC SULPHATE. Thomas J. Hoeppner. Department of Neurological Sciences, Rush Medical College, Chicago, IL 60612

Intravenously injected radiolabeled valproate (VPA) concentrates in the olfactory bulb of mice and rats with highest levels in the medial and dorsal glomerular layer as seen with contact autoradiography. The glomerular layer contains processes of four types of neurons: olfactory nerve terminals, periglomerular cells, mitral and tufted cell dendrites. As a preliminary step to determine if the VPA is contained in a single neuronal population the olfactory epithelium and consequently the olfactory nerve were destroyed by intranasal application of zinc sulphate in mice. Subsequent behavioral testing showed the mice to be anosmic. On intravenous injection of a tracer dose of carbon-14 labeled VPA 4-10 days after the zinc sulphate, autoradiography showed the same distribution and concentration of VPA in zinc sulphate treated mice and control mice that did not receive zinc sulphate. These preliminary findings suggest that VPA does not concentrate in olfactory nerve terminals.

INVERTEBRATE SENSORY SYSTEMS

253.1

WITHDRAWN

253.2

MORPHOLOGICAL PROPERTIES OF THE SENSORY NEURON IN THE COCKROACH TACTILE SPINE. A.S. French, A.R. Klimaszewski* and L.L. Stockbridge*. Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

The cockroach femoral tactile spine contains a single mechanosensory neuron. The neuronal soma is within the spine lumen, and a sensory dendrite passes through the spine wall. Intracellular recordings from the neuron produce a wide range of action potential amplitudes and thresholds, suggesting that the soma and dendrite are electrically passive and the site of action potential initiation is variably distant from the soma.

Tactile spine sensory neuron morphology was reconstructed using 1 μm thick serial sections. Spines were fixed, dehydrated, and embedded before sectioning. Stained sections were photographed and then the outlines of the spine cuticle, the sensory neuron, and the spine trachea were digitized directly from enlarged negatives with a digital graphics tablet. Sections were aligned, reconstructed, and displayed using custom software. The results indicate that the shape, size and position of the dendrite and soma are significantly variable, probably accounting for the electrical observations.

Supported by the Medical Research Council of Canada.

253.3

AN EXTENDED MAP OF SENSE ORGANS OF THE COCKROACH LEG OBTAINED BY APPLYING DiI TO FIXED TISSUES. D. L. MacFarland*, S. F. Frazier*, S.E. Fish and S. N. Zill. Dept. Anat., Marshall Univ. Sch. Med., Huntington, WV 25755.

As a prelude to studies evaluating the functions of proprioceptive sense organs in cockroach walking, running and escape turning, we have mapped the locations of receptors in the legs using the carbocyanine dye, DiI. Animals were anesthetized, decapitated and placed in formalin fixative for 1-2 days. DiI crystals were then positioned at the cut ends of nerve 5 (the main leg nerve) or its branches or nerve 3B. Cockroaches were incubated at 37°C for 10 days to 2 weeks, then returned to room temperature for 1-6 months. Legs were bisected, placed in a clearing solution (Conray) and viewed as whole mounts or sections under both epifluorescent and transmitted light illumination. This technique allows for both the visualization of sensory neuron somata and the cuticular structures which they innervate. We have confirmed the presence of many of the identifiable groups of sense organs previously described including chordotonal organs, campaniform sensilla and hair plates. In addition, we have characterized a number of receptors of the tibia and tarsus that have not previously been mapped. These include new groups of campaniform sensilla and numerous multiply innervated chemoreceptors. In addition, we have also found large sensory neurons in the tarsal segments which resemble "pore" receptors previously described in locusts. These morphological studies will form the basis for future physiological experiments to evaluate the roles of these afferents in behavior.

Supported by a grant from the Whitehall Foundation.

253.4

PROPRIOCEPTIVE INPUTS AND INHERENT MUSCLE TENSIONS CONTRIBUTE TO LOAD COMPENSATORY REACTIONS IN LOCUSTS. S. N. Zill and S. F. Frazier*. Dept. Anat., Marshall Univ. Sch. Med., Huntington, WV 25755.

Load compensatory reactions adapt postures to changes in the environment. We have previously shown that locusts show such reactions when they are placed in a chamber which is repeatedly swayed. Discrete, phase linked bursts of motor activities occur in flexor and levator muscles of the legs but not in the antagonist extensors and depressors when animals stand upon the side of the chamber. In order to evaluate the contributions of proprioceptive inputs to these reactions we have now 1) recorded the changes in joint angle that occur during these responses (by videotaping locusts that had miniature light emitting diodes attached to the leg) and 2) stressed the system (by attaching weights to animals and varying the velocity of swaying). These studies have shown that 1) phase linked changes in angle of the femoro-tibial joint occur during compensatory reactions (increasing during the phase in which the substrate is pulled away from the animal); 2) the onset of muscle bursting is significantly correlated with a decrease in the rate of change of joint angle; and 3) stressing the system by increasing weight of the animal induces reciprocal bursting in muscles that are normally silent. We conclude that both proprioceptive inputs monitoring joint angles and modulators of inherent muscle tensions can contribute to load compensatory reactions in these animals.

Supported by a grant from the Whitehall Foundation.

253.5

SPIKING LOCAL INTERNEURONES: CORRELATION BETWEEN INTERNEURONE MORPHOLOGY AND PHYSIOLOGY AND TACTILE AFFERENT PROJECTIONS IN THE LOCUST. P.L. Newland and M. Burrows, Dept. of Zool., Univ. of Cambridge, Cambridge, England.

In the locust, spiking local interneurones that process mechanosensory information have distinct patterns of central arborizations, and receptive fields consisting of groups of receptors on the leg. We have analysed the relationship between the morphology and the receptive fields of these interneurones by relating them to the central projections of afferents from tactile hairs on a hind leg.

Each hair is innervated by a single sensory neurone that projects to the ipsilateral half of the metathoracic ganglion. The central projections of these afferents form a map of a hind leg in the central nervous system such that the spatial position of hairs on a leg is represented in 3 dimensions in the ganglion.

Interneurones that process mechanosensory information from hairs on the leg have receptive fields formed by direct input from the tactile afferents. There is a strong correlation between the branching patterns of spiking local interneurones, their receptive fields, and the projection patterns of the sensory afferents from which they receive inputs. Interneurones with extensive ventral branching covering most of the afferent map have large receptive fields on the leg. Interneurones with small receptive fields, however, have branching patterns overlapping with small and specific areas of the afferent map.

Supported by NIH grant NS16058 to M. Burrows

253.6

A STUDY OF THE STRUCTURE AND FUNCTION OF TRANSPLANTED CAMPANIFORM SENSILLA IN THE CRICKET REVEALS TWO DISTINCT TYPES OF RECEPTOR. K.A. Killian, D.J. Merritt* and R.K. Murphey. Neuroscience and Behavior Program, Dept. Zoology, University of Massachusetts, Amherst, MA 01003.

Insect campaniform sensilla can be segregated into two classes based on their site of projection within the CNS. Campaniform sensilla (CS) associated with the filiform hair receptors on the abdominal cerci of the cricket Acheta domesticus project to a ventral region of neuropil within the terminal abdominal ganglion (Heusslein and Gnatzy, 1987), while CS located on the thoracic legs project to an intermediate neuropil within each thoracic ganglion. We wondered whether this difference in projection sites was due to a property of the sensory neuron or a regional characteristic of the ganglion under study. We show that CS on cerci transplanted to the position of the metathoracic leg regenerate into the CNS and arborize in a ventral area of neuropil within the host ganglion in contrast to the intermediate arborizations of leg CS. We were interested in determining whether these ectopic afferents could form functional connections in their new location. Interneuron 7-1b, which is excited by both the activation of CS on the cerci and the tactile stimulation of the contralateral thoracic legs, seemed a likely candidate to receive ectopic input. 7-1b's soma is located in the terminal abdominal ganglion and its axon ascends towards the brain to arborize profusely in the ventral neuropil of each of the abdominal and thoracic ganglia. We found that activation of ectopic CS produced a barrage of spikes recorded from the soma of this interneuron comparable to that evoked by tactile stimulation of a normal cercus. This result suggests that transplanted CS can recognize both a local region of neuropil within each ganglion of the CNS and synapse with a normal target within this region. Supported by NRSA 1 F32 NS08847-01 (KAK) and NSF BNS90-96180 (RKM).

253.7

REPRESENTATION OF WIND STIMULUS INFORMATION BY THE RECEPTOR ARRAY OF THE CRICKET CERCAL SENSORY SYSTEM. Michael A. Landolfa and Gwen A. Jacobs. Graduate Group in Neurobiology. University of California, Berkeley, CA 94720.

A combination of morphological and electrophysiological studies were performed to assess the representation of stimulus information by the filiform hair mechanoreceptors of the cricket (*Acheta domestica*) cercal sensory system. Individual receptors give characteristic responses which depend on the direction and velocity of the stimulus. The receptor response adapts to continued stimulation, and the degree of adaptation depends on the stimulus velocity and frequency.

The filiform receptor synaptic terminals form a topographic map of wind stimulus direction within the cercal glomerulus, where they make excitatory synapses onto specific identified interneurons (INs). We have made a 3-D morphological reconstruction of this map from stained afferents representing all receptor directions on the cercus. The directionally selective identified INs which receive input from the receptor afferents were also stained and reconstructed, and the morphological overlaps between the INs and each type of afferent were calculated. We then predicted the directional receptive fields of several different INs by scaling and summing the directional tuning curves of the afferent types which showed anatomical overlap with the dendritic arbors of the INs. The afferent tuning curves were scaled according to: 1) their relative amounts of overlap with the IN; 2) the electrotonic distance between the IN's dendritic arbors and its spike initiating zone (SIZ); and 3) the relative numbers and distributions of presynaptic receptor types on the cercus. These predicted receptive fields were then compared to the physiologically measured directional sensitivity tuning curves of the INs.

253.8

INFORMATION THEORETIC CALCULATION OF DIRECTIONAL ACCURACY IN THE CRICKET CERCAL SENSORY SYSTEM. ENCODING BY SPIKE PATTERNS AND COVARIANCE. F.E. Theunissen*, S. N. Gozani* and J.P. Miller. Dept. of Mol. and Cell Biology. University of California, Berkeley. CA 94720.

The activity patterns of primary sensory interneurons in the cricket cercal sensory system encode information about the direction and velocity of air current stimuli in the animal's immediate environment. In previous work the statistical principles of information theory were used to calculate the maximum directional accuracy attainable from the response ensemble of four low velocity interneurons from this system. We found that 1) the system was capable of encoding wind stimulus direction with a high level of resolution, and 2) the width of the directional tuning curves of the cells were optimal in the sense of maximizing the information theoretic measure of directional accuracy.

In this work, the same principles were used to extend the calculation by considering 1) the encoding by the higher order statistics of the spike train patterns and 2) the effect of the covariance in the response. We found that the higher order statistics of the spike trains could enhance the directional resolution to a negligible extent for the low velocity cells of our previous study but to a significant extent for other cells of the system with higher velocity thresholds. Including the covariance in the calculations further increased the directional accuracy. The covariance was particularly large when the wind stimulus velocity was allowed to vary. The high positive covariance found in those cases did not deteriorate the directional accuracy. However, effective encoding in the high positive covariance situations only occurred if the response curves of adjoining cells had opposite slopes. This was the case in the actual array of cells, and represents another way in which the width and separation of the response curves can be considered as being optimized with respect to the task of discriminating stimulus direction.

253.9

BEHAVIORAL MEASUREMENT OF DIRECTIONAL RESOLUTION IN THE CRICKET CERCAL ESCAPE SYSTEM. R. M. Olberg and J. P. Miller. Dept. of Molecular and Cell Biology, Univ. of Calif., Berkeley, CA 94720.

In response to a rapidly accelerating air current, crickets turn and run or jump away from the source of disturbance. This behavior is mediated by the cercal escape system, consisting of wind sensitive filiform hairs on the cerci and ascending interneurons which receive input from the filiform receptors and project to the thorax. In order to assess the minimum directional resolution of this system we videotaped escape responses of adult crickets (*Acheta domesticus*). We analyzed the escape tracks by digitizing the positions of the head and posterior tip of the abdomen in each of 4 frames (133 msec) after stimulus onset.

To isolate the cercal contribution to the escape behavior, we removed the antennae and painted the eyes. We removed the metathoracic (jumping) legs. Two stimuli were used: 1) a rectangular syrofoam block moving downward, stopping just short of the substrate, 2 cm from the cricket or (2) the raised edge of rectangular plexiglass flap, released from an angle of 40° to the substrate. Both stimuli were more effective than air puffs at eliciting escape.

We plotted direction of the change in head position in freely moving animals from time 0 to 100 msec. The mean vector of head movement thus obtained was directly away from the stimulus with a standard deviation of 16° for the best individuals. A second method of measurement, based on the leg movements of tethered animals whose legs were in contact with a slippery surface, gave similar results. The initial response (latency ca. 30 msec) was a thrust by the prothoracic leg on the stimulus side of the animal.

253.10

CEREBRAL AND TRANSCEREBRAL PROJECTIONS OF OPTIC TRACT FIBERS IN THE CRICKET. D. Moore. Dept. of Biological Sciences, E. Tenn. State Univ., Johnson City, TN 37614

Neuronal projections from the optic lobes in the cricket Teleogryllus commodus were mapped from whole-mount preparations using silver-intensified nickel fills of optic tract axons cut between the lobula and medulla.

At least five separate tracts project to the contralateral lobula, where the terminal branches form distinct lateral and medial dendritic fields. The largest transcerebral tract, however, completely bypasses the lobula and ends in the medulla; its dense terminal branchings form two separate vertical strata. Associated with this tract are cell bodies in the pars intercerebralis and contralateral medulla, as well as extensive arbors descending from its caudal protocerebral traverse into the deuto- and tritocerebrum of both hemispheres.

Cerebral projections include fibers in all three ocellar tracts, an exceptionally dense ipsilateral tract terminating superficially in the frontal protocerebrum, a tract circumventing the pedunculus of the ipsilateral mushroom body & terminating with extensive branches in the caudal protocerebrum, and tracts leading to somata in the frontal deutocerebrum on both sides of the brain.

The results of this study confirm and extend the findings of Honegger & Schürmann (Cell Tiss. Res. 159: 213, 1975) on the cricket Gryllus campestris.

253.11

ASYMMETRIC INHIBITORY INTERACTIONS IN THE ANTENNAL LOBES OF HONEYBEES REVEALED BY REAL-TIME OPTICAL IMAGING. E.E. Lieke. Inst. f. Neurobiol., Königin-Luise-Str. 28/30, D-1000 Berlin 33, FRG.

The antennal lobes are known as the primary olfactory neuropiles of honeybees. They consist of sensory input neurons, output neurons, and several classes of local interneurons some of which are known to be inhibitory. Previous experiments demonstrated the feasibility of real-time optical recording in this neuropile using intrinsic signals. Although the nature of the intrinsic signal is not fully understood it is clearly related to neuronal activity.

Using a 4×4 photodiode array, intrinsic optical signals were recorded from a semi-slice preparation of the bee brain. The antennal nerve of the preparation was stimulated by a train of electrical pulses (100 μAmp, 100Hz). The sizes of the optical signals were different at different locations of the antennal lobes, having largest amplitudes in anterior areas. The slope of the optical signals began with a steep phase followed by a shallow one. After offset of the stimulus the signals returned to their baselines. After treatment with picrotoxine (5 min, 10^{-5} molar) the amplitudes increased in many regions and the time courses became more continuous. However, the increase was not homogeneous - its degree depending on the spatial position of the recorded area. The increase in signal amplitude is consistent with the assumption that GABAergic chloride channels were blocked by picrotoxine. The differences between the optical signals before and after this treatment can therefore be interpreted as the timecourses of the inhibition. The inhibition starts with a 10 msec delay after response onset, and has at least two phases.

Surprisingly, the spatial distribution of this inhibition is extremely inhomogenous. Areas of large inhibition are in the proximity of areas with less inhibition. Thus inhibitory interactions in the antennal lobes are asymmetric with areas that deliver more inhibition than others and areas that receive more inhibition. Such aspect of general network wiring in this neuropile is completely new and should be taken into account in further investigations.

253.12

MANDUCA OPSIN mRNA IN RELATION TO RETINOIDS AND LIGHT/DARK CYCLE. R. H. White, J. Bard*, and R. R. Bennett, University of Mass/Boston, Boston, Massachusetts.

Opsin synthesis in insects is curtailed by vitamin A deprivation and stimulated when the chromophore is provided. J. Schwemer (Julich conference abst., 1990) has found that opsin message is low in the vitamin A deprived blowfly (Calliphora), and increases in response to 11-cis retinal. We have done similar experiments with the tobacco hornworm moth Manduca sexta. Northern blots of moth eye extracts using Drosophila ninaE (opsin) cDNA (W. Pak, Purdue) as probe revealed a single labelled band. The similarity in molecular weights (the labelled band from moth retinas at 1.8 kb, the ninaE message at 1.7 kb) suggests that the probe is binding to the mRNA for one of the moth opsins. Slot blot analysis showed more of this labelled mRNA in the dark phase of the diel cycle than in the light phase, a modulation similar to that found in mouse mRNA (Bowes et al., Exp Eye Res 47:369, 1988). Retinoid deprived photoreceptors, despite low opsin levels, contained similar or larger amounts of the labelled mRNA than did normal cells. Opsin levels and visual function recover slowly when deprived moth photoreceptors are provided with 11-cis retinal. Surprisingly, the labelled mRNA appeared to decrease in these recovering photoreceptors. Thus levels of message appear to be affected by retinoids in both the fly and moth, but in an opposite sense. Supported by NSF grant BNS 8510087.

253.13

RESPONSES OF AN EYE WITHDRAWAL MOTOR NEURON IN THE CRAB. C. E. Diebel*, R. R. Forman, C. I. Abramson*, A. I. Yuan* and R. D. Feinman. SUNY Health Science Center, Brooklyn, NY 11203.

Pavlovian conditioning of eye withdrawal in the crab consists of pairing a puff of air to the eye (which elicits retraction) with an initially ineffective stimulus, vibration of the carapace. After repeated pairings, the withdrawal response is elicited by the vibration. A key question in conditioning is whether the learned response is a new behavior, specifically related to vibration, or whether it is the same motor act caused by air-puff but now brought under the control of the new stimulus. As a first step in answering this question, we studied two nerve tracts, the optic and oculomotor nerves, which contain the axons of the motor neurons controlling eye movement as well as some sensory fibers which mediate this behavior. We performed extracellular recordings from these nerves in a whole animal preparation and measured the responses to air-puff and to vibration of the carapace. Motor output was observed in the optic nerve as rapid, short latency volleys (typically 400 Hz) during 0.5 s air-puffs to the eye. This response (and the withdrawal behavior) was eliminated by section of the oculomotor nerve, suggesting that the afferent fibers mediating air-puff run in this nerve. Consistent with this, activity that correlated with the air-puff stimulus appeared in the oculomotor tract and persisted in the peripheral end of the cut nerve. Motor response to vibration was different from that caused by air-puff and was not affected by section of the oculomotor nerve. The results indicate that pattern of motor activity underlying eye withdrawal depends on the stimulus and suggest the possibility that conditioned responses may be different from unconditioned responses. (Supported by grant BNS-8819830 from NSF and training grant NS 07117 from NIH).

253.14

RECEPTIVE FIELD ORGANIZATION OF CRAYFISH STATOCYST INTERNEURONS. H.Nakagawa* and M.Hisada Zool. Inst., Fac. Sci., Hokkaido Univ. Sapporo 060 Japan

In crayfish, Procambarus clarkii, many statocyst-related interneurons have been identified or characterized in our previous reports. We, however, have known only their responses to simulated body roll. In order to understand how they produce an adaptive equilibrium response, including compensatory eye movement, it is essential to know how their receptive fields are organized. To clarify this point, a group of sensory hairs in different regions of a statocyst was stimulated artificially while activity of an interneuron was recorded using glass microelectrode filled with Lucifer Yellow.

We found two different types of receptive field. Three local spiking interneurons and an identified descending interneuron, C_1, showed either excitatory or inhibitory response to the stimulation of the lateral hairs. On the other hand, two local spiking interneurons showed inhibitory and excitatory response to the anterior and posterior hairs respectively. These observations suggest that the former control the equilibrium responses to roll and the latter control responses to pitch.

253.15

FINE STRUCTURE OF OLFACTORY-GLOBULAR TRACT AXON TERMINALS IN THE CRAYFISH BRAIN. DeF. Mellon, V. Alones*, and M. D. Lawrence*. Department of Biology, University of Virginia, Charlottesville, VA 22903

Globuli cells are interneurons of the arthropod olfactory midbrain, constituting the major projection pathway between the deutocerebrum and the protocerebrum. In decapod crustaceans, globuli cells are relatively numerous; for example, there are at least 10^5 globuli cells on each side of the olfactory midbrain in the crayfish, Procambarus. Globuli have small, uniform somata 7-8 μM in diameter, extensive dendritic arbors in the olfactory and accessory lobes, and their bilaterally-branched axons run out the 'optic nerves' in well-defined olfactory-globular tracts (OGT). OGT axons terminate in the hemi-ellipsoid body (HE). We have used a rapid Golgi technique to study OGT axons and their HE terminals. Light microscopy shows each terminal to be a knot-like structure, 5-7 μM across, consisting of an expanded axon ending that recurves upon and intertwines with itself. Each OGT axon branches several times within the HE, each branch terminating in a separate knot ending. We suggest that these terminals constitute the microglomeruli described in HE by other authors. In gold-toned Golgi sections examined with the electron microscope, the OGT terminals synapse with larger diameter fibers. The contacts occur between OGT terminals and small spines on the postsynaptic neurons. Serial reconstruction of microglomeruli indicates that closely adjacent pairs of spines comprise a frequent configuration targeted by OGT synapses. These data show each OGT axon terminating as several separate microglomeruli and each synaptic focus having many targets. Questions for future physiological analysis include the identity of cells in the HE postsynaptic to the OGT neurons, and the network properties involved with pattern recognition in the identification of complex odorants. Supported by The Whitehall Foundation.

253.16

MULTIPLE TARGETS FOR SEROTONIN, PROCTOLIN, & NEUROPEPTIDE F1 ACTING AS NEUROMODULATORS ON THE LOBSTER MUSCLE RECEPTOR ORGAN. V.M.Pasztor. Biology Department, McGill University, Montreal. Canada. H3A 1B1.

The muscle receptor organ (MRO) of the lobster is a complex proprioceptive system lying in parallel with the axial flexor musculature. Two peripherally located sensory neurons, one tonic and one phasic, extend stretch-sensitive dendrites into individual receptor muscle strands (RM1 & RM2 respectively) . Variable gain is provided by efferent motor innervation to the receptor muscles and efferent inhibitors synapsing upon the sensory dendrites.

A previous study of the isolated MRO in vitro (Pasztor & Macmillan,1990) has shown that proctolin and serotonin enhance the spiking responses to passive stretches. Using a combination of intracellular and extracellular recording electrodes and tension transducers, the current study shows that 5-HT, proctolin, neuropeptide F1, but not octopamine, are significant modulators acting at multiple loci on the MRO to enhance the gain of this important reflex system. In the presence of 10^{-6} mol l^{-1} 5-HT or proctolin: the receptor potentials and excitability of both sensory neurons, as well as the motor junctional potentials and nerve evoked contractions of the receptor muscles, were all enhanced. Neuropeptide F1 had powerful effects upon the receptor muscles but did not modulate the sensory neurons at all. Work on the inhibitory accessory fibers is now in progress.

253.17

TRANSMITTER LOCALIZATION IN THE BRAIN OF THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*. L.S Gammill* and B.S. Beltz. Biology Dept., Wellesley College, Wellesley, MA 02181.

The localization of transmitters in the brain of *Homarus* has never been examined in detail. In this immunocytochemical analysis of the lobster brain, the distributions of staining for serotonin, proctolin, red pigment concentrating hormone (RPCH), FMRFamide, and small cardioactive peptide B (SCPb) are compared. Labelling in synaptic areas such as the accessory lobes (ALs), olfactory lobes (OLs), protocerebral bridge (PB), and central body (CB), as well as in the deutocerebral giant neurons (DGNs), is emphasized.

Immunoreactivity for all five transmitter substances is observed in the OLs, PB, and CB, although the patterns of labelling are distinct. The ALs, which stain for every compound except proctolin, illustrate the diversity of labelling possible within a single region. Serotonin-like immunoreactivity is found in glomeruli throughout the ALs; FMRFamide and SCPb antibodies label only the cortex of the ALs; intense staining for RPCH is localized in glomeruli at the core of the lobe with sparse staining found in glomeruli elsewhere in the lobe. In contrast to this regional localization of transmitters in the ALs, all of the glomeruli in the OLs label with each of the antibodies tested. The intensity and qualitative appearance of the immunoreactivities, however, are distinctive. The DGNs, which innervate the ALs and the OLs, are immunoreactive only for serotonin.

Thus, each transmitter substance has a unique localization pattern throughout the brain. Comparing the distribution of these substances identifies areas of overlap, revealing sites of regional coexistence between two or more substances. Such coexistance suggests areas where transmitters may interact physiologically within a cell or region, providing a framework for future experimentation. (Supported by NSF-BNS-8718938, NSF-BNS-8958169, and NIH-NS-25915)

253.18

EMBRYONIC AND LARVAL DEVELOPMENT OF THE DEUTOCEREBRUM OF THE LOBSTER *HOMARUS AMERICANUS*. M.L. Ruchhoeft*, S.M. Helluy and B.S. Beltz. Bio. Dept., Wellesley College, Wellesley, MA 02181.

Embryonic and larval development of the brain of the American lobster, *Homarus americanus*, is documented histologically using thin plastic sections stained with toluidine blue. The primary focus is on the development and differentiation of the deutocerebrum, specifically the olfactory lobes (OLs) and the accessory lobes (ALs).

The OLs begin to develop much earlier than the ALs; the primordia of the OLs are already present at 25% embryonic development. The characteristic columnar glomeruli of the OLs also appear relatively early, by 43% development. The small ALs are first seen at 50% embryonic development, when they appear medial to the OLs. By the first larval stage, the OLs and the ALs are approximately equal in size. The characteristic spherical glomeruli of the ALs do not appear until the second larval stage. By the fourth larval stage a distinct cortex has developed in the ALs. From late embryonic through early juvenile development the ALs gradually shift position in the brain from medial to posterior to the OLs, and the two neuronal clusters of globuli cells that innervate the lobes shift from posterior and anterior to the lobes to lateral and medial locations. In juvenile lobster brains, the gross differentiation and rotation within the lateral deutocerebrum have been completed.

The ALs develop from their first appearance in the presence of direct serotonergic input from a pair of giant deutocerebral cells. In future studies we plan to investigate the potential role of serotonin as a morphogen via its depletion during development. Aspects of the protracted growth and differen-tiation of the ALs, such as their late appearance, post-hatching acquisition of glomeruli, and shift in position will be used as developmental landmarks to identify potential abnormalities in animals where serotonin has been depleted. (Supported by NSF-BNS-8718938, NSF-BNS-8958169, and NIH-NS-25915)

253.19

BEHAVIORAL DISCRIMINATION OF ODORANT MIXTURES AND THEIR COMPONENTS: EFFECTS OF MIXTURE INTERACTIONS ON QUALITY CODING. J.B. Fine-Levy*, C.D. Derby, and T.J. Bartness.Dept. of Biology, Georgia State Univ., Atlanta, GA 30303.

Elucidation of the mechanisms by which the qualities of chemical stimuli are encoded by the nervous system would be impossible without an understanding of the effects of *mixture interactions* on the *perception* of mixture quality. Mixture interactions have been implicated as the source of mismatches between "predicted" and observed physiological responses to mixtures of components whose individual stimulatory capacities are known. Toward examining this question, we tested the abilities of spiny lobsters to behaviorally discriminate between three odorant compounds (AMP, glutamate (glu), and taurine (tau)) and their binary mixtures through the use of a differential aversive associative conditioning paradigm. Based on the results of ANOVA and multidimensional scaling analysis incorporating observed responses to all stimuli and "predicted" responses to the binary mixtures, the following relationships between the perceived qualities of each binary mixture and its constituent components were determined: AMP+glu was different from either component, as well as its predicted value. AMP+tau was intermediate between both components and no different from the predicted value, a finding consistent with results of binding assays which indicate that these compounds are binding to independent receptor sites. Glu+tau was different from both components and there was some indication that glu was acting to suppress the tau in the mixture. Comparisons between these behavioral results and those of our electrophysiological analysis of the effects of mixture interactions on the coding of stimulus quality and intensity are in agreement, thus lending credence to the use of receptor-based models to predict effects of peripherally initiated mixture interactions on behavioral responses to odorant mixtures.

Supported by NIDCD Grants DC00312 and DC0002.

253.20

BIOCHEMICAL CHARACTERIZATION OF OLFACTORY RECEPTORS OF THE SPINY LOBSTER . K. Olson[1]*H. Trapido-Rosenthal[2], and C. Derby[1]. [1]Dept. of Biology, Georgia State University, Atlanta, GA 30303, [2]Whitney Laboratory, St. Augustine, FL 32086

This biochemical study has two goals: (1) to characterize biochemically the binding of odorant molecules with their receptors; and (2) to investigate the roles played by these receptor-odorant binding events in the sensory perception of odorant mixtures (e.g., mixture interactions). An olfactory tissue fraction enriched in dendritic membrane from olfactory receptor cells was prepared from aesthetasc sensilla on the lateral filament of the antennules. [^{3}H]-Taurine and [^{3}H]-AMP, known to be potent olfactory stimulants, were used as radioligands for the olfactory membrane in a filtration assay. Results suggest that specific binding sites for [^{3}H]-taurine and [^{3}H]-AMP exist in this olfactory membrane. K_m values for both types of binding sites are approximately 3 uM, and B_{max} for taurine sites is 532 pmole/ug protein. Inhibition studies indicate that AMP and taurine bind to different sites: for [^{3}H]-taurine binding, IC_{50} values for unlabeled taurine and AMP are 4.5 uM and >>1 mM, respectively; for [^{3}H]-AMP binding, IC_{50} values for unlabeled AMP and taurine are 0.7 uM and 1 mM, respectively. Glutamate, a known olfactory suppressant, was also tested as an inhibitor and its binding site was characterized. The results elucidate the role of receptor binding in mixture interactions. *Supported by NSF and NIDCD grants.*

REFLEX FUNCTION

254.1

PHASIC VIBRATORY PATELLAR REFLEXIVE RESPONSE OF ABLE-BODIED AND SPASTIC DISABLED ADULTS N.J.Lambert, Department of Physical Therapy, East Carolina University, Greenville, NC 27858

Assessing the magnitude of a phasic tendon tap reflex during vibration as compared to the magnitude of the tendon tap reflex without vibration may give an indication of the amount of remanent Ia presynaptic inhibition in spinal pathways of spastic disabled adults (Delwaide, 1985). The agonist (AG), antagonist (ANT) and reflexive FORCE response to a patellar tendon tap with (VIB) and without (NORM) quadriceps tendon vibration and ONE and TWO minutes post-vibration were compared in spastic disabled (n = 12; 6 SCI, 6 CP) and able-bodied adults (n = 12). The AG rectus femoris and ANT bicep femoris reflexive responses of the disabled group were significantly greater than those of the able-bodied group ($p < .05$). AG, ANT and FORCE reflexive responses during the VIB condition were significantly less than during the NORM, ONE and TWO conditions ($p < .05$). The inhibitory effect of vibration caused a similar absolute decrease in AG, ANT and FORCE response of the patellar tendon tap for both groups, however the relative percent decreases in reflexive response were much greater for the able-bodied group (AG 47%, ANT 40%, FORCE 49%) than the disabled group (AG 21%, ANT 9%, FORCE 20%). This would indicate that the mechanism of inhibitory feedback due to vibration was not necessarily hindered in the disabled group, but that the abnormally large reflexive responses of the disabled subjects were more difficult to suppress. There are mechanisms distinct from a lack of presynaptic inhibition which are also contributing to spasticity.

254.2

REFLEX AND NON-REFLEX MEDIATED STIFFNESS IN THE PLANTAR FLEXORS OF SPASTIC PATIENTS WITH HEMIPARESIS.
T. Sinkjær, K. Larsen*, M. Thomsen*, and I. Magnussen*. Dept. of Medical Informatics and Image Analysis, Aalborg University, Fredrik Bajersvej 7D, DK-9210 Aalborg, and Department of Neuromedicin, Aalborg Hospital, DK-9000, Denmark

It is often proposed that an enhanced stretch reflex in spastic patients contribute to muscular hypertonia, defined as an increased resistance by the muscles to an externally imposed stretch. The objective of this study was to determine how large a fraction of the force increment to an externally imposed stretch is due to intrinsic properties of the contractile apparatus and how large a fraction is due to the stretch reflex.

In this study, 6 spastic patients with hemiparesis were investigated with respect to the mechanical responses to stretch in the plantar flexors around the ankle joint. Each subject sat in a chair, the left foot strapped to a platform. During ongoing voluntary contraction, the platform was rotated around the ankle joint by a motor (Sinkjær et al., J. Neurophysiol. 1988; 60:1110-1121). The stiffness (the torque increment divided by changes in the ankle position), measured during voluntary contraction, evaluated total stiffness. The stiffness measured during electrical stimulation of the tibial nerve, evaluated non-reflex stiffness (intrinsic and passive stiffness). The reflex mediated stiffness was equal to the total stiffness minus non-reflex stiffness. In each subject the stiffness of the spastic joint was compared with the stiffness of the non-spastic joint.

The total stiffness of the spastic joint was on average 3.6 Nm/degrees compared to 2.6 Nm/degrees of the non-spastic joint. The passive stiffness was increased with 162% from 0.63 to 1.65 Nm/degrees. The reflex mediated stiffness amounted to more than 50% of the total stiffness in the spastic and non-spastic leg of the patients, with slightly larger reflex stiffness in the spastic leg.

The higher total stiffness in the spastic leg was primarily caused by a higher non-reflex stiffness. We hypothesize that under the experimental conditions, where hypertonia is usually assessed, the stretch reflex is masked by a simultaneous descending input to the muscle.

254.3

HUMAN SPINAL CORD REFLEX VARIABILITY: NOISE VERSUS CHAOS. S.J.Schiff. Dept of Neurosurgery, Children's Nat Med Ctr, Washington, DC 20010.

Spinal cord monosynaptic reflex variability is striking. Previous analyses have assumed that it has a significant random noise-like component. Recent advances in characterizing complex dynamical systems suggests that a re-evaluation of this phenomenon is warranted. Time series of 1000 human H-Reflex responses to identical 1 Hz stimuli were recorded and integrated. The series were embedded in increasingly higher dimensional space and a box counting algorithm used to compute the correlation integral and approximate a correlation dimension. The Kolmogorov entropy was estimated. Pseudo-random number time series were used as control simulations. Fourier power spectral analysis revealed a broad-band pattern. Enthropy results suggest that the output of this system is not random, but resolution of an attractor with fractal dimension proved difficult. The complexity of this system, as well as practical constraints on data collection, limit the applicability of these techniques in this setting.

254.4

PARTITIONING OF TRIGEMINAL CUTANEOUS AFFERENT REFLEXES BETWEEN FACIAL MUSCLES IN MAN. C. G. Widmer and J. P. Lund, Dental Research Center, Emory University, Atlanta, GA 30322 and Centre de Recherche en Sciences Neurologiques, Univ. of Montréal, Montréal, Canada H3C 3J7.

It has been assumed that all trigeminal cutaneous afferents provide a similar input to facial motoneurons. This study investigated the alternative hypothesis that trigeminal cutaneous afferents have differential reflex effects on various facial motoneuron pools.

In six subjects who gave informed consent, bipolar surface EMG recordings were made from various facial, masticatory and cervical muscles bilaterally and simultaneously for 32 ms prior to and 128 ms after the stimulus (0.2 ms pulse/2 sec). Facial muscles included frontalis, orb. oculi, lev. labii, zyg. major, orb. oris sup., orb. oris inf., and dep. anguli oris; masticatory muscles included masseter and suprahyoids; and cervical muscles included SCM and trapezius. Stimulation sites on the right side of the face included supraorbital, infraorbital, nose, filtrum, upper lip, corner of lip, lower lip, and mental area (all 6 subjects) and intraoral sites (2 subjects) including palate and mucosa over mental nerve. Ten responses were evaluated at non-noxious (4-5T) and at noxious (9-10T) stimulation levels during conditions of relaxation and active muscle contraction.

There were generally four types of responses that were found to be dependent on the site of stimulation: short (13.4±1.4 ms) and long (40.5±2.1 ms) latency excitation in the orb. oculi muscles which was reduced as the stimulus site was moved away from the eye; short latency (14.2±1.5 ms) excitation in the orb. oris with mental nerve stimulation; middle (23.1±3.0 ms) and long (53.1±3.9 ms) latency inhibition in the facial, masticatory and cervical muscles when the stimulus was in the extraoral region; and short (13.1±2.1 ms) and long (51.6±11.3 ms) latency inhibition in the masseter muscles after intraoral stimulation. The excitatory reflexes were elicited at lower (non-noxious) intensities of stimulation while inhibition was more apparent at the noxious stimulation levels.

These findings support the hypothesis of reflex partitioning of trigeminal cutaneous afferents on facial motoneurons

This work supported in part by USPHS grant DE06974 and the Canadian MRC.

254.5

LIP MUSCLE EMG RESPONSES TO MECHANICAL STIMULATION IN A SIMPLE REACTION TIME TASK. M.D. McClean. Audiology and Speech Center, Walter Reed Army Medical Center, Washington, D.C. 20307-5001.

EMG recordings were obtained of upper and lower lip muscle while subjects produced simple speech utterances in response to mechanical stimulation. Subjects were instructed either to produce the syllable "pa" as quickly as possible or not respond when they detected movement of a paddle held between the lips. Stimuli were adequate to elicit reflexes over poststimulus intervals of 15-30 ms (R1) and 30-50 ms (R2). EMG levels were calculated on-line for 240 trials over several poststimulus time intervals. The independent effects of stimulus level, pre-stimulus EMG level, and reaction time on response levels were assessed using multiple regression analysis. R1 and R2 levels were positively correlated with stimulus level, but stimulus level had little modulating effect on intentional responses. Both reflex and intentional response levels were strongly dependent on prestimulus EMG level. Instructional set had significant modulating effects on reflex responses in 9 of 10 subjects, but the nature of these effects varied among subjects. These findings are discussed in relation to similar studies on limb motor systems and lip motor control for speech.

254.6

ELECTROMYOGRAPHIC FINDINGS OF LOCAL TWITCH RESPONSES IN THE RABBIT SKELETAL MUSCLES. C-Z. Hong* and Y. Torigoe*, Dept. of Physical Medicine & Rehabilitation, and Dept. of Anatomy & Neurobiology, University of California Irvine, Irvine, CA 92717.

Local twitch response (LTR) is a brisk twitching of a bundle of muscle fibers elicited by a sudden mechanical stimulation on the sensitive site along palpable **taut band.** It is a characteristic response of myofascial trigger point in skeletal muscle fibers. In this report, electromyographic (EMG) study was performed in adult rabbits under light general anesthesia, preserving most central nervous system mediated reflexes. While LTR was elicited by mechanical tap stimulation (solenoid driven) on the most responsive site of a taut band, EMG activity was recorded from the same band. The EMG recordings showed latency (8-15msec), amplitude (0.3-1.5 mV) and total duration of burst activities (60-100 msec) that are quite different from stretch reflex. After Lidocaine infiltration or transection of the innervating muscle nerve, LTRs were greatly reduced as demonstrated in the decreased EMG activities. These results suggest that the central nervous system is essential for the propagation of LTR, probably via the nociceptive sensory system. Electric simulation of the most mechanically responsive sites along the taut bands elicited EMG activities that were less than 10% of mechanically induced responses. The above findings are compatible with the data of human studies on LTRs. We conclude that it is possible to use this animal model for investigations of myofascial trigger points. (Supported in part by UCI College of Medicine Faculty Research Grant).

254.7

COMPARISON OF THE EFFECT OF CLONIDINE ON REFLEXES RELATED TO URINARY SPHINCTER AND HINDLIMB FUNCTION. G.J. Bialik* and J.W. Downie. Departments of Pharmacology and Urology, Dalhousie Univ., Halifax, Nova Scotia, B3H 4H7.

We have previously shown that clonidine (CLON) diminishes somatic and viscerosomatic reflexes evoked on the pudendal nerve (JPET 246:352, 1988). In this study we compared this effect of CLON to its effect on typical group I, II, and III somatic reflexes. These reflexes were monitored by recording compound action potentials on peripheral nerves in response to electrical stimulation of appropriate nerves in chloralose anesthetized or decerebrate cats acutely spinalized at T10. CLON (1-100μg/kg i.v.) decreased the two pudendal reflexes and the group II and III reflexes, but had no effect on the group I reflex. The ID50 of CLON for suppression of the pudendal reflexes was lower than for group II and III reflexes, however CLON was more efficacious on the somatic reflexes (statistically significant only for group II).

Cord dorsum potentials (CDs) associated with pudendal and group II reflexes were unaffected by CLON. There was a dose-dependent increase in the group III CD. The group I CD was maximally increased at the lowest CLON dose tested (1μg/kg).

Thus it was possible to separate the effect of CLON on pudendal reflexes from its effect on typical group I, II and III somatic reflexes. The suppressive effect of CLON was not likely expressed at the level of the first order interneurons. Based on the CD data, CLON appears to enhance the first order interneuron depolarization.
(Supported by MRC)

254.8

FIELD POTENTIALS PRODUCED BY STIMULATION OF PUDENDAL, SUPERIOR PERINEAL AND PELVIC AFFERENTS IN THE SACRAL SPINAL CORD OF THE CAT. J.W. Downie[1], B. Fedirchuk, L. Song* and S.J. Shefchyk. [1]Dept Pharm, Dalhousie Univ., Halifax NS; Depts of Med. and Physiol., Univ. of Manitoba, Winnipeg, Canada, R3E 0W3.

The location within the lumbosacral spinal cord of neurons activated by sacral afferents was determined by recording the field potentials produced by low threshold electrical stimulation of peripheral nerves. Experiments were conducted on chloralose anaesthetized male cats paralyzed with flaxedil. The sensory branch of the pudendal, the superior perineal cutaneous nerve and branches of the pelvic nerve in close proximity to the bladder were stimulated. Field potentials were recorded within the L7-S3 spinal cord segments using either sodium citrate-filled glass microelectrodes or carbon filament in glass electrodes. Locations of electrode tracks within the spinal cord were histologically confirmed.

Field potentials evoked from both sensory pudendal and superior perineal nerves had a central latency of 0.7-1.1 ms. Fields produced by the sensory pudendal nerve stimulation were maximal at the rostral to mid S1 segment and were in the medial portion of laminae V & VI. Those produced by superior perineal nerve stimulation were maximal at the mid to caudal S1 segment and located more laterally in laminae V & VI than the sensory pudendal fields. Smaller fields from both nerves could be also localized to areas within laminae IV & X depending on the particular segment. Pelvic nerve stimulation (n=2) revealed discrete field potentials located in S2 and caudal S1 spinal segments.

This research was supported by the Canadian National Centres of Excellence (NCE) network for Neural Regeneration and Functional Recovery.

254.9

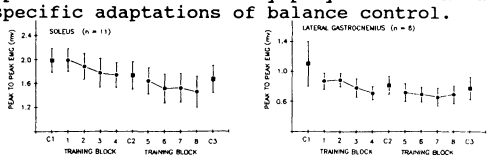
FACTORS CONTRIBUTING TO CYCLICAL MODULATION OF THE SOLEUS H-REFLEX DURING WALKING IN HUMANS. J.F. Yang. Dept. of Physical Therapy and Div. of Neuroscience, University of Alberta, Edmonton, Canada T6G 2S2.

The soleus (SOL) Hoffmann (H) reflex is highly modulated as a function of the time in the walking cycle. The amplitude of the reflex rises and falls with the amplitude of the background EMG from the SOL muscle, suggesting that the reflex amplitude is simply a reflection of the postsynaptic excitability of the SOL motoneuron pool. Do presynaptic mechanisms contribute to this modulation? Human subjects were trained to activate the SOL muscle during the swing phase, when the muscle is normally inactive. The slope of the relationship between the SOL H-reflex amplitude and the background SOL EMG was higher for the stance than the swing phase, suggesting that presynaptic mechanisms may be important in the cyclical modulation of the reflex. Does reciprocal inhibition from the antagonist, the tibialis anterior (TA), contribute to this cyclical modulation? Subjects were trained to keep the TA inactive during walking. The SOL H-reflex continued to be deeply modulated in spite of a lack of TA activity. This suggests that reciprocal inhibition from the antagonist is not essential for the cyclical inhibition of the SOL H-reflex. In summary, both pre and postsynaptic mechanisms appear to contribute to the cyclical modulation of the SOL H-reflex in walking. This modulation is not entirely dependent on the activation of the antagonist muscle.

254.10

DOWNTRAINING OF TRICEPS SURAE H-REFLEXES DURING A BALANCE TASK IN HUMANS. M.H. Trimble* & D.M. Koceja, Motor Control Lab, Indiana University, Bloomington, IN 47405.

Wolpaw and collegues (1989; 1990) and Meyer-Lohmann et al. (1986) have demonstrated that the spinal stretch reflex is modifiable under various conditions. In the present study, training induced reductions of the triceps surae H-reflex during a balance task have been demonstrated. Subjects were instructed to balance on a single axis balance board. H-reflexes, which served as the perturbation, were elicited when the subject was balanced. Activity of the tibialis anterior and triceps surae muscles were monitored throughout. The findings support the notion of a downtraining effect, as shown below. Further, these findings suggest that the plasticity of the spinal stretch reflex may play a role in task specific adaptations of balance control.



254.11

H-REFLEX IN THE FREELY MOVING RAT: METHODS AND INITIAL DATA. J.R. Wolpaw, X.Y. Chen, and J.S. Carp. Wadsworth Labs, NY St Dept Hlth & SUNY, Albany, NY 12201.

Primates can gradually increase or decrease the spinal stretch reflex or its electrical analog, the H-reflex (HR). Such conditioning changes the spinal cord (Wolpaw & Carp, TINS 13:137-142, 1990; and this volume). To develop further this model for defining the substrates of operantly conditioned plasticity, we are studying the HR in freely moving rats.

Male Sprague-Dawley rats (300-400 gm) are chronically implanted with fine-wire EMG electrodes in right ankle extensors, stimulating cuff on right tibial nerve, and bipolar stimulating electrode in left medial forebrain bundle (MFB). Electrodes connect to a head-mounted tether. Monitored and rewarded by computer, each rat learns to keep background EMG in a specified range. At a random time, nerve cuff stim at M response threshold elicits the HR. Under the control mode, reward (60-Hz MFB stim, 20-40 uA, 250 ms) begins 200 ms later. Rats usually perform more than 7,000 trials in 24 hrs.

The HR begins at about 5 ms, lasts about 3 ms, and is of modest size (<1 x background EMG). Its size appears to display a diurnal rhythm different in phase from that of monkeys. Efforts to condition the HR by making reward dependent on HR size are underway. (Supported by NIH NS22189 and Paralyzed Veterans of America Spinal Cord Research Foundation.)

254.12

QUANTITATIVE BEHAVIORAL ASSESSMENT OF CAT TAIL HYPERREFLEXIA AFTER SPINAL CORD LESIONS. R. M. Friedman, C. J. Vierck, Jr., and L. A. Ritz. Departments of Neuroscience and Neurosurgery, University of Florida College of Medicine, Gainesville, FL 32610

Previously we (Friedman et al., 1990) observed that chronic sacrocaudal transection (at Ca1) produces in cat tails the following signs of spasticity: (1) shorter response latencies than normal to mechanical cutaneous stimulation, (2) clonus-like oscillations in response to cutaneous stimulation, and (3) tonic deviation of the tail in a ventroflexed position, suggesting an increase in tone of the ventral muscles. Here we report on responses of the tail to electrocutaneous stimulation. Cats with chronic (> 2 years) Ca1 transections (1) respond with longer durations than normal cats to an electrocutaneous stimulus, (2) and react to trains of electrocutaneous stimulation with a potentiation (summation) of the reflex. We propose that after spinal cord transections, cutaneous reflexes are influenced by abnormally prolonged activity in spinal circuits. Presently, we are studying the development of these signs of spasticity over time after spinal cord lesions. Supported by grant NS27511.

254.13

SUMMATION AND COLLISION BETWEEN ACOUSTIC AND ELECTRICAL SIGNALS THAT EVOKE THE STARTLE REFLEX IN THE RETICULAR FORMATION. J.S. Yeomans and K.A. Cochrane*, Dept. Psychology, Univ. Toronto, Toronto, Canada, M5S 1A1.

To measure when and where the acoustic signals that evoke the startle reflex pass, we delivered brief, loud acoustic stimuli (1-5 ms, 106-110 dB) and tested, at various interstimulus intervals (ISI), the currents required to evoke a startle response with a single 0.1 ms cathodal pulse delivered to the pontine or medullary reticular formation in rats. Acoustic stimulation reduced by 20-90% the required current at ISIs from -10 to +10 ms, suggesting strong summation between acoustic and electrical stimuli. When the electrical pulse was presented 1.5-4.5 ms after the end of the acoustic signal, however, the summation was often blocked for about 0.5 ms, suggesting collision between action potentials evoked by the acoustic and electrical stimuli. Hence, acoustic stimulation evokes a synchronous volley of action potentials that passes through the reticular formation, and this volley evokes most of the acoustic startle reflex. These "acoustic-electric collisions" were similar in form to the "electric-electric collisions" obtained by stimulating pairs of sites in pons and medulla (Yeomans et al., SN Abstr., 1990), but occurred at longer ISIs. These tests define the spatial and temporal properties of the axons and synapses that mediate electric and acoustic startle responses.

254.14

A NEUROMECHANICAL CONTROL MODEL OF THE RABBIT NICTITATING MEMBRANE. **G.T. Bartha* and R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089.**

Nictitating membrane (NM) extension is a passive consequence of eyeball retraction in rabbit. The retractor bulbi muscle is the primary effector for eyeball retraction and the accessory abducens nucleus is the main pool of motoneurons innervating the retractor bulbi muscle. Previous studies have recorded from a small number of isolated accessory abducens neurons in order to functionally characterize their role in NM extension. We have adopted a theoretical approach to define more precisely the relation of motoneuron activity to NM responses.

We have built a computer model of the rabbit eye and retractor bulbi muscle that takes patterns of neural activity in the form of action potentials as input and produces movement of the NM as output. The model is based on known physiological and mechanical properties of the eye and muscle. The retractor bulbi muscle was modeled at the motor unit level to permit direct comparison with experimental data and up to 100 motor units were simulated. The model allows us to investigate what behavior is produced by a given pattern of neural signals and what patterns of neural input are required to produce a given behavior.

Although neural activity is highly correlated with behavior, simulations show that there can be significant differences between the shape of histogrammed motoneuron activity and the shape of the NM response. For example, peak NM extension can occur as much as 70 ms after the termination of a 30 ms burst of spikes. The neural input required for the model to generate NM responses is more consistent with single accessory abducens unit recordings from awake rabbits (Quinn, *et. al.*, Soc. Neurosci. Abstr., 8:314, 1982) than recordings from anesthetized rabbits (Berthier and Moore, Brain Res., 258:201, 1983). The model also supports the hypothesis that NM retraction is generally passive. (Supported by NSF BNS-8718300, ONR N00014-88-K-0112, & McKnight Foundation grants to R.F. Thompson.)

254.15

STRATEGIES FOR THE SIMPLIFICATION OF SENSORIMOTOR TRANSFORMATIONS IN THE WIPE REFLEX OF THE SPINAL FROG. W. Z. Rymer and J. L. Schotland. Department of Physiology, Physical Medicine & Rehabilitation and Neuroscience Institute, Northwestern University Medical School, Chicago, Illinois 60611.

In the wipe reflex of the spinal frog, propriospinal and cutaneous information concerning orientation of the body and limbs is combined with cutaneous information related to the location of the stimulus, to yield a smooth, coordinated movement of one of the limbs to remove an irritating stimulus located at any point on the body surface. Just how this kind of transformation is managed by the nervous systems has been the subject of considerable debate. We sought to investigate the kinds of spinal cord circuitry that might be predicted to simplify the coordination of sensorimotor transformations in the wipe reflex.

We examined the kinematics of wiping movements in four animals, elicited as a sequence of stimuli was moved progressively over the skin. The 3-D coordinates of the position of the foot over time, and the displacement of hip, knee, and ankle joints, were recorded using a WATSMART infrared emitter-detector system. These data were quantified using principal components analysis to provide a measure of the shape (eigenvalues) and orientation (eigenvector coefficients) of the movement trajectories. If an unique neural circuit were invoked to coordinate the response to each stimulus location, smooth changes in kinematic parameters of the movement would be elicited in response to continuous changes in stimulus location. Instead, we found that the movement of the foot during the initial flexion of the wipe appears to be constrained to motion along a single axis that is quite similar for wipes to widely differing stimulus locations, including movement that use very different strategies for removal of the stimulus. Likewise, foot movement during the rhythmic portion of the wipe appears to be limited to motion along one of the two axes. The organization of neural circuits required for sensorimotor transformations involved in the wipe reflex appears to be constrained to a great degree in comparison to the number of stimulus sites and variations of wiping movements. *Supported by PO1NS17489 to WZR and NIMH MH09566-03 to JLS.*

254.16

REPETITIVE BURSTS OF FLEXOR MUSCLE EMG ACTIVATED BY SINGLE CUTANEOUS NERVE STIMULI IN SPINAL CAT. R.G. Durkovic and S.G. Nord. Depts. of Physiology and Neurology, SUNY Health Science Center, Syracuse, NY 13210.

In order to characterize flexion reflex responses, EMG activity was recorded with indwelling needle electrodes from the tibialis anticus and biceps femoris muscles of acute spinal cats (decerebrate, unanesthetized). Single electrical pulses (0.2ms) were presented to the lateral sural, saphenous or superficial peroneal cutaneous nerves. Recordings were monitored for 200 ms following each stimulus.

Stimulation of each cutaneous nerve at an intensity that activated large myelinated (Aα) cutaneous fibers, but just below the threshold of small myelinated (Aδ) cutaneous fibers, produced an initial burst of EMG activity in each muscle (latency 8-24 ms). Additional bursts (usually two) of activity with intervening periods of quiescence often followed the initial one. Cutaneous nerve stimuli that activated both Aα and Aδ cutaneous nerves supramaximally often increased the amplitude and decreased the intervals between EMG bursts. The results demonstrate that cutaneous nerve stimulation can evoke flexion reflexes with multiple EMG bursts in the absence of either supraspinal influences (e.g., startle) or activation of Aδ afferent fibers. Supported by NSF Grant 8808495.

254.17

LARYNGEAL MUSCLE RESPONSES TO PERIPHERAL NERVE AND TRANSCRANIAL MAGNETIC STIMULATION. C.L. Ludlow[1], L.G. Cohen[2], M.Hallett[2], J. S. Yeh*[1] and M. Shibusawa*[1]. [1] Voice and Speech Section, VSLB, NIDCD, and [2] Human Cortical Physiology Unit, HMCS, MNB, NINDS.

Magnetic stimulation has been used to evaluate the conduction time from the cortex to the laryngeal muscles. Because of the close proximity of the laryngeal nerves during transcranial magnetic stimulation, responses may be due to stimulation of either the cortical pathways or the peripheral nerves. Our purpose was to identify responses obtained during stimulation of the peripheral laryngeal nerves at different locations, and the cortex transcranially, in awake humans. Indwelling hooked wire electrodes were used to record from the intrinsic laryngeal muscles, the right and left thyroarytenoids and cricothyroids, while stimulating with a Cadwell magnetic stimulator on each side. Six stimuli were presented at 11 positions on each side: at the angle of the jaw and at 1 cm intervals progressing up the side of the face and transcranially 6 cms anterior to T3 or T4. Five peripheral responses were identified: an early ipsilateral one at a latency of less than 3 ms seen only at the stimulation points close to the larynx; an ipsilateral response to nerve stimulation at 3-4 ms for the cricothyroid and 6.5-8 ms for the thyroarytenoid; an ipsilateral one at 14 ms in the thyroarytenoid; a bilateral brief response in all four muscles at 23 ms; and late responses around 45 ms in both thyroarytenoids. The only response to transcranial stimulation that was not seen during peripheral stimulation was a contralateral response in either muscle, between 15 and 20 ms in the thyroarytenoid, and around 12 ms in the cricothyroid, indicating a cortical to laryngeal muscle conduction time of approximately 12-15 ms.

254.18

VIBRATORY RESPONSES IN SPINAL CORD INJURY SUBJECTS AFTER CUTANEOUS DEAFFERENTATION, A. M. Sherwood, M. R. Dimitrijevic, and P. J. Sharkey*, Div. of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030.

Vibration induces a well-defined tonic vibratory response (TVR) in experimental animals which is abolished by spinal cord transection [Matthews, *J. Physiol.* (Lond.) 184:450-472, 1966]. The TVR is also present in relaxed healthy subjects but has different characteristics in paralyzed spinal cord injury (SCI) subjects [Dimitrijevic et al. *Neurol.* 27:1078-1086, 1977]. Many SCI subjects have exaggerated withdrawal reflexes which can also be elicited by vibration along with the vibratory response (VR). The VR is distinguished by its sustained character time-locked to the vibration. We used multi-channel surface EMG to study responses to vibration at 100 Hz and 2-3mm displacement in four SCI subjects. Recordings were made before and after neurectomy of the sensory branches of the femoral nerve for management of spasticity. We found that the VR did not change, although cutaneous stimuli in the area over the quadriceps no longer elicited withdrawal reflexes. We concluded that, while vibration may elicit a withdrawal reflex mediated by cutaneous afferents, cutaneous receptors do not contribute to the VR itself in paralyzed SCI subjects.

SPINAL CORD AND BRAINSTEM: MOTONEURONS

255.1

STRENGTH OF RECURRENT INHIBITORY POSTSYNAPTIC POTENTIALS BETWEEN CLOSELY SPACED HOMONYMOUS AND HETERONYMOUS MOTONEURONS IN THE CAT. M.L. McCurdy and T.M. Hamm. Div. of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

The strength of recurrent inhibition within homonymous motoneuron pools is reported to be stronger than between heteronymous motoneuron pools, as estimated by average composite recurrent inhibitory postsynaptic potentials (RIPSPs) (Eccles et. al., 1961). Since the distribution of recurrent inhibition produced by stimulating single motor axons in homonymous motoneurons is restricted along the rostrocaudal axis of the spinal cord (Hamm et al., 1987), homonymous-heteronymous differences in the strength of recurrent inhibition may be a result of this topographic distribution and the displacement in the rostrocaudal locations of heteronymous pools. We investigated this possibility by measuring the strength of recurrent inhibition between pairs of closely spaced motoneurons (range= 86-1861 um). Pairs of homonymous or heteronymous motoneurons were impaled with glass micropipettes in the seventh lumbar spinal segment of ischaemically decapitated cats. RIPSPs were recorded in one motoneuron in response to stimulation of a nearby motoneuron by injecting brief current pulses. The RIPSP values in homonymous and heteronymous pairs ranged from 0-339 uV. Preliminary results suggest that motoneurons innervating the anterior-medial biceps femoris produce RIPSPs in nearby homonymous motoneurons that are significantly larger than RIPSPs produced by nearby heteronymous motoneurons. No significant difference was noted between RIPSP amplitude and the distance between motoneurons in a pair over the short range of distances examined. We also examined RIPSP amplitudes in relation to cell size as judged by input resistance and observed a positive correlation (r=.55). These results indicate that recurrent inhibitory pathways from individual motoneurons are distributed preferentially to nearby homonymous motoneurons. Supported by USPHS grants NS22454, NS08773 and NS07309.

255.2

EXCITABILITY OF FACIAL MOTONEURONES IS REDUCED DURING THE ATONIA OF ACTIVE SLEEP. P. J. Soja *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC V6T 1Z3, CANADA.*

During active sleep (AS), lumbar and trigeminal (jaw-closer) motoneurones are subjected to powerful postsynaptic inhibition (see *Int. Rev. Neurobiol.*, 24: 213-258, 1983). Recent studies have demonstrated that the neurotransmitter glycine mediates this inhibitory drive (*J. Neuroscience* 9: 743-751, 1989; *J. Neuroscience*, 1991, *in press*). Facial muscles also become atonic during AS. However, the neural mechanism(s) underlying the atonia of the facial musculature during AS has not been determined. Accordingly, the excitability of facial motoneurones (MotVII) was examined during wakefulness (W), quiet sleep (QS), the non-rapid eye movement (non-REM) episodes of AS, and the REM episodes of AS.

Three adult cats were prepared for recording MotVII activity utilizing chronic head restraint methodologies developed previously by Chase et al. (*J. Neurophysiol.*, 44: 349-358, 1980). Glass micropipettes filled with 165 mM NaCl were directed stereotaxically toward MotVII. Low intensity stimuli (0.04 ms, 4.5 mA, 1.0 Hz) were delivered to the VII nerve via miniature stainless steel screws which were permanently implanted into the mastoid process; such stimuli induced a twitch in the muscles controlling movement of the pinna, eyelid and vibrissae. The MotVII pools were located between 21.0-22.5 mm below the surface of the cerebellum by recording a characteristic negative going antidromic field potential of 3.0-4.5 mV in response to VII nerve stimulation. The stimulus intensity was adjusted during W so that the amplitude of the antidromic field potential was approximately 75% of maximal. Computerized analyses indicated that the amplitude of the antidromic field potential did not change from W to QS; it was, however, markedly suppressed by 45-67% during non-REM episodes of AS when compared to W or QS. During the phasic REM episodes of AS, the antidromic field potential was further suppressed.

Bevelled glass micropipettes filled with 2M K^+-citrate were used to record intracellularly from antidromically identified facial motoneurones. Intracellularly recorded membrane potential activity was characterized by the marked presence of large amplitude spontaneous hyperpolarizing potentials influencing these cells during AS when compared to QS or W; a finding which is reminiscent of the spontaneous glycinergic IPSPs which inhibit lumbar motoneurones during AS (*ibid*). The present results suggest that a process of postsynaptic inhibition contributes to the atonia of the facial musculature which occurs during AS. Supported by grants from the British Columbia Health Research Foundation.

255.3

SHORT LATENCY SYNAPTIC EXCITATION OF FORELIMB AND PHRENIC MOTONEURONS INDUCED BY STIMULATION OF THE RED NUCLEUS AND CEREBROFUGAL FIBERS IN CATS. Y. Fujito, T. Imai* and M. Aoki*. Department of Physiology, Sapporo Medical College, Sapporo 060, JAPAN

Recent histological studies have provided evidence suggesting direct rubro-motoneuronal connections in distal forelimb motoneurons (Mns) and direct cortico-motoneuronal connections in the phrenic Mns in the cat. In the present electrophysiological experiments, we examined whether there are short latency EPSPs suggesting monosynaptic rubro- and cortico-motoneuronal connections in the cervical spinal segments in cats. Under pentobarbital anesthesia and immobilization with gallamine triethiodide, intracellular recordings were made from forelimb and phrenic Mns following stimulation of the red nucleus (RN) and the cerebral peduncle (CP). Single pulse stimulation of RN produced EPSPs in the majority of forelimb Mns (60/86, 70%). Segmental latencies of RN-EPSPs shorter than 1.2 ms were observed in 16 of 24 forelimb Mns in which RN-EPSPs were detected in the C8-T1 segments, while in 3 of 36 forelimb Mns in the C6-7 segments. This result suggests that rubro-motoneuronal connections are, at least in part, monosynaptic in forelimb Mns innervating distal muscles in particular. By contrast, single shocks to RN and CP rarely produced EPSPs in phrenic Mns (6/24) and forelimb Mns (8/86), respectively. There was no obvious evidence suggesting monosynaptic connections between RN and the phrenic Mns, and between cerebral cortex and forelimb Mns. In the phrenic Mns, CP-EPSPs were produced by single shocks in 10 of 24 Mns and their segmental latencies shorter than 1.2 ms were observed in few cells.

255.4

EFFECTIVE SYNAPTIC CURRENTS AND FIRING RATE MODULATION IN CAT TRICEPS SURAE MOTONEURONS PRODUCED BY STIMULATION OF THE RED NUCLEUS. M.D. Binder, R.K. Powers, F.R. Robinson and M. A. Konodi*. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

We have investigated the effects of stimulating the red nucleus on triceps surae motoneurons of the cat both at rest and during repetitive discharge. The modified voltage clamp technique (Heckman and Binder, *J. Neurophysiol. 60*: 1988) was used to measure the steady-state effective synaptic currents produced by 200 Hz stimulation at a fixed site within the contralateral red nucleus. We then injected an amplitude series of depolarizing 1 sec current pulses and repeated the red nucleus stimulation. From these data, we could determine the firing rate-injected current (f-I) relation of each motoneuron and compare the actual effect of stimulating the red nucleus on motoneuron discharge rate with that predicted from the measurements of effective synaptic current and the f-I slope. We have found that the red nucleus generates both net excitatory and net inhibitory steady-state effective synaptic currents, some of which are considerably larger than those produced by other input systems. In most cases, the observed increase or decrease in motoneuron firing rate was matched by the product of the net effective synaptic current and the slope of the motoneuron's f-I relation. (Supported by NIH grants NS26840, NS25206 and EY07991)

255.5

SUMMATION OF EFFECTIVE SYNAPTIC CURRENTS AND FIRING RATE MODULATION IN CAT TRICEPS SURAE MOTONEURONS PRODUCED BY CONCURRENT STIMULATION OF DIFFERENT SYNAPTIC INPUT SYSTEMS. R.K. Powers, F.R. Robinson, M. Konodi* and M.D. Binder. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

We have investigated the summation of effective synaptic currents in triceps surae motoneurons of the cat generated by three input systems; the red nucleus, the sural nerve (5xT), and the homonymous Ia afferent fibers. The modified voltage clamp technique (Heckman and Binder, *J. Neurophysiol. 60*: 1988) was used to measure the steady-state effective synaptic currents produced by activation of each of the inputs alone and the net effective synaptic current when two or all three of the inputs were activated concurrently. We then injected sufficient depolarizing current to elicit repetitive firing in the motoneuron, and repeated the activation of one or more of the inputs. In most cases, the net effective current produced by concurrent activation was within 20% of the predicted algebraic sum of the individual currents. Moreover, the change in a motoneuron's firing rate produced by the concurrent activation of two or more input systems could be predicted from the net effective synaptic current that they generated and the slope of the motoneuron's f-I relation. (Supported by NIH grants NS26840, NS25206 and EY07991)

255.6

STEADY-STATE VS. TRANSIENT SYNAPTIC INPUT FROM THE SURAL AND CONTRALATERAL PERONEAL NERVES IN CAT MOTONEURONS. C.J. Heckman, J.F. Miller and W.Z Rymer. Dept. of Physiology, Northwestern University and Veterans Administration, Lakeside Hospital, Chicago, IL, 60611.

Recent studies have used steady-state synaptic input as a tool for assessing the distribution of synaptic input within the motoneuron pool (e.g. Heckman and Binder, J. Neurophysiol. 60: 1946, 1988). However, it is possible that sustained stimulation may evoke synaptic fatigue, induce plateau potentials, or alter the balance of interneuronal excitability. We investigated the relation between transient and steady-state post-synaptic potentials (PSPs) for the polysynaptic input from the caudal sural and contralateral peroneal nerves. Both inputs often exhibit single, transient PSPs that combine excitation and inhibition.

PSPs were recorded in medial gastrocnemius (MG) motoneurons in the pre-collicular decerebrate preparation. Steady-state PSPs were generated by 1 s of 100-200 Hz electrical stimulation. Steady-state sural PSPs (5 x threshold) were invariably depolarizing even though the transient sural PSPs often included an inhibitory component that was much larger than the short latency excitatory component. Steady-state peroneal PSPs (20 x threshold) were sometimes inhibitory. In addition, both inputs often produced sustained interneuronal afterdischarge or plateau potentials after cessation of stimulation. In our as yet small sample (n = 10), the amplitudes of the steady-state potentials from these inputs showed no systematic relationship with any intrinsic motoneuronal property.

The mechanism by which the depolarizing component of the steady-state sural input comes to dominate the inhibitory component is as yet unknown. However, this phenomenon may relate to recent findings that electrical stimulation of the sural nerve in the decerebrate does not unduly alter recruitment order (Clark and Cope, Soc. Neurosci. Abstr. 16:888, 1990).

255.7

MOTONEURON PHYSIOLOGY AFTER H-REFLEX CHANGE BY OPERANT CONDITIONING. J.S. Carp, X.Y. Chen and J.R. Wolpaw. Wadsworth Labs, NYSDOH/SUNY, Albany, NY 12201.

Monkeys (Macaca nemestrina) can slowly increase or decrease the size of the triceps surae (TS) H-reflex (HR), the electrical analog of the monosynaptic stretch reflex. Such conditioning changes the spinal cord (Wolpaw & Carp, TINS 13:137-142, 1990). In order to define these spinal cord alterations, we are recording intracellularly from motoneurons (MNs) in naive animals and in animals in which one leg's TS HR has been trained up (HR↑) or trained down (HR↓). Animals are deeply anesthetized throughout and sacrificed by overdose.

We have analyzed 95, 77 and 66 TS MNs in 14 naive, 8 HR↑ and 3 HR↓ animals, respectively. Soleus MNs on the trained side of HR↑ animals appear to have smaller homonymous compound EPSPs, lower input resistances and faster conduction velocities than those from naive animals. The opposite trend is seen in HR↓ animals. Also, AHP appears to be briefer on the trained than the control side of HR↑ animals, and the reverse is seen in HR↓ animals. These effects are less apparent in gastrocnemius MNs. Thus, these limited data suggest that HR conditioning changes MNs and/or their afferent input. The relationship between these effects under anesthesia and the HR changes in awake behaving animals is not yet clear. (Supported by NIH NS22189 & Paralyzed Veterans of America Spinal Cord Research Foundation.)

255.8

MOTONEURON MORPHOLOGY IN THE RAT. X.Y. Chen, J.S. Carp, and J.R. Wolpaw. Wadsworth Laboratories, New York State Department of Health and State University of New York, Albany, NY 12201.

In conjunction with work directed at defining physiologic and anatomic substrates of operantly conditioned plasticity in the spinal cord (Wolpaw & Carp, TINS 13:137-142, 1990; and this volume), we are studying morphology of motoneurons in the rat lumbar spinal cord that innervate ankle extensor muscles.

Male Sprague-Dawley rats (300-400 gm) are deeply anesthetized with pentobarbital. Motoneurons identified by antidromic stimulation are labelled intracellularly with HRP. Animals are sacrificed by overdose and perfused.

For 12 motoneurons analyzed to date, soma diameter was 34 ± 5 um (mean ± SD), number of primary dendrites was 8 ± 2, and dendritic spread was 1190 ± 245 um rostrocaudal, 1104 ± 206 um dorsoventral, and 764 ± 121 um mediolateral. Conduction velocity was 43 ± 4 m/s. Additional evaluation of dendritic extent and complexity and correlation of morphologic and physiologic measures are underway.

(Supported by NIH NS22189 and Paralyzed Veterans of America Spinal Cord Research Foundation.)

255.9

FIRING RATE AND FORCE MODULATION OF CONCURRENTLY ACTIVE MOTOR UNITS: IMPLICATIONS FOR THE SYNAPTIC ORGANIZATION OF MOTONEURON POOLS. K.E. Tansey and B.R. Botterman. Dept. of Cell Biology and Neuroscience, Univ. of Texas Southwestern Med. Center, Dallas, TX 75235.

The intrinsic properties of motoneurons and the organization of their synaptic inputs strongly influence their recruitment thresholds and their subsequent firing rate modulation. Here we consider the discharge patterns observed in pairs of type-identified motor units of the cat medial gastrocnemius muscle during graded contractions evoked by brainstem stimulation. Motor units in some pairs were reactivated intra-axonally with their previously recorded discharge patterns to estimate their force contribution to the whole muscle contraction.

Parallel firing rate modulation was observed only in pairs of motor units of the same type and of similar recruitment thresholds. In unit pairs of dissimilar type and recruitment thresholds, however, parallel rate modulation was not seen. As whole muscle force increased, low threshold motor units completed their firing rate modulation before higher threshold units were recruited and subsequently rate modulated. We infer from this that the synaptic drive to the motoneuron pool may be uneven and that higher threshold units may receive increases in synaptic drive that are not shared with lower threshold units. Interestingly, pairs of motor units which demonstrated parallel firing rate modulation rarely produced parallel force modulation. The lower threshold unit usually reached the upper end of its frequency-tension curve before the higher threshold unit reached the steep portion of its frequency-tension curve.

We are presently attempting to sample a larger population of concurrently active motor units by using selective EMG recordings to better assess the distribution of synaptic effects onto selected motoneuron pools. Supported by NIH grant NS17863.

255.10

IN VITRO ELECTROPHYSIOLOGY OF DEVELOPING NEURONS IN THE VENTRAL HYPOGLOSSAL NUCLEUS OF THE RAT. P.A. Núñez-Abades*, J.M. Spielmann and W.E. Cameron. Depts. of Behavioral Neuroscience and Pediatrics, University of Pittsburgh, Pittsburgh, PA 15260

Active and passive membrane properties of presumptive genioglossal motoneurons were studied in rats during postnatal development. These motoneurons are important in the control of the upper airways. Animals were anesthetized with halothane and transcardially perfused with cold oxygenated sucrose-artificial CSF and brainstem slices were cut at 300-400 μm. Intracellular recordings were made in cells located in the ventral hypoglossal nucleus in an area where intense retrograde labeling was found as a result of a prior dextran-rhodamine injection into the genioglossus. Both current-voltage and current-frequency curves were generated for 24 neurons with spike heights >60 mV. Mean input resistance (R_{in}) at 1 week (91.8±30.0 MΩ) was significantly greater than that found at any age including five weeks (32.4±4.1 MΩ; $p<0.02$) while mean rheobase (I_{in}) at 1 week (0.10± 0.06nA) did not become significantly different from older ages until 5 weeks (0.42±0.13 nA; $p<0.01$). The largest change in R_{in} occurred between 1 and 2 weeks while the largest change in I_{in} was found between 4 and 5 weeks of age. The peak (first interspike interval) and steady state firing frequencies (FF) were measured in response to a variety of currents amplitudes. The slope of the steady state FF as a function of current was relatively constant throughout development (~30 Hz/nA). While the steady state FF demonstrated only a primary firing range, a secondary range of the peak FF was expressed in some cells at 1 week. By two weeks, all neurons exhibited a secondary range of peak FF. These data suggest different time courses for the change in ionic mechanisms underlying the active and passive properties of the motoneuron membrane. Supported by NIH (HD 22703) and Magee Womens Hospital.

255.11

MOTONEURON DENDRITIC TREES: DEPENDENCE OF BRANCH DIAMETER ON ORDER PREDICTED BY A SIMPLE MODEL. W. B. Marks and R. E. Burke. Lab of Neural Control, NINDS, Bethesda, MD 20892

Branch diameter has an approximate double exponential dependence on branch order in motoneurons (e.g. Cullheim et al., *J. Comp. Neurol.* 255: 68, 1987). We have been able to fit this dependence by making two simplifying assumptions about dendrites. First, when a dendrite branches, the daughter diameters are random with a mean and standard deviation proportional to the parent diameter. Second, all daughters with diameters greater than a threshold, $\mathbf{d}_0$, branch again, while all with lower diameters terminate. Let G(**d**; mean, sd) be the distribution of daughter diameters, **d**, whose integral over **d** equals 1. Then the change in the distribution at order **k**, num(**d**, **k**), of branch diameters at the next order would be

$$\text{num}(\mathbf{d},\ \mathbf{k}+1) = 2\int_{\mathbf{d}_0}^{\infty} G(\mathbf{d}\ \ \text{mean}=m\ \mathbf{d}',\ \text{sd}=s\ \mathbf{d}')\ \text{num}[\mathbf{d}',\ \mathbf{k}]\ d\ \mathbf{d}'$$

Along with num[**d**, 0] = ι given initial distribution, this is the complete model. Because of the factor 2, at low orders when all diameters are greater than $\mathbf{d}_0$, the total number of branches doubles at each order. On average, the diameters drop by the factor $m < 1$ at each branch point. As branch order increases, the total branch number peaks and declines because increasing numbers of branches terminate as diameters fall below $\mathbf{d}_0$. When all the diameters fall below $\mathbf{d}_0$ the process ends. For gastrocnemius α- and γ-motoneurons, $m \approx .6$, and $s \approx .2$. Adjusting $\mathbf{d}_0 \approx 1.7$ μm gave good fits to plots versus order of the total number of branches, average branch diameter and its variance.

255.12

COMPARATIVE STUDIES OF DENDRITIC MORPHOLOGY USING A COMPUTER SIMULATION APPROACH. R. E. Burke, W. B. Marks, and B. Ulfhake. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892 and Dept. of Anatomy, Karolinska Institutet, Stockholm, Sweden.

We have developed a stochastic computational model to reproduce the statistical features of completely reconstructed gastrocnemius α-motoneuron dendrites from adult cats (*Neurosci. Abstr.* 16:115, 1990). Parameters that govern the probabilities of branching (**Pbr**) and terminating (**Ptrm**) and the diameters of daughter branches at branching points were derived directly from the morphological data. The only free parameter was rate of branch taper, which was allowed to vary near its observed mean value. In mature gastrocnemius motoneurons, **Pbr** and **Ptrm** were found to be exponentially dependent on local branch diameter, **d**, and non-linearly (and less strongly) dependent on path distance from the soma, **p**. The observed distributions of **Pbr(d,p)** and **Ptrm(d,p)** were approximated by algebraic expressions with four parameters each. Simulation of complete dendritic trees with specified stem diameters produced good agreement with a wide variety of morphological attributes of actual motoneuron dendrites. We have extended this approach to examine the dendrites of other species of spinal motoneurons (soleus and lumbosacral γ-motoneurons in adult cats, as well as triceps surae motoneurons obtained from immature kittens). Preliminary observations indicate that the simulation process permits identification of fundamental features that account for the morphological differences between dendrites of different neuronal groups. We assume that the parameter dependencies reflect underlying biological factors that can be studied in detail when identified, giving this approach more than taxonomic interest.

255.13

SHORT-LATENCY EPSPs IN LUMBOSACRAL MOTONEURONS PRODUCED BY STIMULATION IN THE MEDIAL LONGITUDINAL FASCICULUS. M. K. Floeter, G. N. Sholomenko and R. E. Burke. Lab. of Neural Control, NINDS, NIH, Bethesda, MD.

As part of a study of the organization of excitatory last-order interneurons in the cat lumbosacral spinal cord (e.g., *Neurosci. Abstr.*, 16:890, 1990), we have studied the short-latency EPSP produced by electrical stimulation of a medial region of the cat brainstem (P7 - P14, L 0.5, 2mm below the floor of the 4th ventricle; corresponding to the ventral portion of the medial longitudinal fasciculus, or **MLF**) described originally by Grillner and coworkers (*Exp. Brain Res.* 12:457, 1971). The responsible pathway(s) has a narrow range of conduction velocity (~100 m/s) and generates two kinds of short latency EPSPs: (**1**) clearly **monosynaptic** (segmental latency 0.4 - 1.0 ms [mean 0.6 ms], small amplitude [< 0.5 mV], with modest or no temporal facilitation during short stimulus trains [interval 3 - 10 ms]); and (**2**) possibly **disynaptic** (segmental latency 1.0 - 2.0 ms [mode 1.2 ms], generally larger amplitudes [up to 2.5 mV], with usually dramatic temporal facilitation [2-3 fold amplitude increase]). Facilitatory interactions with segmental excitatory reflex pathways have been surprisingly difficult to demonstrate, although a few clear examples have been found. The latter are consistent with attribution of the longer latency EPSPs to disynaptic connection through segmental last-order excitatory interneurons. The nature of the relatively powerful "disynaptic" MLF EPSPs, and their relation to control of excitatory segmental reflex pathways, particularly during fictive locomotion, are being studied.

255.14

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF GUINEA PIG TRIGEMINAL MOTONEURONS *IN VITRO*. S.H. Chandler and L.J. Goldberg. Dept. of Kinesiology, Brain Research Institute, and School of Dentistry, UCLA, Los Angeles, CA 90024

The trigeminal motor nucleus contains the motoneurons responsible for the production of masticatory movements. A detailed characterization of the passive and active membrane properties is essential to understanding the output characteristics of these cells in response to phasic and tonic synaptic input. Therefore, an *in vitro* slice preparation was developed to record from trigeminal motoneurons in the guinea pig. Guinea pigs (150-300g) were anesthetized, the brainstem removed and 400-500μ slices were obtained. Long term intracellular recordings from motoneurons produced average values for membrane potential, action potential amplitude and input resistance of -65mV, 85mV and 13MΩ, respectively. Application of 1 second current pulses produced repetitive firing with a rapid adaptation to a steady-state discharge (SS). In most cells, a plot of instantaneous frequencies of 1st, 3rd, and 5th intervals versus current (F/I) were linear, whereas in some cells distinct primary and secondary range firing rates were observed which approached 300Hz. Steady-state F/I current relationships were linear approaching a plateau level of approximately 10-30Hz. The slopes of 1st and SS F/I intervals ranged from 24-80Hz/nA and 10-30Hz/nA, respectively. Cobalt substituted for Ca^{++} shifted the SS F/I curve to the left. At the same time, slow, long duration AHPs following each action potential in the train were abolished without a change in spike duration or amplitude. This suggests that the long duration AHP and adaptation of repetitive firing are controlled by activation of a Ca^{++} dependent K^+ conductance. In contrast, 5-10mM TEA application increased spike duration and reduced the slope of the SS F/I curve. In addition, combined bath application of TTX, TEA, and barium revealed Ca^{++} spikes and plateau potentials. The experiments demonstrate that the *in vitro* slice preparation is viable for characterizing passive and active membrane properties and has established a foundation for future connectivity and pharmacological studies on masticatory motoneurons. Supported by NIH grants DE09032,DE06193, DE 4166.

255.15

CHANGE IN EPSP AMPLITUDE MODULATION DURING HIGH FREQUENCY STIMULATION IS CORRELATED WITH CHANGES IN EPSP AMPLITUDE - A BACLOFEN STUDY. K.R.Peshori, W.F.Collins,III and L.M. Mendell, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

Large amplitude EPSPs in low rheobase hindlimb motoneurons (MNs) depress during high frequency stimulation, whereas small amplitude EPSPs in large rheobase MNs tend to facilitate (Collins et al, J.Neurophysiol,52:980,1984). We hypothesize that systematic differences in transmitter release onto different MNs account for this frequency dependent behavior. We have assessed this by modifying presynaptic transmitter release using l(-) Baclofen, a GABA-B receptor agonist, in the anesthetized in vivo rat spinal cord. Composite heteronymous EPSPs were recorded intracellularly from gastrocnemius MNs during low frequency stimulation (18Hz) and high frequency (167Hz) 32 shock bursts, before and after Baclofen injection (10mg/kg,i.v.). Baclofen administration reduced the 18Hz EPSP amplitude, with no change in MN input resistance or rheobase. In addition, depending on the initial pattern of modulation, depression of EPSP amplitude during the high frequency burst was diminished (see also Lev-Tov et al, PNAS 85:5330, 1988), facilitation was enhanced, or depression was converted to facilitation. This shift to more positive modulation was seen at all connections regardless of initial (pre-drug) EPSP amplitude, and was directly correlated in a given MN with the decrease in 18Hz EPSP amplitude. This change in facilitation/depression behavior at high frequencies by presynaptic manipulation of EPSP amplitude is an important step towards understanding the underlying mechanism(s) of EPSP amplitude modulation. Supported by NIH NS24206 (WFC) and NS16996 and NS14899 (LMM).

255.16

QUANTITATIVE ULTRASTRUCTURE OF Ia BOUTONS IN THE VENTRAL HORN: VARIABILITY AND POSITIONAL RELATIONSHIPS. J.P.Pierce and L.M.Mendell. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Three MG Ia muscle afferents in the lumbar spinal cord of the cat were physiologically identified and intraaxonally injected with HRP. Serial sections were reacted with DAB, dehydrated, and embedded in EPON. A light level reconstruction of the fiber was generated, and selected regions were excised and serially thin sectioned for ultrastructural examination. High magnification (10,000x) micrographs were taken of every other section through each entire bouton, and provided the basis for quantitative analysis. Thirty-five boutons were characterized in terms of total volume, mitochondrial volume, total vesicle # and density, extent of apposition to the postsynaptic process, active zone area and shape. These features were then related to the position of the bouton within the terminal field and on the collateral arbor, the presence and number of presynaptic inputs and the type of postsynaptic element contacted.

Bouton characteristics varied greatly. Volumes ranged from 0.63 to 38.9 μm^3 with the distribution skewed towards lower values. Total vesicle # varied from 218 to 34700 and was also heavily skewed, with 45% containing fewer than 5000 vesicles. Volume and vesicle # displayed a high level of covariance (correlation: 0.91). Vesicle density ranged from 394 to 1800 vesicles/μm^3, and % mitochondrial volume varied from 5.9 to 39%. Total active zone area ranged from 0.19 to 3.6 μm^2. The extent of variation is almost as great when one examines a group of boutons from the same afferent, contacting the same motoneuron. This suggests that neither the afferent nor the postsynaptic neuron plays an exclusive role in determining these anatomical features. The position of the bouton in the afferent arbor may be related to some bouton features, however. As one moves downstream within an *en passant* chain, the extent of apposition, volume and vesicle # decrease (excluding the terminal bouton, which shows more variability). Supported by NSO8239, NS16996 and NS14899.

255.17

PROPERTIES OF MOTONEURONES, MUSCLE UNITS, AND THEIR RELATIONSHIPS IN RAT HINDLIMB. P.F. Gardiner. Physical Activity Sciences, University of Montréal, Montréal, Québec, H3C 3J7.

These studies extend previous observations (Gardiner and Kernell, Pflugers Arch., 1990) that suggest that motoneuron (MN) membrane properties may be related to muscle unit contractile characteristics in the rat, as they are in cat. Properties of tibial MN (n=123) a subgroup of which innervated gastrocnemius (n=58), were determined in an in situ adult rat preparation using standard microelectrode techniques. For motor units, type slow (S) (twitch half relax time > 28 ms, completely fatigue resistant, absence of sag during unfused tetani) always possessed MN afterhyperpolarization half-decay time (AHPdec) > 20 ms, while fast (F) units always possessed values < 20 ms. Among F units, no significant r values above .5 were noted between MN and muscle unit properties. Similarly, when "slow" MN (AHPdec > 20 ms) were excluded from the total sample, no relationships among MN properties were evident. While slow and fast units in rat muscle can be distinguished based on AHPdec and twitch time-course, subgrouping of F units based on MN properties is less clear than in cat.

255.18

SYNAPTIC ORGANIZATION OF A SPINAL MOTOR POOL STUDIED BY COMPUTER SIMULATION. F.A.Dodge, IBM Research, T.J. Watson Center, Yorktown Heights, NY 10598.

By matching the isometric twitch kinetics, the structure of the motorneurons, and parameters of the Renshaw feedback to distribution measured on specific muscles, a mathematical model can be used to estimate how much excitatory synaptic drive projects to motorneurons of different size during simple reflexes. Tension at recruitment was found to be the most sensitive measure of the projection rules. For tonic reflexes, the excitatory interneurons must project with the same number of synapses independent or motorneuron type or size (S or FR). In this case, the largest motor units are recruited at about 40% or Tmax, at which value about 1/2 the tension can be ascribed to rate modulation. For a soleus muscle, 40% Tmax requires a maintained excitatory drive of 20 na to each neuron, or the firing of 200,000 synaptic endings per second (assuming all endings have the same efficacy as the Ia afferents). The observed frequency of departure from strict size-dependent recruitment order are found to be consistent with stochastic variation in structure measured in a given animal.

In a vibration reflex, the muscle tremor is generated mostly by the few largest active units, whose mean firing rates are seen as peaks in the observed power spectra. The magnitude of the tremor is smaller than that predicted from independent firing because Renshaw feedback introduces some negative correlation between larger and smaller units.

255.19

TRICEPS BRACHII FORCE OF ABLE-BODIED AND SPINAL CORD INJURED SUBJECTS. J.G. Broton, C.K. Thomas, B. Calancie, The Miami Project to Cure Paralysis and Dept. Neurol. Surg., Univ. of Miami, FL 33136.

Whole muscle and motor unit forces in triceps brachii were compared in able-bodied (A-B) subjects and those with cervical (cephalad to C6) spinal cord injury (SCI). SCI subjects were examined at least one year post-injury. Subjects sat in a dental chair. The test elbow was flexed at 90-100°. The forearm was supinated and stabilized in a support with Velcro strips. A transducer attached to the support near the wrist measured the force generated by isometric triceps contractions. EMG was recorded from the surface of the triceps and biceps brachii muscles. A bipolar needle electrode placed within the triceps muscle recorded single unit activity. Whole muscle force was measured from brief (3-5s) maximum voluntary contractions. Motor unit forces were measured by spike triggered averaging (STA). For STA, subjects were given auditory feedback of unit activity, and were asked to fire the unit at a slow steady rate for 1-2 min. All data were digitized and analyzed off-line with computer assistance.

Three A-B and six SCI triceps muscles have been studied. A-B subjects were much stronger than SCI subjects. During maximum voluntary contractions, triceps forces ranged between 180-550 N and 10-57 N for A-B and SCI subjects respectively. However at comparable firing rates, the forces produced by motor units in SCI muscles were either comparable or stronger than those of A-B subjects. Unit forces ranged from 11 to 789 mN for SCI subjects (mean ± SD 165 ± 207 mN; n=25) while those of A-B subjects ranged from 7 to 92 mN (37 ± 24 mN; n=44). Larger than normal unit forces were measured in each SCI muscle studied. We suggest these strong units are due to denervation and subsequent motor axon sprouting because: 1) many of the unit potentials were polyphasic; 2) averages of triceps surface EMG showed that the same unit could be encountered at distant muscle sites; 3) unit activation was often accompanied by visible muscle contractions; and 4) denervation potentials were sometimes present (Supported by The Miami Project to Cure Paralysis).

255.20

CNS MOTOR DRIVE AND MOTOR UNIT FIRING RATES IN TRICEPS BRACHII MUSCLE OF ABLE-BODIED AND SPINAL CORD INJURED SUBJECTS C. K. Thomas, J. G. Broton and B. Calancie The Miami Project to Cure Paralysis, Dept. of Neurol. Surg., Univ. of Miami, Miami FL 33136.

CNS motor drive and motor unit firing patterns were examined during voluntary contractions of triceps brachii in individuals with chronic and incomplete cervical spinal cord injury (SCI) involving levels cephalad to C6. Data from able-bodied (A-B) subjects served as controls. The subject sat in a dental chair with the test elbow flexed at 90-100°. The forearm was supinated and stabilized in a support by velcro straps. The isometric force of triceps brachii was measured with a transducer positioned at the wrist. CNS motor drive was assessed by superimposing single: 1) electrical shocks to the radial nerve and; 2) magnetic transcranial cortical stimuli at Cz on submaximum and maximum voluntary contractions (MVCs) of triceps brachii. All A-B subjects were able to activate triceps brachii maximally by voluntary effort. With either method of stimulation, the amplitude of the superimposed twitch decreased with increasing voluntary effort and the twitch disappeared at maximum force. In SCI subjects, the force evoked by radial nerve stimulation was not eliminated during MVCs. In these same subjects, no force could be evoked by magnetic cortical stimulation during MVCs of triceps. Thus, the force increment seen with radial nerve stimulation during MVCs must represent the contribution of motor units paralyzed by loss of central input to triceps motoneurons.

Motor unit potentials were recorded intramuscularly with a bipolar or tungsten needle. In SCI subjects, motor units were recruited at all force levels. However, unit firing rates differed markedly between subjects. In one SCI subject, minimum and maximum motor unit firing rates varied between 7-45 Hz whereas in another SCI subject, motor unit firing rates never exceeded 13 Hz, even during MVCs. The low motor unit firing rates seen in some SCI subjects may reflect how the CNS (and/or muscle) attempts to compensate for SCI and/or may reflect the nature of the injury (Supported by The Miami Project).

256.1

THE ACTIVITY OF FELINE PROXIMAL FORELIMB MUSCLES DURING LOCOMOTION.C.I.PALMER. Inst.of Physiology, Fribourg Univ.Switzerland.

In order to investigate the fine tuning of muscle activity to the varied requirements of overground locomotion the 3 heads of triceps -:lateral (LaT), long (LoT) and medial (MT) all proximal forelimb extensors and the proximal forelimb flexors biceps brachii (Bi) and brachialis (Br) were recor--ed from chronically implanted EMG electrodes in freely moving cats (LaT n3, LoT n4, MT n2, Bi n4, Br n2) during locomotion on a treadmill with unpredicatble minor variations in treadmill speed.

The extensor burst duration of LaT (n6,82 to 131 steps) and MT (n3, 82 to 131 steps) had a significant (P<0.05, Pit--man's test) positive correlation to step duration. LoT either had a significant positive correlation (n8, 82 steps) or an insignificant correlation either negative or positive to step duration (n3, 82 steps).

The disparate relationship of LoT to step duration is accounted for by 2 strategies to maintain a constant position on the treadmill.1)LoT decreases in amplitude and duration, MT parallels this decrease in amplitude, LaT increases in amplitude and overlaps Bi and Br during the first part of swing. Br and Bi increase in amplitude and duration. LoT has a burst of EMG in swing which is negatively correlated to LoT extensor activity.2) LoT, MT, LaT, Bi and Br increase in amplitude. The amplitude of the LoT swing burst is posit--ively correlated to the amplitude of the LoT extensor burst.

256.2

FORCES GENERATED BY CAT MEDIAL GASTROCNEMIUS MOTOR UNITS DURING SIMULATED WALKING. J.L.F. Weytjens and J.A. Hoffer. Dept. of Clinical Neurosciences, U. of Calgary, Calgary AB, CANADA T2N 4N1.

Muscle force depends on both *muscle fiber length* and its rate of change. In order to study the characteristics of both dependencies during normal locomotion we used a two-pronged approach. First, we recorded muscle length, muscle fiber length, muscle force and electromyograms from the medial gastrocnemius (MG) muscle of chronically implanted cats walking on a treadmill. Second, in terminal acute experiments on the same cats, we replicated chronic conditions, i.e. muscle fiber length and muscle force, by imposing the chronically recorded muscle length changes with a servo-controlled motor and optimizing the stimulus patterns used for distributed stimulation of five small ventral root filaments (L7 and S1). We then ran simulations *with* (active muscle) and *without* distributed stimulation (passive muscle), and *with* and *without* stimulation of an additional ventral root filament containing up to 6 motor units. The latter filament was also stimulated at different constant muscle lengths, with and without distributed stimulation at constant rates.

When muscle length was adequately replicated, reproduction of muscle fiber length was very good in the active muscle. In the passive muscle, however, it was quite different from the chronically recorded measurement. In the passive muscle, stimulation of the filament under study induced appreciable muscle fiber shortening (up to 2 mm), both during simulated walking and under muscle isometric conditions. The time course of shortening was a mirror-image of the time course of force generation. In contrast, in the active muscle, muscle fiber lengths with and without stimulation of the test filament were often indistinguishable. Specifically, with constant background stimulation, fiber length deviated only slightly from isometricity. During simulated walking the forces generated by the small test filament were much smaller in the active than in the passive muscle. Construction of force-length curves indicated that this result could at least in part be accounted for by the fact that MG, during treadmill locomotion, operated on the ascending limb.

These results show that 1) muscle fiber length, a crucial variable on which forces generated by motor units depend, can be well reproduced in acute experiments, 2) length-dependence of forces generated by motor units cannot reliably be studied in isometric passive muscle, because of appreciable fiber shortening, and 3) when small multi-unit filaments are stimulated, fiber length can be held (quasi-)isometric, in intact muscle, by using constant rate background distributed stimulation.

Funded by the Med. Res. Council and the Musc. Dystr. Assoc. (Canada).

256.3

FORCE OUTPUT AND EMG ACTIVITY FOLLOWING EXERCISE INDUCED MUSCLE INJURY. J.N. Howell, G.Chleboun*, J. Cummings*, and R. Conatser*. Ohio Univ. Coll. of Osteopathic Med., Athens, OH 45701.

We examined the relation between force output (FO) and EMG activity in human muscle after exercise (EX). Subjects repeatedly lowered with their elbow flexors a heavy load, normalized to their own strength, until they could no longer control the rate of lowering. This typically took 10 repetitions or less and was repeated 3 times. Isometric strength fell to less than 60% of control on the day after the EX and returned only to 80% 14 days later. The relation between FO and EMG activity was linear over a range of submaximal contractions both before and after EX; the slope gave the ratio of FO to EMG. This ratio dropped by 69% immediately after EX, but gradually returned to normal 4 days later, at a time when the maximum FO capability of the muscle was still 30% below pre-exercise values. A similar pattern occurred with maximum contraction, except that the FO/EMG ratio did not return fully to control values. Injured fibers, initially capable of generating action potentials, but little FO, apparently become electrically silent within 4 days after injury. (Supported by the Am. Osteopathic Assn.)

256.4

CHRONIC CHANGES IN PROPERTIES OF CAT MEDIAL GASTROCNEMIUS (MG) MUSCLE AFTER FUNCTIONAL ELECTRICAL STIMULATION OR HEMISECTION AND DEAFFERENTATION. V.F.Rafuse, M.C. Pattullo* and T.Gordon. Dept. of Pharmacology, Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada, T6G 2S2.

Neuromuscular activity of the cat MG muscle was either increased by functional electrical stimulation (FES; 20Hz, 50% duty cycle), or reduced by spinal hemisection and unilateral deafferentation, to determine the time course of changes in muscle force, speed and endurance. These changes were monitored weekly by coupling the foot to a force transducer via an external boot, and isometric contractions elicited by stimulation of MG nerve via implanted nerve cuffs. In addition, acute experiments were performed after 6-24 weeks to measure the same parameters at the motor unit level. Chronic recordings showed that whole muscle tetanic force was reduced after only 1 week of FES reaching a minimum of 50% of the initial force by 2-3 weeks, while reduction of activity had no effect on force production even after 20 weeks. With FES, the muscle became fatigue resistant (2-minute test) by 4-6 weeks, contrasting the progressive decrease in endurance seen following spinal hemisection and deafferentation. Decrease in muscle contractile speed was gradual but had slowed significantly after 24 weeks stimulation. By this time, force, contractile speed and endurance of isolated units was the same as the normal slow unit sub-population, but at earlier times (4-10 weeks) these parameters were intermediate between the total unit and slow unit populations of normal muscles. Thus, FES has a rapid and dramatic effect on unit size, in addition to the established effect on endurance and speed, in contrast to the small changes observed after inactivation by spinal hemisection and deafferentation. (Supported by MRC, MDAC and Network of Centres of Excellence.)

256.5

EFFECTS OF IMAGINARY MUSCLE CONTRACTION TRAINING ON STRENGTH OF IMMOBILIZED MUSCLE. G. Yue[1], S. Wilson[1], W.G. Darling[1], W.T.C. Yuh[2] and K.J. Cole[1]. Dept. Exercise Science[1] and Radiology[2], University of Iowa, Iowa City, IA 52242.

In a previous study we demonstrated that voluntary strength of a human finger muscle increased with imaginary maximal muscle contraction (IMMC) training, that is, without muscle excitation (Yue, G., Soc Neurosci Abstr, 107.7, 16:244, 1990). However, it is not known if the subjects' activities with the finger outside of the laboratory during the training weeks contributed to the force increases. The purpose of this study was to investigate the effects of the IMMC training on voluntary strength of human abductor digiti minimi (ADM) muscle which was immobilized during the training period.

The subjects' fourth and fifth fingers of the non-dominant hand were immobilized in a plaster cast for 5 weeks in an effort to induce atrophy of the ADM. During this period the subjects were asked to produce 15 IMMCs of the ADM in each training session for a total of 25 sessions. EMG recordings during training indicated that the trained muscle was inactive. Magnetic resonance imaging of the muscle cross-sectional areas exhibited atrophy following immobilization. We found that after immobilization the mean strength of the ADM of the IMMC subjects (N=5) increased 9.2% and that of the control subjects (N=5) whose fingers were also casted showed a 1.4% decrease. The difference between the two groups was statistically significant. Moreover, the integrated surface EMG of the hypothenar muscles increased in both hands in the IMMC group and decreased in both hands in the control group. These results suggest that training which utilizes imaginary muscle contractions is able to maintain or even increase a muscle's strength by strengthening voluntary neural drive to that muscle.

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256.6

LIMB IMMOBILIZATION INDUCES NON-UNIFORM ADAPTATIONS IN SOME MOTOR UNIT PROPERTIES IN A CAT HINDLIMB MUSCLE. M.A. Nordstrom[1], R.M. Enoka[1,2], R.M. Reinking *[1], K.M. Gothard*[1] and D.G. Stuart[1]. Depts. of Physiology[1] and Exercise & Sport Sciences[2], Univ. of Arizona, Tucson AZ 85724.

We have studied cat tibialis posterior (TP) motor units (MUs) after six weeks of immobilization of the right hindlimb by splinting. Physiological properties of single MUs (n=172) in 12 control and 9 splinted cats were examined by stimulating ventral-root filaments. Wet weight of immobilized TP muscle was 87 ± 16% (SD) of contralateral control. Whole-muscle twitch force was unaffected by immobilization but twitch contraction time (CT) was significantly reduced. For all MU types, twitch force, CT and axonal conduction velocity were not significantly different in immobilized TP. Mean tetanic force (TET) was significantly smaller in all MU types (S, 41% of control; FR, 55%; FF 77%). Twitch/tetanus ratio increased significantly in S (173%) and FR (73%) units, but was unchanged in FF units. Both whole-muscle and MU twitch force were unaffected by atrophy despite reduced MU TET and myofibril cross-sectional area (Callister *et al.*, Neurosci. Abstr. 17, 1991). These results are qualitatively similar to those observed following long-term immobilization (17-29 wks; Mayer *et al.*, Neurosci. 6:725, 1981), but differ from those following short-term (3 wks) immobilization (Robinson *et al.*, Muscle & Nerve, 1991, in press). After 3 weeks of immobilization, twitch and tetanic force *increased* in FF units and there were a large number of units that produced no force. Taken together, these data suggest that the temporal effects of immobilization may differ among the three motor unit types. Supported by USPHS grants NS 20544, HL 07249, NS 25077, NS 07309 and RR 05675. M.A.N. is a C.J. Martin Fellow of the NH&MRC of Australia.

256.7

MORPHOLOGIC CHANGES IN IMMOBILIZED TIBIALIS POSTERIOR CAN ACCOUNT FOR DECREASED PEAK FORCE OF FATIGABLE, BUT NOT FATIGUE-RESISTANT, MOTOR UNITS. R.J. Callister[1], M.A. Nordstrom[1], D.H. Laidlaw[2], R.M. Enoka[1,2], R.M. Reinking*[1], and D.G. Stuart[1]. Depts. of Physiology[1]and Exercise & Sport Sciences[2], Univ. of Arizona, Tucson AZ 85724.

Tibialis posterior (TP) muscles of 9 cats were examined following six weeks of immobilization of the right hindlimb by splinting. Transverse sections of right and left TP were stained for myosin ATPase activity (pH 9.4) following preincubation at pH 4.4 - 4.5. Approximately 800 fibers per muscle were classified as type I, IIa or IIb, and fiber-type proportions and cross-sectional areas were assessed from digitized images. Fiber-type proportions were unchanged by immobilization (splinted: I, 28%; IIa, 27%; IIb, 45%; control: I, 29%; IIa, 28%; IIb, 43%). Cross-sectional area was reduced significantly in each fiber type by immobilization (I, 71% of contralateral control; IIa, 77%; IIb, 79%). These changes were compared to MU data obtained from the same animals (Nordstrom *et al.*, Neurosci. Abstr. 17, 1991). The reduced cross-sectional area in IIb fibers in immobilized TP matched the smaller tetanic force of the corresponding type FF MUs (77% of control), suggesting that force reduction in these MUs could be explained by the reduction in myofibrillar contractile elements. In contrast, the reduction in type I and IIa fiber areas were insufficient to explain the smaller tetanic force in the S (41% of control) and FR (55% of control) MUs. In the absence of a change in fiber-type proportions in immobilized TP, these data suggest that additional factors, perhaps related to myofibrillar specific tension or force transmission, are involved in the atrophic response of S and FR MUs to immobilization. Supported by USPHS grants NS 20544, HL 07249, NS 25077, NS 07309 and RR 05675. M.A.N. is a C.J. Martin Fellow of the NH&MRC of Australia.

256.8

DIFFERENTIAL RESPONSE OF CAT MUSCLE FIBERS AND EXTRACELLULAR CONNECTIVE TISSUE TO INCREASED AND DECREASED NEUROMUSCULAR ACTIVITY. **B. Jiang, G.R. Chalmers, R.R. Roy and V.R. Edgerton. Brain Research Institute and Kinesiology Dept., UCLA, L.A., CA, 90024-1527.**

The adaptation of muscle fibers and extracellular connective tissue has been quantified in models of altered neuromuscular activity in cats. The reduced activity models (6 months, soleus) studied were: 1, Spinal Transection (ST) with daily standing (n=4) or no training (n=4); 2, Spinal Isolation (SI) with daily passive hindlimb oscillation (n=5) or no training (n=6). Five normal cats served as controls (S-C). The increased activity model (3 months, plantaris) studied was functional overload (FO, n=6) induced by synergist removal and daily exercise. Six cats served as controls (FO-C). Frozen sections were stained with FITC conjugated lectin (Con A) to identify extracellular space (ES). Mean fiber cross-sectional area (CSA) and ES per fiber, and fiber/ES ratio were calculated. There was no difference between the trained and untrained cats within the ST and SI groups, so these data were combined.

	S-C	ST	SI	FO-C	FO
CSA (pixels/fiber)	5232	3555	1462*	3938	9194*
ES (pixels/fiber)	1084	1103	1028	867	2033*
Fiber/ES	5.2	3.3*	1.6*	5.1	4.9

*differs from control ($p<0.05$).

In FO, there was a similar increase in CSA and ES per fiber, suggesting that muscle fibers and associated connective tissue adapt to overload proportionately. Fiber size decreased in SI, while ES was unaffected in SI and ST, demonstrating that muscle fiber CSA, but not connective tissue, decreases in response to reduced neuromuscular activity. These data demonstrate that muscle fibers and surrounding connective tissue respond differentially to alterations in neuromuscular activity level.

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256.9

ELECTRICAL STIMULATION OF THE SCIATIC NERVE IN RATS INDUCES *C-FOS* PROTEIN EXPRESSION IN HINDLIMB MUSCLE FIBER NUCLEI. B.L. Goldman*, H.H. Ellenberger, R.R. Roy and V.R. Edgerton Dept of Kinesiology and Brain Research Institute University of California Los Angeles, Los Angeles, CA 90024-1527.

The proto-oncogene *c-fos* is thought to encode transcriptional regulatory proteins. Northern blot analyses have demonstrated the presence of *c-fos* protein mRNA in skeletal muscle. In the present study, a muscle-specific selective expression of *c-fos* protein in muscle nuclei was induced via electrical stimulation of the sciatic nerve. Chloral hydrate-anesthetized rats were stimulated for three hours at 5-10 times the threshold needed to elicit muscle contraction. Immediately following stimulation, the rats were perfused with 2% paraformaldehyde, and the soleus and tibialis anterior (TA) muscles were removed and quick frozen in isopentane cooled in liquid nitrogen. Control rats were anesthetized and the sciatic nerve was isolated but not stimulated. Immunohistochemical detection using polyclonal antiserum to the n-terminal region of the *c-fos* protein showed extensive labeling of nuclei in the stimulated, but little or no labeling of nuclei in the non-stimulated soleus. There also appeared to be a greater number of immunoreactive nuclei in the stimulated than in the non-stimulated TA. The proportion of nuclei labeled in the stimulated soleus far exceeded that observed in the stimulated TA. These data are consistent with the hypothesis that myonuclear expression of immediate-early genes such as *c-fos* can play a role in muscle adaptation. Supported by NIH Grant NS16333 and NASA Grant NGT-50562.

256.10

COMPARISON OF TETRODOTOXIN (TTX)- AND DENERVATION-INDUCED DISUSE ON RAT MYOFIBRILLAR PROTEINS. R.N. Michel, G. Cowper*, and H. Falter*. School of Human Movement and Dept. of Chemistry, Laurentian University, Sudbury, Ontario, P3E 2C6.

The purpose of this study was to examine the adaptations of myofibrillar proteins to the removal of neural influences using two different conditions: nerve inactivation with the sodium channel blocker TTX and nerve axotomy causing muscle denervation. The left hindlimb of Sprague Dawley rats was inactivated by either chronic sciatic nerve superfusion with TTX using an osmotic pump and cuff delivery system or denervated (DEN) by excision of a segment of the sciatic nerve. After 2 wks of disuse the plantaris from left and sham-operated right hindlimbs of all animals were removed and myofibrillar protein characteristics compared to each other and to an external control group. Plantaris absolute and relative wet weights were lower by 47% after DEN and TTX. Total protein concentrations (mg/g muscle wt) after DEN and TTX were not different from controls. Myofibrillar protein concentrations (mg/g) after DEN were also similar to controls, whereas values for TTX had a tendency ($p<0.06$) to be 25% lower than controls. Non-specific myofibrillar ATPase activities (μmol Pi/mg/min) at different Ca^{2+} concentrations (pCa range of 9.00 to 3.00) were 26% and 22% lower than controls in the TTX and DEN groups, respectively. At high Ca^{2+} concentrations (pCa 4.42 and 3.00) Ca^{2+}-specific myofibrillar ATPase activities were 44% and 19% lower than controls after TTX and DEN, respectively. There were no clear differences in densitometric scans of SDS-PAGE myofibrillar protein patterns between conditions. In conclusion, although DEN and TTX cause a similar loss of muscle mass, their effect on myofibrillar protein quantity and enzymatic function is different.

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256.11

CRITICAL DIFFERENCES BETWEEN SLOW OXIDATIVE MUSCLE FIBERS IN HETERO- AND HOMOGENEOUS MUSCLES. L. L. Glenn and P.J. Rebeta, Department of Physiology, Ohio College of Pod. Medicine, Univ. Circle, Cleveland, Ohio 44106.

In order to determine how muscle fibers should be grouped, fibers of slow-oxidative (SO) muscles of uniform type (homogeneous) were compared to SO fibers in muscles of mixed type (heterogeneous). Measures of myosin ATPase activity, NADH dehydrogenase activity, fiber density, and microvasculature were taken in the feline knee extensors. The vastus intermedius (VI) muscle, which is a homogeneous type SO muscle, was compared to those for nine subdivisions in rectus femoris, vastus lateralis (VL), and vastus medialis (VM), which are all heterogeneous muscles. Similarity analysis using Euclidean distances linked VI most closely to the glycolytic subdivisions of VL and VM. The most influential factors in this linkage were capillary density and fiber density. Taken in conjunction with published findings in other muscles, these results indicate that type SO fibers of homogeneous muscles and heterogeneous muscles are of fundamentally different type, and these should not, in general, be classified together.

256.12

COMPARISON OF THE PATHOGENESIS OF DIABETES MELLITUS IN SLOW VS. FAST TWITCH MUSCLES.

David J. Porta and Kathleen M. Klueber, Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, KY 40292.

One way that diabetes manifests is progressive weakness in the lower limb presumed to be of neurogenic and myogenic origins. The objective of this study was to compare cytoarchitectural changes in the postural soleus (SOL) muscle with the fast twitch extensor digitorum longus (EDL). Female C57Bl/KsJ-dbm diabetic and control mice were sacrificed (ages: 5 to 27 weeks; N = 5/age). The muscles were fixed in situ, processed, and analyzed by standard electron microscopic procedures. Muscle degeneration was correlated to the time course of the disease in slow twitch (SOL) muscle and to both the time course of the disease and blood glucose levels in the fast twitch muscle (EDL). The data indicated that the slow twitch muscle offers more resistance to the disease. Funded by: NIDDK 1R29DK41553.

256.13

SUSCEPTIBILITY TO NEUROTRANSMISSION FAILURE VARIES ACROSS MUSCLE FIBER TYPES. B.D. Johnson*, M. Fournier and G.C. Sieck. Mayo Clinic and Foundation, Rochester, MN 55905.

The purpose of this study was to examine the susceptibility of rat diaphragm muscle fibers to neurotransmission failure (NF). Susceptibility to NF was assessed by intracellular analysis of evoked end-plate potentials (EPPs) using an *in vitro* nerve/muscle preparation. Failure to evoke an EPP provided evidence of NF. During 10 Hz stimulation, there was no evidence of NF, whereas at 75 Hz, there was a 25% incidence of NF. In a previous EMG study it was suggested that NF may be more prevalent in fast fatigable muscle unit fibers (IIB) compared to fast fatigue resistant (IIA) or slow (I) unit fibers. To evaluate this hypothesis, fiber glycogen depletion patterns were compared after direct muscle and nerve stimulation using an *in vitro* preparation. After direct muscle stimulation at 10 or 75 Hz (1 train/sec at 50% duty cycle for 8 min), the extent of glycogen depletion among fibers varied according to the following rank order: IIB, IIA, I. For all fiber types, the extent of depletion after 75 Hz muscle stimulation was about 30% greater than after 10 Hz. With 10 Hz nerve stimulation, the glycogen depletion pattern among fibers was similar to that observed after direct muscle stimulation. However, after 75 Hz nerve stimulation, the extent of glycogen depletion was less than that observed at 10 Hz, and the rank order of depletion among fiber types was reversed, i.e., I, IIA, IIB. We conclude that at higher stimulation frequencies fast fatigable muscle unit fibers (IIB) are more susceptible to NF than either IIA or I fibers.

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256.14

MYOSIN HEAVY CHAIN ISOFORM COMPOSITION OF DEVELOPING INTRAFUSAL FIBERS IN CHICKEN MUSCLE SPINDLES. A. Maier, Dept. of Cell Biology, Univ. of Alabama, Birmingham AL 35294.

Serial cross sections were incubated with three monoclonal antibodies to determine which myosin heavy chain (MHC) isoforms are present in intrafusal fibers (IF) of chicken leg muscles from embryonic day (E) 13 until 8 weeks *ex ovo*. One antibody reacts with atrial MHC (CA-83), one with both atrial and ventricular MHC (CCM-52) (Sweeney et al., Circ Res. 61:287-295, 1984), and the third with fast MHC (MY32) (Sigma Chemical Co.). Muscle spindles were first recognized at E13 when they contained at least one large IF. This presumptive primary myotube reacted strongly with CCM-52 and weakly with CA-83. At about E14 one other large myotube with immunohistochemical properties that were reciprocal to that of the initial myotube was recognized. Between E14 and E21 (hatching) small myotubes appeared, reacting strongly with CA-83 and mostly weakly with CCM-52. None of the IF did bind with MY32. The number of IF increased continuously until the time of hatching when the full complement had been generated in most spindles. Postnatally MY32 did still not bind with small IF, but reacted with IF of relatively large diameter that were negative for CA-83 and CCM-52. The same immunohistochemical properties seen postnatally in large and small IF were also noted in presumptive fast and slow extrafusal fibers, respectively, suggesting that in chicken leg muscles factors other than innervation may also influence MHC expression in maturing IF and extrafusal fibers, or that sensory and motor innervation exert similar influences.

256.15

CALCIUM-ACTIVATED (Ca^{2+}-)MYOSIN ATPase ACTIVITY OF MUSCLE UNIT FIBERS: RELATION TO MUSCLE UNIT FATIGUE RESISTANCE. C.E. Blanco, M. Fournier, and G.C. Sieck. Department of Anesthesiology and Physiology, The Mayo Clinic and Foundation, Rochester, MN 55905.

Ca^{2+}-myosin ATPase and succinic dehydrogenase (SDH) activities of individual muscle unit fibers in the cat diaphragm (DIA) and tibialis posterior (TP) muscles were determined using quantitative histochemical techniques. Muscle units were isolated by ventral root dissection and classified as fast- (type F) or slow-twitch (type S) using standard physiological criteria. Eight of the 10 units (3 DIA and 5 TP) were classified as type F units with fatigue indices ranging from 0.01 to 1.00. Muscle unit fatigue resistance was positively correlated ($p<0.05$) with the SDH activity of muscle unit fibers in both the DIA and TP ($r=0.974$ and 0.962), respectively). In contrast, a negative correlation ($p<0.05$) was found between muscle unit fatigue resistance and the Ca^{2+}-myosin ATPase activity of DIA muscle unit fibers. Such a correlation was not observed among Tp muscle units. These results would suggest that: 1) muscle unit fatigue resistance is dependent upon the generation of ATP via oxidative metabolism (i.e., SDH) and, 2) muscle unit fatigue resistance may also be dependent on ATP utilization during contraction (i.e, Ca^{2+}-myosin ATPase activity).

This research was supported by NIH grants HL34817 and HL37680.

256.16

MECHANO-LINKAGE OF MYONUCLEI WITH SARCOMERE LENGTH IN ISOLATED RAT SKELETAL MUSCLE FIBERS. B.S. Tseng*, D.L. Allen*, C.K. Sung*, C.E. Kasper* and V.R. Edgerton. Dept. of Kinesiology and School of Nursing. University of California, L.A., CA 90024.

Since skeletal muscle fibers have similar biochemical properties along their length, there must be some level of coordination of protein expression among the hundreds of myonuclei per fiber. In-vitro (Vandenburgh, Science 203:265-268,1979) and in-vivo studies (Sola, Exp. Neurol. 41:76-100, 1973) suggest that mechanical activity has a major role in skeletal muscle protein regulation. To evaluate the possibility of a mechano-linkage transduction pathway, the shape of myonuclei were observed during passive and active fiber length changes. Single rat soleus muscle fiber segments (n=6) were isolated, attached to a micromanipulator and individual myonuclei and sarcomeres were observed under Hoffman modulation (500X). Micromanipulation enabled passive stretch, while a bathing solution [pCa=4.8] was used to initiate submaximal active contractions. The myonuclei were stained with DNA specific dyes, Hematoxylin, Hoechst or Acridine Orange.

One discrete myonucleus was monitored during length changes in six fibers. A correlation of 0.90 ± 0.07 [SD] was observed between myonuclei length and sarcomere length by directly observing the same myonucleus on the same muscle fiber as sarcomere lengths were varied passively (1.33 μm to 4.00 μm) and actively (3.80 μm to 1.75 μm). These data are consistent with the hypothesis that mechanoreceptors, mechanical-biochemical links, and/or stretch-activated nuclear pores are involved in the regulation and coordination of myonuclei within a skeletal muscle cell.

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256.17

BIOCHEMICAL CHARACTERISTICS OF CHOLINESTERASES FROM SEA-BASS TISSUES. F. BACOU, M. YASSINE*, H. ALAMI-DURANTE*+. Département de Physiologie animale, INRA, Place Viala, 34060 Montpellier Cedex 1, and + Département d'Hydrobiologie, INRA, St-Pée sur Nivelle, 64310 Ascain, France.

Sea-Bass (*Dicentrarchus labrax*) tissues contain two types of cholinesterases (ChE) that differ in molecular forms and in substrate specificity. One type of ChE is a true acetylcholinesterase (AChE). The second type of enzyme, called atypical, hydrolyses both acetylthiocholine and butyrylthiocholine. The distribution of both enzymes is tissue-dependent and occurs mainly in the detergent soluble (DS) fraction. In muscle, most of the high ChE activity is related to the atypical enzyme, and to true AChE. In the Central Nervous System (CNS), a high level of AChE only is detected. Whatever tissues, the atypical ChE occurs only as a form sedimenting under our experimental conditions at 4.5S in the presence of Triton X-100. AChE occurs in muscle as collagen-tailed asymmetric forms sedimenting at 17.5S (A_{12}), 13.5S (A_8) and 8S (A_4); globular forms occur as G_4 (10.5S) and a lighter form sedimenting at 5.5S. In CNS, AChE occurs mainly as G_4 form. The 5.5S globular form, and A_{12} and A_8 asymmetric forms are also detected.

AChE appears first during development. In muscle, AChE occurs mainly as A forms. In CNS, AChE shows the same pattern as in the adult.

Our data show that the atypical ChE presents intermediate characteristics of AChE and butyrylcholinesterase which suggest that this enzyme originates from a common polymorphic ChE. These results also show that the classification of ChEs as either "true" (E.C. 3.1.1.7.) or "pseudo" (E.C.3.1.1.8.) is inadequate to the atypical enzyme of muscle sea-fishes.

257.1

THE LATERAL BASAL OPTIC ROOT IS COMPRISED OF AXONS ORIGINATING FROM GANGLION CELLS IN THE PERIPHERAL RETINA OF FROGS. Z. Li, K.V. Fite and J.A. Corsa, Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

We have previously reported that the nucleus of the basal optic root (nBOR) of anuran amphibians is innervated by the basal optic root (BOR) which includes both lateral (BORl) and medial (BORm) fascicles, and the medial portion of nBOR appears to receive input predominantly from the central retina (Fite, et al, 1988; Li, et al, 1990).

In the present study, rhodamine latex microspheres (RLM) were injected into the lateral portion of nBOR which is innervated by BORl in order to examine the distribution of retrogradely labelled ganglion cells in the retina. Three different sizes of retinal ganglion cells were observed following RLM injection; small cells with soma 7 um in diameter, medium cells with soma 10 um in diameter and large cells with soma 13 um in diameter. Retrogradely labelled ganglion cells were mostly (more than 70%) located in the peripheral retina. Medium sized ganglion cells represent the largest component (approximately 2/3's) of the retinal input to the lateral portion of nBOR. Thus, BORm and BORl originate from different portions of the retina. (Supported by NSF Grant BNS 8819870 to K.V.F.)

257.2

HYPOTHALAMIC AFFERENTS IN THE TOKAY GECKO. **T.J. Neary and L.L. Bruce. Anatomy Division, Creighton Univ., Omaha, NE 68178.**

Iontophoretic and needle applications of wheat germ agglutinin/horseradish peroxidase conjugate were made in the medial and lateral hypothalamus of Gekko gecko. Applications in either area labelled cells in the preoptic area, posteroventral thalamic nucleus (ventrolateral to nucleus medialis), isthmal raphé, and two populations ventral to nucleus isthmi, tentatively named parabrachial and pedunculopontine nuclei. Applications centered in the ventral medial hypothalamus also labelled cells in the posterior medial dorsal ventricular ridge (core and shell), lateral and medial amygdalar nuclei, and nucleus sphericus. Applications in the lateral hypothalamus labelled cells in the dorsal cortex, strio-amygdalar area, septal nuclei, diagonal band nucleus, ventral tegmental area, and solitary nucleus. Applications which extended into the dorsal medial hypothalamus labelled cells in the dorsal cortex, septal nuclei, and lateral habenula.

Supported by a BRS Grant to Creighton University.

257.3

NEURAL CIRCUITRY AND IMMUNOCYTOCHEMICAL PROPERTIES OF PRETECTAL INPUT TO THE VISUAL THALAMUS IN A REPTILE. M.B. Pritz and M.E. Stritzel*. Div. Neurol. Surg., Univ. Calif. Irvine Med. Ctr., Orange, CA 92668.

Nucleus rotundus is a prominent nucleus in the dorsal thalamus of reptiles, Caiman crocodilus. It receives retinal input via the optic tectum and projects to a well-defined area of the dorsal ventricular ridge. The present experiments investigated additional connections of nucleus rotundus.

The neural circuitry of pretectal afferents to nucleus rotundus was determined by injections of horseradish peroxidase (HRP) in juvenile Caiman. Retrogradely labeled neurons were observed in the ipsilateral nucleus pretectalis. Furthermore, HRP labeled axons were seen to leave nucleus rotundus, collect on its lateral border, and then course caudal and dorsally to terminate in the ipsilateral nucleus pretectalis. Thus, retrogradely labeled neurons, axons, and terminals were observed together in the same section through nucleus pretectalis. Immunocytochemical experiments used polyclonal antibodies to parvalbumin and calbindin in animals pre-treated with intraventricularly injected colchicine. Neurons immunoreactive to both of these calcium binding proteins were seen in nucleus pretectalis.

These experiments have demonstrated a reciprocal neural circuit between nucleus pretectalis and nucleus rotundus in Caiman. In so doing, additional sources of calcium binding protein immunoreactivity in nucleus rotundus that originated in nucleus pretectalis have been identified. Since classical GABA inhibitory input to nucleus rotundus and the rest of the dorsal thalamus is absent in Caiman, these observations suggest that calcium binding proteins may play an important role in synaptic transmission in the thalamus of these vertebrates.

257.4

CHOLERA TOXIN MAPPING OF RETINAL PROJECTIONS IN BIRDS. T. Shimizu, L.R.G. Britto, H.J. Karten and K. Cox*. Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

Cholera toxin subunit B (CTb) has been recently recognized as a sensitive anterograde and retrograde tracer. We investigated the retinal projections in birds using CTb combined with immunohistochemical techniques. Pigeons (*Columba livia*) were deeply anesthetized and received unilateral intraocular injections of CTb. In the hemisphere contralateral to the injected eye, CTb-immunoreactive fibers and presumptive terminals were found in several known retinorecipient regions: the superficial layers of the optic tectum; ventral lateral geniculate n. (GLv); principal optic n. of the thalamus; n. of the basal optic root (nBOR); suprachiasmatic n. (SCN); and pretectal nuclei. Furthermore, sparse arborizations were seen in several areas which have not been previously described: layers 8-9, and occasionally even deeper portions, of the optic tectum; n. suprarotundus; intergeniculate leaflet (IGL); and the anterior medial preoptic region dorsal to the initial point of attachment of the optic chiasm. This preoptic region appears to be directly comparable to the mammalian SCN. We also found small numbers of fibers and presumptive terminals in the ipsilateral nBOR, GLv, IGL, n. lateralis anterior, area pretectalis, n. pretectalis diffusus, and medial preoptic region. These projections are less likely due to transneuronal transport from the contralateral retinorecipient areas, since even in the contralateral hemisphere no CTb-immunoreactive projections were observed in other areas which receive secondary visual input. These results reveal more widespread retinal projections to the pigeon brain, including the ipsilateral input, than previously reported.

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257.5

GLUTAMATERGIC PHARMACOLOGY OF AUDITORY TRANSMISSION IN BARN OWL OPTIC TECTUM. DE Feldman* and EI Knudsen. Dept of Neurobiology, Stanford Medical School, Stanford CA 94305

Hypotheses of N-methyl-D-aspartate (NMDA) receptor involvement in experience-dependent developmental plasticity in many systems led us to investigate the participation of glutamate receptor subtypes in auditory transmission in barn owl optic tectum. Barn owl optic tectum contains an auditory map of space which is calibrated by visual experience during a critical period in early postnatal development. Multi-barrel iontophoresis electrodes with carbon-fiber recording barrels were used to monitor auditory responses of tectal neurons in adult owls during iontophoresis of glutamatergic agonists and antagonists. pH-equivalent solutions were used as controls. All units tested responded strongly and in a dose-dependent manner to glutamate, NMDA, and quisqualate iontophoresis.

Antagonism of auditory responses was measured with ejections of the nonspecific glutamate receptor antagonist kynurenic acid (KYN) and the selective NMDA receptor antagonist 2-amino-5-phosphono-valeric acid (APV) during presentation of dichotic auditory stimuli. KYN blocked auditory responses effectively and dose-dependently in deep and superficial tectum. When calibrated to a dose which blocked responses to applied NMDA but not to quisqualate, APV antagonized 40-80% of the auditory response in the tectum. In contrast, APV antagonized only 0-40% of the auditory response in the inferior colliculus (IC, external nucleus and lateral shell). These results indicate that auditory transmission in the tectum is at least in part glutamatergic, and that NMDA receptors participate in a large proportion of the adult auditory response. NMDA receptors may play a greater role in auditory transmission in the tectum than in the IC.

Supported by NIH R01 DC00155-12 and an HHMI Predoctoral Training Fellowship.

257.6

NEURAL AND VOCAL DEVELOPMENT IN BUDGERIGARS. S. E. Brauth, D. C. Zocchi, P. L. Cohen, W. Liang and W. S. Hall. Dept. of Psychology, Univ. of MD., College Park, MD. 20742.

Budgerigar (*Melopsittacus undulatus*) contact call learning was investigated during the first six weeks posthatching. Previous work has shown that budgerigars learn to produce contact calls during this period in reference to the calls of cagemates. At hatching, young animals produce small peeps and alarm calls. By two weeks posthatching, budgerigars produce food begging calls, the mean durations of which tend first to increase and then to decrease from 2-4 weeks posthatching. Virtually all budgerigars fledge and learn to produce individually distinct contact calls by six weeks posthatching.

There is considerable brain growth and differentiation of neural circuits during this six week period. At hatching the budgerigar brain is extremely immature with little differentiation of the anterior telencephalon including the hyperstriatum, archistriatum and paleostriatum. Neuronal proliferation and migration continue past the third week posthatching with the most substantial growth and development of auditory and vocal motor circuits occuring during the first 2 weeks. This includes development of auditory projections to the anteromedial hyperstriatum ventrale and archistriatal projections to midbrain and medullary nuclei. Thus the most striking vocal performance changes involving contact calls occur after basic motor and auditory circuits have differentiated by 2-4 weeks posthatching.

257.7

AN ANTEROGRADE STUDY OF CORTICOSTRIATAL PROJECTIONS TO THE BASAL GANGLIA IN PIGEONS. C.L. Veenman and A. Reiner. Dept. of Anatomy & Neurobiol., Univ. of Tennessee, Memphis, TN 38163.

We previously used retrograde labeling methods to clarify the sources of corticostriatal projections in pigeons (Neurosci. Abs., '91). In the present study we used several anterograde markers (PHA-L, TRITC-conjugated dextran amine and biotinylated dextran amine) to examine the extent and distribution of these corticostriatal fibers in the striatum. The anterograde tracers confirmed the observation from the retrograde studies that corticostriatal projections arise from a broad area of the telencephalon, including much of the rostrocaudal extent of dorsolateral telencephalic wall. These areas included the lateral frontal and intermediate neostriatum (NFL, NIL), the temporo-pariatal-occipital area (TPO), the dorsolateral corticoid area (CDL), the caudal dorsolateral neostriatum and the somatic archistriatum. Corticostriatal projections from these regions terminated in lateral striatum (including paleostriatum augmentatum, PA, and lateral lobus parolfactorius, LPO). Neostriatum inferior to olfactory cortex and caudal archistriatum were found to project to medial LPO and ventral striatum-olfactory tubercle. Anterogradely labeled axons in PA and ventral striatum showed few ramifications and possessed many boutons en passant, whereas labeled axons in LPO possessed more extensive arborizations with numerous en passant and terminal boutons. Thus, lateralmost LPO/PA differs from medialmost LPO/ventral striatum in its telencephalic input, with input to the former appearing somatic in nature. Supported by NS-19620 (A.R.)

257.8

CONNECTIONS OF THE HOMING PIGEON OLFACTORY CORTEX STUDIED WITH WGA-HRP AND FAST BLUE.
V.P. Bingman, G. Casini, C. Nocjar* and T.J. Jones*. Department of Psychology, Bowling Green State Univ., Bowling Green, OH 43403.

The avian olfactory cortex receives a direct projection from the olfactory bulb and has been implicated as playing a necessary role in the functioning of the homing pigeon navigational map. We have investigated the origins of afferent projections to, and targets of efferent projections from the olfactory cortex in homing pigeons. Telencephalic afferents included projections from the olfactory bulb, prepiriform cortex, anterolateral neostriatum, hyperstriatum dorsale, parahippocampus, dorsal archistriatum, n. taeniae and contralateral olfactory cortex. Subtelencephalic afferents included projections from the dorsomedial n. of the thalamus, n. subrotundus, mamillary region of the hypothalamus, area ventralis of Tsai, locus ceruleus and the medial raphe. Telencephalic efferents included projections to the hyperstriatum dorsale, septum, bed n. stria terminalis, parolfactory lobe, portions of the paleostriatal area, olfactory tubercle, parahippocampus, n. taeniae, and contralateral olfactory cortex. Subtelencephalic efferents included projections to the region of the lateral hypothalamus and n. intercollicularis. Taken together, the results identify a vast network of brain regions that connect with the olfactory cortex. At least part of this network presumably functions in the processing of olfactory information used for navigation.

257.9

LIMB PRIMARY AFFERENT INPUT TO REGIONS OF CERVICAL AND THORACO-LUMBAR SPINAL CORD CONTAINING NEURONS PROJECTING TO THE CEREBELLUM IN THE PIGEON. J.M. Wild, Dept. of Anatomy, Univ. of Auckland, Auckland, New Zealand.

Injections of cholera toxin B-chain (CTB) conjugated to horseradish peroxidase were made into the radial, median or ulnar nerves of the wing, or the sciatic nerve of the leg, in order to determine the rostrocaudal extent of their central terminations in the spinal grey. Outside the brachial enlargement wing nerve fibers travelled in the dorsal column, both rostrally in the cervical cord as far as the dorsal column nuclei, and caudally in the thoracic and lumbar cord as far as the lumbosacral enlargement. Throughout the entire extent of these cervical and thoracolumbar trajectories, fibers descended from the dorsal column to terminate densely and specifically in medial lamina V, ventrolateral to the apex of the dorsal column. At thoracic levels between the two enlargements, leg nerve fibers terminated in the same location. In order to determine whether cells in medial lamina V were part of a cerebellar projecting system, as has been suggested, injections of CTB were made into Folium VIb from which evoked potentials and multiunit activity of short latency were recorded following either electrical stimulation of the radial nerve, or small displacements of wing feathers. Retrogradely labelled spinal neurons were visualized immunohistochemically and were found in Clarke's nucleus in both enlargements, and in medial lamina V of the cervical and thoracic cord outside the enlargements. Most medial lamina V neurons were located ipsilaterally in the upper five cervical segments. These results suggests the existence of a substantial disynaptic pathway primarily from the wing to the cerebellum in birds, which is possibly involved in the sensorimotor control of flight.

257.10

A MAJOR CLADE WITHIN AVES: EVIDENCE FROM THE SPINAL CORD.
C.J. Woodbury, Dept. of Ornithology, American Museum of Natural History, 79th at Central Park West, New York, NY 10024.

Unlike mammals and pigeons, cutaneous nerves in chickens form two non-overlapping somatotopic maps across the dorsal horn (DH) (Woodbury,Neurosci.Abst.13:1387,1987), each composed of a distinct subset of primary afferents (Woodbury,Neurosci.Abst.15:755,1989). The DH of chickens differs, therefore, from that of all other amniotes described to date, including pigeons, in that lamina III lies medial rather than ventral to lamina II (Brinkman and Martin,Brain Res.56:43,1973).

Comparative studies using HRP methods have revealed that this novel "chicken" character actually has a broad superordinal distribution among birds. This character is found in taxa exhibiting extreme ecological and morphological diversity (e.g., hummingbirds, parrots, loons, et al.), which suggests (in conjunction with physiological studies of chicken and pigeon DH) that it has no obvious adaptive significance. Outgroup analyses indicate that this novel character is derived within birds. In light of the conservative DH lamination patterns in non-avian amniotes, the most parsimonious hypothesis is that this character evolved once, and therefore identifies a major clade within Aves. These data shed new light on the phylogenetic relationships of some historically problematic taxa. (Supported by an F.M. Chapman Fellowship.)

257.11

DISTRIBUTION OF TYROSINE HYDROXYLASE IMMUNOREACTIVE NEURONS IN THE AVIAN FOREBRAIN. D.S. Henshel, J. D. Steeves, and D.M.S. Webster. Department of Zoology and School of Rehabilitation Medicine, University of British Columbia, Vancouver, BC, V6T 1Z4.

The immunocytochemical demonstration of tyrosine hydroxylase (TH), the first rate-limiting enzyme in the catecholamine synthetic pathway, is commonly used as an anatomical indicator of catecholaminergic neurons. We have examined the distribution of TH-immunoreactive neurons in the forebrains of the Great Blue heron (*Ardea herodias*), and chicken (*Gallus gallus*). As previously reported for other species, we found TH-immunoreactive neurons in the olfactory bulbs and pre-optic areas of both chicken and heron. However, we also found relatively sparse populations of TH-immunoreactive neurons in subregions of the heron forebrain including parts of the neostriatum, paleostriatum, archistriatum, the dorsal septal region (rostral to the hippocampus proper), the area corticoidea dorsolateralis (CDL), and the hyperstriatum. This potentially unique species characteristic may be either a "false-positive" due to an unidentified cross-reactive antigen or it may reflect a true species difference.

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257.12

IMMUNOHISTOCHEMICAL LOCALIZATION OF CHICKEN GONADOTROPIN-RELEASING HORMONES I and II (cGnRH I and II) IN TURKEY HEN BRAIN. J.R. Millam[1], P.L. Faris[2], B.K. Hartman[2], O.M. Youngren[3], M.E. El Halawani[4] and R.E. Phillips[3]. [1]Dept. Avian Sciences, UC-Davis, CA 95616, [2]Depts. of Psychiatry, Div. Neurosci. Res., and [3]Ecology, Evolution and Behavior, U of Minnesota, Minneapolis MN 55455 and [4]Dept. of Animal Science, U of Minnesota, St. Paul MN 55108.

The immunohistochemical (IHC) distribution of cells and fibers positively stained for either chicken GnRH I or cGnRH II was determined in brains of turkey hens. Cell bodies immunoreactive (ir) for cGnRH I were located: along the medial aspect of the lateral ventricles and extending into the area of nucleus accumbens and the bed nucleus of the stria teminalis; ventral to the septomesencephalic tract and extending medially to the third ventricle, caudally into the lateral hypothalamic area and rostrally into lobus parolfactorius; and in a diffuse band extending dorsally and medially from the medial preoptic nucleus to the dorsomedial anterior thalamic nucleus. cGnRH I fibers were evident in these areas in addition to the hippocampus, medial subhabenular nucleus, ventromedial hypothalamic nucleus and median eminence. Two groups of ir-cGnRH II cells were observed: a magnocellular group near the oculomotor nucleus and a parvicellular group lying medial to the nucleus of the basal optic root and extending in to lateral hypothalamic area. ir-cGnRH II fibers were prominent in: piriform cortex, lateral to nucleus taeniae, hippocampus, olfactory tubercle, lateral subhabenular nucleus, septal nuclei, medial edge of lobus parolfactorius, and several hypothalamic nuclei. No fibers containing cGnRH II were evident in the median eminence. These results suggest that cGnRH I and II occur in separate neuronal systems and that although cGnRH II remains implicated in reproduction, it is not a physiological secretagogue for gonadotropins.

257.13

CORRELATIONS OF CORPUS CALLOSUM SIZE IN ODONTOCETE CETACEANS. R.J. Tarpley and S.H. Ridgway*. Department of Veterinary Anatomy, Texas A&M University, College Station, TX 77843 and Naval Ocean Systems Center, San Diego, CA 92152

The midsagittal surface area of the corpus callosum was determined by computer-assisted morphometry in four odontocete cetacean families (Delphinidae, Monodontidae, Physeteridae and Ziphiidae) and correlated with brain weight and cerebral cortical surface area. Absolute callosal areas ranged from 41-829 mm^2 in 64 brains from neonates, juveniles and adults in 16 species. Species-averaged callosal area was small in relation to brain weight (.10-.24) and cortical surface area (.047-.097) when compared to other mammal groups. There was a tendency for relative corpus callosum size to be smaller in the larger-brained odontocetes, suggesting that increases in brain size were not necessarily linked with increased callosal trafficking. One delphinid, Tursiops truncatus, for which the largest single-species sample (n=16) was available, was examined for sex differences in callosal size relative to brain weight. Among 10 males and six females the averaged-ratio was identical between sexes.

257.14

A THREE-DIMENSIONAL RECONSTRUCTION OF THE BRAIN OF A FETAL PYGMY SPERM WHALE, Kogia breviceps. E.A. Gastineau, M.S. Jacobs. Maryland Psychiatric Research Center, Univ. of MD. at Baltimore, Catonsville, MD 21228, Glen Arm, MD 21057

A three-dimensional reconstruction of the brain of a fetal pygmy sperm whale, Kogia breviceps, was produced from original magnetic resonance images (MRI). A total of 166 images were obtained in 2 sets (head and body) and 4 planes (sagittal, coronal, oblique, and axial). The head was imaged with: 17 sagittal T2 (TR1500 TE20) weighted images acquired in 5mm thicknesses with a field of view (FOV) of 24cm; 24 coronal T2 weighted images with 5mm thicknesses and a FOV of 24cm; and 10 oblique T1 (TR600 TE20) weighted images acquired in 3mm thicknesses with a FOV of 14cm. These images were retrieved from a 16 track tape using an Ibex tape machine and software programs consistent with processing medical images. Images were then transferred to a Macintosh II with a 4 Mb YARC board and processed with LAI Multi Image Registration and Analysis (MIRA) software. Co-planar images were treated as volumetric data sets. These data sets facilitated 3-D reconstruction of the brain and generation of cortical surfaces. Quantification of brain structures were performed using software applications provided with MIRA. The quantifications were enhanced by the ability to digitally reslice these data sets at variable thicknesses and planes.

257.15

SURFACE FEATURES OF THE CEREBELLUM AND BRAINSTEM OF THE BOWHEAD WHALE, BALAENA MYSTICETUS. D.W. Duffield. Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA 70803.

Only recently has information become available on the brain of the bowhead, a very large baleen whale. Concerns over potential industrial effects have prompted long-term morphological studies utilizing specimens from subsistence-harvested animals. Observations were made on 11 brains from whales obtained under International Whaling Commission guidelines. The brain is removed via the large foramen magnum and placed in 10% formalin. The cerebellum accounts for about 1/5 of the brain weight. The primary fissure, appearing as an inverted "V", separates a relatively small rostral lobe from a massive caudal lobe. The hemispheres meet on the midline caudally, covering the caudal vermal lobules (VIII, IX, and X). The flocculonodular lobe is very small. The wedge-shaped ansiform lobule is wide laterally, filling the space between the simple lobule rostrally and the paramedian lobule caudally. The paramedian lobule is a rounded mass near the midline with transversely arranged folia, limited caudally by the parafloccular fissure which continues rostrally to limit the ansiform lobule laterally. The dorsal paraflocculus extends ventrolaterally from the paramedian lobule, turns rostrally, and loops around the intraparafloccular fissure to continue as the smaller ventral paraflocculus on the ventral surface of the hemisphere. Transversely arranged folia lateral to the lingula and central lobule extend as far laterally as those lateral to the culmen.

Brainstem and cranial nerve anatomy is typical for cetaceans. The cerebellum appears similar to that of the right whale, but interpretation and designations of the hemispheric lobules differ from those previously applied to the cetacean cerebellum.

257.16

ANATOMICAL DISTRIBUTION OF CORTICOTROPIN-RELEASING FACTORS IN EQUUS CABALLUS. M.A. Littlefield-Chabaud & P.A. Melrose. Dept. Anatomy, Louisiana State Univ. School of Vet. Med., Baton Rouge, LA 70803.

In horses, exercise-induced increases in corticotropin-releasing hormone (CRH) and vasopressin (VP) secretion are dissociated; suggesting that two or more cell populations are involved in this response. The present experiment was performed in order to examine localization of various corticotropin-releasing factors (CRFs) in the horse as compared to that described in rats and humans. Thick vibrotome sections of hypothalamus, amygdala and brainstem were taken from ten horses. Alternate sections were stained for CRH, VP or oxytocin (OXY) and projection drawings were made to map the hypothalamic distribution of immunoreactive (ir) neuropeptides. Ir-CRH-containing perikarya were found in the OVLT, suprachiasmatic preoptic nucleus and bed nucleus of the stria terminalis. Scattered CRH perikarya were also localized in the medial and lateral preoptic areas and the supraoptic nucleus. In the PVN of all animals, ir-CRF perikarya were localized primarily in lateral regions. This cell group included both magnocellular and parvocellular neurons that also stained for OXY and VP, respectively. In two of the animals, a large dorsal medial parvocellular PVN group was also found. All cells in this group expressed VP and a smaller subset appeared to include ir-CRH. Ir-CRFs were also colocalized in the central amygdaloid nucleus, dorsal vagal complex and the nucleus tractus solitaris. Thus, the distribution and coexistence of ir-CRFs in Equus caballus appears similar to that in other species. Experiments on the innervation of external median eminence areas and exercise-induced alterations in cellular neuropeptide content may help to improve our understanding of cell groups coordinating endocrine and ANS response to this particular stressor.

257.17

DISTRIBUTION AND MORPHOLOGY OF IMMUNOREACTIVE GONADOTROPIN-RELEASING HORMONE NEURONS IN EQUUS CABALLUS: A SUMMER BREEDING, SEASONALLY-ANOVULATORY MODEL. H.S. Cheramie*, P.A. Melrose & K.M. Knigge, LSU School Vet. Med., Baton Rouge, LA 70803 (HC, PM) & Univ. Rochester Med. Center, Rochester, NY 14642 (KM).

Comparative studies on the topography of gonadotropin-releasing hormone (GnRH) neurons may help to improve our understanding of fertile mammalian reproductive cycles. The present study was performed in order to characterize morphology of GnRH neurons in the basal forebrain of Equus caballus. Thick vibrotome sections from 6 male and 6 female gonadally-intact ponies were stained for immunoreactive (ir)-GnRH, GnRH-associated polypeptide (GAP) or GnRH prohormone (ppGnRH). Projection drawings were then prepared in order to map the distribution of ir-perikarya and fibers as confirmed by microscopic examination. Clusters of ir-perikarya were localized in the OVLT, medial preoptic areas, arcuate nucleus and ventrolateral to the ventromedial nucleus. Most ir-neurons appeared as smooth fusiform cells with beaded axons branching off from proximal dendritic segments. Fewer irregularly-contoured unipolar neurons and occasional multipolar neurons were also present. Further, thin ir-fibers with regularly spaced varicosities were closely applied to all forms of stained perikarya and presumptive dendritic processes. Numerous ir-fibers were also traced into the lateral septum, periventricular areas, perifornical regions and both lateral and medial external median eminence. There were not any obvious differences in cell structure or distribution of ir-neuropeptides with respect to sex or season. Structure of GnRH neurons in Equus caballus therefore appears similar to that found in polyestrous species. However, based on current data, this species apparently lacks projections normally localized close to the optic tracts and terminal fields in the median eminence include medial areas. Whether these regional differences are of functional significance requires further study. Grayson-Jockey Club Research Foundation, AVMA, USDA.

257.18

PARASAGITTAL COMPARTMENTS ARE NOT CONSERVED IN THE MAMMALIAN CEREBELLUM: DIFFERENCES AMONG RODENTS, BATS, AND INSECTIVORES. Lannoo, M. J., J. M. Zook, L. Maler and R. Hawkes. Muncie Cent. Med. Ed., Muncie, IN 47306; Dept. Zool., Ohio Univ., Athens, OH 45701; Dept. Anatomy, Ottawa Univ., Ottawa, Ont. K1H 8M5; Dept. Anatomy, Univ. Calgary, Calgary, Alb. T2N 4N1.

The monoclonal antibody anti-zebrin II has previously been shown to recognize discrete populations of Purkinje cells in the cerebellum. Zebrin II populations are arranged into alternating +/- parasagittal compartments. The general mammalian pattern appears to be seven compartments in each hemicerebellum, as shown by studies on rats, rabbits, guinea pigs, and marsupial opposums. Here we show that zebrin II distinguishes only three compartments in the bats *Eptesicus fuscus* and *Antrozous pallidus*, and at least eleven compartments in the shrew *Blarina brevicauda* and the mole *Parascalops breweri*. In rats and opposums, zebrin II^- regions develop by a suppression of initially zebrin II^+ Purkinje cells. During development, ingrowing afferents remain within zebrin II^+ or zebrin II^- borders. In *Eptesicus*, zebrin II^- compartments also form from a suppression of initially zebrin II^+ Purkinje cells, similar to the rat and the opposum. Therefore, while the mechanism of compartmentation appears to remain constant, compartment numbers vary across species. This variation may extend to regions recognized by ingrowing axons during development.

257.19

DETECTION AND CHROMOSOME MAPPING OF GENES AFFECTING BRAIN WEIGHT IN RECOMBINANT (RI) AND NON-RI INBRED MICE. J.K. Belknap, T.J. Phillips and L.A. O'Toole*, Research Service (151W), VA Med. Ctr., and Dept. of Medical Psychology, OHSU, Portland, OR 97201.

A quantitative trait loci (QTL) method was used, which is potentially capable of detecting and mapping gene loci controlling as little as 20% of the genetic variance. The BXD recombinant inbred (RI) series, derived from a cross between C57BL/6J (B6) and DBA/2J (D2) inbred strains, was used as the initial screen to determine associations between brain weight and 360 known marker gene loci. This yielded five candidate chromosome regions, each reflecting a potential locus (QTL) controlling brain weight. The second step was to test as many of these five as possible with a confirmatory analysis based on standard (non-RI) inbred strain data for brain weight previously reported by Storer (1967) and Roderick et al. (1973). Only those strains possessing either the B6 or D2 allele at relevant marker loci were used. Sufficient data to test two of the five candidate QTL were available. Of these, one was unequivocally confirmed--the D7rp2 region of chromosome 7. The genetic correlations between brain weight and body weight were not significant, indicating that these two traits are virtually genetically independent. (Supported by AA08621, NIDA Contr. 271-90-7405, and a VA Merit Rev.)

257.20

A 3D DIGITAL ATLAS OF A GENERIC RAT BRAIN

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Recently three dimensional (3D) reconstructions of whole brain anatomy have been applied as atlas models. The goals of these efforts are to provide spatially accurate (in 3 dimensions) volumes of brain anatomy that is visually realistic and digitally interactive. The difficulties have been the use of several animals to compose a single representative atlas and the inability to accomodate intersubject variability. We used an averaging approach to compute a generic atlas in order to circumvent these problems.

Four adult (300 g) Sprague-Dawley rats were sacrificed, and sectioned on a macrotome with the brains still in the cranium. The animals were not perfused so that gray/white differences could be easily detected by a digitizing camera. Four hundred 1 megabyte images (20 μm) were collected from the blockface of each brain. Acquisition of blockface imagery eliminated the need for alignment. Each complete 3D brain was oriented to the Paxinos and Watson stereotaxic coordinate space following identification of the bony sutures lambda and bregma and the interaural line. The models were then subjected to global and local deformations using techniques described by (Toga, *et al*, Neurosci Abs. 1990). These warpings placed all four into a common coincident space by weighting the contribution of each of the 4 brains by 25%. The voxels from each of the models were averaged and used to form the generic model of the brain. This model formed the data set for identification of neuroanatomic structures according to Paxinos and Watson.

257.21

STEREOTAXIC MAPPING OF RAT BRAIN:
A NEW DIGITAL APPROACH

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Neuroanatomic atlases have relied upon collections of two dimensional serial maps to describe the shape and location of brain structures. We sought to improve upon this traditional format by employing digital imaging to create a truly three-dimensional (3D) map of the rat brain.

The data used to build this map consists of 400 digitized images of the specimen blockface obtained during the cryoplaning of a whole head obtained from a 300 gram rat. The benefits of this data collection method include the following: (1) direct visualization of bony landmarks; (2) elimination at cutting artifact and distortion; (3) prefect image alignment;and (4) anatomic detail in the z-direction nearly equal to the resolution of the original plane of section.

These data were placed into a standard flat skull orientation on the basis of rotational and translational adjustments calculated from the relative positions of the bony landmarks, lambda and bregma. Transformed images were then rendered by resampling along coronal, sagital, and horizontal planes. This set images form a complete 3D stereotaxic map of the rat brain that can be accessed interactively.

Surface-based models of several brain structures were created from this 3D map and these were morphometrically compared to stereotaxic descriptions provided in previously published atlases. In addition, two dimensional drawings presented in these other studies were subjected to warping algorithms in an attempt to incorporate their anatomical detail into this digital map of the rat brain

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HUMAN COGNITION: EVENT-RELATED POTENTIALS, ATTENTION, METHODS

258.1

ENDOGENOUS ERPS IN MAN FROM FORAMEN OVALE ELECTRODES. M.D. Rugg, D.D. Potter, R.C. Roberts and C.D. Pickles (SPON: European Brain and Behaviour Society). Wellcome Brain Research Group, Dept. Psychology, Univ. of St Andrews, and Dept. Medicine, Univ. of Dundee, U.K.

ERPs were recorded during auditory and visual 'oddball' tasks from 8 patients in whom bilateral foramen ovale electrodes had been inserted to aid localisation of suspected temporal lobe seizures. Both tasks consisted of a random series (inter-stimulus interval 2 sec) of target ($p = 0.25$) and non-target stimuli, with the requirement to make a prompt motor response to each target. The foramen ovale electrodes were of stainless steel wire, and comprised 4 contacts, spaced 1cm apart. The deepest contact was inserted approximately 5 cm beyond the foramen ovale such that the electrode was subjacent to the medial aspect of the temporal lobe. Recordings were made from all 8 contacts, and from 3 midline scalp electrodes, with reference to a sterno-vertebral electrode pair.

In 6 patients, a target-evoked negative-going wave was consistently present in one or more foramen ovale contacts. This deflection occurred in the same latency range as the scalp P3 and, as with the scalp component, was longer in latency in the visual than the auditory task. Typically, the deflection showed a clear amplitude maximum at a single contact (never the most superficial), and was frequently asymmetric.

The distribution of the target-related deflection along the foramen ovale electrodes is suggestive of a local source. The deflection may reflect the endogenous ERP activity known to be generated in medial temporal structures.

258.2

SELECTIVE ATTENTION AND THE SUPRATEMPORAL N1 COMPONENT OF THE AUDITORY EVENT-RELATED POTENTIAL (ERP). W. Teder, R. Näätänen, K. Alho and J. Lavikainen*. University of Helsinki, Department of Psychology, Ritarikatu 5, SF-00170 Helsinki, Finland.

Auditory stimuli (duration 25 ms; 76 dB SPL) of 300 Hz (left ear) and 6000 Hz (right ear) were presented in random sequences to 9 experienced subjects. The interstimulus interval vas very short, varying between 60-200 ms. In the Attend condition, their task was to count, in different blocks, infrequent deviant stimuli of 330 Hz in the left, 6600 Hz in the right ear.

It was found that ERPs to attended tones showed a negative displacement (Nd) in relation to the ERPs elicited by unattended tones. This displacement was of the same amplitude for the two tones. In contrast, the exogenous N1 to the low tones was much larger than the N1 to the high tones during a reading condition. Therefore the present data suggest that the Nd, most importantly its early portion, is caused by an endogenous processing negativity (PN) summating to the exogenous N1 component(s).
This study was supported by The Academy of Finland.

258.3

AUDITORY MAGNETIC EVOKED FIELDS IN RESPONSE TO AN INCONGRUOUS NOTE WITHIN A MUSICAL SEQUENCE IN MUSICIANS AND NON- MUSICIANS.

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Center for Neuromagnetism, Department of Physiology & Biophysics, New York University, Medical Center, New York, NY 10016.

We have investigated whether the presence of an incongruity in a musical sequence generates distinct magnetic auditory responses as compared to other notes, and if so, if this response is lateralized differently for musicians and non-musicians. A familiar musical sequence of four notes (S) (250 to 400 Hz) with same duration (50 ms) and intervals (600 ms) was repeated 300 times and a deviant note (D) (one tone above the highest note of the sequence) was randomly placed with 30% incidence. The sequence was delivered binaurally with earphones. Magnetic recordings were made with the Gemini 14-channel system (BTi), for a total of 35 sites on each hemisphere.

In musically trained subjects the response to the D note demonstrated a significant difference in amplitude, at 100-130 ms. In addition there appeared a different magnetic component peaking at 150-170 ms. These two differences were clearly lateralized to the right hemisphere.

In musicians the analysis of melody may be similar to the analysis of language, at least in the sense that music cognition, as language, is lateralized. The fact that it involves the right hemisphere may indicate that music analysis is functionally quite different from linguistic analysis. In agreement with this view, in non-musicians the response to D notes had no noticeable lateralization. Moreover, these subjects mentioned that they had difficulty in following the melody, specially when the D note was in position 1.

These results suggest that in musically trained subjects, the recognition of melodic differences occurs earlier than in the controls and the responses to the whole musical sequence are more pronounced on the right hemisphere, in agreement with the view that music has a different connotation to professionals and to listeners.

258.4

THE EFFECTS OF TEMPORAL AND PARIETAL LESIONS ON EVENT-RELATED BRAIN POTENTIALS DURING AUDITORY SELECTIVE ATTENTION.

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We examined the effects of lesions of the inferior parietal lobe (IPL, n=7) and the temporal/parietal junction (T/P, n=9) on event-related brain potentials elicited in an auditory selective attention task. Tones were presented randomly to the two ears through headphones (rate 2.5-4/sec), with 1300 Hz tones presented to one ear and 700 Hz tones to the other. Patients and matched controls selectively attended to tones in one ear and responded to occasional targets while ERPs were recorded from 15 scalp electrodes.

T/P lesions reduced the amplitude of sensory ERPs, initially over the damaged hemisphere (N1a, 70-110 ms) and then bilaterally (N1b and N1c, latencies 110-170 ms). T/P lesions also resulted in reduced sensory ERPs to stimuli contralateral to the lesion. Nd components associated with attentional selection were slightly reduced in the T/P group, whereas target and novel P3s were more markedly diminished. The mismatch negativity was unaffected by either T/P or IPL lesions. The results suggest a critical role of the temporal parietal junction in sensory and cognitive operations that underlie auditory selective attention. (Supported by grants from the VARS and the NIDCD to DLW, and NIH to RTK).

258.5

LESIONS OF FRONTAL CORTEX DIMINISH THE AUDITORY MISMATCH NEGATIVITY IN HUMANS.

K. Alho#, D. L. Woods, A. Algazi*, R. T. Knight, and R. Näätänen#*, Dept. of Neurology, U.C. Davis, VAMC, Martinez, CA, 94553; and #Dept. of Psychology, University of Helsinki, Finland.

Deviant auditory stimuli presented in repetitive tone sequences elicit a mismatch negativity (MMN) that is maximal in amplitude over the frontal scalp. We evaluated the role of frontal cortex in MMN generation in 10 patients with unilateral frontal lesions (7 left, mean lesion volume 41.0 cc). Sequences of 1000 Hz tones (standards, probability(p) = 85%) and 1300 Hz tones (deviants, p = 10%) were presented monaurally (ISI 200-400 ms). Patients and matched control subjects ignored the tones, and attended to a visual display to detect occasional targets (p = 5%). ERPs were recorded from 27 electrodes over the scalp. The MMN was isolated by subtracting ERPs to standards from those to corresponding deviant stimuli.

Standard stimuli elicited N1 (120 ms) and P2 (224 ms) deflections that were similar in amplitude, latency, and scalp distribution in patients and controls. In contrast, amplitudes of the MMN were reduced by frontal lesions (from 130-170 ms, $p < 0.05$ at each successive 20 ms interval). The reduction was more marked over the lesioned hemisphere. (Supported by the VARS, the NIDCD, a Fogarty Fellowship, and the Finnish Academy).

258.6

SELECTIVE INHIBITION OF COGNITIVE AEP GENERATION BY THE PHENCYCLIDINE-LIKE NMDA RECEPTOR ANTAGONIST MK-801

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Albert Einstein College of Medicine, Bronx, NY 10461.

Deviant auditory stimuli elicit a mismatch negativity (MMN) that indexes the operation of a comparator located within the superior temporal plane in the vicinity of primary auditory cortex. In order to investigate the neurochemical substrates of MMN generation, auditory event-related potentials (AEPs) were recorded from two cynomologous monkeys in response to soft clicks presented either monotonously or as intensity deviants against a background of more frequent loud clicks. Deviant soft stimuli elicited a long-duration frontocentral negativity that overlay the obligatory AEP components during the 30-105 msec latency range. Intracortical recordings from superior temporal plane demonstrated activity within supragranular cortex that was time-locked to the surface MMN. MK-801, a PCP-like *N*-methyl-D-aspartate (NMDA) channel blocker, selectively inhibited MMN generation following systemic and intracortical injection while having relatively little effect on preceding obligatory AEP components. By contrast, intracortical injection of kynurenic acid, a nonspecific excitatory amino antagonist, inhibited all activity generated in the region of the recording electrode. These findings suggest that NMDA receptor-activation may be selectively involved in mediating MMN and other cognitive as opposed to obligatory AEP components and that NMDA receptor-mediated neurotransmission may be selectively impaired in schizophrenia and other diseases associated with impaired cognitive AEP generation.

258.7

VISUAL P3 LATENCY IS PARTIALLY DEPENDENT ON STIMULUS PARAMETERS.

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Previous studies have shown that the late components of sensory evoked potentials can be enhanced by manipulating the subjective probability of a sensory event, the relevance of the event to an assigned task, and the complexity of the task itself. Most of these studies, and in particular those involving vision, have relied on relatively complex discriminations and/or task requirements to produce such a change.

In this investigation the late components of the visual evoked potential (N2 - P3) were studied in ten subjects using a modification of standard pattern reversal methods.

It was found that i) infrequent and unpredictable changes in contrast evoked a prominent and statistically significant N2 - P3 complex, ii) the temporal and spatial dynamics of the observed enhancement were consistent with the spread of P3 activity from central to more posterior regions of the brain, with the right parietal lobe showing the greatest degree of synchronization with the vertex, and iii) N2 and P3 latencies were dependent, in part, on the nature of the stimulus materials employed.

258.8

EFFECT OF PHARMACOLOGICALLY INDUCED CHANGES IN AROUSAL ON HUMAN VISUAL SEARCH.

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It has been postulated that there are 2 mechanisms underlying visual search: a preattentive, "parallel" mechanism related to processing in earlier visual pathways and a more attention-requiring, "serial" mechanism related to processing in later visual pathways. It was hypothesized that the more difficult, attention-requiring search would be disproportionately affected by changes in arousal. To evaluate this hypothesis, visual search tasks requiring greater and lesser amounts of directed attention were performed by healthy human subjects before and after being given placebo, diphenhydramine and methylphenidate. The visual search task was a 2-choice reaction time paradigm in which the subject was instructed to determine whether or not a given target shape was present on a screen that contained 2, 6, 10, 20 or 30 distractor shapes. Scalp EEG was recorded during performance of the tasks and EEG event-related desynchronization was calculated. As expected, reaction times increased following diphenhydramine and decreased following methylphenidate. However, there was no disproportionate affect on either of the 2 tasks. Additionally, there was no change in the slopes of the number of distractors-reaction time relationship. EEG desynchronization occurred in approximately half the subjects and was not related to either the visual task or the drug condition.

258.9

Attentional Processing During Object Identification.
S.J. Luck, S. Fan, & S.A. Hillyard. Department of Neurosciences, M-008, University of California, San Diego, La Jolla, CA, 92093-0608.

Previous studies of visual-spatial attention using the event-related potential (ERP) technique have shown that stimuli presented at precued locations produce larger P1 and N1 components than stimuli presented elsewhere. This finding has been interpreted as a facilitation of early visual processing due to advance cuing of target location. The present study investigated whether similar effects could be demonstrated during a visual search / object identification task without any advance cuing of attention.

The stimulus arrays consisted of 16 randomly positioned T's, half of which were inverted. 14 of the items were blue and the remaining 2 items were red and green; subjects were instructed to identify the orientation of either the red item or the green item. A probe stimulus (a white square) was presented 250 or 400 msec after the onset of the target array, and surrounded either the red item or the green item. At the shorter delay, probe stimuli elicited enhanced P1 and N1 components when they occurred at the location of the relevantly colored item, consistent with attentional cuing experiments. This finding indicates that the attentional mechanisms that operate when attention is explicitly precued to a particular location are also used during visual search and object identification. Specifically, the P1-N1 enhancement suggests that early sensory processing is facilitated at task-relevant locations in both of these paradigms.

258.10

LOCALIZATION OF THE EARLY COMPONENTS OF THE VISUAL ERP DURING SPATIAL-SELECTIVE ATTENTION.
C. Gomez, V.P. Clark, S. Fan and S.A. Hillyard. Dept. of Neurosciences, UCSD, La Jolla, CA, 92093-0608.

Visual stimuli elicit a series of event-related potentials (ERP) components over the posterior scalp including a negative deflection in the interval 50-90 msec (N70) followed by a positive wave between 80-130 msec (P105). Previous studies have shown that the P105 (also called P1) is increased in amplitude when attention is focused on the location of the evoking stimulus. The present experiment used current source density (CSD) and dipole localization (BESA) techniques to determine the brain sources of these components during spatial attention. Stimuli consisted of geometric figures presented in random order to the left and right visual fields at ISIs of 650-900 msec. Subjects paid attention to one field at a time, ignoring the opposite field, with the task of responding to occasional target figures that varied slightly from the non-targets. ERPs were recorded from 29 channels over the posterior scalp. The P105 was enlarged in the ERPs to attended versus unattended-location stimuli, while the N70 was unaffected by attention. The CSD and BESA analyses indicated that the N70 wave could be accounted for by a source near the occipital pole contralateral to the field of the stimulus, within primary visual cortex. In contrast, the P105 could be modelled by a source in the contralateral prestriate cortex and a delayed ipsilateral source attributed to transfer across the corpus callosum. These results suggest that spatial-selective attention acts to modulate visual processing at a level beyond the primary visual cortex.

258.11

THE EFFECTS OF STIMULUS POSITION IN THE VISUALLY EVOKED POTENTIAL: ANALYSIS AND LOCALIZATION WITH MRI.
V.P. Clark[1], S. Fan*[2], and S.A. Hillyard[1]. [1]Department of Neurosciences, UCSD. La Jolla, CA 92093-0608 [2]Academica Sinica, Beijing, China.

Several of the early components of the visually-evoked potential (VEP) arise from activity in occipital cortex, but the exact locations of their neural generators have been disputed. The present study examined changes in component amplitude and surface topography as a function of stimulus position in order to isolate the activity of different cortical regions. VEPs were recorded to a small (1.5° or 2° diameter) circular checkerboard stimulus randomly placed in the left or right visual fields along positions equidistant from fixation (6° or 8°) spanning 141° of polar angle centered about the horizontal meridian (HM). VEPs were averaged across adjacent stimulus positions. The rising phase of the C1 component (measured at 60-75 msec) achieved its maximum negative amplitude at a polar angle of 20° above HM, with maximum positive amplitudes at approximately 60° above and 50° below HM. The C1 reversed its polarity in the lower visual field at 20° to 40° below HM in most subjects, not at HM as has been assumed in previous research. Subsequent negative and positive components (N150 and P220) were seen to increase in amplitude in the lower visual field, reaching a maximum at the lowest point measured. The Brain Electrical Source Analysis (BESA) system was used to localize the component sources. These sources were localized with respect to MRIs for some subjects. The source of the C1 was slightly lateral to the medial surface of the contralateral occipital lobe, near the position of the calcarine fissure, suggesting a source in striate cortex. The tentative positions of the N150 and P220 source locations were in multiple extra-striate regions.

258.12

RULES OF MULTISENSORY INTEGRATION AND ATTENTION: ERP AND BEHAVIORAL EVIDENCE IN HUMANS.
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The integration of multisensory (auditory and visual) stimuli has been studied at the single-neuron and behavioral levels in animals. These studies report that neuronal responsiveness and behavioral accuracy are enhanced for spatially coincident multisensory stimuli compared to unimodal stimuli. Spatially disparate stimuli also enhance performance and responsiveness but only when they are lateral to the attended target; they depress or do not alter these measures when medial to the attended target. We tested the hypothesis that similar organizing principles govern multisensory integration and attention in humans by measuring behavior and ERPs to attended and unattended unimodal and multisensory stimuli. Stimuli were faint lights either alone or accompanied by broad-band noise bursts that were spatially coincident with or disparate to the light. The sound disparities were either 30° lateral or medial to the light. The Ss fixated straight ahead, and on different runs attended to the lights at left or right 30°. Spatially coincident and laterally disparate stimuli were responded to more quickly and more accurately compared to visual alone, while medially disparate stimuli resulted in little or no change in performance. Additionally, visual ERP components and their modulation by attention were influenced by the simultaneous presence of a sound and a light: spatially coincident and laterally disparate sounds enhanced visual ERP components relative to their amplitudes when lights were presented alone, while medially disparate sounds produced little or no enhancement. Unattended coincident stimuli also showed enhanced ERP components but unattended disparate stimuli did not. These data suggest that the spatial rules of multisensory integration observed in other animals may apply to humans and may operate across many levels of analysis, from the neuron level, to the neuron-systems level, to the behavioral level. (NSF RCD90-54731, NIH DC00128, #NIH NS22543)

258.13

HUMAN INTRACRANIAL POTENTIALS EVOKED BY FACES
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Electrodes were implanted in over 500 sites in 10 patients to localize the seizure focus prior to surgical treatment. Informed consenting patients judged the emotion and familiarity of faces exposed for 300 ms. The P70/N110 were observed near area 19 and are thought to be generated by geniculate afferents to area 17. The P160 had a more anterior and lateral distribution. The large widespread N210 polarity inverted in medial and lateral posterior inferotemporal cortex, with maximal amplitude in the amygdala. The N210 corresponds to the scalp N200 associated with complex sensory analysis. The N420 was largest in basolateral amygdala with steep voltage gradients medially, but with a widespread distribution, including parietal, prefrontal, limbic and inferotemporal cortices. The N420 appears to be identical to the N400 evoked by words and associated with their contextual integration. Thus, faces evoke sequential potentials in primary, secondary visual and supramodal cortices, ending in the amygdala. The large amygdala N420 suggests that emotional processing of faces may be simultaneous and integrated with their cognitive processing in association cortex.
Supported by USPHS(NS18741), NATO, MRT & INSERM.

258.14

BEHAVIORAL AND EVENT RELATED POTENTIAL INDICATORS OF AGING IN WOMEN.
C. A. Christensen, R. J. Compton* and K. J. Drake*. Dept. of Psychology, Vassar College, Poughkeepsie, NY 12601.

Women in four age groups (20,40,60, & 75 ± 2 yrs) were tested on a battery of eight tasks which measure speed of processing, including simple and choice reaction time (RT & CRT), the Sternberg memory task (S), digit symbol substitution, critical flicker fusion, figural synthesis, forward and backward masking. The Beck, Hartford-Shipley and portions of the Cornell medical inventories were used for purposes of group matching. Event-related potentials were recorded during the S, RT, and CRT tasks. The data were evaluated to assess 1) the nature of group differences 2) whether correlations among scores support the notion of a common timing mechanism and 3) whether behavioral and ERP measures provide similar or different characterizations of correlated measures. Significant group differences were observed on most measures, including late ERP components, with the most pronounced effects shown as differences between the two older and two younger groups. Significant correlations of moderate magnitude among many, but not all behavioral measures provide some support for the notion of common timing mechanisms, a conclusion which was also supported by examination of intermeasure correlations observed with ERP indicators. The differences between the ERP and behavioral characterizations which were observed will be discussed.

258.15

EFFECTS OF HIPPOCAMPAL LESIONS ON THE HUMAN P300. R.T. Knight, Dept. of Neurology, University of California Davis, VAMC, Martinez, Ca 94553.

The P300 is generated during detection and encoding of significant environmental events. Intracranial recordings report large hippocampal field potentials associated with scalp P300 activity. However, anterior hippocampal removal has minimal effects on the scalp P300. We recorded P300s in patients with posterior hippocampal lesions and anterograde memory deficits due to posterior cerebral artery occlusion. Patients (n=4, 65.0 +/- 5.0 yrs, mean lesion volume= 20.5 cc.) had unilateral damage in the posterior hippocampus and adjacent inferior temporal cortex. P300s to target and non-target novel stimuli were recorded in auditory, visual and somatosensory signal detection tasks. Hippocampal lesioned subjects and age matched controls generated comparable target and novel P300s at parietal scalp sites in all sensory modalities with no lateralized P300 reduction over lesioned hemisphere. However, earlier latency frontal P300 activity was reduced in all sensory modalities by hippocampal lesions. The hippocampal and neocortical lesion data indicate that the frontal scalp P300 indexes hippocampal and prefrontal cortex activity generated during detection of unexpected events. The parietal P300 may index an implicit memory system dependent on integrity of cortex in the temporal-parietal junction.

258.16

IS UNIT ACTIVITY IN THE LOCUS COERULEUS RELATED TO P300-LIKE POTENTIALS? D. Swick, J.A. Pineda, and S.L. Foote, Depts. of Neuroscience, Cognitive Science, and Psychiatry, University of California, San Diego, La Jolla, CA 92093.

Event-related potentials and locus coeruleus (LC) unit activity were recorded from three awake, untrained macaques during the presentation of an auditory oddball paradigm. We wished to test the hypothesis that novel auditory stimuli lead to phasic increases in LC cell firing, which may be a necessary condition for the occurrence of P300 potentials. Spontaneous EEG and LC unit activity were also recorded to demonstrate the correlation between behavioral state and LC firing rate. Auditory oddball stimuli resulted in P300-like potentials with largest amplitude at centro-parietal sites. Approximately 12 single unit and 4 multiple unit LC recordings were obtained. As previously reported, changes in LC activity precede changes in EEG. Decreased cell firing was associated with periods of synchronized EEG (e.g., during periods of behavioral drowsiness). However, no consistent relationship was found between the presentation of oddball stimuli and LC responses. Post-stimulus time histograms did not indicate a significant increase following stimulus onset. One explanation could be that the oddballs were not significant enough to elicit a shift in attention. The subjects rapidly habituated to the stimuli, which lost their novel or alerting quality. Studies with trained monkeys are in progress and may resolve this issue.

258.17

LOW-DIMENSIONAL CHAOS IN THE HUMAN EVENT-RELATED POTENTIAL: REDUCTION IN THE POINT CORRELATION DIMENSION DURING THE P300 COMPONENT. J.E. Skinner and M. Molnar, Neurophysiology Section, Neurology Dept., Baylor Coll. of Med., Houston, TX 77030.

Low-dimensional chaos may be inherent in noisy-looking signals rather than randomness. We previously analyzed a biological time-series under neural control (heartbeat intervals) and found that a chaotic measure (the point correlation dimension, PD2) was more sensitive to specific changes in the generator (degree of myocardial ischemia) than were stochastic measures (mean and standard deviation, fast-Fourier transform) (Skinner et al., Circ. Res., 1991). We have now analyzed EEG signals, recorded over somatosensory cortex, that were evoked by meaningful stimuli (i.e., counted cutaneous stimuli) and we found that the time-dependent PD2s were transiently reduced during the development of the event-related P300-potential; when the somatosensory stimuli were delivered while the subject was reading, the P300-potential was still present, but reduced, while the PD2-wave was completely abolished. We conclude that, 1) the PD2-measure is more sensitive to specific changes in biological generators than stochastic measures; and 2) the cortex reduces the chaotic dimension of its dynamical process during processing of task-relevant information.

258.18

SIMULATED INTERACTIONS OF ANTERIOR AND POSTERIOR ATTENTION SYSTEMS IN A NETWORK BASED ON CORTICAL-BASAL GANGLIA LOOPS AND CORTICOTHALAMIC LOOPS. D. LaBerge, M. Carter, *University of California, Irvine,* & V. Brown, *University of Texas, Arlington.*

Recent results of brain imaging and neurophysiological studies suggest a role for the pulvinar nucleus of the thalamus in selective visual attention. Computer simulations of the corticothalamic circuit produce selective enhancement (of center) and inhibition (of surround) in cortical units when the corresponding thalamocortical units are given a slight advantage in activation, from either afferent or cortical input (LaBerge, Brown, & Carter, Soc. Neurosci. Abstr. 16:579). The circuitry here assumed to be involved in visual attention is repeated in other thalamic nuclei as well, suggesting that other nuclei may produce selective enhancement in other cortical areas. Recent data implicate anterior cingulate cortex in tasks which require complex attentional operations such as attention to multiple dimensions of a stimulus (Corbetta et al., Science 24:1556-1559). In addition, major inputs to anterior cingulate corticothalamic circuits are inhibitory connections from basal ganglia. It is possible then that attention to a specific task is under control of anterior cingulate via basal ganglia; corticothalamic circuits in anterior cingulate controlling higher-order cognitive procedures may in turn activate specific processes in posterior corticothalamic circuits. A computer simulation of this larger attentional network produces selection of anterior cortical areas via disinhibition by basal ganglia, followed by selection of posterior cortical areas through connections to pulvinar.

(ONR N00014-88-K-0088)

258.19

COMBINING EEG, MEG, PET AND MRI FOR REAL-TIME IMAGING OF CORTICAL ELECTRICAL ACTIVITY. A.M. Dale and M.I. Sereno, Cognitive Science Department, University of California San Diego, La Jolla, CA 92093.

One approach to neuroelectric source localization is to assume a small number of equivalent current dipoles, and then determine their positions, orientations, and strengths using a nonlinear optimization technique (Scherg, 1989). This approach has various problems: 1) the necessary number of "equivalent dipoles" is hard to determine *a priori*, 2) the nonlinear optimization is computationally hard, 3) no general confidence measures exist for the solutions.

The approach we have adopted in a series of model studies is to use MRI data to determine the shape of the cortical sheet, and thus fix the position and orientation of possible current sources (assuming that electric and magnetic fields measured by EEG and MEG are generated by currents flowing perpendicularly to the cortical surface). The problem then reduces to a linear one of finding distributions of current densities on the cortical sheet consistent with the EEG and the MEG data. We find the cortical sheet by shrinking a deformable template onto the MRI images.

To make the linear problem well-posed, it is necessary to add regularization terms (Tikhonov, 1977) in order to obtain a unique pseudoinverse. Possibilities are terms favoring "minimum norm" and/or "minimum norm of the gradient" solutions. One problem with the "minimum norm" constraint is that it always favors superficial, spread-out, low-amplitude solutions over deep, local, strong ones. However, we have found that it can be combined with other regularization terms to improve localization of deep sources.

An extension of this approach is based on the assumption that foci of activation observed with PET techniques are correlated with electrical activity. Another regularization term can be added which favors solutions also consistent with the PET data for particular experimental conditions. This technique will not disallow solutions with activity outside the PET foci if EEG and MEG data point there, but it biases us toward solutions at PET foci, which may be deep and localized.

A major advantage of the linear approach to the problem is that the general inverse solution matrix has to be computed only once for a given brain geometry and sensor placement. Particular solutions (e.g., time steps) can then be computed rapidly by simple matrix multiplications, making real-time imaging possible.

Supported by UCSD McDonnell-Pew Cognitive Neuroscience Center Graduate Fellowship.

258.20

A SENSORIMOTOR PHASE TRANSITION IN THE HUMAN BRAIN REVEALED BY MULTIPLE LOW TEMPERATURE SQUIDS. J.A.S. KELSO, S. BRESSLER, S. BUCHANAN*[1], G. C. DEGUZMAN*, M. DING*, A. FUCHS*, T. HOLROYD*. Program in Complex Systems and Brain Sciences, Center for Complex Systems, Florida Atlantic Univ., Boca Raton, FL 33431 and [1]Biomagnetic Technologies Inc., San Diego, CA 92121.

The recent evolution of multisensor SQUID (Superconducting Quantum Interference Device) instruments allows magnetic field measurement of intracellular current flows over large areas of neocortex, thereby providing a window into the brain's spatiotemporal dynamics. A circular 37-SQUID array of diameter 144mm. was centered over left parieto-temporal cortex. Each of the SQUIDS was sampled continuously at 862 Hz (passband = 1 to 200 Hz). The subject's task was to produce a manual response to an auditory stimulus, the frequency of which was increased every 10 cycles in steps of .25 Hz from 1.0 Hz to 3.25 Hz. At a certain critical stimulus frequency behavior switched from a reactive, syncopated mode of coordination to an anticipatory, synchronized mode (Kelso, DelColle & Schöner, 1990). Analysis of signal frequency, phase and amplitude revealed: a) the behavioral task induced a coherent state over the whole array i.e., brain signals were frequency and phase-locked to the stimulus; b) at a critical stimulus frequency (~ 1.75 Hz), an abrupt qualitative change (phase transition or bifurcation) in signal phase occurred in specific parts of the array; this transition in the brain dynamics coincided with behavioral switching; c) signal power and principal eigenvalues obtained through mode decomposition techniques dropped dramatically across the transition. Coronal, sagittal and axial MR data (contiguous 3mm slices) have been obtained in order to localize the source of these phenomena. Research Supported by NIMH Grant MH42900 and ONR Contract N00014-88-J-1191.

258.21

INTERSUBJECT RELIABILITY OF NEUROMAGNETIC MEASUREMENTS ON THE HUMAN SOMATOSENSORY AND AUDITORY SYSTEMS.
T. T. Yang, C. C. Gallen, B. Schwartz, F. E. Bloom.
Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

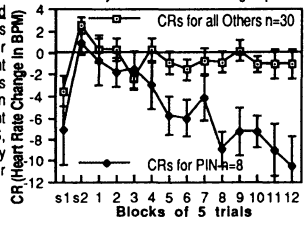
The reliability of neuromagnetic source localization approaches has been demonstrated with intrasubject studies for the auditory and somatosensory systems. An important additional source of localization variability stems from intersubject differences in brain and head morphology. The purpose of this study was to attempt to quantify the intersubject variability of neuromagnetic measurements for the human somatosensory and auditory systems using a BTi 37-channel Neuromagnetometer. In order to quantify the contribution of intersubject variability,we studied the somatosensory area through the repetitious stimulation of the thumb, index, pinky, and cheek. These regions were localized in the human brain using 256 repetitions of a tactile, pressure stimulator upon the respective body regions. We also examined the auditory system using 60 repetitions of four different tones at 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz, respectively. Dipole localizations were done for these evoked fields on 13 neurologically normal adult subjects. The mean X, Y, Z location for each site was computed. The mean and standard deviations for the point to point variation from the mean for all subjects were also calculated. The variability stemming from the intersubject variables was substantially larger than intrasubject variability. The significance and validity of this variability was further examined using neuromagnetic comparisons with magnetic resonance images (MRI) of the respective subjects. The issue of intersubject variability could stem from the increased error due to problems involved with the neuromagnetic recording techniques and/or due to differences inherent within the subjects themselves.

LEARNING AND MEMORY—ANATOMY IV

259.1

MICROSTIMULATION OF A SPECIFIC REGION OF THE MEDIAL GENICULATE(MG) SERVES AS AN UNCONDITIONED STIMULUS(US) FOR AUTONOMIC FEAR CONDITIONING TO A TONE.
Scott J. Cruikshank, Jean-Marc Edeline, and Norman M. Weinberger, Centr. Neurobiol. Learning and Mem. and Dept. Psychobiol, UC Irvine, CA 92717

The medial division of the medial geniculate(MGm) and associated posterior intralaminar nucleus(PIN) receive both auditory and somatosensory input, exhibit discharge plasticity with tone/shock pairing, and are necessary for autonomic fear conditioning(cond) to a tone. However, it remains unclear whether critical convergence of somatosensory and auditory information for fear cond. occurs here, or if this area simply provides auditory information to a center of convergence elsewhere. We reasoned that if convergence is in MGm/PIN, microstimulation (stim) of this area would serve as an US for conditioned bradycardia. Adult male Guinea Pigs were subjected to 40-60 trials of Pavlovian cond. following unpaired sensitization control trials. The conditioned stimulus (CS) was a monaural 6s, 70db tone. The US was a .5-1 s train (100-150uA, 50-75Hz, .5ms) delivered at CS offset to the contralateral MG via bipolar stim electrodes implanted 1 week earlier in PIN(n=8), MGm(n=14), ventral MG(n=9), dorsal MG/SG (n=5) or outside MG(n=2). PIN stim. generally elicited bradycardia Unconditioned Responses, which were maintained throughout training; stim. of other areas elicited responses which usually habituated. The PIN group had significant bradycardia Conditioned Responses(CRs) which developed across cond trials(see blocks 1-12); other placement groups showed no significant CRs, despite similar orienting responses which habituated during sensitization (s1,s2). Thus, stim. of PIN is a sufficient US for cond. bradycardia to a tone CS, supporting that convergence of auditory and somatosensory information for autonomic fear cond. occurs in this area.
Supported by Office of Naval Research



259.2

LESIONS OF THE AUDITORY THALAMUS BLOCK ACQUISITION AND EXPRESSION OF AVERSIVE CONDITIONING TO AN AUDITORY BUT NOT A VISUAL STIMULUS MEASURED WITH THE FEAR POTENTIATED STARTLE PARADIGM.
S. Campeau and M. Davis, Dept. of Psychology and Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, Ct 06508.

Previous research indicated that the auditory thalamus is necessary for the acquisition of auditory fear conditioning (Ledoux et al., Neurosci., 17:615, 1986). To test the generality and specificity of this finding, animals were trained simultaneously with auditory and visual stimuli before or after bilateral lesions of the auditory thalamus, and tested with the fear-potentiated startle paradigm.

Unoperated, sham-operated, and rats sustaining bilateral electrolytic lesions of all the subdivisions of the medial geniculate body, the intralaminar nucleus, and the peripeduncular area, were given 10 pairings each of a 3.7 sec, 70 dB, noise centered at 2 kHz (24 dB/octave attenuation) and a 3.7 sec fluorescent light, each coterminating with a 0.5 sec, 0.4 mA footshock, on each of two consecutive days. All animals were tested 1 day later with 70 startle-eliciting noise-bursts in the presence or absence of the auditory or visual conditioned stimuli. Additional rats, trained as described above, received bilateral electrolytic or sham lesions of the auditory thalamus 24 hrs after the last training session and were tested 10 days later. Bilateral auditory thalamic lesions before or after conditioning nearly completely abolished startle enhancement to the auditory conditioned stimulus. In contrast, the level of visual fear-potentiated startle in lesioned animals was comparable to that of the sham-operated and unoperated rats.

These results suggest that the role of the auditory thalamus in aversive conditioning is specific to auditory stimuli. Experiments investigating the role of subnuclei of the auditory thalamus, as well as other auditory structures, in the mediation of auditory fear-potentiated startle are currently in progress.

259.3

EQUIPOTENTIALITY OF THALAMO-AMYGDALA AND THALAMO-CORTICO-AMYGDALA CIRCUITS IN AUDITORY FEAR CONDITIONING.
L.M. Romanski and J.E. LeDoux, Center for Neural Science, New York University, NY, NY 10003.

The lateral nucleus of the amygdala (AL) is essential for the classical conditioning of fear responses to simple auditory stimuli in rats. AL receives projections from both thalamic and cortical areas of the auditory system. The thalamic projection originates in the medial division of the medial geniculate body (MGm) and in the subadjacent posterior intralaminar nucleus (PIN). The cortical projection is from temporal and perirhinal regions, which in turn are the recipients of auditory thalamo-cortical projections. In the present study we examined the sufficiency of thalamo-amygdala and thalamo-cortico-amygdala auditory pathways as conditioned stimulus (CS) transmission systems in fear conditioning.

Rats were given lesions of auditory cortex (ACX, n=6), MGm/PIN (n=7), or both ACX and MGm/PIN (n=7). After 14-21 days recovery time, they were subjected to fear conditioning (30 pairings of a tone CS with footshock). The next day, CS-elicited changes in arterial pressure and freezing behavior were examined. Lesions of either ACX or MGm/PIN failed to interfere with fear conditioning while combined lesions of ACX and MGm/PIN impaired conditioning. Sensory transmission to the amygdala is essential in fear conditioning but either thalamo-amygdala or thalamo-cortico-amygdala pathways are sufficient as sensory transmission routes.
Supported by MH38774.

259.4

DIFFERENT BRAIN SYSTEMS MEDIATE CONTEXTUAL AND EXPLICITLY CUED FEAR CONDITIONING.
R.G. Phillips and J.E. LeDoux, Center for Neural Science, New York University, NY, NY 10003.

In classical fear conditioning tasks animals develop emotional reactions to a conditioned stimulus (CS) paired with footshock as well as to background or contextual stimuli present during the pairing. In this study we examined whether different brain systems might contribute to the acquisition of conditioned responses to an explicit CS and to contextual stimuli. Lesions were placed in the amygdala (n=8), dorsal hippocampus (n=18) or in the neocortex above hippocampus (n=18). After 10-14 days recovery, blocks of two conditioning trials, consisting of a tone (800 Hz, 80 dB, 20 sec) paired with footshock (0.5 mA, 0.5 sec), were given on three consecutive days. Unoperated controls exhibited freezing during the CS but not prior to it on day 2, whereas on day 3 they exhibited freezing during the pre-CS time and during the CS. Freezing during the CS on day 2 and 3 was used as a measure of conditioning to the CS, whereas freezing during the 20 sec. prior to the CS on day 3 was used as a measure of contextual conditioning. Neocortical lesions had no effect on freezing during the pre-CS time or during the CS. Hippocampal lesions had no effect on freezing during the CS on either day 2 or day 3 but reduced freezing during the pre-CS time on day 3. Amygdala lesions reduced freezing during the CS on both day 2 and 3 and reduced freezing during the pre-CS time on day 3. Thus, the amygdala appears to be involved in the conditioning of fear responses to stimuli explicitly paired with footshock as well as to background or contextual stimuli, whereas the hippocampus only appears to contribute to contextual conditioning. Supported by MH38774.

259.5

NMDA LESIONS OF THE LATERAL AND BASOLATERAL NUCLEI OF THE AMYGDALA BLOCK FEAR POTENTIATED STARTLE AND SHOCK SENSITIZATION OF STARTLE. C. B. Sananes & M. Davis, Yale Univ. Sch. of Med., Dept. of Psychiatry, Ribicoff Res. Fac. of the Conn. Mtl. Hlth. Ctr., 34 Park St., New Haven, CT 06508.

Lesions of the central nucleus of the amygdala block the elevation in startle when this reflex is elicited in the presence of a cue previously paired with shock (fear-potentiated startle). Retrograde and anterograde tracing techniques indicate a direct anatomical connection between the central nucleus of the amygdala and the acoustic startle pathway and lesions at several points along this pathway block the expression of fear-potentiated startle. In addition, lesions of the central nucleus of the amygdala or the pathway between the amygdala and the startle circuit block the elevation in startle that occurs shortly after presentation of a footshock, termed shock sensitization. Finally, electrical stimulation of the amygdala markedly increases the amplitude of the startle reflex using electrical currents and train durations well below those required to produce observable behavioral responses. While these studies strongly implicate the central nucleus of the amygdala in modulating startle, much less is known about the role of other amygdaloid nuclei in fear-potentiated startle.

In the present study, cell bodies in the lateral and basolateral nuclei of the amygdala, but not the central nucleus, were destroyed by local infusion of N-methyl-D-aspartate (NMDA). In Experiment I the lesions were carried out before training and testing, and in Experiment II the lesions were carried out after training but before testing. In both cases the lesions completely blocked fear-potentiated startle. This occurred in each of the 17 animals given NMDA lesions. These lesions also blocked the normal increase in startle observed after a series of footshocks (10, 0.6 mA shocks presented at a rate of 1 shock/sec), but only when the lesion included the ventral aspect of the most anterior part of the basolateral nucleus. We suggest that the lateral and/or basolateral nuclei of the amygdala are involved in relaying visual information to the central nucleus of the amygdala, which is also critical for fear-potentiated startle, and that activation of the anterior part of the basolateral nucleus may be critical for shock sensitization and, perhaps, processing of aversive somatosensory information during fear-conditioning.

259.6

ANATOMICALLY SELECTIVE BLOCKADE OF PAVLOVIAN FEAR CONDITIONING BY APPLICATION OF AN NMDA ANTAGONIST TO THE AMYGDALA AND PERIAQUEDUCTAL GRAY. M. S. Fanselow, J. J. Kim, & J. Landeira-Fernandez, Psychology Dept, UCLA, Los Angeles, CA 90024-1563.

Previously, we reported that ICV injection of the NMDA antagonist, APV, prior to training eliminates fear conditioning. This experiment assessed the anatomical location of that effect by applying APV to various loci known to be involved in conditional fear. Cannulae were implanted bilaterally into the central (ACE) and basolateral (ABL) nuclei of the amygdala and unilaterally into the ventral (vPAG) and dorsal (dPAG) periaqueductal gray. A total of 5μg APV, or its vehicle, was injected prior to training. Conditioning consisted of placing a rat in an observation chamber and presenting 3 (1 s, 1 mA) shocks spaced 20 s apart. Fear was assessed by observing freezing when the rat was returned to the chamber 24 h later. Fear was virtually eliminated in the rats with ABL and vPAG placements. There was no attenuation of fear in the rats with ACE cannulae. A trend toward a reduction in freezing with dPAG placements was not statistically reliable. These data suggest that both the ABL and vPAG are involved in the NMDA dependent plasticity subserving fear conditioning.

259.7

EFFECTS OF DEGREE OF TRAINING ON RETENTION OF AVERSIVELY MOTIVATED LEARNING IN RATS WITH NMDA-INDUCED AMYGDALA LESIONS. M.B. Parent, C. Tomaz, E. Yudko* & J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, Univ. California, Irvine, CA 92717.

It is well documented that retention of aversively motivated learning is impaired by lesions of the amygdala. These experiments were undertaken to determine whether the degree of impairment depends upon the amount of training provided before the lesion. Male Sprague-Dawley rats were given either 1 or 10 training trials in a two-compartment straight alley. On each trial they were placed in a darkened compartment and escaped from footshock (0.75mA) by entering the adjoining illuminated compartment. One week following the training, lesions were induced by microinfusing n-Methyl-d-Aspartic acid (NMDA) into the amygdala (10ug/ml, 0.8ul, 4min). Lesions were verified, using light microscope, by observers blind to the behavioral results. Controls were either unoperated (UO) or received sham-lesion surgery (SL). Retention was tested four days after surgery by placing the rats in the illuminated compartment and recording, during a 600 sec period, the latency to enter the dark compartment (where shock was delivered in training), amount of time spent in each compartment, and the number of crossings between compartments. The amount of training given prior to the surgery significantly affected retention. In the UO and SL controls, as well as the amygdala-lesioned groups, the retention performance of animals given 10 training trials was significantly better than that of animals given one training trial. As was expected, the performance of amygdala-lesioned animals that received one training trial was significantly poorer than that of control animals. However, the retention performance of the lesioned animals given 10 trials was comparable to that of controls given one training trial. Thus, these findings indicate that the retention-impairment produced by post-learning amygdala lesions is partially attenuated by increasing the amount of training given prior to the lesion.

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259.8

ENHANCEMENT OF RAT VISUAL SYSTEM 2-DEOXYGLUCOSE UPTAKE BY AROUSING FOOTSHOCK IN PATTERNED LIGHT AND DARKNESS A.R. McIntosh, F. Gonzalez-Lima & R.M. Cooper[1], Dept Psychol, Univ of Texas - Austin, Austin, TX USA 78712; [1]Dept Psychol, Univ of Calgary, Calgary, AB Canada T2N 1N4.

Autoradiography with [^{14}C]2-deoxy-D-glucose was used to examine metabolic changes in the visual system of hooded rats exposed to patterned light or to darkness following footshock. Primary retinorecipient structures showed a response to light but not to shock. Higher visual sites showed two different shock effects. First, the intermediate grey layer of the superior colliculus and secondary visual cortex were suppressed by the shock regardless of visual condition. Second, in the lateral posterior nucleus and primary visual cortex, the footshock led to significant enhancement of the metabolic responses to the patterned light. Structural models (McIntosh & Gonzalez-Lima, Brain Res., in press) were constructed using the 2-DG data to quantify changes of the covariance relationships among different visual pathways. The models suggested that patterned light led to an increase in the influence of the geniculocortical path relative to models for groups tested in darkness, and that footshock modified extra-visual influences. The models also demonstrated a significant interaction of geniculocortical and tectocortical paths for the group given footshock with the patterned light display. The findings suggest that footshock-induced arousal has significant modulatory effects on the operations of higher visual pathways of behaving rats.

259.9

ACTIVATION OF AUDITORY CORTEX WITH VISUAL STIMULATION THROUGH SENSORY-SENSORY CONDITIONING. *L. Cahill and H. Scheich, Technical University Darmstadt, Institute for Zoology, Schnittspahnstr. 3, D-6100 Darmstadt, Germany.*

We have used the 2-deoxyglucose (2DG) metabolic mapping technique to examine changes in brain activity produced through sensory-sensory associative conditioning in the gerbil. Gerbils received 40 training trials, each consisting of a 10 sec presentation of a light followed by a 10 sec presentation of a tone (1KHz). On approximately every third trial a 1 sec mild footshock was given at the end of the tone to maintain arousal in the subjects. Controls received 40 presentations of the light (no tone), with shock given after the light on the same schedule as the first group. One day later, 2DG was injected, and the subjects given repeated presentations of the light alone. The results show a strong activation of the auditory cortex by the light in the light/tone conditioned group compared to the controls, with the most pronounced activation seen in field AAF. It appears that, through conditioning, the light begins to activate sensory regions normally activated by the tone with which it has been associated.

259.10

ELECTROLYTIC LESIONS OF THE ARCUATE NUCLEUS OF THE HYPOTHALAMUS ATTENUATE CONDITIONAL ANALGESIA BUT DO NOT ENHANCE PAVLOVIAN FEAR CONDITIONING. S. L. Young and M. S. Fanselow. Department of Psychology, University of California, Los Angeles, CA, 90024.

In the rat, analgesia is one of many adaptive responses to fearful stimuli. This analgesia has two roles in Pavlovian fear conditioning. First, it serves as a conditional response (CR) to previously neutral stimuli that have been associated with mild footshock. Secondly, during the course of fear conditioning, analgesia as a CR serves to regulate conditioning by reducing the impact of the noxious unconditional stimulus. Reversal of fear-induced analgesia with opioid antagonists suggests opioid mediation. Reversal of the analgesia during training results in enhanced fear conditioning. However, reversal of the analgesia during testing restores responding to a painful stimulus. The μ opioid receptor is critical in the expression of conditional analgesia. ß-endorphin (ß-EP) has a high affinity for μ receptors and the capacity to produce a profound analgesia. The primary source of brain ß-EP is the arcuate nucleus of the hypothalamus (AN). Electrolytic lesions of the AN were used to deplete brain ß-EP to test its role in conditional analgesia. Response to a subcutaneous injection of dilute formalin was used to indicate pain reactivity and analgesia. Anterior AN lesions attenuated conditional analgesia and posterior AN lesions had no effect. However, neither reliably enhanced fear conditioning as indexed by freezing. These results suggest that the AN plays a role in fear-induced analgesia.

259.11

LEARNED AVERSIVENESS TO A TASTE PAIRED WITH ILLNESS IN RATS LACKING GUSTATORY CORTEX. S.A. Bailey and S.W. Kiefer. Department of Psychology, Kansas State University, Manhattan, KS 66506-5302.

Rats lacking gustatory cortex (GC) and control rats were given four sucrose-illness pairings. Each acquisition trial involved the intra-oral infusion of a sucrose solution while the subjects' taste reactivity was videotaped in a special chamber. Immediately after each infusion, the rats received intubations of lithium chloride (1% body weight of .15 M LiCl). After the training phase, the rats were placed on a schedule of restricted fluid access and tested for sucrose consumption in the home cage. Videotapes of the acquisition trials were scored for orofacial responding, and the two groups' reactivity was compared. Results indicated that while both groups increased aversive responding over the four training trials, the GC animals showed significantly fewer aversive responses than controls. Repeated training trials resulted in reduced ingestive responding for both groups although GC rats' ingestive responses still remained significantly higher than that of control animals. Finally, while the reactivity of the GC rats to sucrose revealed a shift in palatability toward aversiveness, sucrose consumption in the home cage was high. This result indicated that GC rats failed to transfer their learned aversiveness to another context where consumption was used as a dependent measure.

259.12

INJECTION OF A PROTEIN SYNTHESIS INHIBITOR INTO GUSTATORY CORTEX IMPAIRS MEMORY OF CONDITIONED TASTE AVERSION IN RAT. K. Rosenblum*, N. Meiri* and Y. Dudai. Department of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel.

In conditioned taste aversion (CTA) organisms learn to avoid a discrete taste if it is followed by poisoning. In contrast to conventional types of associative learning, CTA tolerates a delay of hours between the sensory percept and the explicit reinforcer. The taste representation must hence be stored in the brain over the delay until aversion occurs. CTA can therefore be used to investigate the formation of a lasting central sensory representation and the subsequent association of this stored representation with a reinforcer. We are investigating molecular and cellular mechanisms that subserve taste memory and aversive association in CTA in rat, using saccharin as the gustatory stimulus and LiCl as the aversive agent. In classical and instrumental learning, consolidation of memory requires protein synthesis. We have tested whether protein synthesis is also required for memory of CTA. Acute infusion of the protein synthesis inhibitor anisomycin to the lateral cerebral ventricles of adult rats before, during and after CTA training, at concentrations that were found by us to inhibit water maze navigation learning, led to only a small decrement in the intensity of memory of CTA, when tested in an extinction procedure up to 5 days after training. In this mode of administration, anisomycin was effective in rapidly and reversibly reducing protein synthesis by >90% in some brain areas, e.g. hippocampus and cingulate cortex, but not in others, including the insular cortex which is a candidate for storing taste representations. In contrast, local injection of anisomycin into gustatory cortex shortly before and during CTA training impaired CTA memory without significantly affecting liquid consumption. Our data thus corroborate the notion that gustatory cortex is required for CTA memory and suggest that protein synthesis in this cortical area is involved. (Supported by the Schilling Foundation, Germany).

259.13

A SIGNALLED BAR-PRESSING PREPARATION FOR STUDY OF THE NEURAL BASES OF APPETITIVE AND AVERSIVE LEARNING IN THE RAT. S. F. Logue, D. P. Miller, and J. E. Steinmetz. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

One interesting aspect of the study of the neural bases of learning and memory is a comparison of how the brain codes appetitive and aversive conditioning situations. Described here is our attempt to develop a rat paradigm that allows for direct comparison of appetitive and aversive learning. We have developed a signalled bar-press conditioning paradigm, in which a bar-press during a 1 sec tone results in either a food reward or the successful avoidance of a footshock. In the appetitive task the rats are shaped to bar-press for food reinforcement and then run on a FR4 partial reinforcement schedule until a high rate of bar-pressing is stabilized. Once baseline bar-pressing is established the rats are given daily sessions of 100, 1 sec tone (2 kHz, 85 dB) presentations. If the rat bar-presses during the tone there is a food reinforcement followed by a 10 sec intertrial interval and a variable pre-CS period (range 1-5 sec). A bar-press during the pre-CS period resets the time interval before tone presentation. This required period of response inhibition increases the distinctiveness of response to the tone. In the aversive task the rats are shaped to bar-press to escape a 0.5 mA footshock and are then run on a Sidman avoidance schedule until a high level of avoidances is established. The tone sessions, using ITI and pre-CS parameters identical to the appetitive preparation, are then begun. The only difference from the appetitive task is that by bar-pressing during the tone the rat successfully avoids a 1 sec 0.5 mA footshock. In addition to describing the neural substrates underlying appetitive and aversive learning using an identical stimulus and response and the same subject, other advantages of this paradigm include the ability to distinguish timed responding from cued responding at a neural level and to distinguish between learning and performance deficits in signalled bar-pressing conditioning. Our initial studies using these tasks have focused on the cerebellum. Early results indicate that large lesions of the deep cerebellar nuclei alter timed responding in the appetitive paradigm through an increase in response preservation but do not affect cued responding. [Supported by NSF Research Training Grant to S.F.L. and J.E.S.]

259.14

THE ROLE OF THE AMYGDALA AND PERI-AMYGDALA REGION IN EARLY OLFACTORY ASSOCIATIVE LEARNING. R.M. Sullivan and D.A. Wilson. Developmental Psychobiology Laboratory, Dept. Psychology, University of Oklahoma, Norman, OK

Olfactory associative learning in infant rats is correlated with a modification of olfactory bulb function and structure. However, recent studies have suggested that acquisition and/or expression of olfactory memories in newborns may involve neural circuits in addition to the bulb (e.g., Kucharski & Hall, 1988). The present experiment focused on the role of the amygdala in olfactory learning in infant rats.

Rat pups were cold anesthetized at PN5 and received either bilateral lesions aimed at the amygdala (1 mA, 40 sec) or sham lesions. On PN6, pups were trained in a classical conditioning paradigm in one of 3 groups: PAIRED-ODOR-STROKE (odors were paired with tactile stimulation from a brush), RANDOM-ODOR-STROKE or ODOR only. On PN7 pups were tested for both conditioned behavioral responding and conditioned odor preferences. After the behavioral testing, pups were perfused through the heart and locations of the lesions confirmed histologically. Preliminary results suggest that bilateral lesions in the region of the amygdala impair performance on an olfactory associative learning task in infant rats.
Supported by BNS8819189 from NSF to DAW and DC00489 from NIH to RMS.

LEARNING AND MEMORY—PHYSIOLOGY III

260.1

OSCILLATORY ACTIVITY RELATED TO SHORT-TERM MEMORY IN THE TEMPORAL POLE OF MONKEYS. K. Nakamura*, A. Mikami and K. Kubota. Dept. of Neurophysiology., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

Neurons in the ventral part of the temporal pole (area TG) of monkeys (Macaca mulatta) showed sustained activity during a delay period of a sequential discrimination task with a time delay, while the monkey memorized particular visual stimuli (Nakamura et al. Soc. Neurosci. Abstr., 760, 1990). These activities were not present after the monkey made erroneous responses. Thus, sustained activity is considered to be related to short-term storage of visual information. When auto-correlograms were computed for sustained activity, oscillatory property was detected in 18 out of 21 neurons. The commonest frequency was 3.0 Hz (range, 2.1-20.0 Hz). They also showed oscillation in response to particular stimuli, but the frequency of oscillation during stimulation was higher than that of oscillation during the delay period. It appears that oscillatory activity is involved in short-term storage of visual information. These neurons were located both in the supragranular layers (n=10) and in the infragranular layers (n=8). The data suggest that the oscillatory activity is generated and maintained within the temporal pole.

260.2

FAILURE OF VISUAL IMAGE PAIRINGS TO PRODUCE CORRELATED SINGLE-UNIT RESPONSES IN INFEROTEMPORAL CORTEX OF A MACAQUE. Stanislaw Sobotka and James L. Ringo, Dept of Physiology, U. of Rochester Med Ctr, Rochester NY, 14642.

In this study we asked if single unit responses to two visual patterns presented separately are made more similar if previously the patterns had often been presented together. During training sessions, 5 pairs of visual patterns were presented (each pair > 1000 times) to a macaque. The monkey distinguished between these 5 pairs and other similarly produced pairs for reward (accuracy > 95%). However, no single member of any pair ever appeared as a member of any other pair. During experimental sessions, responses from 157 well isolated and visually responsive neurons in inferotemporal cortex were recorded to each of the 10 patterns now presented separately. Interlaced sessions continued the pairings training. Spike count analysis as well as a principal components analysis on smoothed response waveforms did not find greater similarity between responses to stimuli presented previously in pairs (compared with responses to stimuli not previously so presented). Interestingly, in response to other stimuli shown in the same sessions, 48 of the 157 cells did show 'recognition' memory (i.e. the response of a cell to an image was significantly altered by previous presentation of that image).

260.3

HIPPOCAMPAL NEURONS IN SHORT-TERM COLOR MEMORY. Joaquín M. Fuster. Department of Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The hippocampus plays a significant role in acquisition and consolidation of memory. In primates, including the human, this role seems to cover a wide range of mnemonic material, whether spatially defined or not. Less understood is the potential involvement of the hippocampus in the retrieval of well-consolidated memory and its temporary retention in short-term working memory. In the course of a study of inferotemporal cortical cells (Fuster, *J.Neurophysiol.*, 64:681,1990), spike records were obtained of 72 hippocampal units from monkeys performing a delayed matching to sample task with familiar compound visual stimuli. On every trial the animal had to attend to a stimulus feature (pattern or color) and retain it for 10-20 sec in order to make a discriminant choice after that delay. Results of statistical spike-frequency analysis can be summarized as follows. Units responsive to the stimulus (sample) were more common in subiculum and CA1-3 than dentate. Latency of response-onset ranged between 120 and 360 ms, mean 217 (SD 62) ms. Responses to color were generally greater when color was relevant (i.e., had to be retained) than when it was not. During the retention period (delay) some cells showed sustained and differential levels of discharge depending on the color to be remembered. These phenomena are similar to those observed in inferotemporal cells of the same animals performing the task. Thus, despite the limited data base, it may be concluded that, in the monkey, at least some hippocampal neurons are part of cortico-limbic circuits that engage in retrieval and short-term memory of nonspatial information.

260.4

ISCHEMIC NEURONAL DEATH IN MONKEY HIPPOCAMPUS. T. Ono, E. Tabuchi*, H. Nishijo* and R. Tamura*. Dept. Physiol., Fac. Med., Toyama Med. & Pharmaceu. Univ., Sugitani, Toyama 93001, JAPAN

To investigate effects of transient cerebral ischemia in the monkey, eight monkeys were subjected to temporary occlusion of 8 (bilateral common carotid, internal and external carotid, and vertebral) major arteries. The monkeys were sacrificed by perfusion-fixation 5 days after the operation. Slight increment of glial cells in the striatum and neocortices was the only neuronal change produced by 5 min occlusion. After 10-15 min occlusion, there were ischemic cell changes restricted to the CA1 subfield of the hippocampus. More prominent ischemic neuronal necrosis in the CA1 subfield of the hippocampus was evident after 18 min occlusion. Two other monkeys with ischemic lesion confined to the hippocampus, and which had been well trained pre-operatively, performed a delayed nonmatching to sample (DNMS) task with 60 to 600 sec delays (declarative memory), with scores 10 to 30% below their pre-operative scores until the 10th day of post-operative testing. The monkeys performed object discrimination and motor skill tasks (procedural memories) with their pre-operative scores. The results suggest that the CA1 subfield of the monkey hippocampus is most susceptible to a relatively mild ischemic insult, and transient ischemic insult by 10-15 min occlusion of 8 arteries in the monkey could produce a model comparable to human amnesia.

260.5

AUTOIMMUNITY AND AVOIDANCE LEARNING IN NZB X RF RECOMBINANT INBRED LINES. L.M. Schrott, R.E. Wimer, C. Wimer, G.F. Sherman, G.D. Rosen, A. M. Galaburda and Y.H. Denenberg Biobeh. Sci. Grad. Prog., U.Conn., Storrs, CT 06269; City of Hope National Med. Center, Duarte, CA 91010; and Beth Isreal Hospital and Harvard Med. School Boston, MA 02215.

NZB and BXSB mice perform poorly on avoidance conditioning (AC) tasks but well in other behavioral tests. Degree of autoimmunity, especially levels of brain reactive and anti-DNA antibodies influences AC performance (Denenberg et al., submitted; Nandy et al., 1983). To further investigate this relationship, 70 mice from 4 NZB x RF recombinant inbred (RI) lines (Lines 1, 8, 17, and 18) were studied. NZBs are autoimmune and have cortical ectopias, while RFs have neither anomaly. The RI mice received a battery of behavioral tests including two-way AC. Blood was drawn for immune assays and brains were examined. All lines had one or more ectopias. Since in prior studies ectopias have not influenced AC, data were summed across this condition. All lines were poor in AC, and did not differ in avoidance responses. However, Line 18 mice had the slowest escape time ($p<.0001$) and most null responses ($p<.04$). Line 18 also had the greatest degree of immune reactivity. They differed from the other 3 lines on IgM-RF ($p<.004$) and immune complexes ($p<.03$); from Lines 8 and 17 on IgG ($p<.001$) and anti-dsDNA antibody ($p<.01$); and from Line 8 on IgM ($p<.03$). The learning deficit in Line 18 was specific to AC. They performed well in discrimination learning, water escape learning and the Morris maze. Line 18 appears to have received a complement of genes associated with autoimmune disease. Concomitantly, it also has a deficit in some AC parameters. This is the fourth time we have found an association between degree of autoimmunity and AC using 3 different paradigms. This suggests a common mechanism is involved. This work was supported in part by NIH grant HD 20806. We thank Drs. P. Behan and L. Morrison for doing the immune assays.

260.6

BRAIN AGING AND AUTOIMMUNITY: ACCELERATED DECLINE OF MEMORY, LEARNING, AND SENSORIMOTOR PROCESSES IN BXSB/MpJ, NZB/BINJ, AND C57BL/6NNIA MICE. M. J. Forster and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine Fort Worth, TX 76107-2690.

In order to investigate the influence of autoimmunity on functional brain aging, we compared rates of decline in learning, memory and sensorimotor capacities in non-autoimmune C57BL/6NNia mice and two short-lived autoimmune strains, BXSB/MpJ and NZB/BINJ. In accordance with previous studies (Forster & Lal, *Br. Res. Bull.*, *25*:503-516, 1990), the autoimmune strains showed a rapid decline in their ability to learn an avoidance task between 2 and 8 months of age, whereas C57BL/6NNia mice showed no decline until after 12 months. When mice were tested for rates of forgetting under a discriminated escape paradigm (Forster et al., *Drug. Dev. Res.11*:97, 1987), a clear pattern of accelerated memory decline was observed for NZB/BINJ mice. However, in contrast to the findings with avoidance learning, BXSB/MpJ mice failed to show impaired memory for up to 8 months. Both autoimmune strains exhibited an accelerated decline with age in the speed and average duration of spontaneous horizontal locomotion in Digiscan activity chambers. Additionally, the autoimmune genotypes had earlier declines in their capacity to remain on an accelerating rotorod both before and after practice. These findings suggest that brain aging processes involved in memory and sensorimotor functions may be accelerated in genetically autoimmune mice. [Supported by NIH grant AG06182 (MJF)].

260.7

OVERSHADOWING EFFECTS IN AGED NUCLEUS BASALIS MAGNOCELLULARIS-LESIONED RATS. A. E. Butt, B. G. Cooper*, D. J. Hardy*, & G. K. Hodge. Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

Recovery of function typifies most studies of learning and memory in animals with lesions of the nucleus basalis magnocellularis (nbm). We examined the extent to which behavioral recovery is dependent upon stimulus salience.

The nbm was infused bilaterally with ibotenic acid in rats that were subsequently tested in a bar-press operant discrimination learning task. Although acquisition was initially impaired, enduring behavioral recovery ensued (Butt et al., *Soc. Neurosci. Abstr.*, 1990, 16, 905). Testing was suspended for 11 months. When animals were retested at 18 months post-lesion, operant performance had not changed in either the lesioned or control groups.

The original conditioned stimulus (CS) for food reward consisted of a light accompanied by a "clicking" sound at the light's onset and offset. Overshadowing procedures involved removing the light (CS_L) while retaining the auditory component of the stimulus (CS_A). Although both groups showed less conditioning to CS_A alone than to CS_L and CS_A in compound, lesioned animals showed significantly poorer conditioning to CS_A compared to controls ($p<.002$).

Results suggest that while nbm-lesioned animals are capable of learning about salient aspects of a given cue, their ability to process relevant cues of lesser salience is dramatically impaired.

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260.8

IMMEDIATE AND DELAYED MEMORY PROCESSES ARE DIFFERENTIALLY AFFECTED BY AGING. L Nielsen-Bohlman & R T Knight, Neurology Dept, UC Davis, VAMC, 150 Muir Rd, Martinez, CA 94553

We examined long latency ERPs in young (n=9, mean age=22 years) and old (n=6, mean age=70 years) adults in a recognition memory paradigm. Stimuli were line drawings of familiar objects presented in quadrafields (duration 800 msec, ISI 1200 msec). Eighty percent of the images were presented twice and 20% were presented once. The second presentation occurred at 1.2 sec with no intervening images (immediate), or at 4-6, 22-58, or 100-158 sec (delayed). Subjects indicated whether an image had been previously presented by pressing a 'yes' or 'no' button. Older subjects showed enhanced frontal positivity to all stimulus presentations. The first presentation generated a parietal P600 component of comparable amplitude in young (9.3uV) and old (8.7uV) subjects. The P600 generated by immediate stimulus repetition was also comparable in amplitude in young (15.9uV) and old (14.8uV) subjects. Delayed stimulus repetition generated a central negative component at 330 msec in both groups, with reduced amplitude in the elderly (young=5.0uV, old=3.4uV; $p<0.05$). The P600 was reduced at all repetition delays in the old group, while the young group showed P600 reduction only beyond 100 sec. These data indicate that while immediate memory processes remain intact, delayed recognition memory is altered by aging.

260.9

ANALYSIS OF MEMORY TESTS AND REGIONAL NEUROPHYSIOLOGY IN DEMENTED PATIENTS USING HMPAO AND XENON SPECT SCANS

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Analysis of the relationships between specific psychometric tests, regional cerebral blood flow (rCBF), and regional uptake of HMPAO in patients with various dementias was done to better understand possible physiopsychologic differences for different diseases as well as to explore regions of brain that are physiologically related by either rCBF or HMPAO uptake to specific cognitive functions.

Underlying assumptions include: 1) the impairment of a cognitive function is related to the physiological alteration of one or a set of physiologically connected regions; 2) the control of a function does not change loci in the brain as that function deteriorates; 3) the Xenon and HMPAO SPECT methods repsectively represent rCBF and regional perfusion of the brain; and 4) a given psychometric test is processed in similar ways by different persons.

We evaluated all patients coming to the UCI Dementia Assessment Program in a similar way. Each received a neurologic and physical exam, a comprehensive battery of psychometric tests that included the CERAD (Consortium for the Evaluation and Research on Alzheimer's Disease), an MRI, an HMPAO and Xenon SPECT scan done at UCLA-Harbor Medical Center by Dr. Ishmael Mena, and a standard set of laboratory tests to exclude dementias other than vascular or Alzheimer's. The regions of interest (ROIs) were selected on axial slices of the HMPAO and Xenon SPECT scans. The psychometrics used for this analysis pertained to memory, and included the CERAD 10 item 3 trial immediate free recall with delayed free recall and recognition, digit span, metacognition, and Mattis nonverbal recognition.

We predict that these tests will have correlations with specific ROIs. We loaded the data onto a massively parallel processing database, the TERADATA DBC, which was then used to select appropriate subsets for statistical analysis. We used BMDP to look for correlations between psychometrics and SPECT measures.

260.10

EXPOSURE TO COLD DISRUPTS THE ACQUISITION AND PERFORMANCE OF A FOUR MEMBER RESPONSE CHAIN IN RATS.

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Cold temperature exposure disrupts memory processes. The extent to which this is caused by a failure to acquire tasks in the cold is controversial. This study examined the effect of acute exposure to 2° C air on the acquisition and performance of four member response sequences in rats. Four animals were trained on a four lever, four member repeated acquisition procedure in which each day of the week was associated with a different required sequence of responses. For example, the correct sequence on Monday was 2314 while on Tuesday it was 3241. Each correct response advanced the sequence to the next member and the fourth correct response resulted in the delivery of a food pellet. Incorrect responses resulted in a three second period of timeout. Following the development of baseline stability the animals were exposed to cold air while performing the procedure. Each animal was exposed twice on each day of the week with a minimum of 48 hours between exposures. The procedure was then changed to a performance condition where the required sequence was the same each day. The animals were subsequently exposed to cold twice on this procedure. The results show that cold exposure disrupted both the acquisition and performance of sequences. Accuracy of responding decreased on both the acquisition and performance baselines; however, the magnitude of the change was greater during acquisition. Timeout responding was also disrupted in a similar fashion. The results indicate that cold exposure interferes with the acquisition and memory of response sequences.

260.11

TASTE AVERSION INDUCING EFFECTS OF COCAINE IN SELECTIVELY-BRED TASTE AVERSION PRONE AND RESISTANT RATS

R.L. Elkins[1], P.A. Walters[1], T.E. Orr[1*], E.F. Kolbe[1*], F.Westbrook[2*] and S.H. Hobbs[2*]; [1]VA Medical Center and Medical College of Georgia; [2]Augusta College, Augusta, Georgia, USA 30910.

Twenty-two generations of bidirectional selective breeding for efficiency of taste aversion (TA) conditionability have produced markedly different strains of TA prone (TAP) and TA resistant (TAR) rats. Strain selection methodology paired a saccharin flavored conditioned stimulus (CS) with the aversive unconditioned stimulus (US) consequences of a cyclophosphamide injection. The strain difference in TA conditionability is not restricted to the cyclophosphamide US. Other previously identified strain segregating USs include lithium chloride, emetine hydrochloride or ethanol injections and rotationally induced motion sickness. Within the present experiment, a range of cocaine hydrochloride US injections (10, 20 and 30 mg/kg, ip), likewise all produced significant strain differences in conditioned saccharin TAs. A pseudo-conditioning control procedure supported an associative (conditioning) interpretation of the strain differences. The TAP and TAR strains may provide a useful model for studies of biological bases of genetically mediated differences in cocaine reactivity as revealed via the TA conditioning paradigm. (Supported by the VA Medical Research Service.)

260.12

TASTE AVERSION RESISTANT RATS HAVE HIGHER NE AND LOWER 5-HT LEVELS IN BRAIN THAN TASTE AVERSION PRONE RATS

T.E. Orr*, R.L. Elkins, P.A. Walters and G.F. Carl; Psychology Research, VA Medical Center and Medical College of Georgia, Augusta, Georgia, USA 30910.

Twenty-two generations of bidirectional selective breeding for taste aversion (TA) conditionability have yielded two strains of rats that show virtually no overlap during behavioral testing of TA conditioning. Following a conditioning pairing of a novel saccharin solution with a cyclophosphamide, ethanol or cocaine hydrocloride i.p. injection, TA prone (TAP) rats prefer water to saccharin, whereas TA resistant (TAR) rats drink the saccharin solution almost exclusively. In an initial study of biological bases the strain differences, whole brains of TAP and TAR rats were analyzed for levels of neurotransmitter amines. The amines were separated by HPLC and measured by electrochemical detection. No strain differences were found with respect to DOPAC, DA or 5-HIAA. However, TAP brains had higher levels of 5-HT and lower levels of NE than TAR brains. Planned studies of regional concentrations and receptor densities as well as the effects of attempted pharmacological and/or dietary manipulations of these and other neurotransmitter systems should clarify the biological bases of TA conditioning. The findings may be applicable to improved treatment and prevention of alcoholism and drug abuse. (Supported by the VA Medical Research Service.)

260.13

PRENATAL MALNUTRITION AND POSTNATAL NUTRITIONAL REHABILITATION EFFECTS ON CA1 HIPPOCAMPAL PYRAMIDAL CELLS IN ADULT RATS.

S. Díaz-Cintra, L. Cintra, A. Aguilar*, L. Granados*, T, Kemper* and P.J. Morgane. Inst. Invest. Biomédicas, UNAM, México, D.F.04510, Neurol. Unit. Boston City Hosp. Boston MA, 02118 and Worcester Foundation for Experimental, Biology. Shrewsbury, MA, 01545.

We have previously morphometrically analyzed the effects of prenatal protein malnutrition on the dentate gyrus of the hippocampal formation. We used the same material of previous studies with nutritional rehabilitated animals that were born from mothers that were fed by a 6% casein diet before mating and during gestation. At birth, pups were cross-fostered to normal mothers fed in similar conditions with 25% casein diet. Using the rapid Golgi method we studied the CA1 hippocampal pyramidal cells in rehabilitated rats at 90 and 220 days of age. We found significant reductions in the major axis at both ages in the prenatally malnourished rats. The number of dendritic spines measured in medial and terminal segments showed significant reductions at 90 days while the terminal dendritic spines showed a significant reduction in dendritic spines at 220 days of age. These results indicate a long-term effect of prenatal protein malnutrition on the stratum radiatum and stratum lacunosum-moleculare where fibers from the perforant and Shaffer collaterals synapse on the apical dendrites of CA1 pyramidal neurons.(Supported by NIH Grants HD-22539-04 and HD 23338-03).

260.14

SLEEP DEPRIVATION IN NORMAL AND MALNOURISHED RATS OF 60 DAYS OF AGE.

L. Cintra, A. Galván* and S. Díaz-Cintra. Depto. de Fisiolología, Instituto de Investigaciones Biomédicas, UNAM, México, D.F. 04510.

In a previous study we reported significant decreases of REM sleep before and after total sleep deprivation in malnourished rats of 30 days. The objective of the present study was to evaluate the effects of protein malnutrition induced by a 6% casein diet, instituted before mating and continued during gestation and into postnatal life, on the sleep-waking cycle before and after sleep deprivation. A base line-recording day was followed by one day of total sleep deprivation in a continuously rotating cylinder (diam. 32 cm; rotation rate: 1 turn/2.40 min) and three recovery days in normal (25% casein diet) and malnourished rats of 60 days of age. We found a significant increase of SWS in malnourished rats on days 1 and 3 during the activity phase (lights-off) and also during day 3 of the rest phase (lights-on). REM sleep was significantly decreased in the malnourished animals during the rest phase on day 1 and the next 3 recovery days and during the activity phase, these rats showed a significant increase of REM sleep on days 1 and 5. These data reveal a significant delay in the mechanisms that regulate SWS and REM sleep recovery after sleep deprivation as well as a change in the circadian phase shifted in both sleep states. (Supported in part by CONACyT Grant No. P219CCOA 880341. México.)

260.15

PRENATAL PROTEIN MALNUTRITION ALTERS VIGILANCE STATE MODULATION OF INHIBITION AND FACILITATION IN THE DENTATE GYRUS. P.J. Morgane, K. Austin, R. Austin-LaFrance, J. Bronzino, J. Tonkiss*, and J.R. Galler*. Worcester Found. for Exp. Biol., Shrewsbury, MA 01545, Trinity College, Hartford, CT 06106 & Boston Univ. Med. School, Boston, MA 02118.

The effects of prenatal protein malnutrition on interneuronally-mediated inhibition and facilitation in the dentate gyrus were examined across several vigilance states using the paired-pulse technique. The paired-pulse index (PPI), computed by dividing the amplitude of the second population spike (p2) by the amplitude of the first population spike (p1), was used as a measure of the net short-term facilitation or inhibition. PPIs were classified on the basis of p1 amplitude to make comparisons between vigilance states and dietary treatment. Testing was performed during slow-wave sleep (SWS), paradoxical sleep (REM), immobile waking (IW) and active waking (AW) using IPIs of 20 - 400 ms. Magnitude and duration of interneuronally-mediated inhibition was significantly increased in rats of the malnourished group. Tests at IPI 20 ms. under high p1 conditions (p1 > median) showed significantly smaller PPIs in malnourished rats regardless of vigilance state. At IPIs > 20 ms. PPIs were consistently smaller in the malnourished group during SWS and IW. No consistent diet-related differences were found during AW or REM at IPIs > 20 ms. No consistent vigilance state effect was observed for either the facilitatory (IPI = 40 - 80 ms.) or late inhibitory (IPI = 400 ms.) periods in either group. Under low p1 conditions (p1 < median) during AW the PPIs at IPI 40 and 50 ms. were smaller that those obtained at IPIs of 30 and 60 ms. This depression interrupts the facilitatory phase and may indicate an interaction between perforant path stimulation and hippocampal theta rhythm which is masked in the high p1 condition. Alterations in interneuronally-mediated inhibition may help to explain the resistance to kindling and inability to maintain LTP previously demonstrated in our malnourished rats. **Supported by NIH Grants HD-22539-04 & HD-2338-03**

260.16

PRENATAL PROTEIN MALNUTRITION EFFECTS ON THE CHOLINERGIC SYSTEM OF THE RAT HIPPOCAMPAL FORMATION. G.J. Blatt, D.L. Rosene, K.J. Rhodes and A. Virga. Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118

Prenatally protein malnourished rats born to dams on a 6% casein diet during pregnancy and cross-fostered at birth to normal females on a 25% casein diet show adult alterations in hippocampally-mediated behaviors and in anatomy, physiology and neurochemistry of the hippocampal formation (HF). Previously we reported a marked decrease in the serotonergic (5-HT) innervation as well as a concomitant decrease in high affinity uptake sites for 5-HT in the HF of adult (day 220) malnourished rats. We report here alterations in the cholinergic system of three matched pairs of adult (day 220) malnourished rats utilizing on-the-slide *in vitro* ligand binding and histochemical techniques. High affinity choline uptake sites (HACU) revealed a 15% decrease in density in the infragranular and granule cell layers in the dentate gyrus (DG) in malnourished rats compared to normals. Histochemical staining using the Tago method for acetylcholinesterase also showed decreased reaction product in the infragranular and granule cell layers in malnourished rats but this marker also showed a decrease in the outer third of the molecular layer of the DG. Using ^{3}H-oxotremorine to assay the muscarinic M2 cholinergic receptor revealed a 20% decrease in malnourished rats in the pyramidal cell layer of CA1-3 and the prosubiculum and subiculum and a 10-15% decrease in the infragranular layer of the DG. In contrast, ^{3}H-pirenzepine labelling of M1 cholinergic receptors revealed no difference in density between normal and malnourished rats. Similar to the 5-HT system, alterations in the cholinergic system were prominent in and around the granule cell layer. This demonstrates that there are corresponding changes in at least two subcortical neurotransmitter afferent systems in the DG of prenatally protein malnourished rats. Such neurochemical changes could effect this first step in the hippocampal 'trisynaptic circuit' and may ultimately underlie some of the observed physiological and behavioral alterations produced by prenatal protein malnutrition. "Supported by NIH HD22539".

260.17

NEUROCHEMICAL EFFECTS OF PRENATAL PROTEIN MALNUTRITION ON HIPPOCAMPAL SEROTONIN ACTIVITY. J.-C. Chen, J. Galler*, J. Tonkiss* and L. Volicer.
Dept. Pharmacology and Ctr. for Behav. Development, Boston U. Med. Sch., Boston, MA 02118.

The effect of prenatal protein malnutrition on central serotonin (5-HT) metabolism was assessed in adult rats. These subjects (6/25 group) were males born to dams fed a 6% casein diet during gestation and fostered at birth to dams fed a control (25% casein) diet. Tissue content of 5-HT, 5-hydroxyindoleacetic acid, 5-hydroxytryptophan, l-tryptophan and catecholamines in the hippocampal formation was similar to that of well-fed controls (25/25 group). However, a two-fold increase in basal 5-HT efflux from hippocampal slices was observed in 6/25 rats as compared with 25/25 rats during a 30 min incubation period. [^{3}H] paroxetine binding indicated that there was no alteration of apparent maximal binding and affinity of the 5-HT transporter in the 6/25 rats. In addition, the release rate of pre-taken [^{3}H]5-HT from isolated platelets was similar in the two groups. The results indicate that prenatal protein malnutrition causes selective changes in central 5-HT metabolism. (Supported by NIH Grant HD-22539-04).

260.18

PRENATAL PROTEIN MALNUTRITION AND HIPPOCAMPAL FUNCTION: SPATIAL LEARNING AND LONG-TERM POTENTIATION. **R.J. Austin-LaFrance, J. Tonkiss*, J.R. Galler*, J.D. Bronzino and P.J. Morgane.** Trinity College, Hartford, CT 06106, Boston Univ. School of Medicine, Boston, MA 02118 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

At 70 days of age, male rats born to dams fed a 6% casein diet during gestation and fostered at birth to lactating dams fed a control diet (25% casein) were assessed for their ability to acquire and retain the location of a submerged, fixed-position platform in the Morris water maze. Twelve trials, four from each of three different start positions, were run on each of two consecutive days. Latency scores over the first day's trials indicated significantly impaired acquisition in these animals compared with well-nourished controls of the same age. Scores recorded during the first three trials of day two revealed a significant deficit in the ability of malnourished animals to maintain the previous day's performance levels. All animals were subsequently tested for their ability to establish and maintain LTP of the perforant path/dentate granule cell synapse over a 24 hr period. Although malnourished animals showed potentiation of the EPSP slope this occurred without the parallel enhancement of population spike amplitude characteristically observed in controls. This suggests that, in the malnourished animals, granule cell activity is under strong inhibitory modulation which serves to limit the translation of synaptic activation (EPSP) to cellular discharge (population spike) resulting in a decrease in the overall efficacy of neuronal transmission. A causal relationship between these behavioral and neurophysiological measures of hippocampal function remains to be determined. **(Supported by NIH Grant HD-22539)**

260.19

PRENATAL PROTEIN MALNUTRITION ALTERS DENTATE GRANULE CELL RESPONSE TO TETANIC STIMULATION. **J.D. Bronzino, R.J. Austin-LaFrance and P. J. Morgane.** Trinity College, Hartford, CT 06106 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Male rats born to dams fed a 6% casein diet during gestation and fostered at birth to lactating dams fed a control diet (25% casein) were tested at 90 days of age for their ability to establish and maintain LTP of the perforant path/dentate granule cell synapse. Prior to tetanization, input response measures were obtained from each animal to determine the stimulus intensity required to evoke population responses equal to 75% of maximum. Quantification of the population EPSP slope and population spike amplitude (PSA) were used to assess the effects of tetanization on these measures of the evoked field response. The ratio of PSA/EPSP slope was computed to provide a measure of the overall efficacy of neuronal transmission. Following tetanization, 10 evoked waveforms were recorded in response to stimulation at the 75% level at each of 8 time periods ranging from 5 min. - 24 hrs. The percent change in EPSP slope and PSA were determined for each time point with reference to the prepotentiation measures. Following potentiation, malnourished animals showed an immediate and significant enhancement of EPSP slope which was maintained over the entire 24 period. In contrast, the PSA failed to show significant enhancement until 18 - 24 hrs. post-potentiation. The level of PSA enhancement in the malnourished group remained significantly lower than that of controls across all time points. Ratio measures from the malnourished animals indicated a significant decline in the overall efficacy of neuronal transmission in response to tetanization, whereas controls showed a significant enhancement in this measure. The data suggest that prenatal malnutrition results in enhanced inhibition of granule cell activity, effectively limiting the conversion of potentiated synaptic activation into enhanced cellular discharge. **(Supported by NIH Grants HD-22539 & HD-23338 and NSF Grant BCS9010616)**

260.20

EFFECTS OF RESTRAINT AND ADRENALECTOMY ON BEHAVIORALLY - RELATED SYNAPTIC EFFICACY CHANGES IN RAT FASCIA DENTATA. E. J. Green, P.M. McCabe, and M. A. Nichols*. Department of Psychology, University of Miami, Coral Gables, FL 33124.

The transfer of rats between environments is normally associated with an increase in exploratory behavior and a number of alterations in perforant path - evoked population responses, including a large increase in evoked EPSP slope, and a substantial reduction in both the onset latency and area of the population spike. These response changes have a gradual onset and outlast the exploratory behavior. The present experiments were designed to assess 1) whether the expression of these synaptic efficacy changes requires an animal to move through the environment, and 2) the possible role of peripheral stress hormones in the mediation of these effects.

Chronically implanted rats were subjected to bilateral adrenalectomy (ADX) or sham procedures (SHAM) and maintained on 20 µg/ml corticosterone and 0.9% saline. A sound-attenuating chamber served as the animals home environment. Perforant path - evoked responses, hippocampal EEG, and behavior were sampled from the animals during a baseline period in the chamber. Following the baseline period, animals were either allowed to explore an open field containing junk objects, or they were restrained in the same environment. The animals were then deeply anesthetized with methoxyflurane, and blood was collected for subsequent determination of plasma corticosterone. Baseline responses fluctuated in accordance with the animal's behavioral state at the time of the stimulus. Both ADX and SHAM rats exploring the open field exhibited gradual decreases in the onset latency and area of the evoked population spike, relative to values observed in a similar behavioral state during the baseline period. ADX and SHAM animals also showed robust efficacy changes during and subsequent to restraint. Serum corticosterone levels indicated that bilateral adrenalectomies were complete. The present results indicate that substantial movement through an environment is *not* necessary for the expression of synaptic efficacy changes following environmental transfer. They also indicate that such alterations are not simply a consequence of stress - related adrenal hormone release. Supported by BNS 9021632 to EJG.

261.1

HUMAN BRAIN ACTIVATION DURING DYSPHORIA

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Dysphoria describes a negative mood state-- feelings of sadness, anguish, misery, and mental malaise. Dysphoria occurs in normal humans if transient and limited. When severe and persistent, dysphoria is a symptom of psychiatric disease, most notably major depression.

We have used CBF PET to visualize cortical regions activated during dysphoria. Seven right-handed volunteers (4 male & 3 female), who were normal psychiatrically and who did not have first degree relatives afflicted with affective disorder, were studied under two conditions for intrasubject paired subtractions. First, the control state was resting with eyes closed. Second, the experimental condition (dysphoria) was transient self-induced sadness, also with eyes closed. All subjects reported the subjective experience of feeling sad during the mood induction. All three women had visible tearing; two of four men teared to a slight degree.

The major responses occurred in the orbital frontal cortices. Women had bilateral orbitofrontal activation, while men had left orbitofrontal activation.

We conclude that the orbital frontal cortices play an important role in normal emotional cognitive processing.

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261.2

THE NUCLEUS ACCUMBENS IN MONKEYS (*MACACA FASCICULARIS*): INCENTIVE AND EMOTION.

C.E. Stern and R.E. Passingham. Dept. of Experimental Psychology, Univ. of Oxford, S. Parks Rd., Oxford, England, OX1 4JF. C.E.S. currently at Massachusetts General Hospital NMR Center, 13th St. Bldg.149, Charlestown, MA 02129.

The nucleus accumbens (NA) receives inputs from the amygdala. In order to better understand the behavioural significance of the NA, studies were undertaken which compared the emotional and motivational behaviour of monkeys with NA lesions with previous studies on amygdala lesioned monkeys (Aggleton and Passingham, 1981, 1982). In addition, results of a button press extinction task and a frustration task are reported.

A comparison was made between 3 unoperated control monkeys and 3 monkeys which received ibotenic acid lesions of the NA. The NA lesioned monkeys maintained normal food preferences between 5 food types and did not show signs of hyperorality in a food vs. non-food discrimination task. There was no significant difference between the food preferences of the two groups pre-operatively ($F(1,4)=1.4$) or post-operatively ($F(1,4)=0.3$). These results contrast with the effects of total amygdala lesions, which result in a flattening of food preferences, a willingness to eat previously aversive foods such as meat, and hyperorality (Aggleton and Passingham, 1981).

The NA lesioned monkeys were capable of working for reinforcement on a button press task (15 presses per nut, 900 presses per session), but they extinguished their behaviour faster than the control monkeys when reinforcement for button pressing was withheld. We suggest that the extinction results may be explained by an increased reaction to frustration in the NA lesioned monkeys. Response to frustration was recorded when peanuts were visible but unobtainable. In this testing situation, as in the extinction task, the NA lesioned monkeys exhibited hyperactive and aggressive behaviors such as pacing, cage rattling, and cage banging. In contrast, it may be significant that lesions which do result in perseveration on button press extinction tasks, such as lesions in the orbitofrontal cortex (Butter, 1969) and amygdala (Weiskrantz, 1956) produce monkeys which exhibit decreased aggressive responses in other testing situations.

261.3

ABSENCE OF KLÜVER-BUCY SYMPTOMS AFTER NEONATAL LIMBIC LESIONS IN INFANT RHESUS MONKEYS. V. Nalwa and J. Bachevalier. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Reactions to familiar vs novel objects were assessed in four monkeys with neonatal amygdalo-hippocampal lesions (AH) and 10 normal controls when the animals were 9 months old. Four of the normal controls were pair-reared with monkeys from Group AH (N/AH) and 6 were pair-reared with each other (N/N). All monkeys saw the same set of 4 familiar and 8 novel objects, presented either one, two, or three objects at a time in a fixed, pseudorandom order for 3 min each at 30-s intervals, in each of 6 daily sessions. All infants looked at and manipulated the familiar stimuli more than the novel and showed displacement activity to the novel stimuli, as indicated by an increase in cage manipulation equivalently in all three groups. However, animals in Group N/AH showed more aggressive responses towards all stimuli than animals in Groups N/N and AH and those in Group AH displayed more temper tantrums and were more passive than the others. It thus appears that early damage to limbic structures does not yield the Klüver-Bucy symptoms of loss of fear and indiscriminate approach to objects, often orally, seen in monkeys with limbic lesions done in adulthood.

261.4

EEG AND STIMULUS SALIENCE IN SCHIZOPHRENIA. C.C. Kessler* and A.S. Kling. Dept. of Psychiatry, 116A-8A, V.A. Med. Center, Sepulveda, CA 91343.

Eighteen unmedicated chronic schizophrenic patients and 13 normal controls were tested in a paradigm designed to examine changes in EEG activity following presentation of emotionally salient auditory stimuli and control tones. Five standard bands of EEG spectral power were examined at bilateral frontal and temporal recording sites. The schizophrenic subjects were assigned to diagnostic subgroups on the basis of DSMIII-R criteria following independent clinical examination by 2 staff psychiatrists. Those subjects who met DSMIII-R criteria for paranoid schizophrenia were assigned to one subgroup (PS subgroup), while those who met DSMIII-R criteria for either residual or undifferentiated schizophrenia were assigned to a second subgroup (R/US subgroup). Analysis of Variance of EEG activity revealed significant diagnosis-related differences at bilateral temporal recording sites (T3 and T4). Post-hoc tests revealed that the R/US subgroup manifested the greatest degree of change following emotionally salient stimuli, including a bilateral decrease in temporal alpha and an increase in temporal beta-2 power. Changes in EEG power following each of 8 categories of emotional stimuli were ranked together, forming a hierarchy of temporal lobe response. Alterations in this response hierarchy were observed in the R/US subgroup. These findings are interpreted as being indicative of subgroup-specific alterations in the processing of emotionally salient information within the schizophrenic spectrum. They suggest that temporal lobe mechanisms may be disturbed in certain forms of schizophrenia.

261.5

EMG SPECTRAL ANALYSIS DISTINGUISHES SPONTANEOUS FACES FROM FACES TO COMMAND.

M. M. La Cerra and A. J. Fridlund*. Psychophysiology Laboratory, Department of Psychology, University of California, Santa Barbara, CA 93106.

Global differences between faces emitted spontaneously and those emitted to command have been noted in the behavioral neurology literature on pyramidal vs. extrapyramidal lesions, as well as in the neuropsychology literature on cerebral asymmetry. The present study was designed to investigate precise differences in the patterns of muscle fiber contraction between these two types of facial displays. EMG recordings were obtained from facial sites on the cheeks directly over the right and left zygomatic major muscles of subjects while they 1) viewed a comedy video (the spontaneous condition) and 2) responded to commands to emit smiles of varying intensity (the face to command condition). Spectral analyses of these recordings indicated a significant difference in the spectral coherence (degree of synchronization) of right and left cheek muscle recruitment between experimental conditions. These findings suggest an in vivo method for parsing pyramidal vs. extrapyramidal influences on facial behavior, and have implications for neurodiagnosis and forensic polygraphy.

261.6

UNILATERAL POSTERIOR PARIETAL CORTEX LESIONS ALTER EMOTIONALITY IN RATS.

Susan E. Maier & Douglas P. Crowne, Dept. Psychology, Univ. Waterloo, Waterloo, Ontario, Canada N2L 3G1

In both human and non-human primates, unilateral injury to the posterior region of parietal cortex (PPC) produces disturbances of spatial ability and affect. In rats however, more is known about PPC effects on spatial ability than emotionality. In this experiment, we asked whether removal of PPC would alter emotionality in the rat and if unilateral lesions would produce a reliable behavioural deficit. We also asked about the influence of infantile stimulation (IS) on emotional behaviour of animals with PPC lesions. Male rats were stimulated in infancy and sustained lesions as adults. Emotionality was measured by ratings of reactivity and the open field. Consistent with human findings, rats receiving unilateral lesions were significantly less emotional than those with an intact PPC, but left lesions produced a larger effect than right lesions. IS changed behaviour consistent with the literature but IS/lesion interactions were not found. We demonstrate the role of PPC in emotionality and confirm that unilateral lesions alter behaviour in rats.

261.7

GABA BLOCKADE IN THE REGION OF THE ANTERIOR BASOLATERAL AMYGDALA (BLA) ELICITS "ANXIETY" IN THE ABSENCE OF CARDIOVASCULAR STIMULATION. S.K. Sanders and A. Shekhar. Program in Medical Neurobiol. & Dept. of Psychiatry, Indiana Univ. Sch. of Medicine, Indianapolis, IN 46202.

Intracerebral microinjection of the GABA antagonist, BMI (25 ng/250 nl) into the region of the anterior basolateral amygdala (BLA) elicits significant increases in heart rate (HR) and blood pressure (BP). To study the possibility that this physiological arousal may be part of an "anxiety" response, rats trained in the conflict paradigm were implanted bilaterally with chronic microinjection cannulae for i.c. injections and arterial catheters for direct measurement of HR and BP. Preliminary data show that 1 ng/250 nl BMI microinjected into the BLA enhances punishment-induced supp ression, while 5 ng/250 nl BMI generalized the suppression to all responding. Based on the previous study, doses greater than 5 ng BMI were necessary to elicit cardiovascular stimulation. This data suggests that the degree of GABA blockade in the BLA necessary to produce detectable "anxiety" is significantly lower than that which causes minimal cardiovascular stimulation. (Supported by R 29 MH 45362-02).

261.8

QUISQUALIC ACID LESIONS OF THE SEPTUM INHIBIT FEAR REACTIONS IN THE ELEVATED PLUS-MAZE AND SHOCK-PROBE BURYING TESTS. C. Pesold and D. Treit. University of Alberta, Canada T6G 2E9.

Recent studies in our laboratory have shown that both electrolytic and kainic acid lesions of the posterior septum inhibit fear reactions in the elevated plus-maze. The purpose of the present experiment was to further characterize the anatomical and behavioral specificity of these lesions, by comparing the behavioral effects of excitotoxic (quisqualic acid) and electrolytic lesions of the posterior septum in the elevated plus-maze and shock-probe burying tests. Following sodium pentobarbital anaesthesia, rats were given either quisqualic acid, electrolytic or sham lesions of the posterior septum and then tested 12-16 days later in the elevated plus-maze and shock-probe burying paradigms. Compared to sham lesions, both electrolytic and quisqualic acid lesions significantly reduced fear reactions (i.e, increased open arm activity in the plus-maze, and decreased burying behavior toward the shock-probe). These results provide further evidence that the posterior regions of septum play an important role in the modulation of anxiety-related behaviors.

261.9

REPEATED STRESSFUL EXPERIENCES DIFFERENTLY AFFECT INCREASED DOPAMINE RELEASE IN MESOLIMBIC SYSTEM DURING AND FOLLOWING STRESS. A. Imperato, S. Puglisi-Allegra°, P. Casolini* and L. Angelucci. Farmacologia Medica 2a, Università di Roma "La Sapienza"; ° Ist. di Psicobiologia e Psicofarmacologia (CNR), Roma, Italia.

Brain dopamine (DA) release was studied in awake rats during 1h restraint stress and immediately after the animals were freed. Restraint induced a rapid increase in DA release in the nucleus accumbens and in prefrontal cortex, while no changes in the striatum were observed. Forty min after the onset of restraint, DA went gradually back to basal levels, while a pronounced increase of DA release was again evident in both areas as the animals were freed. The effects of repeated stressful experiences (1h restraint once a day for six days) on DA release in the nucleus accumbens were also studied. Starting from the third day no increase in DA release was anymore observed during restraint. On the opposite, the increase of DA release produced by freeing the animal was always present. These findings suggest that increased DA release in mesolimbic and mesocortical areas may be a response to emotional arousal produced by sudden environmental changes, either aversive (restraint) or positive (freeing). Moreover, since increased DA release related to aversive experience undergoes to tolerance while that related to the end of it does not, different adaptation patterns for aversive and positive repeated experiences may be envisaged.

261.10

EFFECTS OF REPEATED RESTRAINT ON THE TIME-DEPENDENT RESPONSES OF MESOLIMBIC DOPAMINE SYSTEM TO STRESS Stefano Puglisi-Allegra and Simona Cabib ,Istituto di Psicobiologia e Psicofarmacologia (C.N.R.), via Reno 1, I-00198 Roma, Italy.

Stressful experiences (restraint or footshock) induce similar biphasic alterations of 3-Methoxytyramine (3-MT) in the nucleus accumbens septi (NAS) of mice. In particular, 3-MT increases dramatically during the first 10 min and then decreases after 30 min (up to 120 min). These data suggest biphasic alteration of dopamine (DA) release during prolonged stress exposure, and are in agreement with in vivo microdialysis study in rats which show biphasic changes in DA outflow (increase followed by decrease) produced by restraint. Moreover, they support the hypothesis that biphasic alteration of DA transmission in the mesolimbic system is a general response to stress and suggest that the initial increase DA release represents an arousal response while the subsequent decrease in DA release may be related to coping failure.

Repeated exposure to restraint (5 to 120 min daily for 10 consecutive days) abolishes the increase in 3-MT concentrations induced by 5-10 min restraint in the NAS. By contrast the decrease in 3-MT levels produced by longer exposure to stress is unchanged by repeated exposure to the stressor. These results point to a different effect of repeated stressful experiences on the two phases which characterize the response of the mesolimbic system to stress. While the phase of arousal characterized by increased DA release undergoes tolerance, the phase characterized by decreased DA release does not; thus indicating different adaptation patterns of the two responses possibly depending on their adaptive value.

BIOLOGICAL RHYTHMS AND SLEEP II

262.1

EXPRESSION OF RHYTHMICITY BY SCN TRANSPLANT IS AFFECTED BY SIZE OF HOST SCN LESION. M. A. Vogelbaum and M. Menaker, Department of Biomedical Engineering and Department of Biology, University of Virginia, Charlottesville, VA 22901

The circadian oscillators responsible for the temporal control of locomotor activity in mammals are located in the suprachiasmatic nuclei (SCN) of the hypothalamus. While the rhythms expressed by golden hamsters (*Mesocricetus auratus*) normally have periods close to 24 hours, a mutation has been found which in the homozygous form reduces the value of the freerunning period to about 20 hours. Complete lesions of the SCN result in circadian arrhythmicity, and transplants of fetal SCN restore locomotor rhythmicity to fully SCN lesioned, arrhythmic hosts with periods corresponding to those expected from the genotype of the donor.

Partial lesions of the SCN often result in disrupted rhythmic activity patterns with clear circadian components. To investigate the ways in which the locomotor output system would respond to .iple rhythmic inputs, we transplanted fetal SCN tissue from donors of one genotype into adult hosts of a different genotype, bearing partial SCN lesions. By altering the size of the host lesion and the time of transplantation after lesioning, we have attempted to vary the relative amount of host and donor input to the locomotor output system. We have found that individual hamsters with both 24 hour and 20 hour circadian oscillators can express both host and donor circadian rhythms. Consequently, we have referred to these animals as "temporal chimeras".

The results of our experiments show that the response of the locomotor output system to two rhythmic inputs depends upon the size of the partial SCN lesion made in the host. Large lesions reduce the frequency of appearance of host derived rhythms and increase the frequency of expression of donor derived rhythms. Smaller lesions often result in the expression of both. In no case was a donor derived rhythm seen in cases where implants were placed into hosts that had no prior SCN lesion. We believe that an output of the host's circadian oscillators suppresses expression of rhythmicity by oscillators in the transplanted SCN, and vice versa. As a larger portion of the host's SCN is destroyed, the amplitude of this suppression decreases and an increasing percentage of donor derived rhythms are expressed.

262.2

RAT-TO-HAMSTER HETEROGRAFTS OF FETAL ANTERIOR HYPOTHALAMIC (AH) TISSUE DO NOT DIFFER FROM MOUSE-TO-HAMSTER HETEROGRAFTS IN THE RESTORATION OF CIRCADIAN PERIOD. P.J.Sollars, S.I.Sollars and G.E.Pickard. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403 and Depts. of Psychiatry and Anat., Univ. of Penn., Phila., PA 19104.

To determine whether the circadian period restored by fetal AH transplantation is donor-species-typical, we have implanted fetal AH tissue containing the SCN into the third ventricle of arhythmic SCN-lesioned hamsters, using mouse, rat or hamster donors. The periods restored by hamster homografts (n=6) and mouse heterografts (n=9) were similar to the species-typical period of the intact donor (hamsters, 23.99 ± 0.08 vs 24.07 ± 0.01; and mice, 23.47 ± 0.08 vs 23.62 ± 0.03). However, those restored by rat heterografts (n=17) were significantly different from that of the intact rat (23.60 ± 0.07 vs 24.34 ± 0.04, $p<0.001$). The results suggest either that the organization of the rat circadian system differs from that of mouse and hamster or, since the rat and mouse heterografts do not differ from one another, that the period of the restored rhythm may be an effect of a host extra-SCN oscillator coupling to the grafted SCN. Supported by GM 07257 (PJS) and NS 21165 (GEP).

262.3

TIME-COURSE OF FIBER OUTGROWTH FROM FETAL ANTERIOR HYPOTHALAMIC (AH) HETEROGRAFTS. G.E.Pickard and P.J.Sollars. Depts. of Psychiatry and Anatomy, Univ. of Pennsylvania, Phila., PA 19104 and Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

We have reported that heterografts of fetal AH tissue are capable of restoring circadian rhythmicity to arhythmic SCN-lesioned hamsters (Soc. Neurosci. 14:49, 1988). We also presented evidence of neuronal outgrowth from the implant into the host brain, demonstrated by the mouse specific anti-M-6 antibody (supplied by Dr. Carl Lagenaur). We have now conducted a time-course study to determine whether this fiber outgrowth precedes the time usually required for the restoration of behavioral rhythmicity (generally more than two weeks for heterografts). Animals were killed at 2, 4, 7, 14, 30 and 45 days after implantation. By 4 days, fibers were observed extending more than 600 μm into the host brain. By day 14, a dense fiber plexus was observed in the host hypothalamus, which suggests that the restoration of circadian rhythmicity may be mediated by neuronal outgrowth from the graft to the host brain. Supported by NS 21165 and MH 47501 (GEP) and GM 07257 (PJS).

262.4

SUPRACHIASMATIC NUCLEUS (SCN) LESIONS ABOLISH AND FETAL SCN GRAFTS RESTORE CIRCADIAN GNAWING RHYTHMS IN SCN-LESIONED ADULT HAMSTERS. J. Le Sauter, and R. Silver. Dept. Psychol., Barnard College of Columbia Univ., New York, NY 10027.

Lesion studies indicate that the SCN controls daily activity cycles of a number of behavioral and physiological rhythms. Detailed anatomical studies indicate that (at least in rats) rhythms of drinking and eating (Van den Pol & Powley, 1979) and of locomotor activity and drinking (Brown & Nunez, 1986) can be dissociated following SCN lesions (SCN-X). Previous work on restoration of circadian rhythmicity by SCN grafts in SCN-X adult hamsters has shown that the restoration of locomotor rhythmicity and gonadal response to photoperiod can be dissociated (Lehman et al.,1987; Silver et al., 1990). It is not known whether other behavioral rhythms can be restored by fetal tissue grafts, nor whether all responses are restored simultaneously.

Circadian activity of gnawing activity has been demonstrated in adult hamsters (Morin, 1978). We measured both gnawing and wheel running activity in intact, in SCN-X, and in SCN-X hamsters bearing fetal grafts of SCN. Rhythmicity of gnawing activity is abolished by SCN lesions. Fetal SCN transplants into the 3rd ventricle of lesioned hamsters restore locomotor activity and gnawing rhythms simultaneously, suggesting control by a common coupling signal from the SCN. (Support: NIH NS24292)

262.5

^{14}C-2-DEOXYGLUCOSE (2DG) UPTAKE REVEALS BILATERAL ASYMMETRY IN THE SUPRACHIASMATIC NUCLEUS (SCN) OF HAMSTERS WITH SPLIT CIRCADIAN ACTIVITY RHYTHMS. S.F.Glotzbach,P.J.Sollars and G.E.Pickard. Dept. of Pediatrics, Stanford Univ., Stanford, CA 94305, Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403, and Depts. of Psychiatry and Anat., Univ. of Pennsylvania, Phila., PA 19104.

Under constant illumination, the circadian activity rhythms of some hamsters split into two components. 2DG uptake in the SCN was used to indicate relative metabolic activity in the nucleus in splitters and non-splitters. 2DG was administered intracardially at circadian times (CT) 2 and 14 to anesthetized hamsters. At 45 minutes post-injection, brains were removed and processed for 2DG autoradiography. Right/left relative optical density (R/L ROD) ratios in the SCN were quantified on a DUMAS image analysis system. Significant differences in R/L ROD ratios were observed between splitters and non-splitters in the caudal half of the SCN at both CT's. The bilateral asymmetry in 2DG uptake in the SCN of split animals suggests that an alteration in the normal pattern of metabolic activity in the SCN may underlie the phenomenon of splitting. Supported by NIH grants NS 21619 (SFG), GM 07257 (PJS) and NS 21165 (GEP).

262.6

EXAMINATION OF THE PHOTOSENSITIVITY OF THE CIRCADIAN SYSTEM IN AGED *rd/rd* MICE. R.G. Foster, I. Provencio & C.A. Cziesla*, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

We have been investigating the photosensitivity of the circadian system of *rd/rd* mice. This mutation results in the progressive loss of photoreceptor cells; the rods degenerate rapidly while cone degeneration is more protracted. Despite the loss of visual function in *rd/rd* mice at 80 days of age, we have found that the photosensitivity of the circadian system is identical in *rd/rd*, *rd/+* and +/+ genotypes. While no photoreceptor outer segments have been seen within the *rd/rd* retina at 80 days of age, cone cell perikarya have been identified. It is possible that these cone cell bodies mediate circadian photoreception. Bowes et. al. (Exp Eye Res 47: 369-390, 1988) report that all cone perikarya disappear in the *rd/rd* mouse retina between 180 - 360 days of age. We have examined the effect of a half-saturating 15 min. light pulse (515 nm) on phase shifting the circadian rhythm of locomotor behavior in *rd/rd* and +/+ mice aged between 180 - 700 days. Throughout this age range the magnitude of the phase shift showed no difference between the two genotypes and aged mice showed phase shifts that were not significantly different from animals 80 days of age. At 80 days of age the *rd/rd* eye contains 2% of the rhodopsin chromophore (11-cis retinaldehyde) found in the eyes of +/+ individuals. The chromophore content of aged *rd/rd* eyes was not significantly different from that of *rd/rd* animals 80 days of age. Chromophore content of the eye does not therefore parallel the decline of cone perikarya. These results suggest that circadian photoreception is not mediated by the "remnant" cone perikarya and that another, as yet unidentified, photoreceptor may mediate circadian light detection within the mammalian eye. (Supported by Jeffress Memorial Trust J-213 to RGF & NSF Grad. Fellowship to IP).

262.7

PATTERNS OF CIRCADIAN BODY TEMPERATURE RHYTHMS IN AGING RATS. H. Li*, M. Price*, and E. Satinoff. Psychology Dept and Neuroscience Program, University of Illinois, Champaign, IL 61820.

Many aging studies use a cross-sectional design, comparing young rats at a particular age with old rats at a particular age. This assumes that old rats are uniform with respect to the variable under investigation. Our strategy is longitudinal: it takes into account that aging proceeds at different rates in separate individuals. Long-Evans rats of both sexes were maintained in a 12:12 light-dark cycle at an ambient temperature of 23 ± 1°C. When they were at least 18 months old, they were anesthetized and implanted intraperitoneally with temperature telemeters and housed individually. Their body temperatures (Tb) were computer-recorded every 10 min until they died. We analyzed changes that occurred during the aging process in 4 variables: 1) daily mean Tb; 2) daily amplitude of the rhythm; 3) acrophase (peak of the rhythm; and 4) mean Tb during light and dark. Old rats of the same chronological age were not homogeneous with respect to their circadian Tb rhythms (CTR). Individual rats showed differences in both their CTR patterns and the way the patterns changed as they aged. Rats younger than 18 months old generally had a high amplitude CTR with little variance in the acrophase. Some rats older than 18 months maintained a fairly good CTR, with clear differences between daily Tb means in the light and the dark, and relatively regular acrophases even at very old ages. Others began to show unstable CTR's, with amplitudes varying over days from flat to ones resembling those of young rats, and greater variance in the phase. These patterns correlate with old rats' ability to maintain Tb during thermal challenges.

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262.8

A PHASE-RESPONSE CURVE FOR THE BENZODIAZEPINE CHLORDIAZEPOXIDE AND THE EFFECT OF GENICULO-HYPOTHALAMIC TRACT ABLATION. E.L. Meyer*, M.E. Harrington and T. Rahmani*, Department of Psychology, Smith College, Northampton, MA 01063.

A phase-response curve (PRC) is used to describe how a particular stimulus can shift the biological clock throughout the circadian cycle. The hamster PRC to light pulses indicates light can phase shift the biological clock during the subjective night, but cannot induce shifts during the subjective day. Dark pulses generate a different PRC in hamsters, with phase-advances during the subjective day and delays during the subjective night. The PRC for the benzodiazepine triazolam is very similar to that for dark-pulses.

The geniculo-hypothalamic tract (GHT) provides a major input to the mammalian circadian pacemaker in the suprachiasmatic nuclei. Lesions of the GHT cause a compression of the phase advance portion of the dark pulse PRC. GHT lesions totally block phase-shifts to the benzodiazepines triazolam and chlordiazepoxide (CDZ) at a few circadian phases. In this study, we measured the complete PRC to CDZ pre- and post- GHT ablation.

Twenty-four male Syrian hamsters (*Mesocricetus auratus*) were housed individually under constant dim red light with continuous access to a running wheel. Intraperitoneal injections of CDZ (100mg/kg dissolved in 0.9% saline) were administered to twelve hamsters at various circadian phases. Other hamsters served as vehicle-injected phase-matched controls. Chlordiazepoxide induced phase-advances during the subjective day, and phase-delays during the early subjective night. Unlike triazolam, during the late subjective night CDZ can induce phase advance shifts. Following GHT ablation, CDZ did not induce phase shifts at any phase tested. The results suggest that the geniculo-hypothalamic tract is a component of the neural mechanism by which CDZ induces phase shifts of circadian rhythms. This work was supported by NIH grant NS26496 to M.E.H. and a grant from the Howard Hughes Medical Institute to E.L.M.

262.9

EFFECTS OF THE DOPAMINE AGONIST, BROMOCRIPTINE, ON THE CIRCADIAN CLOCK OF GOLDEN HAMSTERS. C. Wickland and F. W. Turek, Dept. Neurobiol. & Physiol., Northwestern Univ., Evanston, IL 60208.

Administration of dopamine agonists, either systemically or centrally, results in a variety of motor effects in rats and mice. Relatively high doses of dopamine agonists induce a short period of hypoactivity, followed by a longer period of hyperactivity. Since a number of stimuli which result in acute increases in locomotor activity in golden hamsters have been found to induce phase shifts in their circadian rhythms, studies were conducted to determine whether bromocriptine (Br), a dopamine agonist, could induce increases in locomotor activity in this species, and whether this treatment could have an effect on the hamster's circadian clock. Male golden hamsters, 7 to 8 mos. of age were housed in constant light in cages equipped with running wheels. The animals were injected I.P. with 5 or 10 mg/kg Br at either circadian time (CT) 6 or CT 22 (CT 12 = predicted onset of activity). Animals were observed to be awake and actively grooming, eating and pouching for several hours after the injection, and other animals, for which activity was measured by telemetry, showed sustained increases in total activity for up to 20 hours after injection. However, in spite of the general increase in activity, there was usually little or no increase in wheel running at this time. Injections of Br at CT 6 induced phase advances in the wheel running rhythm (mean = +55.0±10.6 min., n=6), while injections given at CT 22 induced phase delays (mean = -27.0±11.4 min., n=5). These results indicate that I.P. injections of Br can induce increases in locomotor activity in golden hamsters, and that these injections also result in phase-dependent shifts in circadian rhythms which are similar in direction to those induced by other agents which are associated with acute increases in locomotor activity. At the present time, it is not known whether the effect of Br on the circadian clock is mediated via dopamine receptors in the SCN region or via the accompanying increased locomotor activity.

262.10

THYMUS VASOPRESSIN CONTENT IN ACTIVITY-BASED ANOREXIA. S. Downing, L.E. Doerries, T.S. Rieg, E. Farrar*, A. Johnson* & P.F. Aravich. Dept. of Anatomy/Neurobiology, Eastern Virginia Medical School, Norfolk, VA 23501; Department of Psychology, Christopher Newport College, Newport News, VA 23606; Veterans Affairs Medical Center, Hampton, VA 23667.

Anorexia nervosa (AN) is associated with a variety of immunological deficits, including deficiencies in cell-mediated immunity. Unfortunately, it is difficult to distinguish the primary features of AN from the secondary consequences of weight loss. Because of interest in the relationship between exercise and AN, we have been exploring activity-based anorexia (ABA) in the rat (1.5 hr/day access to food; 22.5 hrs/day access to running wheels). ABA is associated with reduced delayed-type hypersensitivity (DTH) skin responses to keyhole limpet hemocyanin (KLH) and reduced relative thymus weights. Both effects are due to the secondary consequence of weight loss (Aravich et al., Neurosci Abs 16:1199, 1990). This investigation determined the effects of ABA on thymus VP, which is synthesized within the gland. Thymi were taken from rats in the DTH study cited above (sacrificed 1-2 days post challenge and 14-15 days post sensitization) and from rats not exposed to KLH. Within each condition there were ABA (defined after a 25% weight loss), weight-matched (BWM) and non-exercised freely fed (ADL) subgroups. The KLH treatment caused an overall increase ($p<.04$) in thymus VP/mg protein compared to the non-KLH treatment; neither the ABA or BWM groups differed from their respective ADL controls. When thymic VP was expressed per gland wet weight, there was an overall KLH effect with VP/gland weight comparably elevated ($p<.01$) in the ABA and BWM KLH groups relative to the ADL KLH rats. It is concluded that 1) thymic VP/mg protein is elevated during a DTH reaction irrespective of weight loss and 2) this effect is unrelated to the efficacy of a DTH skin response, which is compromised by weight loss. Support: V. A. Merit Award (PFA).

262.11

CONSTANT ILLUMINATION INCREASES SUSCEPTIBILITY TO ACTIVITY-BASED ANOREXIA. **E.Z. Stanley, L.E. Doerries, T.S. Rieg and P.F. Aravich. College of William and Mary, Williamsburg, VA 23185; Christopher Newport College, Newport News, Va 23606; Eastern Virginia Medical School, Norfolk, VA 23501; and Veteran Affairs Medical Center, Hampton, VA 23667.**

Circadian rhythms have been implicated in anorexia nervosa and can be disrupted in a variety of species by constant illumination. The purpose of this investigation was to determine the effects of constant illumination on susceptibility to activity-based anorexia. Male and female rats were subjected to activity-based anorexia (ABA) (restricted food intake 1.5hrs/day and voluntary access to a running wheel 22.5hrs/day) and exposed to three different lighting conditions. Two of these conditions involved animals maintained on a 12/12 hour light/dark cycle and fed 1.5 hrs/day at either the midpoint of the dark phase or the midpoint of the light phase. The final circadian condition involved animals fed 1.5 hrs/day under constant illumination, which was utilized to functionally disrupt circadian rhythms. The females required the least number of days to lose 25±1.5 percent of their original body weight in the constant illumination condition (3.1 SE±0.1) and the most days in the diurnally fed condition (4.8 SE±0.3) with the nocturnally fed condition (3.9 SE±0.2) falling in between the two. Analysis of terminal wheel revolution and food intake data revealed no significant differences between any of the conditions regardless of gender. The results suggest that disrupted circadian rhythms increase susceptibility to ABA in female rats. Information regarding the contribution of circadian rhythms to ABA may be useful for the identification of a novel risk factor for anorexia nervosa in humans. Support: Veterans Affairs Merit Award (PFA).

262.12

24-HOUR TIMED-FEEDING SHORTENS THE FREE-RUNNING PERIOD OF THE LIGHT-ENTRAINED CIRCADIAN PACEMAKER IN RATS UNDER LL. J.E. Ottenweller*, W.N. Tapp, S.D. Cook, and B.H. Natelson. Neurobehavioral Unit (127A), VA Medical Center, East Orange, NJ 07019 and Department of Neurosciences, New Jersey Medical School, Newark, NJ 07103.

One circadian pacemaker in rats is entrained by the light-dark cycle (LEP) and another by the time of food availability (FEP). We have shown that phase-shifting the LEP resets the phase of the FEP. In the present study, we examined the influence of the FEP on the LEP by limiting food access to 2 hours a day in constant light (LL). Male rats were housed in running wheels with free access to powdered Purina lab chow. Wheel turns were recorded by computer. Rats were entrained to a 12hr:12hr light:dark (LD) cycle for 27 days and then transferred to LL. After 23 days in LL, food access was limited to 0900-1100h for 20 days. Rats were then returned to ad lib feeding. All rats had large amplitude, well entrained activity rhythms in LD. Spectrum analysis revealed that 15 of 24 rats had significant free-running rhythms in LL with periods ranging from 24.1 to 26.4 hr (mean±sem = 25.0±0.2 hr). In 22 of 24 rats (including all 9 rats without significant circadian rhythms in the prior LL), timed-feeding induced a 1-3 hr burst of activity before the time of food availability. There was also a very prominent period of inactivity for 4-6 hours before this anticipatory burst. The free-running period of the LEP after timed-feeding was shorter than before by 0.53±0.18 hr ($P < 0.01$). This change in period made it difficult to determine whether entrainment of the FEP by timed-feeding caused entrainment of the LEP or masking. However, the data do suggest that the LEP was clearly entrained by timed-feeding in 5 rats and clearly not entrained in 3 others. These data suggest that imposing a 24-hour feeding schedule shortens the free-running period of the LEP from 25.0 to 24.5 hr. The coupling between the FEP and LEP may also be such that entrainment of the FEP can produce apparent entrainment of the LEP. Supported by VA Medical Research funds.

262.13

ALKALI METALS CONSOLIDATE CIRCADIAN ACTIVITY RHYTHMS IN SYRIAN HAMSTERS **H. Klemfuss, T.A. Kylstra*, T. T. Bauer*, and D.F. Kripke* Veterans Affairs Medical Center, San Diego, CA 92161, Department of Psychiatry, University of California at San Diego, and Universitaet Bonn.**

A role for potassium ions in the control of circadian rhythms has been suggested by work in plants, algae, invertebrates, and more recently, mammals. We have previously reported that chronic administration of K^+ in the diet advances entrained wheel-running rhythms in hamsters and increases the amplitude of phase advances in response to light, while lithium delays entrained rhythms without affecting phase response. In the present studies, we examined effects of dietary K^+, Li^+, and rubidium (Rb^+) on the period (τ) and duration (α) of wheelrunning in hamsters, as estimated by a blind rater.

Adult male Syrian hamsters were housed in running wheel cages in constant darkness. In the first study, hamsters were given either a control diet (171 mmol K^+/kg diet), the same diet containing 1104 mmol K^+/kg, or the same diet containing 0.4% Li_2CO_3. Dietary K^+ supplementation decreased τ from 24.13±.03 (SEM) to 24.03±.02 h (N=49 and 54, $p<.05$ by Newman-Keuls) and shortened α from 12.7±.26 to 11.8±.25 h ($p<.05$). Addition of Li^+ lengthened τ to 24.27±.03 h and further shortened α to 11.1±.25 h (N=70, $p<.05$). In the second study RbCl (20mM in drinking water), like K^+ in the diet, shortened τ (from 24.10±.03 to 23.92±.03 h, N=22 and 12, $p<.001$ by t-test) and decreased α (from 12.8±.38 to 11.3±.31 h, $p<.05$).

All three alkali metals significantly shortened the duration of activity. Circadian period was either decreased (K^+, Rb^+) or increased (Li^+). This may suggest that a common ionic mechanism is involved in coupling between oscillators involved in onset and offset of hamster locomotor activity, and that a different ionic mechanism regulates period.

Supported by the Dept. of Veterans Affairs and NIMH MH000117.

262.14

DAILY AND CIRCADIAN MODULATION OF THE RAT ACOUSTIC STARTLE RESPONSE. C.C. Chabot*, D.H. Taylor* and R.G. Sherman, Zoology Dept., Miami Univ., Oxford, OH 45056.

When presented with a loud acoustic stimulus, mammals exhibit a stereotyped neuromuscular reflex or "startle". While the acoustic startle response (ASR) of the rat is currently being used in many laboratories to explore questions in pharmacology, toxicology, and reflex and sensory physiology, only one study (Horlington, 1970) has attempted to determine the potential daily modulation of this response. We investigated the daily and circadian modulation of the ASR using female Sprague Dawley rats. The rats (n=12), housed under a 12:12 light:dark (LD) cycle for 2 weeks, were placed into a small cage in a dark anechoic chamber. Ten minutes later they were exposed to bursts (40 msec) of white noise of varying intensity (80, 90, 100, 110, 120 dB; 10 trials each) that were delivered every 20 sec in a semi-random but balanced fashion. This procedure was repeated every 4 h for 24 h. Two weeks later, the ASR of the same rats was measured on the second day of exposure to constant darkness (DD) using the same procedure as above. In LD conditions, dark phase response amplitudes (g) were significantly higher than light phase response amplitudes at stimuli above 90 dB (peak, trough means ±SE; 120 dB: 205 ±20, 121 ±11; 110 dB: 166 ±16, 90 ±8; 100 dB: 98 ±15, 42 ±6). In DD, subjective night response amplitudes were significantly higher than subjective day response amplitudes at 110 dB (145 ±16, 59 ±8), 100 dB (78 ±13, 46 ±7) and 80 dB (7 ±1, 4 ±1). These results clearly demonstrate that the ASR amplitude of the rat is modulated on a daily and circadian basis. Studies are currently underway to determine the sensory or motor nature of this modulation.

262.15

STRAIN-DIFFERENCES IN PERIOD OF GROSS CAGE MOVEMENT VS RUNNING WHEEL ACTIVITY IN MICE.
J.R. Hofstetter*, A.R. Mayeda*, and J.I. Nurnberger, Jr. Institute for Psychiatric Research, Indiana University Medical School, and Roudebush Veteran Affairs Medical Center, Indianapolis, IN.

In an earlier study of circadian locomotor activity in mice under constant light (LL), we measured the period of gross cage movement by counting photoelectric beam crossings. For two strains of mice we obtained periods different from those reported for running wheel (Possidente and Hegmann, 1982). We compared running wheel activity and gross cage movement without a wheel in 7 mice of C57BL/6J and BALB/c strains under 200 lux LL. In one protocol, 2 mice of each strain were in each condition for 26 d, then switched for 26 d, and switched back for 10 d. In a second study 3 mice of each strain had running wheels for 18 d then no wheels for 15 d. C57BL mice had clear activity periods in all conditions. The free-running period was greater than 24 hr and similar when measured with either technique. BALB mice had erratic activity patterns and ran less on the wheel. The period for gross cage movement was greater than 24 hr in all BALBs but in 3 the running wheel period was less than 24 hr. There is a clear difference between strains on the running wheel but not in gross movement. This suggests that the two kinds of activities may have both different genetic inputs and different behavioral significance.

BIOLOGICAL RHYTHMS AND SLEEP III

263.1

EFFECTS OF LIGHT AND MELATONIN ON EXPRESSION OF C-*FOS* AND NGFI-A IN THE RAT SUPRACHIASMATIC NUCLEUS.
E.L. Sutin and T.S. Kilduff, Sleep Research Center, Department of Psychiatry, Stanford University, Stanford, CA 94305.

Recent studies have demonstrated the expression of the immediate early genes (IEGs), c-*fos* and NGFI-A, in the mammalian suprachiasmatic nucleus (SCN) after photic stimulation at circadian times (CT) which are known to induce phaseshifts of the biological clock. We have previously shown using immunocytochemical methods that the hormone melatonin will induce the Fos protein in the rat SCN at CT 22. The present study intended to evaluate the effects of light and melatonin on expression of c-*fos* and NGFI-A mRNAs in the rat SCN by *in situ* hybridization. Male Sprague-Dawley rats (n=24) housed under LD 12:12 were transferred into darkness for 2 days before being divided into one of four groups. At CT 22, animals were given either a subcutaneous injection of melatonin (100 µg/kg; n=6) or saline vehicle (n=6) and were sacrificed 45 minutes later by decapitation. Another group of animals were given a 30 min pulse of light (n=6) or left in the dark (n=6) for 30 min prior to sacrifice. *In situ* hybridization was performed on alternate sections with either a sense or antisense ^{35}S-labeled riboprobe to murine c-*fos* or similar oligonucleotide probes to NGFI-A. Optical density measurements of the ventral and dorsal regions of the SCN, anterior hypothalamus and optic chiasm were taken from film autoradiograms. Light induced both c-fos and NGFI-A mRNA at CT 22 in the ventrolateral SCN but in no other brain region examined. Both IEGs were induced in some animals in the melatonin and vehicle-treated groups, suggesting that non-photic stimuli can also induce IEG expression in the ventrolateral SCN. Supported by a grant from the Upjohn Company.

263.2

DIURNAL AND PHOTIC REGULATION OF C-FOS EXPRESSION IN TRANSGENIC MICE.
L. M. Robertson, R. J. Smeyne, D. Luk*, J. I. Morgan, and T. Curran* Departments of Molecular Oncology and Neurosciences, Roche Institute of Molecular Biol., Nutley, NJ 07110

The protooncogenes, *fos* and *jun*, are cellular immediate-early genes that are induced in response to a variety of extracellular stimuli. Their protein products (Fos and Jun) form heterodimers that regulate transcription of a number of target genes. To study the molecular basis of c-*fos* regulation in vivo, we developed a transgenic mouse line containing a c-*fos*-*lac*Z fusion gene. A bacterial ß-galactosidase (*lac*Z) gene was fused, in frame, into the carboxyl-terminal region of c-*fos*, without disrupting the known regulatory sequences. The purified fragment was then introduced into mice by microinjection and founder strains were derived and characterized for ß-galactosidase (ß-gal) activity. In animals sacrificed during the day, basal expression of ß-gal activity in the central nervous system (CNS) was restricted to a few discrete areas. In contrast, high basal ß-gal activity was observed in many regions of the CNS of animals sacrificed during the night. Northern analysis of mRNA levels in nontransgenic animals revealed a similar temporal pattern of c-*fos* expression. Exposure to a pulse of light in the middle of the night dramatically induced ß-gal activity in the suprachiasmatic nucleus of the hypothalamus and in the retina. The regulation of expression of c-*fos*-*lac*Z in transgenic animals was similar to that of the endogenous c-*fos* gene under a variety of physiological conditions. Future studies will involve the characterization of transgenic mice that contain mutations of different known regulatory elements of the c-*fos*-*lac*Z gene.This transgenic system should prove useful for the study of c-*fos* regulation by physiological stimuli in vivo.

263.3

LOCAL ADMINISTRATION OF CNQX ATTENUATES LIGHT-INDUCED PHASE ADVANCES OF THE CIRCADIAN ACTIVITY RHYTHM AND FOS EXPRESSION IN THE HAMSTER SCN.
M. A. Rea, B. Buckley, A.M. Michel* and L. M. Lutton. Armstrong Laboratory, Brooks AFB, TX.

Male, Syrian hamsters were fitted with guide cannulas stereotaxically aimed at the SCN and were housed in constant darkness. Five minutes prior to photic stimulation (10 min pulse; 20-40 lux) at CT18-18.5, animals received 300 nl injections of either 1 mM CNQX or vehicle into the suprachiasmatic hypothalamus. Light pulses induced phase advances of 81 ± 8 min. Vehicle injections reduced shifts ($p<0.01$) by 33% to 54 ± 10 min. However, CNQX completely blocked light-induced phase advances in most animals (12 ± 9 min; n=9). Furthermore, CNQX reduced the number of c-fos protein (FOS)-immunoreactive cells in the SCN of light-stimulated hamsters by 30% ($p<0.05$). This effect was most pronounced on the side of the injection (329 ± 63 vs 548 ± 55 cells). Vehicle injections did not affect light-induced c-fos expression in the SCN. These results are consistent with the hypothesis that light-induced phase alterations of the SCN circadian pacemaker are mediated by excitatory amino acids and may require the expression of FOS-dependent genes. Supported by AFOSR 2312W6 (MAR).

263.4

THE NMDA RECEPTOR ANTAGONIST MK-801 INHIBITS LIGHT-INDUCED CHANGES IN FOS-LIKE IMMUNOREACTIVITY IN THE MOUSE SUPRACHIASMATIC NUCLEUS.
C.S Colwell and R. Foster. Dept of Biology, Univ. of VA, Charlottesville, VA 22901.

Recent work has shown that light causes an increase in Fos-like immunoreactivity (Fos-LI) in the suprachiasmatic nucleus (SCN) of rodents. Since antagonists of NMDA receptors are known to block the effects of light on the circadian system, we sought to determine whether the NMDA receptor antagonist, MK-801, could block light's induction of Fos-LI in the SCN of mouse. This experiment consisted of three groups: vehicle plus light, MK-801 alone, and MK-801 plus light. Vehicle plus light led to an increase in Fos-LI compared to vehicle treated controls. MK-801 alone had no obvious effect on Fos-IR. Treatment with MK-801 reduced but did not completely block the light-induction of Fos-IR. Treatment with the optical isomer of MK-801 did not appear to have this effect. The results suggest that NMDA receptors mediate some of the effects of light on Fos-LI in the SCN.

263.5

COMPETITIVE NMDA RECEPTOR ANTAGONISTS INHIBIT PHOTIC INDUCTION OF FOS-LIKE PROTEIN IN THE SUPRACHIASMATIC NUCLEUS OF HAMSTERS. H. Abe[1], B. Rusak[1,3], and H.A. Robertson[2]. Depts. of [1]Psychology and [2]Pharmacology, Dalhousie Univ., Halifax, Nova Scotia, Canada, [3]Dept. of Biomedical Sci., Faculty of Health Sci., McMaster Univ., Hamilton, Ontario, Canada.

At some circadian phases, retinal illumination induces expression of several immediate-early genes (including c-*fos*) in cells in the hamster suprachiasmatic nucleus (SCN), a dominant pacemaker in the circadian system. Systemic treatment with non-competitive antagonists affecting the NMDA subtype of excitatory amino acid receptor, MK-801 and ketamine, inhibits photic induction of Fos-like immunoreactivity (Fos-lir) in the hamster SCN (Abe, et al., 1991). Because these drugs block the Ca channel associated with the NMDA receptor, they may also influence the activity of other transmitters acting through Ca channels. To assess the specificity of these effects to the NMDA receptor, we used central injections of a competitive NMDA antagonist, CPP, and of an antagonist of the AMPA/kainate receptor subtype, DNQX, to attempt to block photic induction of Fos-lir in the hamster SCN. Fos-lir was produced in SCN cells of vehicle-injected hamsters by appropriately timed light pulses. Pretreatment with MK-801 (>3 mg/kg), ketamine (>100 mg/kg) or CPP (>1 mM), however, caused a dose-dependent inhibition of light-induced Fos-lir in portions of the SCN. These treatments blocked photic induction of Fos-lir in the rostral SCN and ventrolateral portions of the caudal SCN, but not in a discrete region of the dorsolateral SCN. Pretreatment with DNQX (up to 10 mM) had no effect on light-induced Fos-lir in the SCN. These results indicate that activation of an NMDA-type receptor is critical to the induction of c-*fos* expression in a portion of the SCN, and that blocking the AMPA/kainate receptor does not affect such expression in any region of the hamster SCN.

263.6

DNQX AND CPP PREVENT LIGHT-INDUCED PHASE SHIFTS OF THE CIRCADIAN SYSTEM OF THE HAMSTER.

M. Menaker and C.S. Colwell. Biology, Univ. of VA, Charlottesville VA 22901.

We report here the results of experiments designed to evaluate whether the whether the intraventricular administration of EAA receptor antagonists prevent light-induced phase shifts of the circadian rhythm of wheel-running activity in the hamster. Administration of the non-NMDA antagonist 6,7-dinitro-quinoxaline-2,3-dione (DNQX) blocked light-induced phase advances and delays. Similarly, administration of the NMDA receptor antagonist, 3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) prevented light-induced phase advances. Neither drug, by itself, caused any consistent effect on the phase of the rhythm. These data provide further evidence that EAA receptors mediate the effects of light on the circadian system of the hamster and suggest that both NMDA and non-NMDA receptor types may be involved.

263.7

INTRACRANIAL NERVE GROWTH FACTOR INJECTIONS PHASE SHIFT ACTIVITY RHYTHMS IN SYRIAN HAMSTERS. K.G. Bina[1] and B. Rusak[1,2]. [1]Dept. of Psychology, Dalhousie Univ., Halifax, Nova Scotia, and [2]Dept. of Biomedical Sciences, McMaster Univ., Hamilton, Ontario, Canada.

The mammalian suprachiasmatic nucleus (SCN) functions as a pacemaker for the generation and photic entrainment of circadian rhythms. In rats, receptors for nerve growth factor (NGF) are found in a region of the SCN which receives photic input, suggesting a potential role for NGF in the circadian entrainment mechanism. We examined whether application of NGF to the SCN region could affect free-running, circadian activity rhythms in hamsters. Hamsters maintained in constant darkness were injected through indwelling cannulae directed at the SCN with either NGF (2µl) or vehicle at circadian times (CT) 6, 14 or 22. In general, NGF produced phase advances of the wheel-running activity rhythm at CT6 (32 +/- 10.24 min (mean+/- SEM); n=19) and CT22 (44.4 +/- 15.2 min; n=21); however, a few animals with very short free-running periods showed phase delays at these times. At CT14, NGF produced small phase delays (17.1 +/- 11.96 min; n=12) as well as changes in period which made assessment of shifts difficult. Saline vehicle administration did not produce similar phase shifts at these phases. NGF-induced shifts at these phases were similar to those elicited by the cholinergic agonist, carbachol, suggesting a possible influence of NGF on cholinergic neurons which project to the SCN. Supported by NSERC and MRC of Canada and the US AFOSR.

263.8

RESPONSE OF HAMSTER SUPRACHIASMATIC NEURONS TO NICOTINIC STIMULI IN VITRO. L.R. Waldman* and C.A. Fuller, University of California, Department of Animal Physiology, Davis, CA 95616-8519.

This study examined the nicotinic cholinergic sensitivity of neurons recorded *in vitro* from the suprachiasmatic nucleus (SCN) of the hamster. Three hundred fifty micron slices were cut and incubated in artificial cerebrospinal fluid. Action potentials recorded were 200 to 800 µvolts in amplitude with durations of 2 to 5 msec. The mean baseline firing rate was 2.0±1.5 Hz.; range, 0.2 to 6.8 Hz. Seventy five cells were given nicotine (30 µmol). Fifty three (71%) showed significant changes in firing rate while 22 (29%) did not. Of those that responded to nicotine, 33 (44%) increased their firing rate while 20 (27%) decreased. The mean absolute value of the change of the responsive group was high ($t=7.118$, $p<0.0001$) as compared to the nonresponsive population ($t=0.883$, n.s.). Eighteen cells recovered from the nicotine and were given mecamylamine (100 µmol) with nicotine (30 µmol). Mecamylamine was effective in blocking the response to nicotine in 15 of the 18 cases. Six showed no significant change in firing rate ($t=0.844$, n.s.) while 9 responded with changes opposite to those from nicotine ($t=6.906$, $p<0.001$). In 3 cases, mecamylamine was not able to block the response to nicotine ($t=2.855$, $p<0.05$). Supported in part by the Smokeless Tobacco Research Council Grant #0167.

263.9

PHOTIC REGULATION OF THE PEPTIDES LOCATED IN THE VENTROLATERAL SCN OF RATS. K.Shinohara*, K.Tominaga*, Y.Isobe* & S.T.Inouye. Lab. Integrative Brain Function, Mitsubishi Kasei Inst. Life Sci. Machida 194 Tokyo Japan.

The ventrolateral (VL) SCN is characterized by chemically different neurons containig vasoactive intestinal polypeptide (VIP), gastrin releasing peptide (GRP) and neuropeptide-Y (NPY) and also by the terminal arborization of visual afferents. To ellucidate the proper function to the VL SCN (photic entrainment), we measured VIP-, GRP- and NPY-like immunoreactivity (LI) in the rat SCN under various lighting conditions.

SCNs were punched out from 1mm section of the brain in a diameter of 400 µm. The tissues were homogenized in acetic acid and lyophilized. Peptides levels of the samples were assayed by enzyme immunoassay.

The present study demonstrates that VIP- and GRP-LI, which are synthesized in the VL SCN, does not show circadian rhythms under constant dark (DD) conditions. Under light dark (LD) conditions, GRP-LI and VIP-LI display a steady rise and fall during light period, respectively and then they gradually recover throughout night. NPY, which is transported from intergeniculate leaflet of lateral geniculate body, shows a circadian rhythm with a peak at CT 12. Apparent circadian rhythms of NPY-LI observed under DD conditions are modulated by light exposure. NPY-LI display a bimodal rhythm with peaks at both the dark-light and light-dark transition periods under LD conditions. Therefore, VIP, GRP and NPY distributed in the VL SCN may be involved in the process of photic entrainment because levels of them are regulated by photic stimulation.

263.10

ELECTRICAL HETEROGENEITY OF SUPRACHIASMATIC NUCLEUS (SCN) NEURONS THAT RECEIVE OPTIC NERVE INPUT. Y.I. Kim and F.E. Dudek. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

The electrical activity of SCN neurons receiving retinal input is likely to be important in the phase-shifting and entrainment of various circadian rhythms in mammals. To test the hypothesis that the intrinsic electrical properties of SCN neurons are homogeneous, we obtained intracellular recordings (n=30) in parasagittal hypothalamic slice preparations from adult male rats. The action potentials (57±1 mV; measured from threshold) of SCN neurons were very similar in shape; they were short in duration (0.83±0.03 ms; at half amplitude) and followed by pronounced hyperpolarizing afterpotentials (10-25 mV). In most of the neurons, an afterhyperpolarization followed a burst of action potentials evoked by a depolarizing current pulse. However, some intrinsic properties were only observed in a subpopulation of the neurons; low-threshold spikes (LTS) (Llínas & Yarom, J. Physiol., 315:549, 1981) were evident in about 50% of the neurons, and inward rectification was present in 60% of the neurons. Those neurons with inward rectification had a higher spontaneous firing rate than neurons with no rectification or with outward rectification [12.2±2.0 Hz (n=16) vs. 4.2±1.6 Hz (n=11), $p<0.01$]. In addition, neurons with inward rectification were more excitable; i.e., the slope of the frequency-current (F-I) plot was steeper [418±65 Hz/nA (n=15) vs. 222±33 Hz/nA (n=10), $p<0.05$]. Neurons with LTS potentials were not significantly different from neurons without LTS potentials in spontaneous firing rate and excitability.

These results suggest that 1) SCN neurons receiving retinal input are not electrically homogeneous, and 2) heterogeneity in certain intrinsic properties is associated with the difference in spontaneous firing rate and/or excitability. Supported by U.S. Air Force Grants 90-0056 to F.E.D.

263.11

SIBERIAN HAMSTERS WITH SUPRACHIASMATIC LESIONS EXPRESS TORPOR IN RESPONSE TO FOOD RESTRICTION.
N.F. Ruby. Dept. of Psychology, University of California, Berkeley, CA 94720.

The role of the suprachiasmatic nucleus (SCN) in mediating torpor induced by restricted feeding was studied in adult Siberian hamsters housed in a short photoperiod (8 h light/day, ambient temperature = 15°C). Body temperature (T_B) and activity were recorded at 10 min intervals beginning 8 weeks after initial short day exposure. Animals manifesting ≥4 torpor bouts (T_B<30°C) in a 14 day interval received lesions of the SCN (SCNx) or a sham-operation. Expression of torpor was eliminated in 8/11 SCNx animals and unchanged in sham-operated hamsters. Torpor was reestablished in all SCNx hamsters by limiting daily food intake to 75% of normal for 3-7 days. These torpor bouts were temporally random, occurring at all times of day for several weeks even after resumption of *ad libitum* feeding; several animals repeatedly expressed multiple bouts of torpor in a single 24 hour period. Circadian rhythms in T_B and activity were undetectable in 73% and 100% of SCNx hamsters, respectively, as determined by cosinor analysis.

These findings implicate the SCN in timing of torpor bouts. Ablation of the SCN may inhibit torpor by altering hormone secretion, and restricting food intake may reinstate torpor in SCNx hamsters by reversing these hormonal changes.

BIOLOGICAL RHYTHMS AND SLEEP IV

264.1

THE HUMAN SUPRACHIASMATIC NUCLEUS (SCN); DIFFERENCES IN RELATION TO SEXUAL ORIENTATION AND SEASON. D.F.Swaab* and M.A. Hofman*, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.
Morphometric analysis of SCN in the human hypothalamus revealed differences between hetero- and homosexual men and seasonal variations in the reference group. The volume of the SCN in homosexual men was 1.7 times as large as that of a reference group of male subjects and contained 2.1 times as many cells. In another hypothalamic nucleus which is isolated in the immediate vicinity of the SCN, the sexually dimorphic nucleus (SDN), no such differences in either volume or cell number were found, indicating the selectivity of this finding (1). The volume and cell number of the SCN appeared also to fluctuate significantly over the year, with the lowest values during the summer and the highest values during the autumn. Again, no such fluctuations were observed in the SDN. Material was obtained from the Netherlands Brain Bank (Coordinator Dr. R. Ravid). (1)/D.F. Swaab and M.A. Hofman, Brain Research 537 (1990) 141-148.

264.2

RETINOHYPOTHALAMIC PROJECTIONS IN THE MACAQUE MONKEY. J.C.Speh and R.Y.Moore. Depts. of Psychiatry, Neurology and Behavioral Neuroscience and the Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Recent studies of retinohypothalamic projections in the rat and hamster (Johnson et al, 1988) using cholera toxin-horseradish peroxidase (CT-HRP) have demonstrated extensive projections to the the suprachiasmatic nucleus (SCN), anterior hypothalamic area (AHA), lateral hypothalamic area (LHA) and retrochiasmatic area (RCA) with the projection much more extensive in the hamster than in the rat. In the present study the organization of retinohypothalamic projections was analyzed in the macaque monkey using the CT-HRP method. The projection to the SCN is the densest of all projections to hypothalamus. It is particularly dense in the ventral portion of the nucleus in a zone occupied by vasoactive intestinal polypeptide-containing neurons. Labeled fibers are about twice as dense on the side ipsilateral to the eye injection, a pattern differing from rodents where the contralateral projection is most dense. Labeled fibers extend beyond the SCN into the adjacent AHA where they form a sparse plexus. There is also a limited input to the LHA. Labeled fibers extend caudally beyond the SCN into the retrochiasmatic area where they form a dense plexus. This is a restricted pattern of hypothalamic projections similar to that observed in the rat. The pattern of projections to the perigeniculate region (ventral lateral geniculate, intergeniculate leaflet) also will be described. (Supported by a grant from AFOSR).

264.3

THE RAT RETINOHYPOTHALAMIC PATHWAY REVEALED BY DiI INJECTIONS. D. M. Murakami, I-H. Tang* and C. A. Fuller. Department of Animal Physiology, University of California, Davis, California 95616-8519.

The mammalian retinohypothalamic pathway is critical for mediating the phase-shifting and entrainment effects of light on circadian rhythms. However, little is known about the characteristics of this retinal input. This study examines the general anatomy of the retinal terminal projections to the hypothalamus. Wistar rats were sacrificed and perfused with 10% formalin. The brains were removed and DiI crystals were placed in either the optic nerve or optic chiasm. The brains were kept in 10% formalin for approximately 3 months for transport. Each brain was coronally sectioned at 100 microns on a vibratome and examined for fluorescence. Individual axons and axonal arbors could be distinguished as they projected dorsal from the optic chiasm to the suprachiasmatic nucleus (SCN). Terminals were relatively dense in the ventral SCN, while sparse terminals were found in other areas of the SCN. In addition, retinal axons extended outside the SCN, including the region ventral to the paraventricular nucleus. This research was supported in part by NASA Grant NAGW-2195.

264.4

AN ULTRASTRUCTURAL STUDY OF THE RETINOCORTICAL AND RETINOHYPOTHALAMIC TRACTS OF THE SYRIAN HAMSTER. A.S. Elliott, M.L. Weiss and A.A. Nunez. Dept. of Psych., Neuroscience Program, Michigan State University, East Lansing, MI 48824.

It has been reported that a direct retinal projection exists to the pyriform cortex (PYR; *J. Comp. Neurol.* 1981, **196**:155-172; *Brain Res. Bull.* 1991, **26**:403-411). The primary aim of this study was to determine if these retinofugal fibers made synaptic contacts with the neurons in that region. Bilateral, intraocular injections of cholera toxin-horseradish peroxidase conjugate were made into Syrian hamsters (*Mesocricetus auratus*), and the animals were allowed to survive for 48 hours. The tissue was then processed for visualization with tetramethylbenzidine (TMB), and the TMB was subsequently stabilized with diaminobenzidine. This procedure allowed both light microscopic and ultrastructural analysis of the labeled fibers. At the light-microscope level, labeled fibers were seen in the superficial layer of the PYR as well as in the suprachiasmatic nuclei (SCN) and other hypothalamic and basal forebrain regions. Ultrastructurally, fibers appeared to make *en passant* synaptic contacts with dendrites in area Ia of the PYR, where no neuronal cell bodies were seen. Post-synaptic densities and vesicles were clearly visible. However, the labeling was too dense to determine the shape of the vesicles. The number of labeled fibers was sparse when compared to the labeling in the SCN, which had numerous fibers containing label that made axo-somatic contacts as well as axo-dendritic. The presence of retinal projections to the PYR may provide a possible mechanism for photoperiodic modulation of chemosensory inputs to the hamster brain. Supported by grants BNS8908576 and BNS8919898 from the NSF, NS 07279 and by Biomedical Research Funds from M.S.U.

264.5

SUPRACHIASMATIC NUCLEUS (SCN) GLUCOSE UTILIZATION IN LATE DAY IS NORMALIZED BY SEROTONIN OR cAMP. F. E. Hospod, H. Qi, A. DeMontagnac*, K. Grundmann and G. C. Newman. Neurology Service, VAMC at Northport, NY, 11768 and Dept. of Neurology, SUNY at Stony Brook, 11777.

Brain slice glucose utilization (SGU) parallels *in vivo* SCN glucose utilization at every time of day except late subjective day when slice glucose utilization is considerably above that found *in vivo*. We have proposed that this occurs because an innibitory pathway is removed upon slice isolation. Based on the work of others, we have hypothesized that serotonin (5HT) will inhibit SCN SGU in late subjective day through mechanisms involving cAMP. To test this hypothesis we have measured SGU in the SCN and anterior hypothalamic area (AHA) in early day, late day and at night with 5HT or cAMP analogues.

Brain slices containing the SCN were prepared from male Sprague-Dawley rats (300g) entrained to a 12:12 light:dark cycle (Lights on CT00-CT12). SCN slices were pre-incubated for 75 min, exposed to K-R containing ^{14}C-2DG for 45 min, rinsed for 10 min and frozen. The 2DG incubation midpoints occurred during early day (CT03), late day (CT09) or nighttime (CT21). Cryostat sections were image-analyzed to obtain SGU in μmole/100g/min (U).

Serotonin reduces SCN SGU at CT09 from 185 ± 20 U to 94 ± 7 U ($p<.002$). Similarly, cAMP reduces SCN SGU at CT09 to 109 ± 42 U, while forskolin, methylxanthine and the combination reduce SGU at CT09 to a slightly lesser extent. Neither 5HT nor cAMP had any effect on SGU at CT03 or CT21. AHA SGU at CT09 was not affected by 5HT.

These results are consistent with the hypotheses that 1) serotonin inhibits SCN neural activity in late subjective day through a mechanism involving cAMP and 2) the high SCN SGU of late day does reflect loss of an inhibitory pathway, probably from the dorsal raphe, during slice isolation.

264.6

SEROTONERGIC AGONISTS ADVANCE THE CIRCADIAN RHYTHM OF NEURONAL ACTIVITY IN RAT SCN IN VITRO. M. Medanic and M.U. Gillette. Dept. of Physiol. & Biophys. and Dept. of Cell & Struct. Biol., Univ. of Illinois, Urbana, IL 61801.

Serotonin (5-HT) directly affects the SCN pacemaker *in vitro*. Brief application of 5-HT to ventrolateral (VL) SCN during the subjective day phase-advances ($\varnothing_A$) the time of peak neuronal activity with a maximal shift of 6.9±0.1 hr at CT 7. 5-HT is ineffective at night. This temporal sensitivity matches that for cAMP analogs. To confirm the specificity of 5-HT-induced $\varnothing_A$ and to investigate the mechanism by which 5-HT acts on the SCN, the effects of two 5-HT$_1$ agonists, 5-CT and 8-OH DPAT, were tested.

Hypothalamic brain slices containing the paired SCN were obtained from male Long-Evans rats (8wks old, raised in 12L:12D) and maintained *in vitro*. The slices were treated with 10^{-6}M 5-CT at CT 9 (n=3) or 15 (n=2), or with 10^{-6}M 8-OH DPAT at CT 9 (n=3) on day 1, by a 30 μl drop to the VL-SCN for 5 minutes. The time-of-peak in the rhythm of neuronal activity was accessed the following day.

While treatment of the VL-SCN with 5-CT at CT 15 did not significantly alter the rhythm, exposure at CT 9 resulted in a 6.0±0.1 $\varnothing_A$ of the time-of-peak. Similarly, administration of 8-OH DPAT at CT 9 induced a 6.9±0.1 $\varnothing_A$ of the peak time. This confirms the specificity of the 5-HT-induced $\varnothing_A$ and lends support to the hypothesis that 5-HT may affect the SCN via a 5-HT$_1$ receptor-linked pathway. (Supported by AFORS grant 90-0205.)

264.7

TEMPORAL CHANGES IN PROTEIN KINASE A SUBSTRATES IN THE RAT SUPRACHIASMATIC NUCLEUS. L.Faiman and M.U. Gillette. Dept. of Cell and Structural Biology, Univ. of Illinois, Urbana IL 61801

Specific stimulation of cAMP-dependent pathways induces a 4-5 hr advance in the phase of the circadian rhythm of neural activity of the suprachiasmatic nucleus (SCN) *in slice*. Sensitivity is limited to the subjective day of the SCN circadian pacemaker. Additionally, endogenous cAMP levels change *in slice*, peaking late in the day. These results suggest that stimulation of protein kinase A (PKA)-mediated phosphorylation and the level and regulation of PKA itself may be important to the mechanisms underlying time-keeping and phase-shifting.

To address this hypothesis, changes in PKA substrates in SCN maintained *in slice* were examined at hourly intervals. Punches obtained from SCN *in slice* were examined for protein phosphorylation in the presence of exogenous cAMP and EGTA. The resulting phosphorylated proteins of a single punch (~6 μg protein) per circadian time were separated by SDS-PAGE. The identity of these proteins is being established by a) their apparent M.W. and b) by Western blots. Of special interest are the levels of synthesis and phosphorylation of the regulatory and catalytic subunits of PKA across the circadian cycle. (Supported by PHS grant NS22155.)

264.8

QUANTITATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) AND TYROSINE HYDROXYLASE (TH) IN SINGLE SUPRACHIASMATIC NUCLEI (SCN) ACROSS THE CIRCADIAN CYCLE. D. Richard*, L. Faiman & M.U. Gillette. Dept. of Cell & Struct. Biol., Univ. of Illinois, Urbana, IL 61801

To elucidate the organization of substrates underlying the circadian pacemaker, we are quantitating identifiable proteins in single SCN. The SCN are derived from hypothalamic slices of rat brain maintained in glucose/$NaHCO_3$-supplemented minimal salts, conditions under which the SCN continue the circadian rhythm of neuronal firing observed *in vivo*. At a desired timepoint and after a fixed incubation period, a slice is quick-frozen on dry ice and one SCN (~5 μg protein) punched out. Proteins are separated by SDS-PAGE and identified immunologically by Western blot.

Using this technique, we determined levels of GAD and TH, the biosynthetic enzymes for GABA and dopamine, respectively, throughout the circadian cycle. Since TH is a protein kinase A substrate, we titrated phosphorylatable sites by cAMP-stimulated *in vitro* phosphorylation in the same samples. The level of TH relative to the degree of PKA phosphorylation was compared directly to assess the endogenous TH phosphorylation state.

Levels of GAD and TH were studied to develop the parameters for quantifying other regulatory proteins and their phosphorylation states in single SCN over the circadian cycle.

264.9

TEMPORAL CHANGES IN PROTEIN KINASE G SUBSTRATES IN THE RAT SUPRACHIASMATIC NUCLEUS. E.T. Weber and M.U. Gillette. Dept. of Physiology and Biophysics, Dept. of Cell and Structural Biology, Univ. of Illinois, Urbana, IL 61801.

Endogenous cGMP levels in the suprachiasmatic nucleus (SCN) oscillate *in slice* with a circadian period, with a peak around the night-day transition. Additionally, the circadian rhythm of neuronal activity of the SCN *in slice* is subject to 4-5 hr phase advancement with subjective night-time treatment with cGMP analogs, possibly stimulating pathways primed and waiting for the endogenous rise in cGMP levels. This suggests that night-time phase-shifting may involve stimulation of protein kinase G (PKG)-mediated pathways.

Phosphorylation stimulated by exogenous 100μM cGMP in low Ca^{++} conditions was examined in lysates of SCN and hypothalamus (HT) micro-punches quick-frozen hourly over the course of a 24 hr cycle *in vitro*. Protein kinase A activity was inhibited with a specific peptide inhibitor. PKG-phosphorylated proteins were identified in single SCN and HT after SDS-PAGE. We are comparing PKG substrate phosphorylation over the course of the day to identify phase-dependent substrates which may underlie circadian rhythm modulation. (Supported by PHS Grant NS22155.)

264.10

THE CIRCADIAN CLOCK IN THE RAT SCN HAS PHASE-DEPENDENT SENSITIVITY TO PROTEIN KINASE INHIBITOR H7. C. Liu & M.U. Gillette. Neurosci. Program, Dept. of Cell & Struct. Biol., Univ. of Ill., Urbana, IL 61801

Both cAMP and cGMP induce a phase advance of the circadian rhythm of neuronal activity of the suprachiasmatic nucleus (SCN) *in vitro*. Sensitivity to cAMP and cGMP is limited to the subjective day and night, respectively. To further elucidate the mechanisms by which these cyclic nucleotides affect the SCN clock, the effect of a nonspecific protein kinase inhibitor, H7, at different circadian times (CT) was examined.

Coronal hypothalamic slices containing the SCN were prepared from 8 wk old female Long Evans rats housed in a 12L:12D cycle. The media in the brain slice chamber was replaced with fresh media containing the H7 for 1 hr at different CTs. All measurements of phase were made on day 2 by monitoring firing rates and determining the time-of-peak of neuronal activity in SCN *in vitro*.

Incubation in H7 at CT 6-7 or 18-19 phase advanced the circadian rhythm. The time-of-peak was advanced by 5-6 hr at 100 μM and 2 hr at 10 μM. Incubation in 100 μM H7 at CT 11.5-12.5 had no effect. These results show that the circadian clock has phase-dependent sensitivity to inhibition of activity of protein kinases. We are using relatively specific protein kinase inhibitors to test the possibility that the effect of the H7 during day and night is mediated by different kinases, possibly cGMP and cAMP kinases, respectively. (Supported by NS 22155).

264.11

CHARACTERIZATION OF REGIONAL NEURONAL ACTIVITY IN THE SUPRACHIASMATIC NUCLEUS USING A CURVE-FITTING TECHNIQUE **T.K. Tcheng and M.U. Gillette. Neuroscience Program and Department of Cell & Structural Biology, University of Illinois, Urbana, IL 61801.**

The rat suprachiasmatic nuclei (SCN) contain an endogenous circadian pacemaker that is expressed in the brain slice as a 24 hr oscillation in ensemble neuronal firing rate (ENFR). We are studying the anatomical distribution of the pacemaker within the SCN by analyzing the ENFR in isolated dorsomedial (DM) and ventrolateral (VL) subregions.

We have previously demonstrated empirical changes in the ENFR of the DM-SCN after surgical isolation. Our current work quantifies these changes and characterizes neuronal activity in the isolated regions. Single unit activities were recorded over >24 hr to examine the circadian pattern of ENFR for each region. A parameterized curve was constructed that describes the 24 hr ENFR of control SCN in an intact slice. Goodness-of-fit for ENFRs from isolated DM- and VL-SCN were calculated after fitting the curve to 24 hr data from control SCN. Significant differences ($p < 0.05$) were found between controls (N=4) and isolated DM-SCN (N=2) but not isolated VL-SCN (N=3). Spectral analysis will be performed on control and experimental data to identify the major periodicities expressed by each group. (Supported by AFOSR grant 90-0205.)

264.12

IN VITRO RESETTING OF THE SUPRACHIASMATIC CIRCADIAN PACEMAKER BY SPECIFIC SEROTONERGIC AGONISTS AND ANTAGONISTS. R.A. Prosser, J.D. Miller, and H.C. Heller*. Dept. Biological Sciences, Stanford University, Stanford CA 94305.

The mammalian circadian clock in the suprachiasmatic nuclei (SCN) produces a 24 hr rhythm of spontaneous neuronal activity *in vitro*. We have used the SCN slice to investigate the ability of specific serotonergic (5-HTergic) compounds to reset the SCN circadian clock. In these experiments slices prepared from adult male Wistar rats were maintained *in vitro* under constant perfusion conditions.

We previously found that 1 hr bath application of the nonspecific 5-HT agonist Quipazine (Quip) shifts the SCN clock *in vitro*, inducing phase advances in the day (circadian time (CT) 6 & 9) and phase delays at night (CT 15, 18 & 21) (Brain Res. 534:336). These effects are mimicked by 5-HT and blocked by the non-specific 5-HT antagonist metergoline. Here we find that the advances induced at CT 6 are also mimicked by the 5-HT_{1A} agonists 8-OH-DPAT and Buspirone, but not by the 5-HT_{1B} agonist CGS 12066B or the $5\text{-HT}_{1C/2}$ agonist DOB. Also Quip-induced advances were blocked by the 5-HT_{1A} antagonist NAN-190 but not by the 5-HT_{1A} antagonist ICS 205-930. None of the specific 5-HT agonists mimicked the delays induced by Quip at CT 15; neither did the specific antagonists block Quip-induced delays. These results suggest that Quip resets the SCN during the day by stimulating 5-HT_{1A} receptors but acts on undetermined 5-HT receptor type(s) at night to reset the clock. (Supported by NIH postdoctoral fellowship NS 08905 and The Upjohn Co.)

264.13

TETRODOTOXIN DOES NOT BLOCK IN VITRO RESETTING OF THE SUPRACHIASMATIC CIRCADIAN PACEMAKER BY QUIPAZINE. J.D. Miller, R.A. Prosser, and H.C. Heller*. Dept. of Biological Sciences, Stanford University, Stanford CA 94305.

The mammalian suprachiasmatic nuclei (SCN) contain a circadian pacemaker that produces a 24 hr rhythm in spontaneous neuronal activity *in vitro*. Previous work shows that this *in vitro* rhythm can be permamently reset by 1 hr applications of the non-specific serotonin (5-HT) agonist Quipazine (Quip; Brain Res. 534:336). To begin addressing the issue of whether Quip acts directly on pacemaker cells or on afferents to the clock, we investigated whether blocking Na^+-dependent action potentials with tetrodotoxin (TTX) prevents Quip-induced phase shifts of the SCN clock.

Brain slices prepared during lights-on from adult male Wistar rats housed in 12:12 LD were maintained *in vitro* under constant perifusion conditions. Slices were treated for 1 hr with TTX (1 uM) alone or TTX + Quip (1 uM) at either CT 6 or 15 during the first day *in vitro*. Neuronal activity was recorded on day 2. TTX treatment by itself at either CT did not affect the phase of the SCN clock. TTX also did not block Quip-induced advances at CT 6 (X=2.5±0.0 hr; N=2) or delays at CT 15 (X=2.5±0.0 hr; N=2). Therefore, Na^+-dependent action potentials do not appear to be required for Quip to reset the SCN clock. These results suggest that 5-HT receptors are located on SCN pacemaker cells or on cells that communicate with pacemaker cells by means other than Na^+-dependent action potentials. (Supported by NIH postdoctoral fellowship NS 08905 and The Upjohn Company)

264.14

EFFECTS OF CHOLINERGIC AGENTS ON CELLULAR METABOLISM IN CULTURED SUPRACHIASMATIC NUCLEUS. V.Cao*, J.Owicki*, D.Edgar and J.D.Miller. Depts. of Psychiatry,Biology,Stanford Univ., Stanford, CA 94305, and Molecular Devices Corp., Menlo Park, CA 94025.

The suprachiasmatic nucleus (SCN) of the hypothalamus is necessary and sufficient for circadian rhythmicity in mammals. Cholinergic stimulation *in vivo* generates phase shifts in such rhythms, perhaps via a nicotinic receptor. However, high affinity nicotine binding has not been observed in the SCN. We examined the effects of cholinergic agents on cellular metabolism, as measured in a microphysiometer, in cultured SCN cells from two day old Sprague-Dawley rats. The rate of extracellular acidification, following a one min pause in perfusion, was the index of cellular metabolism. Eight cultures underwent a 20 min baseline recording period, followed by three 10 min exposures to either 10μM carbachol or 10μM nicotine, in counterbalanced order, with 20 min recovery periods between each drug exposure. Subsequently each culture was exposed to an additional three trials of the other drug. An additional four cultures were exposed to six trials of 5μM nicotine plus the nicotinic antagonist, mecamylamine (5μM). Immunohistochemical validation of the cultures was provided by antibodies against VIP, GFAP and MAP2. Carbachol produced no alteration in cellular metabolism whereas nicotine induced a reliable and significant decrease in metabolism compared to baseline (-12.5±2%). Mecamylamine did not significantly affect the nicotine-induced decrement in cellular metabolism. These results suggest that nicotine's effects in the SCN are probably not mediated by a classical cholinergic receptor.

This research was supported by a grant from the Upjohn Company.

264.15

EFFECT OF NICOTINE AND CARBACHOL ON THE CIRCADIAN RHYTHM OF THE SUPRACHIASMATIC NUCLEI *IN VITRO*. **L. Trachsel*, H.C. Heller*, W.C. Dement, and J.D. Miller. Dept. of Biol. Sciences, Stanford University, Stanford, CA 94305-5020.**

Cholinergic drugs have been reported to phase-shift circadian rhythms *in vivo*. However, cholinergic mechanisms in the suprachiasmatic nuclei (SCN) proper have remained unclear. We thus investigated effects of cholinergic compounds on the SCN *in vitro*. SCN brain slices were prepared from adult male Wistar rats housed in 12:12 LD. At a designated circadian time (CT), the slice perfusion medium was replaced for 1 hr with medium supplemented with carbachol (10 μM), or nicotine (10 μM). To determine whether the treatment shifted the SCN clock, the CT of peak neuronal activity in the SCN monitored on day 2 *in vitro* was compared to the control CT 6 (CT 0: lights-on). Nicotine phase-advances the SCN clock: +2.5-3 hrs at CT 9, 12, 15, 21; +1-1.5 hrs at CT 6 and 18. Carbachol had no effect at CT 15 (-1 and +1 hr). The results suggest that nicotine generally accelerates the SCN clock. Nicotine may act on a binding site in or near the SCN that is dissimilar from the classical cholinergic receptor. (Supported by The Upjohn Co. & the Swiss National Foundation.)

264.16

IN VIVO EVIDENCE FOR A DIURNAL RHYTHM IN SCN SEROTONERGIC ACTIVITY IN THE SIBERIAN HAMSTER. S.A. Ferreira, W.W. Randolph and J.D. Glass. Dept. Biological Sciences, Kent State University, Kent, OH 44242.

In vivo microdialysis was used to characterize the daily pattern of serotonergic (5-HT) activity in the SCN and to explore the relationship between interstitial 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the male Syrian hamster. Freely-behaving animals with a dialysis probe in or near the SCN exhibited an apparent circadian rhythm in 5-HIAA release, with the peak (147±5% of the daily mean) occurring 2-3 h after lights-off. A direct relationship between 5-HIAA and 5-HT was shown through increased dialysate 5-HIAA (27±6% of baseline; $p<0.05$) in response to localized application of 5-HT (3 uM). Tryptophan loading (25 mg/kg i.p.) increased 5-HIAA in SCN dialysates by 42±6% ($p<0.05$), and perfusion with high $[K^+]$ (150 mM) or veratridine (100 uM) decreased 5-HIAA by 62±5 and 49±11%, respectively (both $p<0.05$). The effect of K^+ was not blocked in the presence of Ca^{++} channel blockers. Localized application of tetrodotoxin (5 uM) significantly decreased 5-HIAA in the dark phase, but had little effect in the light phase (42±8% vs. 8±4% suppression, respectively; $p<0.05$). These results are evidence that synaptic release of 5-HT in the SCN is increased at night and is reflected by a rise in interstitial concentrations of 5-HIAA. Supported by AFOSR 440785 (J.D.G.).

264.17

NAAG in the Retinohypothalamic Projection System: Metabolism and High Affinity Binding. M. A. Aryan Namboodiri, H. M. Valivullah and John R. Moffett. Molecular Neurobiology Laboratory, Department of Biology, Georgetown University, Washington, DC 20057.

We have recently reported immunohistochemical data indicating that N-acetylaspartylglutamate (NAAG) may act as a transmitter in the retinohypothalamic tract (RHT) in the rat (PNAS 87, 8065-8069, 1990). In the present study, we have investigated uptake, metabolism and high affinity binding of NAAG using hypothalamic preparations containing all the RHT projection areas.

To study uptake and metabolism of NAAG, ^{3}H-glutamate labelled NAAG (50nM, 52Ci/mmole) was incubated with hypothalamic slices in artificial cerebrospinal fluid equilibrated with 95%O_2/5%CO_2 for different duration at 37°C. ^{3}H-NAAG was found to be taken up rapidly, with gradual increase up to 120 min. HPLC analysis (Partisil SAX column, 0.2M potassium phosphate, pH 4.9) of the radioactivity in the tissue showed that about 75% of the ^{3}H-NAAG has been converted to ^{3}H-glutamate at 30 min incubation. To study high affinity binding of NAAG, a hypothalamic membrane preparation (200 ug protein) was incubated (23°C, 5 min and 30 min) with ^{3}H-NAAG (50nM,52Ci/mmole) in Tris HCl buffer (50 mM, pH 7.4) in a total volume of 100ul, and the bound radioactivity was separated from free by membrane filtration. Nonspecific binding was determined using 50uM nonlabeled NAAG and identity of the bound radioactivity was determined by HPLC analysis. Significant specific binding was detected at both the early (5 min) and late (30 min) incubation times. However, HPLC analysis showed that almost all the bound radioactivity was associated with glutamate at both times. The extraordinarily rapid conversion of NAAG to glutamate in the hypothalamic preparation combined with the high affinity binding of NAAG being exclusively through glutamate suggest that NAAG may act in the RHT system via glutamate by a mechanism that is likely to be specific to NAAG. (Supported by the NIH grant DK37024 to MAAN).

264.18

VIP AND ANGIOTENSIN II BINDING IN RODENT SCN: CIRCADIAN AND DEVELOPMENTAL STUDIES. M. Li and J.L. Fuchs. Dept. Biological Sciences, University of North Texas, Denton TX 76203

VIP and angiotensin II (Ang II) binding are present in high levels in the suprachiasmatic nucleus (SCN) relative to adjacent hypothalamus. Diurnal variation has been reported in the rat SCN for VIP mRNA and for VIP immunoreactivity. In this study, we tested for the presence of diurnal differences in VIP and Ang II receptor levels in 12-17 adult hooded rats at each of two times, mid-L and mid-D (LD 12:12). SCN sections were incubated in [^{125}I]-VIP or [^{125}I-Sar^1-Ile^8]-angiotensin II. Autoradiographs revealed no diurnal differences in ratios of binding levels in SCN vs. adjacent hypothalamus. In addition, neither VIP nor Ang II showed evident changes in hamster SCN examined at 3 time points (5-6 animals per group). The results do not support the hypothesis that circadian regulation of these SCN receptors is critical for the generation of rhythmicity.

Rat and hamster SCN had similar levels of VIP binding, with patterns resembling cell distributions in Nissl sections. However, these species showed marked differences in Ang II binding: in rat, label was dense, particularly in ventral SCN except in caudalmost regions, whereas in hamster, levels were considerably lower and did not delineate the SCN.

Developmental studies used 1-3 rats at each age: VIP: E18, E19, P0, P10; Ang II: E16, E17, E18, E20, P1. VIP binding matured early, reaching nearly adult levels in the SCN by E18. In contrast, Ang II levels were very low prenatally and rose substantially from P1 to adulthood. The early appearance of VIP binding suggests that it could be involved in fetal entrainment, while the later development of SCN Ang II binding suggests that this neuropeptide may play a role in postnatal ontogeny of circadian characteristics. Supported by NIMH MH41864.

BIOLOGICAL RHYTHMS AND SLEEP V

265.1

ENTRAINMENT OF RAT ACTIVITY RHYTHMS BY MELATONIN DOES NOT DEPEND ON WHEEL-RUNNING ACTIVITY J.R.Redman and C.M.Roberts*, Dept. Psychology, Monash University, Clayton, Vic. 3168, Australia

Daily injections of melatonin (MT) entrain rat activity rhythms free-running in constant dark when the time of MT administration coincides with the time of activity onset (Redman et al. 1983). This circadian phase (activity onset) is also the phase at which hamsters respond to social zeitgebers and it was suggested that the effects of MT on the circadian system may be mediated by some modification of general arousal mechanisms (Mrosovsky 1988). To determine whether entrainment by MT is mediated by alterations in activity levels, we examined the effects of daily injections of MT when wheel-running was prevented. Twenty nine male rats were held in very dim constant light (<0.1Lux) until rhythms in running-wheel activity were established. Rats were injected daily with MT (n=18; 100µg/Kg/2ml 1% ethanolic-saline vehicle) or with vehicle (V) (n=11). On day 37 of the injection regime the running-wheels of 10 MT-injected and 5 V-injected rats were blocked to prevent wheel rotation while still allowing access. Injections were continued for a furter 29 days, then wheels were unblocked and wheel-running activity was again monitored. The phase of the rhythm when wheel-running resumed indicated that entrainment to MT persisted even when running-wheel activity was prevented. Further investigations will look at the effects of MT injections on general activity and body temperature.

265.2

EFFECT OF LIGHT INTENSITY AND MELATONIN (MEL) ON THE RATE OF REENTRAINMENT OF LOCOMOTOR ACTIVITY IN THE C3H/HeN AND C57BL/6J MOUSE AFTER A PHASE ADVANCE IN THE L/D CYCLE.
J. M. Fang and M.L. Dubocovich.. Dept. Pharmacol., Northwestern Univ. Medical School, Chicago, IL 60611.

Melatonin binding sites are found in the SCN of the C3H/HeN and C57BL/6J mice (Eur. J. Pharmacol. 180: 387, 1990). The C57BL/6J does not produce melatonin in the pineal gland, and has a lower threshold for entrainment by light (0.01 lux) than the C3H/HeN mouse (5-10 lux). Mice (4-5 weeks old) were kept on a 12/12 L/D cycle. When illumination during the light period was 45 lux the transitory period to reach reentrainment of activity rhythms after 8 h phase advance of the L/D cycle was significantly longer in the C3H/HeN [6.58 ± 0.36 days (12)] than in the C57BL/6J mouse [4.33 ± 0.31 days, (12), $p < 0.05$]. Daily injections (12 days) of melatonin (1 mg/kg, i.p.) at the pre-phase shift dark onset decreased the rate of reentrainment in the C3H/HeN mouse [10 ± 0.37 days (11), $p < 0.001$]. The effect of melatonin was less pronounced in the C57BL/6J mouse [5.25 ± 0.28 days, (12), $p < 0.05$]. The transitory period was significatively shorter in both the C3H/HeN [3.4 ± 0.4 days, (6)], and C57BL/6J [2.75 ± 0.48 days, (4)] mice, with illumination of 300 lux. Under these conditions melatonin (1 mg/kg, i.p.) significantly decreased the rate of reentrainment in the C3H/HeN mouse [5.38 ± 0.3 (6), $p < 0.001$). In the C3H/HeN mouse melatonin and light intensity may partially influence the lenght of the transitory period.

265.3

NON-PHOTIC ENTRAINMENT IN SUPRACHIASMATIC NUCLEI ABLATED HAMSTERS. Ralph Mistlberger, Psychology, Simon Fraser University, Burnaby, BC Canada.

This study examined the role of the SCN in non-photic entrainment in the hamster. SCN ablated hamsters were recorded in constant dark and under schedules of 2h daily cage changes, restricted food availability and/or light-dark. Some hamster with very large lesions subsuming SCN and local areas exhibited significant but unstable circadian rhythms in DD. Some with similar lesions showed entrained rhythms to daily cage changes. All hamsters showed robust rhythms entrained to a daily feeding schedule, but no hamster entrained to LD cycles. Competent circadian oscillators evidently exist outside of the SCN, at least .5mm or more away, and at least some are non-photically entrainable. Weaker entrainment in animals with larger lesions suggests that non-photically entrainable oscillators also exist within the SCN or its immediate vicinity.

Supported by NSERC, Canada.

265.4

THE EFFECTS OF EXOGENOUS MELATONIN OR PINEALECTOMY ON SEVERAL SIMULTANEOUSLY MONITORED CIRCADIAN RHYTHMS. C.W. Coen, M.C. Ruiz de Elvira*, R. Persaud*, R. Stoughton*, D. Sugden* & C. Demaine*. Division of Biomedical Sciences, King's College, London, UK.

Studies designed to test the possibility that melatonin acts as an internal Zeitgeber for circadian rhythms have been undertaken on Long-Evans rats. Four parameters have been monitored every 5 minutes under constant dim red light: wheel-running and drinking (by circuit closure), and core temperature and general locomotor activity (by radiotelemetry; Mini-Mitter Co.). The data were analyzed using Dataquest and Tau software. When daily injections of melatonin coincided with the onset of activity, each of the free-running rhythms was entrained in synchrony; doses of 500, 100 or 50 µg/kg were completely effective, but only 33% of the animals showed entrainment with 10µg/kg, and 17% with 5µg/kg (n=6-8). Each of the parameters remained synchronized during the loss of entrainment associated with the relatively low doses of melatonin. Furthermore, the free-running circadian rhythms failed to show any desynchronization when either pinealectomy (n = 8) or the appropriate sham procedure (n = 8) was carried out at the mid-point of a six-month monitoring period. Thus, although pharmacological doses of melatonin can entrain several rhythms (including that of core temperature which persists in the absence of a rhythm of locomotor activity and even following ablation of suprachiasmatic nuclei), it has not been possible to demonstrate a physiological role for the pineal in the synchronization of the circadian phenomena examined here.

265.5

MELATONIN INJECTION AFFECTS THE CIRCADIAN RHYTHM OF WHEEL RUNNING AND THE PROFILE OF SCN NEURONAL FIRING IN VITRO IN PHOTO-NONRESPONSIVE DJUNGARIAN HAMSTERS. R.R. Margraf, P. Zlomanczuk‡, A. Morisette‡, and G.R. Lynch. Department of Biology, Wesleyan University, Middletown, CT 06459.

The role of melatonin (MEL) feedback on circadian behavior and SCN function is poorly understood. In this study, we investigated the effects of daily MEL injections on phase angle of entrainment, duration of wheel-running activity, and frequency of SCN neuronal discharge in two phenotypes of the Djungarian hamster, Phodopus sungorus. These phenotypes, photo-responsive and photo-nonresponsive, differ in their photoinductive responses to short day (LD 9:15 lights on 0800 h) and in a variety of circadian parameters including phase angle of entrainment, duration of wheel-running activity, and timing of the nocturnal MEL pulse. Further, in this species, these behavioral differences correlate with the daily profile of SCN neuronal activity. Twenty photo-nonresponsive hamsters were identified following 12 weeks of short day exposure and were divided into MEL- and saline-injected groups. Animals from both groups were housed in cages equipped with running wheels, and activity was continuously measured. Following 12-14 weeks of injection (at 2000 h), coronal hypothalamic slices containing the SCN were prepared, and SCN electrical activity from both groups was measured in vitro for 24-36 hours (after Margraf et al., 1991, Brain Res 544, 42-48).

MEL-injections transformed formerly photo-nonresponders hamsters into photo-responsive hamsters in that they showed molt, body weight loss, gonadal regression and torpor. Mel-injected hamsters also exhibited an expansion in alpha and an entrainment to lights off, similar to responsive hamsters. Saline-injected animals remained in the nonresponsive state, as characterized by a compressed alpha and delayed activity onset. They did not experience molt, weight loss, gonadal regression or torpor. SCN neuronal activity recorded from MEL-injected animals strongly correlated with behavioral data. In all cases, SCN discharge frequency was high during periods of locomotor suppression. These results demonstrate that MEL induces photoresponsiveness in previously nonresponsive hamsters, that MEL modifies some circadian behaviors to resemble responders, and that MEL affects the daily firing profile of the SCN. Supported by NSF DCB 87-19120.

265.6

MELATONIN DURATION CODES FOR DAYLENGTH IN MALE SYRIAN HAMSTERS. J.D. Karp and J.B. Powers. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

These experiments investigated the response of pinealectomized (PX) male Syrian hamsters to programmed administration of melatonin (MEL). Hamsters were castrated, given s.c. testosterone (T) implants and housed in 8L:16D. After 7 weeks, T was removed and the hamsters were either PX or sham operated. Daily s.c. MEL (250ng) or vehicle infusions began the night after pineal surgery. Infusion durations were long (11 or 12 hours) or short (6 hours) and ended 1 hour before lights-on each night. Measurement of serum luteinizing hormone (LH) after 3 weeks indicated that long duration MEL infusions maintained short photoperiodic conditions, (LH levels were low), but short duration MEL infusions did not (LH levels were elevated). In a second experiment, PX gonadally intact hamsters were housed in 12L:12D and injected s.c. with MEL or vehicle, once daily for 11 weeks. Injections were timed to coincide with a circadian phase reported to be maximally sensitive to the effects of MEL in 12L:12D. Injections 3 or 5 hours after dark did not induce gonadal regression; testes size and serum T levels between the MEL and vehicle treated groups were not different. These results support the hypothesis that the duration of MEL secretion each night is an important variable in conveying daylength information; the circadian phase of MEL availability appears to be less relevant.

265.7

COMPARISON OF MATERNAL PINEALECTOMY AND MELATONIN-CLAMPS ON PRENATAL TRANSFER OF PHOTOPERIODIC INFORMATION. T.H. Horton‡, S.L. Ray‡, R.V. Dorman and M.H. Stetson‡. Dept. of Biological Sciences, Kent State Univ., Kent, OH 44242 (THH, RVD) and School of Life and Health Sciences, Univ. of Delaware, Newark, DE 19716 (SLR, MHS).

Maternal pinealectomy (PINX) and melatonin-clamps (MEL) alter photoperiodic information transfered from female Siberian hamsters to their fetuses. Female Siberian hamsters were PINX'ed or sham-operated (SHAM), allowed ten days to recover, then received two 7 mm pellets containing beeswax (CONT) or beeswax+melatonin (MEL, @1.3 mg MEL/pellet) and were paired with a male. Females were housed in 16L:8D until parturition when they and their litters were moved to constant light. Male young were sacrificed at 28 days of age to assess the effects of the maternal treatments. MEL altered the effects of PINX, indicating that the young received different prenatal photoperiodic information (ANOVA: Surg x Imp, df 1 / 58, F = 22.81, p < 0.001). Paired testicular weights of young born to SHAM-CONT dams are larger than those born to SHAM-MEL dams (252 mg vs 139 mg, p < 0.001); those of young born to PINX-CONT dams were significantly smaller than those born to PINX-MEL dams (109 mg vs 219 mg, p < 0.001). PINX and MEL alter the prenatal transfer of photoperiodic information through different mechanisms and not simply by obscuring of the day-night difference in serum melatonin levels. (Supported by NSF Grant DCB87-14638 to MHS.)

265.8

AGE-RELATED DECREASES IN THE DENSITY OF SPECIFIC MELATONIN BINDING SITES IN THE HAMSTER SUPRACHIASMATIC NUCLEI (SCN) AND MEDIAN EMINENCE/PARS TUBERALIS (ME/PT). M.J. Duncan and F.C. Davis. Dept. of Anatomy & Neurobiol., Univ. of Missouri Med. School, Columbia, MO 65212; Dept. of Biology, Northeastern Univ., Boston, MA 02115

In Syrian hamsters, the circadian timing system is very sensitive to melatonin during gestation while the reproductive system is most responsive during adulthood. Specific binding of 2-[^{125}I]-iodomelatonin (IMEL; 115 pM) was prominent in the ME/PT at all ages but only in the SCN of fetuses and neonates (Duncan et al, Soc. Neurosci. Abst. 16:773, 1990). Quantitative saturation autoradiography examined if the age-related loss in specific IMEL binding in the SCN represents a change in the affinity (K_d, pM) and/or maximal density (B_{max}, fmoles/mg protein) and if a developmental change also occurs in the ME/PT. In the neonatal SCN, the specific IMEL sites showed K_d=74.9±6.9 and B_{max}=0.637±0.019. Very few sections of adult SCN showed specific IMEL binding (<0.35 fmol/mg protein). In the ME/PT, B_{max} decreased significantly ($p<0.005$) from 6.99±0.76 in neonates to 2.78±0.64 in adults but K_d did not change (neonates: 91.7±17.8; adults: 102.4±16.3). Thus, the density of specific IMEL binding sites in the SCN and the ME/PT decreases during development.

265.9

AUTORADIOGRAPHIC LOCALIZATION OF MELATONIN RECEPTORS IN RHESUS MONKEY HYPOTHALAMUS. I.H. Stehle*, D.R. Weaver, and S.M. Reppert. Laboratory of Developmental Chronobiology, Children's Service, Massachusetts General Hospital, Boston, MA 02114.

Rhesus monkeys housed in a natural lighting environment limit reproductive activity to the fall and early winter. This photoperiodic regulation of reproduction appears to be mediated by the pineal hormone, melatonin (Wilson & Gordon, J Reprod Fertil 86:435-444, 1989). In the present work, we used 2 [^{125}I]-iodomelatonin (I-MEL, 2000 Ci/mmol, 30-40 pM) to examine the distribution of high-affinity melatonin receptors in the hypothalamus of an adult male rhesus monkey (Macaca mulatta). Specific I-MEL binding (defined as I-MEL binding displaced by 1 µM melatonin) was most concentrated in the hypophysial pars tuberalis (PT) and the suprachiasmatic nuclei (SCN). Specific binding was also apparent in low levels throughout the mediobasal hypothalamus. Melatonin potently inhibited I-MEL binding in the PT and SCN, with half-maximal inhibition at melatonin concentrations ca. 1 nM. The PT is the only site of high-affinity I-MEL binding in every photoperiodic species examined to date, and we have suggested that the PT is a prime site of melatonin action in regulating reproduction. The present results are consistent with this hypothesis. It is interesting to note that while I-MEL binding is present in the human SCN (Reppert et al., Science, 242:78-81, 1988), I-MEL binding is not readily detected in the human PT. This apparent difference in PT I-MEL binding correlates with the clear photoperiodic influence on reproduction in the rhesus vs. the subtle influence of season on human reproductive function.

265.10

EFFECTS OF PINEALECTOMY ON DAILY PATTERN OF CEREBRAL 2(125I)IODOMELATONIN BINDING IN THE HOUSE SPARROW, PASSER DOMESTICUS. LU JUN* AND VINCENT M. CASSONE. Department of Biology, Texas A&M University, College Station. TX 77843

The pineal gland and its hormone melatonin are important for the generation of circadian rhythms in house sparrows. The application of in vitro binding of 2(125I)iodomelatonin(IMEL), and autoradiography have indicated binding is widely distributed in the visual, auditory and limbic systems. This binding is rhythmic: low during subject night and high during subject day. To test the hypothesis that the rhythm is generated via down-regulation by endogenous pineal melatonin rhythms, we have determined the effects of pinealectomy(PINX) on IMEL binding rhythms within the house sparrow brain. 24 house sparrows were maintained in L:D=12:12. 12 birds were pinealectomized and 12 birds received sham operation. After recovery, two pinealectomized and two sham birds were decapitated every four hours, brains were removed, frozen and thaw-mounted on gelatin-coated slides. Brains were sectioned at 20 uM from the rostral to caudal extents. Slides were incubated in 100 pM IMEL exposed to autoradiographic film for 7 days. Binding was measured densitometrically using a computer image analysis system. In sham birds, the binding clearly showed a daily change with maximum at ZT8 and minimum at ZT20. In PINX birds, however, the binding rhythm was not abolished. Its phase was same as that of sham birds. However, the amplitude of rhythm was elevated in all structures 6-20 fold. Characterization of IMEL binding indicated that Bmax varied significantly but neither the Ki nor Hill coef. of binding were affected by day or PINX. The melatonin binding rhythm is not driven by endogenous melatonin. The source of this rhythmicity is currently under study. Supported by NSF GRANT 88-96225 and AFOSR 90-0244.

265.11

2-[^{125}I]-IODOMELATONIN BINDING SITES IN THE CHICK BRAIN: DECREASED BINDING IN VISUAL AREAS FOLLOWING UNILATERAL OPTIC NERVE TRANSECTION. D. Krause[2], J.A. Siuciak[1], and M. Dubocovich, Dept. of Pharmacology, [1]Northwestern University Medical School, Chicago, Il. 60611 and [2]University of California, Irvine, CA., 92717.

Previous autoradiographic studies have shown wide distribution of ML-1 melatonin receptors throughout the chicken brain, predominantly in areas associated with the visual system (Siuciak et al., J. Neurosci., 1991). The aim of this study was to examine 2-[^{125}I]-iodomelatonin binding in discrete visual areas of the chick brain following unilateral transection of the optic nerve.

Chicks were sacrificed 1 or 7 days post transection with all animals 3 weeks old at the time of sacrifice. Quantitative autoradiography was performed in coronal brain sections incubated with 75 pM 2-[^{125}I]-iodomelatonin in 150 mM Tris-HCl buffer for 1 h as previously described. Specific binding was defined with 3 uM melatonin.

One day after nerve transection, no significant changes in 2-[^{125}I]iodomelatonin binding were observed. However, seven days following optic nerve transection, significant reductions in specific binding were found contralateral to the lesion in primary retinorecipient areas such as the optic tectum (46%) and the ventrolateral geniculate nucleus (40%). Significant unilateral reductions were also seen in second order sites receiving no direct retinal input. i.e., the N. rotundus (43%) which receives input from the tectum and the Edinger-Westphal nucleus (31%) which receives input from the suprachiasmatic region. The latter finding may reflect trans-synaptic degeneration in visual pathways. No changes in binding were found in the ventral supraoptic decussation (suprachiasmatic area) or in the N. triangularis.

Support: NRSA MH09997 (JAS) and MH42922 (MLD).

265.12

2-[^{125}I]-IODOMELATONIN BINDING SITES IN THE CHICK BRAIN: DIURNAL VARIATIONS AND EFFECT OF PINEALECTOMY. J.A. Siuciak, R. Stewart and M.L. Dubocovich, Dept. of Pharmacology, Northwestern University Medical School, Chicago, Il. 60611

In the chicken brain, melatonin levels show a diurnal rhythm, with high levels at night. Melatonin receptor sites (ML-1), which are widely distributed throughout the chick brain, are found predominantly in areas associated with the visual system (Siuciak et al., J. Neurosci., 1991). The aim of these studies was to investigate the regulation of melatonin receptor sites by the light/dark cycle and by endogenous melatonin using quantitative autoradiography. Scatchard analysis was performed in coronal brain sections of the optic tectum incubated with 20-300 pM 2-[^{125}I]-iodomelatonin. Specific binding was defined with 3 uM melatonin.

For diurnal rhythm studies, chicks were maintained on a 14:10 L:D cycle (light on 0400) until 2 weeks old and sacrificed at 4 hour intervals beginning at 0400 h. Dark phase chicks were sacrificed under dim red light. The density of binding sites follows a diurnal rhythm. B_{MAX} values were 147 and 203 fmol/mg protein at 1200 and 2400 h, respectively.

Pinealectomies (PNX) and shams were performed on two week old chicks sacrificed at 1, 7 or 14 days post surgery. One day after PNX, scatchard plots were linear and indicated a single class of high affinity binding sites: SHAM: K_D = 88.35 $\pm$ 2.61 pM, B_{MAX} = 122.51 $\pm$ 18.12 fmol/mg protein and PNX: K_D = 91.40 $\pm$ 2.13 pM, B_{MAX} = 127.13 $\pm$ 15.07 fmol/mg protein. No changes were found in either the affinity or density of binding sites at either 7 or 14 days following PNX. These data suggest that endogenous melatonin may not play a role in the regulation of melatonin receptor site density.

Supported by NRSA MH09997 (JAS) and USPHS MH42922 (MLD).

265.13

OVERLAP OF N-ACETYLASPARTYLGLUTAMATE (NAAG)-LIKE IMMUNOREACTIVITY AND 2-[^{125}I]-IODOMELATONIN BINDING IN VISUAL AREAS OF THE TURTLE BRAIN. J.R. Moffett[1], J.A. Siuciak[2], L.J. Larson-Prior[3], N.T. Slater[3] and M.L. Dubocovich[2]. [1]Dept. Biology, Georgetown Univ., Washington, D.C. 20057, and Dept. of [2]Pharmacology and [3]Physiology, Northwestern University Medical School, Chicago, Il. 60611.

Previous reports suggest a role for both NAAG and melatonin in the visual areas of the vertebrate brain. The aim of this study was to examine the distribution of NAAG and melatonin in discrete brain regions in order to determine areas of overlap.

NAAG was detected immunohistochemically using an antiserum which recognizes carbodiimide-fixed NAAG as previously described (Moffett et al., 1989). 2-[^{125}I]-Iodomelatonin binding sites were examined using in vitro autoradiography. Coronal brain sections were incubated with 75 pM 2-[^{125}I]-iodomelatonin in 150 mM Tris-HCl buffer (pH 7.4, 25°C) as described previously (Siuciak et al., 1990, 1991).

2-[^{125}I]-Iodomelatonin binding and NAAG-like immunoreactivity were widely and unevenly distributed throughout the turtle brain. Overlap was found namely in regions of the brain receiving visual information which include areas of the midbrain: optic tectum, isthmal nuclei, optic nerve, optic tracts, nucleus of the optic tract; the diencephalon: dorsolateral and dorsomedial anterior thalamic areas, geniculate nuclei, habenula; and the telencephalon: striatum, dorsal ventricular ridge, parahippocampal region. These data open the possibility that both NAAG and melatonin may play a role in the processing of visual information in the turtle brain. Supported by NRSA MH09997 (JAS), NS17489 (NTS), MH42922 (MLD).

265.14

DEVELOPMENTAL RESPONSES OF HAMSTER PINEALS TO NOREPINEPHRINE. C.M. Kaufman and M. Menaker. Department of Biology, University of Virginia, Charlottesville, VA 22901.

The Syrian hamster pineal displays age dependent changes in melatonin output when maintained and measured *in vitro*. Pineal melatonin generation in response to 10μM norepinephrine (NE) increased about 34 fold betwen 4 and 19 days of age. When incubated with 10μM NE for 6hr in static culture, glands from 11 day old neonates had over 3 times higher serotonin N-acetyltransferase specific activity and produced 20 times more melatonin than did glands from 4 day old animals. Production of melatonin by pineals of one week old hamsters exposed to NE showed a clear dose responsive relationship. The most effective dose was 10μM; the response declined with higher or lower doses. Pineals from 7 day old neonates were perifused *in vitro* and were exposed to four NE cycles consisting 10hr 10μM NE alternating with 14hr 0M NE; their melatonin output followed the imposed NE rhythm with a rising lag-time of approximately 8hr and a falling lag of about 4hr. Pineals from animals of different ages took approximately the same amount of time to rise to half maxmimal melatonin levels after NE stimulation or to fall to half minimal melatonin level after NE removal. The long lag time between the introduction of NE and the induction of melatonin synthesis is consistent with the idea that transcription events are required.[1,2] In the absence of exogenous NE, melatonin from most glands dropped to undetectable levels in just over 2 days. However, even after 3 days *in vitro* without exogenous NE, pineals produced high levels of melatonin when NE was added. Our results show that the hamster pineal is capable of producing melatonin as early as 4 days of age. Furthermore, although the gland's response to NE increases with age, the time course of this response to exogenous stimulation does not change throughout postnatal development.

[1]*Gonzalez-Brito, ME, Troiani, A, Menendez-Pelaez, A, Delgado, MJ, Reiter, RJ (1990) J. Cell. Bioch. 44:55-60.*

[2]*Santana, C, Menendez-Pelaez, A, Reiter, RJ, Guerro, JM (1990) J. Neurosci. Res. 25:545-548.*

265.15

EFFECTS OF THE TRANSCRIPTION INHIBITOR, 5,6-DICHLORO-1-ß-D-RIBOFURANOSYLBENZIMIDAZOLE (DRB), ON THE CIRCADIAN MELATONIN RHYTHM OF CULTURED CHICK PINEAL CELLS. K. Ohi* and J.S. Takahashi*. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Chick pineal cells contain circadian oscillators that regulate rhythmic melatonin release in vitro. Previous work has shown that the protein synthesis inhibitor, anisomycin, can induce phase-shifts of the melatonin rhythm in a phase-dependent manner. Because control of protein synthesis could occur at either transcriptional or post-transcriptional levels, we have investigated the effect of DRB which is a selective and reversible inhibitor of transcription by RNA polymerase-II.

Six-hour pulses of DRB (CT 0-6, 100 uM) produced a phase shift (7.0 $\pm$ 0.42 hr) in the melatonin rhythm of pineal cells under constant darkness. Pulses given at 3-hour intervals showed that the effects of DRB were phase-dependent. The phase-shifts were all delays with maximum delays occurring between CT 18 and CT 6. No phase-shifts were detected at CT 9 and CT 12. Treatment with 100 uM DRB between CT 0 and CT 6 inhibited TCA precipitable ^{3}H-uridine incorporation by 75 % and TCA precipitable ^{35}S-methionine incorporation by 25 %. Continuous treatment with several doses of DRB between 5 uM and 50 uM lengthened the period in a dose-dependent manner (29.6 $\pm$ 0.62 hr, 50 uM). Treatment with 100 uM DRB blocked both the phase-advancing and delaying effects of light pulses (100 nWatt/cm^2).

Our results demonstrate that DRB has three effects on the circadian system in chick pineal cells: 1) phase dependent phase-shifts; 2) lengthening of circadian period; 3) blockade of the resetting effects of light. These results suggest that transcriptional events are involved in the generation and regulation of the circadian oscillator.

265.16

THE EFFECTS OF MELATONIN ON THE ACTIVATION OF PROTEIN KINASE A IN CULTURED OVINE *PARS TUBERALIS* CELLS. David G. Hazlerigg* , Peter J. Morgan+, Wilfred Lawson+* and Michael H. Hastings. Department of Anatomy, University of Cambridge, Downing St. CB2 3DY and +The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB.

The cellular mechanisms which mediate the photoperiodic and circadian actions of melatonin are not known. The ovine *pars tuberalis* contains a very high density of specific, high affinity melatonin binding sites which have been extensively characterized. In this tissue, melatonin acts through a receptor coupled to an inhibitory G-protein to suppress forskolin-induced activation of adenylate cyclase. This study sought to extend this finding by investigating the effect of melatonin upon the effector enzyme of the c-AMP signal transduction pathway, protein kinase A (PKA). Primary cultures of ovine *pars tuberalis* were prepared as described previously (Morgan *et al.* 1989, J. Mol. End. 3, R5) and cytosols produced by hypotonic lysis. Photoaffinity labelling of native PKA revealed the presence of both type I and type II isozymes in these cells. The degree of activation of cellular PKA prior to lysis was determined with a phospho-transferase assay in which cytosols were incubated with an acceptor peptide specific to PKA (Kemptide) and γ ^{32}P-ATP. Enzyme activation was expressed as the ratio (A.R.) of activity within the cytosol in the absence and in the presence of excess amounts of exogenous c-AMP. PKA activation was low in unstimulated cells (A.R. <0.20) and unaffected by incubation with melatonin (10^{-6}M). Incubation with forskolin produced a rapid (<10 mins) and dose-dependent (10^{-7}>10^{-4}M) activation of the enzyme (A.R. 0.70). Coincubation with melatonin (10^{-6}M) blocked the effect of forskolin and inactivated PKA already stimulated by prior exposure to forskolin. The effects of melatonin were rapid and dose-dependent (EC50 10^{-9}M). This study has demonstrated that melatonin has a potent inhibitory influence upon c-AMP dependent signal transduction in the ovine *pars tuberalis*. Suppresion of PKA activation suggests that the cellular basis to the actions of melatonin may depend upon altering the phosphorylation state of specific substrate proteins within neuronal and hypophyseal target tissues.

265.17

IONIC CURRENTS IN ACUTELY ISOLATED CHICK PINEAL CELLS. D. Henderson and S. E. Dryer, Program in Psychobiology and Neuroscience, Department of Biological Science, Florida State University, Tallahassee, FL 32036.

Previous research on cultured chick pineal cells suggests that melatonin production is modulated by Ca^{++} influx via voltage-dependent Ca^{++} channels (Harrison and Zatz, 1989). The possible existance of other channels was investigated using whole-cell recordings from acutely isolated cells at room temperature. Several distinct inward and outward currents were identified. Inward currents included L-type Ca^{++} currents and voltage-dependent TTX-sensitive Na^{+} currents. Sodium currents have not been reported previously in pineal cells. These two inward currents were observed in the majority of cells studied. Considerable heterogeneity was apparent in the amplitude and characteristics of the outward current. Cells expressed several types of voltage-dependent and Ca^{++}-dependent outward currents. Calcium-dependent K^{+} currents were observed with physiological levels of external Ca^{++}. Single channel recordings also revealed several distinct populations of ionic channels. These channels may be important for the modulation of melatonin secretion and circadian rhythmicity.

Harrison, N. and Zatz, M. (1989). Journal of Neuroscience, 9, 2462-2467.

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MONOAMINES AND BEHAVIOR: D_1 AND D_2 MECHANISMS

266.1

BEHAVIORAL EFFECTS OF THE FULL EFFICACY D_1 AGONIST DIHYDREXIDINE IN MODELS OF DOPAMINE DENERVATION. K.J. Darney, Jr., M.H. Lewis, W.K. Brewster*, D.E. Nichols, S. Southerland* and R.B. Mailman. University of North Carolina, Chapel Hill, NC, 27599, and School of Pharmacy, Purdue University, West Lafayette, IN, 47907.

We have previously shown that dihydrexidine (DHX; trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine) has behavioral effects in control rats that may be explained by its properties as a potent, full efficacy D_1 dopamine agonist having some D_2 activity. These characteristics make it useful for testing hypotheses concerning D_1/D_2 dopamine receptor interactions. The present studies investigated how dopamine denervation may cause, as previously reported, a loss of functional D_1/D_2 interactions. We assessed the behavioral effects of systemic DHX (0.1-1.0 mg/kg) in rats with central DA lesions produced by intracisternal administration of the neurotoxicant 6-OHDA (IC). Responses were assessed using an observational method that quantified the modified frequency of multiple behavioral topographies. Unoperated, sham, and IC rats had similar dose response curves for sniffing. There was, however, a significant leftward shift in the dose-response curve for locomotion in the IC vs. sham or untreated rats. Conversely, the dose-response relationship for DHX-induced grooming was absent in IC rats. IC rats showed somewhat elevated baseline grooming, but no increases in grooming at doses of DHX that stimulate grooming in sham or unoperated rats. This effect on grooming may be the result of behavioral competition from the DHX-induced increases in locomotion. We have reported previously that the D_1 antagonist SCH23390 blocks DHX-induced locomotion, grooming, and sniffing (as expected for a D_1 antagonist). Conversely, the D_2 antagonist remoxipride completely blocked only locomotion. We therefore performed preliminary competition studies using these same IC and sham rats. The preliminary data suggest that the pattern of inhibition of DHX-induced behaviors seen in control rats was not significantly altered by the IC lesion. This suggests that a drug like DHX (having both D_1 and D_2 agonist properties) may elicit different functional responses then are seen with receptor selective drugs. Further studies (e.g., in rats with unilateral 6-OHDA lesions, and using drugs like SKF38393 that do not have full D_1 efficacy) will provide important data to understand how lesions affect D_1/D_2 interactions.

266.2

ASYMMETRIC ELEVATION OF STRIATAL DOPAMINE D2 BUT NOT D1 RECEPTORS IN THE CHAKRAGATI (CIRCLING) MOUSE: AGE DEPENDENCE AND RESPONSE TO QUINPIROLE. L.W. Fitzgerald[1], K.J. Miller,[1], A.K. Ratty[2], S.D. Glick[1], M. Teitler[1], and K.W. Gross[2*]. [1]Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208 and [2]Dept. of Molecular & Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263

The Chakragati (Chak) circling mutant displays lateralized circling, postural asymmetry, hyperactivity, and body weight deficits without microcephaly. Previously, we reported that homozygous Chak mice have asymmetric elevations in striatal dopamine D2 receptors in the absence of presynaptic denervation. To investigate the specificity and permanence of these changes, we measured D1 and D2 densities in striatal and extrastriatal regions of mice at postnatal day (PN) 30-40 and PN 180-200 using receptor autoradiography. Chak mice showed characteristic asymmetries in striatal D2 receptors at PN 30-40; however, these asymmetries were attenuated in older mice. D1 receptors in striatum, nucleus accumbens, and olfactory tubercle of young Chak mice were neither asymmetric nor elevated compared to heterozygous normal mice; however, D1 levels in these regions were bilaterally lower in PN 180-200 Chak mice. We examined the functional relevance of striatal D2 asymmetries by measuring rotation following quinpirole challenge (0.0-2.5 mg/kg,s.c.). Most Chak mice showed dose-related increased rotation, while normal mice never rotated above baseline. Older Chak mice showed a diminished rotational response to quinpirole. The finding that receptor asymmetry and rotational responses to a D2 agonist show a similar age-related profile suggest that this receptor alteration may underlie the circling phenotype; however, some Chak mice do not respond to quinpirole, suggesting that other transmitter systems may play a role.

266.3

D1 AND D2 RECEPTORS IN MPOA DIFFERENTIALLY AFFECT GENITAL REFLEXES IN MALE RATS. E. M. Hull, R. C. Eaton, V. P. Markowski, L. A. Lumley, J. Moses, R. Dua*, and J. A. Loucks*. Department of Psychology, SUNY at Buffalo, Buffalo, NY 14260

D1 and D2 receptors in the MPOA differentially control genital reflexes. A D1 agonist (THP) in the MPOA increased ex copula erections (parasympathetically controlled) and decreased seminal emissions (sympathetically controlled). A D2 agonist (quinelorane, LY-163502) in the MPOA decreased erections and increased seminal emissions. Thus, the D2 agonist favored sympathetically mediated seminal emission.

A low dose of the mixed (D1 and D2) agonist apomorphine in the MPOA increased erections, as had the D1 agonist. This increase was blocked by a D1 antagonist (SCH-23390). The low dose of apomorphine did not affect seminal emissions. A high dose of apomorphine increased seminal emissions, as had the D2 agonist, but did not affect erections. The increase in seminal emissions was blocked by a D2 antagonist (raclopride). Thus, low doses of the mixed agonist apomorphine may preferentially stimulate erections via D1 receptors, whereas high doses elicit seminal emission via D2 receptors.

The differential effects of low and high doses of apomorphine in the MPOA may reflect similar functions of rising levels of dopamine during copulation (Eaton et al., 1991; Pfaus, 1990). The smaller increases early in the copulation may enhance erections and penile movements via D1 receptors. D1 receptors also may suppress ejaculation early in the bout. However, peak dopamine concentrations observed just prior to ejaculation may switch from parasympathetic erectile processes to sympathetic seminal emission and ejaculation. These observations may explain the progression during copulation from parasympathetic facilitation of erection to sympathetic elicitation of seminal emission and ejaculation.

266.4

HIPPOCAMPAL MODULATION OF LOCOMOTION: ROLE OF D1 AND D2 RECEPTORS. G. Mittleman, P. LeDuc and I.Q. Whishaw. Depts. of Psychology, Memphis State University, Memphis, TN, 38152 and University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

This experiment examined locomotion and stereotyped behavior in mature, male Long Evans rats that had received aspirative lesions of the hippocampus (HPC), control lesions of the overlying parietal cortex, or were unoperated controls. Locomotion, measured as photocell beam breaks, was measured during 2 or 3 hour test sessions. Behavioral stereotypy was simultaneously rated. In agreement with previous results, HPC lesioned rats exhibited a selective enhancement in locomotion following d-amphetamine (0.0-5.6 mg/kg) when compared to animals in the control groups (Whishaw & Mittleman, Behav. Neural Biol. in press). Similar results were observed following injections of apomorphine (0.0-0.25 mg/kg), a mixed D1&D2 agonist. In order to determine if D1 or D2 receptors were involved in this increased locomotion the D1 agonist SKF 38393 (0.0-15 mg/kg) and the D2 agonist Quinpirole (0.0-0.5 mg/kg) were tested alone and in combination. HPC-ablated rats showed significantly increased locomotion only in response to Quinpirole, suggesting that these lesion-induced increases were largely mediated by D2 receptors. When both drugs were administered together, SKF 38393 further enhanced the locomotor stimulating effects of Quinpirole in HPC lesioned rats, indicating a synergistic interaction between D1 and D2 receptors in the modulation of locomotion. As the different groups were not distinguishable on the basis of stereotypy, these results provide further evidence of HPC modulation of locomotion and suggest that dopaminergic mechanisms in the nucleus accumbens are involved.

266.5

BEHAVIOURAL SENSITIZATION AND TOLERANCE TO A DOPAMINE D2 AGONIST IS A FUNCTION OF FREE-RUNNING CIRCADIAN RHYTHMS. M.T. Martin-Iverson and N. Yamada*. Neurochemical Research Unit, Dept. of Psychiatry, Univ. of Alberta, Edmonton, Alberta, T6G 2B7, Canada.

Daytime tolerance and nocturnal sensitization occur to the locomotor effects of a direct dopamine D2 agonist, (+)-4-propyl-9-hydroxynaphthoxazine (PHNO). We investigated whether this phenomenon is a function of the lighting schedule or to the circadian rhythms in locomotor activity, and further investigated interactions between D2 and D1 agonists. 48 male Sprague-Dawley rats (300-400 g) were maintained under constant dark (infrared illumination of 2 lux), and an additional 48 rats were maintained under constant light (55 lux) for 5 weeks prior to drug treatments. Groups of rats (n=12) in each experiment were given 14 days of continuous subcutaneous infusions of vehicle (double-distilled water), PHNO (5 μg/h), a direct D1 agonist (SKF 38393, 336 μg/h) or both PHNO and SKF 38393, using Alzet osmotic minipumps (Model 2ML2). Circadian periodicity under constant lighting averaged 25 h. PHNO was without effect in rats in constant light. Rats in constant dark exhibited behavioural sensitization to the locomotor effects of the D2 agonist (PHNO) during subjective "night" (3 h peak activity in each 25 h period). On the other hand, the same animals exibited tolerance to the locomotor stimulant effects of PHNO during their subjective "day" (3 h nadir of activity in each 25 h period). Therefore, it can be concluded that the differential development of daytime tolerance and nocturnal sensitization is dependent upon the circadian rhythms in activity. SKF 38393 was without effect when given alone. When given with PHNO in rats under constant light, the D1 agonist interacted synergistically with PHNO to increase locomotion. In rats in constant dark, SKF 38393 increased PHNO-induced activity on the first subjective "day" of treatment, but this effect developed tolerance. Under certain conditions, the D1 agonist blocked the locomotion induced with PHNO. These data indicate that the effects of D1 and D2 agonists on behaviour are a function of endogenous activity rhythms and lighting conditions.

Funding from AHFMR, MRC, AMHRF.

266.6

OPPOSING EFFECTS OF D1 AND D2 RECEPTOR STIMULATION ON LOCOMOTION, SNOUT CONTACT, MOUTHING AND GROOMING. D. Eilam, H. Talangbayan', K. Clements', G. Canaran', & H. Szechtman. Dept. Biomedical Sci., McMaster Univ., Hamilton, Ontario, CANADA L8N 3Z5.

The study compares the behavioral profiles induced in rats (N=118) by the D2-dopaminergic receptor agonist quinpirole (0.03 mg/kg; QLow, and 0.5 mg/kg; QHigh), and the D1- agonist SKF38393 (1.25 - 40 mg/kg), and both agonists administered together. The amount of locomotion and the frequency of snout contacts were reduced by QLow but increased by QHigh; SKF38393 also reduced these behaviors and attenuated the effect of QHigh. Only QHigh increased the duration of snout contact bouts and the frequency of mouthing; SKF38393 had no effect but in combination with QHigh, it enhanced the performance of these behaviors greatly. The duration of mouthing bouts was not affected by either agonist but was greatly extended when SKF38393 was administered together with QHigh. Grooming was inhibited by both QLow and QHigh, and stimulated by the injection of SKF38393 or its addition to QLow. Spatial distribution of locomotion was transformed from routes that cross the center of the open field under QHigh to travel only along the edge with QHigh+SKF38393 (1.25-2.5 mg/kg). These findings indicate that some behaviors are controlled by modulating the frequency and others by modulating the duration of episodes and that such control can be modelled by an oppositional interaction between D1 and D2 receptors.

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266.7

ANATOMICAL LOCALIZATION OF DOPAMINE D1 RECEPTOR-MEDIATED BEHAVIORS IN RATS USING EEDQ. J. L. Neisewander, A. Ong*, and P. McGonigle. Depts. of Pharmacology and Psychiatry, Univ. of Pennsylvania, Philadelphia, PA 19104.

Systemic injection of the D1-selective agonist SKF-38393 (SKF) increases grooming. In contrast, direct injection of SKF into brain regions produces neurotoxicity and an increase in locomotion. We have employed an alternative approach for localizing SFK-induced grooming, using the irreversible antagonist EEDQ to inactivate monoamine receptors in discrete brain regions. Either vehicle or EEDQ was injected bilaterally into various brain regions. Forty-eight hr after surgery, the rats were placed into an observation cage for 30 min both before and after injection of SKF (10 mg/kg, SC). Grooming was measured during the last 15 min of each period. EEDQ (1.5 μl/side) injected into the nucleus accumbens did not alter SKF-induced grooming. In contrast, EEDQ (0.15-1.5 μl/side) injected into the lateral caudate-putamen (CPu) dose-dependently decreased SKF-induced grooming. To determine whether this decrease was due to inactivation of D1 receptors, rats were injected with vehicle or SCH-23390 (3 mg/kg, SC) 15 min prior to injection with EEDQ (1.5 μl/side) into either the medial or lateral CPu. In animals pretreated with SCH-23390 to prevent D1 receptor inactivation, SKF-induced grooming was not disrupted by EEDQ injected into the lateral CPu. Furthermore, EEDQ injected into the medial CPu did not disrupt SKF-induced grooming. Quantitative autoradiographic analysis confirmed the loss of D1 receptors in the injected regions of the CPu. We conclude that SKF-induced grooming involves D1 receptors in the lateral CPu. (Supported by USPHS grant MH 14654 (JLN) and GM 34781 (PM)).

266.8

WITHDRAWN

266.9

THE EFFECTS OF THE D_2 DOPAMINE RECEPTOR AGONIST, QUINELORANE (LY163502), ON THE SEXUAL BEHAVIOR OF MALE RATS . M.A. Charles', A.R. Lumia' and M.Y. McGinnis. Dept. of Cell Biology and Anatomy, Mount. Sinai Sch of Med., New York, NY 10029 and Dept. of Psychology, Skidmore College, Saratoga Springs, NY 12866.

Recent research has demonstrated that dopamine (DA) plays an important role in the mediation of male rat sexual behavior. DA receptor stimulation has varied effects on male rat copulatory behavior due to the presence of three DA receptor subtypes. Quinelorane (LY), a selective D_2 receptor agonist, has produced increases in male rat sexual behavior when given systemically and has been shown to stimulate behavior in sexually inactive male rats.

Two experiments were performed in the first study. The purpose of the first experiment was to determine if the effects seen with systemic LY injections could be reproduced upon intracranial infusion of LY into the medial preoptic area, a brain region implicated in the control of male sexual behavior. Long Evans male rats, which were proven copulators, were castrated and allowed to rest for three weeks. After a post-castration test, noncopulators were implanted with bilateral intracranial cannulae. Following the surgery, all animals received two 10mm Silastic T capsules and were tested twice weekly for the restoration of behavior. Immediately prior to each test, half of the animals were infused with 25ug/kg of LY in 5ul of saline. The results of this experiment showed an acceleration in the onset of behavior with the LY group. In addition, there was a tendency for LY to increase mount latency and post ejaculatory interval, decrease intromission frequencies and increase the hit rate. Using the same paradigm, the second experiment examined whether LY, either alone or in combination with suboptimal T exposure (one 5mm capsule), restores male sexual behavior. The results show that LY alone does not stimulate male rat sexual behavior but requires the presence of T for its effects to be expressed.

A second study examined whether LY injections could stimulate copulation in sexually inactive rats. Intact males received systemic injections of either LY(25ug/kg s.c.) or saline 30 minutes prior to copulatory testing. Our results suggest that LY does not induce high levels of sexual behavior in sexually inactive male rats.

From these results, we conclude that the D_2 receptor is an important mediator of male rat sexual behavior and that its actions require the presence of T.

266.10

DOPAMINE D1 and D2 ANTAGONISTS BLOCK L-DOPA-ELICITED AIR-STEPPING IN NEONATAL RATS. A. E. Sickles*, D. J. Stehouwer and C. Van Hartesveldt. Psychology Dept., University of Florida, Gainesville, FL 32611.

Previous studies (Van Hartesveldt et al., 1991) have shown that L-DOPA elicits highly stereotypic coordinated locomotor activity in developing rats if they are fitted into slings and suspended in air. In order to determine the role of dopamine in air-stepping, we attempted to block L-DOPA-induced air-stepping with either SCH 23390, a D1 antagonist, at doses of 0.25, 0.5, 1, 2, 4, 8, and 16 mg/kg s.c., or with spiperone, a D2 antagonist, at doses of 0.0625, 0.125, 0.25, and 0.5 mg/kg s.c. Antagonists were administered prior to the injection of 50 mg/kg L-DOPA s.c. in 5-day old rat pups that were suspended in slings. Either antagonist independently inhibited expression of air-stepping in a dose-dependent manner. Therefore, dopamine plays an integral part in air-stepping. However, the additional involvement of numerous other neurotransmitter systems cannot be ruled out.

266.11

RESTORATION OF SELF-STIMULATION BY A COMBINATION OF D1 AND D2 DOPAMINE AGONISTS IN DOPAMINE-DEPLETED RATS. S. Nakajima, C. L. Lau, and X. H. Liu, Dalhousie University, Halifax, Nova Scotia, Canada.

A dopamine antagonist, either D1 or D2, reduces the reinforcing effect of brain stimulation. Recent study in our laboratory suggested that a D2 agonist facilitates brain self-stimulation but a D1 agonist does not (Nakajima & O'Regan). To examine the interaction of D1 and D2 receptors, rats were tested after depleting dopamine in the brain. Rats were implanted with bipolar electrode into the lateral hypothalamus and trained to press a bar for a 0.5 s train of 0.3 ms pulses. The frequency of stimulation was varied from 200 to 12 pulses per s, and a frequency-response curve was plotted. Injection of reserpine (1 mg/kg) was followed by alpha mthyl-p-tyrosine 16 hours later. When the animals were tested 4 hours after the second injection, they responded to the highest frequency of stimulation only (shift of the cureve to the right). Subsequent injection of either a D1 agonist (SKF 38393, 0.4 mg/kg) alone or a D2 agonist (quinpirole HCl, 1.0 mg/kg) alone did not facilitate self-stimulation. Injection of quinpirole followed by SKF 38393 1 hour later restored self-stimulation to the level prior to reserpine injection. The results suggest that a D2 agonist enhances the reinforcing effect of brain stimulation only when D1 receptors are activated by endogenous dopamine. When dopamine is depleted, an exogenous D1 agonist is necessary to enable the D2 agonist to mediate the reinforcing effect of brain stimulation.

266.12

DIFFERENTIAL EFFECTS OF CENTRALLY-ADMINISTERED QUINPIROLE ON LOW AND HIGH BASAL LEVELS OF LOCOMOTION. G.J. Mogenson, and M. Wu* Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Quinpirole, a dopamine D_2 agonist, was injected bilaterally into the nucleus accumbens of rats to investigate D_2 mechanisms in locomotor activity. A comparison was made of the effects of quinpirole (4 μg in 0.2 μl) when basal locomotion (recorded for 30 min in an open field apparatus equipped with lights, photo cells and automated counters) was relatively low and high. A low level of locomotor activity was increased by quinpirole from 203.7 ± 67.5 to 385.9 ± 99.6 ($t_{15}=2.40$, $p<0.05$). High levels of locomotor activity resulting from administering amphetamine to the accumbens and from adding novel stimuli to the open field were reduced by quinpirole. Amphetamine-elicited locomotion was reduced from 1057.1 ± 127.6 to 717.2 ± 109.8 ($t_{12}=4.16$, $p<0.002$) and novelty-elicited locomotion was reduced from 671.1 ± 60.3 to 400.4 ± 60.1 ($t_8=3.07$, $p<0.02$). These observations are consistent with the suggestion that the effects of the D_2 agonist depend on the initial level of locomotion. It appears that quinpirole increases locomotor activity by postsynaptic action and decreases locomotor activity by presynaptic action.

(Supported by Medical Research Council of Canada)

266.13

CLINICAL INVESTIGATION OF THE DOPAMINE HYPOTHESIS OF SELF-INJURIOUS BEHAVIOR IN THE LESCH NYHAN SYNDROME. J Harris, DF Wong, M Yaster*, R Dannals*, W Nyhan*, S Hyman*, S Naidu*, W Fischer*, C Piazza*, A Wilson*, H Raverty* H Wagner, Jr.*, Div Child Psych, Kennedy Inst, Nuclear Med, Johns Hopkins, Baltimore, MD 21205

To investigate the hypothesis that self-injurious behavior in the Lesch Nyhan syndrome is related to dopamine receptor density in the basal ganglia, we have evaluated 6 affected males (ages 4-23) who have received in vivo dopamine receptor PET scan studies (Wong et al, Soc Neuro Sci, 1991). Five of the 6 subjects showed hand and lip biting and had nondetectible levels of HPRT. One case, an adolescent male, has low levels of HPRT activity (1.1%) and has never self-injured. D2 dopamine density (Bmax) was highest in the youngest case (age 4) but D2 density was also high relative to 95% confidence levels in an adolescent male who had never self-injured. Consequently, the significance of the D2 elevation to self-injury remains unclear. The D1 dopamine system has also been implicated in the LN syndrome through an animal model of SIB proposed by Breese (1984). A trial of fluphenazine HCl, a D1/D2 antagonist thought to have adequate D1 blocking effects for a clinical trial, was carried out in two subjects (ages 5, 23). Fluphenazine HCl (Blood level 4.4 ng/ml) showed minimal effects on D1 receptor blockade of 11C SCH23390 in one case, suggesting that it is a poor D1 antagonist in vivo. This was consistent with the clinical trial of fluphenazine HCl (0.3-1 mg/day) where no improvement in self-injury, disruption, or in other LN symptoms was found in a 4-week trial. Future investigations of the self-injury hypothesis in LN syndrome will require utilization of D1 antagonists with greater receptor specificity.

266.14

BEHAVIORAL CHARACTERIZATION OF QUINPIROLE AND SKF38393 UNDER VARYING SOCIAL AND ENVIRONMENTAL DEMANDS. J.W. Tidey and K.A. Miczek. Dept. of Psychology, Tufts University, Medford, MA 02155.

D1 and D2 dopamine receptors appear to be of critical importance in mediating motor functions, stress reactions and reinforcement processes. The present experiments examined conditioned and unconditioned behaviors of mice under different demands and reinforcing contingencies. We compared the behavioral effects of the D2 agonist quinpirole and the D1 agonist SKF38393 in mice that were pair-housed with a female for four weeks and received behavioral tests in the home cage versus mice that were singly-housed and examined for motor activities, aggressive behavior and schedule-controlled responses. Singly-housed males tested during a schedule-controlled behavioral session were more motorically active than males housed with females. The singly-housed mice were less sensitive to the suppressive effects of quinpirole and SKF38393 on motor behavior. However, in the presence of an intruder challenge, the different housing and testing conditions did not alter the behavioral effects of quinpirole and SKF38393. Quinpirole decreased the display of aggressive behaviors similarly in both groups of mice, while the singly-housed mice were less sensitive than the pair-housed mice to the suppressive effects of SKF38393 on aggressive behaviors. Finally, following the motor and aggressive behavioral tests, mice completing a schedule-controlled behavioral session were less sensitive to the FR and FI rate-decreasing effects of quinpirole and SKF38393 than they were preceding the motor and aggressive behavioral test. We conclude that mice in resting conditions are more sensitive to the motorically suppressive effects of D1 and D2 receptor agonists than when under intense behavioral demands.

266.15

DIFFERENTIAL EFFECTS OF DIRECT- AND INDIRECT-ACTING DOPAMINE AGONISTS ON GNAWING BEHAVIOR. E. Tirelli and J. M. Witkin. Psychobiology Lab, NIDA Addiction Research Center., P. O. Box 5180, Baltimore, MD, 21224.

Indirect- and direct-acting dopamine agonists were differentiated on the basis of their ability to induce intense gnawing in C57BL/6J mice. Gnawing was assessed by the total damaged surface of a corrugated cardboard floor during a 75-min session. The indirect-agonists, methylphenidate, methamphetamine, cocaine, GBR-12909, nomifensine, amfonelate, and bupropion facilitated gnawing in a dose-dependent manner. In contrast, neither D1 (SKF 38393), D2 (piribedil, RU-24213), D1/D2 (apomorphine), nor selective D2 agonists which stimulate adenylate cyclase (pergolide, (-)-NPA) induced any gnawing even at very high doses. Both the D2 antagonist, eticlopride, and the D1 antagonist, SCH 23390, blocked the gnawing induced by either methylphenidate or cocaine. Non-sedative doses of the GABA-A agonist THIP potentiated gnawing primed by a moderate dose of methylphenidate or methamphetamine as previously reported, but did not potentiate gnawing induced by GBR-12909 or cocaine, suggesting a differential role for GABA in modulating the stimulatory effects of indirect dopamine agonists. THIP did not alter the inefficacy of either apomorphine or RU-24213. These data suggest a rapid in vivo screening method for differentiating direct- and indirect-acting dopamine agonists.

267.1

CHANGES IN THE DOPAMINERGIC RECEPTOR SYSTEM AFTER CHRONIC ADMINISTRATION OF COCAINE. M.E. Alburges, M.A. Hunt, N. Narang and J.K. Wamsley. Neuropsychiatric Research Institute, 700 First Avenue South, Fargo, ND 58103.

In order to understand the neurochemical consequences of chronic cocaine administration, groups of rats were injected (15 mg/kg, i.p., b.i.d.) for 1,3,7,14 or 21 days. Binding of [^{3}H]cocaine, [^{3}H]SCH23390 (D_1), [^{3}H]raclopride (D_2) and [^{3}H]BTCP (DA-uptake) in striatal and cortical tissue was compared to controls. [^{3}H]cocaine binding was significantly increased in the striatum and cortex at day 14 and 21, respectively. The binding of [^{3}H]SCH23390 was significantly increased after day 3. In striatal membranes, [^{3}H]BTCP binding was significantly increased after day 7; whereas, binding in cortical membranes was increased from day 1. [^{3}H]Raclopride binding was significantly higher only at day 7 in cortical tissues. These results indicate that repeated exposure to cocaine produces a time-dependent supersensitivity in cortical D_1, D_2, cocaine and DA-uptake receptor sites coupled with an increase in striatal D_1, cocaine and DA-uptake receptor sites; with no changes in striatal D_2 receptor sites.

267.2

THE EFFECTS OF COCAINE AND OTHER NEURONAL UPTAKE INHIBITORS ON CULTURED MESENCEPHALIC DOPAMINE NEURONS. BA Bennett and JE Clodfelter*. Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

Cocaine inhibits the neuronal uptake of dopamine (DA) as do certain other neuronal uptake inhibitors. The purpose of this study was to assess dopaminergic function after chronic exposure to the neuronal uptake inhibitors, cocaine (COC), methamphetamine (METH) and mazindol (MAZ) in a tissue culture model of the ventral mesencephalic area. Previous in vivo studies of the effects of these agents on the dopamine uptake system have provided conflicting results. In this study, we utilized a tissue culture model that allows a direct means of drug application to the mesencephalic cells and minimizes peripheral influences.

Brains from fetal rats (19 days gestation) were removed and the mesencephalic area obtained and dispersed into single cells. Neuronal uptake inhibitors, COC and METH, (10^{-4},10^{-5}M) or MAZ (10^{-6}, 10^{-7}) were added to the cultures on day 2 and fresh daily thereafter. The effect of chronic exposure to the uptake inhibitors on high affinity DA uptake was determined. In addition, cultures were fixed with 5% acrolein and immunostained for the presence of tyrosine hydroxylase (TH) positive cells. Lactate dehydrogenase (LDH) activity was measured as an indicator of cell death. Preliminary results show that COC (10^{-4}M) causes a slight, but insignificant, decrease in high affinity DA uptake (18%) while 10^{-5}M has no effect. Chronic exposure to METH, on the other hand, decreases DA uptake at both 10^{-4} and 10^{-5}M (>50% at 10^{-4}M). MAZ also decreased high affinity DA uptake, but not as vigorously as METH. These results will be discussed in relation to data obtained from immunocytochemical and neurotoxicological analyses. Supported by grant DA05073 (BAB).

267.3

EFFECTS OF COCAINE ON THE TRANSPLANT-REINNERVATED STRIATUM. J. Graham[1], R. Meloni[1], I. Hanbauer[2], M. Valchar[2]* and K. Gale[1]. [1]Dept. of Pharmacology, Georgetown Univ. Medical Center, Washington, D.C. 20007, and [2]Lab. Chem. Pharmacology, NHLBI, Bethesda, Md. 20892.

Cocaine (COC) (25mg/kg i.p.) elicits asymmetrical locomotor activity in rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the dopaminergic (DA) nigrostriatal pathway. This behavior is directed towards the lesioned side (i.e. ipsiversive). As transplants of fetal substantia nigra innervate the lesioned striatum and release DA in an amphetamine (AMPH) sensitive fashion, we were interested in determining the sensitivity of the reinnervated striatum to COC. One month after implantation of solid fetal tissue containing substantia nigra, the ipsiversive rotational behavior induced by COC was significantly reduced, as compared to the pretransplantation baseline. More importantly, a strong contraversive rotational behavior appeared in response to COC in all rats with successful transplants. This behavior, which occurred in several transplant-bearing rats which showed no contraversive rotation with AMPH, was never observed after COC in lesioned rats without transplants. Other DA uptake blockers (GBR 12909, nomifensine) induced the same response as COC, whereas uptake inhibitors for norepinephrine or serotonin were without effect.

Examination of the striatum for ^{3}H-COC binding revealed that the transplant restored a significant amount of the binding lost as a result of the DA lesion. Moreover, the restoration of COC binding sites in the host striatum did not appear to be limited to the region adjacent to the transplant.

These data suggest that the ability of COC to potentiate DA transmission in the transplant reinnervated striatum may be more pronounced than in the contralateral intact striatum. The distribution of cocaine-sensitive DA uptake sites may be a determinant of this action.

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267.4

CORRELATION BETWEEN NUCLEUS ACCUMBENS NEURONAL ACTIVITY AND COCAINE SELF-ADMINISTRATION BEHAVIOR IN RATS. J.Y. Chang, S.F. Sawyer, R.-S. Lee and D.J. Woodward, Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center & Callier Center, University of Texas at Dallas, Dallas, TX 75235.

The goal of this study was to obtain detailed correlations between neuronal activity in the nucleus accumbens (NAc) and components of behavior that precede lever pressing for cocaine self-administration in rat. Single unit recording was performed in the NAc of freely moving rats using chronically implanted microwires. Rats were trained to self-administer cocaine (0.1 mg/kg, i.v.) by pressing a lever. The animal's behavior during lever pressing was divided into 7 categories in which the animal 1) turns from a corner towards the lever; 2) faces lever with no overt movements; 3) raises head towards lever; 4) rears towards lever; 5) touches and depresses lever with forelimb; 6) moves forelimb from lever to floor; 7) turns away from lever. Some NAc neurons exhibited an increase or decrease in spike activity beginning 5-20 seconds prior to lever pressing. These responses were termed excitatory and inhibitory anticipatory responses, respectively. For neurons that exhibited an excitatory anticipatory response, the increase in spike activity was associated only with categories 3, 4 and 5, i.e., the initiation and completion of movements directly related to lever pressing. Such alterations in neuronal activity were not secondary to movements *per se* since no alterations in unit activity occurred during equivalent rearing behaviors on the other side of the chamber or during treadmill-enslaved locomotion. The inhibitory anticipatory response, on the other hand, often began before and persisted throughout the behaviors leading to lever press. The dopamine D2 antagonist pimozide (0.25 mg/kg, i.p.) failed to block either type of anticipatory response even though it elicited an extinction response. The results of this study provide further evidence that the NAc participates in the expression of reward seeking behaviors through distinct anticipatory responses that are contingently linked to movement. *Supported by DA02338, MH44337 and Biological Humanics Foundation.*

267.5

THE EFFECTS OF CHRONIC COCAINE ON BEHAVIOR, MONOAMINES AND OCCUPATION OF DOPAMINE RECEPTOR SUBTYPES. L.Y. Burger*, G.B. Baker and M.T. Martin-Iverson. Neurochemical Research Unit, Dept. of Psychiatry, University of Alberta, Edmonton, Alberta, Canada T6G 2B7

The effects of chronic administration of male Sprague-Dawley rats with each of 4 doses of cocaine (0.0 [vehicle], 2.5, 7.5 and 22.5 mg/day) on locomotion, brain regional monoamine levels and striatal binding to selective dopamine D1 and D2 antagonists were investigated. Cocaine was administered using Alzet osmotic minipumps (Model 2ML2), as was the D1 receptor antagonist, SCH 23390 (80 μg/day) given to some groups. Locomotion in the rats was assessed by counting interruptions of infrared photobeams in hourly blocks throughout the 16 days of drug administration. There was an initial stimulant effect for the middle and high doses of cocaine. Unlike direct D2 agonists or amphetamine, which produce nocturnal sensitization and daytime tolerance, chronic cocaine administration produced tolerance over successive days and nights. SCH 23390 was found to attenuate cocaine effects at all doses regardless of light or dark cycle. On Day 15, the monoamine receptor inactivator, N-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 5 mg/kg) was injected IP. Interestingly, EEDQ produced hyperlocomotion in vehicle controls. Cocaine-treated rats exhibited hypolocomotion after EEDQ. The D1 antagonist did not significantly influence the effect of cocaine on EEDQ's actions on locomotion. 24 h post-injection with EEDQ, the rats were killed by guillotine decapitation, and their brains were removed and dissected. Since the degree of receptor inactivation by EEDQ is inversely related to the degree of occupancy of the receptors by drugs and endogenous ligands, the density of binding sites after EEDQ treatment is related to the number of occupied sites. The affinity and Bmax of sites that bind to [3H]-SCH 23390 and [3H]-spiperone in striatal tissue were determined in all groups. Levels of monoamines and their major acidic metabolites were determined in striatum, nucleus accumbens and olfactory tubercle. It is concluded that chronic cocaine alters the effects of EEDQ on receptors that regulate locomotor activity. Funding by AMHRF, MRC, CPRF and NSERC.

267.6

ELECTROPHYSIOLOGICAL EFFECTS OF COCAINE IN AMYGDALA NUCLEI: RELATIONSHIP TO DOPAMINE AND SEROTONIN ACTIONS. **P.M. Callahan and K.A. Cunningham, Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston TX, 77550.**

Implicated in psychological processes (mood, emotion) affected by psychoactive drugs, the amygdala may be an important site of action for cocaine, particularly as it is sensitive to cocaine-induced kindling. Cocaine (iv) inhibits and excites the spontaneous activity of amygdala neurons (Cunningham et al., *Soc. NS. Abst. 15*: 1098, 1989), possibly due to dopamine (DA) and/or serotonin (5-HT) uptake inhibition. In the present study, extracellular recording and microiontophoretic techniques were employed to investigate the direct actions and interactions of cocaine with DA and 5-HT in amygdala neurons of male rats (n=16) anesthetized with urethane (1.5 g/kg). Of the 24 neurons recorded to date, 13 were in the basolateral, 8 in the central, 2 in the medial dorsal and 1 in the lateral amygdala. As observed previously, the spontaneous activity of these neurons was slow (range: 0.1-8.5 Hz) and irregular. The majority of both spontaneously-active and glutamate-driven cells were inhibited by microiontophoretic DA (0.1 M, pH 4) and 5-HT (0.04 M, pH 4); 83% (10/12) and 95% (20/21) of cells tested were inhibited by DA and 5-HT, respectively. Maximal inhibitions (50-70%) occurred with ejection currents between 10-40 nA. Cocaine (0.01 M, pH 4) inhibited 66% of the cells tested (n=4/6); remaining neurons were unaffected by cocaine (5-20 nA). Intravenous cocaine (1 or 2 mg/kg) also potentiated the duration of the inhibitory response to microiontophoretically-applied DA or 5-HT. These data suggest that cocaine has direct actions on a proportion of amygdala neurons which is probably a consequence of DA and 5-HT uptake inhibition. We are currently studying the role of potential "synergistic" actions of DA and 5-HT in mediating the inhibitory effects of cocaine on amygdala neurons (White, *PB&B 24*: 365, 1986). *Support by NARSAD, DA05708, DA06511.*

267.7

COCAINE-INDUCED BEHAVIORAL SENSITIZATION AND D-1 DOPAMINE RECEPTOR FUNCTION IN RAT NUCLEUS ACCUMBENS AND STRIATUM. R.D. Mayfield*, G. Larson* and N.R. Zahniser. Dept. Pharmacol., Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Repeated cocaine administration produces a persistent sensitization of dopamine (DA)-mediated behaviors. It also produces a persistent enhancement of the electrophysiological sensitivity of D-1 DA receptors in nucleus accumbens (Henry and White, Soc. Neurosci. Abstr. 15:1013, 1989). Our goal was to determine if repeated cocaine administration induces long-lasting increases in D-1 DA receptor density, in agonist (DA) affinity at D-1 receptors and/or in the ability of DA to stimulate D-1 receptor-coupled adenylate cyclase activity. Male Sprague-Dawley rats were treated once daily for 6 days with either 15 mg/kg cocaine-HCl (i.p.) or saline. Locomotor and stereotypic behaviors were rated on treatment days 1 and 6 to confirm that the rats were behaviorally sensitized. Assays were conducted on tissue taken 1 week after the last injection. Quantitative autoradiographic analysis of [3H]-SCH 23390 saturation curves in nucleus accumbens and striatum detected no differences in D-1 receptor density or affinity between control and cocaine-treated rats. Furthermore, competition curves indicated that the affinity of D-1 receptors for DA was similar between brain regions and between treatment groups. Dose-response analysis of DA-stimulated adenylate cyclase activity also revealed no changes induced by repeated cocaine treatment in nucleus accumbens or striatum. While not ruling out changes in other D-1 receptor mediated events, these results indicate that repeated cocaine administration does not produce persistent changes in D-1 DA receptor density, affinity of D-1 receptors for DA or D-1 receptor-stimulated adenylase cyclase activity in nucleus accumbens or striatum. Supported by DA 04216 and AA 07464.

267.8

DIFFERENCES IN DOPAMINE CLEARANCE/DIFFUSION IN STRIATUM AND NUCLEUS ACCUMBENS FOLLOWING PERIPHERAL COCAINE ADMINISTRATION. W. A. Cass, G. A. Gerhardt, P. Curella* and N. R. Zahniser. Depts. of Pharmacology and Psychiatry, Univ. of Colorado Hlth. Sci. Ctr., Denver, CO 80262.

In vivo electrochemistry was used to determine whether or not dopamine (DA) nerve terminals in striatum and nucleus accumbens respond differently to a single, systemic dose of cocaine. Nafion-coated carbon fiber electrodes were positioned in dorsal striatum or nucleus accumbens in urethane anesthetized rats. When a finite amount of DA (25-50 nl, 200 μM barrel concentration) was pressure-ejected every 5 minutes from a micropipette positioned 300 $\pm$ 30 μm from the electrode, transient ($t_{1/2}$ = 15-32 sec) and reproducible increases in DA (0.35-2.35 μM) were detected. In response to a 15 mg/kg (i.p.) injection of cocaine-HCl, no change in DA clearance/diffusion was detected in dorsal striatum. In contrast, pressure-ejected DA concentrations increased 57% above baseline in nucleus accumbens indicating significant inhibition of the DA transporter. The time course of the DA increase paralleled the behavioral changes observed in nonanesthetized rats injected with 15 mg/kg cocaine (see R. D. Mayfield, this meeting). Analysis of competition curves (^{3}H-mazindol binding to the DA transporter) revealed that the affinity of the transporter for cocaine did not differ between the striatum and nucleus accumbens. However, similar to previous reports, the density of ^{3}H-mazindol binding sites in the nucleus accumbens was 40% of that in the dorsal striatum. Our results suggest that the greater apparent sensitivity of the nucleus accumbens to cocaine is due to a lesser number of DA transporter molecules for cocaine to inhibit in nucleus accumbens, as compared with dorsal striatum, rather than a higher affinity of the DA transporter for cocaine in the nucleus accumbens. Supported by USPHS DA04216 and NS09199.

267.9

DEVELOPMENT OF TOLERANCE OR SENSITIZATION TO INHIBITION OF DOPAMINE UPTAKE BY COCAINE IS DEPENDENT ON TREATMENT REGIMEN. B.M. Cox and S. Izenwasser. Dept. of Pharmacology, Uniformed Services University, 4301 Jones Bridge Road, Bethesda, MD 20814.

We have previously shown that repeated daily injections of cocaine (one injection of 15 mg/kg/day for three days, a dose regimen which produces behavioral sensitization) produced a decrease in [^{3}H]dopamine uptake in rat nucleus accumbens but not in striatum (caudate-putamen). Additionally, the potency of cocaine in inhibiting [^{3}H]dopamine uptake in the nucleus accumbens was increased by this treatment. In the present study, male rats were chronically treated with either cocaine (50 mg/kg/day, expressed as free base) or saline (0.9% sodium chloride, 24 µl/day) via subcutaneously implanted osmotic mini-pumps. After 7 days, the effects of cocaine on [^{3}H]dopamine uptake in chopped tissue slices of striatum and nucleus accumbens were examined. Total uptake of [^{3}H]dopamine was not influenced by these treatments in either tissue. However, tolerance to the inhibition of [^{3}H]dopamine uptake by cocaine was observed in both the nucleus accumbens and striatum of cocaine treated rats. Thus, chronic cocaine has different effects than intermittent cocaine on [^{3}H]dopamine uptake. The occurrence of sensitization or tolerance depends on the schedule of administration. (Supported by a grant from NIDA).

267.10

TOLERANCE TO INHIBITORS OF DOPAMINE UPTAKE: DIFFERENTIAL EFFECTS OF CHRONIC COCAINE OR NICOTINE. S. Izenwasser and B.M. Cox. Department of Pharmacology, Uniformed Services University, 4301 Jones Bridge Road, Bethesda, MD 20814-4799.

We have previously shown that nicotine inhibits [^{3}H]dopamine uptake in striatum (caudate-putamen) *in vitro* via a mechanism that appears to differ from that of cocaine. In the present study, male rats were chronically treated with either nicotine (6 mg/kg/day, expressed as free base), cocaine (50 mg/kg/day), or saline (0.9% sodium chloride, 24 µl/day) via subcutaneously implanted osmotic mini-pumps. After 7 days, the effects of nicotine and cocaine on [^{3}H]dopamine uptake in chopped tissue slices of striatum and nucleus accumbens were examined. Chronic nicotine treatment produced tolerance to the inhibition of [^{3}H]dopamine uptake by nicotine and cross-tolerance to cocaine in the striatum. Chronic cocaine, however, produced tolerance only to itself. Nicotine did not inhibit uptake of [^{3}H]dopamine in the nucleus accumbens of saline treated animals. In this tissue, both cocaine and nicotine treatments produced tolerance to cocaine, as in striatum. Neither chronic nicotine nor cocaine led to changes in the total amount of [^{3}H]dopamine accumulated in either brain region. These findings provide further evidence that nicotine and cocaine inhibit dopamine uptake via different mechanisms. (Supported by a grant from NIDA).

267.11

CHARACTERIZATION IN VIVO OF THE NEUROCHEMICAL STATE OF THE DOPAMINE NERVE TERMINAL IN THE NUCLEUS ACCUMBENS AFTER CHRONIC COCAINE. **J.W. Brock, L.H. Parsons, J.P. Ng, and J.B. Justice Jr., Department of Chemistry, Emory University, Atlanta, GA 30322**

The effects of repeated administration of cocaine on synthesis and metabolism of dopamine (DA) in the nucleus accumbens after 10 days of abstinence were determined. Male rats were pretreated with cocaine (10 mg/kg i.p.) or saline once daily for 10 days followed by 10 days of abstinence. On the tenth day of abstinence, microdialysis perfusion of NSD 1015 was used to assess DOPA accumulation. Reduced DOPA formation (73% of control) was found in the cocaine-treated animals (N = 4/group). Rates of DOPA clearance after synthesis inhibition by an acute AMPT injection (200 mg/kg i.p.) were similar for both groups further suggesting a difference in DOPA production. Prior to NSD 1015 perfusion, DOPAC in dialysate was monitored to determine the effect of repeat cocaine injections on basal extracellular DOPAC. Reduced DOPAC levels (73% of control) were found in the cocaine-treated animals (N = 4/group). Previously, using the same paradigm, similar decreases were found after one day of abstinence(Brock et al. Neurosci. Lett. 117, 234-239, 1990). Thus, the effect on synthesis and metabolism persists longer than an increase in maximal uptake and stimulated release seen at day 1 which then relaxes to control values by day 10 (Ng et al., J Neurochem. 56, 1485-1492, 1991). The decreased synthesis rates may contribute to decreased basal extracellular DA levels seen at day 10 (Parsons et al., Synapse, in press, 1991).

267.12

ANALYSIS OF ACUTE AND CHRONIC COCAINE EFFECTS ON EXTRACELLULAR DOPAMINE PEAK-TO-BASAL RATIOS. F. Weiss, M. Paulus[1], M.T. Lorang*, and G.F. Koob. Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037 and [1]Department of Psychiatry, University of California, San Diego, CA 92161.

Recent evidence suggests that basal dopamine (DA) activity can predict the behavioral reponse to psychostimulant treatment. These findings suggested a possible relationship between basal DA release and the magnitude of stimulant-induced increases in extracellular DA. To test this hypothesis basal and peak DA levels in reponse to an acute injection of cocaine (10 mg/kg; IP) were determined in the nucleus accumbens of anesthetized rats on Days 1, 3, and 7 following a 10-day chronic cocaine (30 mg/kg; IP) or saline pretreatment period. Both basal (10.7 $\pm$ 2.55 nM vs. 3.55 $\pm$ 0.56 nM) and peak (23.6 $\pm$ 4.36 nM vs. 12.61 $\pm$ 1.76 nM) extracellular DA concentrations were significantly elevated in chronic cocaine-pretreated rats on Day 1 post cocaine, but decreased to control levels by Day 7. The percent increase in peak levels was inversely related to basal concentrations in both chronic cocaine and saline treatment groups at all postchronic intervals tested. Compared to saline pretreatment, chronic cocaine produced a significant decrease in peak-to-basal ratios on Day 1 suggesting some tolerance. This effect was no longer evident on Day 7. Regression analyses of logarithmically (ln) transformed data revealed the existence of a significant non-linear relationship between basal and peak DA concentrations independent of chronic pretreatments. All regression line slopes (0.58 to 0.73) were significantly less than 1. No statistical differences among slopes of individual treatment groups were found. These results indicate that a) chronic cocaine can increase the absolute levels of basal and peak DA, b) the magnitude of the cocaine-induced increase in extracellular DA decreases proportionally with increasing basal levels, and c) the DA response to cocaine can show tolerance in spite of elevations in absolute DA levels. This work was supported in part by NIDA grant DA 05843.

267.13

OPPOSITE EFFECTS OF DOPAMINE D_1 AND D_2 AGONIST PRETREATMENT ON COCAINE SELF-ADMINISTRATION.

D.W. Self and L. Stein. Dept. of Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92717.

D_1 and D_2 receptor antagonists both block cocaine reward, but animals will self-administer only D_2 and not D_1 receptor agonists. To further clarify the role of D_1 and D_2 receptors in reward, we pretreated rats with D_1- and D_2-selective agonists 30 min before cocaine self-administration tests. The D_2 agonist N-0923 (0.03, 0.1, and 0.3 mg/kg s.c.) dose-dependently reduced cocaine self-administration rates by lengthening the interinfusion interval between successive self-injections ($p < .001$). The same effect is produced by increasing the self-administered dose of cocaine, suggesting that N-0923 prolongs cocaine's reinforcing efficacy. In contrast, the D_1 agonist SKF 38393 (3 and 10 mg/kg s.c.) increased cocaine self-administration rates by shortening the interinfusion intervals ($p = .001$). This effect resembles decreasing the cocaine dose, suggesting that SKF 38393 reduces the reinforcing efficacy of cocaine. The combination of SKF 38393 and the D_1 antagonist (+)SCH 23390 failed to produce additive increases in cocaine intake; hence, the rate-increasing action of SKF 38393 alone did not result from D_1 receptor antagonism (due to the partial agonist properties of the drug). Opposing effects of D_1 and D_2 receptors on cocaine reward is consistent with their opposing roles in cyclic AMP formation, perhaps implicating cyclic AMP in the regulation of reward.

(Supported by DA 05107, DA 05379 and AFOSR 89-0213)

267.14

MICRODIALYSIS RECOVERY IN VIVO IS ALTERED INDEPENDENTLY OF CONCENTRATION: EFFECTS OF CHRONIC COCAINE

L.H. Parsons, A.D. Smith and J.B. Justice, Jr. Dept. Chemistry, Emory Univ., Atlanta, GA 30322.

The basal state of the dopaminergic system was examined during abstinence from chronic cocaine using the quantitative microdialysis method of Lonnroth et al. (1987). Rats were treated for ten days with cocaine (20 mg/kg, i.p.) followed by either one or ten days abstinence. After one day of abstinence there was no significant difference in basal extracellular dopamine (DA) levels in the nucleus accumbens (N ACC) between cocaine treated (4.1±0.3 nM) and saline treated (3.9±0.2 nM) groups (mean±SEM; n = 5/grp). There was, however, a significant increase in the in vivo probe recovery of the cocaine treated group (91±4% vs 67±8%; $P < 0.03$). After ten days of abstinence, basal extracellular levels of DA were reduced in cocaine treated animals (2.1±0.3 nM vs. 3.9±0.2 nM; n = 5/grp), while the in vivo recoveries were not significantly different for the cocaine (63±7%) and saline (64±9%) groups. These results suggest not only that basal levels of DA are altered by chronic cocaine, but also that the dynamics of release and uptake are independently altered as well.

To examine the effect of active processes on in vivo recovery, the same experimental procedure was performed to compare the in vivo recovery for DA and the metabolite DOPAC. The in vivo recovery of DA was found to be significantly higher that of DOPAC (77±10% vs 22±1%; $P < 0.0007$; n = 5/grp). There was no significant difference in the in vitro recoveries for DA or DOPAC. The difference in in vivo recovery is thought to be caused by the active processes of release and uptake associated with DA, but not DOPAC. Rats which displayed 80% DA depletion (caused by unilateral 6-OHDA lesions) had significantly lower in vivo recoveries for DA than control animals (30±3% vs 60±3%, respectively) indicating that reduced numbers of DA nerve terminals result in reduced in vivo recovery. The basal extracellular DA levels between the groups were unchanged by the lesions.

267.15

COCAINE CAN INCREASE EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS IN THE PRESENCE OF GAMMA-BUTYROLACTONE.

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Acute peripheral injection of cocaine stimulates motor activity in parallel with an increase of extracellular dopamine in the nucleus accumbens, as measured by in vivo microdialysis. We have previously demonstrated that injection of the GABAB agonist baclofen into the A10 dopamine region blocked the motor-stimulant response to cocaine. However, the cocaine-induced increase of accumbal dopamine was not blocked by baclofen pretreatment. It has been suggested that the cocaine-induced increase in accumbal dopamine is dependent on intact firing activity of dopamine neurons. Baclofen has been reported to inhibit firing of dopamine cells, and thus would be expected to block the cocaine-induced increase of dopamine in the nucleus accumbens. In order to further investigate the role of neuronal impulse activity in the increase of dopamine following cocaine, male rats were injected with gamma-butyrolactone (GBL), a compound which has been reported to inhibit dopamine impulse activity, before acute cocaine treatment. One week after being implanted with guide cannulae for in vivo microdialysis monitoring of dopamine in the nucleus accumbens animals were attached to a dialysis probe. Four 20 min baseline samples were collected before injection of saline or GBL (750 mg/kg). 3 additional samples were collected after which animals were injected with saline or cocaine (15 mg/kg) followed by collection of 9 samples. Preliminary data showed that GBL led to a greater than 50 percent reduction in basal dopamine in the nucleus accumbens. Dopamine returned to baseline levels 20-40 min after injection of cocaine. Similar to saline-pretreated animals, when compared to basal concentrations after GBL-pretreatment, cocaine led to an approximate 100 percent increase of dopamine. These data suggest that cocaine can increase dopamine in the nucleus accumbens independent of intact impulse activity of dopamine neurons.

267.16

MICRODIALYSIS STUDIES ON MDMA-INDUCED NEUROTRANSMITTER RELEASE.

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The effect of the serotonin (5-HT) neurotoxin, MDMA (3,4-methylenedioxymethamphetamine), on the extracellular concentrations of dopamine (DA) and 5-HT was studied using in vivo microdialysis. Infusion of MDMA (1-1000 μM) directly into the dialysis probe produced a dose-dependent increase in the extracellular concentration of DA in the striatum. Peripheral administration of the DA uptake inhibitors GBR 12909 (10 mg/kg) or mazindol (5 mg/kg) 30 min prior to the infusion of MDMA (10 μM) inhibited MDMA-induced DA release. In contrast, neither the 5-HT uptake inhibitor, fluoxetine (10 mg/kg), nor the 5-$HT_{2/1C}$ antagonist, ketanserin (3 mg/kg), affected the increase in DA release produced by the local infusion of MDMA (10 μM). The peripheral administration of MDMA produced a dose-dependent increase in the extracellular concentration of DA and 5-HT. Pretreatment with the tyrosine hydroxylase inhibitor, α-methylparatyrosine (250 mg/kg), partially inhibited the increase in the extracellular concentration of DA produced by MDMA (20 mg/kg). Similarly, infusion of the sodium channel blocker, tetrodotoxin (TTX, 10 μM), attenuated MDMA-induced DA release. Pretreatment with the 5-HT_2 antagonist, pipamperone (3 mg/kg), significantly attenuated MDMA-induced DA release whereas the peripheral 5-HT_2 antagonist, xylamidine (5 mg/kg), had no effect on MDMA-induced DA release. These data are suggestive that MDMA increases the extracellular concentration of DA in the striatum, in part, via a carrier mediated mechanism which is largely independent of its effects on 5-HT release. However, the ability of TTX to partially antagonize the effect of MDMA coupled with the inability of α-MPT to completely abolish the effects of MDMA is suggestive that MDMA also affects impulse-mediated release of DA, perhaps via a 5-HT_2 receptor mechanism.

267.17

INTERACTIONS BETWEEN COCAINE AND THE PRESUMED PREFERENTIAL DOPAMINE AUTORECEPTOR ANTAGONISTS, (+)-AJ 76 AND (+)-UH 232.

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(+)-AJ 76 and (+)-UH 232 are stimulant dopamine (DA) antagonists that may preferentially act at DA nerve terminal autoreceptors (Svensson et al., 1986, Arch. Pharmacol., 334:234 and Hoffmann et al., 1988, Neurosci. Abs. 14:524). In ventral tegmental area (VTA), both (+)-AJ 76 and (+)-UH 232 antagonized cocaine-induced depressions in firing rates of DA neurons, with (+)-UH 232 being significantly more potent (ED_{50}=22 µg/kg i.v.) compared to (+)-AJ 76 (ED_{50}=1360 µg/kg i.v.). (+)-AJ 76 and (+)-UH 232 moderately increased locomotor activity (LMA) in habituated rats, but antagonized the stronger stimulant effects of cocaine. (+)-AJ 76, but not (+)-UH 232, partially generalized to the cocaine cue in drug discrimination tests. Neither agent blocked the cocaine discriminant cue. In 2-deoxyglucose autoradiography studies, (+)-AJ 76 antagonized cocaine-induced increases in energy metabolism in most brain regions. It is concluded that these preferential autoreceptor antagonists have unique interactions with cocaine that could be useful in treating cocaine abuse.

267.18

DOPAMINE AND DOPAC EFFLUX DURING WITHDRAWAL FROM CHRONIC COCAINE

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Previous research conducted in this laboratory indicated that subjects receiving chronic subcutaneous injections of cocaine exhibited sensitization to a subsequent challenge dose of cocaine. In contrast, rats implanted with coca ne osmotic minipumps exhibited tolerance to a subsequent challenge dose of cocaine. The present study examines whether tolerance and sensitization under these conditions are correlated with differences in dopamine efflux in slices of the caudate nucleus. On the seventh day of withdrawal from the chronic dosing regimes, the caudate was removed by decapitation, and then rapid dissection using a vibratome. The slices were then perfused with artifical CSF for two hours, at which time baseline samples were collected. At the end of the basline period, the slices were perfused with either 10, 20, or 40 micromolar cocaine for ten minutes and samples collected every two minutes during drug perfusion. At the end of the perfusion period, the drug was removed, and samples collected for a subsequent 45 min. The samples were assayed for dopamine (DA) and 3,4-dihydroxypheynlacetic acid (DOPAC) using HPLC-EC. The results indicate that the baseline levels of DOPAC are similar across all three dosing regimes. However, the basal levels of DA are depressed in the subjects pretreated with the osmotic minipumps. Dopamine efflux, in the presence of cocaine, in the injection pretreatment group is significantly higher than in the saline control; the dopamine efflux, in the presence of cocaine in the cocaine pump pretreatment group is significantly less than in the saline control groups. Therefore, the present results indicate that the behavioral differences produced by a cocaine challenge are correlated with differences in dopamine efflux in the caudate nucleus. Supported by NIDA DA 05303.

267.19

BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF REPEATED COCAINE ADMINISTRATION IN RATS. J. I. Javaid and J. M. Davis . Illinois State Psychiatric Institute, University of Illinois at Chicago, Chicago, IL 60612.

Repeated administration of cocaine causes augmentation in behavioral effects induced by acute administration. Present studies were designed to examine the changes in dopaminergic systems in various areas of rat brain after repeated cocaine administration. Repeated daily injections of cocaine (10 mg/Kg, IP) resulted in progressive increases (behavioral sensitization) in locomotor activity and stereotypy scores (repetitive movements, sniffing). The basal levels of DA and its metabolites, DOPAC and HVA in nucleus accumbens and caudate putamen from sensitized rats were not significantly different than control animals. However, DA levels in hippocampus from sensitized rats were significantly higher than controls. ^{3}H-Spiroperidol binding, with butaclamol as a displacer, was decreased in nucleus accumbens but remained unchanged in caudate in sensitized rats. However, with sulpride as a displacer, ^{3}H-spiroperidol binding was decreased in both brain regions. Thus, repeated cocaine administration results in differential effects on dopaminergic systems in different brain areas.

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267.20

COCAINE'S EFFECT ON DOPAMINERGIC AND SEROTONERGIC ACCUMBENS NEURONS ORIGINATES IN A PRESYNAPTIC MECHANISM FOR RELEASE. P.A. Broderick and F.T. Phelan, Dept. Pharmacol., CUNY Medical School (Rm. J-910), Convent Ave. & W. 138th St., N.Y., N.Y. 10031, U.S.A.

Gamma-butyrolactone (γBL) is a known impulse flow inhibitor. γBL increases dopamine (DA) synthesis and predicts a reduced dopamine release; γBL does not increase serotonin (5-HT) synthesis and its effects on serotonin release are not known (Walters, J.R. and Roth, R.H., *Biochem. Pharmacol.* 21(1972) 2111). Since presynaptic mechanisms for release are dependent on impulse flow, γBL was used to assess possible DA and 5-HT releasing properties of the psychostimulant, cocaine. *In vivo* voltammetric studies, (semiderivative electroanalysis and stearate working electrodes) provided selective detection of DA and 5-HT in nucleus accumbens of chloral hydrate anesthetized, male, virus free, Sprague-Dawley rats (cf. Broderick, P.A., *Brain Res.* 495 (1989) 115). In the γBL studies and in the cocaine studies, voltammograms were recorded one hour before and after administration of either γBL (750 mg/kg ip) or cocaine (20 mg/kg sc). In the γBL plus cocaine studies, after impulse flow was significantly blocked by γB L ($p<0.006$), cocaine (20 mg/kg sc) was administered and synaptic DA and 5-HT concentrations were measured for one hour. The results from time course data show that cocaine's effects on synaptic concentrations of DA were significantly inhibited ($p<0.002$) and synaptic concentrations of 5-HT were significantly inhibited ($p<0.0001$) by γBL. It is concluded that cocaine-induced alterations in synaptic concentrations of DA and 5-HT originate in presynaptic mechanisms for release. (Supported by NIDA, 1 R01-04755-01, and PSC/CUNY 6-61188).

267.21

EFFECTS OF AMPHETAMINE AND COCAINE ON EXTRACELLULAR DOPAMINE LEVELS IN THE DORSOLATERAL PREFRONTAL CORTEX AND CAUDATE NUCLEUS IN THE RHESUS MONKEY: A MICRODIALYSIS STUDY. B.S. Kolachana, R.C. Saunders, and D.R. Weinberger. Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.

Extracellular dopamine (DA) levels were measured in the caudate (cd) and dorsolateral prefrontal cortex (pfc) of the Rhesus monkey using in vivo microdialysis coupled with HPLC electrochemical detection. Magnetic resonance imaging was used in conjunction with a specialized guide implant to permit accurate probe placement. Dialysis probes with a 5 mm exposed membrane tip were perfused constantly with artificial CSF at a rate of 1 ul/m. Basal levels of DA were consistantly found in the cd 180 fmol/20ul and pfc 3.5 fmol/20ul. Amphetamine infusion through the dialysis probe (7 ng/ul) increased DA 25-40 fold in the cd (2000-8000 fmol/20ul) but only a 5 fold increase in the pfc (18-25 fmol/20ul). Cocaine infusion (40 ng/ul) induced a moderate release of DA in the cd (1500 fmol/20 ul) and a smaller increase in pfc. In one monkey amphetamine infusion in pfc resulted in marked decrease in cd DA (55 fmol/20ul) and later cocaine infusion resulted in total depletion of DA in cd. The results demonstrate that reliable levels of DA can be measured in the monkey pfc and cd, and these levels can be manipulated locally via infusion through the dialysis probe.

DRUGS OF ABUSE--COCAINE: MONOAMINES AND BRAIN STIMULATION

268.1

COMPARATIVE INTERACTIONS OF IBOGAINE WITH OPIATE AND STIMULANT DRUGS: *IN VIVO* MICRODIALYSIS AND MOTOR BEHAVIOR. I.M. Maisonneuve, K.L. Rossman* and S.D. Glick. Department of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208.

Ibogaine, an indolalkylamine, has been claimed to be effective in treating opiate (patent No 4,499,096) and stimulant addiction (patent No 4,587,243). In order to determine if there is a rational basis for these claims, neurochemical and behavioral studies of potential interactions between ibogaine and either morphine, d-amphetamine or cocaine were conducted in rats. When administered 19 hr before a morphine challenge (5 mg/kg, i.p.), ibogaine (40 mg/kg, i.p.) prevented the expected rise in extracellular dopamine levels in the nucleus accumbens. Using photocell activity cages, the same ibogaine pretreatment was found to block the stimulatory motor effects induced by morphine (0.5 to 30 mg/kg, i.p.). In contrast, ibogaine pretreatment enhanced the rise in extracellular dopamine levels induced by d-amphetamine (1.25 mg/kg, i.p.) in the nucleus accumbens and potentiated the motor activity induced by d-amphetamine (0.625 to 5 mg/kg, i.p.). Lastly, ibogaine pretreatment did not appear to modify the increase in dopamine levels normally induced by cocaine (20 mg/kg, i.p.) in the nucleus accumbens; however, effects of cocaine on dopamine metabolite (DOPAC and HVA) levels were enhanced. A small enhancement of cocaine (5 to 40 mg/kg, i.p.) induced motor activity was also observed. In summary, ibogaine induced neurochemical and behavioral changes that could be consistent with its having anti-addictive effects.
(Supported by NIDA grant DA03817).

268.2

INTERACTIONS BETWEEN COCAINE AND DOPAMINERGIC OR SEROTONERGIC ANTAGONISTS: EFFECTS ON PATTERNS OF MOTOR ACTIVITY M.P. Paulus and M.A. Geyer. UCSD Dept Psychiatry, Lab of Biol Dynamics and Theoret Med, CA 92093

Cocaine's effects on geometric patterns of motor activity can be distinguished clearly from patterns observed for amphetamine treated animals. In this investigation, we used a video system to track the movements of animals in an 30.5 × 61 cm rectangular enclosure. Based on other investigations showing that a 5-HT 1B agonist, RU-24969, led to extremely repetitive straight paths of locomotion, we hypothesized that similar path patterns observed after relatively high doses of cocaine may reflect influences on the serotonergic system. In addition, previous results with the D1/2 agonist apomorphine and specific combinations of the D1 agonist, SKF-38393, and the D2 agonist, quinpirole, indicated that the synergetic activation of D1 and D2 receptors may also lead to consecutive straight movements. Thus, the changes of path patterns could also result from specific activation of D1 or D2 receptors. Therefore, we tested the effects on patterns of motor activity of the interaction between 20.0 mg/kg cocaine and 20.0 mg/kg propranolol or 2.0 mg/kg ritanserin, and 5.0 μg/kg SCH-23390 or 25.0 μg/kg raclopride, respectively. The animals were injected 10 min prior to exposure to the videotracker; the behavior was recorded by a video camera; and the movements were reconstructed with a resolution of 0.3 cm in the x and y direction and a sampling frequency of 18 Hz. The patterns of activity were assessed using a previously introduced scaling approach which was augmented by additional calculation of the fluctuation of scaling exponents and the susceptibility of changes of one scaling exponent with respect to another. The results indicate that some components of highly local movements induced by cocaine can be specifically influenced by the 5-HT 1 (and β) antagonist propranolol, suggesting that the behavioral movement patterns observed for cocaine result from a combined activation of dopaminergic and serotonergic systems.

268.3

SELF-ADMINISTERED vs. PASSIVE COCAINE INTAKE: EFFECTS ON MONOAMINE NEUROTRANSMITTERS IN THE RAT BRAIN. J.M. Wilson, J.N. Nobrega, W.A. Corrigall, K. Shannak* and S.J. Kish. Dept. Pharmacology, Univ. Toronto, Clarke Inst. Psychiatry, Addiction Res. Fnd., Toronto, Ont.

Recent evidence suggests the possibility that self-administered and experimenter-given cocaine (COC) have different effects on brain amine systems. To test this hypothesis, one group of adult, male Long-Evans rats (responders, n = 6) was allowed to self-administer COC (1 mg/ kg/ i.v./ infusion) for 1 h/ day, 5 days/ week for 4 weeks. Each responder was yoked to a partner which received the same dose of COC at the same time as the responders, but had no control over the drug administration schedule. Mean daily COC intake was 8.6 ± 0.4 mg/ kg. Control animals in a third group were sham-operated but received no drug. Four weeks following the last COC session, rats were killed and brains dissected into discrete areas, including striatum, n. accumbens, medial prefrontal cortex and hypothalamus. For both responders and yoked groups, chronic COC exposure failed to alter levels of the monoamines DA, NA and 5-HT or their metabolites HVA, DOPAC, MHPG and 5-HIAA in the brain areas studied as compared with controls ($p > 0.05$). The level of the dopamine metabolite 3-methoxytyramine was elevated in the striatum (+44%, $p < 0.05$) of responders but not of yoked animals. These data suggest that, irrespective of the method of administration, cocaine at the doses employed does not produce permanent degeneration of brain monoaminergic neurones. Self-administration may, however, be associated with long-lasting changes in striatal dopaminergic metabolism and/or activity.

268.4

CONDITIONED INCREASES IN MESOLIMBIC DOPAMINE OVERFLOW BY STIMULI ASSOCIATED WITH COCAINE.
D. J. Fontana, R.M. Post, and A. Pert. BPB /NIMH, Bethesda, MD

Stimuli associated with the pharmacological effects of cocaine acquire the ability to elicit actions similar to those produced by the drug itself. The purpose of this study was to evaluate the neurochemical bases underlying such conditioned effects of cocaine — specifically in the mesolimbic dopamine (DA) system. Three groups of rats, implanted with microdialysis guide cannulae aimed for the n. accumbens, were employed in the initial study. On Day 1, the first group (conditioned) was injected with cocaine (40 mg/kg, i.p.) and placed in locomotor activity chambers. One hour following return to their home cages, the rats were injected with saline. The second group (pseudo-conditioned) was treated in a similar fashion but received saline in the activity chambers and cocaine in the home cage. The third group (control) received saline in both environments. On Day 2 microdialysis probes were introduced into the nucleus accumbens. Two hours following insertion, all rats received a low dose of cocaine (10 mg/kg, i.p.) and were placed in the locomotor chamber. Activity counts were recorded at 20-min intervals. Dialysate samples also were collected at the end of each interval and assayed for DA and its metabolites with microbore HPLC-EC procedures. Conditioned rats were significantly more active on Day 2 compared to the pseudoconditioned and control groups (1206 vs. 685 vs. 486 horizontal activity counts at peak effect, respectively). Similarly, extracellular DA levels were also elevated significantly in the conditioned group in comparison to the pseudoconditioned and control groups (218 vs. 176 vs. 162%, respectively). Such elevations in extracellular DA cannot be attributed to increases in locomotor activity per se, however. Systemic administration of MK801, which similarly elevated locomotor activity, had no effect on extracellular DA in the n. accumbens. These findings indicate that conditioned stimuli are capable of altering extracellular DA in the mesolimbic system.

268.5

THE EFFECTS ON COCAINE SELF-ADMINISTRATION OF DIFFERENT DOPAMINE ANTAGONISTS INJECTED INTO THE NUCLEUS ACCUMBENS.
R. Maldonado, P. Robledo, A.J. Chover and G. F. Koob.
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The peripheral administration of D1 and D2 dopamine receptor antagonists has been shown to produce an increase in cocaine self administration. Animals are thought to compensate for decreases in the magnitude of the reinforcer by increasing their self-administration behavior. In response to changes in the injection dose, animals typically show an inverse relationship between dose and number of injections per sessions, and thus the effect of dopamine antagonism resembles decreasing the dose of cocaine. In this study, the effects of several dopaminergic antagonists injected into the nucleus accumbens on cocaine self administration behavior were tested in rats. Animals were trained to perform operant responses to self administer cocaine (0.75 mg/kg/inj) via an intravenous catheter on a FR5 schedule of reinforcement. Bilateral intra-accumbens injections (1µl total) were performed through indwelling cannulae. Fluphenazine, a non specific but preferentially D2 dopamine receptor antagonist, when injected intraperitoneally significantly increased the number of lever presses for cocaine at the dose of 0.03 mg/kg ($p<0.05$), and decreased them at the dose of 0.1 mg/kg ($p<0.01$). When fluphenazine was injected into the nucleus accumbens (1 and 2 µg) no significant effects were observed, but a higher dose (4µg) significantly decreased lever presses for cocaine ($p<0.02$). Haloperidol, another preferential D2 dopamine antagonist, significantly increased the self-administration of cocaine when injected into the nucleus accumbens (1 µg; $p<0.05$). Injections of the selective D1 dopaminergic antagonist SCH 23390 into the nucleus accumbens significantly increased response rate for cocaine (1 µg; $p<0.01$). These data suggest that both D1 and D2 receptors in the nucleus accumbens are important for the reinforcing actions of cocaine (supported by NIDA Grant number DA 04398).

268.6

SENSITIZATION TO COCAINE'S REINFORCING AND MOTOR ACTIVATING EFFECTS: DIFFERENT RESPONSE TO QUINPIROLE PREEXPOSURE. B.A. Horger and S. Schenk, Texas A&M Univ., Dept. Psychol., College Station, TX 77843

We have previously reported more rapid acquisition of cocaine self-administration in rats that had been preexposed to moderate levels of amphetamine, caffeine, cocaine or nicotine. The present study was designed to assess the hypothesis that the sensitized response to cocaine could be attributed to an alteration in the sensitivity of D2 dopamine receptors produced by the pretreatment regimen. Rats received daily injections of the specific D2 agonist quinpirole (1.5 mg/kg) or saline. Although this dose of quinpirole failed to increase motor activity after the first exposure, it reliably elevated activity after 5 daily injections, suggesting that the rats had become sensitized to this behavioral effect of the drug. Cross sensitization to cocaine's motor activating effect (10 mg/kg) was also apparent in that quinpirole exposed rats were more responsive to this dose of cocaine. A separate group of rats was also preexposed to quinpirole or saline, as above, and was then tested for intravenous self-administration of a low dose of cocaine (0.25 mg/kg/infusion). Although quinpirole had sensitized rats to the motor activating effects of cocaine, the same preexposure regimen failed to facilitate the acquisition of cocaine self-administration. These data suggest that (1) sensitization to cocaine's motor activating effect may be related to enhanced sensitivity of D2 dopamine receptors, (2) sensitization to the reinforcing effects of cocaine does not appear to be similarly related to changes in D2 dopamine receptor sensitivity under the same parameters, and (3) the motor activating and reinforcing effects of cocaine may rely on different dopaminergic mechanisms.
Supported by DA05548.

268.7

EFFECTS OF SELECTIVE DOPAMINE D_1 AND D_2 AGONISTS ON THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION TO APOMORPHINE.
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Recent work suggests that behavioral sensitization to amphetamine and apomorphine (APO) develops as a result of repeated D_1 stimulation. In the present study we tested this view by determining the effects of selective dopamine agonists on locomotor activity and subsequent sensitivity to APO. In two experiments, male Wistar albino rats (250-350 g) were injected SC daily with either the D_1 agonist SKF 38393 (SKF; 0-, 4-, 8-, or 16- mg/kg) or the D_2 agonist quinpirole (QUIN; 0-, .3-, or 3.0- mg/kg) and tested for locomotor activity in photocell arenas for ten days. On day 11, all rats were tested with a 1.0 mg/kg dose of APO. Both SKF and QUIN depressed activity when first injected. This inhibition of activity persisted across sessions for SKF but progressively decreased with QUIN. Indeed, rats injected with QUIN were more active than vehicle control rats by day 10. When challenged with APO on day 11, rats pretreated with SKF were more active than control rats. Rats pretreated with QUIN were also more active than control rats, but these rats were less active after APO than they were after the last QUIN injection. These results suggest that although D_1 receptor stimulation may be sufficient to induce behavioral sensitization, D_2 receptors also play a role.

268.8

SELECTIVE SIGMA LIGANDS BLOCK STIMULANT EFFECTS OF COCAINE J. M. Witkin, M. Menkel, P. Terry, M. Pontecorvo, and J. L. Katz. Psychobiology Lab, NIDA Addiction Res. Center, Box 5180, Baltimore, MD 21224 and Nova Pharmaceutical Corporation, Baltimore, MD 21224

Compounds which bind to sigma receptors are being developed as novel antipsychotic agents which may be devoid of the side-effects associated with dopamine antagonist agents. We evaluated the ability of the sigma ligands BMY 14802 and NPC 16377 to block the locomotor stimulant effects of cocaine in male, SW mice. The effects of haloperidol and (+)-3PPP which have both dopamine and sigma receptor affinity, and the novel non-sigma antipsychotic clozapine were studied for comparison. When given alone, IP, the compounds produced dose-related decreases in activity with a rank order of potency of haloperidol > clozapine > (+)-3PPP > BMY 14802 > NPC 16377. Behaviorally-inactive doses of BMY 14802 (10 mg/kg) and NPC 16377 (40 mg/kg) decreased the locomotor stimulant effects of cocaine and shifted the dose-response curve to the right without reducing maximal effect. In contrast, behaviorally-active doses of haloperidol (0.1 mg/kg) or clozapine (1 mg/kg) were required to dampen the stimulant effects of cocaine; higher doses of (+)-3PPP were still ineffective. These results suggest that behaviorally-inactive doses of some sigma ligands may provide protection against the stimulant effects of cocaine.

268.9

EFFECTS OF INDIRECT DOPAMINE AGONISTS ON BRAIN STIMULATION REWARD THRESHOLD AT MULTIPLE CURRENTS C.S Maldonado-Irizarry, D. Garity*, J.R. Stellar
Dept. Psychology , Northeastern University , Boston , MA 02115.

The rate-frequency curve paradigm of measuring brain stimulation reward thresholds is well accepted in the literature. However, it has been recently discovered that MFB lesions produce different rate-frequency threshold shifts at multiple currents in the same subject. Stellar et al. (Neuroscience Abstracts,1990) showed that at low currents, LOR was dramatically increased by the lesion, but at higher currents this LOR shift was smaller. If true for pharmacology, this effect would require testing at multiple currents, and could undermine many past results. To address this issue, dopamine indirect agonist like GBR-12909 (5-20 mg/kg), cocaine (5-30mg/kg) , and amphetamine (1.0 mg/kg) were administered IP acutely to rats self-stimulating in the rate-frequency paradigm at multiple currents (200-501 uAmps). Results show no systematic variation in drug-induced threshold shift at different currents.
(supported by the Whitehall Foundation)

268.10

EFFECTS OF REPEATED COCAINE ADMINISTRATION ON INTRACRANIAL SELF-STIMULATION (ICSS) THRESHOLDS IN RATS. Athina Markou, Ilham Y. Polis and George .F. Koob.
Dept. of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

It has been hypothesized that human cocaine users tend to increase their cocaine dose due to the development of tolerance to cocaine's euphorigenic effects. The purpose of the present study was to investigate whether there is tolerance or sensitization to the rewarding effects of cocaine as measured by ICSS current-thresholds. Rats were injected (IP) with 10 mg/kg/8 hr for 10 consecutive days. Thresholds were assessed daily, 10 min after one of the 10 mg/kg injections. The results indicated the development of sensitization to the threshold lowering effect of cocaine. This finding suggests that repeated, intermittent administration of cocaine results in sensitization to the rewarding effects of cocaine. The influence of chronic self-administered (IV) cocaine on ICSS thresholds was also investigated. The effect of self-administered cocaine on thresholds was dose-dependent. Low doses of cocaine (i.e., short intravenous self-administration sessions) lowered thresholds, reflecting cocaine's rewarding effect. However, higher doses of cocaine (i.e., longer self-administration sessions) raised thresholds. These higher thresholds may reflect the post-cocaine anhedonia experienced by human cocaine users during cocaine withdrawal.
Supported by awards DA05365 to AM and DA04398 to GFK.

268.11

THE EFFECT OF A MIXED $D_2/5\text{-}HT_2$ ANTAGONIST, MDL 28133, ON COCAINE-INDUCED DECREASES IN BRAIN STIMULATION REWARD THRESHOLDS. S. Panicker, P. Z. Manderscheid, R. A. Frank, J.H. Kehne, C. J. Schmidt & S. M. Sorensen, Dept. of Psychology, Univ. of Cincinnati, Cincinnati, OH 45221 & Marion Merrell Dow Research Inst., Cincinnati, OH 45215.

Recent neurochemical & electrophysiological studies have shown that the mixed $D_2/5\text{-}HT_2$ antagonist MDL 28,133 can modulate stimulant-induced changes in central dopaminergic neurotransmission. The ability of this compound to reverse cocaine-induced decreases in intracranial self-stimulation thresholds was assessed in the present experiment. Cocaine-induced shifts in thresholds were evaluated following 5, 15 and 30 mg/kg cocaine HCl (IP) in ten rats with ventral tegmental area electrodes. The magnitude of cocaine's effects was also assessed following pretreatment (IP) with 5 and 10 mg/kg MDL 28,133. The effects of cocaine and MDL 28,133 were mutually antagonistic, as might have been predicted for the interaction of a neuroleptic and psychomotor stimulant drug. This research was supported by NIDA grant DA04483 to R. A. Frank.

268.12

WITHDRAWAL FOLLOWING COCAINE SELF-ADMINISTRATION DECREASES REGIONAL METABOLISM IN CRITICAL BRAIN REWARD REGIONS. R.P. Hammer, Jr., W.S. Pires*, D.W. Clow, A. Markou, and G.F. Koob, Dept. Anatomy & Reprod. Biol, Univ. Hawaii Sch. Med., Honolulu, HI 96822, and Dept. Neuropharm., Res. Inst. of Scripps Clinic, La Jolla, CA 92037.

Psychostimulant withdrawal produces biological changes in brain activity which are reflected in elevated intracranial self-stimulation thresholds and reduced local cerebral glucose utilization (lCGU) in the basal forebrain. LCGU was examined at 30 hr after a 3 hr cocaine self-administration session, at either 6 or 72 hr after a 12 hr session, or in drug-naive rats. LCGU was significantly reduced following cocaine self-administration compared to drug-naive animals in the nucleus accumbens (–21-32%), olfactory tubercle (–14-31%), rostral striatum (–15-26%), lateral hypothalamus (–14-22%), amygdala (–16-22%), substantia nigra (–13-17%), ventral tegmental area (–12-15%) and several cortical and brain stem regions. Moreover, a main effect of time was observed in many brain regions, suggesting that cocaine withdrawal produced a sustained reduction of lCGU which is greater at 72 than at 6 hr post-drug. The results indicate that drug experience produces significant metabolic changes even in the absence of a 12 hr drug "binge." In addition, the amount of drug self-administered during the 12 hr session was significantly correlated with lCGU in medial prefrontal cortex, olfactory tubercle, substantia nigra and locus coeruleus at 6, but not 72 hr post-drug. Reduction of regional metabolic activity may represent a biological substrate for the motivational aspects of the cocaine withdrawal syndrome. Supported by USPHS awards DA06645 and HD01161 to RPH, DA05365 to AM, and DA04398 to GFK.

268.13

AN ANIMAL MODEL OF COCAINE WITHDRAWAL USING BRAIN STIMULATION REWARD. Michael A. Bozarth & Cindy M. Pudiak.
Department of Psychology, University at Buffalo, Buffalo, NY 14260.

An animal model of cocaine withdrawal was developed based on studying the function of the brain system mediating cocaine reinforcement. Measurement of reinforcement from electrical activation of this system was used to examine changes in the system's functional activity following the termination of daily cocaine injections. Male, Long-Evans rats were implanted with monopolar electrodes aimed at the lateral hypothalamic level of the medial forebrain bundle. After 7 days recovery from surgery, rats were trained (during daily 60-min test sessions) to press for monophasic cathodal stimulation pulses using the threshold tracking method.

Subjects were administered daily cocaine injections (40 mg/kg, i.p.) for one week, and their brain stimulation reward thresholds pre- and post-treatment were compared. A marked elevation of brain stimulation reward thresholds was seen following the cocaine injection series. This threshold elevation persisted for 3 days with thresholds gradually returning to pretreatment levels. The decreased efficacy of electrical brain stimulation (demonstrated by an increase in the minimum stimulation frequency necessary to maintain responding) probably corresponds to the diminished dopamine levels seen following intravenous cocaine self-administration. It is also likely that diminished functioning of this important brain reward system may produce subjective experiences of anhedonia, depression, and drug craving in human cocaine users.

268.14

THE VENTRAL STRIATOPALLIDAL SYSTEM AND CONDITIONED REINFORCEMENT IN RATS. B.J. Everitt, G. Phillips*, J. Le Noury*, T.W. Robbins[1], G. Wolterink* & J. Wolterink*. Depts Anatomy & [1]Experimental Psychology, Cambridge University, Cambridge CB2 3DY, U.K.

We have investigated the interaction between cholecystokinin (CCK) and dopamine (DA) in the ventral striatum (VS), as well as the effects of excitotoxic lesions to the ventral pallidum (VP) and cholinergic nucleus basalis (nbm), on the control over behaviour by conditioned reinforcement (CR). In the training phase, rats received pairings of a light/noise compound stimulus, and 10% sucrose. In the test phase, in the absence of sucrose, a response on one of two novel levers produced the CR, but on the other had no programmed consequences.

Intra-accumbens infusion of d-amphetamine (AMPH) enhanced responding selectively on the CR lever. Co-infusion of CCK8 potentiated the impact of AMPH, and this potentiation was blocked by the CCK-A antagonist, devazepide.

Lesions of the VP induced by ibotenic acid completely prevented the acquisition of a new response with CR and also the effects of intra-accumbens AMPH, but did not affect consumption of sucrose. These lesions were associated with 25% reductions in cortical cholinergic markers. Lesions of the nbm induced by AMPA reduced cortical cholinergic markers by >65%, but spared many VP neurons. These lesions caused a small impairment in the acquisition of a new response with CR independently of the effects of intra-accumbens AMPH. The results will be discussed in terms of the modulation by DA/CCK8 of limbic afferents to the VS and the involvement of VP and/or nbm cholinergic targets of VS outflow in reward-related processes.

268.15

COMPARISON OF THE EFFECTS OF AMPHETAMINE WITH NOMIFENSINE ON A BRAIN SELF-STIMULATION REINFORCEMENT THRESHOLD TASK IN RATS. G.J. Schaefer and R.P. Michael, Department of Psychiatry, Emory University, School of Medicine, Georgia Mental Health Institute, 1256 Briarcliff Rd., Atlanta, GA 30306.

Rats (N=12) were implanted with stimulating electrodes in the lateral hypothalamus and trained in the auto-titration brain self-stimulation (ICSS) procedure. After reinforcement thresholds stabilized, animals were administered saline, d-amphetamine (0.1, 0.3, 1.0 mg/kg) or nomifensine (1.0, 3.0, 10 mg/kg) and tested in the ICSS procedure. Amphetamine produced a graded decrease in reinforcement thresholds to approximately 30% at the highest dose ($p < 0.01$). All three doses, increased responding by about 25% above baseline levels ($p < 0.01$). Nomifensine also produced a graded decrease in reinforcement thresholds, but the slope was steeper and, at the highest dose, there was a 70% decrease in thresholds ($p < 0.01$). Nomifensine produced no significant increase in rates but, at the highest dose, rates declined to 35% below baseline ($p < 0.01$). We have previously shown that amphetamine lowers reinforcement thresholds in the auto-titration procedure, and we now extend this to a second stimulant, nomifensine. While both drugs lowered the reinforcement threshold, they had opposite effects on rates of responding under these conditions. This provided further evidence that in the auto-titration procedure changes in reinforcement threshold can occur independently of changes in response rates. (Supported by the Georgia Department of Human Resources.)

ANTIPSYCHOTICS I

269.1

TYPICAL VERSUS ATYPICAL NEUROLEPTICS: DIFFERENCES IN REGIONAL CEREBRAL GLUCOSE UTILIZATION IN RATS. N.G. Cascella*, F.I. Tarazi, O. Shirakawa, C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228

Typical neuroleptics, like haloperidol, are potent antidopaminergic antipsychotic agents. Recently, new atypical neuroleptics have been developed with low extrapyramidal side effects and unique antipsychotic actions. An understanding of the biologic basis of atypical drug action may be informative about the nature of psychosis and may encourage new drug development. We have used regional cerebral glucose metabolism (rCMRglu) to functionally localize the action of several neuroleptic drugs, typical and atypical. Haloperidol (H) (1.0mg/kg), raclopride(R) (1.5mg/kg), and savoxepine (S) (0.005 mg/kg and 0.5mg/kg) were administered individually to rats just prior to 14C-2DG injection. rCMRglu was measured quantitatively using 2DG autoradiography technique. (H) reduced rCMRglu in neocortical, limbic and extrapyramidal systems. (R) reduced rCMRglu only in limbic and neocortical areas but not in extrapyramidal regions. This suggests that raclopride has little functional effects in motor regions of brain, while exerting a haloperidol-like effects in areas traditionally linked to cognitive and affective behaviors. At a low dose (0.005mg/kg) selective for hippocampal D2 receptors, (S) was found to have functional effects in all three cerebral systems, limbic, neocortex and extrapyramidal. (S) produced (H)-like changes in striatum at doses too low to block postsynaptic D2 striatal receptors, but at doses which nonetheless elevated HVA and DOPAC, two major metabolites of DA in rat striatum (Baumann,P.A. et al. Psychopharmacology 96,S-339. 1988). This is consistent with an action of (S) as a D2 autoreceptor antagonist.

269.2

DIFFERENTIAL EFFECTS OF CLOZAPINE AND HALOPERIDOL ON LIMBIC SYSTEM KINDLING. S. Reeves*, T.B. Borowski*, and L. Kokkinidis. Dept. of Psychology, Univ. of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0.

Kindled seizure activity elicited by repeated intermittent electrical stimulation of limbic structures has shed considerable light on the mechanisms involved in neuronal plasticity and sensitization. Given that limbic system dysfunction is thought to play a significant role in clinical psychopathology, we investigated the effects of clozapine (10.0 mg/kg) and haloperidol (1.0 mg/kg) on the evolution of kindled seizures after stimulation of the central nucleus of the amygdala, and the ventral dentate gyrus of the hippocampus. Neither drug affected the minimum current intensity necessary to elicit an afterdischarge from these limbic structures. However, administration of clozapine 30 min prior to each daily electrical stimulation of the amygdala and ventral hippocampus substantially retarded the development of kindled seizures. Haloperidol treatment did not influence epileptogenesis from either limbic site. Subsequent experiments evaluated the function of the anticholinergic effects of clozapine on its seizure suppressing properties. It was found that pilocarpine administration in doses that did not facilitate kindling genesis (20.0 and 40.0 mg/kg), partially antagonized the clozapine-elicited inhibition of amygdaloid kindling. Injection of this ACh muscarinic receptor agonist together with clozapine induced some catalepsy indicating a synergism on ACh activity. These results suggest that the clinical efficacy of clozapine might involve specific effects on limbic system functioning not shared by the typical neuroleptics.

Supported by the National Sciences and Engineering Research Council of Canada.

269.3

RITANSERIN AND HALOPERIDOL FAIL TO SUBSTITUTE IN CLOZAPINE-TRAINED RATS. J.L. Wiley and J.H. Porter. Department of Psychology, Virginia Commonwealth University, Richmond, VA 23284.

Fifteen naive adult male Sprague-Dawley rats (85% BW) were trained to discriminate clozapine (5.0 mg/kg, i.p.) from vehicle in a two-lever drug discrimination procedure under a FR30 schedule of food reinforcement. Daily session duration was 15 minutes. Following testing for acquisition of the discriminative cue, stimulus generalization tests with clozapine (0, 1.25, 2.5, 5.0, 10.0, & 20.0 mg/kg), haloperidol (0, 0.015, 0.03, & 0.10 mg/kg), and ritanserin (0, 2.5, 5.0, 10.0, & 15.0 mg/kg) were conducted.

Percent drug-lever responding and response rate were calculated for each dose of the test drugs. Linear regression on the linear portion of the clozapine dose-effect curve (X = log10 transformation of the dose and Y = % drug-lever responding) revealed that clozapine generalized to the training dose (5 mg/kg) in a dose-dependent manner (ED50 = 1.99 mg/kg). The D2 antagonist, haloperidol, and the 5-HT2 antagonist, ritanserin, failed to substitute for clozapine at any of the doses tested. These results suggest that the clozapine-cue is not mediated by D2 or 5-HT2 receptors.

269.4

FOS IS INDUCED BY THE ANTIPSYCHOTIC DRUG CLOZAPINE IN THE RAT STRIATUM N. Hiroi[1], H. A. Robertson[2] and A. M. Graybiel[1]. [1]Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139, and [2]Dept. of Pharmacology, Dalhousie Univ., N.S., Canada B3H 4H7.

The atypical neuroleptic clozapine (CLZ) is a unique antipsychotic drug in that, unlike classical neuroleptics, it alleviates schizophrenic symptoms without causing extrapyramidal side effects. We have investigated the effect of CLZ on Fos-like immunoreactivity (Fos) by immunohistochemistry. Rats (n=4 for each group) were treated with vehicle or CLZ (5.0, 10.0, 20.0, and 40.0 mg/kg, i.p.), and patterns of Fos induction were studied in relation to immunostaining for calbindin D28k (calbindin), a marker for the extrastriosomal matrix and the core of the nucleus accumbens (NA). Fos was induced in the caudoputamen (CP) and NA of CLZ-treated animals in a dose-dependent manner. At 10 mg/kg, Fos was induced in the medial CP and medial NA anteriorly; caudally, the CP and NA were devoid of Fos. As doses of CLZ increased (20 and 40 mg/kg), Fos induction spread laterally in the anterior CP and, in the middle CP, extended dorsally from medial to lateral in a curving marginal band. Interestingly, Fos induction in the center of the CP tended to be clustered in striosomes, identified as calbindin-poor zones. More caudally, Fos induction was mainly distributed in the dorsomedial corner of the CP. In the NA, abundant Fos was present in the rostral pole, and more caudally, Fos was mainly confined to the shell of the NA; both zones are calbindin-poor. Thus, there was an inverse relation between Fos induction and calbindin immunoreactivity in the NA and, to some extent, in the CP. Fos was also induced, *inter alia*, in parts of the prefrontal cortex, septum, VTA and medial substantia nigra pars compacta. (Supported by the Human Frontier Science Program and Javits Award NS 25529).

269.5

EFFECTS OF AMFONELIC ACID AND GBR 12909 ON THE CLOZAPINE- AND HALOPERIDOL-INDUCED STIMULATION OF STRIATAL DOPAMINE SYNTHESIS, METABOLISM AND RELEASE. G.A. Gudelsky, E. Nwajei and J.F. Nash, Dept. of Psychiatry, Case Western Reserve Univ., Cleveland, OH

It has been shown that amfonelic acid (AFA) potentiates the increase in striatal DOPAC concentrations induced by haloperidol (HLD), but attenuates that induced by clozapine (CLZ). In the present study, the stimulation of dopamine (DA) synthesis, metabolism and release induced by HLD and CLZ were examined in rats treated with AFA or the dopamine uptake inhibitor GBR 12909. DA metabolism was assessed from the concentrations of DOPAC in post mortem tissue. DA synthesis was evaluated from the in vivo accumulation of DOPA after decarboxylase inhibition. DA release was assessed by quantitation of its extracellular concentration using in vivo microdialysis. Whereas treatment with AFA potentiated the HLD-induced increase in DOPAC in the striatum and n. accumbens, the CLZ-induced increase was markedly attenuated. The CLZ-induced increase in striatal DOPAC also was prevented by treatment with GBR 12909. Although AFA did not alter the HLD-induced increase in DOPA accumulation in the striatum or n. accumbens, it attenuated the CLZ-induced increase in both brain regions. In contrast, the CLZ-induced increase in DA synthesis in the median eminence was not altered by AFA. Both HLD and CLZ increased the extracellular concentration of DA and DOPAC in the striatum. Results from the microdialysis studies with AFA were consistent with those from the post mortem experiments. It is suggested that inadequate blockade of DA autoreceptors by CLZ may account for the suppression of CLZ-stimulated DA synthesis and metabolism by DA reuptake blockers.

269.6

EFFECT OF AMPEROZIDE UPON DOPAMINE (DA) RELEASE IN VITRO FROM THE CORPUS STRIATUM (CS) & OLFACTORY TUBERCLE (OT) OF MALE RATS. J.L. McDermott and H.Y. Meltzer, Depts. of Geriatrics and Psychiatry, Case Western Reserve University, Cleveland, Ohio 44106

Amperozide, a novel atypical antipsychotic agent, has been shown to block uptake and increase basal release of [^{3}H] DA both in striatal and limbic rat tissue (Eriksson and Christensson, Pharmacol & Toxicol. Supp. 1:45, 1990). Utilizing in vitro superfusion the effect of direct infusion of 10 uM amperozide upon endogenous DA release from CS tissue slices was examined. DA release was significantly increased in response to amperozide infusion (areas under stimulated curves = 26.5 ± 6.4 ng/40 min, N=7) as compared to Krebs-Ringer-Phosphate (KRP) superfused control CS (AUC 8.1 ± 2.5 ng/40 min, N=6). We next examined amphetamine (AMPH - 10 uM)-stimulated DA release from the CS and OT in the presence or absence of 10 uM amperozide in KRP. In the presence of amperozide, AMPH-stimulated DA release from the CS was significantly ($P < .05$) decreased (AUC 164 ± 35.6, N=9 vs 313.7 ± 53.4 pg/40 min, N=8) whereas there was no effect in the OT (AUC 260.9 ± 59.3, N=7 vs 231.5 ± 76.3, N=6). These results are, in main, consistent with the results of in vivo microdialysis techniques (Ichikawa and Meltzer, submitted). However, amperozide did inhibit amphetamine-stimulated DA release in the nucleus accumbens using in vivo microdialysis. Further study of possible differences of the effect of amperozide on amphetamine-stimulated DA release in different limbic areas is required.

269.7

EVALUATING THE PAW TEST AS A PARADIGM FOR DIFFERENTIATING BETWEEN CLASSICAL AND ATYPICAL NEUROLEPTICS. A. T. Shropshire and K.L. Marquis. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

The paw test, originally developed as a motor assay (Vrijmoed-deVries *et al.* Psychopharmacology 92:73-77, 1987), was proposed by Ellenbroek *et al.* (Psychopharmacology 93:343-348, 1987) as a behavioral paradigm for differentiating between classical and atypical neuroleptics. While classical neuroleptics were reported to be equipotent in prolonging both the forelimb (FRT) and hindlimb retraction time (HRT) in rats, atypical neuroleptics were more potent in prolonging HRT than FRT. These data suggested that FRT may reflect parkinsonian side-effect potential with classical neuroleptics, while HRT predicts the antipsychotic effect of both classical and atypical neuroleptics. In the present study, we attempted to replicate these data using the paw test to differentiate between classical and atypical neuroleptics. Moreover, we determined whether drugs which produced false positives in the conditioned avoidance test also appeared as false positives in the paw test.

The classical neuroleptics, haloperidol (.1, .5, 1 & 5 mg/kg ip) and chlorpromazine (1, 2.5, 5, 7.5 & 10 mg/kg ip) significantly prolonged HRT and only marginally affected FRT. The atypical neuroleptics, thioridazine (7.5, 15, 30 & 60 mg/kg ip), clozapine (1, 10, 20 & 50 mg/kg ip) and gevotroline (5, 10 & 20 mg/kg ip), significantly prolonged HRT and had no effect on FRT. Other drugs which produced false positives, B-HT 920 (5, 10 & 20 mg/kg ip), clonidine (.06, .125 & .25 mg/kg ip) and prazosin (10 mg/kg ip), in the conditioned avoidance test, also appeared as false positives in the rat paw test. These results failed to support the conclusions drawn by Ellenbroek *et al.* concerning the utility of this test. Moreover, using HRT as an index of antipsychotic activity, the paw test offers no distinct advantage over the conditioned avoidance test which is highly predictive of clinical efficacy in this therapeutic area.

269.8

DOES THE ATYPICAL ANTIPSYCHOTIC CLOZAPINE BLOCK THE REWARDING PROPERTIES OF AMPHETAMINE? D.C. Hoffman and H. Donovan. Neurogen Corp., Branford, CT 06405.

It has been suggested that the antipsychotic efficacy of clozapine is due to it's blockade of dopamine (DA) receptors within the mesocorticolimbic system. Thus, one might expect clozapine to be effective in blocking the rewarding properties of amphetamine which are mediated by an increase in DA levels within the nucleus accumbens, one target area of the mesocorticolimbic DA neurons. The present study investigated this prediction using the conditioned place preference paradigm. Male Sprague-Dawley rats received amphetamine (2.0 mg/kg IP) paired with one side of a two-compartment box and saline paired with the other compartment. During these pairings, locomotor activity was measured by photocell beam breaks. On the test day, the amount of time drug-free rats spent in each compartment was measured. Rats trained with amphetamine alone showed a significant increase in time spent on the drug-paired side from pre- to post-conditioning, indicating a place preference. When rats were injected with clozapine (1, 5, 10 mg/kg SC) 30 min prior to the amphetamine pairings, a dose-dependent decrease in locomotion was observed, but only the highest dose of clozapine blocked the establishment of place conditioning. The typical antipsychotic haloperidol prevented place conditioning at a dose of 0.1 mg/kg. These data suggest that acute injections of clozapine may not possess potent DA antagonist actions within the nucleus accumbens.

269.9

EFFECTS OF CLOZAPINE ON GLUTAMATERGIC TRANSMISSION. T.I.Lidsky, E. Alter, S.P.Banerjee. Inst. for Basic Research, Staten Island, NY 10314

Clozapine (C), an atypical antipsychotic, differs from conventional neuroleptics in having no appreciable liability for significant extrapyramidal side effects including tardive dyskinesia. The present study is the first in a series from this laboratory investigating the bases of C's unique action.

Both C and haloperidol (H) attenuate glutamatergically mediated electrophysiological responses in the striatum. H's action is dependent on the presence of dopamine: lesions of the nigrostriatal tract abolish H's inhibitory effect. In contrast, C continues to attenuate striatal responses after nigrostriatal lesions. To evaluate the possibility of a more direct influence, C and H were compared with regard to displacement of [^{3}H] MK-801 binding to glutamate receptors in the striatum. C was approximately 3x more effective than H in this regard; at clinical doses, C would be about 600x more potent. Taken together, the electrophysiological and binding data indicate that C is an effective glutamate blocker.

269.10

REGIONALLY SELECTIVE EFFECT OF CHRONIC CLOZAPINE ON EXTRACELLULAR DOPAMINE LEVELS. K.D. Youngren, R.H. Roth, B.S. Bunney, and B. Moghaddam. Neuroscience Program and Depts. of Pharmacology and Psychiatry, Yale Univ. School of Medicine, New Haven, CT 06510, and West Haven VA Medical Center, West Haven, CT 06516.

Previous studies in this laboratory have suggested that preferential activation of dopamine (DA) release in the medial prefrontal cortex (mPFC) occurs following acute clozapine administration. In order to investigate this preferential effect of clozapine on the mesocortical DA system further, microdialysis was used to measure the extracellular level of DA after chronic administration of clozapine. Dialysis measurements were performed in mPFC, striatum, and nucleus accumbens of the rat. Chronic clozapine administration (20 mg/kg in drinking water) had no effect on basal levels of DA in striatum. However, it did produce a significant increase in the basal levels of DA in mPFC in comparison to control. Upon injection of a challenge dose (20 mg/kg i.p.) of clozapine, a significant tolerance effect in mPFC was observed, while no tolerance was seen in striatum. Study of the effect of chronic clozapine on DA release in nucleus accumbens is ongoing. These results provide additional support to the hypothesis that the clinical profile of atypical antipsychotic drugs like clozapine may relate to a regional specificity of action.

Supported in part by PHS grants MH-28849, MH-14092.

269.11

THE EFFECTS OF THE PUTATIVE ATYPICAL ANTIPSYCHOTIC AMPEROZIDE ON EXTRACELLULAR DOPAMINE LEVELS IN THE RAT MEDIAL PREFRONTAL CORTEX. E.A. Pehek, H.Y. Meltzer and B.K. Yamamoto. Dept. of Psychiatry, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Others have shown that the atypical neuroleptic clozapine, but not the typical neuroleptic haloperidol, has a greater effect on extracellular dopamine (DA) in the medial prefrontal cortex (MPFC) relative to the caudate-putamen (CP). The present study compared these effects to those of amperozide, a novel agent that may have antipsychotic efficacy with few extrapyramidal side effects. *In vivo* microdialysis coupled with HPLC/EC was used to measure DA and its metabolite DOPAC in the freely-moving rat. Each rat was implanted with a guide cannula aimed at the MPFC. Two days later, a 4 mm concentrically designed probe was inserted through this cannula into the MPFC. A modified Ringer's medium was perfused through the system at a rate of 2.0 ul/minute. Samples were collected every 30 min and immediately injected onto the HPLC for analysis by electrochemical detection. Amperozide (10 mg/kg) increased extracellular levels of DA and DOPAC. These effects were greater in the MPFC (mean DA = 551.71% of basal levels) relative to the CP (mean DA = 202.68%). In contrast, administration of haloperidol (0.5 mg/kg) produced a smaller increase in cortical DA (mean = 169.04%) that was not greater than that observed in the CP (mean = 225.93%). These results suggest that a common property of atypical antipsychotics may be their ability to preferentially increase cortical DA.

269.12

RATIO OF POTENCY OF ANTIPSYCHOTIC DRUGS (APD) IN DISPLACING DA-2 AND 5HT-2 RECEPTOR BINDING IN VIVO DISCRIMINATES BETWEEN TYPICAL AND ATYPICAL ANTIPSYCHOTIC DRUGS. C.A. Stockmeier, J.J. DiCarlo*, Y. Zhang* and H.Y. Meltzer. Depts. of Psychiatry, Neuroscience and Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

Atypical APD, as opposed to typical APD, have higher in vitro ratios of the pK_i values for 5HT-2 binding in cortex divided by the pK_i values for DA-2 binding in striatum (JPET 254:238, 1989). We sought to determine if this in vitro distinction between atypical and typical APD could also be obtained in vivo in rat brain. Rats were pre-treated with increasing doses of APD followed by ^{3}H-n-methyl-spiperone (^{3}H-NMSP), and killed 60 min later. Cinanserin, cyproheptadine and ritanserin, but not pindolol, blocked in vivo binding only in frontal cortex; raclopride potently blocked in vivo binding only in striatum and olfactory tubercle. The putative atypical APD clozapine, melperone, amperozide, tiospirone, HP 370, fluperlapine, risperidone, perlapine, FG 5803 and RMI 81,582 were all more potent against 5HT-2 than DA-2 binding sites. Atypical APD were slightly but significantly more potent at olfactory tubercle than striatal DA-2 receptors. The ratios of 5HT-2 (cortex)/DA-2 (striatum or olfactory tubercle) pED_{50} values were significantly greater for atypical than typical APD. It appears that the ratio of the potency at 5HT-2 to DA-2 binding sites rather than the absolute potency is crucial in a drug being classified as an atypical APD. Supported by MH 41684 and Laureate Foundation/NARSAD grants.

269.13

CLOZAPINE DISSOCIATES COGNITION AND PSYCHIATRIC SYMPTOMS IN PATIENTS WITH SCHIZOPHRENIA. T.E. Goldberg, R.D. Greenberg*, S.J. Griffin*, J.M. Gold*, J.E. Kleinman, D. Pickar*, S.C. Schulz*, D.R. Weinberger. Clinical Brain Disorders Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

Neuropsychological deficits in schizophrenia, while frequently reported, are often thought to be an epiphenomenon of psychopathology and as such not a valid indicator of reduced cognitive capacities. In the present study, we attempted to "dissect" psychiatric symptoms and cognition in schizophrenia with the atypical neuroleptic clozapine. Assessments of psychiatric symptoms and cognition were made in 14 patients with schizophrenia and one patient with psychosis not otherwise specified while they received a conventional neuroleptic medication and then again after an average of 15 months treatment with clozapine. Despite impressive improvements in psychiatric symptoms, cognitive function in such areas as attention, memory, and higher level problem solving remained impaired and essentially unchanged. This suggests that certain cognitive deficits are relatively independent of psychotic symptoms in schizophrenia and are probably central and enduring features of the disorder. In fact, cognitive disability appeared to have been rate limiting in the sample's rehabilitation as patients' social and vocational adjustment remained marginal during the course of the study. We also observed that clozapine treatment was associated with a decline in some memory functions; the potent anticholinergic properties of the drug may have been responsible.

269.14

THE EFFECT OF AMPEROZIDE (AMP) ON BASAL AND AMPHETAMINE-(AMPHET)-STIMULATED DOPAMINE (DA) RELEASE IN RAT STRIATUM (STR) AND NUCLEUS ACCUMBENS (NA) J. Ichikawa* and H.Y. Meltzer, Dept. of Psychiatry, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

Amperozide, a piperazine carboxamide, is a putative atypical antipsychotic drug with strong serotonin ($5\text{-HT})_2$ and no apparent DA_2 or DA_1 antagonist properties *in vivo*. It is a potent DA uptake blocker. Its ability to modulate the availability of DA *in vivo* was studied by means of *in vivo* microdialysis using awake, freely-moving rats. AMP (2.5-10 mg/kg, s.c.) significantly increased extracellular DA levels in both regions (maximum levels 145% of basal value), whereas AMP (1-5 mg/kg, s.c.) dose-dependently decreased D-AMPHET- (1.0 mg/kg, s.c.) -induced DA release (maximum increase 43-fold) in both regions. AMP alone had only a slight effect on extracelleular DOPAC levels. AMP had no effect on the AMPHET-induced decrease (70% of basal) DOPAC levels in either region. The results suggest that amperozide acts on a carrier-mediated mechanism to block the effect of AMPHET on DA release. Chronic administration of AMP (2 mg/kg/day) in drinking water for 21 days followed by a 3 day washout increased basal extracellular DA in the STR but decreased it in the NA. It enhanced the D-AMPHET-induced DA release in the STR but inhibited it in the NA. The effect of AMP to inhibit DA release in the NA may be related to its antipsychotic action.

269.15

REGULATION OF CELLULAR IMMEDIATE-EARLY GENES BY TYPICAL AND ATYPICAL ANTIPSYCHOTIC DRUGS. B. M. Cohen, T.V. Nguyen,* R. Birnbaum* & S. E. Hyman, Mailman Research Center, McLean Hospital, Belmont, MA 02178, and Massachusetts General Hospital, Boston, MA 02214

Cellular immediate-early genes (IEG's) have been studied as sensitive indicators of cellular activation and as potential third messengers involved in long-term changes in neural function. The slow onset of therapeutic effectiveness of antipsychotic drugs suggests that slowly developing neural plasticity, perhaps involving changes in gene expression, is critical to their mechanism of action. We report that, as determined by Northern analysis, expression of mRNA for the cellular IEG's *c-fos* and *zif/268* was increased 2-4 fold in rat striatum after a single dose of the typical antipsychotic drug, haloperidol, or the psychostimulant, amphetamine. These effects were rapid, transient, and dose-dependent. In contrast, the atypical antipsychotic drug, clozapine, induced *zif/268* mRNA but not *c-fos*. With chronic (2 weeks) administration the IEG responses to haloperidol, clozapine, and amphetamine were partly desensitized. The apparent paradox that both a dopamine antagonist (haloperidol) and an indirect agonist (amphetamine) activated striatal *c-fos* expression prompted us to perform an immunohistochemical analysis with an antibody specific for *c-fos* protein. This revealed marked differences in the pattern of *c-fos* expression. A single dose of haloperidol preferentially induced expression in cells in the dorsolateral striatum while amphetamine increased expression in cells in the ventro-medial region.

269.16

COMPARISON OF THE EFFECTS OF STANDARD NEUROLEPTICS ON THE REFLEX RESPONSES TO BILATERAL CAROTID OCCLUSION: A MODEL OF ORTHOSTATIC STRESS. C.M. Detig*, S.E. Aschoff*, D.M. Howard*, J.W. Hubbard, P.L. Wood, A.J. Gorman*. Department of Biological Research, Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ, 08876

It has been clinically recognized that a prominent side-effect of neuroleptic treatment is orthostatic hypotension due to an inhibition of compensatory circulatory mechanisms. The present study utilizes a chloralose-anesthetized dog model of orthostatic stress and compares the relative potency of risperidone (RIS), clozapine (CLOZ), and haloperidol (HAL) on cardiovascular reflex responses to bilateral carotid occlusion (BCO). Each agent was administered intraduodenally at 1x and 4-5x the oral ED_{50} value for inhibition of pole climb avoidance in rats. RIS (0.5 mg/kg; 1x ED_{50}) attenuated reflex increases in mean arterial pressure (MAP), total peripheral resistance (TPR), and cardiac output (CO) by -84%, -72%, and -100%, respectively. At 5x ED_{50}, CLOZ (60 mg/kg) elicited similar inhibitory effects on reflex responses to BCO (MAP: -66%; TPR: -73%; CO: -100%). In fact, CO decreased by 48% and 44% during BCO after RIS and CLOZ, respectively. Less of an inhibitory influence on BCO-mediated responses was exhibited by HAL at 2 mg/kg, 4x ED_{50} (MAP: -34%; TPR: -65%; CO: -83%). Therefore, the relative potency of the standard neuroleptics for inhibiting circulatory reflex responses to BCO is RIS > CLOZ > HAL. This model may be useful for preclinical assessment of the relative potential of neuroleptic agents to produce orthostatic hypotension.

270.1

THE REVERSAL OF EXTRAPYRAMIDAL SIDE EFFECTS (EPS) WITH SCH 39166, A D1 ANTAGONIST, IN HALOPERIDOL-SENSITIZED MONKEYS. D.T. McHugh*, A. Barnett and V.L. Coffin. Schering-Plough Research, Bloomfield, NJ, 07003.

Cebus monkeys (female n=5), with a history of haloperidol administration (daily for 28 wks), produced EPS when given either haloperidol or SCH 39166 acutely. EPS was characterized by limb extensions, facial grimacing, chewing and/or tongue protrusions. In agreement with prior work these monkeys are sensitized. This sensitization does not fade with time. Even after 100 drug free days, both dopamine antagonists given acutely still could produce EPS in these monkeys. However, if SCH 39166 is given repeatedly, the EPS observed lessened and then completely disappeared within 5 days. The sedation observed with SCH 39166 was still present after repeated administration, thus tolerance did not develop to this central effect.

Cebus monkeys (female n=6) with a history of SCH 39166 administration (daily for 28 wks.), never produced EPS with SCH 39166, Furthermore, SCH 39166 did not sensitize the monkeys to the effects of haloperidol on EPS. SCH 39166 treated monkeys showed significant sedation compared to vehicle treated monkey (n=6) throughout the 28 weeks.

Thus these studies further emphasize the differences between D1 and D2 antagonists with respect to EPS liability.

270.2

ENHANCED PROLACTIN ELEVATION WITH CHRONIC LOW-DOSE HALOPERIDOL IN THE RAT. H.I.Ryer, J.Woo* and M.R.Lynch, Dept. Psychiat. NYU, VAMC, New York, NY 10010 and Depts. Psychiat. and Pathol., SUNY HSC, VAMC, Syracuse, NY 13210

Typical neuroleptics such as haloperidol (H) are antipsychotic by virtue of central D2 receptor blockade. TIDA DA exerts tonic inhibitory regulation over prolactin (PRL) secretion. In the absence of direct receptor measurements, PRL has been used as an index of central DA antagonism. The clinical significance of this measure remains controversial; PRL response may be correlated with clinical variables in certain schizophrenic subpopulations. Moreover, there are reports of both tolerance to PRL elevation and a lack thereof. More dorsal A9 and A10 areas show an enhanced response to chronic H, mimicking delayed-onset clinical effects. The question addressed here was whether PRL elevation shows tolerance or sensitization to chronic H in a therapeutic dose (0.1 mg/kg). N=6-8/group male rats received daily injections of H or vehicle for 21 days. A separate group of N=5 received acute H 90-min prior to PRL assay with a double antibody RIA (NIDDK-r-PRL-RP-3). Acute H increased PRL from 14.9±3.4 to 28.3 ±3.0 ($\bar{X}$±SEM,$p>.05$); the final dose in chronic H animals (day 21) induced an almost two-fold greater stimulation (49.8±6.2,$p<.01$) which returned to baseline at 48 hr withdrawal. As challenge with 0.07 mg/kg apomorphine failed to reveal evidence for upregulation, the mechanism for this enhanced response remains to be determined.

270.3

NEUROLEPTIC-INDUCED STRIATAL NEURON LOSS: RELATIONSHIP TO AGE. JB Lohr, J Browning*, and DV Jeste. Department of Psychiatry, UCSD, and Psychiatry Service, VA Medical Center, V-116A, San Diego CA 92161.

Tardive dyskinesia (TD) is a potentially irreversible movement disorder caused by long-term use of neuroleptics. We have previously demonstrated that there is a loss of large neurons in the striatum after 8 months of neuroleptic treatment in rats. Because advanced age is a well-established risk factor for the development of TD, we studied whether age is also a risk factor for striatal neuron loss in rats treated long-term with neuroleptics. Methods: We administered fluphenazine decanoate 5 mg/kg or sesame oil every two weeks for four months to three groups of animals; aged 3 months (n=12), 9 months (n=10), and 18 months (n=10). The brains were removed and fixed in formalin and 12 micron thick sections were stained with cresyl violet. Neuron density was calculated in the central striatum using an IBAS 2000 image analysis system. Results: There was a loss of both medium-sized and large neurons with age in both treated and control animals, although the loss was greater in the large cells. With fluphenazine treatment there was a significant decrease in large cell density ($t=2.37$, $df=8$, $p<0.05$) in the 9 month old group only. In the 18 month old group, there was no difference between treated and control animals, but there were very few large neurons in either group. Discussion: In conclusion, age appears to be a risk factor for neuroleptic-induced neuron loss, and neuroleptics appear to accelerate a process of age-related large cell loss in the striatum. This study was supported by VA Merit Review Grants to JBL and DVJ, and NIMH Grant R29MH45142 to JBL.

270.4

BEHAVIORAL SENSITIZATION TO INTERMITTENT COCAINE ADMINISTRATION: EFFECTS OF CHRONIC HALOPERIDOL. P.A. LeDuc and G. Mittleman. Dept. of Psychology, Memphis State Univ., Memphis, TN 38152.

This experiment examined the effects of chronic haloperidol (HAL) treatment on locomotion and stereotyped behavior elicited by intermittent injections of cocaine (CHCL). Female Long Evans rats received injections of HAL (0.2 mg/kg, ip) or vehicle for 6, 12, or 18 days prior to the start of testing with CHCL. Daily injections of HAL were continued throughout CHCL testing. All rats received 4 doses of CHCL (0.0, 3.0, 7.5, or 15.0 mg/kg) in random order with an intervening vehicle day between successive drug days. The 4 dose sequence of CHCL was repeated a total of 4 times. Initial CHCL administration produced dose dependent increases in locomotion and stereotyped behavior. When the 4 dose sequence of CHCL was repeated, differences among treatment groups emerged. Specifically, groups treated with HAL exhibited heightened locomotion in response to CHCL (all doses) in comparison to vehicle treated controls. Additionally, with repeated injections, CHCL-induced locomotion showed a higher rate of sensitization in HAL treated rats. In order to examine the mechanisms underlying the heightened responsiveness to CHCL, locomotion in response to Apomorphine (0.0-250 ug/kg, sc) was determined. Regardless of dose, rats treated with HAL showed significantly more locomotion in response to apomorphine suggesting changes in the sensitivity of pre- and postsynaptic receptors. Overall, these results indicated that chronic treatment with HAL results in heightened responsiveness to acute CHCL as well as more rapid behavioral sensitization to repeated administration. This increased responsiveness is related to apparent autoreceptor down-regulation combined with post-synaptic up-regulation.

270.5

INTERACTIVE EFFECT OF HALOPERIDOL, DEXAMETHASONE AND HYPOTHERMIA STRESS ON HALOPERIDOL INDUCED CATALEPSY. H.L. Schreiber and Carole Slotterback Oakes*. Dept. of Psychology, Univ. Texas at Tyler, Tyler, TX, 75701 and Dept. of Behav. Sci., Highlands Univ., Las Vegas, NM 87701

In a 2X2X2 factorial design, 96 male rats, matched by weight, received dexamethasone, 400 ug/kg or the corn oil vehicle, followed 30 min later by a high dose of haloperidol (3 mg/kg) or saline, which in turn was followed 30 min later by forced cold water immersion (2 min, 3°) or by being left in their individual home cages (N/cell=12). Three days later, all rats received haloperidol (1mg/kg, IP) one hour prior to holeboard and latency-to-remove-forepaw catalepsy testing. Analyses of variance revealed significant interactions (dexamethasone history by haloperidol history by immersion history) in catalepsy, $F(1,72)=4.22$, $p<.05$, and in locomotion, $F(1,72)=6.13$, $p<.02$. Among saline history rats, catalepsy was significantly increased by a history of dexamethasone treatment, cold water immersion, or both, in relation to control rats. These effects were not seen among haloperidol history rats. Also, a history of dexamethasone administration, cold water immersion or both significantly diminished locomotion among saline history but not haloperidol history rats. These results indicated a discernible effect of adrenocortical perturbations on catalepsy which was abolished by a single haloperidol administration.

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270.6

A RAPID COLOR-BASED VIDEOTRACKER FOR BEHAVIORAL STUDIES: APPLICATION TO STUDIES OF TARDIVE DYSKINESIA IN HUMANS. S. ZEIGLER, A. KEYS*, G. ELLISON and W. WIRSCHING*. Department of Psychology, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024

The form of the altered Oral movements (OMs) in rats administered chronic neuroleptics have been successfully measured using computerized spot-detection circuitry involving fluorescent dyes placed on the rat's muzzle. We now report an extension of these procedures to humans using a new rapid color-detection circuit called the "Multitracker".

In these studies small colored spots were placed on the upper and lower lips of human patients receiving chronic neuroleptics. The resulting video records (30/sec) were analyzed by computer using fast-fourier and other analytic methods and the results compared with those of normal controls. This method permits the study of both vertical and lateral components of the altered oral movements observed in tardive dyskinesia without any attached device, permitting minimal disturbance of the patient. In a further refinement of this methodology, two video cameras can be used to record simultaneously oral movements and movements of the hands and feet so that the exact temporal relationship between different body movements can be quantified. This "Multitracker" system is applicable to a wide range of behaviors in humans and animals.

270.7

N-CYCLOHEXYLBENZAMIDES: A NOVEL CLASS OF DOPAMINE AUTORECEPTORS AGONISTS WITH POTENTIAL ANTIPSYCHOTIC ACTIVITY. L.D. Wise, J.C. Jaen*, B.W. Caprathe*, S.J. Smith*, T.A. Pugsley and T.G. Heffner. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor MI 48105.

As part of a project to develop a selective dopamine (DA) autoreceptor agonist as an improved agent for the treatment of schizophrenia, *trans*-N-[4-[4-(2-pyridinyl)-1-piperazinyl]-cyclohexyl]benzamide (PD136515) was identified as a chemical lead. As expected of a DA autoreceptor agonist, this compound was found to have affinity for DA D-2 receptors, to reverse gamma-butyrolactone-stimulated DA synthesis and to inhibit spontaneous locomotor activity in rodents. SAR studies demonstrated that while activity was retained with the *trans*-4-(piperazinylethyl)cyclohexyl analog, the *trans*-piperazinylmethyl and propyl analogs were much less active as were all of the *cis*-isomers. In addition, the pyridinylpiperazine could also be replaced with a phenyltetrahydropyridine group and the benzamide phenyl group with various heterocycles without a loss of activity. PD138276, *trans*-N-[4-(3,6-dihydro-4-phenyl-1(2H)-pyridinyl)-cyclohexyl]-4-fluorobenzamide, based on its overall profile was selected for further studies.

270.8

EFFECTS OF THE DOPAMINE AUTORECEPTOR AGONIST PD 138276 IN BEHAVIORAL, NEUROCHEMICAL AND NEUROPHYSIOLOGICAL TESTS. A.E. Corbin, C. Christoffersen, L.T. Meltzer, S. Whetzel*, T.A. Pugsley, J.N. Wiley*, Y.S. Shih* and T.G. Heffner. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.

The benzamide PD 138276 was found to be a dopamine (DA) autoreceptor agonist in preclinical tests. PD 138276 bound to DA D_2 receptors in vitro (IC_{50} = 13.8 nM), inhibited the firing of substantia nigra DA neurons and reversed gamma- butyrolactone-stimulated brain DA synthesis in rats. Consistent with activation of brain DA autoreceptors in vivo, PD 138276 reduced locomotor activity in mice (ED_{50} = 0.2 mg/kg, i.p.). At higher doses, PD 138276 reduced the stimulation of locomotor behaviors in mice caused by the DA releasing agent amphetamine (ED_{50} = 2 mg/kg, i.p.) and by the direct DA agonist apomorphine (ED_{50} = 7.4 mg/kg, i.p.), effects that may stem from weak partial agonist properties. Relative selectivity for presynaptic DA receptors was evidenced by the lack of locomotor stimulation and stereotypy at high multiples of the ED_{50} for locomotor inhibition in rats. Postsynaptic DA agonist effects were revealed in rats only after co-administration of high doses of PD 138276 with a DA D1 agonist. The effects of PD 138276 in rats persisted for up to 12 h after a single oral dose. Collectively, these results indicate that PD 138276 is an orally-active DA autoreceptor agonist with relative selectivity for presynaptic DA receptors.

270.9

EFFECTS OF THE NOVEL DOPAMINE AUTORECEPTOR AGONIST PD 135222 IN NEUROPHYSIOLOGICAL, NEUROCHEMICAL AND BEHAVIORAL TESTS IN RODENT AND MONKEY. L.M. Ball, L.W. Cooke, A.E. Corbin, T.A. Pugsley, F.W. Ninteman, L.T. Meltzer, T.G. Heffner and M.D. Davis Dept. of Pharmacology, Parke-Davis Pharm. Research Division, Warner Lambert Co.,Ann Arbor, Michigan 48105.

PD135222, (±)1-(2-pyridinyl)-4-[4-(2-pyridinyl)-3-cyclohexenyl]-piperazine, is a dopamine (DA) autoreceptor agonist as demonstrated in neurophysiological, neurochemical and behavioral tests. PD 135222 bound selectively to DA D_2 receptors (IC_{50} = 100nM). In the gamma- butyrolactone (GBL) model, PD 135222 reversed the GBL- induced increase in striatal DA synthesis with an ED_{50} of less than 10mg/kg i.p. In neurophysiological tests, PD 135222 (2.5 mg/kg i.p.) produced a 78% inhibition in firing of A9 DA neurons in chloral hydrate-anesthetized rats. Behaviorally, PD 135222 inhibited spontaneous locomotion in mice (ED_{50} = 0.9mg/kg i.p.) and rats (ED_{50} = 5.8mg/kg p.o.), consistent with presynaptic activation of DA receptors. PD 135222 also inhibited conditioned avoidance responding after oral dosing in squirrel monkeys (Saimiri Sciurea) (ED_{50} = 3.5mg/kg). Intracerebral microdialysis (ICMD) of the striatum/putamen was used to assess changes in extracellular neurotransmitter levels in both anesthetized and awake Sprague Dawley rats, and squirrel monkeys implanted with guide cannula. As measured by HPLC-EC, PD 135222 (3-10 mg/kg p.o. and i.p.) decreased DA overflow 40-60%. In rats, this effect was reversed by the DA antagonist haloperidol. BHT-920, another DA D_2 agonist, also decreased DA overflow to a similar degree. PD 135222 decreased acetylcholine (ACH) levels using ICMD in conscious and anesthetized rats, an effect seen with DA D_2 agonists such as LY171555 (quinpirole), but not with DA antagonists such as remoxipride. These results indicate that PD 135222 is a potent and efficacious DA autoreceptor agonist.

270.10

GABAERGIC AND DOPAMINERGIC DYSREGULATION IN THE BASAL GANGLIA FOLLOWING CHRONIC HALOPERIDOL TREATMENT: PUTATIVE RELATIONSHIP TO PATHOPHYSIOLOGY OF TARDIVE DYSKINESIA O. Shirakawa, X.M. Gao, F.I. Tarazi, T. Kakigi*, H. Kaneda, C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD. 21228

Chronic neuroleptic treatment in rats often leads to spontaneous orofacial movement, described as vacuous chewing movements (VCMs). Many characteristics of VCMs are similar to tardive dyskinesia (TD) in humans (Tamminga, C.A. Psychopharmacol. 102, 474, 1990). Coadministration of the GABA receptor agonist progabide with haloperidol (H) inhibits the development of VCMs (Shirakawa, O. Neurosci. Abst., 1989). To identify the neurochemical pathophysiology of VCMs, we studied several measures in 6 month H-treated rats: (1) quantitative regional cerebral glucose metabolism; (2) GABA-A and D1 and D2 receptor densities; (3) correlations between regional neurochemical changes and VCMs. H treatment decreases glucose utilization in extrapyramidal areas and nigral efferent structures, except for the caudate-putamen (CP). H treatment increases D2 receptor binding in CP and GABA-A receptor binding in substantia nigra reticulata (SNR), but without a direct correlation with VCMs. However, VCMs show a significant correlation with the ratio of D2/D1 receptor density in CP and GABA-A/D1 receptor density in SNR. These results suggest that chronic H treatment alters D2/D1 receptor sensitivity in striatum, which leads to VCMs by altering GABA release at D1-regulated synapses in SNR. Thus, hypofunction of nigral efferent pathways, occasioned by a cascade of neurochemical changes in extra-pyramidal structures, appears to underlie VCMs, and perhaps TD.

270.11

EFFECT OF SERTINDOLE, CLOZAPINE AND HALOPERIDOL ON THE DISCRIMINATIVE STIMULUS PROPERTIES INDUCED BY D-AMPHETAMINE, D-LSD AND ST 587 IN RATS.

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Classical neuroleptics induce extrapyramidal side effects (EPS), and have preferential dopamine (DA) antagonistic activity. The atypical neuroleptic clozapine has preferential inhibitory effect on serotonin (5-HT_2) receptors and α_1-adrenoceptors, compared with its weak effect on DA receptors. In this study the relative effects on the stimulus properties induced by AMPH (1 mg/kg, i.p.), the 5-HT_2 agonist d-LSD (0.16 mg/kg, i.p.) and the α_1-adrenoceptor agonist St. 587 (1 mg/kg, i.p.) were studied for haloperidol, clozapine and the putative antipsychotic sertindole (Skarsfeldt & Perregaard, Eur. J. Pharmacol. **182**, 1990, 613). For each training drug discrimination vs. saline was acquired in water-deprived rats responding in a FR 32 schedule in 2-lever chambers. Haloperidol (2 h s.c. pretr.) antagonized the effect of AMPH (ED_{50} 0.3 µmol/kg), but did not block the effect of d-LSD or St 587 in doses increasing reaction times. Clozapine (1 h s.c. pretr., ED_{50} values in µmol/kg in parentheses) inhibited the effect of AMPH (7.2), d-LSD (6.4) and St 587 (0.78). Sertindole (2 h s.c. pretr.) inhibited the effect of d-LSD (0.17) and St 587 (0.52), but did not antagonize the effect of AMPH in doses up to 23 µmol/kg. Thus neuroleptics differ markedly in their relative in vivo effects on DA, 5-HT_2 and α_1-adrenergic receptors studied by drug discrimination. This may have importance for the different clinical profiles of neuroleptics.

270.12

EFFECT OF CHRONIC TREATMENTS WITH NICOTINE AND HALOPERIDOL ON NICOTINE POTENTIATION OF HALOPERIDOL INDUCED CATALEPSY. M.D. Zanol*, P.R. Sanberg, D.F. Emerich, B.J. McConville*, L.M. Ford and A.B. Norman. University of Cincinnati College of Medicine, and Cellular Transplants, Inc., Providence, Rhode Island 02906.

Nicotine markedly potentiates the locomotor inhibiting effects of neuroleptics such as haloperidol. Furthermore, coadministration of nicotine with haloperidol has been found useful in further reducing tic frequency and severity in patients with Tourette's Syndrome. We investigated whether chronic treatment with haloperidol or nicotine could modify the interaction between these drugs.

Male Sprague-Dawley rats received 21 daily injections of haloperidol (0.8 mg/kg/day i.p.) or saline. One day after the last injection haloperidol and saline treated rats were divided into 4 groups and received saline only, saline + nicotine, haloperidol + saline, or haloperidol + nicotine. Catalepsy was monitored 2 or 4 hours following the last injection. Nicotine alone produced no significant catalepsy in either group. The catalepsy produced by haloperidol was reduced demonstrating tolerance. Although the magnitude of the nicotine potentiation of haloperidol catalepsy was reduced in the chronic haloperidol-treated rats there was a significant potentiation of the haloperidol catalepsy proportionately similar to that observed in control rats. In a separate experiment rats received either nicotine (5 mg/kg/day p.o.) for 28 days via drinking water or water alone. Rats were then divided and treated as in the previous experiment. Nicotine produced no catalepsy in either group. There was no significant potentiation of haloperidol catalepsy in the chronic nicotine treated rats. Continuous administration of nicotine may produce a tolerance to the beneficial effects in Tourettes patients receiving haloperidol. Intermittent administration of nicotine may avoid this.

270.13

PARTIAL DOPAMINE AGONIST -3PPP: PHARMACOKINETIC AND PHARMACODYNAMIC CHARACTERISTICS IN MAN. C.A. Tamminga, N. Cascella, R. Lahti, A. Carlsson. Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228 and Department of Pharmacology, University of Göteborg, Göteborg, Sweden

The partial DA agonist -3PPP acts at the D_2 autoreceptor to reduce DA turnover, diminish rat locomotor behavior, and inhibit DA neuronal firing. Based on its antidopaminergic properties, it is being studied as a novel drug treatment for schizophrenia. It has currently been administered to eight schizophrenic patients for safety and preliminary efficacy study, in doses up to 40 mg IM. Kinetic analysis suggests a plasma half life of 2-2.5 hours, with peak plasma levels of 200-500 nmolar after 30 mg IM. Growth hormone (GH) levels increase in a linear fashion through a dose of 30 mg IM, then precipitously fall to basal GH levels with higher -3PPP doses. Additional endocrine response data will be reported. Four schizophrenic subjects without other CNS medication, were given -3PPP doses up to 40 mg/kg. After the highest dose administered, mental status changes were seen in each subject which resembled a "calming" effect. Two of the subjects failed to respond to -3PPP with any specific antipsychotic effect; one subject had a mild antipsychotic effect, while a single subject had a robust antipsychotic response. These data suggest the feasibility of -3PPP use in schizophrenia and are consistent with its potential therapeutic activity.

270.14

MEASUREMENT OF DRUGS USED IN PSYCHIATRY BY FREE ZONE CAPILLARY ELECTROPHORESIS WITH UV DETECTION. M.A. Javors, M.S. De Buysere*, T.S. King, and C.L. Bowden. Departments of Psychiatry, Pharmacology, Cellular and Structural Biology, and Biochemistry, University of Texas HSC, San Antonio, TX 78284-7792.

The purpose of this study was to evaluate the use of capillary electrophoresis with UV detection for the separation and quantitation of psychiatric drugs and their metabolites and to make a preliminary evaluation of the usefulness of this method for clinical and research studies in which these drugs would be measured in biological fluids. We used free zone capillary electrophoresis with a capillary tube that was 75 microns in diameter and 50 cm long, UV detection at 214 nm, and 100 mM phosphate buffers at pH's between 7 and 10. Depending on the running buffer, we varied the voltage between 10 and 25 kV so that the current was constant between 200 and 250 uA. All of the compounds we tested were amines. For all compounds tested, the peak area was linear with concentration between 2.5 and 25 ug/ml (25 nl constant sample volume). Peak height was curvilinear with concentration in the same range. Compounds were best separated from their structurally similar metabolites at running buffer pH's near the pKa's of the compounds. Not all compounds could be separated at any given running buffer pH. We successfully quantitated fluoxetine and its metabolite norfluoxetine in plasma and compared our results to measurements made with HPLC and UV detection. A disadvantage of this method in its present form was that absolute identification of the compounds was not possible. However, in patients that were taking only a specific drug, we were able to quantitate the drug and its metabolites. The development of more sensitive methods of detection would permit therapeutic drug monitoring from very small plasma sample volumes.

270.15

PHARMACOLOGICAL PROFILE OF A NOVEL DOPAMINE-FATTY ACID BIOCONJUGATE WITH CNS ACTIVITY. G.W. Hesse and V.E. Shashoua. Ralph Lowell Laboratories, McLean Hospital, Harvard Medical School, Belmont, MA, 02178.

The behavioral pharmacology of a novel bioconjugate of dopamine was investigated. The compound, which consists of a fatty acid amide linked to dopamine, readily crosses the blood-brain barrier of mice and rats. It inhibits the spontaneous locomotor activity of mice with an ED50 dose of about 12 μmol/kg. No evidence of hyperactivity, catatonia, stereotypy or toxicity has been observed at doses up to 100 times the ED50. The effects of the compound are antagonized in a dose dependent manner by sulpiride, but not by domperidone. The compound appears to have little or no post-synaptic dopaminergic activity. It does not reverse reserpine induced depression of spontaneous locomotor activity, and it does not induce rotation in rats unilaterally depleted of dopamine with 6-OHDA. Finally, daily administration for three weeks produced little or no tolerance. This novel dopamine bioconjugate has behavioral effects which are similar to those of dopamine agonists selective for dopamine D2 autoreceptors.

270.16

EFFECTS OF DOPAMINE (DA) ANTAGONISTS AND AGONISTS ON DENTATE GYRUS FIELD POTENTIALS IN ANESTHETIZED RATS. K.A. Serpa and L.T. Meltzer. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Multiple lines of evidence provide a rationale for studying the hippocampus as a site involved in schizophrenia and the action of antipsychotic agents. Thus, we have begun to evaluate the effect of DA antagonists and agonists on dentate gyrus field potentials (FPs) elicited by electrical stimulation of the perforant path in urethane anesthetized rats. The effects of the D-2 antagonist, haloperidol (HAL), and D-2 agonist, quinpirole (QUIN), on dentate FPs were variable. HAL (100 μg/kg i.v.) increased the population spike (PS; 5 of 8 experiments) but did not effect the EPSP. QUIN (100 μg/kg i.v.) increased the PS (3 of 6 experiments) and produced decreases and increases in the EPSP. Despite the variability, the magnitude of the changes were much greater than occurred during vehicle treatment. It is unclear why both a D-2 antagonist and agonist produce similar effects. In contrast, neither the D-1 agonist, SKF 77434 (10 & 20 mg/kg i.v.; N-allyl analog of SKF-38393), nor the D-1 antagonist, SCH 23390 (100 μg/kg i.v.), had little effect on dentate gyrus field potentials. These initial data suggest that DA D-2 agents but not DA D-1 agents modify dentate FPs.

ALZHEIMER'S DISEASE: NEUROPATHOLOGY II

271.1

MIDLINE THALAMIC PATHOLOGY IN ALZHEIMER'S DISEASE. V.E. May*, W.G. Tourtellotte and G.W. Van Hoesen. Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

The midline thalamic nuclei are known to have direct connections with structures critical for learning and memory such as the hippocampal formation and entorhinal cortex. Damage to the mediodorsal and midline thalamic nuclei has been shown to impair memory in humans and non-human primates. For these reasons, and because memory impairment is a hallmark of Alzheimer's disease (AD), we investigated the integrity of thalamic nuclei in 15 AD cases and 8 age-compatible controls.

Formalin fixed tissue blocks containing the thalamus were cut on a freezing microtome and stained with Thioflavin S, Nissl and Alz-50 (8 cases, 4 controls). The salient findings are as follows: (1) There were numerous neurofibrillary tangles in both the midline mediventral (reuniens) and paraventricular nuclei of the thalamus; (2) Neuritic plaques were observed in these same nuclei in 7 AD cases; (3) There were abundant NFTs and NPs in the mediodorsal nucleus in 6 AD brains, and in anteroventral nucleus of 2 AD brains; (4) Neither NFTs nor NPs were observed to any degree in other thalamic nuclei; and (5) None of the control cases had NFTs or NPs in any thalamic nuclei.

The paraventricular and mediventral nuclei of the thalamus appear to have a selective vulnerability in AD. The NFTs and NPs seen in these nuclei are likely to disrupt the connections that link these structures with the medial temporal region, thus further deafferenting them and compromising the circuits on which memory processing depend. (Supported by NS 14944 and PO NS 19632.)

271.2

MODULAR ORGANIZATION OF THE HUMAN ENTORHINAL CORTEX. Solodkin, A., Kuljis, R.O. and Van Hoesen, G.W. Depts. of Anatomy and Neurology. The University of Iowa College of Medicine. Iowa City, IA 52242.

The entorhinal cortex (EC; Brodmann's area 28) that forms the anterior part of the parahippocampal gyrus, is an important connectional link between sensory and multimodal neocortices and the hippocampal formation. By gross inspection of the ventromedial temporal lobe, a modular anatomical organization of this area is suggested by the presence on its surface of *warts* or *verrucae*.

We have examined the EC using cyto- and myeloarchitectonic stains, immunolabeling for various neurochemicals (SP, NPY, GAD) and histochemistry for oxidative enzymes (NADPH, AChE) in blocks of human tissue sectioned in the coronal and tangential planes. For the latter, we have used a method to prepare flat mounts of EC that are cut parallel to the pial surface.

Our results reveal that the EC is formed by a mosaic of columnar components whose most conspicuous elements are the islands of cells of layer II and myelinated fibers that form columns around these islands. The impressions of these cell islands and their surrounding neurites constitute the surface *verrucae*. Additional observations showed the presence of cellular bridges interconnecting two adjacent islands. Module-like compartments are also revealed by AChE histochemistry, in contrast to other subpopulations of local circuit cells (NADPH, SP, NPY, GAD).

The unique organization of the EC has several important implications: a) anatomically evident modules are present not only in sensory and motor cortices; b) these modules could be the basis of physiologically distinct operations; c) since EC is the area most affected in Alzheimer's disease, these data provide a basis to study its pathology in the context of modular organization. Supported by NS 14944 and PO NS 19632.

271.3

PARVALBUMIN IMMUNOREACTIVE INTERNEURONS ARE DIFFERENTIALLY LOST IN HIPPOCAMPAL FORMATION OF ALZHEIMER'S DISEASED BRAIN. D.R. Brady and E.J. Mufson. Inst. Biogeront. Res., Sun City, AZ 85351; NIA/NIH, Bethesda, MD 20892; Rush Med. Ctr., Chicago, IL 60612.

Projection neurons of the entorhinal cortex and subiculum/CA1 subfields of the hippocampal formation undergo severe degenerative changes in Alzheimer's disease (AD). However, the fate of interneurons in the hippocampal formation remains unclear. Using a monoclonal antibody against parvalbumin, which distinguishes a subset of fast-firing GABAergic interneurons, we ascertained the topography of PV immunoreactive neurons in postmortem, paraformaldehyde fixed hippocampal formation from normal (X=64 yrs; n=8) and AD (X=81 yrs; n=12) brains. In normal hippocampus, PV interneurons were aspiny and pleomorphic, with extensive dendritic arbors. In dentate gyrus, PV cells, as well as a dense plexus of fibers and puncta, were associated with the granule cell layer. A few cells also occupied the molecular layer. In strata oriens and pyramidale of CA1-CA3 subfields, PV neurons gave rise to dendrites that extended into adjacent strata. Densely stained puncta and beaded fibers occupied stratum pyramidale, with less dense staining in adjacent strata oriens and radiatum. Virtually no PV profiles were observed in stratum lacunosum-moleculare or the alveus. Numerous polymorphic PV neurons and a dense plexus of fibers and puncta characterized the deep layer of the subiculum and the lamina principalis externa of the presubiculum. In AD hippocampus, there was a significant decrease in the number of PV interneurons in the dentate gyrus/hilar subfield ($P<.05$) and subfields CA1-CA2 ($P<.01$). In contrast, PV neurons did not appreciable decline in subfields CA3, subiculum or presubiculum in AD brains relative to normals. Staining with thioflavin-S did not reveal any degenerative changes associated with PV stained profiles. These findings indicate that PV interneurons within specific hippocampal subfields are selectively vulnerable in AD. This vulnerability may be related to their differential connectivity. Support: ADCRC, NIH and AHAF.

271.4

DISTRIBUTION OF PATHOLOGICAL NEURITES ACROSS THE SURFACE OF ALZHEIMER'S NASAL EPITHELIUM: A NEW WHOLE-MOUNT IMMUNOCYTOCHEMICAL METHOD. W.H.Feng, J.S.Kauer and B.R.Talamo. Tufts Univ. School of Med., Boston, MA 02111.

Previous immunocytochemical studies of frozen sections of autopsied nasal tissue from Alzheimer's patients showed that normal and abnormal sensory areas were not uniformly distributed across the olfactory epithelium (oe). For the purpose of targeting areas for sampling by biopsy and understanding how neuritic fibers are misdirected into the oe, a whole-mount immunocytochemical technique was developed to reveal the surface distribution of abnormal neuritic patches and degenerating olfactory receptor neurons (ORN) in the oe of patients with Alzheimer's Disease. Antibody to MAP5 (Sigma) or tubulin (J1, A. Frankfurter) is used to stain normal and abnormal neurons; a second antibody is selected that is specific for abnormal somata and neurites (e.g. to phosphorylated neurofilament proteins or to tau). Whole mount tissue can be rehydrated and sectioned and/or restained for further analysis. In dehydrated and cleared whole mounts from neonates, uniformly distributed normal ORNs and their axons are clearly visible. In AD tissue, normal areas may be present while other areas show abnormally stained ORNs and densely matted or complicated swirled neurites and neuron-free islands circumscribed by a thickened border of neuritic fibers which retrace their circular pathway repeatedly. The pattern of abnormal neurite aggregation suggests that the axons are diverted from their route to the olfactory bulb (thereby failing to make their normal connections), while they have an enhanced affinity for each other and an apparent attraction to the oe. This lack of normal connectivity may lead to altered expression of macromolecules such as tau. This interpretation is consistent with our other experiments in monkeys in which tau is expressed only in ORNs not showing olfactory marker protein (OMP); low levels of OMP are a feature of ORNs that are deprived of their synaptic targets.

271.5

PRELIMINARY CHARACTERIZATION OF A NOVEL ALZHEIMER'S DISEASE ASSOCIATED PROTEIN. D.J. Gunnersen, B.E. Haley, and J.T. Slevin. Departments of Neurology and Biochemistry, University of Kentucky, Lexington, KY 40536.

To characterize some of the biochemical changes in the Alzheimer's Diseased (AD) brain, two-dimensional gel electrophoresis was performed. Use of this technique resulted in the observation of a constellation of proteins near the β-tubulin band that appeared in confirmed AD samples only. The constellation consisted of 5-7 proteins with approximate M_r values of 55-56 kDa and a pI ranges of pH 4-5. These proteins were present in 18 of 18 AD cases examined and absent in 11 of 11 age-matched controls (including other neurological diseased controls). Preliminary characterization of this protein constellation has begun. For example, a. the proteins do not react with α-tubulin antibodies in 2-D Westerns, and b. these proteins can be phosphorylated by [γ-^{32}P]ATP. Procedures used to purify, characterize, and sequence these proteins will be presented. To determine if the presence of the protein constellation represents a systemic metabolic defect or if the phenomenon is unique to AD brain, fibroblast cell lines were also examined. Initial experiments indicate that the protein constellation is not present in AD fibroblasts. (Supported by NIH Grants GM35766 and PO1AG05199 and the VA Research Service)

271.6

GANGLION CELL LOSS IN RETINAL WHOLE-MOUNTS FROM PATIENTS WITH ALZHEIMER'S DISEASE. J. C. Blanks, Y. Torigoe and R. H. I. Blanks. Doheny Eye Institute, Dept. of Ophthalmology, USC Sch. of Med., Los Angeles, CA 90033, and Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, Irvine, CA 92717.

Previous studies in this laboratory have shown differences in the number of neurons in the ganglion cell layer (GCL) in the macula of patients with Alzheimer's disease (AD) compared with age-matched controls. The object of this study was to examine the extent of neuronal loss across the entire GCL. The remaining portion of the retina was prepared as a whole-mount and counter-stained with toluidine blue. Seven retinas from 7 AD patients (age 69-88) and 11 retinas from 9 age-matched controls (age 64-85) were examined. All neurons in the GCL were drawn with the aid of a camera lucida and digitized; neuronal density maps were generated by sampling the entire retina at 1 mm or 2 mm increments. A total loss of 37 ± 12% (mean ± s.d.) of neurons in the GCL of AD patients was observed compared with age-matched controls. The total loss of neurons in the GCL was remarkably constant across the retinas of AD patients (range = 20-58%) and between both eyes from two AD patients: an 82-year-old (right = 22%; left = 26%) and an 85-year-old (right = 48%; left = 44%). Cell loss in the peripheral retina was correlated (correlation coefficient r = 0.53) with loss of neurons in the macula of the same patient. Cell loss occurred equally among both large and small diameter neurons in the GCL. These results confirm earlier reports of retinal degeneration in patients with AD and demonstrate that the defect extends across the entire retina.

271.7

RECIPROCAL PATTERNS OF UBIQUITIN IMMUNOREACTIVITY IN NORMAL AGED AND ALZHEIMER'S DISEASE BRAIN A.E.Singer, N.W.Kowall, A.C.McKee, Harvard Medical School, Boston MA 02114

A wide variety of intracellular inclusions are ubiquitin immunoreactive (imr) including dot-like structures (DLS), an age-related feature of normal brain. We examined ubiquitin imr in the temporal lobes of 10 aged controls and 10 patients with AD. In control brains, ubiquitin imr DLS were found in CA3, CA2, and to a lesser extent, CA4 and white matter. Occasional smaller DLS were present in the dentate gyrus. Scattered punctate and threadlike ubiquitin imr dystrophic neurites (DN) and rare neurofibrillary tangles (NFT), morphologically similar to typical tau positive DN and NFT but distinct from DLS, were found in CA1 and subiculum. DLS were infrequent in these areas. Occasional ubiquitin imr DN were found in layer 2 of lateral and intermediate entorhinal cortex, whereas dense DLS were prominent in layer 2 of medial entorhinal cortex. DLS were evenly and densely deposited in the superficial layers of transentorhinal and temporal neocortex. In all regions, a few diffusely positive neurons were found, often with intensely positive granular nuclear staining. In AD, diffusely imr neurons with intense nuclear staining were frequent in every region. DLS were more prominent in white matter and less evident in cerebral cortex. In CA1 and subiculum, intensely imr DN and occasional NFT were present. Round and threadlike DN and occasional DLS were found in the vicinity of senile plaques. All entorhinal areas, transentorhinal and temporal cortex contained dense deposits of intensely imr DN, occasional NFT and DLS. Ubiquitin imr DN are most likely neuronal in origin given their topographic distribution, whereas DLS are not similarly distributed and may represent aging changes within astrocytes or microglia. DLS are scant in areas prone to the development of DN and NFT, such as CA1, subiculum, lateral entorhinal cortex, and numerous in relatively less affected areas, such as CA2, CA3, and medial entorhinal cortex. The reciprocal relationship between these imr structures suggests topographically specific variations in degeneration.

271.8

LOSS OF BASAL GANGLIA DOPAMINE IN THE LEWY BODY VARIANT OF ALZHEIMER'S DISEASE. P.J. Langlais,[1,2] L.T. Thal,[1,2] L. Hansen,[1] D. Galasko,[1,2] M. Alford[1] and E. Masliah.[1] [1]Dept. of Neurosciences, UCSD 92093 and [2]Neurology Research Services, VA Medical Center, San Diego, CA 92161

A recent study described diffuse cortical and subcortical Lewy bodies (LBs) in a large proportion (13/36) of clinically diagnosed and pathologically confirmed cases of Alzheimer's disease (AD) (Hansen et al. Neurology 40:1-8, 1990). These Lewy body variant (LBV) cases did not appear to represent the coexistence of AD and Parkinson's disease (PD) since clinical parkinsonism and neuronal loss in substantia nigra were mild.

The present study compared monoamine levels and choline acetyltransferase (ChAT) activity in cortical regions and basal ganglia from LBV (N=7), pure AD (N=6), and control (N=7) post-mortem brains. Values are expressed as % of control value. Comparable reductions of ChAT activity were observed in caudate (48-51%) and putamen (14-31%) of the LBV and AD cases. However, ChAT was much lower in the LBV compared to AD cases in frontal (19 vs 68%), medial temporal (22 vs 49%) and parietal (14 vs 45%) cortex. These data suggest a greater destruction of cholinergic basal forebrain neurons and/or cortical interneurons in the LBV cases which may contribute to the selective cognitive impairments observed in the LBV cases. Significant reductions of dopamine in caudate (19%) and putamen (5%) and homovanillic acid (HVA) in caudate (40%) and putamen (25%) were observed in the LBV but not the AD cases. These reductions are comparable to those observed in PD and suggest that the LBV biochemically resembles PD. The reason for milder parkinsonism in LBV than in PD is unclear. The effect of reduced nigrostriatal dopamine activity may be mitigated by the basal ganglia cholinergic deficit in LBV, or possibly by damage to other motor areas, e.g., subthalamic nucleus. Supported by funds from Dept. of Veterans Affairs and NIH (#S10RR04754-01).

271.9

ABERRANT LOCALIZATION OF MAP5 IMMUNOREACTIVITY IN THE HIPPOCAMPAL FORMATION IN ALZHEIMER'S DISEASE.
J.W. Geddes and K. Lundgren*. *Sanders-Brown Center on Aging and Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536.*

The cause of the cytoskeletal abnormalities in Alzheimer's disease (AD) is uncertain. One possibility is that some of the alterations may result from an aberrant sprouting response. We have previously observed that sprouting in the rat CNS results in the re-expression of the embryonic form of α-tubulin mRNA and that expression of the corresponding human α-tubulin mRNA is increased in Alzheimer's disease (*Neurosci. Lett.* 109:54, 1990). Hasegawa and colleagues recently reported that antibodies against phosphorylated epitopes of a fetal microtubule-associated protein, MAP5, label plaques, tangles, and curly fibers in AD (*Neuron* 4:909, 1990). We used monoclonal antibodies against non-phosphorylated MAP5 epitopes to examine the hippocampal formation obtained postmortem from four control individuals, six individuals with AD, and two 'transition' cases which did not have a history of dementia but did exhibit significant AD pathology. In both AD and control cases, axonal staining was restricted to the mossy fibers. In control cases, MAP5 immunoreactivity (IR) was observed in the neuronal cytoplasm and the the proximal portion of the apical dendrites of pyramidal and granule cells. In AD, numerous CA3 pyramidal neurons and the occasional neuron in CA1 and subiculum were intensely immunostained. Immunoreactivity filled the neuronal perikarya and the apical dendrite. In transition cases, immunostaining was similar to that observed in AD.

The increase and altered distribution of MAP5 IR in AD may reflect an aberrant sprouting response. The prominent staining in CA3, an area that is relatively uninvolved in AD pathology, suggests that many more neurons are affected in AD would be predicted based on neuropathological alterations. The results further suggest that the increased expression of cytoskeletal proteins may be tolerated in some regions such as CA3, but not in others including CA1 where the increased expression appears to precede aberrant phosphorylation, proteolysis, and incorporation of cytoskeletal proteins into AD pathology.

271.10

REGIONAL SYNAPTIC CHANGES IN ALZHEIMER'S DISEASE
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Regional aspects of synaptic pathology in Alzheimer's disease (AD) were studied using an antibody (EP10) which recognizes a synaptophysin like antigen. Homogenates of hippocampus, caudate and temporal and occipital cortices were studied from 10 cases of AD and 7 age and sex matched controls using an ELISA. The EP10 antigen was significantly reduced in AD ($F_{1,51}$=13.05, p=.002), with a 77% decrease in hippocampus (U=3, p=.001) and a 54% decrease in temporal cortex (U=11, p=.009). In temporal cortex, the reductions in the antigen were inversely correlated with the number of neurofibrillary tangles present (rho=.778, p=.007). This relationship was not observed in the hippocampus. Pallor of the outer two-thirds of the molecular layer of the dentate gyrus was observed with EP10 immunocytochemistry in AD. Western blotting studies qualitatively supported the ELISA findings. These results suggest that synaptic loss in AD occurs to a different extent in different brain regions. Synaptic loss in the temporal cortex may be related to local pathology. The disproportionately large loss of a synaptic marker in the hippocampus suggests an additional contribution of deafferentation to the mechanism of loss in this region.

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271.11

EXPRESSION OF SULFATED GLYCOPROTEIN-2 (SGP-2) IN HUMAN GLIOMAS, EPILEPTIC FOCI AND RAT BRAIN TISSUES.
J.-G. Chabot, M. Danik*, C. Chauvin*, C. Mercier*, A.-L. Benabid*, R. Quirion and M. Suh*. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Verdun and Maisonneuve-Rosemont Hospital Research Centre, Department of Medicine, Montreal University, Montreal, Quebec, Canada.

SGP-2 was first identified as the major secretory protein of rat Sertoli cells. We have isolated the clone pTB16, the human homologue of rat testicular SGP-2. By RNA blot analysis, pTB16 was elevated by 11-fold in gliomas compared to a primitive neuroectodermal tumor. These results were supported by *in situ* hybridization studies. In malignant gliomas, cells expressing pTB16 mRNA were markedly increased as compared to benign tumors. In metastasis to brain (adenocarcinoma of digestive origin), hybridization signal was found only in normal CNS tissue. Heavy but diffuse specific labeling was also observed in neuronal and glial cells from surgical specimens of epileptic foci. However, the pTB16 gene expression is not restricted to pathological brain tissues. We have also found hybridization signals in temporal cortex from normal control as well as in normal and lesioned rat brain tissues. In normal temporal cortex, clusters of silver grains were detected throughout the gray matter. In normal rat brain tissues, a widespread distribution of pTB16 transcripts was observed. The highest level of expression was found in the choroid plexus and ependyma. Prominent labeling was also observed over the pyramidal cell layer of the hippocampus, the granular cell layer of the dentate gyrus and the hippocampal fissure. In kainate-lesioned striatum and hippocampus, hybridization signals were markedly increased. Our studies demonstrate the presence of pTB16 gene expression in normal and pathological brain tissues. In brain tumors and lesioned tissues, SGP-2 may play a role in mediating apoptosis while its location in the hippocampus and choroid plexus raises interesting possibilities in regard to cognition and production of CSF fluids.

271.12

SELECTIVE EXPRESSION OF SULFATED GLYCOPROTEIN-2 (SGP-2) BY HIPPOCAMPAL NEURON AND EPENDYMA IN HUMAN BRAIN ENRICHED IN β-AMYLOID SENILE PLAQUES OF THE ALZHEIMER-TYPE.
E. Moysse[1], D. Michel[1]*, N. Choi[2]*, Y. Robitaille[3], G.Brun[1]*, R. Quirion[3], and J.G. Chabot[3]. [1]E.N.S. of Lyon, France; [2]Dept of Physiol. Chem., Showa Univ., Tokyo, Japan; [3]Douglas Hosp Res. Ctr, Dept of Psychiatry, McGill Univ, Verdun, Canada.

SGP-2 was first recognized as the major secretory protein of rat Sertoli cells. Overexpression of its gene was recently shown in Alzheimer's disease (AD) hippocampus, human gliomas and transformed neuroretina cells. The present study aimed to compare the distribution of SGP-2 and β-amyloid proteins in the forebrain of AD patients (n=8) and normal controls (n=8) using immunohistochemistry on serial sections. Occurrence and density of SGP-2-like immunoreactivity (IR) in hippocampus were correlated with density of β-amyloid-immunostained senile plaques in hippocampus and temporal cortex. However, the distributions of SGP-2- and β-amyloid-IRs were quite different. SGP-2-IR was mainly detected in hippocampal ependymal cells and neurons. The predominant ependymal localization of SGP-2-IR is in keeping with the high density of SGP-2 transcripts observed in rat choroïd plexus and ependyma (Chabot et al., this meeting). β-amyloid IR was exclusively found in senile plaques and blood vessel walls. Overproduction of SGP-2 by periventricular elements may play a role in the normal aging process as well as in its pathological acceleration in AD, especially since this protein has been proposed as a marker of cell death.

271.13

LONG TERM EFFECTS OF ALUMINUM AND ALUMINOSILICATES ON CULTURED NEURONS OF RAT CEREBRAL CORTEX: RELATIONSHIP TO ALZHEIMER'S DISEASE.
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Several reports have suggested that aluminum, an ubiquitous element, has a strong relation to Alzheimer's disease. In particular, aluminosilicate was found to exist in the senile plaques of patients with Alzheimer's disease. Therefore, we investigated the neurotoxicity of aluminum derivatives on dissociated neurons of cerebral cortex derived from fetal rats. After 22 days in culture, neurons which were exposed to 100μM of $Al(NO_3)_3$ showed extensive outgrowth of axon-like processes. Accumulation and fasciculation of neurofilaments with twisted shapes were observed. Similar results were also obtained with application of other aluminum salts. Using a functional assay system for synapse formation between cortical neurons, long term application of aluminum salts, microparticulates of aluminum oxide, silica gel, and aluminosilicates (zeolites) to cultured neurons was found to influence the frequency of spontaneous oscillations of intracellular Ca^{2+} in neurons which was correlated with the numbers of synapses formed in the culture.

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271.14

CORTICAL DISTRIBUTION OF ABNORMAL MICROVASCULATURE AND VASCULAR HEPARAN SULFATE PROTEOGLYCAN POSITIVE PLAQUES IN ALZHEIMER'S DISEASE (AD).
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The heparan sulfate proteoglycan of the vascular basement membrane (vHSPG) may play a role in the formation of amyloid deposits in AD. Using a monoclonal antibody specific to the vHSPG protein core, we studied the distribution of microvessels and vHSPG-containing amyloid deposits in the cerebral cortex of AD and age matched control cases. The distribution of vHSPG+ amyloid deposits was similar to that of thioflavin S (TS) staining in layer IV of the primary visual cortex and in the dentate gyrus. However, there was a differential plaque distribution in the temporal and frontal neocortex: a high density of vHSPG+ plaques was found mainly in deep layer VI, whereas the TS+ plaques were mainly observed in layers III and V. The number of shrunken and tortuous vessels was low or absent in control cases, whereas these pathological profiles were commonly observed in AD cases. Also, in these areas, their density was correlated to TS+ structures. These results suggest that severe damage to the cortical microvasculature occurs in AD. In addition, the specific laminar distribution of vHSPG+ plaques may be related to the specific angioarchitecture of a given cortical region. The pathogenesis of vHSPG+ plaques may be different from other plaques that do not share the same immunoreactivity profile, supporting the notion of the existence of a vasculature-derived subgroup of plaques. Supported by the Gould Foundation, the Brookdale Foundation, and NIH grants AG06647, AG05138 and AI24876.

271.15

BINDING OF THE SECRETED FORM OF THE ALZHEIMER BETA AMYLOID PRECURSOR PROTEIN TO HEPARAN SULFATE. L. Buée[1,4], J. Anderson[2], N.K. Robakis[2,3], A. Delacourte[4] and H.M. Fillit[1,3] Dept of [1]Geriatrics, [2]Psychiatry and [3]Neurobiology, Mt Sinai Med. Ctr, New York, NY 10029 and [4]U156 INSERM, 59045 Lille France.

Glycosaminoglycans (GAGs) are the proteoglycan (PG) moiety shown to be consistently present in all types of amyloid deposits. In Alzheimer's disease, heparan sulfate (HS) is the main GAG found in amyloid deposits. To investigate the potential physiological significance of this colocalization, we are studying the binding of the amyloid precursor protein (APP) to HS. APP containing the Kunitz protease inhibitor (KPI) insert (APP-PNII) was purified from PC12 cell conditioned medium containing NGF. To study the binding of APP-PNII to HS, a 1ml HS-column was made by coupling AH-Sepharose CL-4B gel and 5mg of HS using the carbodiimide technique. For each experiment, APP-PNII was loaded onto the HS-column. A stepwise gradient elution with NaCl released APP-PNII with 0.75N NaCl. APP-PNII was also eluted at approximately the same normality of Na_2SO_4. A linear gradient of dextran sulfate (average MW 5kDa) eluted APP-PNII at a concentration of 7mg/ml. These data indicate a high affinity binding of APP-PNII to HS, supporting the hypothesis that interactions between HS-PG and APP play a role in normal physiological processes and amyloidogenesis. Supported by the Florence J. Gould Foundation and NIH grants AG05138 and AI24876.

ALZHEIMER'S DISEASE: NEUROPATHOLOGY III

272.1

IMMUNOCHEMICAL LOCALIZATION OF HEPARAN SULFATE GLYCOSAMINOGLYCANS IN ALZHEIMER'S DISEASE

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Recent studies have demonstrated that heparan sulfate proteoglycan (HSPG) promotes neuritic outgrowth *in vitro* and has affinity to FGF and APP. In this study, two monoclonal antibodies were used in AD and control brains; one recognizes a glycosaminoglycan epitope present in heparan sulfate (HSGAG) and the other recognizes the core protein of a basement membrane HSPG. Sections of the hippocampal formation, temporal and occipital cortex were examined. Anti-HSPG antibody immunostained only the walls of arterioles and a few neuritic plaques (NPs). Anti-HSGAG also stained arterioles and NPs. In addition, immunoreactivity was medium to strong in neurofibrillary tangles (NFTs), some of which were not co-localized with Tau-1 positive NFTs. The cytoplasm and/or nuclei of pyramidal and non-pyramidal neurons were also stained. Some plaques contained HSGAG-positive neurites and/or were surrounded by HSGAG-positive astrocytes in AD tissue. The number, intensities, and extent of distribution of NFTs, NPs, and positive neurons were variable in different AD brains, but seemed to have a predilection for entorhinal cortex, CA1, and layers III, V and VI of cortex. A few NFTs, NPs, and positive neurons were also demonstrated by anti-HSGAG antibody in control brains, but their numbers were dramatically less than those seen in the AD brains. These results confirm a recent report (Snow, A.D., 1990) and extend these observations. Furthermore, this study suggests that HSGAG is a major component within specific lesions of AD. HSGAGs may play an important role in the pathogenesis of characteristic lesions and could serve as a marker reflecting pathological changes in AD.

272.2

THE RELATIONSHIP BETWEEN DENDRITIC EXTENT AND PLAQUES IN THE HIPPOCAMPUS IN ALZHEIMER'S DISEASE (AD). D. G. Flood, Y.-G. Lin*, A. M. Kazee*, T. A. Eskin and L. W. Lapham*. Depts. of Neurology, Neurobiology & Anatomy, and Pathology & Laboratory Medicine, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

Recent attention has focused on the ability of amyloid protein (β/A_4) to induce neurite outgrowth *in vitro* after application of synthetic β/A_4 fragments and *in vivo* around plaques. In order to study the possible effect of plaques on the dendritic extent of neurons in the hippocampus, we have undertaken a correlative study of Golgi-stained dendrites and of Bielchowsky-stained plaques. Data have been collected from 22 cases of the Rochester Alzheimer's Disease Project (RADP): 14 cases with AD (age range 59-96 yrs) and 8 normal control cases (age range 51-89 yrs). Total dendritic extent of 15 randomly chosen neurons was quantified for apical trees of dentate gyrus granule cells and basal trees of CA2/3 and CA1 pyramidal cells. Plaques were counted in the dentate molecular layer, hilus, CA3 and CA1. Dendritic extent was positively correlated between pairs of the cell types. Similarly, plaques between pairs of the regions were positively correlated, especially those of the dentate molecular layer and CA1. Plaques of the dentate molecular layer were negatively correlated with dendritic extent of all 3 cell types. Plaques of CA1 correlated negatively with dendrites of only the granule cells. Plaques in the hilus were seen in only a few of the cases but correlated negatively with dendrites of granule cells and CA2/3 pyramids. Plaques in CA3 were also limited and did not correlate with dendritic extent. Dendritic extent of neurons is either unaffected by plaques within the region (CA2/3 and CA1) or is lessended (dentate gyrus). Because dentate molecular layer plaques correlated with dendritic extent of all 3 cell types, it can be suggested that disruption of the initial input to the hippocampus in the dentate gyrus by plaques has the greater influence on dendritic extent of neurons in later processing stations in the hippocampus. Supported by NIH grant AG 03644.

272.3

QUANTITATIVE CHEMOARCHITECTONIC ANALYSIS OF THE CINGULATE CORTEX IN ALZHEIMER'S DISEASE (AD). P.R. Hof, P. Hsu*, and J.H. Morrison. Fishberg Research Center for Neurobiology and Dept of Geriatrics, Mount Sinai School of Medicine, New York, NY 10029.

Analyses of chemically-identified neurons in the human prefrontal and temporal cortex have demonstrated that pyramidal cells that contain high levels of nonphosphorylated neurofilament protein (SMI32+) are highly vulnerable in AD, whereas parvalbumin (PV)+ interneurons are resistant to degeneration. Further, in these areas, vulnerabilty is linked to cell size in that the large (>350 μm^2) SMI32+ are particularly vulnerable. SMI32+ cells in cingulate cortex are predominantly small or intermediate in size (150-350 μm^2). Variations of the distribution of both SMI32+ and PV+ cells across the cingulate cortex clearly delineate the subareas within both anterior (ACg) and posterior (PCg) cingulate cortex. While PV+ cells are unaffected in the AD cases, SMI32+ cells are decreased in both supra- and infragranular layers (30-40%) in all PCg subfields, whereas these cells remain unaffected in the ACg. Although neurofibrillary tangles (NFT) are present in both ACg and PCg, their density across cases is highly variable and unlike temporal and prefrontal cortex, does not correlate with SMI32+ cell loss. Moreover, both ACg and PCg are less affected in AD than prefrontal and temporal neocortex. The relative vulnerability of PCg as compared to ACg suggests that both the neuron type profile and connectivity of these areas may be fundamentally different. These data further support the hypothesis that specific chemically-identifiable elements of the cortical circuitry are selectively affected in AD. Supported by NIH AG06647, AG05138, N° 2 T35 AM07420, and the Brookdale Foundation.

272.4

MOLECULAR AND NEUROPATHOLOGIC STUDIES OF AMYLOID AND CYTOSKELETAL PROTEINS IN THE LEWY BODY VARIANT OF ALZHEIMER'S DISEASE. V.H. Mah[1], P.F. Reyes[1], L.L. Doyle*[1], G.A. Higgins[2]. [1]Dept. of Neurology, Thomas Jefferson Univ., Philadelphia PA, 19107 and [2]Mol. Neurobiol., NIA, NIH, Baltimore MD 21224

A subset of Alzheimer's Disease (AD) is the Lewy body variant which is pathologically characterized by increased numbers of Lewy bodies in addition to the typical findings of AD. Recently a mutation in the APP gene was described in two kindreds of familial AD. In these cases cortical Lewy bodies were noted by some observers. The question arises as to the relationship between abnormal amyloid expression and deposition and Lewy body formation in AD. Initial studies using conventional histochemical stains suggest increased amyloid deposition in both plaques and blood vessel walls in cerebral cortex in association with Lewy bodies. Lewy bodies tend to be prominent in the nucleus basalis of Meynert (NBM), amygdala and substantia nigra (SN). In these areas, neuronal cell loss appears to be greater with the presence of Lev , bodies. The NBM and amygdala show conspicuous amyloid deposition, in contrast to the SN. Current studies are focused on examination of amyloid precursor protein and neuronal cytoskeletal protein expression in areas affected by Lewy body accumulation in AD.

272.5

LOCALIZATION OF THE PROTEASES CHYMASE AND CATHEPSIN G IN ALZHEIMER'S DISEASE BRAIN. M.J. Savage[1], N. M. Schechter*[2], J.Q. Trojanowski[3] and R. Siman[1]. [1]Cephalon, Inc. West Chester, PA. 19380, [2]Dept. of Dermatology, and [3]Dept. of Pathology, Univ. of Pennsylvania, Philadelphia, PA. 19104

Amyloid deposition into neuritic and cerebrovascular plaques is a characteristic of Alzheimer's disease (AD), and results from abnormal proteolysis of the amyloid precursor protein (APP) to form ß/A4. Indeed, chymotrypsin-like protease activity is significantly elevated in AD parietal cortex (Soc.Neurosci. Abstr., 16:787). At least two proteases contribute to this activity: chymase, found in mast call granules, and cathepsin G, found in neutrophil granules. The distribution of these enzymes in AD and control temporal cortex was studied using immunohistochemistry. Mast cell secretory granules were stained selectively with anti-chymase. Mast cells were present in both AD and control brains, usually inside blood vessels. Occasional mast cells were lodged within the brain parenchyma, and a few were intimately associated with neuritic plaques. Neutrophils, containing secretory granules stained by anti-cathepsin G, were plentiful in the brain and are located inside blood vessels. Both types of granulocytes, containing chymotrypsin-like proteases potentially important for ß/A4 liberation from APP, are present in brain, and could contribute to pathological deposition of ß/A4, especially within the vasculature. Whether an imbalance exists in the numbers of these cells in AD brains is currently being examined.

272.6

THE FIRST APPEARANCE OF AMYLOID PLAQUES IS IN THE NEOCORTEX, NOT IN LIMBIC AREAS. J.L. Price and J.C. Morris, Depts. of Anat./Neurobiol. and Neurology, and the Alzheimer's Disease Research Center, Washington Univ. Sch. Med., St. Louis, MO 63110

The borderline between healthy aging and the early stages of Alzheimer's Disease is being studied in a series of very well characterized cases. Cognitive status was determined by clinical evaluation and psychometric testing before death, or by a structured retrospective interview with a close relative, and a Clinical Dementia Rating was assigned to each case. Fifteen nondemented (CDR=0), six very mildly demented (CDR=0.5) and eleven more severely demented (CDR=1-3) cases were studied.

As reported previously, all of the CDR=0.5 cases had large numbers of plaques in the neocortex as well as in the hippocampus and amygdala, most of which were primitive in type. It is apparent that the earliest development of plaques must be in non-demented cases. Of the non-demented cases, no plaques were found in the seven cases younger than 75, with either the Bielschowsky stain or antibody staining for ß-amyloid. In the eight older cases (75-89), plaques were found in only five. Two of these had a single area of primitive plaques restricted to a small part of the temporal cortex, without any plaques in limbic structures. One had more widespread clusters of primitive plaques, although these were also restricted to the neocortex. These plaques were distributed through the grey matter, without any relation to blood vessels. The last two cases had plaques in limbic areas, but the highest density of plaques was in the neocortex. One of these resembled the CDR=0.5 cases with some mature as well as primitive plaques, although the overall plaque density was lower. These observations suggest that ß-amyloid deposits first develop as primitive plaques in the neocortex, in contrast to tangles, which are first seen in limbic structures. (Supported by NIH grants AG03991 and AG05681.)

272.7

ALZHEIMER NEUROPATHOLOGY IN THE BRAINSTEM TEGMENTUM PARALLELS SUBSTANCE P INNERVATION. J.M.Lee*, N. Kowall, and E.T. Hedley-Whyte. Departs. of Neuropathology and Neurology, Massachusetts General Hospital, Boston, MA 02114.

Neuronal loss and globose neurofibrillary tangles (NFT) are found in the noradrenergic locus coeruleus (LC) in Alzheimer's disease (AD). Previoulsy, we found a decrease in the number of 5-HT neurons in the dorsal raphe (DR) (-23%) and B9 raphe (- 31%) but not in the median raphe (MR) nuclei (JNEN, 1990). Tau antibodies recognized globose NFT's and neuritic changes in the DR and B9 nuclei, the dorsal and ventral but not the middle part of the MR, and the lateral parabrachial nuclei.

Yankner et al. (Science 1990; 250:279-82) have shown that B-amyloid (1-40) which is abnormally deposited in the neuropil in AD is neurotoxic. The active moiety is B-amyloid (25-35) which has a structural homology to the human tachykinin, substance P (SP), and therefore may mediate its effect through a SP receptor. We investigated the SP fiber distribution in five human brainstem tegmentums as a possible marker for post-synaptic SP receptor localization. Immunocytochemical staining of SP revealed a dense fiber innervation in the dorsal aspects of the tegmentum, including the dorsal raphe, LC, dorsal to the medial lemniscus in the region of the B9 nuclei, and in the lateral parabrachial nucleus. Fibers were sparse in the MR. Therefore, the SP fiber distribution in the human brainstem parallels the distribution of tau immunoreactive NFT's and cell loss in AD. Receptor binding studies will be necessary to confirm the presence of as well as the ability for B-amyloid to bind at SP receptors in the tegmentum.

272.8

NEURONS DISPLAY PROMINENT LYSOSOMAL ABNORMALITIES IN ALZHEIMER'S DISEASE AND DOWN'S SYNDROME. R. A. Nixon and A. M. Cataldo. McLean Hospital, Harvard Medical School, Belmont, MA 02178

Lysosomes are a major site of intracellular protein metabolism and membrane turnover. Many types of hydrolytic enzymes, including over a dozen proteases of wide-ranging specificity, reside in these vesicular organelles. Using immunocytochemistry and histochemistry on sections of neocortex and hippocampus from mouse brain and postmortem human brain, we found neurons to be the richest source for 7 of 8 acid hydrolases examined. Hydrolase contents and lysosomes were particularly high in neuronal perikarya and proximal dendrites. Certain of the hydrolases (e.g., cathepsin B and α-hexosaminidase) were barely detectable in glia; the others were represented to varying degrees. In sections of neocortex from individuals with Alzheimer's disease and Down's syndrome, immunoreactivity to each of the hydrolases was massively increased in neurons displaying degenerative changes. The cell bodies of some neurons were completely filled with darkly immunostained lysosomes. Within "at-risk" neuronal populations of the neocortex and hippocampus, increased levels and altered distributions of acid hydrolases were also seen in many neurons that appeared normal by other criteria. This finding suggests that hydrolase accumulations may be an early marker of metabolic derangements or compensatory responses in these neurons. Apparent transitions were often seen between the degeneration of hydrolase-laden neurons and the appearance of thioflavin-positive senile plaques containing high levels of the same acid hydrolases. The altered patterns of intracellular degradation implied by these findings may be relevant to the abnormal processing of amyloid precursor protein. Neurons appear to be the principal source of the abundant acid hydrolases in senile plaques, which we consider candidate enzymes in the formation of β-amyloid (PNAS 1990; 87:3861-3865). Supported by the NIH.

272.9

LYSOSOMAL HYDROLASE DISTRIBUTIONS IN BRAINS OF PATIENTS WITH ALZHEIMER DISEASE AND DOWN SYNDROME. A.M. Cataldo, P.A. Paskevich, D.A. Mann, R.A. Nixon. McLean Hospital, Harvard Medical School Belmont, MA, and Department of Pathology, University of Manchester, U.K.

Our previous studies, showing an abnormal extracellular localization of lysosomal hydrolases with β-amyloid deposits in senile plaques (SP) of Alzheimer brains, support a mechanism involving neuronal degeneration and consequent dysregulation of lysosomal mechanism(s) leading to the altered breakdown of amyloid precursor protein. In this study we have localized selected lysosomal hydrolases in 25 brains from individuals with Down Syndrome (DS) (age 31-71). Neocortical sections were analyzed immunocytochemically at the light and EM levels with antisera to cathepsin D (CD), cathepsin B (CB), α-galactosidase (GAL) and β-hexosaminidase-A (HEX). SP were identified with Bielschowsky silver stain and thioflavin histofluorescence. CD, CB, HEX and GAL were abundant within SP of DS brains. Certain neurons were intensely labeled with these hydrolase antisera and many were associated with SP. By EM, immunoreactivity in SP was predominantly in lipofuscin granules of abnormal types and dense bodies of degenerating neurites. Immunoreactive SP in DS were not limited to specific cortical layers, as in AD, but instead were observed in all laminae. All immunoreactive SP contained thioflavin histofluorescence. In DS, as in AD, therefore, a strong relationship exists between prominent amyloid deposition and high extracellular concentrations of lysosomal hydrolases. Neurons appear to be a principal source of these enzymes. The variations observed in the laminar distribution of hydrolase-positive SP in DS and AD brains may reflect differences between these disorders in rates of neurodegeneration.

272.10

QUENCHING LIPOFUSCIN AUTOFLUORESCENCE IN AGED AND ALZHEIMER'S BRAIN: A DOUBLE-LABEL FLUORESCENCE METHOD WITH THIOFLAVIN COUNTERSTAIN. Gisele Styren, W. Harold Civin, and Joseph Rogers. Institute for Biogerontology Research, Sun City, AZ 85372.

Fluorescence sometimes offers advantages over colorimetric detection--especially when two different antigens must be co-localized within the same cellular elements. Unfortunately, in aged nervous (and cardiac) tissues, lipofuscin and other auto-fluorescent pigments accumulate markedly, frequently obscuring labeling with a specific fluorescence marker. The emission/excitation spectra of these "aging pigments" span those for most commercial fluorophores. Although there may be differences in color between a fluorophore and lipofuscin at a particular wavelength, the differences are subtle (e.g., with a conventional fluorescein filter set lipofuscin appears yellow-green and fluorescein appears lime-green). Moreover, at exposure times appropriate for micrographs of specific fluorophore labeling, lipofuscin autofluorescence in the field will often overexpose the image. We have attempted to surmount this problem by several methods. Custom filter sets were not helpful. Subjecting tissue sections to a strong UV light for several days did not appreciably quench lipofuscin. Fluorophores in the blue range (e.g., AMCA, Vector Laboratories) were successful, but one still needs a higher wavelength marker for double-labelling.

In the course of counterstaining Alzheimer's tissue with thioflavin S, we had previously noted that lipofuscin auto-fluorescence was greatly diminished. We therefore modified the thioflavin protocol to maximize lipofuscin quenching and to permit fluorescence double-labelling (the complete protocol will be available at the meeting as a handout). The procedure gives excellent results with AMCA, fluorescein, rhodamine, and Texas Red in aged brain. In Alzheimer's tissue, amyloid deposits are counterstained by the thioflavine, providing a triple label.

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272.11

SOME ALZHEIMER'S PATIENTS SHOW ATROPHIED OPTIC NERVES CLINICALLY. T. Russell Nelson,*[1] R. Angel Cruz,*[1,2] S. Arnold Adler,*[1] Douglas McNinch,*[1] Monique J. Leys,*[3] and J. Vernon Odom.[4] [1] Bay Pines VA Medical Center, Bay Pines, FL; [2] Department of Neurology, USF Medical Center, Tampa, FL; [3] Department of Ophthalmology, University Hospital, Ghent, Belgium; [4] Department of Ophthalmology, WVU Health Sciences Center, Morgantown, WV.

Eighty percent of patient's with probable Alzheimer's disease (AD) have reduced numbers of optic nerve (ON) fibers (Sadun and Bassi, Ophthalmol 1990; 97:9-17). Therefore, AD patients should show clinical signs of ON atrophy. We photographed the ONs of 13 patients (23 ONs) with probable AD and 10 normal patients (20 ONs) with the same mean age. An ophthalmologist, masked as to the diagnosis of the patients, reviewed the photographs, measured the cup-to-disc (c/d) ratios and rated the discs as normal, questionably normal with larger than normal cup excavation, questionably normal with arteriosclerosis, or abnormal with ON atrophy.

The c/d ratios were similar in the two groups. Their ratings differed ($X^2 = 19.02$, $p < 0.001$). ON atrophy was more prevalent in the AD group (8 eyes in 7 patients) compared to the normal group (1 eye in 1 patient) as was a rating of questionably normal with arteriosclerosis (4 eyes in 4 patients vs 1 eye in 1 patient). Although ON atrophy and sclerotic vessels are not diagnostic of AD, their presence may assist the physician in making a diagnosis.

272.12

ACETYLCHOLINESTERASE-RICH PYRAMIDAL NEURONS IN ALZHEIMER'S DISEASE. S. Heckers, C. Geula, M-M. Mesulam. Harvard Medical School, Boston, MA 02215

The distribution of Acetylcholinesterase (AChE)-rich pyramidal neurons was studied in the cortices of six Alzheimer's Disease patients and four normal aged subjects.

Both groups showed a characteristic distribution of these neurons with the highest density in motor and premotor areas, moderate density in association cortices and low density in limbic-paralimbic areas. Three areas (Brodmann areas 6, 22, and 24) were chosen for quantitative analysis. The density of pyramidal AChE-rich neurons in layers III and V was significantly reduced in AD patients, most pronounced in anterior cingulate cortex. Nerve cell density was not significantly different in adjacent Nissl-stained sections. The density of AChE-positive fibers was also decreased in all three areas of the AD patients but was not correlated with the number of AChE-rich neurons. Loss of AChE-rich neurons was more pronounced in cases with high counts for plaques and tangles but was also found in one case with no tangles and low plaque count in the areas of interest.

These findings demonstrate a dramatic loss of AChE-rich cortical neurons in layers III and V in Alzheimer's Disease which can not be attributed to nerve cell loss or loss of cholinergic fibers. It may occur in the early disease stages, perhaps even prior to the formation of plaques and tangles.

272.13

CHEMOARCHITECTURE OF THE STRIATUM IN ALZHEIMER'S DISEASE. N. Selden, C. Geula, L. Hersh and M-M. Mesulam, Harvard Medical School, Boston, MA 02215

The neuronal distributions of choline acetyltransferase (ChAT), somatostatin (SOM) and calcium-binding protein (calbindin D_{28k}) were mapped in striatal tissue from Alzheimer's disease (AD) cases and controls using specific antibodies. In controls, ChAT-positive neurons were large (area: $475u^2$), multipolar in shape, intensely stained and evenly distributed throughout the striatum. ChAT-positive neurons in the dorsal striatum of AD cases were similar in size, morphology and distribution to controls. By contrast, ChAT-positive neurons in the ventral striatum of AD cases were reduced in size to 77% and in density to 24% of control values. SOM-positive neurons were of medium size ($230u^2$), richly stained, with variable perikaryal morphology and more densely distributed in the caudate nucleus than in the putamen or ventral striatum. Calbindin-positive neurons were either medium-sized ($100u^2$), lightly stained neurons distributed most plentifully over medial caudate nucleus or large ($165u^2$), richly stained neurons frequently encountered in the lateral putamen. No significant change was observed in either SOM-positive or calbindin-positive neurons in AD cases as compared with controls. Selective loss and shrinkage of ChAT-positive neurons in the ventral striatum of AD cases is consistent with the preferential involvement of the limbic system in this disease.

Antibodies to somatostatin and calbindin generously provided by Drs. R. Benoit and M. Celio, respectively.

272.14

SELECTIVE ACCUMULATION OF ALUMINUM AND IRON IN THE NEUROFIBRILLARY TANGLES OF ALZHEIMER'S DISEASE: A LASER MICROPROBE (LAMMA) STUDY. D.P. Perl[1,2], P.F. Good[1], L.M. Bierer[2*], and J. Schmeidler[2*]. [1]Fishberg Research Center for Neurobiology, [2]Dept. of Psychiatry, Mt. Sinai School of Medicine New York, NY 10029

We report the results of an examination of the elemental content of neurofibrillary tangle (NFT)-bearing and NFT-free neurons identified within the hippocampus of 10 Alzheimer's disease cases and 4 clinically and neuropathologically intact age-matched controls. The study employed laser microprobe mass analysis (LAMMA), a technique which provides extremely sensitive multi-element detection in plastic embedded semithin sectioned tissues. Evidence for selective aluminum accumulation within the NFT-bearing neurons was obtained in all ten Alzheimer's disease cases. The site of aluminum deposition within these cells was the NFT itself, and not the "nuclear region" as we previously reported (Science 208:297,1980). Iron accumulation was also detected within NFTs. Evaluation for the accumulation of other elements within the NFT-bearing neurons failed to reveal any other metallic or non-metallic biologically active element as being consistently present. In addition, probe sites directed to neurons identified in snap frozen cryostat sections of two of the Alzheimer's disease cases, revealed similar spectra with prominent aluminum-related peaks, confirming that our findings are not related to exogenous contamination through fixation, embedding or other procedures prior to analysis. This study further confirms the association of aluminum and NFT formation in Alzheimer's disease. Supported by NIH grants AG-5138, AG-6833 and ES-928.

272.15

LASER MICROPROBE MASS ANALYSIS OF ALUMINUM, COPPER AND ZINC IN DIFFERENT NEURODEGENERATIVE DISORDERS. C. Bouras[1], P.R. Hof[2], P.F. Good[2], A. Hsu[2*] and D.P. Perl[2]. [1]Dept of Psychiatry, Univ. of Geneva, Switzerland, and [2]Fishberg Research Center for Neurobiology, Mt Sinai Sch. Med., New York, NY 10029.

The aluminum (Al, M/Z 27), copper (Cu, M/Z 63) and zinc (Zn, M/Z 64) content was analyzed in plastic-embedded semithin sections from the brains of 3 Alzheimer's disease (AD), 4 Pick's disease (PD), 1 dementia pugilistica (DP) and 6 control cases using laser microprobe mass analysis (LAMMA). In the hippocampus of AD cases higher levels of Al were detected in neurofibrillary tangles (NFT), in the nuclei of NFT-bearing cells, as well as in the nuclei of non-NFT-containing cells and neuropil as compared to controls. The Al accumulation was highest in the NFT. In the hippocampal hilus and fascia dentata no differences were observed for Cu and Zn between AD and controls, however values for these elements were higher in the CA1 region in the AD cases. Al was present in higher amounts in the nuclei and neuropil of PD cases than in controls. The Pick bodies contained higher Al levels than the nuclei of the affected neurons in the fascia dentata and CA1. Cu and Zn levels were generally higher in PD than in controls, and were comparable to the level of AD cases. In DP, the nuclei of NFT-bearing neurons contained Al, Cu and Zn levels comparable to those observed in AD cases, however higher Al levels were observed in NFT of the DP case as compared to AD. These results demonstrate the abnormal accumulation of Al, Cu and Zn in brain areas that are severely affected in neurodegenerative disorders, and suggest that Al may play a key role in the formation of lesions such as NFT and Pick bodies. Supported by NIH grants AG05138, AG06833 and ES-928.

273.1

REPEATED MEASURES OF ChAT ACTIVITY ARE HIGHLY CORRELATED WITH COGNITIVE FUNCTION AMONG 23.5mo F-344 RATS. M.D. LINDNER AND T.L. MARTIN. Bristol-Myers Squibb Pharmaceutical Res. Inst., P.O. Box 5100, Wallingford, CT 06492-7660

Animal studies of aged versus young rats (Decker, 1987), and studies of cognitive function among aged rats (Fischer et al., 1989) have failed to replicate the high correlations with ChAT activity reported clinically (r=.81, Perry et al., 1978). We correlated cognitive function, using the Morris water task, with measures of hippocampal and cortical ChAT activity in aged rats (23.5mo). There was a large range in both learning ability (from 3.4 ± 1.3m to 35.1 ± 1.1m) and in ChAT activity (the rat with the lowest ChAT activity was 40% less than the highest). Both behavioral and neurochemical measures were very reliable as determined by split-half reliability coefficients on the 10 trials in the water maze (r=.83), and the 6 measures of ChAT activity in the cortex (r=.80) and hippocampus (r=.62). Cortical and hippocampal ChAT activities were both correlated with learning ability (r=.61 and .62 respectively), and the combined ChAT activities were even more highly correlated with learning ability (r=.75). However, even though the variability in our ChAT data was no greater than Fonnum (1975), single measures of ChAT activity were not correlated with learning ability (n=10, r=.11 and r=.36) for hippocampus and cortex respectively. Despite the accuracy of Fonnum's (1975) ChAT assay, these results suggest that large correlations with cognitive function are dependent on further reducing measurement error by taking means of repeated measures.

273.2

DELAYED LATE COMPONENT OF VISUAL GLOBAL FIELD POWER IN PROBABLE ALZHEIMER'S DISEASE. K.L. Coburn, J.W.Ashford and M.A.Moreno*. SIU School of Medicine, Springfield, Il 62708.

Late components of flash visual evoked potentials (FVEP's) are delayed in at least some forms of dementia in the elderly. The delay is selective in that earlier components are not affected. Recent work suggests that this selective late component delay may be characteristic of Alzheimer's disease (AD) rather than an inevitable feature of dementia in general. This study uses reference free Global Field Power (GFP) analysis to more objectively define FVEP components and finds that the GFP peak corresponding to the late P2 component is delayed in a probable AD group but not in a demented unlikely AD group, relative to age equivalent normals. The late component delay is again found to be selective in that the GFP peak corresponding to the earlier P1 component does not differ between groups. These findings further strengthen the evidence for electrocortical changes in the visual system of AD patients.

273.3

THE EFFECT OF NORMAL AGING AND ALZHEIMER'S DISEASE ON SPATIAL ATTENTION. Patricia M. Mueller, Steven P. Tipper* and Gordon C. Baylis. Dept. of Psychology, U. C. San Diego, CA 92093, and Dept. of Psychology, McMaster University (S.P.T.)

Normal young adults show an action-based attentional system when tested on a task that requires them to reach for a target stimulus. Distractor objects on the action path towards the target cause very much more interference than those located beyond the target (Tipper et al., *Psychonomic Soc. Meeting*, 1990). Data suggest that a process of distractor inhibition is used in order to mimimize interference from such distractor objects. This is manifest as an increased reaction time to a target which was at the location of the distractor on the previous trial.

We examine the extent to which normal aging and Alzheimer's Disease affect the ability to make a speeded reach to the correct target. It was found that normal aged people show increased interference from distractor objects, but a pattern of reaction times that broadly mirrors that in young adults. Patients suffering from Alzheimer's Disease, however, show a different pattern of interference that suggests a fundamental disruption in the basic mechanisms of spatial attention.

This work was supported by grants from N.I.M.H., the U.S. Office of Naval Research, and N.A.T.O.

273.4

EARLY BEHAVIORAL SYMPTOMS IN ALZHEIMER'S DISEASE: PATHOLOGICAL CORRELATES. J.M. Verhein*, D.M. Freed, and V.W. Henderson. Departments of Psychology and Neurology (Div. of Cognitive Neuroscience & Aging), Univ. of Southern Calif., Los Angeles, CA 90089

Clinical heterogeneity of Alzheimer's disease (AD) is well documented, but the pathological substrate of AD symptoms is poorly understood. We hypothesized that AD patients with early behavioral abnormalities would show particularly severe neuropathological alterations within frontal lobe association areas. We retrospectively studied 60 demented patients followed by the Alzheimer's Disease Research Center at the University of Southern California with autopsy-confirmed "definite" AD. Based on initial symptoms as reported by a subject's primary caregiver on a structured intake interview, AD patients were dichotomized into those with behavioral changes at the onset of their dementia and those without early behavioral abnormalities. Targeted behavioral symptoms were depression, delusions, hallucinations, or paranoid ideation. Twenty-one index AD subjects (35%) were reported to have early behavioral changes, and 39 (65%) comparison subjects did not; index and comparison subjects did not differ significantly with regard to gender or to mean age at the time of symptom onset, age at death, or symptom duration. At autopsy, numbers of neurofibrillary tangles (NFT) and neuritic plaques (NP) were semiquantitatively estimated in the middle frontal gyrus (Brodmann's area 9) and hippocampus. NFTs (chi sq. [1] = 8.90, $P = 0.003$) and NPs (chi sq. [1] = 9.22, $P = 0.002$) were significantly more abundant in frontal cortex for the index cases than for comparison subjects, whereas the histopathological burden in the hippocampus was similar for the two subgroups. Findings suggest that early behavioral abnormalities in AD are, at least in part, determined by the distribution and severity of the associated pathological changes. [*NIA P50-AG05142*]

273.5

SELECTIVE AND DIVIDED ATTENTION TO VISUAL FEATURES ARE IMPAIRED IN PATIENTS WITH EARLY DEMENTIA OF THE ALZHEIMER TYPE. J.V. Haxby*, R. Parasuraman, J. Gillette*, K. Raffaele. Lab. of Neurosciences, Natl. Inst. on Aging, Bethesda, MD 20892

Selective and divided attention to visual features was investigated in 8 patients with mild dementia of the Alzheimer type (DAT) and twelve age-matched controls by measuring reaction time (RT) on visual matching tasks with varying attentional demands. Subjects indicated which of two choice stimuli had the same color, shape, or number as a sample stimulus. On a baseline choice RT task, DAT patient RT was 23% slower than that of controls. On the simplest visual matching task, in which all items in a block matched on the same feature and other features did not vary, DAT patient RT was 26% slower than control RT, indicating they had no difficulty discerning these features beyond what could be attributed to general psychomotor slowing. By contrast, DAT patient RTs were disproportionately slower on tests of selective and divided attention. On the selective attention task, in which irrelevant, non-matching features varied creating distraction, DAT patient RT was 40% slower than control RT. On the divided attention task, in which the matching feature changed every item, DAT patient RT was 115% slower than control RT. On a task that examined primed shifts of selective attention, in which changes in matching feature were followed by 2 to 7 items with the same matching feature, DAT patients demonstrated priming that reduced their disproportionate slowing on divided attention to the level of slowing on selective attention. Mean accuracy was greater than 95% on all tasks for both groups. These results indicate that attentional impairment in early DAT involves decreased ability to filter out irrelevant attributes of visual stimuli and marked difficulty dividing attention among different attributes of compound stimuli.

273.6

GLUCOSE ENHANCEMENT OF MEMORY IN ALZHEIMER'S PATIENTS. M.E. RAGOZZINO, C.A. MANNING, W.K.K. LAM, and P.E. GOLD. Department of Psychology, U. Virginia, Charlottesville, VA 22903.

Recent findings indicate that administration of glucose facilitates memory in elderly rodents and humans. In healthy elderly humans who demonstrate normal age-related memory changes, glucose improves performance on secondary verbal memory tasks but does not affect performance on non-memory tasks or on visual memory or implicit memory (stem completion) tests. The present experiment examined the effects of glucose on memory in Alzheimer's patients.

Subjects, aged 61-92 (n=14), were tested on two consecutive mornings. Subjects took a series of tests assessing orientation, explicit secondary verbal and visual recall and recognition, and implicit verbal memory after ingesting 75 g of glucose or 32 mg of saccharin. Blood glucose levels were measured at several times during testing. On Day 2, the procedure was repeated with alternate treatments and test forms, permitting within-subject comparisons.

Recall of contextual and noncontextual verbal material was significantly improved after glucose ingestion (p's < 0.01) as was recognition of verbal material (p < 0.005). In addition, facial recognition was significantly enhanced by glucose (p < 0.05). Performance under glucose on these tests was still substantially below that seen in non-demented aged individuals. Scores on the implicit memory task were unaffected by glucose. These findings provide evidence that glucose enhances both verbal and visual explicit memory in Alzheimer's patients. As in healthy elderly subjects, glucose did not enhance implicit memory in Alzheimer's patients. (Supported by ONR N0001489-J-1216, NIA AG 07648, NSF BNS 9012239, and the Virginia Center on Aging. CAM is a Postdoctoral Fellow on NIH Training Grant HD 07323).

273.7

VISUOMOTOR CONTROL IN ALZHEIMER'S DISEASE:RELATION TO PARIETAL ASSOCIATION CORTEX

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Pathological changes in visual association area and inferior parietal cortex,has been reported in the patients with Alzheimer's Disease(AD) even in early stage,so that the investigation of visuomotor control in the patients was planned to demonstrate behaviorally specified deficit of the visual perceptual function related to SPECT and MRI findings.Gazing,eye movement trajectory and visual field in front of subject during drawing or copying a cube were recorded and analyzed in 10 patients with AD by means of TV camera and Vision Analyzer(TKK939).They showed the reduced uptake of I-123-IMP SPECT and shrinkage MRI in parietal association area.When they drew a cube according to their memory or without visual guidance,drawing was almost successful. But when they copied a cube by using visual guidance a picture became poor.Gazing shift away from the original and copied figures indicated disorder of accurate eye movement towards visual targets.In our quantitative analysee,the distribution of eye movement velocities during copying was diffuse to wide range from slow to fast and had no peak,and the amount of high velocity eye movements which mean back and forth eye movements between a model and a copied figure showed no difference within head fixed and free condition.It is suggested that parietal association cortex is needed to get accurate eye movement and that abnormal one in AD towards visual targets make negative effects to do something.

273.8

SEMANTIC FLUENCY IN PARKINSON'S DISEASE AND ALZHEIMER'S DISEASE: EFFECTS OF CUING. C. Randolph, A.R. Braun, T.E. Goldberg, T.N. Chase, and D.R. Weinberger. Clinical Brain Disorders Branch, NIMH Neuroscience Center, Washington DC 20032

It was hypothesized that poor performance on semantic fluency tasks might be due to either 1) a degradation of semantic memory, in which exemplars are no longer available for retrieval, or 2) an ineffective retrieval strategy. Patients with Parkinson's disease (PD), patients with Alzheimer's disease (AD), and normal controls were compared on two versions of a semantic fluency task. One version consisted of uncued retrieval from a relatively broad semantic category (e.g. animals). In the other version subjects were provided with a retrieval strategy, which consisted of prompting them with subordinate categories in which to search (e.g. household pets, farm animals). Each version was administered to all subjects in a counterbalanced design using two semantic categories. Both PD and AD patients were significantly impaired relative to controls on the uncued version. PD patients improved significantly on the cued version (to control levels), whereas the AD patients exhibited no improvement as a result of cuing. The results suggest that deficits exhibited by PD patients on semantic fluency tasks may be a result of ineffective retrieval strategies, whereas AD patients may perform poorly as a result of semantic memory loss.

273.9

MAGNETIC AUDITORY STEADY-STATE RESPONSE INCREASES WITH AGING AND IS GREATEST FOR SUBJECTS WITH MILD ALZHEIMER'S DISEASE. S.B.Baumann*,A.C.Papanicolaou*,H.S.Levin, L.A.Bertolino*,R.L.Rogers*,B.E.Masel*, H.M.Eisenberg. MEG Lab, Div. of Neurosurgery, University of Texas Medical Branch and the Transitional Learning Community, Galveston, TX 77550.

As part of a project to determine if magnetoencephalography (MEG) might contribute to improvements in the diagnosis of early Alzheimer's disease, we have used an auditory steady-state paradigm to test three groups of subjects - young normals (n=11, mean age=31), elderly normals (n=4, mean age=77), and elderly subjects with probable, mild, Alzheimer's disease (n=5, mean age=79). A seven-channel Neuromagnetometer was used to make recordings just above the right ear as subjects lay on a padded table within a shielded room. Tone bursts of a 500 Hz carrier, 4 msec rise/fall time and 2 msec plateau time, were delivered to the left ear in 5 Hz intervals at tones of 20-50 Hz. Each average of 500 epochs was Fourier transformed and analyzed for power at frequencies of 20-60 Hz. When power for the resonant frequency was measured (e.g. power measured at 35 Hz for 35 Hz tone bursts), at nearly every stimulus frequency the young subjects had the least power and the mild Alzheimer's subjects had the greatest power. The effect was greatest in the frequency range of 30-45 Hz where the normal elderly subjects had 21%-57% more power than the young subjects (N.S.) and the Alzheimer's subjects had 117%-165% more power than the young subjects ($p<.05$). This effect is in agreement with recent reports that many disease processes lead to greater periodicity in physiological signals.

273.10

CANTAB PARALLEL BATTERY: A SUITABLE TOOL FOR INVESTIGATING DRUG EFFECTS ON COGNITION?

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The Cambridge Automated Neuropsychological Test Battery (CANTAB) was devised to assess various aspects of cognition in patients with brain damage, including conditions associated with dementia. CANTAB comprises 3 "clinical" batteries - Working Memory and Planning, Visual Memory and Attention. A further battery based on elements of these is available in 4 parallel forms and was designed for phase I volunteer studies. The tests can be run on an IBM computer and utilise a Touch Sensitive Screen. Forty eight healthy volunteers completed all four versions of the test battery. Results indicated comparable performance on all versions though ceiling effects may be a problem with young volunteer populations.

273.11

COGNITIVE DEFICITS IN EARLY ALZHEIMER'S DISEASE RELEVANT TO DRIVING SKILLS. L. J. Fitten, C. J. Wilkinson*, M. Burns*, K. Perryman*, S. Ganzell*, and D. Ganzell*. Lab of Cognitive Neurophysiology, VAMC Sepulveda, CA 91343 / UCLA School of Medicine, CA 90024 and Southern California Research Institute, Los Angeles, CA 90066.

In recent preliminary work, we have shown that generally healthy patients with early Alzheimer's disease (AD) perform significantly worse on an actual road test than either age-matched healthy controls or age and Folstein Mini-mental State Exam (MMSE) score-matched, non-stroke diabetics and multi-infarct (MID) patients. We are presently attempting to define which cognitive deficits present in early AD are responsible for such a decrement in driving ability. In the laboratory, we are measuring driving related skills by various tests of attention (e.g., sustained, divided) perception, information processing and decision-making. These include tests for visual search, tracking, divided attention, vigilance, response time, short term memory and visual evoked potentials. Our preliminary findings show a consistent pattern of differential performance as a function of group, the poorest scores originating from the AD patients followed by MID, diabetics and controls. AD patients performed particularly poorly on high demand perceptual and attentional tasks as well as on short-term memory tests. This was true even for AD patients with relatively high MMSE scores (24-26). Cognitive defects in mild AD appear to include sizable deficits in the early phases of information processing which appear to impact significantly on driving performance.

273.12

CYTOCHROME OXIDASE INHIBITION BY SODIUM AZIDE INFUSION IMPAIRS LEARNING IN THE MORRIS WATER MAZE. M.C. Bennett and G.M. Rose. Department of Pharmacology, C-236, UCHSC; and VAMC, Denver, Colorado

Cytochrome oxidase activity is significantly reduced in blood platelet mitochondria from Alzheimer's disease (AD) patients [Parker *et al., Neurol., 40*, 1301 (1990)]. Previously, we reported that chronic and selective inhibition of cytochrome oxidase by sodium azide impaired learning on two behavioral tasks and impaired hippocampal plasticity [Bennett *et al., Neurosci. Abst., 16*, 1346 (1990)]. We now report that sodium azide treatment impairs learning in the Morris water maze.

Adult male Sprague-Dawley rats were implanted with Alzet osmotic minipumps (2ML4) containing 0.9% saline (CONTROL) or 160 mg/ml sodium azide (AZIDE) 7-21 days prior to training. Rats were given 4 daily trials for 7 days in a tank filled with opaque water maintained 24-25° C. Swim time to a hidden platform, which occupied a fixed position relative to extramaze cues, was the performance measure. The AZIDE rats exhibited overall poorer performance across trials [MANOVA main effect: $F(1,23) = 16.0$; $p < 0.001$). First day performances did not differ significantly between groups ($t = 0.78$, NS), indicating that the deficit was not due to a motor impairment.

These results extend our previous findings and indicate that chronic cytochrome oxidase inhibition produces a generalized learning deficit. The present finding is consistent with the hypothesis that tonic infusion azide in rats models some characteristics of AD.

273.13

BLUE COLOR DEFICIT IN ALZHEIMER'S DISEASE.

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Our findings with the City University Colour Vision Test (Cronin-Golomb et al., Ann. Neurol., 1991, 29, 41-52) suggested that selective color deficits on the blue-yellow axis occur in Alzheimer's disease (AD). We now report a replication and extension of these findings, using two measures of color vision that are more subtle and sensitive than that used in the previous study. The Farnsworth-Munsell D-15 Test and the Luneau-Lanthony New Colour Test require subjects to discriminate hues on color caps and place them in the proper sequence. We tested 29 patients with AD and 25 age-matched control subjects (CS). Of the AD group, 13 consecutive patients had received a complete neuro-ophthalmological examination that ruled out significant disorders of the anterior visual structures. Statistical analyses showed that the AD patients made more tritan (blue) errors than did the CS ($p<.01$ for each test) but not more deutan (green) or protan (red) errors. Within the AD group, there were more tritan than other error types ($p<.05$). The results support the conclusion that there is a deficit in blue hue discrimination in AD. Such a deficit may result from the lesions of extrastriate visual cortex that are known to occur in this disease.

273.14

INTACT PRIMING OF PICTURES IN DIFFERENT ORIENTATIONS IN PATIENTS WITH ALZHEIMER'S DISEASE.

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In a prior study, we found that patients with early-stage Alzheimer's disease (AD) showed normal priming on a picture-naming task despite their impaired recognition memory of the pictures. In the present study, we sought to discover if the preserved priming capacity extends to pictures shown in different orientations. Thirteen mildly-to-moderately demented AD patients and 12 normal control (NC) subjects participated in this 3-phase study. In Phase I, subjects named 84 pictures of common animals and objects presented in 12 different orientations in 30° increments around the picture plane; naming latencies were recorded. In Phase II, subjects performed a three-choice recognition test with 12 of the pictures named in Phase I. In Phase III, subjects renamed the remaining 72 pictures that were presented in an orientation different from that in Phase I. AD patients named pictures slower and made significantly more naming errors than NC subjects. AD patients were also significantly impaired on the recognition test. AD and NC groups showed similar and significant picture-naming repetition priming as measured by faster naming of Phase III than Phase I pictures. These results replicate our previous finding of preserved picture-naming despite impaired recognition memory of pictures in AD. Moreover, AD patients demonstrated intact priming despite cha.ges in orientation of the pictures. Thus, these results show that picture-naming priming in AD extends to pictures shown in noncanonical positions and across different orientations. (Supported by grants from the Alzheimer Association and Illinois Department of Public Health.)

273.15

PERFORMANCE ON ATTENTIONAL TASKS IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE IMPROVES FOLLOWING ORAL ADMINISTRATION OF THE COGNITIVE ENHANCER BMY-21502.

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Studies of BMY-21502 in laboratory animals indicate that it may prevent the disruption of memory, enhance the acquisition of new learning, and improve spatial memory. The objective of this 12 week open trial was to identify a safe and effective dose range for the treatment of symptoms of mild to moderate Dementia of the Alzheimer Type (DAT). Nine DAT patients (mean MMS = 21.4 ± 4.03, range = 15-26) received escalating doses of BMY-21502 according to the following schedule: one week at 100 mg once a day, one week at 200 mg once a day, 5 weeks at 300 mg twice daily (300x2), and 5 weeks at 300 mg three times daily (300x3). Baseline psychometrics were administered during two sessions preceding the beginning of the study, and testing on drug was conducted after 1 week at 100 mg once a day, 1 week at 200 mg once a day, and after 1, 3 and 5 weeks at the 300x2 and 300x3 doses. For the 300x2 and 300x3 doses, mean neuropsychological scores were used for analysis. No significant improvements were found at any dose on tests of dementia severity (MMS, $0.05 < p < 0.15$ for doses 300x2 and 300x3) and memory (Buschke Selective Reminding, $0.05 < p < 0.10$ for doses 300x2 and 300x3). Significant improvements were found at the 200, 300x2 and 300x3 doses on tests of attention (Stroop Color Naming: 300x3, mean improvement = 5.9, $t = 4.378$, $p < 0.008$ (n=6); Stroop Interference: 200 mg, mean improvement = 4.722, $t = 2.895$, $p < 0.05$ (n=9); 300x2, mean improvement = 4.656, $t = 3.135$, $p < 0.02$ (n=9); 300x3, mean improvement = 4.017, $t = 5.3$, $p < .004$ (n=6)) and short-term memory span (Digit Span: 300x3, mean improvement = .683, $t = 3.555$, $p < 0.02$ (n=6)). The present results indicate that BMY-21502 may act as a cognitive enhancer by improving visuomotor speed and by increasing the general level of arousal and attention.

273.16

EYEBLINK CLASSICAL CONDITIONING AND NEUROPSYCHOLOGICAL TESTS IN DOWN'S SYNDROME ADULTS.

M. Papka,[1,2] E. Rozette,[3*] J. M. Coffin,[1,2] D. A. Rappaport,[3*] E. W. Simon,[3*] & D. S. Woodruff-Pak.[1,2] Dept. of Psychology[1], Temple Univ., Philadelphia Geriatric Center[2], Philadelphia, PA 19141 & Elwyn[3], Elwyn, PA 19063.

Virtually all adults with Down's syndrome (DS) develop Alzheimer's-like neuropathology by age 35, but most do not have accompanying behavioral signs of dementia. We demonstrated that eyeblink conditioning discriminates patients with probable AD from controls. Here we used the 400 msec delay paradigm to see if eyeblink conditioning would detect Alzheimer-like changes in DS adults in the absence of behavioral signs of dementia. Data were collected on 20 DS adults, ranging in age from 19 to 64 years. To assess dementia, the Down Syndrome Mental Status Examination (DSMSE) and the Slosson Intelligence Test (SIT) were used. There was a significant difference in CR percentage between DS adults below 35 (CR average=36.1%) and those 35 or older (CR average=17.5%; $t=2.26$;$p<.05$). Analysis based on a sub-group of the DS adults indicated that the DSMSE and the SIT failed to discriminate between older and younger subjects. Eyeblink conditioning may index neuropathological expression of AD in DS adults when other tests are insensitive to the changes. *Supported by an NIH Biomedical Research grant from PGC.*

273.17

CLASSICAL EYEBLINK CONDITIONING AND THE ASSESSMENT OF MEMORY DISORDERS.

R.G. Finkbiner & D.S. Woodruff-Pak. Dept. of Psychology, Temple University & Philadelphia Geriatric Center (PGC), Philadelphia, PA 19141.

We examined the diagnostic utility of eyeblink classical conditioning in differentiating memory disorders of varying etiologies. Patients (N=38; Mean age=76 years) underwent full diagnostic evaluations at the Baer Consultation and Diagnostic Clinic at PGC which included physical, neurological, psychiatric, and radiological examinations, neuropsychological testing, and eyeblink classical conditioning in a delay paradigm (500 msec, 1 KHz, 80 dB tone CS followed 400 msec after its onset by a 100 msec, 5 psi corneal airpuff US). Following audiometric testing, patients received 10 blocks of acquisition training (90 trials). Learning (measured by total percentage CRs) was significantly poorer for patients meeting NINCDS-ADRDA criteria for probable Alzheimer's disease (AD) or Mixed dementia (17.9%) compared to patients with dementia secondary to alcohol abuse (36.5%). In this sample, the two patients with cerebrovascular dementia (CVD) conditioned more poorly (6.7%) than both AD/Mixed patients and personality disordered patients (25.8%). Eyeblink classical conditioning is a useful measure in differentiating memory disorders. *Supported by NIMH CRC 40380 and a grant from the Alzheimer's Association.*

273.18

TRACE CLASSICAL CONDITIONING IN ALZHEIMER'S DISEASE.

J. M. Coffin, M. Papka., & D. S. Woodruff-Pak. Department of Psychology, Temple University and Philadelphia Geriatric Center, Philadelphia, PA 19141.

We have previously reported that patients diagnosed with probable Alzheimer's Disease (AD) condition more poorly in the delay classical conditioning paradigm than do non-demented, age-matched elderly. Our goal is to identify a classical conditioning paradigm that differentiates non-demented elderly from AD patients to an even greater extent than does the 400 msec delay paradigm. The present study was undertaken to explore performance on the 750 msec trace paradigm in patients who met NINCDS-ADRDA criteria for diagnosis of probable AD and normal elderly. Following a brief hearing test, subjects were presented with 90 acquisition trials. Each trial consisted of a 250 msec, 85 dB tone CS, a 500 msec blank period, and a 100 msec, 5 psi corneal airpuff US (750 msec CS-US interval). The probable AD group had a mean age of 86 years and the non-demented control subjects had a mean age of 79 years. Percentage of CRs for probable AD patients were 15.2, and for normal elderly were 34.4. Classical conditioning of the eyeblink response in the trace paradigm reliably discriminates between normal elderly and patients with probable AD. *Supported by a grant from the Alzheimer's Association.*

274.1

PHYSIOLOGIC RESPONSES TO THYROTROPIN-RELEASING HORMONE (TRH) INFUSION IN ALZHEIMER'S DISEASE AND NORMAL AGING. T.H.Lampe*, R.C.Veith*, S.C.Risse*, S.R.Plymate* and M.A.Raskind. Univ. of Wash. Sch. of Med. and American Lake VA Medical Center, Tacoma WA 98493.

Physiologic responses to TRH in normal man primarily reflect TRH-evoked activation of the sympathetic nervous system (SNS). To clarify possibilities that evoked SNS activity is altered in aging and blunted in Alzheimer's disease (AD), prompt physiologic responses (i.e., maximum increments over baseline in blood pressure [BP], heart rate [HR] and plasma norepinephrine [NE]), occurring over the first 5 minutes after an i.v. bolus of 0.1 mg TRH (protirelin), were assessed in 11 otherwise healthy men with probable AD (age 64.8 ± 6.3; mean ± SD), 10 Old Normal (ON) men (61.4 ± 9.8) and 8 Young Normal (YN) men [27.1 ± 2.9). Methods employed were similar to our previous study which had identified physiologic responses to TRH as relatively blunted in men with advanced AD vs. matched normals (**Psychoneuroendocrinology** 14:311-320, 1989).

Results: In Old Normal compared to YN cohorts, mean HR response to TRH was significantly blunted (9.3±4.5 bpm vs. 17.9±6.4; $p < 0.05$ [ANOVA]) yet mean plasma NE response was elevated (98.0±64.9 pg/ml vs. 36.9±35.6; $p < 0.05$). In AD subjects, physiologic responses (most notably HR) correlated positively with measures of their cognitive performance on established tests (e.g., correlation of HR responses with Folstein MMS scores, $r = 0.75$ [$p < 0.01$]; HR with Boston Naming, $r = 0.70$ [$p < 0.02$]; HR with Mattis' DRS scores, $r = 0.77$ [$p < 0.01$]).

Conclusions: The impact of age on normal subjects' responses (↓HR,↑NE in ON vs. YN) accords with prior reports of this pattern of SNS-related alterations with advancing age; this reinforces the potential utility of employing TRH to assess human SNS reactivity. Present findings suggest that physiologic responses to TRH may be relatively blunted as a function of dementia severity in AD; the mechanism(s) underlying this phenomenon and its potential utility as a severity-related pathophysiologic biomarker in AD await resolution.

274.2

HP 290: A POTENTIAL ALZHEIMER'S THERAPEUTIC AGENT WITH REDUCED CARDIOVASCULAR LIABILITY. D. Howard*, S Aschoff*, G. Bores, C. Detig,* C. Errico*, D. Fink*, E. Glamkowski, R. Hamer*, F. Huger, C. Smith, and A. Gorman*. Depts. of Biological and Chemical Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

HP 290 is a potential agent in the treatment of Alzheimer's disease displaying potent cholinesterase inhibition (IC_{50}: 31 nM), oral bioavailability and a long duration of action. In this report, the cardiovascular profile of HP 290 was evaluated. In anesthetized beagles, HP 290 (0.1 mg/kg/min, iv) elicited increases in mean arterial pressure (MAP: +54%), cardiac output (CO: +40%), and total peripheral resistance (TPR: +61%) with heart rate unchanged. In contrast, heptylphysostigmine (HEP) caused overall cardiovascular depression (MAP: -24%; CO: -28%; HR: -56%; TPR: -19%). During α- and β-adrenergic blockade, HP 290 elicited responses similar to HEP in the intact state, whereas HEP induced pronounced cardiac depression (MAP: -72%; CO: -89%; HR: -56%) with A-V blockade at cumulative doses of 7.6-10.2 mg/kg. Similar degrees of potentiation of HR responses to electrical stimulation of vagal efferent fibers was observed for HEP and HP 290 at cumulative i.v. doses of 1.2 mg/kg and 12.0 mg/kg, respectively. Cumulative doses of HP 290 (8 mg/kg/day x 4 days) in conscious dogs did not result in prohibitive changes in MAP and HR although AChE in RBC's was inhibited by as much as 90-100%. Therefore, HP 290 exhibits a more desirable therapeutic index in the potential symptomatic treatment of Alzheimer's disease in the elderly.

274.3

HP 290: A POTENT, ORALLY ACTIVE CHOLINESTERASE INHIBITOR FOR THE TREATMENT OF ALZHEIMER'S DISEASE. G.M. Bores, F.P. Huger, R.S. Hsu*, S.M. Chesson*, B. Gorney*, K.C. Bradshaw*, R.R.L. Hamer*, E.J. Glamkowski* and V. Haroutunian. Depts. of Biological and Chemical Research, Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ, 08876 and Mt Sinai Medical Center, New York, NY, 10029.

Cholinesterase inhibitors, such as physostigmine have been proposed for the treatment of Alzheimer's disease (AD). HP 290, (3aS-cis)-1,2,3,3a,8,8a- hexahydro-1,3a,8-trimethyl- pyrrolo[2,3-b]indol-5-ol (1,2,3,4- tetrahydroisoquinolinyl) carbamate (ester), is a physostigmine analog with an improved profile. HP 290 was found to be a potent inhibitor of rat brain AChE (IC_{50}= 0.032 μM) *in vitro*, with selectivity for inhibition of AChE versus BChE (IC_{50}= 0.88 μM). *In vivo* experiments demonstrated that HP 290 was orally bioavailable, had a longer duration of action and had a more favorable brain-to-plasma ratio than physostigmine. Doses of HP 290 required to produce acute lethality in rats after i.p. adminstration were much higher than those of physostigmine. HP 290 (0.16 mg/kg) caused significant (+108%), scopolamine-sensitive increase in wave VI of the brainstem auditory-evoked potential in rats. HP 290 significantly enhanced the performance of rats in a passive avoidance task at doses of 0.32 and 0.64 mg/kg and reversed the effect of a memory deficit induced by a lesion of the nBM at a dose of 0.64 mg/kg. Increased stability and duration of action, improved oral bioavailability, good brain penetration and decreased acute lethality make HP 290 an interesting therapeutic candidate for the treatment of AD.

274.4

ACETYL-L-CARNITINE: A NEUROPROTECTIVE THERAPY FOR ALZHEIMER'S DISEASE. M. Calvani[1], M.T. Alderdice[2], M. Iannuccelli[1], A. Koverech[1], A. Carta[1]. [1]Sigma-Tau S.p.A., Pomezia (Rome) Italy; [2]Sigma Tau Pharmaceuticals, Gaithersburg, MD 20878, USA.

Acetyl-L-Carnitine was originally tested as a potential treatment of Alzheimer's Disease (AD) because of its cholinomimetic properties. Interestingly, a series of controlled clinical trials performed in Europe on Alzheimer's patients demonstrated that Acetyl-L-Carnitine, compared to placebo, was able to slow down the progression of the disease. This was demonstrated by the fact that there was less deterioration in cognitive function in the Acetyl-L-Carnitine group during the period of double-blind treatment. This action appears to be in contrast to nootropic compounds which may enhance cognitive function temporarily, while the underlying degenerative disease process continues to progress.

In particular, a one-year multicenter study on 130 AD patients showed a statistically significant "protection" of deterioration in subjects treated with Acetyl-L-Carnitine, compared to placebo, with respect to decline in cognitive function over time. The first controlled data obtained from clinical trials performed in the United States support these encouraging results. Details of results of changes in cognitive function data will be presented.

274.5

NEW INSIGHTS INTO THE MECHANISM OF ACTION OF ACETYL-L-CARNITINE (ALCAR) IN NEURO-DEGENERATION. A. Carta[1], M.T. Alderdice[2], G. Caruso*[1], A. Marchionni*[1], N. Benedetti[1], M. Calvani[1]. [1]Sigma-Tau S.p.A., Pomezia (Rome) Italy; [2]Sigma-Tau Pharmaceuticals, Gaithersburg, MD 20878, USA.

Acetyl-L-Carnitine, an endogenous compound with the primary function of assuring the availability of Acetyl-CoA, the key factor of mitochondrial metabolism, is currently under extensive evaluation in patients with Alzheimer's Disease. Animal studies have shown both in aged models of neuronal degeneration and in pathological models of neuronal damage that Acetyl-L-Carnitine prevents neuronal loss and protects cells from oxidative insults. Data on brain microvessels metabolism also yield new insights into a better understanding of the therapeutic activity of the compound.

Basic research data in animal models will be presented, along with discussions to help explain the basic cellular mechanism(s) of Acetyl-L-Carnitine.

274.6

A STUDY ASSESSING THE SAFETY AND EFFICACY OF PHOSPHATIDYLSERINE IN PATIENTS WITH MILD TO MODERATE ALZHEIMER'S DISEASE. M. Miernicki[a], M. Bradshaw[a*], W. Petrie[b*] & T. Crook[c*]. [a]FIDIA Pharmaceutical Corporation, Washington, DC 20006, [b]Vanderbilt University School of Medicine, Nashville, TN 37240 and [c]Memory Assessment Clinics, Inc., Bethesda, MD 20814.

Phosphatidylserine extracted from bovine cortex (BC-PS) has been shown to reduce age-related declines in certain cognitive functions in both animal models and open-label human trials. BC-PS also exerts modulatory influences on several neurochemical parameters that have been implicated in Alzheimer's disease (AD). Here we report the results of a double-blind, placebo-controlled clinical trial conducted to examine the safety and efficacy of BC-PS in patients with AD.

Fifty-one patients meeting the clinical criteria for AD were treated for 12 weeks with either BC-PS (100 mg capsules, t.i.d.) or placebo. BC-PS treated patients showed statistically significant improvement on objective cognitive performance tests and psychiatric rating scales compared to patients treated with placebo. An analysis of various subgroups suggested that patients with less severe cognitive impairments at the beginning of the study responded more favorably to BC-PS than did patients with more severe cognitive impairments.

These results suggest that BC-PS may be a promising candidate for further study in early, and perhaps prophylactic, treatment of AD.

274.7

PERFORMANCE IMPROVEMENTS IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE FOLLOWING TREATMENT WITH THE MUSCARINIC CHOLINERGIC AGONIST ARECOLINE. K.C. Raffaele, A. Berardi, S. Asthana,* P. Morris,* M.B. Schapiro,* J.V. Haxby,* and T.T. Soncrant. Laboratory of Neurosciences, National Institute on Aging, Bethesda, MD 20892.

Alzheimer's disease (AD) is consistently associated with deficits in cholinergic function. In the current study, 8 patients with probable or possible AD (NINCDS/ADRDA criteria) with mild or moderate dementia (MMSE scores 15-26, ages from 50-81 years) were treated with arecoline (a direct muscarinic cholinergic agonist) in a two part study. First, each patient received a 2-week continuous i.v. infusion of escalating doses of arecoline. Cognitive testing was performed at 5 doses (1, 4, 16, 28, and 40 mg/day). An optimal dose was selected individually for each patient and each patient was then retested in a double-blind crossover manner during 1-week infusions of the optimal dose and placebo. A significant improvement was found in verbal memory (Buschke selective reminding test, p=0.03) at low doses during dose-finding. That improvement was replicated for responders during the double-blind study. Improvement in figure-copying (p=.053) was also demonstrated during the double-blind study. Thus, improvement of verbal memory and of visuo-spatial function can be demonstrated in DAT patients in response to treatment with the direct cholinergic agonist, arecoline.

274.8

CHANGES IN VERBAL MEMORY DURING CHRONIC ARECOLINE INFUSION ARE CORRELATED INVERSELY WITH AGE IN ALZHEIMER'S DISEASE. T.T. Soncrant, K.C. Raffaele, A. Berardi, S. Asthana*, M.B. Schapiro*, J.V. Haxby*. Lab. Neurosci., NIA, NIH, Bethesda, MD 20892.

Preliminary results of a study employing chronic infusion of arecoline, a muscarinic cholinergic agonist, suggest that verbal memory can be improved by this drug in a majority of patients with dementia of the Alzheimer type (DAT). The present analysis was performed to identify predictors of a positive response. Eight patients (5 male, age 50-81, MMSE 15-26) underwent a 2 wk continuous i.v. infusion of arecoline, escalating from 0.2 to 40 mg/day. The dose producing optimal cognitive enhancement on 10 neuropsychological tests was then administered for 5 days in a double-blind, placebo-controlled, crossover design. Long-term recall (LTR), measured with the Buschke Selective Reminding Test, was improved ($P<0.05$) during dose finding in 6 of 8 subjects, with an inverted U-shaped relation to dose. During optimal dose infusion, 5 of 6 responders again improved (mean increase 6.0±1.0 [SEM] words). Change in LTR during optimal dose arecoline infusion was not correlated with MMSE or baseline LTR, but was inversely correlated with age ($r= -0.77$, $P<0.05$). Whether the greater cognitive responses of younger subjects to arecoline is due to differential pathophysiology of DAT by age or to underlying age-dependent changes in cholinergic function (independent of DAT) is unknown.

274.9

PHYSOSTIGMINE PHARMACOKINETICS FOLLOWING INTRAVENOUS ADMINISTRATION IN PATIENTS WITH DEMENTIA OF ALZHEIMER TYPE. **S. Asthana,* L. Hegedus,* N.H. Greig,* H.W. Holloway,* K.C. Raffaele, A. Berardi, M.B. Schapiro,* P. Pietrini, T.T. Soncrant. Lab of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.**

Physostigmine, a reversible cholinesterase inhibitor, temporarily influences memory in patients with dementia of the Alzheimer type (DAT). As part of a study to evaluate the effects on cognition of physostigmine administered by continuous i.v. infusion, its plasma pharmacokinetics were measured in four patients with DAT (age 63, 66, 72, 83; 2 males, 2 females). Physostigmine 0.025 mg/min was infused i.v. over 45-60 min. Venous blood samples were collected, at several times during and after completion of the infusion, into tubes containing an excess of the cholinesterase inhibitor octylcarbamoyl eseroline. Plasma was separated by centrifugation and assayed for physostigmine, using high pressure liquid chromatography. Plasma physostigmine levels rose to a peak of 11.9± 2.8 (S.E.) nM at completion of infusion, then fell monoexponentially with an elimination half-life of 17.9± 5.9 min. Thus, steady state plasma levels of physostigmine will be achieved only after at least 90 min. of continuous i.v. infusion of physostigmine.

274.10

Cholinomimetic Activity of RS86, AF102B and L-689,660 in Primates

NMJ Rupniak*, SJ Tye* and SD Iversen, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR, U.K.

Failure to demonstrate convincing beneficial effects of RS86 in Alzheimer patients may be attributable to constraints on dose escalation due to adverse cholinergic side-effects, including cardiovascular disturbances mediated via M_2 receptors. Muscarinic partial agonists with functional selectivity for M_1 and M_3 receptors might exhibit an improved therapeutic window but retain central cholinergic efficacy. We now compare the central and peripheral effects of RS86 with the M_1/M_3 partial agonists AF102B and L-689,660 ((-)-3-[2-(6-chloropyrazin)yl]-1-azabicyclo[2.2.2]octane) in primates.

Using a visuospatial task in rhesus monkeys, administration of RS86 (1.5 -2.25mg/kg i.m.) partially reversed a scopolamine-induced cognitive impairment. Animals exhibited pallor, tremor, salivation and emesis shortly after treatment with high doses. In squirrel monkeys, centrally mediated hypothermia was induced by 0.05mg/kg p.o. of RS86; intermittent emesis was the only observable side effect in this species ($\geq$ 0.25mg/kg p.o.). Unlike RS86, treatment with AF102B (up to 6mg/kg i.m.) failed to reverse the effects of scopolamine on cognition. Salivation was the most prominent side-effect. The minimum hypothermic dose of AF102B was 7mg/kg p.o.; emetic episodes were observed at a slightly higher dose (9mg/kg). In contrast, L-689,660 (0.05-0.3mg/kg i.m.) caused partial reversal of the effects of scopolamine on cognition. Transient salivation was the most marked side-effect. Hypothermia and emesis respectively, were induced by 0.01mg/kg and 0.05mg/kg p.o. of L-689,660.

In conclusion, central cholinomimetic activity comparable to that induced by RS86 may be achieved using the M_1/M_3 partial agonist L-689,660 in primates.

274.11

COMPARISON OF BRAIN TO PLASMA METABOLIC DISTRIBUTIONS OF TACRINE AND 1-HYDROXYTACRINE IN RAT. W. F. Pool*, S. M. Biorge*, B. Windsor*, A. Black, T. Chang*, and T. F. Woolf* Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, MI, 48106.

Tacrine (THA, Cognex, CI-970) and 1-hydroxytacrine (1-HOTHA) are acetylcholinesterase inhibitors currently under development for treatment of Alzheimer's disease. The following study was designed to assess the CNS penetration capabilities of THA and 1HOTHA in rats after single equimolar (79 umole/kg) oral doses of ^{14}C-THA or ^{14}C-1HOTHA. Animals were sacrificed at 0.5, 2, 4, and 8 hr with brain and plasma collected. Brain homogenates and plasma were analyzed by liquid scintillation spectrometry and profiled by HPLC radioactivity detection. Results clearly demonstrate that THA readily crosses the BBB and concentrates in the CNS. Rapid concentration of THA-derived radioactivity in brain was also observed in autoradiographic studies (McNally et al. this meeting). Brain to plasma THA concentration ratios between 5.2 and 6.4 were observed throughout the study. The major THA metabolite present in plasma, namely 1-HOTHA, was also detected in brain. Concentrations of 1-HOTHA in the CNS were lower than corresponding plasma concentrations throughout the study (0.2 to 0.7). Based on metabolic profiling data, app. 80% of THA-derived radioactivity in brain was comprised of unchanged THA. Brain to plasma 1-HOTHA ratios after 1-HOTHA dosing ranged from 0.3 to 1.1 with nearly 100 % of the radioactivity in brain corresponding to unchanged 1-HOTHA. In conclusion, THA concentrations achieved in rat brain were several fold higher than those observed for 1-HOTHA indicating greater CNS penetration capabilities of THA compared to 1-HOTHA.

274.12

A COMPARISON OF IN VIVO DISTRIBUTION OF RADIOACTIVITY IN RAT BRAIN FOLLOWING ORAL ADMINISTRATION OF TACRINE OR ITS 1-HYDROXY METABOLITE. W.P.McNally, P.DeHart*, W.Pool*, and T.Chang*. Parke-Davis Pharmaceutical Research, 2800 Plymouth Rd., Ann Arbor, MI 48105

A previous report on the distribution of tacrine(THA) in rat tissues (McNally et al. 1989) showed penetration of radioequivalents into brain with preferential uptake in gray matter, notably cortex, hippocampus, striatum and thalamus. In rats a major metabolite of THA is 1-OH-tacrine(1-HOTHA). However, Pool et al.(this meeting) have shown that following THA or 1-HOTHA dose the primary radioactive species in brain is parent drug. The present study was undertaken to compare, by quantitative autoradiography, regional disposition and relative concentration of ^{14}C-THA and ^{14}C-1-HOTHA radioequivalents in brain following equimolar oral dose. The table below provides comparative data (μg equivalents/g) for blood and several brain regions:

	30 MINUTES		4 HOURS	
	THA	1HOT	THA	1HOT
BLOOD	1.9	6.1	2.7	1.7
CORTEX	3.6	1.4	4.8	1.7
THALAMUS	3.5	1.3	4.5	1.6
STRIATUM	3.1	1.1	4.1	1.7
HIPPOCAMPUS	2.9	1.1	4.7	1.8
BRAIN STEM	2.5	1.1	2.8	0.9

1-HOTHA appeared to be more rapidly absorbed and reached higher blood levels than THA. Following administration of either substance radioactivity penetrated the blood-brain barrier, but levels in THA animals were up to 3 times higher than in those dosed with 1-HOTHA. At 24 hours postdose, no activity could be detected in brains of 1-HOTHA treated animals, while low but quantifiable levels were found in the THA group. No activity remained in brain at 48 hours for either compound, although blood levels were measurable through 96 hours. Tacrine appears to be better able to penetrate into brain tissue and may have a longer effective half-life there than 1-hydroxy tacrine.

274.13

DOES TACRINE BIND TO THE ACTIVE CENTER OF ACETYLCHOLINESTERASE (AChE)? C.J. Moore, V.E. Gregor*, C. Lee* and M.R. Emmerling. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105

The anticholinesterase Tacrine (THA, Cognex®, CI-970) is a potential palliative therapy for Alzheimer's Disease. Tacrine is a reversible, noncompetitive inhibitor of electric eel AChE (Ki = 17 nM). The relation of Tacrine binding to the active center of eel AChE was studied using irreversible inhibitors that covalently bind to the catalytically active serine of the enzyme. We observe that Tacrine protects AChE in a dose-dependent manner from inhibition by diisopropyl fluorophosphate (DFP) and paraoxon (diethyl-4-nitrophenylphosphate), but not from the smaller methanesulfonyl fluoride (MSF). An active center inhibitor, N-methylacridinium, which has a structure similar to Tacrine, also protects from DFP, but not MSF. In contrast, the active center inhibitors edrophonium and galanthamine protect against both MSF and DFP in a dose-dependent fashion. Addition of bulk to the saturated ring of Tacrine can increase its protection of AChE from MSF. PD 141078 (1,2,3,4-tetrahydro-1,4-methanoacridin-9-amine) protects from MSF inhibition of AChE in a manner similar to edrophonium and galanthamine. Both N-methylacridinium and Tacrine antagonize PD 141078 protection of AChE from MSF. The data imply that Tacrine binds to the active center of AChE, protecting the enzyme from irreversible inhibitors through steric hindrance. Tacrine binding appears to overlap with that of N-methylacridinium, leaving the serine in the active center accessible to small irreversible inhibitors. The active center binding of Tacrine agrees with results from the circular dichroism studies of Wu and Yang (1990, Mol. Pharm., 35, 85) and from the enzymological studies of Dr. Harvey Berman at SUNY in Buffalo (personal communication).

274.14

THE EFFECTS OF ALLOSTERIC INHIBITORS ON TACRINE BINDING TO ACETYLCHOLINESTERASE (AChE). M.R. Emmerling, C.J. Moore, V.E. Gregor*, and C. Lee*. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105

Tacrine (THA, Cognex®, CI-970) is an anticholinesterase which is reported to improve the cognitive deficits associated with Alzheimer's Disease. Our previous study (see preceding abstract) suggests that Tacrine binds to the active center of electric eel AChE, based upon its almost complete protection of the enzyme from inhibition by diisopropyl fluorophosphate (DFP). In contrast, the allosteric inhibitors propidium and gallamine, which bind to sites on AChE peripheral to the active center, only partially protect (about 40%) AChE from DFP inhibition under the same assay conditions. Gallamine and propidium, at doses up to 10 mM and 100 μM, respectively, do not antagonize protection from DFP in the presence of Tacrine, or in the presence of the active center inhibitors edrophonium and galanthamine. This lack of antagonism is consistent with the active center inhibitors binding to sites on AChE different from the site to which the allosteric inhibitors bind. Gallamine and propidium, however, do produce a dose-dependent decrease in the protection of AChE by edrophonium and galanthamine from irreversible inhibition by methanesulfonyl fluoride (MSF). The allosteric inhibitors also antagonize protection from MSF by the Tacrine analog PD 141078 (1,2,3,4-tetrahydro-1,4-methanoacridin-9-amine). Because they have no effect on protection from DFP, it seems unlikely that the allosteric inhibitors antagonize protection from MSF by displacement of PD 141078, edrophonium, or galanthamine from the active center of AChE. Thus, the observed antagonism of MSF protection in the presence of active center inhibitors may result from the allosteric inhibitors changing the conformation of the active center, making the active center serine more accessible to small irreversible inhibitors.

274.15

INTERACTIONS BETWEEN NEUROPEPTIDES AND ACETYLCHOLINESTERASE (AChE). R.T. Carroll, T.W. Hepburn* and M.R. Emmerling. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105

A number of neuropeptides from the enkephalin, dynorphin and tachykinin families were tested for their ability to inhibit electric eel AChE activity. Only Big Dynorphin, α-neoendorphin, BAM-22P, and Substance P inhibit AChE activity with IC_{50}s of 100 μM or less. The effect of the interaction with purified AChE on the neuropeptides was studied by incubating the peptides (30 μg) with affinity purified eel AChE (5 μg) at 37°C for up to 24 hrs. Samples were withdrawn from the mixture at various times during the incubation and subjected to reverse-phase high pressure liquid chromatography (HPLC). No detectable effect by AChE on Substance P is seen. Slight digestion of α-neoendorphin is detected only after 24 hrs. Digestion products in the mixture containing Big Dynorphin are present at 4 hrs and complete loss of the parent peptide occurs after 24 hrs. Cleavage products of BAM-22P appear after 1 hr, and parent peptide is lost completely by 24 hrs. Digestion of BAM-22P does not occur spontaneously, and is dependent upon the amount of AChE added and the time of incubation. The turnover rate for BAM-22P is slow, about 0.05 sec^{-1}. Other met-enkephalin containing peptides (Met-enkephalin-RRVG and Peptide E) are also hydrolyzed upon incubation with purified AChE. These results indicate that inhibition of AChE activity by neuropeptides is not predictive of their hydrolysis by the enzyme preparation. Moreover, our studies show that the neuropeptidase activity is not unique to the purified AChE but is also present in abundance in the starting material used for AChE purification. Thus, our data do not exclude the possibility that the digestion of neuropeptides by purified AChE results from contaminating proteases rather than by AChE itself.

PARKINSON'S DISEASE: ANIMAL STUDIES

275.1

A HISTOCHEMICAL ANALYSIS OF THE BRAINS FROM MPTP-TREATED ANIMALS. J. Harper, R. Philip, J.S. Schneider and F.J. Denaro. Slippery Rock University, Slippery Rock, PA 16057. Dept. of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430. Dept. of Neurology, Hahnemann University, Philadelphia, PA 19102.

A battery of histochemical stains were used to analyze the neuropathology of MPTP treatment across species. Mouse (C-57, Swiss-Webber), rat, cat and monkey were studied. Periodic Acid Schiff revealed granular inclusion bodies in some residual neurons in the substantia nigra of the monkey. Hematoxylin and eosin stains revealed variable gliosis in the caudate/putamen and substantia nigra of all animals studied confirming the findings of previous studies using antibodies against glial fibrillary acidic protein (GFAP) (*J. Neuropath. & Exp. Neurol.* Vol. 47, #4, 1988). Chromatolysis and neurophagia were found for some residual neurons of the substantia nigra in the cat and monkey, but not in the mouse or rat. Bielschowsky stain, which provides a sensitive measure of plaques and neurofibrillary tangles, did not reveal any such neurocytological changes in striatal or nigral neurons in any of the species examined. Immunocytochemistry to ubiquitin revealed positively stained neurons in the substantia nigra in the cat and monkey. Occasional ubiquitin positive neurons were also found in the mouse. In all cases ubiquitin immunopositivity was not associated with inclusion bodies. C-fos immunocytochemistry has been examined in the acute mouse model. Animals were treated with MPTP for one week and were sacrificed within 24 hours of their last MPTP injection. Under these conditions, some neurons of the substantia nigra were found to be C-fos positive. The production of C-fos by these nigral neurons may be a response to the loss of their terminal fields due to MPTP.

275.2

EXTENSIVE LOSS OF BRAIN SEROTONIN INDUCED BY MPTP IN THE MARMOSET. J. Del Río, C. Oset*, I. Pérez-Otaño*, M.L. De Ceballos*, M.T. Herrero*, R. Luquin and J.A. Obeso. Departments of Pharmacology and Neurology, University of Navarra, Pamplona and Cajal Institute, CSIC, Madrid, Spain.

The neurotoxic effects of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) on several non-dopaminergic brain neurotransmitter systems are controversial. In the present study, MPTP (1.25-2.5 mg/kg s.c.) was given twice a week for 5 or 10 consecutive months to common marmosets (n = 6) and the animals were sacrificed 15 days or 6 months later. Six other marmosets served as controls. At any of the two survival times, MPTP produced a strong depletion of serotonin (5-HT), approximately 90%, in all regions studied: caudate nucleus, putamen, n.accumbens, hypothalamus, septum, amygdala, frontal and cingulate cortex. Fifteen days after the chronic treatment, 5-hydroxiindoleacetic acid (5-HIAA) concentration was also decreased by 90% in the striatum, the depletion being less profound, about 75%, in all other regions. At 6 months, the 5-HIAA decrease was lower, especially in extrastriatal regions. The 5-HIAA/5-HT ratio, an index of 5-HT turnover, was much higher than in control monkeys at this time. The results suggest that a treatment schedule with MPTP involving multiple injections spread out over months may induce lasting serotonergic lesions in the marmoset brain. (Supported by grants from E.E.C., Schering España S.A. and Fundación J.A.L.S.).

275.3

EFFECTS OF MPTP ON SELECTIVE DOPAMINERGIC FIBERS IN THE STRIATUM AND ON NEURONS IN THE PARS COMPACTA. Y. Gohda, D.L. Felten and S.N. Haber, Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642

Systemic injection of MPTP damages the dopaminergic(DA) nigrostriatal system in C57BL/6 mice. Recently it has been demonstrated that there are variations in the extent of DA terminal injury between striatal regions. To determine the relationship between the pattern of TH-immunoreactive(IR) fiber depletion in the striatum and the subset of nigral neurons involved, we injected WGA-HRP after MPTP treatment. MPTP (4x20mg/kg i.p.) was given to young adult C57BL/6 mice at 3 h intervals. Three days after treatment WGA-HRP was injected iontophoretically into the different regions of the striatum, and into control mice. They were sacrificed 2 days after WGA-HRP injection.

As expected, MPTP treatment resulted in the disappearance of TH-IR fibers in the striatum. However, regional pattern of TH-IR fiber depletion was more complex than a simple dorsal-to-ventral gradient. The central portion of the striatum was preferentially affected by MPTP, often leaving the dorsolateral rim rich in TH-IR fibers. After injections of WGA-HRP into this central striatal region in MPTP treated mice, fewer labelled cells were found in the central nigra than in control mice. These observations indicate that the regions within the dorsal striatum may be differentially affected by MPTP and that this may be a reflection of degeneration of a subset of DA neurons within the A9 region of the substantia, pars compacta. Alternatively the lack of labelled cells may be the results of interference with the transport mechanism of the effected cells. Supported by NIH NS22511, NIH AG06060 and the PEW Foundation.

275.4

SPONTANEUS RECOVERY OF THE MPTP-DAMEGED CATECHOLAMINE SYSTEM$ IN GOLDFISH BRAIN AREAS. A. Poli, O. Gandolfi, R. Rimondini*, L. Villani* and O. Barnabei*. Dept. of Biology, University of Bologna, 40126-Bologna, Italy.

We have previously reported that the neurotoxin MPTP (10mg/Kg i.p.for 3 days)causes a marked disappearance of tyrosine hydroxylase immunoreactivity and degeneration of neurons in several areas of the goldfish brain, with concomitant depletion of DA and NA levels. To better assess damage we measured a number of different dopaminergic parameters at 3 days and six weeks following cessation of MPTP administration. At 3 days, a marked fall in high affinity [3H]-dopamine uptake by synaptosomal preparations of telencephalon (50%), diencephalon (68%) and medulla (46%) was observed, together with increase number of postsynaptic D2 receptors labelled by [3H]-spiperone. Surprisingly at six weeks, the MPTP - induced depletion of DA and NA, and the fall in 3H - dopamine uptake were completely reversed in all dameged areas. In addition, no changes were found in Bmax for [3H]-spiperone as compared to controls. All these results suggest that catecholamine systems in goldfish brain show a remarkable power of recovery after MPTP lesion.

275.5

Kinetics of Elimination of MPP+ in the MPTP-treated Squirrel Monkey. I. Irwin, L.E. De Lanney*, D. D. DiMonte* and J. W. Langston*. California Institute for Medical Research and California Parkinson's Foundation, San Jose, CA 95128.

We have previously reported the behavioral, neurochemical and neuropathological effects of MPTP on a group of squirrel monkeys (Br Rsrch, 1990). Because the effects of MPTP are thought to be mediated by the toxic metabolite MPP+, we undertook the analysis of MPP+ in 18 brain regions and 8 peripheral tissues of these animals. The purpose of this study was to characterize the distribution and kinetics of elimination of MPP+ to detect and evaluate the relationship to toxicity. All animals received a single 2.5 mg/kg, subcutaneous injection of MPTP. Groups of animals (n=3/group) were sacrificed 1, 3 or 5 days after MPTP and MPP+ was assayed using GC/MS. There were striking differences between the patterns of elimination for MPP+, both inside and outside the CNS, with tissues falling into one of two groups. Seventeen of the 24 tissues studied exhibited first order kinetics of elimination, with half-lives being between 16 and 24 hours. In contrast, the caudate, putamen, substantia nigra, globus pallidus, nucleus accumbens, heart and adrenal medulla all showed virtually no elimination of MPP+ between 1 and 5 days. Indeed, the concentration of MPP+ was found to increase in some of these tissues. Analysis of MPTP-induced neurotransmitter changes in certain brain and peripheral catecholaminergic tissues, and the values for peak concentrations, clearance or area under the curve for MPP+, failed to reveal simple relationships between concentration and toxicity.

275.6

GM1 GANGLIOSIDE THERAPY REVERSES PARKINSONIAN MOTOR DEFICITS IN MPTP-TREATED MACAQUE MONKEYS. Anne Pope and J.S. Schneider, Dept. of Neurology, Hahnemann Univ., Phila., PA. 19102.

GM1 ganglioside has been shown to reverse motor deficits in dopamine lesioned rats and to accelerate functional recovery in cats made parkinsonian by MPTP. Due to certain limitations of the previously mentioned models, the present study was conducted to assess whether GM1 ganglioside might have therapeutic effects on MPTP-induced model of parkinsonism in the macaque monkey. Prior to MPTP administration, five cynomolgus monkeys were rated on a 19 item behavioral/neurological assessment and performed a reward retrieval task (in which the time taken to remove raisins placed in 16 holes on a tray was measured) and an object retrieval task which assessed both motor and cognitive abilities. After collection of normal data, monkeys received MPTP-HCl (0.35 mg/kg, i.v.) until they became severely parkinsonian. Three monkeys were randomly chosen to receive GM1 treatment and two to receive saline blindly in daily injections begun 48 hrs. after the last MPTP dose. All monkeys became severely parkinsonian and needed to be maintained on dopamine D2 agonist therapy to sustain life. GM1-treated monkeys recovered sufficiently to be taken off agonist therapy after 14 days (±6) while control animals needed to be maintained on agonist therapy for 28 days (±4). Behavioral/neurological scores for GM1-treated animals improved consistently over the 8 wk. treatment period and by wk. 3 of the study, these animals appeared significantly different from control animals. GM1-treated monkeys improved significantly on motor task performance and showed no cognitive deficits while control monkeys performed tasks poorly or not at all. Preliminary examination of the striatum by tyrosine hydroxylase immunohistochemistry has shown evidence of a possible sprouting response in GM1-treated animals. These data suggest that GM1 therapy may be effective in reversing parkinsonian symptoms in primates and may stimulate repair of the nigrostriatal dopamine system in this species. The results suggest the potential usefulness of trophic factor therapies for the treatment of degenerative disorders such as Parkinson's disease. Supported by the National Parkinson Fdn. and Fidia Research Laboratories.

275.7

GM1 GANGLIOSIDE TREATMENT PROMOTES RECOVERY BUT DOES NOT PROTECT FETAL DOPAMINERGIC NEURONS FROM MPP+-INDUCED DAMAGE. N. Stull, L. Iacovitti, L. DiStefano and J.S. Schneider, Dept of Neurology, Hahnemann University, Philadelphia, PA 19102

Exposure of fetal rat dopaminergic neurons to MPP+ causes the death of some neurons and largely irreversible damage to others. The present study was performed to examine whether MPP+-induced damage to such cultures could be reversed or prevented by GM1 ganglioside treatment. The dopaminergic ventral mesencephalon was dissected from E15 rat embryos and plated at a density of 1x106 cells/well. Control cultures were maintained for a total of 9 days in standard media containing heat inactivated fetal calf serum. To assess the reparative effects of GM1, cells were grown for 3 days in standard media before supplementation with MPP+ (100μM) for an additional 3 days. Some cultures served as lesion controls and the toxic media was replaced with standard media for a final 3 day incubation. In others, the toxic media was replaced with standard media supplemented with 100μM GM1. Possible protective effects of GM1 were assessed in cultures pre-incubated in GM1-supplemented media for 2 days prior to incubation with toxin and standard media as described above. All cultures were then fixed and processed for the immunocytochemical localization of tyrosine hydroxylase (TH). Exposure to MPP+ only resulted in a 41% (± 11) loss of TH positive neurons compared to controls. In contrast, the loss of TH neurons was reduced to only 12.5% (± 9.5) in cultures treated with GM1 after MPP+ exposure. Cultures pre-treated with GM1 before MPP+ contained 31% (± 8.5) fewer TH-positive neurons than controls. TH activity (pmol/culture/hr) was greatly decreased in MPP+ cultures (1.87 vs. 4.60 in normal cultures), minimally protected in GM1-pretreated cultures (2.03) and greatly restored (3.45) in cultures treated with GM1 after MPP+ exposure. Measures of dopamine from these cultures showed a similar pattern. These data suggest that, under the present experimental conditions, pre-treatment of dopaminergic cultures with GM1 has minimal protective effects against MPP+ toxicity. Nonetheless, GM1 ganglioside administered after toxic insult may have significant reparative effects on dopamine neurons. Supported by Fidia Research Laboratories.

275.8

EFFECTS OF LONG-TERM MK-801 TREATMENT ON NEUROTOXICITY OF METHAMPHETAMINE AND MPTP IN MICE AND RATS. P.K. Sonsalla, L. Manzino and A. Giovanni, Dept. of Neurol., UMDNJ-RWJ Med. Sch., Piscataway, NJ 08854

Both MPTP and methamphetamine (METH) are neurotoxic to nigrostriatal dopaminergic neurons. We have previously shown that MK-801, an antagonist at N-methyl-D-aspartate receptors, prevents METH- but not MPTP-induced neurotoxicity in ***mice***. However, it has recently been reported that long-term treatment (24 h) of ***rats*** with MK-801 prevented nigral cell loss produced by an intranigral infusion of MPP^+, the neurotoxic metabolite of MPTP. Possible reasons for the apparent discrepancies of these findings are: 1) an inadequate duration of MK-801 effects in our MPTP mouse studies, 2) different mechanisms responsible for neurotoxicity produced by an intranigral bolus of MPP^+ versus systemic MPTP whereby MK-801 exerts some protective effect in the former situation or 3) a rat versus mouse difference in response to MK-801. To test these possibilities, ***mice*** received MK-801 (6 injections, 4 h apart) starting just before the first of 4 injections of MPTP at 2 h intervals. This treatment enhanced lethality and only slightly attenuated the neurotoxic effects of MPTP in the survivors (as measured by neostriatal DA levels and tyrosine hydroxylase activity one week later). To determine if rats differed from mice in their response to the protective effect of MK-801 against METH-induced neurotoxicity, ***rats*** were treated with MK-801 just prior to each injection of METH (5 injections, 6 h intervals). This treatment with MK-801 protected the ***rats*** against the METH-induced loss of neostriatal DA and TH activity. In contrast, long-term MK-801 treatment failed to attenuate nigral or striatal DA loss following intranigral MPP^+ (see Giovanni et al., this meeting). Thus, we conclude that MK-801 provides little if any protection against MPTP or MPP^+ in mice or rats, although it protects against METH toxicity in both species.

275.9

FAILURE OF MK-801 TREATMENT TO PROTECT AGAINST MPP⁺-INDUCED NEUROTOXICITY IN RATS. A. Giovanni, L. Manzino, G.D. Zeevalk, R.E. Heikkila and P.K. Sonsalla. Dept. of Neurol., UMDNJ-RWJ Med. Sch., Piscataway, NJ 08854

It has recently been reported that long-term MK-801 administration to rats can prevent the loss of dopaminergic neurons in the substantia nigra produced by the intranigral infusion of MPP⁺, the neurotoxic metabolite of MPTP. In this study we have examined the effects of long-term MK-801 treatment on MPP⁺-induced neurotoxicity in both the nigra and the striatum following either an intranigral or an intrastriatal infusion of MPP⁺. One week following an intranigral infusion of MPP⁺, there was a marked decrement in both neostriatal and nigral DA content. MK-801 did not prevent this effect of MPP⁺ in either brain area. In addition, we examined the selectivity of the lesion induced by intranigral MPP⁺ infusion and observed decrements in GABA and serotonin levels in the nigra at one week post infusion, indicating damage to these neurotransmitter systems. Furthermore, MK-801 treatment failed to alter the MPP⁺-induced decrements in serotonin and GABA. Intrastriatal infusion of MPP⁺ via a microdialysis probe (10 mM, 15 min) resulted in a dramatic increase in dopamine efflux (150x basal levels) which could not be repeated upon re-exposure to MPP⁺ the following day. The fact that subsequent exposure to MPP⁺ did not elicit a dramatic rise in dopamine efflux indicated a loss of dopaminergic nerve terminals. MK-801 treatment failed to block both the initial increase in dopamine efflux seen upon exposure to MPP⁺ and the dopaminergic nerve terminal loss assessed by the subsequent re-exposure of the striatum to MPP⁺. We conclude that, under these conditions, MK-801 provides no protection against MPP⁺-induced dopaminergic neurotoxicity in the nigra or the striatum.

275.10

INTERACTION OF R(+)MK-801 AND COCAINE IN THE ROTATIONAL RESPONSE TO D1 AND D2 AGONISTS BY RATS WITH 6-OHDA LESIONS. P. Curzon and D.R. Britton. Neuroscience Research, 47U, Pharmaceutical Discovery, Abbott Labs., Abbott Park, IL 60064

Rats with 6-OHDA lesions were tested for rotation to various dopamine agonists following pre-treatment with the NMDA non-competitive antagonist, MK-801, or cocaine at doses which, by themselves, produced equivalent ipsilateral rotation. MK-801 at 0.3, but not at 0.1 umol/kg, sc decreased contralateral rotation to the D2 agonist, LY171555 (0.6 umol/kg, sc) ($p<.05$). The rotation response to the D1 agonist , SKF38393 (0.75 umol/kg, sc) was increased ($p<.05$) by MK-801 at 0.1 but not at 0.3 umol/kg, sc. Cocaine (50 umol/kg, ip) was without effect on the response to either LY171555 or SKF38393. The rotation response to the full D1 agonist, A68930 (0.75 umol/kg, sc) was enhanced ($p<.05$) by MK-801 (0.1 umol/kg, sc). The response to l-dopa (125 umol/kg, po) which acts through D1 and D2 receptors was not altered by pre-treatment with MK-801. These findings are consistent with those of Morelli and Di Chiarra (1990) and suggest differential effects of MK-801 on D1 and D2 responses in lesioned rats.

275.11

METHYLATION OF DOPAMINE BY 1-METHYL-4-PHENYLPYRIDINIUM (MPP^+) BUT NOT 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP): A POSSIBLE MECHANISM OF ACTION. C. Charlton. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

MPTP and MPP^+ caused Parkinson's disease-like toxicity, but analogs lacking the N-methyl group are essentially devoid of toxicity, which means that the methyl group of the pyridine ring plays a role in the toxicity. This is of interest because S-adenosylmethionine (SAM), which is the endogenous methyl donor and requires a methyl group for its action also caused MPP^+-like motor deficits. Therefore, the requirement of a methyl group by MPP^+ for it action suggest that, like SAM, MPP^+ may serve as a methyl donor. This hypothesis was tested by reacting SAM, MPP^+ or MPTP with dopamine (DA) in the presence of catechol-O-methyltransferase. The results showed that similar to SAM, MPP^+ but not MPTP, donated a methyl group to DA to produce 3-methoxytyramine (3-MT). MPP^+ and SAM at 62.5, 250 and 1000 nmoles/tube increased the 3-MT recovered by 13.9, 50.58, 121.31 and 6.88, 44.55, 129.47 nmoles above controls, respectively. These values are equivalent to 7.3, 28.1, 67.3 and 3.2, 25.1, 72.8 %. DA was proportionately decreased. These findings suggest that the ability to serve as a methyl donor may represent a mechanism of action for MPP^+, the active metabolite of MPTP. These are additional support for the possible involvement of methylation in parkinsonism.

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275.12

THE INDUCTION OF METHIONINE ADENOSYL TRANSFERASE (MAT) AND CATECHOL-O-METHYLTRANSFERASE (COMT) BY L-DOPA: DEPENDENCE ON FREQUENCY AND DURATION OF ADMINISTRATION. R. Benson, B. Hill*, K. Doonquah*, and C. Charlton. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

When injected into the lateral ventricles of mice S-adenosylmethionine (SAM), an endogenous methyl donor, causes Parkinson's disease (PD)-like symptoms. Hence, the positive effects of l-dopa therapy may be due to its conversion to dopamine (DA), as well as, the ability of l-dopa and DA to utilize SAM. Protracted exposure to l-dopa may increase the production of MAT and COMT which may relate to the decreased efficacy of chronic l-dopa therapy. As a partial test for this hypothesis, we exposed mice to several schedules of l-dopa treatments and determined the brain activities of MAT and COMT. using chromatographic, spectrophotometric and radioenzymatic methods.

L-dopa (200mg/kg) treatments of 1, 2, and 3 times/day for 4 days increased MAT activity by 0.2%, 4.9% (not significant) and 21.3%, respectively. A treatment of 3 times/day for 8 days further increased (28.4%) MAT activity (Vmax). The L-dopa treatments of 1 and 3 times/day for 4 days increased the COMT activity by 27.9% and 29.1% above that of the pooled controls. These results show that high frequency and chronic l-dopa treatments will induce increases in the activities of MAT and COMT. This may help to explain the decreased efficacy of chronic l-dopa therapy in PD patients. (Supported by: NIH RCMI #RR3032, NSF RII-8704121 and NSF 871-4805).

275.13

S-ADENOSYL-METHIONINE (SAM)-INDUCED HYPOKINESIA BLOCKED BY L-DOPA BUT NOT D-DOPA: RELEVANCE TO PARKINSON'S DISEASE (PD). B.Crowell,Jr.,*S.Spector and C.Charlton. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208 and *Dept. of Psychiatry, Vanderbilt University, Nashville, TN 37232

The symptoms of PD include hypokinesia, tremor and rigidity. SAM, when injected into the lateral ventricles of rats caused PD-like symptoms. For the effects of SAM to represent a model for PD, the PD therapeutic agent, L-dopa, should antagonize the effects of SAM. We tested the "SAM induction of PD Model" by studying the ability of L-dopa and D-dopa to block the hypokinetic effects of SAM.

Rats were anesthetized, cannulated and injected, i.p., with L-dopa, D-dopa or saline and challenged, icv, with hypokinetic doses of SAM or saline. SAM dose-dependently decreased motor activity in the rats. The onset was within 1 min and lasted for about 1.5 hr. At 1 hr 1.0 µmole of SAM decreased the mean distance traveled (TD) and the number of movements by 65 and 75%. SAM at 6.25 nmoles/rat was without effect, whereas 9.38, 50 and 400 nmoles decreased the TD by 65, 80 and 94.9%, respectively. The effects of the 50 nmoles of SAM was 40% blocked and completely reversed by 200mg/kg of L-dopa, given 2 hr and 4 hrs before SAM. At the same pretreatment times 400 mg/kg of L-dopa completely blocked the effect of SAM. D-dopa had no effect.

The study showed that SAM, at low doses can induce hypokinesia, which may represent a model for PD. L-dopa is an effective therapy for PD and also antagonizes the effect of SAM. D-dopa, the inactive stereoisomer, had no effect. The time and dose-dependent effects of l-dopa are probably related to the rate of absorption for achieving the effective brain level.

(Supported by:NIH RCMI #RR3032, NSF RII-8704121 and NSF 871-4805)

275.14

STRAIN-DEPENDENT LOSS OF NIGROSTRIATAL DOPAMINE NEURONS AFTER 3-ACETYLPYRIDINE TREATMENT OF RATS. Ann C. Smith[1], Menek Goldstein[2], and Ariel Y. Deutch[1]. [1]Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06510 and [2]Department of Psychiatry, NYU Medical Center, NY, NY 10016.

We have previously demonstrated that 3-acetylpyridine (3-AP) treatment of rats results in a loss of the dorsolateral striatal dopamine (DA) innervation, as well as lesioning the cerebellar climbing fiber innervation. However, the number of DA neurons in the substantia nigra (SN) of rats sacrificed 6 weeks after 3-AP treatment is not decreased, despite a 40% decline in DA concentrations in the dorsolateral striatum. In an attempt to determine if the presence of midbrain DA perikarya in the face of terminal field degeneration represents a slow retrograde degeneration which ultimately involves the perikarya, or alternatively is due to strain differences, we treated Sprague-Dawley (SD) albino rats (in which our previous studies had been conducted) with 3-AP and sacrificed these animals one year later. We also compared the effects of 3-AP administration to SD and Long-Evans (LE; pigmented) rats on the nigrostriatal dopamine system. No appreciable loss of midbrain DA neurons in SD rats was seen one year after 3-AP treatment, although a significant decline in striatal DA was observed. In SD animals examined one week after 3-AP adminstration there was also no significant loss of DA neurons. In contrast, LE rats sacrificed one week after 3-AP treatment had a marked loss of SN DA neurons, and a decrease in striatal DA concentrations. Supported by the Amer. Park. Dis. Assn. and by the NPF Center at Yale University.

275.15

EQUINE NIGROPALLIDAL ENCEPHALOMALACIA (ENE) AND *CENTAUREA SOLSTITIALIS* (CS) : SEARCH FOR CULPABLE NEUROTOXIN(S). D.N. Roy, M. Craig*, L. Blythe*, M. Lefor*, M. Seelig*, R.E. Kayton* and P.S. Spencer. Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR 97201 (DNR, ML, MS, REK, PSS), and School of Veterinary Medicine, Oregon State University, Corvallis, OR 97331 (MC, LB).

The basal ganglia disorder ENE manifests as somnolence, hypokinesia, and impaired mastication after heavy ingestion of the Yellow Star Thistle (CS), a neurotoxic weed native to the western United States. Although cattle are refractory, entry of the neurotoxin(s) into milk consumed by humans is not excluded. To isolate and evaluate the culpable CS agent(s), dried weed was homogenized in 30% ethanol and the supernatant subjected to Dowex-50W-H^+ column chromatography, with 0.5M ammonia elution. Eluates (designated by pH ranges) were administered to mouse cortex explant cultures to evaluate neurotoxic potential. Crude CS extracts and Fraction VI (pH 8-9) induced edematous vacuolation, chromatin clumping, and dense shrunken neurons, a picture compatible with excitotoxicity. Fraction VI (most active) contains 10-12 ninhydrin-positive spots identified by two-dimensional thin-layer chromatography. While identification of the active neurotoxic principle(s) is in progress, it seems unlikely that this fraction contains the CS sesquiterpene lactones identified by Ying Wang et al. (*Helvetica Chimica Acta* 74: 117, 1991) as toxic for rat fetal brain cultures. [Supported by NS-19611]

275.16

DEGENERATION OF NIGRO-STRIATAL NEURONS INDUCED BY FREE IRON. G.J. Sengstock*[1], C.W. Olanow[2], R. Menzies[3], A.J. Dunn[4] and G.W. Arendash[1]. Depts. of Biology[1], Neurology[2] and Psychiatry[3], University of South Florida, Tampa, FL 33620 and Dept. of Pharmacology[4], LSU Medical Center, Shreveport, LA 71130.

Oxidation reactions have been implicated in the pathogenesis of **Parkinson's Disease** (PD). Postmortem PD brains have increased quantities of iron within the substantia nigra (SN). Since iron facilitates oxidation reactions resulting in free radical formation, iron may be a causative factor in the degeneration of nigro-striatal dopaminergic neurons seen in PD. To mimic this spontaneous accumulation of iron in the SN, we infused various amounts of iron (0.63 - 7.3 nmol) unilaterally into the SN of young adult male rats utilizing two separate surgical protocols *(Double and Side-by-Side Infusions)*. The iron was combined in an iso-osmotic, pH-balanced, citrate-bicarbonate-Tris-HCl buffer solution. Animals were sacrificed through two months post-infusion. Histological examination of thionine- and iron- stained *(PERL's+DAB intensification)* brain sections revealed iron diffusion essentially limited to the infused SN and a selective degeneration of neurons within the zona compacta of SN at low-moderate iron amounts; surviving zona compacta neurons stained for increased iron. Consistent with the degree of neuronal loss, **striatal dopamine** and its catabolites *(DOPAC and HVA)* were significantly reduced in a dose-dependent fashion (by over 80% at the highest iron amounts). Interestingly, animals infused with an iron solution containing excess transferrin did not have decreased striatal dopaminergic markers. Non-dopaminergic markers in striatum were largely unaffected by free iron infusions into SN. In addition, **apomorphine-induced rotational behavior** was evaluated; animals receiving higher amounts of iron exhibited a 5-fold increase in ipsilateral turning compared with vehicle-infused controls. Furthermore, the number of ipsilateral turns shown by high iron-infused animals were significantly greater at two months than at one month post-infusion. These data indicate that neurons within the SN, particularly those of the zona compacta, are sensitive to free iron-induced degeneration; thus, the data are supportive of iron as an etiological factor in the pathogenesis of PD.

275.17

THE EFFECT OF ANTI-PARKINSONIAN MEDICATION ON THE ADRENAL MEDULLA OF THE MOUSE. S.L. Stoddard, G.M. Tyce, J.A. Cook, G.J. Merkel*, A.R. Zinsmeister*, and S.W. Carmichael, Depts. of Anatomy and Microbiology, Indiana University School of Medicine, Fort Wayne, IN 46805, and Depts. of Physiology, Biostatistics, and Anatomy, Mayo Clinic, Rochester, MN 55905.

We have previously shown that catecholamines are significantly lower in the adrenal medulla of parkinsonian patients (Stoddard et al., *Exp. Neurol. 104*:22 and 218, 1989). These patients had been taking L-dopa and carbidopa (Sinemet®) for an average of 11 years. To investigate whether chronic drug treatment with Sinemet® could alter adrenal medullary catecholamine levels, we fed male C57Bl mice L-dopa and/or carbidopa at ten times a common clinical dose (on a per weight basis) for up to 4 months (16% of expected life span; equivalent to 11 human years). The animals were sacrificed at monthly intervals; the adrenal glands were removed and analyzed for catecholamines using HPLC with electrochemical detection. Catecholamine levels were expressed as ng/adrenal pair. There were no differences in the content of norepinephrine or epinephrine with any drug treatment at any time. Dopamine content was significantly elevated at 12 weeks in the mice receiving both L-dopa and carbidopa. These results suggest that the decreased medullary catecholamines observed in patients do not result from chronic Sinemet® treatment.

275.18

IRON CHELATION MODIFIES 6-HYDROXYDOPAMINE (6-OHDA) PARKINSONIAN ANIMAL MODEL. J. Hubble, F. Shi, and B. Skikne and W. Koller. University of Kansas Medical Center, Kansas City, KS 66103.

Iron may play a role in the pathogenesis of Parkinson's disease by participating in the generation of free radicals, ultimately leading to nigral cell death; if this hypothesis is correct, relative depletion of brain iron may protect against nigrostriatal degeneration. To test this concept, we gave the iron chelator desferrioxamine (250mg) via s.c. pump over 7 days to rats (n=8). 6-OHDA was injected into the left substantia nigra on day 4. Apomorphine (.25mg/kg s.c.) was given on day 25 to assess turning behavior (reflecting unilateral dopamine [DA] depletion). Striatal iron and DA content was quantitated. Non-chelated 6-OHDA lesioned rats served as controls. Controls turned more than chelated rats (means:182 vs 65 turns/10min, $p \leq .065$, Student t-test). Striatal iron was less in the chelated group than in controls (mean ugFe/mg protein: left chelated=.50, right chelated=.52, left control=.88, right control=.82; $p<.05$ ANOVA & Newman Keuls post-hoc). Right striatal DA content did not differ between groups; left striatal DA was greater in the chelated vs controls but did not reach significance by ANOVA (mean DA pmole/mg protein: left chelated=182, right chelated=638, left control=65, right control=718). This latter finding may reflect over-chelation in some animals as a positive correlation existed between left striatal DA and iron in chelated rats but a negative correlation was found in controls (Pearson correlation coefficient r=.83 vs r=-.73). In summary, iron chelation appeared to marginally improve functional outcome (turning) in this parkinsonian animal model. Significant sparing of striatal DA was not established; since DA synthesis is iron-dependent, the neuroprotective effects of iron chelation may occur within a relatively narrow range, that is, normal iron stores may participate in neurotoxic cell damage while markedly reduced iron stores may prohibit residual nigrostriatal DA function.

275.19

PHARMACOLOGICAL CHARACTERIZATION OF THE NEW IRREVERSIBLE MAO-B INHIBITORS p-F-DEPRENYL, MDL 72,974-A AND REVERSIBLE INHIBITOR RO 19-6327 IN MOUSE CEREBRUM. I.A Terleckyj, R.E. Heikkila and W.J. Nicklas, Dept. of Neurology, UMDNJ-RWJ Med. School, Piscataway, NJ 08854.

Clinical studies evaluating daily administration of l-deprenyl (DEP), a selective and irreversible MAO-B inhibitor, to newly-diagnosed Parkinsonian patients indicate the need for l-dopa therapy can be delayed by one year (DATATOP). However, chronic DEP treatment is associated with inhibition of MAO-A and metabolism to the CNS stimulant amphetamine in animals and humans. The aim of the present study was to evaluate the pharmacological properties of MDL 72,974A (974A), p-F-deprenyl (F-DEP) and RO 19-6327 (RO 19) in mouse cerebrum. RO 19 and 974A are not metabolized into amphetamine. Also, RO 19 is a highly selective reversible inhibitor of MAO-B and should not inhibit MAO-A with time. In vitro IC_{50} values for inhibition of MAO-B and A by DEP, F-DEP, 974A and RO 19 are, respectively, 4.2 nM, 1.3uM; 1.7nM, 2.1uM; 0.49nM, 464nM; and 3.53nM, 210uM. IC_{50} values for DEP, F-DEP and 974A following a single administration in vivo are 1.9, 296 umol/kg; 2.1, 425 µmol/kg; and 0.3, 107 µmol/kg. These compounds will be useful tools in understanding the possible role of MAO in the neurodegenerative process of Parkinson's Disese and the exact nature of deprenyl's beneficial effects.

275.20

EFFECTS OF DOPAMINE D1 AND D2 RECEPTORS AGONISTS IN A LONG TERM MODEL OF UNILATERALLY MPTP-TREATED CEBUS APELLA MONKEYS. M.E.Emborg, J.A.Colombo and A.Yañez*. Prog. Unidad de Neurobiologia Aplicada -Av.Galvan 4102-(1431) Bs.As.-Argentina.

D1 and D2 receptor agonists were evaluated in an hemiparkinsonian model induced by MPTP.Five 13-15 years old Cebus Apella monkeys received an intracarotid infusion of 1.2mg/kg MPTP-HCL(SIGMA) in 50 ml of saline,under ketalar-fluothane.After 48-72 hs all animals developed a hemisyndrome contralateral to the side of MPTP administration: postural abnormalities,hipokinesia,bradikinesia, tremor,reduction of prensile strength, circling behaviour ipsilateral to lesion with inversion after apomorphine administration (0.4mg/kg, im). After 8-10 months 6 monkeys(five treated and one normal)received Bromocriptine Mesilate(D2) or CY 208-243(D1)(0.5-1.0 mg/kg,im) (Sandoz). Animal care and experimental procedures followed W.H.O. regulations. Both drugs affected motor behavior (reduction/elimination of bradi-hipokinesia and postural changes, with an inversion of circling activity). In our conditions,D1 and D2 agonists were effective to reduce the syndrome induced by MPTP,although individual variations were present. Support:CONICET,CEMIC,CIRHA,Petrol.Arg."S.Jorge".

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SYMPOSIUM. DYNAMICAL BEHAVIOR OF NEURAL SYSTEMS. E. Kaplan, Rockefeller Univ. & R.M. Siegel, Rutgers Univ. (Chairpersons); D. Farmer, Los Alamos National Lab.; L. Liebovitch, Columbia Univ.; J. Rinzel, NIH; M.C. Mackey, McGill Univ. & J.G. Milton, Univ. Chicago Hosp.; R. Llinas, NYU Sch. Med.

Technological advances in the electrophysiological recording from neurons are revealing new dynamical behaviors in complex neural systems. These behaviors can be simple oscillations or enigmatic patterns in space and time. Non-linear dynamical theory, colloquially known as chaos theory, may contribute the matching theoretical advances necessary to classify the behavior of such systems and extract essential mechanisms and principles. The objective of the symposium is to present some modest, yet rigorous, examples of the application of chaos theory to questions in Neuroscience at several different levels.

Siegel will review early work and the strengths of the dynamical approach in the analysis of complex neural systems. **Farmer** will review recent progress in the use of dynamical system theory to analyze data from neural systems. **Liebovitch** will analyze the kinetics of single ion channels to delimit the differences between stochastic and deterministic mechanisms. The mechanisms underlying rhythmic oscillations of single and pairs of neurons will be explored by **Rinzel** using phase space analysis. **Mackey & Milton** will consider the significance of nonlinearities and time delays in generating a diversity of behaviors from simple, yet physiologically realistic models of neural networks. **Llinas** will describe the spatio-temporal patterns of neuronal ensembles in cerebellar cortex using different mathematical approaches. **Kaplan** will summarize and discuss directions for future exploration.

All the presentations will be non-technical in nature and will strive to impart an intuitive grasp of the conceptual framework of dynamical system theory and its application to the brain.

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SYMPOSIUM. NEURON DOCTRINE 1891-1991. G.M. Shepherd, Yale University (Chairperson); E.G. Jones, UC,Irvine; L.W. Swanson, University of Southern California; Albert Aguayo, Montreal General Hospital; K. Tyler, Harvard Medical School.

This symposium, organized by the Ad Hoc Committee on the History of Neuroscience, commemorates the centenary of the doctrine that forms the foundation of all work on the nervous system. Formulated by Wilhelm Waldeyer in December, 1891, the neuron doctrine stated that the nerve cell is the anatomical, functional, genetic and metabolic unit of the nervous system. It was an affirmation of the cell theory for the nervous system and was based on the work of several prominent neuroanatomists, especially that of Ramón y Cajal. The symposium examines the evidence upon which the neuron doctrine was based; the laws of connection by contact and of dynamic polarization that formed integral parts of it; the controversy between Cajal and Golgi and the confirmation of the theory by later investigations up to the present day. The importance of the theory for the understanding of neural development, behavior and neurological disorders will be highlighted, along with those revisions of the theory that appear necessitated by current research at the cellular and molecular level.

CARDIOVASCULAR REGULATION II

280.1

DIFFERENTIAL EFFECTS OF BRAINSTEM LESIONS ON THE 10-HZ AND 2- TO 6-HZ RHYTHMS IN SYMPATHETIC NERVE DISCHARGE (SND). G.L. Gebber, S.M. Barman, and S. Zhong. Dept. of Pharmacol. & Toxicol., Mich. State Univ., East Lansing, MI 48824.

The discharges of postganglionic sympathetic nerves often contain a mixture of two rapid rhythms (10-Hz and 2- to 6-Hz) in baroreceptor-denervated, unanesthetized-decerebrate cats. The question arises whether these two rhythms are dependent upon the same brainstem regions. We approached this problem by comparing the frequency components in SND, the strength of correlation between the discharges of inferior cardiac (CN) and renal (RN) postganglionic nerves, and the level of blood pressure before and after lesions of the medullary raphe (R), the parabrachial pontine region (PB), or the rostral ventrolateral medulla (RVLM). SND was characterized with autospectral and coherence analyses. Lesions placed in R or PB produced the following effects: 1) elimination of the 10-Hz rhythm in SND and a marked decrease in coherence of CN and RN discharges at this frequency, 2) an increase in sympathetic nerve power between 2 and 6 Hz without a change in CN-RN coherence values at these frequencies, 3) no change in total power (0-100 Hz band) in SND, and 4) a statistically significant decrease in blood pressure. In contrast, RVLM lesions essentially eliminated the power in the 2- to 6-Hz and 10-Hz bands of SND. Blood pressure in these animals fell to a greater extent than in those cats in which the R or PB were lesioned. These results indicate that the brain stem networks responsible for the 10-Hz and 2- to 6-Hz rhythms are not identical, although they may share some of the same components (e.g., RVLM). Furthermore, the results demonstrate that the level of blood pressure is dependent not only on total power in SND, but also on its rhythmic pattern (10-Hz vs 2- to 6-Hz). (Supported by NIH grants HL-33266 and HL-13187.)

280.2

RAPHE AND ROSTRAL VENTROLATERAL MEDULLARY (RVLM) NEURONS WITH ACTIVITY CORRELATED TO THE 10-HZ RHYTHM IN SYMPATHETIC NERVE DISCHARGE (SND). S.M. Barman and G.L. Gebber. Dept. Pharmacol. & Toxicol., Mich. State Univ., East Lansing, MI 48824.

Autospectral analysis shows that in most unanesthetized-decerebrate, baroreceptor-denervated cats the power in SND is distributed in two frequency-bands: in a narrow band surrounding a peak at ~10 Hz ("10-Hz rhythm") and in a wider band at frequencies <6 Hz ("2- to 6-Hz activity"). Medullary neurons that control the 2- to 6-Hz activity have been extensively studied, but those responsible for the 10-Hz rhythm have not been identified. Since the 10-Hz rhythm is eliminated by ablation of the raphe or RVLM (Gebber et al., this volume), we searched for raphe and RVLM neurons with activity correlated to the 10-Hz rhythm in inferior cardiac SND in nine unanesthetized-decerebrate cats. Spike-triggered averaging and coherence analysis showed that the extracellularly recorded activity of 23 of 88 raphe neurons and 8 of 20 RVLM neurons was correlated to the 10-Hz rhythm in SND. The interval between unit spike occurrence and the peak of the 10-Hz inferior cardiac sympathetic nerve slow wave was 50 ± 8 ms for raphe neurons and 64 ± 5 ms for RVLM neurons. These values are significantly shorter than those reported in anesthetized cats for the intervals between raphe or RVLM unit discharges and the peak of the 2- to 6-Hz slow wave in inferior cardiac SND. Raphe and RVLM neurons missed firing during many 10-Hz sympathetic nerve slow waves (firing rates: 2.5 ± 0.3 and 3.6 ± 1.0 spikes/s, respectively). Since the activity of some of these neurons also cohered to the 2- to 6-Hz component of SND, in part a common pool of medullary neurons governs both components of SND. The activity of other raphe and RVLM neurons cohered to only the 10-Hz rhythm in inferior cardiac SND. These data support the view that some elements of the 10-Hz rhythm generator are not the same as those responsible for the 2- to 6-Hz activity. (Supported by NIH grants HL-33266 and HL-13187.)

280.3

ORGANIZATION OF CENTRAL SOURCES OF RHYTHMIC SYMPATHETIC NERVE DISCHARGE (R-SND): PARTIAL COHERENCES. B. Kocsis. Center for Complex Systems, Florida Atlantic Univ., Boca Raton, FL 33431.

It has been shown earlier that there is a strong correlation between simultaneously recorded R-SNDs. In this study the analysis was extended to three sympathetic nerves with different functions and anatomical locations, i.e. nerves controlling the heart (inf. cardiac nerve), and visceral (renal nerve) and muscle circulation (vertebral nerve). The resting nerve recordings of chloralose-urethane anesthetized cats were taken from earlier studies in which unequal involvement of these nerves in the cerebral ischemic response was indicated. Examination of ordinary coherences of each pair of these nerve triples revealed common and specific sources contributing to the regional R-SND. The results of such analysis, however, in case of three signals may lead to invalid conclusions. To estimate the overlapping sources of three R-SNDs the coherences have to be partialised. This means separation of coherent components in a nerve pair that are contributed by a source common for all three nerves from those that remain after removal of the influence of the third signal. Five different patterns were observed. They appeared with different frequencies in intact and baroreceptor denervated animals and changed during cerebral ischemia. The results point to the flexibility of central sympathetic networks and an important role of the baroreceptor input in organizing the central map of R-SND sources.

280.4

EFFECTS ON ARTERIAL BLOOD PRESSURE FOLLOWING MICROINJECTION OF EXCITATORY AMINO ACIDS INTO THE MEDIAL MEDULLARY RETICULAR FORMATION. S. A. Aicher and D. J. Reis. Div. of Neurobiol., Cornell Univ. Med. Coll., NY, NY 10021.

We examined the effects of chemical stimulation of the rostroventromedial medulla (RVM), a region of the reticular formation which extends from the ventral reticular nucleus to the ventral subdivision of the gigantocellular n., in anesthetized, paralyzed rats (urethane = .9 g/kg i.p.; α-chloralose = 45 mg/kg s.c.). This region appears to be topographically distinct from other vasoactive reticular zones such as the RVL and CVL. Microinjections of glutamate (22 mM; 20 nl) into this region consistently produced decreases in mean arterial blood pressure (MAP) (-15.3 ± 2.1 mmHg; n=9) and heart rate (-12.2 ± 3.8 bpm). The rostral portion of this vasodepressor region is localized immediately adjacent to vasopressor regions lying dorsally and laterally, since injections centered laterally or dorsally or in larger volumes (100 nl) elevated MAP. Microinjections into RVM of either ibotenic acid (IBO) or kainic acid (KA) at neurotoxic doses (65 mM; 20 nl) initially elicited depressor responses (-28 and -30 mmHg, respectively). At 30 min following KA injections, MAP was significantly elevated ($MAP = 147 \pm 10.8$; n=5). In contrast, at 30 min following IBO, pressure was significantly reduced and remained down for the duration of the 2 hr test period ($MAP = 60 \pm 6.0$; n=5). We conclude that the RVM is a distinct medullary sympathoinhibitory region which is functionally and anatomically distinct from RVL and CVL. Further, the RVM may contain heterogeneous populations of neurons which are differentially sensitive to different classes of excitatory amino acids. (Support:NIH #F32HL08251-01)

280.5

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF NEURONS IN THE CAUDAL VENTROLATERAL MEDULLA (CVL) WHICH MAY MEDIATE THE VASODEPRESSOR RESPONSE TO BARORECEPTOR ACTIVATION. I. Jeske, S.F. Morrison*, and D.J. Reis Cornell Univ. Med. Coll., New York, NY 10021 and *Northwestern Univ., Chicago, IL 60611.

The CVL is thought to be an obligatory synapse in the baroreceptor reflex arc, interposed between barosensory neurons in the nucleus tractus solitarius (NTS), and sympathoexcitatory reticulospinal neurons in the rostral ventrolateral medulla (RVL). We sought to identify baroreceptor "interneurons" in the CVL of urethane-anesthetized, paralyzed rats. In 28 experiments, 13 spontaneously active neurons were identified in the CVL which responded antidromically (5 ± 2.5 ms onset latency) to RVL stimulation. All 13 units were orthodromically excited by electrical stimulation of the aortic depressor nerve (ADN), with Group I (n=3) interneurons responding with a mean onset latency of 20.7 ± 3.1 ms, Group II (n=8) at 45.5 ± 5.3 ms, and Group III (n=2) with two excitatory peaks at 20.5 ± 3.5 ms and 43.5 ± 0.7 ms. The spontaneous discharge (1-10 Hz) of neurons within each group was locked to the cardiac cycle, and increased during the pressor response to i.v. phenylephrine. Three CVL baroreceptor interneurons were silent at arterial pressures below 60, 80 and 120 mm Hg. All CVL neurons were localized at the level of obex, distributed dorsoventrally between the parvocellular reticular formation and the lateral reticular nucleus. The findings provide electrophysiological evidence for an NTS-CVL-RVL relay as the intramedullary neural substrate of the baroreceptor reflex arc. Baroreceptor information may be processed by parallel networks of CVL neurons projecting to the RVL.

280.6

DIRECT EFFECTS OF 8-OHDPAT ON SYMPATHETIC NEURONS IN THE LATERAL TEGMENTAL FIELD M.E. Clement and R.B. McCall, Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The lateral tegmental field (LTF) of the cat contains neurons which exhibit a temporal relationship to the 2-6 Hz slow-wave pattern of sympathetic nervous discharge (SND). These neurons can be designated as sympathoexcitatory (SE) or sympathoinhibitory (SI) depending on their response to alterations of blood pressure. It has recently been suggested that these neurons are a source of the basal activity of similarly sympathetically related neurons in the rostral ventrolateral medulla (RVLM), a site critical to the maintenance of sympathetic tone. We recently demonstrated a high degree of correlation between inhibition of RVLM neurons and the sympatholytic action of intravenous 8-OHDPAT. In this study we have shown that LTF-SE neurons are inhibited in a dose dependent manner by intravenous 8-OHDPAT. This decrease in LTF-SE activity is closely correlated to the inhibition of SND. However, unlike RVLM-SE neurons, LTF-SE neurons are also inhibited by direct application of 8-OHDPAT by microiontophoresis. In contrast, LTF-SI neurons are excited by intravenous 8-OHDPAT. In addition, we demonstrated the existence of sympathetically related neurons in the LTF which can be activated by microiontophoretically applied 8-OHDPAT. These data support the hypotheses that the LTF exerts a sympathoexciatory influence on neurons in the RVLM and suggests that this area is crucial in the sympatholytic action of 8-OHDPAT.

280.7

TONIC CARDIOVASCULAR CONTROL BY NEURONS IN THE PONTINE RETICULAR FORMATION IN RATS. K. Hayes and L.C. Weaver. The John P. Robarts Research Inst. & Department of Physiology, University of Western Ontario, London, Ontario, Canada.

The search for specific brainstem nuclei responsible for generating sympathetic nerve activity has focused on the rostral ventrolateral medulla (RVLM). However, we explored a new area, the pontine reticular formation (PRF), for regions providing tonic control of arterial pressure (AP), heart rate (HR) and activity of sympathetic nerves. In Saffan-anesthetized rats, discharge of PRF neurons was inhibited by unilateral injections of glycine (.01-1M;65nl). Thirty-one injections (1M) produced large, brisk (duration 124±14s) decreases in AP (-28±2mmHg), HR (-21±3bpm) and renal (-47±4%) and splenic (-45±4%) nerve activity indicating that PRF neurons are a powerful source of sympathoexcitatory drive. Seven glycine injections in rats with sino-aortic denervation caused cardiovascular and sympathetic responses as brief (132±27s) as those in intact rats. Therefore baroreflex compensation was not responsible for the brief nature of PRF responses. Moreover, the short duration was not due to compensation by contralateral PRF neurons since bilateral glycine injections (3 rats) produced sympathetic responses as short (87±14s) as those following unilateral injections. Responses were not transient because of short actions of glycine since unilateral injections (10mM;36nl) of the long-lasting GABA agonist muscimol (3 rats) caused responses which recovered as rapidly (70±15s) as those produced by glycine. We conclude that following PRF blockade, another brain area such as the RVLM must compensate for the loss of important excitatory sympathetic drive originating from the PRF.(Support: Ont. Heart & Stroke Fdn).

280.8

A5 NORADRENERGIC (NE) NEURON ACTIVITY AND SYMPATHETIC NERVE DISCHARGE (SND) IN RATS. D. Huangfu, N. Koshiya*, Lie-Ju Hwang* and P. G. Guyenet. Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908

We sought to determine if A5 NE neurons contribute to the generation of the vasomotor SND in halothane-anesthetized rats. Presumed A5 cells were selected on the following criteria: location in ventrolateral tegmentum rostrolateral to facial nucleus, antidromic (AD) activation from thoracic spinal cord and inhibition by i.v. clonidine (<15 μg/kg). These cells had low discharge rates and slow conduction velocities. AD activation of 7 out of 8 neurons was abolished after intraspinal microinjection of 6-hydroxydopamine (i.sp. 6-OHDA, 4 μg, 1 hr). AD mapping located the terminal fields of 16 A5 area neurons in the intermediolateral cell column of the spinal cord. Most neurons (37/59) were inhibited by raising arterial pressure and/or by stimulation of the aortic depressor nerve. The discharge of 12 out of 29 cells was correlated to the splanchnic SND and preceded a peak of SND by 69±6 ms. Microinjection of NMDA (0.5 nmol) in A5 area produced excitation of splanchnic and renal SND and slight inhibition of lumbar SND. The excitation of splanchnic SND was reduced by at least 80% i) 1 hr after i.sp. 6-OHDA, ii) 3 weeks after administration of this drug into the A5 area, or iii) by i.v. prazosin (1 mg/kg). Thus many A5 cells have a visceral vasomotor sympathoexcitatory function. (HL 28785).

280.9

INFLUENCE OF THE MIDBRAIN CENTRAL GREY ON MEDULLARY SYMPATHOEXCITATORY NEURONS AND THE BAROREFLEX IN THE RAT. P.G. Guyenet and A.J.M. Verberne. Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

The influence of the central grey (CG) on the activity of barosensitive, sympathoexcitatory neurons of the rostral ventrolateral medulla (RVL) and the sympathetic baroreflex was studied in halothane-anesthetized, paralysed rats. Electrical stimulation (50Hz, 25-75 uA, 0.3 ms, 10s train) of the CG produced sympathoexcitation and a pattern of hemodynamic responses characteristic of the defence reaction. Splanchnic sympathetic activation produced by CG stimulation exceeded that of the lumbar nerve. CG stimulation produced elevation of the baroreflex gain and baroreflex cut-off pressure. Intermittent stimulation (0.5 Hz, double pulse, 3 ms interval) of the CG produced splanchnic and lumbar sympathoexcitation with latencies of 46±2 and 80±2 ms, respectively. RVL barosensitive neurons were readily activated by train stimulation of the CG (18/19 units tested). Non-barosensitive units were not excited by CG stimulation (5 units tested). Inhibition of neurons within the RVL by microinjection of the GABA-mimetic agent muscimol (10 ng/50 nl) abolished the pressor responses to CG stimulation, while the accompanying sympathetic activation was converted to inhibition. These findings support the hypothesis that sympathoexcitatory responses elicited from the CG are mediated by RVL barosensitive, sympatho-excitatory neurons. (HL 28785)

280.10

PROJECTIONS FROM THE CARDIOVASCULAR RESPONSIVE REGION OF BED NUCLEUS OF THE STRIA TERMINALIS (BST) TO MEDULLARY CATECHOLAMINERGIC CELL GROUPS. S.A. Janssen and J. Ciriello, Department of Physiology, University of Western Ontario, London, Canada, N6G 5C1.

We have previously shown that microinjection of the excitatory amino acid, L- glutamate into a crescent shaped region of BST hugging the anterior commissure on it's dorsal, lateral, and ventral surfaces resulted in decreases in both arterial pressure and heart rate. In this study, the efferent pathways that maybe involved in mediating these cardiovascular responses were investigated using the anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHA-L). PHA-L was iontophoresed into the cardiovascular responsive region (CRR) of the ventral component of the lateral BST, and after a survival period of 13 days, transverse sections of the brainstem were processed immunohistochemically for the demonstration of PHA-L and/or tyrosine hydroxylase containing fibers, terminals and perikarya. PHA-L labelled fibers and terminals resulting from the injection within the CRR of BST were observed ipsilaterally within the A5 noradrenergic cell group and bilaterally within the dorsal motor nucleus of the vagus (DMV), rostral nucleus of the solitary tract (NTS-C2), caudal NTS (NTS-A2), rostral ventrolateral medulla (VLM-C1) and caudal VLM (VLM-A1). However, only in the caudal VLM and the caudal NTS were BST fibers and terminals, labelled with PHA-L, observed in close apposition to the noradrenergic cells. As both NTS and the caudal VLM function as vasodepressor sites, these data suggest that both medullary regions may be components of the descending pathway that mediates the decreases in blood pressure seen following stimulation of the CRR of BST. (Supported by MRC of Canada and HSFO).

280.11

HYPOTHALAMIC CONNECTIONS OF THE PARAVENTRICULAR THALAMIC NUCLEUS. M. E. Puigbonet and J. Ciriello. Department of Physiology, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

The paraventricular nucleus of the thalamus (PV) has previously been shown to receive afferent projections from brainstem structures involved in visceral and autonomic regulation. However, the efferent projections of PV have not been completely elucidated. In this study, pathways originating from neurons in PV were identified in the rat using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L). PHA-L was iontophoretically injected into PV and after a survival period of 7-13 days, forebrain sections were processed immunohistochemically for the demonstration of PHA-L in fibers and terminals. Within the hypothalamus, PHA-L labelled fibers and terminals were observed bilaterally. The densest projections were observed in the lateral aspects of the lateral hypothalamic nucleus and ventromedial hypothalamic and suprachiasmatic nuclei. Moderate PHA-L labelling was found in the dorsal and medial parvocellular components of paraventricular nucleus of the hypothalamus (PVH), the area of the tuber cinereum, dorsal medial hypothalamic nucleus and the posterior hypothalamic area. Additionally, scattered fiber and terminal PHA-L labelling was also found in and around supraoptic nucleus and the magnocellular component of PVH. These data suggest that PV may function as an important link in the relaying of visceral afferent information to hypothalamic sites involved in behavioural, autonomic, and endocrine integration. (Supported by MRC of Canada and HSFO).

280.12

ALTERED NEURONAL ACTIVITY IN THE POSTERIOR HYPOTHALAMUS OF SPONTANEOUSLY HYPERTENSIVE RATS. C.A. Shonis and T.G. Waldrop. Dept. of Physiology & Biophysics, Univ. of Illinois, Urbana, IL 61801.

Previous studies have shown that GABAergic activity in the posterior hypothalamus (PH) of the spontaneously hypertensive rat (SHR) is decreased compared to normotensive Wistar-Kyoto rats (WKY). The purpose of these experiments was to determine if this loss of inhibition elevates the discharge frequency of neurons in the PH, a known pressor region. Extracellular single unit activity was recorded from the PH of anesthetized, adult SHR (mean systolic pressure= 205±13.3 mmHg) and age-matched WKY (mean systolic pressure= 141±6.8 mmHg). PH neurons in the SHR possessed a higher spontaneous firing rate (3.66±0.55 Hz) compared to the cells in the PH of the WKY (2.11±0.29 Hz). Many of the neurons in the SHR (38%) had a "bursting" discharge; only 16% of the WKY neurons had this discharge pattern. Computer averaging revealed that 87.5% of the neurons from the SHR had a cardiac-related discharge compared to 64% of those in the WKY. The basal firing rate of these neurons with a cardiac-related discharge was higher in the SHR (5.86±1.56 Hz) compared to those from the WKY (2.32±0.38 Hz). SHRs had a blunted heart rate response to baroreceptor stimulation and a smaller percentage of the PH cardiac-related neurons were responsive to baroreceptor stimulation in the SHR (24%) compared to the WKY (66%). This study supports the hypothesis that the SHR has elevated neuronal activity in the posterior hypothalamus, a region involved in modulating cardiovascular activity; this alteration may contribute to the development and/or maintenance of hypertension in this animal model. (Supported by NIH 38726; American Heart Association).

CELL LINEAGE II

281.1

ISOLATION OF LEECH HOMEOBOX GENES RELATED TO THE SEGMENT-IDENTITY GENES OF *DROSOPHILA*. M. Shankland & D. Nardelli-Haefliger*. Dept. of Anatomy, Harvard Med. School, Boston, MA 02115.

In both insects and vertebrates, segmental identity is determined in large part by the action of a particular set of homeobox genes, known in *Drosophila* as the homeotic or segment-identity genes. The cellular events which give rise to segmental diversification are especially tractable in embryos of the leech *Helobdella robusta*, and we have therefore chosen to isolate and characterize the homologous leech genes. Candidate homeobox gene sequences were initially isolated by PCR amplification, and the amplified sequences were then used to screen a genomic DNA library to obtain restriction fragments containing the entire homeobox plus portions of the flanking coding region. Sequence analysis suggests that 4 of the leech homeobox genes obtained in this manner -- designated as *Lox5, Lox6, Lox7* and *Lox2* -- are respectively homologous to the Drosophila homeotic genes *Antp, Dfd, lab* and *Ubx/AbdA*. (The gene *Lox2* has previously been described in another, distantly related leech species, see Wysocka-Diller *et al., Nature* 341:760, 1989). The presence of discrete *Antp* and *Ubx/AbdA* homologues suggests that these particular genes diverged prior to the separation of annelids and arthropods, contrary to some current models of homeotic gene evolution (Akam, *Cell* 57:347, 1989). Oligopeptides were synthesized to correspond to specific regions of each leech homeodomain protein, and antibodies were raised against those peptides in rabbits. These antisera are currently being used to examine the spatiotemporal pattern of gene expression in the developing leech embryo.

281.2

DETERMINANTS OF CELL FATE: CHARACTERIZATION OF *NOTCH*-RELATED MOUSE CDNA CLONES DERIVED FROM EARLY POSTNATAL GRANULE NEURONS ME Ross[1] and N Heintz[2] Univ. of Minnesota, Minneapolis MN, 55455[1] and Rockefeller Univ., NY NY, 10021[2]

In *Drosophila*, the neurogenic locus *Notch* (*N*) plays a pivotal role in the differentiation of neuroectodermal cells as they adopt a neuronal or epithelial fate. Its 10.2 kb mRNA encodes a membrane protein with cysteine rich repeated units in the extracellular domain which are homologous to epidermal growth factor (EGF) and to EGF-like repeats in the cell lineage control gene, *lin-12* of *C. elegans*. Other neurodevelopmental genes in *Drosophila* possess EGF-like repeats, including *Delta (Dl), slit (sli)* and *Serrate (Ser)*. At least two, *Dl* and *Ser*, may interact with *N*, suggesting that proteins containing EGF-like regions represent a class of developmentally important genes, many working via cell-cell interactions. To identify mammalian members of this gene family and determine their role in CNS development, we have used probes encompassing the EGF-like repeats of *N* to screen a mouse cDNA library (Ross et.al., Soc. Neurosci. Abs., 16:151, '90) made in λgt11 from postnatal day (P) 3 to 5 cerebellar granule neurons. Of $2x10^6$ recombinants screened, 34 plaques were identified on duplicate filters. Sequence data (~500 bp ea) from the first 6 clones reveals that 5 are unique with respect to GenBank. Translation of open reading frames from the 5' ends of at least 3 clones reveal cysteine rich regions. Pustell DNA matrix comparisons (65% minimum score) align these ends within the EGF-like repeats of *N* and its *Xenopus* homolog, *Xotch*. At least three of these mRNAs are developmentally regulated. Computerized image analysis of Northern autoradiograms, normalized to GAPDH, reveal that MN20 (~9.5 kb) is expressed at levels >200 fold higher in P6 than in adult mouse cerebellum, while MN51 (~4 kb) is 6 fold higher and MN22 (~4.5 kb) is 4.8 fold higher in P6 than adult cerebellum. Our data suggest that several *N* related genes are expressed in mouse cerebellar granule neurons. The temporal regulation of MN20, 22, and 51 indicates a role for these genes in mammalian neuronal development.

281.3

STRUCTURE AND EXPRESSION OF TES-1: A HOMEODOMAIN-CONTAINING GENE THAT IS EXPRESSED IN THE NEUROEPITHELIUM OF THE EMBRYONIC MOUSE VENTRAL FOREBRAIN. J.L.R. Rubenstein#, M.H. Porteus#, A. Bulfone*# and R.D. Ciaranello. Nancy Pritzker Laboratory, B002,Stanford School of Medicine, Stanford, CA 94305. #Present address: Neurogenetics Laboratory, LPPI, Box F, 401 Parnassus, UCSF, San Francisco, CA 94143-0984.

Using subtractive hybridization and screening with homeodomain-encoding probes, we isolated, from an E14.5 mouse telencephalon cDNA library, a 2.1 kb cDNA encoding a novel homeodomain-containing protein called TES-1. The TES-1 homeodomain is identical in 53 out of 61 amino acids with the homeodomain of the Drosophila *distal-less* gene. Unlike other vertebrate homeodomain-containing genes, TES-1 is only expressed in the head. Within the brain of the midgestational embryo, TES-1 is expressed in the ventral forebrain. At E11.5 it is strongly expressed in the ganglionic eminence and in a separate patch of neuroepithelium that is probably in the diencephalon. At E13.5, it is expressed continuously along the floor of the ventral forebrain, as well in a patch of cells in the ventral thalamus. By E18.5, TES-1 expression can also be seen in the developing olfactory bulb, vomeronasal organ and preameloblasts. At this time, TES-1 is also expressed in the dorsal ventricular zone that gives rise to the cerebral cortex. We suspect that TES-1 may be an important transcriptional regulator that controls differentiation of the mammalian forebrain.

281.4

THE NESTIN PROMOTER: DNA SEQUENCES FLANKING AN INTERMEDIATE FILAMENT GENE DIRECT HIGH-LEVEL EXPRESSION OF EXOGENOUS GENES TO NEURAL AND MUSCLE PRECURSORS. L.B. Zimmerman*, M.J. Marvin*, A. McMahon*# and R.D.G. McKay. Dept. of Biology, M.I.T., Cambridge MA. 02139; #Roche Inst. of Molec. Biology, Nutley NJ 07110-1199.

The mitotic neuroepithelium and somites transiently express the intermediate filament nestin. Nestin regulation is effected at the RNA level both *in vivo* and in the teratocarcinoma P19, in which expression of nestin transcripts and protein increases in response to retinoic acid treatment. Reporter constructs containing the lacZ or CAT genes flanked by sequences upstream of the nestin translation intitiation site are appropriately expressed by nestin-positive and -negative cell lines in transient transfection assays, but do not show high-level, specific expression in transgenic mice. The addition of sequences from nestin's introns downstream of lacZ confers such expression in the transgenic mouse assay. This provides a general strategy for directing the expression of a wide variety of experimental genes to neural precursor cells both *in vitro* and in transgenic animals.

281.5

A Brain-Derived Oligotrophic Factor

Barbara Q. Kreider, Judith B. Grinspan, Lawrence G. Wrabetz and David E. Pleasure

A brain-derived oligotrophic factor (BDOF) with an apparent molecular weight of 50,000 daltons, was prepared from the 100,000 x g pellet from neonatal rat brains by salt extraction, ion exchange and molecular sieve chromatography. Like platelet-derived growth factor (PDGF) and the fibroblast growth factors (FGFs), BDOF is mitogenic for rat O2A oligodendroglial progenitor cells but, unlike PDGF and the FGFs, BDOF also enhances the proliferation of galactocerebroside+ (galC+) oligodendroglia. BDOF is antigenically distinct from PDGF and the FGFs. Its effects on oligodendroglial differentiation are also distinctive. In the presence of BDOF, cultured neonatal rat O2A cells become galC+, A2B5- oligodendroglia, but expression of myelin basic protein (MBP) and MBP mRNA is inhibited. This is in contrast to PDGF, which induces accumulation of MBP+ oligodendroglia in such cultures, and of basic FGF, which drives proliferation of O2A cells but inhibits expression of both galC and MBP. Thus, BDOF has the following unique properties: it is a mitogen for galC+, as well as galC- cells of the oligodendroglial lineage; and it inhibits expression by galC+ oligodendroglia of the MBP gene. Further purification of BDOF will be necessary to determine if a single protein is responsible for both the mitogenic and phenotypic effects on the oligodendroglial lineage, and to learn its site of origin in brain.

281.6

LAMININ INDUCES A RAPID PHENOTYPIC AND BIOCHEMICAL DIFFERENTIATION OF EMBRYONIC RODENT BRAIN CELLS. P.Liesi and S.Murtomäki*. Institute of Biotechnology,University of Helsinki,Valimot. 7,Helsinki 38,Finland.

Laminin promotes neurite outgrowth of cultured neurons and localize in the brain during early development(1). Retinal pigment epithelium and murine brain cells extend processes on laminin substrate(2,3)4). We show that very early rodent brain cells differentiate into postmitotic neurons in less than 12 hrs when plated on laminin. Neuronal phenotype is accompanied by cessation of cell division and expression of the 200 kDa neurofilament protein. The neurite outgrowth domain of laminin(5) has similar ifferentiating effects whereas fibronectin supports expression of neurofilament light and middle chains without inhibition of cell division. These results indicate that laminin is a differentiation factor for early brain cells and may direct their development into neurons.

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281.7

THE RAT INSULINOMA CELL LINE, RINm5F, RESPONDS TO NERVE GROWTH FACTOR (NGF) AND LAMININ BY EXTENDING NEUROFILAMENT POSITIVE PROCESSES. M. Polak*, B. Seilheimer, G.S. Eisenbarth*, H. Potter. Department of Neurobiology and Joslin Diabetes Center, Harvard Medical School, Boston, MA 02115.

Although insulin-secreting cells do not derive from the neural crest, they share with neurons the expression of certain proteins such as glutamic acid decarboxylase, and both possess the surface ganglioside defined by its reactivity with the A2B5 antibody.

We therefore postulated that extracellular matrix components (ECM) and neurotrophic factors could alter the phenotype of a rat insulinoma cell line, RINm5F, in culture. RINm5F cells grown in defined, serum-free medium were plated at low density on laminin or poly-L-lysine (pLl)-coated plastic or glass coverslips (GC) in the presence of NGF (100 ng/ml) or laminin (10 µg/ml). BetaTC3 cells, derived from a transgenic mouse SV40-induced insulinoma and functionally more differentiated than the RIN cells, were also tested. At day 3 the cells were photographed and prepared for immunocytochemistry of the 160 kD neurofilament (NF) and the NGF receptor (NGFR).

RINm5F cells cultured either with NGF (60%) or with laminin (65%) or both (75%) displayed cellular projections (spikes), in contrast to typical small round cells cultured in defined medium on pLl-coated culture dishes. BetaTC3 remained round in the presence of NGF. RINm5F cells, round on pLl-coated GC, stained for NF and NGFR. On laminin or pLl-coated GC, in the presence of NGF, most of the RINm5F cells displayed NF+ spikes and stained for the NGFR. On laminin-coated GC, the betaTC3 cells had NF+ spikes.

In conclusion, the de-differentiated RINm5F cells respond in a similar way as PC 12 cells to ECM and NGF, which has previously been shown to affect neurons or neural-crest derived cells only. By contrast the more differentiated betaTC3 cells are able to respond to ECM with NF+ spikes but not to NGF. This suggests that neuronal survival factors and ECM may play important regulatory roles in the early differentiation and growth of pancreatic beta cells.

281.8

TRANSFORMING GROWTH FACTOR BETA-1 AND CHICK EMBRYO EXTRACT DIFFERENTIALLY AFFECT NEURAL CREST CELL NEURONAL DEVELOPMENT *IN-VITRO*. M.J. HOWARD and M.D. GERSHON. Dept. of Anat.& Cell Biol., Columbia University, New York, NY 10032.

Neural crest cells give rise to neurons of the peripheral nervous system. Previous studies showed that transforming growth factor beta-1 (*TGF-β1*) could substitute for chick embryo extract (CEE)-derived factors in supporting catecholaminergic differentiation by some neural crest cells *in-vitro*. The current studies test the possibility that the responsible factor in CEE might be *TGF-β1*. The time-course of the response to *TGF-β1* was studied by analyzing formaldehyde induced fluorescence, (FIF) in neural crest cells plated in medium supplemented with 2% CEE (a non-permissive growth condition for catecholaminergic differentiation) and 3 ng/ml *TGF-β1* from 1 to 4 days. After removing *TGF-β1*, the cells were maintained in the non-permissive condition for the remainder of the 7 day growth period. Exposure to *TGF-β1* for 1-4 days supports expression of FIF in approximately 50% of explants. This finding contrasts with previous results showing that only 12 to 20% of explants maintain the capacity to respond to CEE-derived factors with catecholaminergic differentiation when initially exposed to non-permissive conditions for 1 to 4 days. Even after the 4 day critical period for CEE-derived factors, *TGF-β1* supports catecholaminergic expression, suggesting that CEE and *TGF-β1* may activate different signalling mechanisms. Antibodies to *TGF-β1* block its effect *in-vitro* but do not block CEE-derived factors suggesting that CEE-derived factors and *TGF-β1* are not the same. Persistence of CEE-supported catecholaminergic expression following early exposure to the RNA synthesis inhibitor 5,6-dichlorobenzimidizole riboside suggests that transcriptional regulation is probably not initially required for catecholaminergic phenotypic expression. The possibility that the inhibition observed later is due to effects on protein synthesis can not be ruled out at this time. These studies suggest that the CEE-derived catecholamine-supporting factor is not *TGF-β1* (This work was supported by NS12969).

281.9

BASIC FIBROBLAST GROWTH FACTOR INDUCES RETINAL PIGMENT EPITHELIUM TO GENERATE NEURAL RETINA IN VITRO. Catrin Pittack*, Micheal Jones* and Thomas A. Reh, Dept. of Biological Structure, University of Washington, Seattle, Washington 98115.

During embryogenesis, the cells of the eye primordium are initially capable of giving rise to either neural retina or pigmented epithelium (PE), but become progressivily restricted to one of these potemtial cell fates. However, following surgical removal of the retina in embryonic chicks and larval amphibians, new neural retina is generated by the phenotypic switching, or transdifferentiation, of PE cells into neuronal progenitors. A recent study has shown that basic fibroblast growth factor (bFGF) stimulates neural retinal regeneration in chicks *in vivo*. To further characterize the mechanisms by which this factor regulates the phenotype of retinal tissues, we added bFGF to enzymatically dissociated chick embryo PE cultured as a monolayer. We found that bFGF stimulated proliferation and caused several morphogenic changes in the PE, including loss of pigmentation; however, no transdifferentiation to neuronal phenotypes was observed. By contrast, when small sheets of PE were cultured as aggregates on a shaker device, preventing growth as a monolayer, we found that a large number of retinal progenitor cells were generated from the PE treated with bFGF. The bFGF treated explants proliferated extensivily to form large regions of a nonpigmented pseudostratified columnar epithelium that resembled a neuronal epithelium (NE) characteristic of the neural retina. Immunocytochemical analysis of these explants showed positive immunoreativiry for the following neuronal marker; NCAM, NSE and NF-M in the NE. Negative control explants were negative for these markers. These results indicate that bFGF promotes retinal regeneration *in vitro* as well as *in vivo* and suggest that the ability of chick PE to undergo transdifferentiation to neuronal progenitors appears to be dependent on the physical configuration of the cells. NIH R01 NS28308.

281.10

EPIDERMAL GROWTH FACTOR IS MITOGENIC FOR RAT RETINAL NEUROEPITHELIAL CELLS. Raymond M. Anchan and Thomas A. Reh. Department of Biological Structure, University of Washington, Seattle, Washington 98105.

The factors that regulate the number of neurons and glia during histogenesis of the central nervous system (CNS) are not well understood. We recently reported that EGF and TGFα are mitogens for multipotent retinal neuroepithelial cells in disscoiated primary cultures. In addition, EGF and TGFα messages, as well as the EGF receptor, are also present in the developing rat retina (Anchan et al, 1991, Neuron, in press). We have now extended our studies to whole retinal cultures. [^{3}H]-thymdine pulse labelling studies of embryonic day 14 rat whole retinas cultured for up to 11 days *in vitro* show a two fold increase in the number of mitotically active neuroepithelial cells when cultured with EGF. In addition, the number of differentiated neurons is concomitantly reduced in the EGF treated cultures; eg. the number of rod photoreceptor cells in the control cultures is approximately that of the appropriate *in vivo* age (postnatal day 1), while only a few rods are present in the EGF treated retinas. Upon removal of the growth factor and continued culture of the whole retinas for another 3 to 4 days, the majority of neuroepithelial cells withdraw from the cell cycle and differentiate into rods. This data suggests that EGF and TGFα normally regulate retinal cell numbers by stimulating the proliferation and suppressing the differentiation of neuroepithelial cells. NIH RO1 NS28308.

281.11

CONTROL OF PROLIFERATION OF RETINAL CELLS IN VITRO. *L.E. Lillien and C. L. Cepko. Dept.of Genetics, Harvard Medical School, Boston, MA 02115.*

As in other parts of the CNS, progenitor cells in the retina divide for a limited period of time before they differentiate. In order to begin to understand how proliferation is controlled in the retina, we have tried to identify factors that alter the proliferation of retinal cells in vitro. We have found that proliferation in explant and monolayer cultures of embryonic and postnatal retinal cells is enhanced by aFGF, bFGF, EGF and TGFa. The dose-dependence of the mitogenic response to aFGF and bFGF, however, changes with age: higher concentrations of the factors are required to elicit maximal responses at later stages. As this change precedes loss of the capacity to divide, this observation suggests that a decline in responsiveness to FGF may be one of the mechanisms that restricts proliferation in the retina.

SUBCORTICAL VISUAL PATHWAYS

282.1

MORPHOLOGY OF GANGLION CELLS INNERVATING THE MEDIAL INTERLAMINAR NUCLEUS OF THE LATERAL GENICULATE BODY. M. Pu* and D.M. Berson. Div. Biology & Medicine, Brown Univ., Providence, RI, 02912

Correlations between morphology, physiology and central projections, well established for alpha (Y) and beta (X) cells of the cat retina, are still largely unexplored for W-cells. We have examined the morphology of ganglion cells innervating a major thalamic target of W-cells, the medial interlaminar nucleus (MIN) of the LGN. Living MIN-projecting ganglion cells, labeled by retrograde transport of fluorescent beads, were stained in vitro by intracellular injection of Lucifer Yellow and biocytin. The intracellular markers were converted to a stable, high-resolution stain using anti-Lucifer Yellow immunohistochemistry and an avidin-biotin procedure. We stained more than 350 cells in the retinas of two cats. These included alpha cells, beta cells and various presumed W-cells. The beta cells (both ON and OFF varieties) were encountered with surprising frequency, making up about a third of our non-alpha sample. Labeled beta cells were widely distributed over the retina even though our bead deposits apparently spared all of the laminar LGN, confirming earlier evidence for some X-cell input to MIN (Dreher & Sefton, JCN '79; Sur and Sherman, Science '82). About half of the stained W-cells formed a relatively homogeneous group with medium-sized somas (18-28 μm) and huge dendritic fields (400-600 μm diam. centrally and >1000 μm peripherally) reminiscent of the epsilon cells of Leventhal et al. (JCN '80). Involvement of the geniculate wing (to which epsilon cells project) was minimal, but could have accounted for the labeling of some of these cells. The remaining W-cells were extremely diverse morphologically, including small-field cells with fine, densely interwoven processes; medium-field cells with tortuous or crooked spiny dendrites; and some that resembled the delta cell of Boycott and Wässle (J.Physiol. '74).

282.2

Frequency Facilitation and Long-Term Potentiation of Retinogeniculate and Corticogeniculate EPSPs of Cat Lateral Geniculate Neurons Recorded in Thalamic Slices. H.E. Scharfman[1,2], M.E. Bickford[2], S-M. Lu[2], W. Guido[2], P.R. Adams[1,2], and S.M. Sherman[2]. The Howard Hughes Medical Institute[1] and Department of Neurobiology and Behavior[2], SUNY at Stony Brook, Stony Brook, NY 11794-5230.

We examined the effects of physiologically relevant stimulus frequencies on retinogeniculate and corticogeniculate EPSPs of cat lateral geniculate neurons recorded in thalamic slices. We were particularly interested in the possibility that brief, high frequency afferent stimulation could cause a long lasting enhancement of EPSPs (long-term potentiation; LTP), since NMDA receptors contribute to EPSPs of relay cells, and NMDA receptors appear to be fundamental to LTP in other systems. We found that pairs of stimuli to either optic tract or optic radiations demonstrated strong facilitation, and brief stimulus trains (3-100Hz, 0.5-2sec) to either input produced long lasting increases in EPSP amplitude. When identical stimuli were presented in pairs with short interstimulus intervals (10-50 msec), the response to the second stimulus was a larger EPSP, which often triggered an action potential. At a surprisingly wide range of membrane potentials, when a single stimulus evoked an EPSP, the second stimulus could evoke a LTS. During 3-10Hz trains that lasted 1-2sec, EPSP amplitude increased greatly and triggered action potentials. Following these trains the EPSP amplitude remained elevated for as long as 5 minutes, and then returned to its original amplitude. The response to 100Hz trains was more dramatic. During these trains, cells depolarized and discharged at high frequency, and the train was followed by an afterhyperpolarization. After 3-10 trains the EPSP amplitude often remained elevated (2-5 fold) for long periods (0.5-2 hours). The ability of cat thalamic neurons to increase their responses to retinal or cortical excitation provides a mechanism for enchancement of retinal or cortical input that may occur during normal signal processing. Supported by The Howard Hughes Medical Institute and grant EY 03038 to S.M.S.

282.3

EFFECT OF STIMULUS CONTRAST AND SIZE ON NMDA RECEPTOR ACTIVITY IN THE CAT LATERAL GENICULATE NUCLEUS (LGN). Y. H. Kwon, S. B. Nelson, L. J. Toth* and M. Sur. Dept. of Brain and Cognitive Science, MIT, Cambridge, MA 02139.

39 cells from layers A or A1 of cat LGN were recorded with extracellular iontophoretic electrodes, and were classified as X or Y, and lagged or non-lagged based on standard physiological criteria. We displayed a spot of optimal size in order to visually drive the cells, and examined the cell's response to contrast variation before, during and after application of APV. In the same manner, we recorded responses to maximal contrast stimuli presented at sizes smaller and larger than optimal.

The NMDA-dependent (APV-sensitive) component of the response was found to be a *linear* function of response for most cells studied, regardless of cell type. APV application changed only the gain and saturation response. The actual NMDA contribution, however, varied between cells and cell types. Lagged X cells were found to have a significantly greater NMDA component, as a population. The NMDA fraction of the response decreased with increasing stimulus size, suggesting that surround inhibition can preferentially decrease the NMDA component. These data suggest that while retinal excitatory drive alone does not affect the percentage of NMDA-dependent response, intrageniculate inhibition can modulate that percentage. Supported by EY 07023.

282.4

SUBLAMINAR ORGANIZATION OF METABOLIC ACTIVITY IN THE OFF PATHWAY THROUGH CAT LATERAL GENICULATE NUCLEUS (LGN): D,L-2-AMINO-4-PHOSPHONOBUTYRIC ACID (APB) INTRA-OCULAR INJECTION AND 2-DEOXYGLUCOSE (2-DG) AUTORADIOGRAPHY. G.A. Thurlow, D.B. Bowling and R.M. Cooper. Depts. of Medical Physiology and Psychology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

In the visual system, ON and OFF pathways show a degree of separation that differs with anatomical location and with species. In cat LGN the ON and OFF cells have been reported to form opposing density gradients through the depth of layer A: OFF cells highest at the bottom of the layer, ON cells highest at the top. However, the evidence for this is entirely based on microelectrode recordings which are subject to sampling errors and positional inaccuracies.

To obtain a measure of ON/OFF activity that is independent of microelectrode techniques we eliminated retinal ON responses with intraocular injections of APB. Using 2-DG autoradiography, we examined the spatial distribution of the remaining OFF activity in awake and freely-moving cats. Tetrodotoxin injected controls allowed assessment of baseline LGN metabolic activity. The results showed a simple gradient of OFF activity that was highest at the bottom of layer A. Little or no gradient was detected in layer A1.

In addition to the metabolic gradient in the OFF-pathway, previous work has demonstated that X/Y cell density, response amplitude (Bowling & Wieniawa-Narkiewicz, J. Physiol. 1986; 1987), response latency (Bowling, Vis. Neurosci. 1989), and projections to cortex (Humphrey et al., J. Neurosci. 1985; Bowling, Brain Res. 1989), all exhibit a sublaminar organization within the A layer. Such an organization is beyond that implied solely by X/Y and ON/OFF channels and may contribute to the unique response properties of cortical cells. Supported by MRC (Canada), NSERC and AHFMR.

282.5

STIMULATION OF THE BRAINSTEM RETICULAR FORMATION DOES NOT ABOLISH THE LAGGED/NON-LAGGED CELL DISTINCTION IN THE CAT LGN A.L. Humphrey and A.B. Saul. Dept. of Neurobiology, Anatomy and Cell Science, U. of Pittsburgh, Pittsburgh PA

Two groups of X-cells in the cat LGN are distinguished by their responses to flashing spots (Humphrey and Weller, *J.Comp.Neurol.* 268:429). Non-lagged cells (X_N) respond to spot onset with a short latency (30-60ms) excitatory transient followed by a sustained discharge. Lagged cells (X_L) respond with an early dip in firing, due to GABA-mediated inhibition, and a long latency to discharge (70 to >250ms). Uhlrich et al. (*PNAS* 87: 2560) recently reported that X_L-cells could be induced to respond like X_N-cells by stimulating the peribrachial region (PB) of the brainstem reticular formation. We have reinvestigated this issue using a similar paradigm. Spot stimuli flashing at 0.25-2Hz were paired with short trains (1100ms) of electrical pulses delivered to the PB region in cats anesthetized with halothane in nitrous oxide/oxygen.

For 33 X_N-cells, PB stimulation led to a moderate increase in both the transient and sustained portions of the spot-elicited discharge but did not alter the cells' latencies to discharge. For 40 X_L-cells, PB stimulation significantly increased spot-elicited firing rates, as much as 3 times above control levels, and reduced the cells' latencies (mean reduction in latency: 40%; range:10-70%). Despite these shorter latencies, the spot-induced early dip in discharge was still present in most X_L-cells and all of the cells had longer latencies than any X_N-cell (range: 66-250ms). For 3 additional cells (1 X_L and 2 partially lagged) PB stimulation shortened the latencies from the lagged to non-lagged range. Interestingly, none of these 3 cells had an early dip in their control response to spots, suggesting that the inhibition making these cells lagged may have been minimal and readily counteracted by PB activation. In summary, PB stimulation greatly facilitates the responsiveness of X_L- and X_N-cells but, with very few exceptions, these two cell groups remain clearly distinguishable in response timing. (*Supported by EY06459*)

282.6

THE EFFECT OF BRAINSTEM PERIBRACHIAL STIMULATION ON THE CONTRAST-RESPONSE PROPERTIES OF CELLS IN THE CAT LATERAL GENICULATE NUCLEUS. E. Hartveit and P. Heggelund. Department of Neurophysiology, University of Oslo, Oslo, Norway.

Most cells in the retina and LGN respond primarily to stimulus contrast. In the LGN, the response can also be strongly influenced by input from the midbrain peribrachial region (PBR). The functional importance of the input from the PBR is not known. To address this question, we have investigated the influence of input from the PBR on the relationship between stimulus contrast and visual response of LGN cells. We recorded the response of single cells to a series of stationary flashing light spots of different contrasts. The spots approximately filled the receptive field center. The response was recorded both with and without electrical stimulation of the PBR. From contrast-response curves we determined response threshold, contrast gain (the slope of the initial part of the curve), contrast giving half-maximal response (C_{50}), and dynamic range (the range of contrasts over which the cell changed its response).

The visual response of most cells increased almost linearly with increasing contrast over a large part of the dynamic range. In this range, an enhancement of the visual response by PBR stimulation could be elicited at all contrasts. On the nonlagged cells the proportional increase of the response at the various contrasts was approximately constant. Accordingly, the effect of the PBR stimulation was mainly an increase of the contrast gain. Response threshold, C_{50} and dynamic range changed little.

The finding that the PBR stimulation primarily enhances the contrast gain suggests that the mechanism of response enhancement on nonlagged cells is a multiplicative one, rather than an additive one, e.g. by an increase in the spontaneous activity. Preliminary results suggest that the contrast-response curves of lagged LGN cells may change in a different manner during PBR stimulation.

282.7

EFFECT OF MEMBRANE VOLTAGE AND PARABRACHIAL ACTIVATION ON LAGGED AND NONLAGGED CELLS IN THE CAT LGN. S.-M. Lu, W. Guido, and S.M. Sherman Dept. of Neurobiology, SUNY Stony Brook, NY 11794

Lagged and nonlagged responses among cat LGN cells represent may different response modes of the same cells rather than different cells *per se*. A factor in this phenomenon may be a voltage-dependent A-current (I_A) due to a K^+ conductance that offsets and retards EPSPs, thus creating the lag. We tested this hypothesis during intracellular recording of LGN cells in cats by measuring responses to flashing spots centered on the receptive field as a function of Vm. Among other parameters, we determined the latency to reach half maximum response following stimulus onset. In support of the I_A hypothesis, depolarization of V_m in LGN cells did reduce this response latency, and this effect was more prominent in lagged than in nonlagged cells. In some lagged cells, the latency declined into the nonlagged range with V_m depolarization from -70 to -50 mV, but in other cells the latency change was less dramatic. We also repeated an earlier observation that electrical activation of the parabrachial region (PBR), which provides a largely cholinergic input to the LGN, converts lagged responses to nonlagged. We thought this might occur because PBR activation depolarizes LGN cells, thereby inactivating I_A. However, depolarization alone due to PBR activation was insufficient to explain the reduction in response latency: comparable depolarization via current injection into the cell produced much less latency reduction. Perhaps PBR activation has indirect effects on LGN cells through its action on local GABAergic circuits, or perhaps ACh has other effects on LGN cells that are not obvious in our recording. We conclude that I_A may well play a role in the lagged/nonlagged response dynamics, but that other factors, presently unknown, also contribute. Furthermore, the ability of PBR input to convert lagged to nonlagged responses is not a simple consequence of its effects on V_m. (Supported by USPHS grants EY03038 and EY06082.)

282.8

LOW THRESHOLD CALCIUM SPIKES PARTICIPATE IN LAGGED AND NONLAGGED RESPONSES OF CAT LGN CELLS. W. Guido, S.-M. Lu, and S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Voltage-dependent low threshold (LT) Ca^{2+} spikes in cat LGN cells are visually-evoked and comprise a large proportion of responses to visual stimuli. Some have suggested that the pattern of LT spiking might be different for lagged and nonlagged cells, and we tested this possibility. In cat LGN cells, we recorded visually evoked activity to a square-wave modulated bright or dark spot of light centered on its receptive field. Roughly 20% of the sample showed a lagged response ($\geq$70 msec latency to half-peak of response). During intracellular recording, both lagged and nonlagged cells exhibited stimulus-evoked LT spikes with bursts of action potentials riding their crests. We could recognize these responses extracellularly as action potentials with interspike intervals $\leq$4 msec, and we refer to them as *burst responses*; we define action potentials with longer interspike intervals as part of the *tonic response*. Nearly all cells displayed burst responses. For both lagged and nonlagged cells, the initial response to stimulus onset tended to be dominated by burst responses, which were then followed by more tonic responses. The latency of the first stimulus-evoked burst (or LT spike) was significantly longer and more variable among lagged cells than among nonlagged cells. Nonlagged cells typically showed a period of poor tonic responsiveness following the initial burst response, and this may reflect a refractory period following most LT spikes. Lagged cells may display a similar refractory period, but in the average histograms this is less evident because of more temporal scatter in the burst responses. Finally, for those lagged cells showing the property, the "anomalous off" discharge was dominated by tonic rather than burst responses. Overall, these data do not suggest any dramatically different involvement of LT spikes between lagged and nonlagged cells. (Supported by USPHS grants EY03038 and EY06082.)

282.9

ELECTROPHYSIOLOGICAL ANALYSES OF SINGLE NEURONS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF SQUIRREL MONKEYS. James R. Wilson, Yerkes Research Center, and Dept. of Anatomy and Cell Biology, Emory Univ., Atlanta, GA 30322.

Over 200 neurons were recorded in the LGNs of squirrel monkeys. All 58 of the parvocellular cells (P-cells) tested had linear responses to grating patterns, and only 3 of 16 magnocellular cells (M-cells) showed a nonlinear or doubling response. No cell gave a response that would be considered of the lagged type. Also, no cells with non-dominant eye inhibition were observed. Of the 64 P-cells tested, 44 had tonic responses (>5 sec) to standing center contrast, while 13 of 18 M-cells had phasic (<1 sec) responses. Qualitatively, very few LGN cells gave strongly differential responses to red, green or blue spots, and some of those were magnocellular cells, in distinct contrast to LGN cells of macaque monkeys. Most ON-center P-cells were located in the outer or dorsal portion of the parvocellular laminae, while most OFF-center P-cells were in the inner or ventral portion. One P-cell interneuron with an OFF center was observed in the most dorsal lamina. Therefore, the squirrel monkey's LGN probably has laminae physiologically separable into ON and OFF response type cells. It is concluded that squirrel monkey LGN neurons are very similar in their response properties to those of the macaque except for the relative lack of wavelength selective P-cells. Supported by NIH Grants EY04976 and RR00165.

282.10

COUPLING OF NEURONAL OSCILLATORS *VIA* INTRANUCLEAR COLLATERALS IN THE DORSAL LATERAL GENICULATE NUCLEUS (dLGN). Ivan Soltesz and Vincenzo Crunelli. Dept. of Visual Science, Inst. of Ophthalmology, London, U.K.

Recent in vitro studies have shown that some, though not all, thalamocortical (TC) neurons are capable of generating TTX-resistant oscillations in the frequency range of the EEG during the deep stages of slow wave sleep, by the periodic activation and inactivation of two inward currents, I_T and I_H (Leresche et al, Neurosci.Lett.,1990 & J.Physiol. 1991; McCormick & Pape, J.Physiol. 1990; Soltesz et al. J.Physiol. 1991). Here we studied the periodic excitatory and inhibitory synaptic interactions taking place between neurons of the cat dLGN in vitro.

In TC cells 3 types of rhythmic synaptic events could be observed: 1) EPSPs which were abolished by TTX (1μM); 2) IPSPs which were blocked by bicuculline (50μM); 3) complex, TTX-sensitive synaptic potentials consisting of a fast (15-25ms) depolarizing-hyperpolarizing sequence followed by a slower (50-70ms) depolarization. The frequency of these events was insensitive to changes in the membrane potential (-40 to -90mV). Between -65 and -80mV they could rhythmically evoke low-threshold Ca^{2+} spikes while above -50mV the EPSPs and the complex potentials directly gave rise to action potentials. Since all three types of periodic forcing were observed in slices which did not contain the perigeniculate nucleus, it is suggested that the intranuclear collaterals of TC cells might serve as one of the means of thalamic synchronization. Current pulses, injected intracellularly at different phases of the oscillations, designed to mimic EPSPs and reversed IPSPs revealed the existence of weak type 1 as well as strong type 0 phase resettings, and that critically timed (T^*) exposure to a stimulus of critical strength (S^*) could drive the oscillator towards its singularity (Winfree, J.Theor.Biol.,1970).

282.11

RETINAL GANGLION CELLS THAT PROJECT TO THE PULVINAR NUCLEUS IN MACAQUE MONKEYS. P.Stoerig[1], A.Cowey[2*], and M.Bannister[2*], Institute of Medical Psychology, LMU, Munich, FRG[1], and Department of Experimental Pychology, Oxford, GB[2]

To see which of the three classes of ganglion cells project to the pulvinar in primates, we injected HRP iontophoretically into various parts of the inferior, lateral, and medial pulvinar of 3 monkeys (2 injections 0.5mm apart in each hemisphere). We studied the retrogradely labelled cells in retinal wholemounts, disregarding the focus of dLGN-projecting cells seen when the region of uptake involved the caudal tip of the dLGN. Cells labelled from the pulvinar were found at all eccentricities including the parafoveal region. The vast majority were small, with cell bodies within the size range of Pβ and Pγ cells. On the basis of dendritic morphology we could identify well-filled cells as Pβ or Pγ. A small number of large cells, including classifiable Pα cells, were also labelled. There was no noticeable difference in the population when the region of uptake included or excluded the brachium. The results show that it is primarily Pβ and Pγ cells that project to the pulvinar. The subpopulation of Pβ cells that survives transneuronal retrograde degeneration up to 8 yrs. after striate cortical ablation may at least partially correspond to the pulvinar-projecting Pβ cells. *(Supported by DFG, MRC and MacDonell-Pew)*

282.12

FORM AND LOCATION PROPERTIES OF SINGLE UNITS IN THE PULVINAR OF AWAKE MACAQUE MONKEYS. Port, J. D. and L. A. Benevento. Department of Anatomy, University of Maryland College of Dental Surgery, Baltimore, MD 21201 and Department of Anatomy and Cell Biology University of Illinois, Chicago, IL 60612.

Recent anatomical studies have shown the pulvinar is composed of a mosaic of cell groups which are defined by their specific connections with functionally and anatomically defined cortical and midbrain areas. These cell groups, located within or across traditional pulvinar nuclear boundaries, are thought to form part of the distributed systems involving the occipitotemporal and occipitoparietal cortical visual systems for the analysis of form/color and location/motion respectively. We designed single unit experiments to examine functional correlates of this anatomical model. We recorded from trained rhesus monkeys performing a number of visual tasks. We found units which responded selectively to different forms. Some of these responded preferentially to simple stimuli such as a circle or square, while others responded preferentially to complex stimuli such as hands or stars. These units were located in clusters. Other units responded preferentially to certain non-foveal locations of point stimuli (0.5°) in the visual field. None of these cells responded differentially to different intensities of a visual stimulus (0.25°) indicating that they are not responding to light alone. The cells responded to form or location with either a suppression or excitation.

Attention effects were also found in some cells. The general firing rate seen throughout the intertrial interval (when the animal is expecting a trial) and the trial itself is suppressed or enhanced relative to the background firing seen in the "no task" state (in which the animal is not expecting any trials). During the trial, firing rate could also be further modulated by fixation and manipulanda action.

NERVE GROWTH FACTOR III

283.1

REGULATION OF C-FOS TRANSCRIPTION BY NGF IN CULTURED SENSORY NEURONS. N. Gabellini*, C. Minozzi*, A. Leon and R. Dal Toso. Fidia Research Labs, 35031 Abano Terme, Italy.

To investigate the possible influence of nerve growth factor (NGF) on c-fos expression in peripheral neurons, dorsal root ganglia (DRG) neurons from chicken embryos (E_8-E_{12}) were transfected with plasmids containing the mouse c-fos promoter linked to the chloramphenicol acetyltransferase CAT gene. Transfected DRG neurons expressed high CAT activity which could be enhanced by adding NGF, fetal calf serum or forskolin to the culture medium. After 24hr of NGF and serum deprivation CAT expression decreased to 30%. Readdition of NGF increased c-fos promoter activity linearly, with a two-fold stimulation after 13hr. The NGF EC_{50} on c-fos transcriptional activation was approximately 40pM suggesting the involvement of high-affinity NGF receptors. Deletion mutagenesis of the c-fos promoter showed the serum response element (SRE) to be relevant for basal and NGF-induced CAT expression. This experimental paradigm represents an appropriate tool for exploring the mechanisms underlying NGF effects in primary neurons, as well as their modulation by pharmacological agents (e.g. GM1).

283.2

EXPRESSION OF NGF *IN VIVO*, FROM A DEFECTIVE HSV-1 VECTOR ALTERS THE RESPONSE TO PERIPHERAL NERVE AXOTOMY. H.J. Federoff, M.D. Geschwind, A.I. Geller and J.A. Kessler. Depts. of Medicine, Neuroscience and Neurology, Albert Einstein Col. Med., Bronx, NY 10461, Div. Cell Growth and Regulation, Dana Farber Cancer Inst., Boston, MA 02115.

In adult rats, sympathetic neurons in the superior cervical ganglion (SCG) depend on target-derived NGF for maintenance of their adrenergic phenotype; one such action is the stimulation of the activity of the catecholamine biosynthetic enzyme, tyrosine hydroxylase (TH). Axotomy of the SCG results in NGF deprivation, causing a decline of TH activity within that ganglion. This effect can be prevented by the local application of NGF to the axotomized SCG. We used this SCG axotomy paradigm to test whether a HSV-1 vector expressing NGF (pHSVngf) could prevent the decline in TH activity that follows axotomy. Adult rats had one SCG exposed and injected with either pHSVngf virus (N=10) or pNFlac virus (N=9) which expresses *E. coli* ß-galactosidase gene instead of NGF. Four days later, animals underwent axotomy of the ipsilateral (virus injected) SCG. After another 10 days, all animals were sacrificed and both the injected-axotomized and contralateral-control ganglia were harvested and assayed for TH activity. In animals injected with pNF lac virus, axotomy produced a 50% decline in TH activity relative to the control ganglion (P= 0.02). In contrast, animals injected with pHSV ngf virus did not show a decline in TH activity and instead, manifested an 18% increase in TH levels relative to the control ganglion. These data indicate that defective HSV-1 vectors can be used to modify neuronal physiology *in vivo*.

283.3

INTRODUCTION OF NGF INTO CULTURED NEURONS AND NON-NEURONAL CELLS FROM A DEFECTIVE HSV-1 VECTOR. M. D. Geschwind, J.A. Kessler, A.I. Geller, and H.J. Federoff Depts. of Medicine, Neuroscience and Neurology, Albert Einstein Col. of Med., Bronx, N.Y. 10461 and Div. Growth and Regulation, Dana Farber Cancer Institute, Boston, MA. 02115.

An NGF minigene has been cloned into a defective HSV-1 vector, termed pHSVngf (Science 241:1667, 1988; Proc. Natl. Acad Sci., 87:1149,1990; Biochem. Pharm. 40: 2189,1990). pHSVngf virus has been tested for its ability to infect neurons and other cells, to produce NGF, and to modify the physiology of neurons. To ascertain whether the virus can cause the production and secretion of functional growth factor, established cell lines were infected, and the amount of NGF secreted into the medium was quantitated by an ELISA. NGF production rates ranged from 1-3 ng/hr. Moreover, the secreted NGF was functional, since media from these infected cell lines supported the survival of cultured rat sympathetic neurons in a dose-dependent manner. Infection of PC12 cells with the virus stimulated neurite outgrowth in the absence of exogenously added NGF. Furthermore, direct infection of cultured neonatal rat sympathetic neurons (which fail to survive in culture without addition of NGF) allowed survival without exogenous growth factor. Finally, infection of cultured striatal or septal neurons increased levels of the cholinergic enzyme, choline acetyl transferase (ChAT). These studies suggest that pHSVngf virus can be used to modify the physiology of neurons in culture and perhaps *in vivo*.

283.4

MOLECULAR CLONING AND DEVELOPMENTAL ANALYSIS OF NEUROTROPHIC FACTORS AND THEIR RECEPTORS IN ZEBRAFISH. **S.C. Martin* and G. Heinrich University Hospital and Boston University School of Medicine, Boston, MA 02118**

The Zebrafish (Brachydanio rerio) has been used as a model system to study neuron process outgrowth, growth cones and guidance mechanisms. We have sought to integrate this background with a molecular genetic analysis of the expression of nerve growth factor (NGF) and its low and high affinity receptors during neurodevelopment. Firstly, we used pools of degenerate oligonucleotide primers and the polymerase chain reaction to clone the Zebrafish homologs of NGF, the low affinity NGF receptor and the high affinity NGF receptor, the trk proto-oncogene. It has been found that these genes are closely similar to their mammalian homologs. We have used Northern blot analysis to study the expression of these genes during development. Secondly, we are using in situ hybridization techniques to study the temporal and spatial distribution of these mRNAs during Zebrafish neurodevelopment.

283.5

WIDESPREAD INCREASE OF mRNAs FOR NERVE GROWTH FACTOR AND BRAIN DERIVED-NEUROTROPHIC FACTOR DURING KINDLING EPILEPTOGENESIS

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Kindling, induced by repeated subconvulsive electrical or chemical stimulations leads to progressive and permanent amplification of seizure activity, culminating in generalized seizures. Acetylcholine act as a dis-inhibitory neurotransmitter and increased activity facilitates kindling epileptogenesis. Nerve growth factor (NGF) supports survival of, and induce sprouting from, cholinergic neurons of the basal forebrain, and recently, brain-derived neurotrophic factor (BDNF) have been shown to support cholinergic neurons of the basal forebrain.

Two hours after focal seizure, induced by electrical stimulation in the ventral hippocampus, lead to increases of mRNAs for NGF and BDNF in the dentate gyrus granule cells. Generalized seizures led to further increases of BDNF mRNA in the dentate gyrus, and an increase was also seen in the pyramidal layer of the hippocampus, the piriform cortex, the amygdaloid complex and the outer pyramidal layer of the parietal cortex 2 hrs after the last sstimulation. Generalized seizures also led to further increases of NGF mRNA in the dentate gyrus granule cells after 2 hrs. At 4 hrs after the last stimulation an increase in NGF mRNA was seen in the piriform cortex and in the outer pyramidal layer of the parietal cortex. The increased expression of NGF and BDNF mRNA was not blocked the NMDA receptor antagonist MK801 but partially blocked by the quisqualate, AMPA receptor antagonist NBQX. The presumed subsequent increase of the trophic factors themselves may be important for kindling-associated plasticity in specific neuronal systems in the hippocampus, which could promote hyperexcitability and contribute to the development of epileptic syndromes.

283.6

MECHANISMS OF INTERLEUKIN-1 ACTION IN HIPPOCAMPAL CULTURES

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Regulation of nerve growth factor (NGF) expression in hippocampus has been examined using a dissociated primary culture system. We have previously demonstrated that inflammatory agents such as interleukin-1 (Il-1) and prostaglandin E_2 increase levels of NGF mRNA.

Further work has proceeded to investigate possible second messenger pathways mediating the influence of Il-1 on NGF expression. cAMP does not appear to play a role as treatment of hippocampal cultures with dbcAMP or forskolin elicited no change in NGF mRNA. Moreover, exposure to Il-1 did not cause any change in cAMP levels. Nor did Il-1 alter PI turnover or calcium flux. Exposing cultures to phorbol ester resulted in a large increase in NGF mRNA, however pretreatment with the phorbol ester to desensitize the protein kinase C pathway only partially blocked the subsequent ability of Il-1 to induce NGF mRNA. This suggests that Il-1 may act partly through a PKC-dependent mechanism, but that other second messengers yet to be defined must also be involved.

We are currently investigating the role of other cytokines in regulation of trophic factor expression in the brain.

283.7

NGF BIOACTIVITY: ROLE OF THE AMINO TERMINUS

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The amino terminal octapeptide of NGF is highly conserved between species suggesting it might contribute important structural information; moreover, it is cleaved in a physiological setting in mouse submandibular gland. We wished to re-investigate whether or not NGF lacking this peptide (des^{1-8}NGF) is active.

NGF was prepared from male mouse submandibular glands as previously described (Biochem 15:5543, 1976). Des^{1-8}NGF was prepared by addition of ß- NGF endopeptidase which cleaves the NH_2-terminus. It was purified by ion-exchange chromatography; SDS-PAGE showed that > 97.5% chains were des^{1-8}NGF. Bioactivity was assessed using neurons from the ED8 chick dorsal root ganglion. Dissociated neurons were cultured with NGF for 24 hours and the number of neurite bearing cells counted. NGF activity was also assessed by measuring c-fos kinase activation in intact PC12 cells. (Method of L Taylor and G Landreth, Case Western Reserve University.)

In both assays NGF was significantly more active than des^{1-8}NGF. Neurite outgrowth from chick DRG neurons had an ED50 (50% maximum activity) of 80 pg/ml for routinely prepared NGF(35% des^{1-8}NGF) whereas for des^{1-8}NGF it was 2000 pg/ml. The c-fos kinase assay gave an ED50 of 10 ng/ml for NGF and an ED50 of greater than 400 ng/ml for des^{1-8}NGF.

These results contradict earlier data using intact dorsal root ganglia (Biochem 15:5543, 1976) which indicated that removal of the NH_2-terminal octapeptide had no effect on NGF activity. We are examining further the mechanism whereby the octapeptide confers activity - does it facilitate dimer formation, does it contribute directly to binding, or does it influence binding through allosteric effects? On the basis of our results it will be important to characterize NGF preparations for the extent of NH_2-terminal modification.

283.8

A RATE-LIMITING REPRESSOR NUCLEAR PROTEIN SPECIFICALLY REGULATES NGF GENE TRANSCRIPTION.

S. R. D'Mello and G. Heinrich. University Hospital and Boston University School of Medicine, Boston, MA 02118

Analyses of the mouse NGF gene promoter using transient expression assays in L929 cell fibroblasts indicate that the region from -250 to -500 contains an element associated with transcriptional repression. Deletion of this region results in an 8-fold increase in NGF promoter activity. Insertion of this region upstream from the mouse metallothionein gene promoter does not affect transcription suggesting that the repressor element is specific for the NGF gene. DNAse-1 footprint analysis indicate that the segment from -269 to -296 is bound by L929 nuclear proteins and suggests that the protected segment serves as the transcriptional repressor element. Cotransfection of a NGF promoter construct with a pUC-18 plasmid containing only 4 copies of the footprinted segment results in a 6-fold increase in transcriptional activity supporting this possibility. Additionally, this result suggests that the nuclear protein(s) that binds to this element is present in limiting amounts. Gel retardation assays show that the protein(s) that bind to the repressor-element are less abundant in L929 cells than in NIH 3T3 fibroblasts and B-16 melanoma cells. Since B-16 cells do not express the NGF gene and NIH 3T3 cells express lower amounts of NGF mRNA as compared with L929 cells, this result suggests that the cellular level of repressor protein(s) may in part influence the level of NGF mRNA expressed. The repressor-element lies within a 170 bp region of the NGF gene that has been implicated in glucocorticoid regulation raising the possibility that this element may be involved in both basal and modulated NGF gene expression.

283.9

Biological activity of NGF is mediated through a novel subclass of high affinity receptors. G.Weskamp* and L.F.Reichardt, Dept. of Physiology an HHMI, UCSF, San Francisco CA 94143.

Trophic factors, such as NGF, regulate survival and differentiation of neurons by binding to specific receptors. Two types of NGF receptors have been identified, which bind NGF with low and high affinity. However, the molecular relationship between both receptors is still unclear. To determine whether the low affinity receptor is necessary for generating a NGF-high affinity receptor complex, a polyclonal antibody to the low affinity NGF receptor was generated using a recombinant extracellular domain of the receptor as an antigen. The antibodies eliminate binding of NGF to the low affinity receptor expressed in L cells. On PC12 cells, these antibodies inhibit binding of NGF to the low affinity receptor as well as to one class of high affinity receptors. Suprisingly, a second subclass of high affinity NGF receptors was not affected by the antibody. In cross-linking experiments, these antibodies precipitated the complex of NGF with the low, but not the high affinity receptor. Furthermore, NGF-dependent survival or neurite outgrowth by rat sensory neurons or PC12 cells was not affected by the antibodies. Our data suggest that one class of high affinity receptors is derived from the low affinity receptor. However, a second, antigenically distinct class of high affinity receptors sufficient for mediating the major biological actions of NGF exist on PC12 cells. Recently, the product of the *trk* proto-oncogene was shown to be a constituent of a high affinity NGF receptor complex which may correspond to the high affinity receptor complex that is not recognized by the anti-low affinity antibodies.

283.10

TRKB: A FUNCTIONAL RECEPTOR FOR BDNF AND NT-3 BUT NOT NGF.

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A variety of findings seem to functionally link brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), while distinguishing both of these factors from a third member of the neurotrophin gene family, nerve growth factor (NGF). Here we demonstrate that all three of these neuronal survival molecules similarly bind to the low affinity NGF receptor (LNGFR), but that BDNF and NT-3 unlike NGF do not act via the high affinity NGF receptor HNGFR). However, both BDNF and NT-3, but not NGF bind to full-length and truncated forms of a receptor-like tyrosine kinase, trkB, for which no ligand had previously been identified. In addition to binding BDNF AND NT-3 with relatively hgih affinity, trkB can also mediate phenotypic responses to these two neurotrophins. For example, BDNF and NT-3 induce neurite outgrowth from trkB-expressing PC12 cells. BDNF and NT-3, but not NGF, can function as both mitogenic and survival-promoting molecules for trkB-expressing NIH 3T3 cells grown in serum-free defined media (growth factor-dependent 3T3 clone). Also, it appears that trkB alone, in the absence of the LNGFR, can serve as a functional receptor for the mitogenic/survival phenotype induced by BDNF and NT-3. Finally, we have detected trkB mRNA in ratE14 DRG and in some human neuronal cell lines and further expression data predicts the existence of additional functional receptors for the neurotrophins.

283.11

STRUCTURE-FUNCTION STUDIES OF NGF AND BDNF: IDENTIFICATION OF ELEMENTS DETERMINING NEUROTROPHIC SPECIFICITY, BIOLOGICAL ACTIVITY, RECEPTOR BINDING AND PROTEIN STABILITY. Carlos F. Ibáñez[1], Ted Ebendal[2], Wilma Friedman[3], Ira Black[3] and Håkan Persson[1]. [1] Department of Medical Chemistry, Laboratory of Molecular Neurobiology, Karolinska Institutet, S-10401 Stockholm, Sweden, [2] Department of Developmental Biology, Uppsala University, Uppsala, Sweden and [3] Department of Neuroscience and Cell Biology, UMDNJ, Piscataway, New Jersey.

Using site-directed mutagenesis, chimeric molecules were constructed in which different combinations of sequences from BDNF replaced the corresponding sequences in NGF. The resulting molecules were transiently expressed in COS cells and were assayed for biological activity in explanted chick sympathetic, spinal and nodose ganglia. Our results show that the biological specificities of the two proteins are obtained by specific combinations of a set of sequences that differ between the two molecules. Some of these combinations allowed us to engineer molecules which display multiple neurotrophic activities recruited from both the NGF and BDNF proteins. These molecules promote survival and induce neurotransmitter synthesizing enzymes in neuronal cultures of specific brain regions.

Alanine-scaning mutagenesis was applied to a highly conserved hydrophylic region in the rat NGF molecule (residues 25 to 36). The remarkable conservation of this region, together with its hydrophilic character, has led to the speculation that it could be important for biological activity and receptor binding. Our results show that some of these residues, notably those located between Asp30 and Gly33, are important for binding and biological activity. Our approach has also allowed us to identify residues important for production and/or stabilization of the protein, particularly Lys25, Asp30, Gly33 and Val36. Interestingly, this residues are also conserved in the other three members of the NGF family (BDNF, NT-3 and NT-4), indicating that they may contribute to the formation of a structural frame common to all members of the family.

283.12

EVOLUTIONARY STUDIES OF THE NERVE GROWTH FACTOR FAMILY REVEAL A NOVEL MEMBER NAMED NEUROTROPHIN-4 ABUNDANTLY EXPRESSED IN *XENOPUS* OVARY. F Hallböök, C. F. Ibáñez and H. Persson. Department of Medical Chemistry, Laboratory of Molecular Neurobiology, Karolinska Institute, Box 60400, S-104 01 Stockholm, Sweden

Evolutionary conservation of members of the NGF family in vertebrates was studied by DNA sequence analysis of PCR fragments for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) from human, rat, chicken, viper, *Xenopus*, salmon and ray. The results showed that the three factors are highly conserved from fish to mammals. Phylogenetic trees reflecting the evolution and speciation of the members of the NGF family were constructed. In addition, the gene for a fourth member of the family, neurotrophin-4 (NT-4), was isolated from *Xenopus* and viper. The NT-4 gene encodes a precursor protein of 236 amino acids which is processed into a 123 amino acid mature NT-4 protein with 50 to 60 % amino acid identity to NGF, BDNF and NT-3. The evolutionary analysis showed that NT-4 is closer related to BDNF than to NGF or NT-3. The NT-4 protein was shown to interact with the low-affinity NGF receptor and elicited neurite outgrowth from explanted chick dorsal root ganglia with comparable activity as NGF, BDNF and NT-3. Lower activity was seen on nodose ganglia and no activity was seen on sympathetic ganglia. Northern blot analysis of different tissues from adult *Xenopus* showed NT-4 mRNA only in ovary where it was present in more than 100-fold higher levels as NGF mRNA in adult xenopus heart.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION I

284.1

EFFECT OF TIME OF DAY ON LEVELS OF NUCLEAR AND CYTOPLASMIC POMC mRNA IN PROESTROUS AND ESTROUS RATS. K. Scarbrough, M. Jakubowski, N. Levin and J. L. Roberts. Fishberg Research Center for Neurobiology, The Mount Sinai School of Medicine, New York, NY 10029.

Previous studies showed a diurnal rhythm in the level of POMC mRNA in the peri-arcuate region of the hypothalamus of proestrous rats. The aim of the present study was to determine whether this rhythm of mRNA results, at least in part, from changes in the level of transcription of the POMC gene. In addition, we wished to determine if the level of primary transcript and/or POMC mRNA differs in rats on estrus compared to proestrus. We examined changes in the level of these nuclear and cytoplasmic RNA species using a solution hybridization/RNase protection assay.

Young rats exhibited at least two consecutive 4-day estrous cycles before they were killed at 0600 and 1300 h on proestrus or estrus. The medial basal hypothalamus (MBH) was dissected, frozen in freon, and stored at -70°C. Two MBH were pooled in each sample (5-6 samples per group), nuclei were pelleted and the cytoplasmic fraction was separated from the nuclear fraction. RNA was isolated from nuclear and cytoplasmic samples and hybridized to an excess of ^{32}P-labeled riboprobe complementary to a segment of intron A, extending through exon 2 and covering a segment of intron B of the POMC primary transcript. This probe was designed to distinguish between POMC mRNA (spliced), primary transcript (unspliced), as well as processing intermediates. We observed a similar pattern of change in POMC mRNA in the nucleus (expressed per µg DNA) and cytoplasm (expressed per µg cytoplasmic RNA). Levels of nuclear and cytoplasmic POMC mRNA decreased significantly on proestrus between 0600 and 1300 h. By 0600 h on estrus they returned to levels similar to those observed on the morning of proestrus. We were able to visualize a band corresponding to the POMC primary transcript, however, the signal was too low to quantitate reliably. We are currently pooling more animals/sample in order to quantitate changes in the levels of hnRNAs of low abundance. (Supported by HD07170, HD15955)

284.2

SEXUALLY DIMORPHIC EXPRESSION OF DYNORPHINS AND NEUROKININS IN THE RAT MEDIAN EMINENCE. P. Ciofi, D. Croix*, J.C. Beauvillain*, G. Tramu* and M. Mazzuca*. U.156 INSERM, Place de Verdun, 59045 Lille (France) & Laboratoire de Neurocytochimie Fonctionnelle (GT), URA.CNRS 339, Université de Bordeaux I, 33405 Talence (France).

Brain sexual differentiation results in the dimorphic organization of neural circuits controling reproduction. Few morphological data are available to explain the marked sex differences in the regulation of neuroendocrine functions.

Antisera specific for gonadoliberin (GnRH), α-neo-endorphin, dynorphin A, dynorphin B, substance P or neurokinin A (NKA, cross-reacts fully with neurokinine B) were used in immunocytochemical studies of the rat mediobasal hypothalamus. Antisera to dynorphins and to NKA all visualize a same population of nerve fibers distributed around blood vessels in the arcuate nucleus and in the palisade zone of the median eminence (ME) in normal adult male animals. This prodynorphin/neurokinin (DYN/NK)-containing innervation is codistributed in these areas with the GnRH-containing fiber plexus. Double-staining electron microscopic studies in the ME with preembedding immunoperoxidase DYN/NK labeling and postembedding immunogold GnRH labeling revealed the close apposition of DYN/NK-immunoreactive boutons with either tanycytes or GnRH-immunoreactive nerve endings. This DYN/NK ME staining pattern was neither observed in normal cycling, lactating or 35-day ovariectomized female rats, nor in 35-day orchidectomized adult males. Implantation of Silastic capsules filled with either testosterone, dihydrotestosterone or estradiol prevented the castration-induced depletion of ME DYN/NK-immunoreactivity in males. During development, the staining pattern typical of adult animals is installed progressively over the week preceding puberty and is also observed in the ME of 22-month old males.

This pronounced sexual dimorphism in the expression of neuropeptides in a circuit with neurohemal projections may be related to the dimorphic pattern of GnRH secretion which is phasic in females and tonic in males. The metabolism of dynorphins and neurokinins in the afore-mentioned circuit appears to be under the dual influence of testosterone and its aromatized metabolite estradiol.

284.3

PITUITARY PRODYNORPHIN mRNA LEVELS ARE SUPPRESSED BY ANDROGEN TREATMENT, BUT NOT BY ESTRADIOL OR PROGESTERONE. Alan H. Kaynard and Michael H. Melner. Divisions of Neuroscience and Reprod. Biology, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Prodynorphin(proDYN)-derived peptides can inhibit hypothalamic GnRH release thereby reducing pituitary gonadotropin secretion. The high levels of proDYN expression and/or peptide content within neurons of the hypothalamus and gonadotropes of the anterior pituitary gland suggest a role for proDYN-derived peptides in the negative feedback regulation of GnRH and gonadotropin secretion. The present study examined whether gonadal steroid hormones, which exert important negative feedback actions on the neuroendocrine axis, are capable of modulating pituitary proDYN expression in intact, immature rats. Steroids were administered via s.c. Silastic implants; rats were killed at 29 days of age. Northern blot analysis of anterior pituitaries using cDNA fragments of the rat proDYN, LHß, FSHß, and common α-subunit genes as probes was used to measure mRNA levels (normalized to 18S ribosomal RNA). Treatment groups (n=6) consisted of control (CNT; empty implants), estradiol (E_2; 8 days @ ~365 pg/ml), E_2+progesterone (E_2/P_4; 8 days @ ~370 pg/ml and 4 day @ ~25 ng/ml, respectively), and dihydrotestosterone (DHT; 8 days @ ~0.95 ng/ml). Pituitary proDYN mRNA was significantly suppressed in only the DHT treated animals (56 ± 12% of CNT, $p<0.05$) although slight reductions were seen in the E_2 and E_2/P_4 groups (75 ± 8% and 87 ± 9%, respectively). LHß mRNA was suppressed by all steroid treatments ($p<0.01$), FSHß was lower in only the E_2 group, and α-subunit was reduced in both the E_2/P_4 and DHT groups ($p<0.01$). Serum LH was suppressed by all steroid treatments but FSH was reduced in only the E_2 and E_2/P_4 groups ($p<0.01$). Since treatment of rats with continuous high levels of androgens is known to severely reduce endogenous hypothalamic GnRH release, these data suggest that diminished GnRH stimulation of the pituitary reduces proDYN expression. The absence of such an effect in E_2 and E_2/P_4 treated rats may be attributed to stimulatory effects of E_2 either directly on the pituitary or indirectly via alterations in the release of ovarian non-steroidal products (e.g. inhibin). Supported by NIH DK41035, RR00163, HD18185, and ONR NN00014-90-J-1122.

284.4

STEROIDAL MODULATION OF HYPOTHALAMIC PreProNPY mRNA AND IN VIVO NPY SECRETION INTO THE ANTERIOR PITUITARY OF MALE RATS. A. Sahu, [1]C. Phelps, [2]J. White, S.P. Kalra, [3]W.R. Crowley and P.S. Kalra. Dept. of OB-Gyn, Univ. of Fla. Gainesville, FL 32610, [1]Univ. So. Fla., Tampa, FL, [2]SUNY, Stony Brook, NY, [3]Univ. Tenn, Memphis, TN.

Neuropeptide Y (NPY) is an excitatory signal to LHRH and LH secretion. In male rats, castration (CAST) decreased and testosterone (T) replacement restored hypothalamic NPY levels and release in vitro in response to KCl (Endo. 124:410, 1989). To test whether androgens enhance NPY neurosecretion (release and synthesis) in vivo, we assessed the effects of T replacement on expression of NPY mRNA in the medial basal hypothalamus (MBH) of CAST rats and the effects of CAST on NPY secretion reaching the anterior pituitary (AP) as determined by push-pull cannula (PPC) technique. Expt. 1: Male rats received either T-filled or empty Silastic capsules (60mm, 3x20) s.c. immediately after CAST and were killed two weeks later. The content of PreProNPY mRNA was determined from total RNA extracts from the MBH by solution hybridization/RNase protection assay using cRNA probe against NPY. Expt. 2: One week after CAST or sham CAST, AP PPC were implanted. One week later, AP was perfused for 4 h (1100-1500 h) with artificial CSF and samples were collected at 10 min intervals for NPY analysis by RIA. Rats showing methylene blue dye penetration within the AP parenchyma at the end of the experiment were used for analysis. The results show that PreProNPY mRNA levels in the MBH were 2-fold higher in CAST, T-replaced as compared to control CAST rats. Further, NPY release in the AP was significantly decreased after CAST (sham: 33 ± 8.8 vs CAST: 10.4 ± 2.5 pg/10 min). These results indicate that gonadal steroids enhance hypothalamic NPY gene expression and secretion, and for the first time show an existence, in vivo, of a close relationship between secretion and gene expression of a neuropeptide involved in regulation of gonadotropin release (NIH HD11362, HD13703; NSF BNS9007573; MH46808).

284.5

NEUROPEPTIDE Y (NPY) GENE EXPRESSION IN THE ARCUATE NUCLEUS IS INCREASED DURING PREOVULATORY LH SURGES. A.C. Bauer-Dantoin, J.H. Urban and J.E. Levine. Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208.

NPY infusions have been shown to potentiate LHRH-induced LH surges in proestrous and steroid-treated, OVX rats. Augmented NPY neurosecretion may serve to sensitize the pituitary to the actions of LHRH on proestrous, and thereby act as an important component of the LH surge-generating process. The hypothesis that NPY neurons in the arcuate nucleus may operate in this capacity was tested in the present experiment. In situ hybridization was used to measure NPY mRNA levels at 0900h and every 2h from 14-2200h on the day of proestrous. Comparisons between groups showed a clear, stepwise increase in NPY gene expression throughout the day of proestrous. At 1600h, when LH values were significantly greater than 0900h values, NPY mRNA labeling intensities in the arcuate nucleus were significantly greater than 0900h intensities ($p<0.01$). By 1800h, the time at which the LH surge peaked, NPY mRNA levels also peaked, and were 3-fold greater than levels observed at 0900h ($p<0.01$). NPY mRNA levels at 2000h and 2200h remained elevated above 0900h levels ($p<0.01$), but by 2200h had decreased significantly from 1800h levels ($p<0.05$). No significant changes in NPY mRNA levels were observed in cerebral cortex during the same period. Our results support the hypothesis that NPY neurons participate in the generation of LH surges through increased production of NPY and subsequent potentiation of the release and/or actions of LHRH. (NIH HD20677 & HD21921)

284.6

STEROID DEPENDENCY OF NEUROPEPTIDE Y (NPY) mRNA-CONTAINING CELLS IN THE ARCUATE NUCLEUS OF THE MALE RAT. J.H. Urban, A.C. Bauer-Dantoin, J.E. Levine. Dept. Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208.

We have recently shown that NPY mRNA levels are increased in the arcuate nucleus (ARC) of female rats on the afternoon of proestrous, suggesting a dependency of NPY gene expression on periovulatory, ovarian steroid secretion. To determine whether testicular steroids regulate the expression of the NPY gene in male rats, in situ hybridization and quantitative autoradiography were used to measure NPY mRNA levels in the ARC of intact, castrated (2 wks) and castrated male rats treated with low (30mm capsules) and high doses (4x30mm capsules) of testosterone (T). The intensity of labelled cells was measured unilaterally throughout the ARC and compared between groups. Plasma T levels were significantly lower in the castrate group (<0.1 ng/ml) than in the intact (2.1±0.2 ng/ml), low T (1.3±0.1 ng/ml) or high T (5.3±0.4 ng/ml) treated groups. LH levels were inversely related to T values. NPY mRNA levels were significantly reduced in the castrate group (75% of control; $p<.05$). Both low and high T treatment restored NPY mRNA levels, but treatment with high T did not significantly enhance NPY gene expression in the ARC beyond control levels. Our observations support previous immunochemical findings and suggest that in the male rat, T may regulate NPY gene expression in the ARC. Studies are continuing to further investigate the role of NPY on reproductive functions in the male. (NIH HD20677 & HD21921)

284.7

NPY Y_1 RECEPTOR MEDIATES THE EFFECTS OF NPY ON LH AND LHRH SECRETION: STRUCTURAL REQUIREMENTS. S.P. Kalra, M. Fuentes, [1]A. Fournier and [2]W.R. Crowley, Dept. of OB-Gyn., Univ. Fla. Gainesville, FL 32610, [1]CNRS, Point-Claire, Quebec, [2]Dept. Pharm., Univ. Tenn., Memphis, TN 38163

Recent studies show that hypothalamic neuropeptide Y (NPY) is involved in the regulation of pituitary LH secretion. We have now assessed the effects of NPY Y_1 and Y_2 receptor agonists and several COOH- and NH_2-terminal NPY fragments on LH release in vivo and hypothalamic LHRH release in vitro. Since the in vivo excitatory and inhibitory LH responses to intraventricular NPY depended upon ovarian steroidal milieu, the LH response to these agents was evaluated in ovariectomized (ovx) and ovx rats pretreated with estrogen and progesterone (EP) and on the in vitro LHRH release from the hypothalami of ovx EP rats. Like NPY, a specific Y_1 receptor agonist, [Leu^{31},Pro^{34}]NPY, inhibited LH release in ovx rats, but it readily stimulated LH release in ovx EP rats. On the other hand, a specific Y_2 receptor agonist, NPY_{13-36}, was completely ineffective in these two models. Similarly [Leu^{31}, Pro^{34}]NPY and not NPY_{13-36} stimulated hypothalamic LHRH release. Interestingly, NPY_{2-36} was also active in these three experimental models. However, with the exception of NPY_{11-36} which stimulated LH release in ovx EP rats, other COOH-terminal peptides, NPY_{5-36}, NPY_{13-36}, NPY_{16-36}, NPY_{18-36} and the amidated NH_2-terminal fragment, NPY_{1-24}, were completely inactive. Thus, a distinct NPY Y_1 receptor mediates the in vivo excitatory and inhibitory LH and the in vitro excitatory LHRH responses. Further, these central agonistic responses are fully retained only after deletion of a single amino acid residue at the NH_2-terminal. (Supported by NIH grant HD 08634 and HD 13703).

284.8

NEUROANATOMY OF THE NEUROPEPTIDE Y SYSTEM IN THE MONKEY FOREBRAIN. K.K. Thind, J.E. Boggan* and P.C. Goldsmith. Reproductive Endocrinology Center, Univ. Calif., San Francisco, CA 94143.

Neuropeptide Y (NPY), a 36 amino acid peptide, promotes steroid-dependent release of pituitary gonadotropins, as well as gonadotropin-releasing hormone (GnRH) secretion from the medial basal hypothalamus (MBH). To help elucidate direct and indirect neuroendocrine (NEU) NPY systems in primates, we examined NPY neuroanatomy in the forebrain of female cynomolgus monkeys. Neuroendocrine (NEU) neurons were retrogradely labeled by microinjection of tracer into the median eminence. After aldehyde perfusion, series of 40 um frontal vibratome sections at 500 um intervals were collected and immunostained for NPY with PAP. NPY fiber densities were highest in the area olfactoria, medial septal, and ventromedial nuclei; high in the tuberculum olfactorium, MBH, tuber cinereum, and the arcuate, dorsomedial, lateral septal, medial preoptic (MPO), accumbens, and paraventricular (PVN) nuclei; moderate in the vertical portion of the diagonal band of Broca, the anterior and lateral hypothalamic, lateral preoptic, and anterior ventral periventricular areas, as well as the anterior commissural, suprachiasmatic, and dorsolateral supraoptic nuclei; and low in the nucleus and horizontal portion of the diagonal band, and in the supraoptic nucleus (SON). NPY neurons were scattered throughout the caudate nucleus, but none were NEU. However, in a sample series of sections, 65% of 133 NPY neurons in the SON, 41% of 112 in the PVN, 32% of 28 in the MPO, 14% of 118 in the MBH, and 55% of 11 in the inferior thalamic peduncle were NEU. We conclude that NEU NPY neurons occur in classical NEU regions, and that numerous NPY neurons contribute to an extensive forebrain fiber network in primates. The extent to which these diffuse and partially coextensive systems interact and regulate GnRH and other NEU systems remains to be determined. (Supported by NIH HD10907).

284.9

A SUBSET OF LHRH-IMMUNOREACTIVE NEURONS COLOCALIZE TYPE II (GLUCOCORTICOID) RECEPTORS IN THE RAT BRAIN. R.S. Ahima and R.E. Harlan. Dept. Anatomy and Neuroscience Training Program, Tulane University Medical School, New Orleans, LA 70112

Although estrogen and progesterone regulate LHRH release, the mechanism is still debatable, since LHRH neurons do not have estrogen (ER) or progesterone (PR) receptors. Excess glucocorticoids administered peripherally or intracerebroventricularlly inhibit gonadal function, presumably by interacting with LHRH neurons. The distribution of Type II corticosteroid receptors (GR) in the CNS overlaps with the LHRH system. This raises the possibility that the effect of glucocorticoids on gonadal function might be mediated via a direct action on LHRH expression and release. Progesterone can bind to GR *in vitro*. Hormone-activated PR and GR bind to the same DNA response element. Activated ER can act synergistically with GR to regulate multiple gene networks. GR may therefore play a major role in mediating the central effects of sex steroids and glucocorticoids on gonadal function.

Using the anti-GR monoclonal antibody BUGR2 and the anti-LHRH antiserum LR1, we have demonstrated, using immunoperoxidase or immunofluorescence double labeling, that about 20% of LHRH neurons in the median and medial preoptic regions colocalize GR. Few double-labeled neurons were observed in the diagonal bands, medial septum or anterior preoptic regions. These regions are also major targets for estrogen and progesterone. We suggest that the subset of LHRH neurons which colocalize GR may play a central role in linking the hypothalamo-hypophyseal adrenal axis to gonadal function. Supported by NS-24148.

284.10

GABA-OPIOID-ADRENERGIC INTERACTIONS IN THE REGULATION OF GONADOTROPIN SECRETION IN THE FEMALE RAT. D.W. Brann*, P.L. Zamorano*, W.J. Jackson, V.B. Mahesh*. Dept. of Physiol. & Endocrinol. Medical College of Georgia, Augusta, GA 30912

Previous studies in the male rat has demonstrated that GABA acting via $GABA_B$ receptors can abolish naloxone-induced LH secretion (Endocrinology 121:2251-2255). The purpose of the present study was to determine if an analogous situation exists in the female rat. Naloxone administered to ovariectomized (ovx.) immature rats had no effect on LH release. In contrast, naloxone potently stimulated LH release in estrogen-primed ovx. immature rats. Elevation of endogenous brain levels of GABA by administering aminooxyacetic acid, an inhibitor of GABA catabolism, prevented the naloxone-induced release of LH. This effect appeared to be both $GABA_A$ and $GABA_B$ receptor mediated, since exogenous administration of either muscimol ($GABA_A$ agonist) or baclofen ($GABA_B$ agonist) prevented the naloxone-induced release of LH. Neither GABA agonist had any effect on LHRH-induced LH release in vivo, suggesting that their effect was specific and achieved at the level of the CNS. GABA has been previously shown to rapidly reduce catecholamine concentration and turnover in the hypothalamus and this effect may explain GABA's ability to prevent naloxone-induced LH release in our studies. Along these lines, naloxone's effect on LH release does appear to involve catecholamine mediation since we found in the present study that administration of an α_1 or an α_2 adrenergic antagonist (prazosin or yohimbine, respectively) potently blocked naloxone's ability to stimulate LH release. In conclusion, these studies, as a whole, demonstrate that activation of *either* $GABA_A$ *or* $GABA_B$ receptors can abolish naloxone-stimulated LH release in the female rat. This effect is specific and appears to be achieved at the level of the CNS. Furthermore, naloxone-induced LH release appears to involve catecholamine mediation through α_1 and α_2 adrenergic receptors. *Supported by Research Grant HD-16688, NIH.*

284.11

HORMONAL REGULATION OF [^{125}I]IGF-I BINDING IN HYPOTHALAMUS AND PITUITARY OF THE RAT. K.M. Michels, M. Viswanathan, A.M. Seltzer* and J.M. Saavedra. Lab. Clin. Sci., NIMH, Bethesda, MD 20892.

Brain regions shown in autoradiographic studies to exhibit high levels of [^{125}I]insulin-like growth factor-I ([^{125}I]IGF-I) binding in the adult rat include the pituitary and several areas of the hypothalamus involved in endocrine regulation. In this study we use quantitative autoradiography to investigate the effect of endocrine manipulations on [^{125}I]IGF-I binding. In male rats, thyroidectomy decreased the binding of 250 pM [^{125}I]IGF-I in the suprachiasmatic nucleus (SCN), medial preoptic area and anterior and posterior pituitary compared to sham operated animals. Continuous infusion of a constant amount of T4 (48µg/kg/day) for 3 weeks restored the binding to sham operated levels. Binding in the median eminence (ME) was not affected by thyroidectomy. In female rats, ovariectomy decreased the level of binding in the SCN, ME and anterior and posterior pituitary compared to animals receiving continuous estradiol replacement. Binding levels in the SCN and ME of males were similar to estrogen-replaced females in the hypothalamus and to ovariectomized females in the pituitary. These results suggest hypothalamic and pituitary [^{125}I]IGF-I binding is affected by blood thyroid hormone levels in the male and by estrogen levels in the female.

284.12

COMBINATION OF IN SITU HYBRIDIZATION WITH RETROGRADE MARKER TO STUDY HORMONE EFFECT ON OXYTOCIN mRNA-EXPRESSING NEURONS WHICH PROJECT TO SPINAL CORD. Sookja K. Chung, Jaya Haldar and Donald Pfaff, The Rockefeller University, New York, NY 10021.

Ovarian hormones have been reported to increase oxytocin synthesis and release, and in turn this peptide facilitates reproductive and maternal behaviors. Using in situ hybridization we found increased levels of oxytocin mRNA, following estradiol (E), in SON and ACN neurons (J. Comp. Neurol., 307:281-295, 1991). Now we have combined in situ hybridization with retrograde tracer technique to study effects of E on oxytocin mRNA-expressing cells projecting to cord. Fluorogold pellets were applied to high thoracic cord in ovariectomized female rats treated with E (low dose, 1x2µg; or high dose, 2x10µg) or control (sesame oil vehicle). In situ hybridization was performed with a specific oxytocin oligonucleotide probe, end-labelled as previously described. Oxytocin mRNA-containing neurons which project to cord were found predominantly in middle and posterior levels of the paraventricular hypothalamic nucleus of female rats, comprising 12%-14% of the retrograde-labelled cells there. In frequency distributions of grains per oxytocin-labelled cell, it was seen that high but not low doses of E were followed by significantly greater amounts of oxytocin mRNA in a subpopulation of these neurons which project to cord. These results indicate that high E levels can stimulate oxytocin synthesis in a class of paraventricular neurons which might be important for controlling certain behavioral or autonomic functions.

NEUROTOXICITY I

285.1

GLUCOCORTICOIDS AGGRAVATE THE CHOLINERGIC DEFICIT INDUCED BY ETHYLCHOLINE AZIRIDINIUM (AF64A) IN RAT HIPPOCAMPUS. H.Hörtnagl*, M.L.Berger* and O.Hornykiewicz. Institute of Biochem. Pharmacol., Univ.Vienna, A-1090 Vienna, Austria

Glucocorticoids increase the vulnerability of hippocampal neuronal systems to various noxious stimuli (Sapolsky, TINS 10,346, 1987). In the present study we addressed the question whether glucocorticoids influence the extent of a cholinergic lesion in the rat hippocampus induced by ethylcholine aziridinium (AF64A). Male Sprague Dawley rats (300-350g) received bilateral stereotaxic infusions of 1nmol AF64A or vehicle into the lateral ventricle. Glucocorticoid treatment was started 7 days before AF64A and continued until sacrifice, 7 days after AF64A (corticosterone: 10mg daily or dexamethasone: 1mg/kg every second day; in sesame oil s.c.). Treatment with dexamethasone significantly increased the AF64A-induced loss of choline acetyltransferase (ChAT) activity in the hippocampus (dissected into CA1, CA3 and dentate gyrus), but did not affect ChAT activity in vehicle-treated rats. Reduction in ChAT activity in % ± SEM of corresponding control group (*$p < 0.05$;**$p < 0.01$ vs AF64A(sesame oil)

	(n)	CA1	CA3	dentate gyrus
AF64A/sesame oil	(5)	46 ± 2	57 ± 3	45 ± 2
AF64A/dexamethasone	(7)	55 ± 3	74 ± 3**	59 ± 4*

Similar but less pronounced results were obtained with corticosterone ($p < 0.05$ in CA1). The data indicate that glucocorticoids have the potential to increase the toxicity of AF64A on the septohippocampal cholinergic pathway.

285.2

NEUROTOXICITY OF CENTRAL NERVOUS SYSTEM THERAPY FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA. P. J. Mullenix, A. Schunior*, W. J. Kernan*, D. P. Waber*, A. Howes* and N. J. Tarbell*. Dept. of Toxicology, Forsyth Research Inst., Boston, MA 02115.

As part of the treatment for acute lymphoblastic leukemia, children receive central nervous system (CNS) prophylactic therapy to improve long term survival. Neurotoxic side effects, i.e. cognitive impairment, have been associated with this therapy. We studied a rat model to determine which of the agents in CNS therapy, cranial radiation (XRT), methotrexate (MTX) or prednisolone (PDN), was responsible for the neurotoxity. All experiments included Sprague Dawley rats exposed on postnatal days 17 and/or 18. The number studied was 15-20/treatment/sex, and each treatment group had matched controls given the appropriate sham radiation and/or saline (ip) injections. Spontaneous behavior was tested in the rats at 6 weeks and 4 months of age. A computer pattern recognition system automatically recorded and classified the behavioral acts performed while exploring a novel environment. Behavioral initiations, total time and time structure were compared in the treatment and control groups. We demonstrated (Mullenix et al., Cancer Res. 50:6461-6465, 1990) that exposure to combined therapy including 1000 cGy XRT, 18 mg/kg (ip) PDN and 2 mg/kg (ip) MTX permanently altered behavioral time structure in males. The XRT given alone did not produce the same effect, and the present study shows that MTX and PDN given alone are without significant behavioral impact. In contrast, when XRT is combined with MTX, steroid omitted, significant changes in behavioral time structure ($p<0.04$), total time and number of initiations ($p< 0.05$) appear in females. We conclude that the neurotoxicity induced by CNS therapy is due to an interaction of XRT and MTX, that females are more sensitive to this interaction, and that steroid modulates neurotoxic response, increasing susceptibility in males while decreasing susceptibility in females. Supported by NIH CA53858.

285.3

CELL ADHESION MOLECULES IN THE CEREBELLUM: TARGETS OF METHYLMERCURY TOXICITY? L.A. Lagunowich, S. Bhambhani*, R.D. Graff* and K.R. Reuhl*. Neurotoxicology Labs, Rutgers College of Pharmacy, Piscataway, NJ 08855.

Developmental neurotoxicity of methylmercury (MeHg) may be mediated, in part, by interactions of the toxicant with cell adhesion molecules. To test this hypothesis N-CAM and N-cadherin were examined biochemically and immunocytochemically in cerebella of Swiss Webster mice given 3mg/kg/day for 5 days, beginning on PN day 1 and collected on PN6, and in neurons derived from murine embryonal carcinoma cells exposed to 1-3 µM MeHg for 2 hours. Immunoblots reacted with antibodies for N-CAM revealed persistence of high molecular weight N-CAM form in the MeHg treated tissues. Immunoblots reacted with antiserum to N-cadherin revealed no differences between treated and untreated tissues. Results indicate that: 1) MeHg had no observable effect on N-cadherin; 2) MeHg inhibited conversion of N-CAM from a sialic acid-rich embryonic form to a sialic acid-poor adult form and; 3) both cerebellum *in vivo* and cultured neurons show similar patterns of toxic effect on N-CAM. These data suggests that N-CAM may be an important molecular target for MeHg. Supported by NIH grant ES-04976.

285.4

IMMUNOCYTOCHEMICAL LOCALIZATION OF METALLOTHIONEIN IN HUMAN SPINAL CORD. E. J. Kasarskis, X. Xie*, and W. R. Markesbery. VA and Univ. of Kentucky Med. Ctrs.; Sanders-Brown Aging Ctr.; Lexington KY 40536.

The accumulation of toxic heavy metals, such as mercury, has been detected in amyotrophic lateral sclerosis and Alzheimer's Disease. In non-neural tissues, metallothionein (MT), a cysteine-rich soluble protein, detoxifies heavy metals and contributes to zinc and copper homeostasis. We have studied the localization of MT in human spinal cord to establish if MT could potentially play a similar role in the human CNS.

The distribution of MT was examined in formalin-fixed spinal cords from 10 middle aged persons using a monoclonal antibody to human liver metallothionein-II, a gift of Dr. Ananda Prasad. MT-immunoreactivity was visualized in the cytoplasm of motor neurons at all spinal levels. Immunoreactivity was also detected in astrocytes, capillary endothelia, and arachnoid.

These findings indicate that MT is widely distributed in human spinal cord. Although the function of MT in spinal cord has not been studied, its localization to neuronal cytoplasm suggests that MT could be important for metal homeostasis and detoxification in motor neurons.

285.5

N-METHYL-4-PHENYLPYRIDINIUM (MPP+) POTENTIATES THE KILLING OF CULTURED HEPATOCYTES BY CATECHOLAMINES. J.W.Snyder*, T.N.Ferraro, M.E.Kyle*, J.R.Grothusen*, G.M.Alexander and J.L.Farber*. Departments of Medicine, Neurology, and Pathology, Jefferson Medical College, Philadelphia, PA 19107. Catabolism of catecholamines can lead to oxidative stress predisposing cells to injury not encountered in cells lacking catecholamines. The glutathione reductase inhibitor BCNU accelerated the death of the cells by dopamine (DA) or 6-hydroxydopamine (6-OHDA), while deferoxamine, catalase or superoxide dismutase reduced cell death. Incubation of hepatocytes with combinations of 1-methyl-4-phenylpyridine (MPP+) and either 6-OHDA, DA or norepinephrine (NE) (order of potency: 6-OHDA > DA > NE) accelerated cell death under conditions where MPP+ alone or catecholamines alone did not kill cells. Cultures treated with combinations of rotenone plus 6-OHDA also displayed increased cell death under conditions where rotenone alone or 6-OHDA alone did not kill cells. Pretreatment with deferoxamine, glycine or fructose delayed death of cells treated with catecholamines plus MPP+. Inhibitors of monoamine oxidase had no protective effect. These data suggest: 1) cells containing catecholamines are more vulnerable to site I (NADH dehydrogenase) inhibition; 2) cell vulnerability is catecholamine dependent; 3) the mechanism of death by catecholamines may involve oxidative stress, but does not require MAO-dependent oxidation.

285.6

ACUTE EFFECT OF MPTP ON ATP IN MOUSE BRAIN. P. Chan*, L.E. DeLanney, I. Irwin*, J.W. Langston and D. Di Monte. California Institute for Medical Research and California Parkinson's Foundation, San Jose, CA 95128.

The cytotoxic effects of the parkinsonism-inducing neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are dependent upon its conversion to the 1-methyl-4-phenyl-pyridinium (MPP+) metabolite. Then, MPP+ is thought to block mitochondrial respiration at the level of complex I and to cause cell damage by depleting ATP. In this study, ATP levels were measured in mouse brain after rapid fixation of cerebral tissue in situ by microwave irradiation. Administration of MPTP (40 mg/kg s.c.) caused a rapid (within 30 min) decrease (approxim. 10-20%) in striatal ATP. This decrease was prevented by mazindol, a catecholamine uptake blocker, and was enhanced (ATP levels were approxim. 40% lower than in control animals) by pretreatment of mice with 2-deoxyglucose. In mice exposed to MPTP after treatment with 2-deoxyglucose, a gradual recovery of ATP was observed during the following 24 hours. The time course of ATP changes was similar to the time course of MPP+ accumulation and elimination in the striatum, while dopamine depletion seemed to follow these events. These results suggest that ATP depletion plays an important role in MPTP-induced neurotoxicity and support the hypothesis of a defect of mitochondrial function in the MPTP model of parkinsonism.

285.7

TOXICITY OF MPTP IN PRIMARY CULTURES OF MOUSE ASTROCYTES. D. Di Monte, E. Y. Wu*, L.E. DeLanney, I. Irwin* and J.W. Langston. California Institute for Medical Research and California Parkinson's Foundation, San Jose, CA 95128.

Astrocytes are likely to be a primary locus for the bioactivation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to the toxic 1-methyl-4-phenyl-pyridinium (MPP^+) metabolite. However, degeneration of glial cells does not appear to be a neuropathologic feature of MPTP exposure *in vivo*. In order to identify possible protective mechanism(s) that may make glial cells relatively resistant to MPTP injury, biochemical and toxic changes were studied in primary cultures of astrocytes exposed to this compound. MPTP caused a concentration-dependent loss of cell viability assessed as leakage of lactate dehydrogenase in the incubation medium. An early metabolic change following MPTP (250 µM) exposure was an increase in the rate of utilization of glucose and accumulation of lactate in the culture medium. The ratio between the rates of lactate production (0.37 mM/hour) and glucose consumption (0.2 mM/hour) was 1.85, thus very close to the ratio of 2 expected if all glucose was stoichiometrically converted to lactate. Exposure of astrocyte cultures to MPTP also resulted in a dramatic depletion of ATP which occurred before the onset of cytotoxicity. ATP depletion and loss of cell viability occurred more rapidly in cultures incubated in the absence than in the presence of glucose. Finally, cytotoxicity was prevented when MPTP was washed from the incubation medium even just before the onset of cell death. This is probably due to the fact that, once formed within cells, MPP^+ can cross astrocyte membranes toward the extracellular space. Thus, "escape" of MPP^+ from glial cells may represent a protective mechanism against irreversible damage following MPTP exposure *in vivo*.

285.8

MK 801 PREVENTS MPTP-INDUCED NIGROSTRIATAL NEURONAL DEATH IN MONKEYS AND MICE. A. Zuddas, F.Vaglini, F.Fornai, F.Fascetti, G.U.Corsini. Institute of Pharmacology, University of Pisa, Italy

An anatomical and biochemical lesion similar to that observed in monkeys can be obtained in mice combining MPTP treatment with acetaldehyde (ACE) (Brain Res. 501:1-10,1989, Eur.J.Neurosci. 3:72-85,1991). Here we investigated whether excitatory aminoacids may modulate the MPTP-induced dopamine depletion and cell death. In both mice and monkeys, MK 801, a non-competitive antagonist of the NMDA receptor, was systemically administered together with MPTP. The behavioral, biochemical and histological effects were evaluated 7 days after treatment. In C57BL mice, MK 801 (2.5 mg/Kg administered i.p. 30 min before and 90 min after MPTP), completely prevented ACE-induced enhancement of MPTP toxicity. Striatal dopamine content was 75.6±6.3 and 6.7±3.1 ng/mg protein in MPTP and ACE+MPTP-treated animals, respectively (controls: 166.5±13.7). When MK 801 was also administered, striatal dopamine was 89.5±3.9 and 59.4±8.5 ng/mg protein in MPTP and ACE+MPTP-treated animals, respectively. Tyrosine hydroxylase immunostaining revealed that the extensive loss of DA neurons observed in the SNc of ACE+MPTP-treated mice was completely prevented by MK 801 administration.

In monkeys (*Macaca fascicularis*), MK 801 (0.010 mg/kg administered i.v. 7 times per day during MPTP treatment), was able to completely prevent the parkinsonian syndrome induced by i.v. administration of MPTP (0.3 mg/kg/die per five days). Catecholamine analysis showed how MK 801 significantly attenuated the MPTP-induced dopamine depletion in the basal ganglia. The protective effect of MK 801 was more evident in the ventral portion of the caudate and putamen than in the dorsal part of these brain regions (DA in Dorsal Caudate: 132.4±13.3, 0.8±0.8 and 27.6±11.8 ng/mg protein in control, MPTP and MK 801+MPTP-treated monkeys, respectively; DA in Ventral Caudate: 84.0±9.5, 2.1±1.6, and 75.0±17.8, respectively). Taken together, these findings indicate that NMDA receptors play a crucial role in MPTP-mediated cell death and suggest that ACE administration make the MPTP toxicity in mice similar to that observed in primates.

285.9

MPP+ PRODUCES EXCITOTOXIC LESIONS IN RATS. E. Storey, B.T. Hyman, J.M. Miller* and M.F. Beal. Neurology Service, Mass. General Hospital, and Harvard Medical School, Boston, MA 02114.

MPP^+ is the active metabolite of MPTP, and is an inhibitor of mitochondrial complex I. Turski et al. (Nature 349, 414) showed that MPP^+ nigral lesions were blocked with NMDA antagonists. In the present study we examined whether local striatal injections of MPP^+ produce excitotoxic lesions. MPP^+ was stereotactically injected into the left striatum. One week after injection animals were sacrificed and the left and right striata dissected. Neuropeptides were measured by radioimmunoassay and GABA and monoamines were measured by HPLC with electrochemical detection. Increasing doses of MPP^+ (7.5 - 90 nmol) resulted in dose-dependent reductions of substance P and GABA, with relative sparing of somatostatin and neuropeptide Y. Relative sparing of somatostatin neurons was confirmed by immunocytochemistry. At a dose of 85 nmol of MPP^+, substance P and GABA were depleted 50%, dopamine 85% and serotonin 40%. Prior decortication protected significantly against MPP^+ neurotoxicity, however the protection was only partial for dopamine. Immunocytochemistry for glial fibrillary acidic protein and Nissl stains confirmed a protective effect with decortication. Freeze-clamp experiments showed that ATP was 2-fold decreased and lactate was increased 4 fold on the injected side at 3, 24 and 48 hr. These experiments show that MPP^+ can produce excitotoxic lesions, presumably by lowering membrane potential which relieves the voltage dependent Mg^{++} block of the NMDA receptor, leading to persistent receptor activation.

285.10

IS AMPHETAMINE (AMP) INDUCED NEUROTOXICITY THERESULT OF REDUCED NEUROTROPHIC FACTOR PRODUCTION? C.M.Buhrfiend, J.Z. Fields [1], G.E. Drucker[1], D.H. Lin*, E.S. Lo, L.R. Ptak*, and P.M. Carvey. Rush U. and [1]Hines V.A., Chicago, IL.

Using an established AMP neurotoxicity model (3 daily treatments of 9.2 mg/kg AMP + 10 mg/kg imprindole) we examined the effect of striatal extracts on rostral mesencephalic tegmentum (RMT) culture growth to determine if toxic AMP treatment reduces the production of a striatal-derived neurotrophic factor (SdNTF). At 7 and 14 days following the last treatment, striatal DA content was decreased 23 and 48% respectively by AMP treatment relative to imprindole treated controls suggesting neurotoxicity. Forty hours after the addition of striatal extracts to E13.5 RMT cultures, the number of viable cells with processes was assessed. Striatal extracts taken from AMP treated animals 7 and 14 days following the last treatment reduced RMT culture growth by 94 and 84% respectively relative to controls. These data suggest that acute neurotoxic doses of AMP reduce the production of SdNTF and that it is therefore possible that the neurotoxicity observed following AMP treatment could result from AMP-induced reductions in SdNTF.

285.11

CARBAMAZEPINE INDUCES NEUROTOXICITY OF CEREBELLAR NEURONS BY A N-METHYL-D-ASPARTATE (NMDA) REVERSIBLE MECHANISM. X.M. GAO and D.-M. Chuang, Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

Treatment of cerebellar granule cells with carbamazepine (CBZ) in growth medium resulted in a time- and dose-dependent decrease of carbachol-induced phosphoinositide turnover and of muscarinic cholinergic receptor sites. To investigate whether this effect is due to neurotoxicity, we studied long-term effects of CBZ on ^{3}H-ouabain binding to neuronal Na^+, K^+-ATPase, which recently was shown to provide a means to quantify neurons in a mixed culture (Markwell et al, Brain Res 538:1-8, 1991). Exposure of cerebellar granule cells to CBZ for 3 days induced a dose-dependent decrease of ^{3}H-ouabain binding to intact neurons. The IC_{50} was approximately 40 μM and maximal loss occurred at 100 μM. CBZ's effect was totally blocked by the co-presence of 100 μM NMDA. The EC_{50} of NMDA for reversing CBZ (50 μM)-induced toxicity was approximately 30 μM and this protection was nullified by 200 μM APV. The reversal of CBZ-induced toxicity by NMDA was visible by morphological examination. MK-801, a NMDA antagonist, also displayed a dose-dependent neurotoxicity and its effect was less than additive to that produced by CBZ. Long-term CBZ treatment resulted in significant up-regulation of NMDA-induced phosphoinositide turnover. Our results indicate that CBZ is capable of inducing slow toxicity in cerebellar neurons. Moreover, this effect might be due to a constant blockade of NMDA receptor by CBZ.

INTERACTIONS BETWEEN NEUROTRANSMITTERS I

286.1

EFFECTS OF COCAINE, AMPHETAMINE, AND GBR 12909 ON THE GENE EXPRESSION OF STRIATAL NEUROPEPTIDES. Y.L. Hurd and M. Herkenham. Section on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Psychomotor stimulants such as cocaine and amphetamine acutely potentiate the overflow of dopamine (DA) from presynaptic terminals in the striatum. The major post-synaptic targets of DA in this region are medium spiny neurons which contain the neuropeptides dynorphin (DYN), substance P (SP), and enkephalin (ENK). Using *in situ* hybridization histochemistry in rats, the effects of a single acute injection (i.p.) of cocaine, amphetamine, or GBR12909 on the gene expression of striatal neuropeptides were investigated 2 hrs (acute) or 2 weeks (delayed) following drug administration. In the dorsal striatum, acute cocaine and GBR 12909 elevated DYN mRNA and SP mRNA levels. The increases were greater in the dorsal than in the ventral striatum. The acute elevations returned close to control values at 2 weeks. A second, challenge injection of the same stimulants, given 2 weeks after the first, re-elevated mRNA levels of the neuropeptides. However, the increase, e.g. of DYN mRNA by cocaine and GBR 12909, was attentuated compared to the response induced by the acute initial exposure to the stimulant, and although amphetamine had the weakest effect in acute and delayed groups, a challenge injection of the drug greatly potentiated DYN mRNA levels. Thus, differential initial as well as long-term consequences of a single injection can be revealed at the genetic level. Differential effects on striatal DYN, SP and ENK gene expression were also found following daily repeated (2 weeks) administration of the stimulants.

286.2

DIFFERENTIAL EFFECTS OF PARTIAL DOPAMINE DEPLETION ON STRIATAL PROENKEPHALIN AND DOPAMINE D2 RECEPTOR mRNA LEVELS IN THE RAT. Jean Lud Cadet [1], Shu-ming Zhu [*,1], and Jesus A. Angulo [2]. [1]Columbia University, and [2]Rockefeller University, New York, New York

Nigrostriatal (NS) dopaminergic (DA) neurons are thought to exert an inhibitory influence on striatal enkephalinergic systems through their DA D2 receptors. In order to investigate the effects of partial lesions of the NS DA on striatal proenkephalin (PEK) and D2 receptor mRNAs, animals were divided into *High*, *Intermediate*, and *Low* rotators on the basis of amphetamine-induced rotation observed after intrastriatal injections of 6-hydroxydopamine (6-OHDA). There were significant increases in PEK mRNA in all three groups on the ipsilateral side of the lesions; these changes correlated positively with increases in rotation rate. Unexpectedly, there were significant increases in PEK mRNA on the contralateral side as well. On the other hand, only the *High* rotation group showed any increases in D2 receptor mRNA which occurred only on the side of the lesions. These results indicate that partial lesions of NS DA projections are sufficient to cause increases in PEK mRNA but not in D2 receptor mRNA. These data also provide evidence that the two NS DA projections and the systems which they modulate might be interdependent. The present experiments also suggest that the striatal enkephalinergic molecular machinery may actually be under a set of controls that are more complex than a simple inhibitory influence by DA acting via its D2 receptors.

286.3

GLYCINE-EVOKED RELEASE OF [^{3}H]-ACETYLCHOLINE FROM NUCLEUS TRACTUS SOLITARII. W.T. Talman, H. Ohta, and D. Martinez.* Department of Neurology, University of Iowa and VAMC, Iowa City, IA 52242.

Our previous studies have demonstrated that microinjection of glycine (GLY) into nucleus tractus solitarii (NTS) produces depressor and bradycardiac responses that are blocked by strychnine or muscarinic antagonists and prolonged by the cholinesterase inhibitor physostigmine injected into NTS. Our hypothesis that the responses to GLY were mediated through release of acetylcholine (ACh) from NTS was supported by release of [^{3}H]-ACh from NTS tissue punches exposed to GLY. In the current study we have sought to determine (1) whether GLY-mediated release of [^{3}H]-ACh from NTS is Ca^{2+}-dependent and (2) whether GLY acts through strychnine-sensitive receptors to mediate release of ACh. [^{3}H]-ACh release evoked by GLY (1mM) from micropunches of NTS was significantly reduced by removal of Ca^{2+} from Kreb's bicarbonate buffer bathing the tissue (control: 136±38 dpm/5 min. vs. Ca^{2+}-free: 4±24 dpm/5 min., $p<0.05$). When 10 μM strychnine was added to Ca^{2+}-containing incubation medium prior to and during application of GLY (1mM), the release of [^{3}H]-ACh was inhibited. These results indicate that GLY causes release of ACh from neurotransmitter pools in NTS and release is mediated through activation of strychnine-sensitive GLY receptors. Support: VA Merit Review; NIH HL32205; HL14388, and NS24621.

286.4

BETA ADRENERGIC REGULATION OF EXTRACELLULAR CYCLIC AMP AND ADENOSINE LEVELS IN CEREBRAL CORTEX IN CULTURE. K.K. Petrison, R.B. Knowles, and P.A. Rosenberg. Dept. of Neurol. and Program in Neuroscience, Children's Hospital and Harvard Med. Sch., Boston, MA 02115.

Stimulation of astrocyte-rich rat cortical cultures with the beta adrenergic agonist isoproterenol (ISO) caused secretion of cyclic AMP into the extracellular medium with an EC50 of 70 ± 40 nM [n = 5, 29-34 days in culture (DIC)]; peak extracellular concentration of cyclic AMP with maximal stimulation (1-10 μM) was 71 ± 33 nM (n=9, 28-67 DIC), determined by radioimmunoassay. In order to characterize the fate of secreted cyclic AMP, high pressure ion pair liquid chromatography, using pre-column chloroacetaldehyde derivatization, was employed to characterize the time course of the appearance of cyclic AMP, AMP, and adenosine in the extracellular medium following ISO stimulation of cultures. A significant increase (214 ± 23%; n=3, 47-61 DIC) in extracellular adenosine was observed within thirty minutes following addition of 1 μM ISO. The concentration of adenosine measured in the medium surrounding control cultures was 32 ± 7 nM. Secretion of cyclic AMP by astrocytes may be a significant source, in cerebral cortex, of extracellular adenosine, which might in turn mediate some of the actions of norepinephrine by a paracrine mechanism. Supported by NIH grant NS 26830.

286.5

INTRACISTERNALLY COINJECTED GALANIN AND A 5-HT_{1A} RECEPTOR AGONIST ACT SYNERGISTICALLY TO PRODUCE VASODEPRESSOR RESPONSES IN THE α-CHLORALOSE ANAESTHETIZED MALE RAT. J.A. Aguirre, P. Hedlund*, J.A. Narváez* and K. Fuxe. Dept. of Histology and Neurobiology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

Interactions between the galanin (GAL) and the 5-hydroxytryptamine receptors of the 1A type (5-HT_{1A}) have previously been demonstrated in *in vitro* studies. The present work has been undertaken to evaluate the possible functional implications of such interactions. DPAT (0.1-30 nmoles/rat) was given intracisternally (i.c., 10 µl) in α-chloralose (100 mg/kg i.v.) anaesthetized male Sprague-Dawley rats (200-250 gr). Coinjections of DPAT and the selective 5-HT_{1A} receptor antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthlimido)butyl]piperazine (NAN-190) were carried out. Simultaneous i.c. coinjections of DPAT and GAL were performed to evaluate any interactive effects of GAL and DPAT. Measurements of mean arterial blood pressure (MAP) were made during a 1 h time interval. In receptor autoradiographical experiments coronal 20 µm thick cryostate sections from the region of the dorsal medulla (bregma -13.8 mm) were incubated with the radioligand [^{125}I]GAL (0.4 nM) in the presence or absence of DPAT (10 nM). DPAT given i.c. produced a dose-dependent reduction of MAP, the peak action being 32% at 10 nmol. The vasodepressor action of DPAT could be counteracted by NAN-190. A threshold dose (1nmol) of GAL given i.c. was shown to enhance the vasodepressor effect both of an ED_{50} dose and a threshold dose of DPAT. Quantitative receptor autoradiography showed that the IC_{50} value for GAL was reduced by approximately 40% within the dorsal region of the nucleus of the solitary tract, the area postrema and the raphe pallidus and obscurus nuclei in the presence of DPAT (10 nM). The results give evidence for a synergistic interaction between DPAT and GAL in cardiovascular regulation upon their central administration, possibly related to an ability of DPAT to enhance the affinity of the galanin receptor within cardiovascular regions of the medulla oblongata.

286.6

INTERACTION OF PRESYNAPTIC HISTAMINE H_3 RECEPTORS WITH PRESYNAPTICα_2-AUTORECEPTORS AND NMDA HETERORECEPTORS IN THE BRAIN CORTEX. M. Göthert, E. Schlicker* and K. Fink*. Inst. of Pharmacol. and Toxicol., Univ. of Bonn, D-5300 Bonn 1, Germany.

We have recently shown that presynaptic histamine H_3 receptors mediate inhibition of noradrenaline (NA) release in the rat and mouse brain cortex. The aims of the present study were (1) to determine the effects of α_2-adrenoceptor ligands on the histamine (H)-induced inhibition of the electrically evoked NA release and (2) to examine whether H also inhibits the NA release in response to activation of the presynaptic NMDA receptor. In superfused rat or mouse brain cortex slices preincubated with tritiated NA, tritium overflow was evoked either electrically (mouse brain slices) or by NMDA 100 µM (rat brain slices superfused with Mg^{2+}-free medium). The electrically evoked tritium overflow was inhibited by H 1 and 10 µM. The effect of H 10 µM was not modified by α_1-adrenoceptor ligands but decreased by the α_2-agonist B-HT 920 1 µM and increased by the α_2-antagonist rauwolscine 1 µM. These effects still occurred, when stimulation parameters were adjusted to obtain the same tritium overflow as in the absence of α_2-adrenoceptor ligands. In slices superfused in the presence of the α_2-antagonist idazoxan 1 µM, H 0.1-10 µM inhibited the NMDA-evoked tritium overflow. This effect was abolished by the H_3 receptor antagonist thioperamide 0.32 µM, but was not affected by the H_1 and H_2 receptor antagonists dimetindene 32 µM and ranitidine 320 µM, respectively. In conclusion, the presynaptic inhibitory H_3 receptor interacts with two further presynaptic receptor systems on the same noradrenergic nerve terminals of the brain cortex. (1) Activation of the α_2-autoreceptor decreases, whereas its blockade increases, the ability of the H_3 receptor to mediate inhibition of NA release. (2) The NA release in response to activation of the presynaptic excitatory NMDA receptor is inhibited by the stimulation of the H_3 receptor.

286.7

PROJECTIONS OF GABAERGIC AND CHOLINERGIC BASAL FOREBRAIN NEURONS TO THE POSTEROLATERAL HYPOTHALAMUS. I. Gritti*, L. Mainville, B.E. Jones. Lab. of Neuroanatomy, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

GABAergic neurons are intermingled with cholinergic neurons within the basal forebrain and may correspond to the cells that are active during slow wave sleep which are intermingled with those that are active during waking. Putative sleep active basal forebrain neurons could promote sleep by damping the activating system of the brain stem reticular formation and posterior hypothalamus. To assess the potential influence of forebrain GABAergic and cholinergic neurons upon this system, Cholera-Toxin-B (CTb) was injected into the posterolateral hypothalamus and its retrograde transport was examined in combination with immunohistochemistry for GAD (glutamic acid decarboxylase) and ChAT (choline acetyl transferase) in the rat. Of a total large population of retrogradely labeled cells within the basal forebrain, an important contingent (11.5%) were GAD-immunoreactive, whereas only a small portion (0.5%) were ChAT-immunoreactive. At least 25% of the GAD+ whereas less than 5% of the ChAT+ cells within the basal forebrain were found to send descending projections to the posterolateral hypothalamus. The GAD-immunoreactive cells were found intermingled with ChAT-immunoreactive cells within the basal forebrain, and exceeded the ChAT+ cells in a ratio of 3:2. The results suggest that GABAergic neurons of the basal forebrain could promote sleep through a descending projection onto the posterolateral hypothalamus. (Supported by the Canadian MRC)

286.8

MONOSYNAPTIC INPUT TO DOPAMINERGIC NEURONS IN RAT SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA FROM DYNORPHIN-IMMUNOREACTIVE TERMINALS. V.M. Pickel, J. Chan, and S.R. Sesack. Div. of Neurobiology, Dept. of Neurology and Neuroscience, Cornell University Med. Center New York, N.Y. 10021

We sought to determine whether there is a direct synaptic basis for interactions between terminals containing dynorphin-like immunoreactivity (DYN-LI) and tyrosine hydroxylase (TH) containing dopaminergic neurons in the substantia nigra (SN) and ventral tegmental area (VTA). Sections through the adult rat midbrain were immuno-labeled using respectively, peroxidase and gold-silver to identify a rabbit anti-dynorphin (1-17) and mouse anti-TH. Terminals containing DYN-LI were numerous in the SN and rarely detected in the VTA. However, in both regions these terminals were characterized by either all small clear vesicles or a mixture of small, clear and intensely labeled large dense core vesicles. The majority of the synapses were symmetric and the targets included both TH-labeled and unlabeled dendrites in both regions. The rarity of the terminals containing DYN-LI in the VTA prevented further quantitative analysis of their cellular associations. However, from 1191 observed terminals containing DYN-LI in SN, 20% formed synapses with TH-labeled and 40% with unlabeled dendrites. Many of these terminals also were in direct apposition to other terminals with or without detected DYN-LI. These findings provide ultrastructural evidence that in both the SN and VTA, dynorphin may elicit changes in locomotor activity, feeding and other behaviors directly through synaptic input to dopaminergic and non-dopaminergic neurons and possibly through presynaptic associations. (Grant support: MH40342, DA04600, NS08193).

286.9

COMPARISON OF CHOLINERGIC PROJECTIONS TO MESENCEPHALIC DOPAMINERGIC CELL GROUPS: SUBSTANTIA NIGRA (SN) AND VENTRAL TEGMENTAL AREA (VTA). S.A. Oakman, E.H. Overstreet*, C. Cozzari*, P.L. Faris, and B.K. Hartman. Dept. of Psychiatry, Division of Neuroscience Research, University of Minnesota Medical School, Minneapolis, MN 55455.

It has been established that the dopaminergic cells in the mesencephalon are innervated by cells from the pontomesencephalic cholinergic group. This relatively large and diffuse cholinergic group is composed of cells in the lateral dorsal tegmental nucleus, the parabrachial nucleus, and the pedunculopontine nucleus. This study was initiated to determine whether cholinergic projections to two functionally divergent dopaminergic cell groups (SN and VTA) reflect a similar organization within the cholinergic cell group. Stereotaxic injections of retrograde tracer (Fluorogold, 10-20 nl) were administered in rats to the SN and VTA. Choline acetyltransferase was localized using immunohistochemistry (Texas Red fluorochrome) in sections from the midbrain and pons, and locations of double-labelled cells were analyzed at multiple levels through the cholinergic group. Initial results of this analysis are consistent with a functional organization of the cholinergic group. Cells which project to the VTA were concentrated toward the dorsal/caudal parts of the group. Those projecting to the SN were more evenly distributed through the cholinergic group. Supported by NS12311 (BKH), MH47189 (PLF).

286.10

OLFACTORY RECEPTORS SHARE ANTAGONIST HOMOLOGY WITH OTHER G-PROTEIN COUPLED RECEPTORS. Stuart Firestein and Gordon M. Shepherd. Section of Neurobiology, Yale University School of Medicine, 333 Cedar St., New Haven, CT, 06510

It is generally believed that olfactory transduction is initiated by the interaction of an odor molecule with a membrane bound receptor. The olfactory receptor has been shown to share homology with receptors belonging to a family marked structurally by possessing 7 transmembrane spanning domains and functionally by coupling to G-proteins. These include, among others, muscarinic, adrenergic and serotonergic receptors. We hypothesized that the olfactory receptor might share sufficient homology with these other members of this family that some identified antagonists might also effect olfactory receptors. The muscarinic antagonists atropine, scopolamine and QNB, applied extracellularly in concentrations of 20-200 uM, acted as reversible antagonists, attenuating the odor elicited current by one-third to one-half. The inhibitory EC50 ranged from 70-100 uM. Muscarine, at similar concentrations, had no direct effect on the cell but also antagonized the odor response. The β-adrenergic and $5HT_1$ receptor antagonist propranolol was more effective, blocking the odor response at a concentration of 80 µM with an EC50 of 20 µM. Similar effects were seen with alprenolol. Although these concentrations are higher than those effective in the native systems, no non-specific effects, such as alterations in input resistance, resting potential or voltage gated currents, were observed. Additionally these antagonists had no effect on the current elicited by application of membrane permeable cyclic nucleotides, suggesting that the effects were upstream of the ion channel. The glutamate channel blocker APV had no effect on odor elicited currents. These results provide added evidence that the odor receptor belongs to the family of 7 transmembrane domain, G-protein coupled receptors. Additionally, since there are no known odor antagonists, these neurotransmitter antagonists may play a role in analyzing odor receptor mechanisms, and in classifying odor receptor families.

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286.11

ANTAGONISM OF RESPIRATORY DEPRESSION BUT NOT ANALGESIA PRODUCED BY THE SELECTIVE μ-OPIOID AGONIST DERMORPHIN BY THE NON-IMIDAZOLINE α_2-ADRENOCEPTOR ANTAGONIST, SK&F 86466. A.-L. Sirén, and S. Vonhof. Dept. of Neurology, USUHS, Bethesda, MD 20814.

Picomole doses of the selective μ-opioid agonist dermorphin (DM) produced analgesia and respiratory stimulation after intracerebroventricular (i.c.v.) administration by an μ_1-opioid receptor mechanism while nanomole doses of DM i.c.v. induced respiratory depression which was related to activation of μ_2-receptors (Paakkari, P. et al., J.Pharm. Exp. Ther. 252: 235-240, 1990). In the present study the influence of α_2-adrenoceptor antagonism on the respiratory and antinociceptive effects of DM i.c.v. was examined in conscious male Sprague-Dawley rats (270-350 g, n=48) using SK&F 86466 (6-chloro-N-methyl-2,3,4,5,-tetrahydro-1-H-3-benzazepine), a potent and selective non-imidazoline α_2-adrenoceptor antagonist (Hieble, J.P. et al., J. Pharm. Exp. Ther. 236: 90-96, 1986). Administration of DM at a dose of 3 nmol/rat i.c.v. decreased respiration rate and ventilation minute volume by 38% and 50% of baseline, respectively. SK&F 86466 (1 or 5 mg/kg i.v. 20 min before DM) had no effect on ventilation minute volume but blocked the DM-induced respiratory depression in a dose-related manner. In contrast, SK&F 86466 had no effect on analgesia (increase in tail-flick latency) produced by low doses of DM (30 and 100 pmol/rat i.c.v.). These data suggest that α_2-adrenoceptors selectively interact with μ_2-opioid receptor associated effects such as respiratory depression but are not involved in the modulation of supraspinal analgesia mediated by μ_1-opioid receptors.

SEROTONIN RECEPTORS I

287.1

CLONING OF A HUMAN 5-HT$_{1D}$ SEROTONIN RECEPTOR GENE AND ITS RAT HOMOLOG. M.W. Hamblin and M.A. Metcalf*. GRECC, Seattle VA Medical Center and the Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98108.

We have isolated the human gene for a 5-HT$_{1D}$ type serotonin receptor. The gene contains an apparently intronless open reading frame encoding a 377 amino acid polypeptide with the seven hydrophobic domains characteristic of G protein linked receptors. The 5-HT$_{1D}$ receptor deduced amino acid sequence is 88% identical to that for RDC4 (a canine thyroid cDNA encoding a heretofore undetermined member of the G protein linked receptor family) and 43% identical to that for the human 5-HT$_{1A}$ receptor. Expression of the human gene product in transfected cell lines results in the appearance of saturable, high affinity [^{3}H]5-HT binding with 5-HT$_{1D}$ type pharmacological specificity. It has been suggested that the pharmacologically distinct 5-HT$_{1B}$ receptor found in the rat is the homolog of the 5-HT$_{1D}$ receptor expressed in many mammals. We have cloned a homologous rat gene whose nucleotide sequence is approximately 85% identical to that of the human 5-HT$_{1D}$ receptor. Further studies may help clarify several ambiguities in the classification and action of serotonin receptor subtypes.

Supported by the Department of Veterans Affairs.

287.2

EXPRESSION AND CHARACTERIZATION OF A CLONED RAT 5-HT$_{1B}$ RECEPTOR: COMPARISON WITH A CLONED HUMAN 5-HT$_{1D}$ RECEPTOR T. Branchek, N. Adham*, M. Macchi*, P. Hartig, and R. Weinshank* Neurogenetic Corporation, 215 College Road; Paramus, N.J. 07666

The serotonin receptors, 5-HT$_{1B}$ and 5-HT$_{1D}$, display similarities in their pharmacology, second messenger coupling, and CNS distribution which have led to the proposal that they are species homologs (Hoyer and Middlemiss, 1989). The recent cloning of a 5-HT$_{1D}$ receptor (Branchek et al., 1990) has enabled a direct evaluation of this proposal. To isolate the putative rat homolog of the 5-HT$_{1D}$ gene, a rat library was screened at high stringency using a human 5-HT$_{1D}$ sequence as a probe. A strongly hybridizing signal was selected, subcloned, and transfected into Cos- 7 cells for study. [^{125}I]Iodocyanopindolol was found to bind to membranes derived from the transfected cells with high affinity [K_d = 0.16 nM]. In contrast, no specific binding of [^{125}I]I-CYP was measured using membranes derived from cells transfected with the cloned human 5-HT$_{1D}$ receptor gene. Rauwolscine was essentially inactive (K_i > 10,000 nM) at the rat receptor as was DP-5-CT. The rank order of potencies for the compounds studied was 5-HT > (-) propranolol > 5 methoxytryptamine > tryptamine > DP-5-CT = rauwolscine. Therefore, the rat gene isolated by homology with the human 5-HT$_{1D}$ receptor encodes a protein with pharmacological properties which identify it as a 5-HT$_{1B}$ receptor. Thus, these receptors are species homologs which exhibit significant differences in pharmacology. The rat 5-HT$_{1B}$ receptor messenger RNA was detected in the brain as well as in several peripheral locations including kidney and aorta. Ongoing studies of second messenger coupling will be presented.

287.3

PROMOTER ACTIVITY OF THE 5'-FLANKING REGION OF THE SEROTONIN-1A RECEPTOR GENE. Paul R. Albert, Caroline Saucier*, Alain Charest* Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Canada, H3G-1Y6.

The 3-kb Pst I/BamHI fragment of 5' flanking region of the rat receptor gene was investigated for the presence of *cis*-acting transcriptional regulatory elements. Sequential 5' deletion fragments of this region were linked to the firefly luciferase reporter gene; the KS+ plasmid constructs were transiently-infected into P19 embryonic stem cells, and luciferase activity measured. Sequences between -1065/-1 displayed a 10-fold enhancement of basal luciferase activity, and contained 2 TATAA boxes preceded by a CCAAT box, as well as putative SP-1, AP-1, EGF-responsive elements. Fragments from 1258/-1 or -1391/-1, which contained a poly-GT repeat, suppressed the promoter activity of the -1065/-1 fragment to one-third of basal activity. The largest fragment, containing additional promoter elements (eg., CCAAT and TATAA boxes) enhanced luciferase activity by 2-fold basal. Our results indicate that the 5'-flanking region of the rat 5-HT1A receptor contains an alternating array of promoter and represser elements which control downstream transcriptional rate. A novel GT-repeat element is associated with the transcriptional repression.

287.4

5-HT DEPLETION ALTERS THE LEVELS OF 5-HT$_{1a}$ RECEPTOR mRNA. **D. Brousseau, S. Wieland, I. Lucki, P. McGonigle, Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.**

The regulation of mRNA for the 5-HT$_{1a}$ receptor was studied following depletion of serotonin in the CNS. Twenty-four male Sprague-Dawley rats (225-235 g) were injected ICV either with saline or with 10 μg of the serotonin selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) and sacrificed eleven days later. Half of the brains were hemisected along the midline. One half of each hemisected brain was further dissected and used for an assessment of regional serotonin levels. Alternate cryosections from the remaining brains were labelled with [^{3}H]-8-OH-DPAT, a selective 5-HT$_{1a}$ agonist, or with a 183 base ^{35}S-CTP labelled riboprobe selective for the N-terminal of 5-HT$_{1a}$ receptor mRNA. HPLC analysis confirmed that 5,7-DHT produced significant depletion of serotonin throughout the CNS with losses of 75% in the hippocampus, 50% in the neocortex and olfactory area, and 40% in the anterior raphe area. Regional serotonin depletions were associated with changes in mRNA levels as determined by densitometric measurement of autoradiograms. Except for the dorsal raphe, where there was a 40% decrease in 5-HT$_{1a}$ mRNA, increases in mRNA levels were observed in most 5-HT terminal fields which were examined including increases of 20-40% in the entorhinal cortex, dentate gyrus, and CA1 and CA2 fields of the hippocampus. No increases in the density of sites labelled with [^{3}H]-8-OH-DPAT were observed in any of these terminal fields, however a 60% decrease was observed in the dorsal raphe. (Supported by USPHS MH 43821 and GM-07170)

287.5

ALTERATION OF LIGAND BINDING PROFILES OF THE 5-HT_{1A} RECEPTOR WITH A SINGLE POINT MUTATION.

X.-M.Guan, B.K.Kobilka* and S.J.Peroutka. Depts of Neurology, Medicine, Molecular and Cellular Physiology, HHMI, Stanford Univ. Sch. of Med., Stanford, CA 94305.

5-HT_{1A} receptor is a member of the G protein-coupled receptor family, which is characterized by the proposed seven transmembrane topology. Previous studies have indicated that the VI and VII transmembrane domains of the adrenergic receptors are important in determining the specificity of antagonist bindings. Certain beta-adrenergic antagonists such as pindolol also bind to 5-HT_{1A} receptors with high affinity. In the present study, a single amino acid residue, Asn_{385}, in the VII transmembrane segment of the human 5-HT_{1A} receptor was changed to Val. Both the wild type and mutant receptor genes were expressed in COS-7 cells. Radioligand binding studies are performed by using ^{3}H-5-HT and ^{3}H-8-OH-DPAT. The affinity of the mutant receptor for pindolol is significantly decreased by about 100 fold while its affinities for 5-HT and 8-OH-DPAT are essentially unchanged as compared to the wild type receptor. The data suggest that Asn_{385} plays an important role in the specific interaction between 5- HT_{1A} receptor and pindolol.

287.6

CHARACTERIZATION OF THE TRANSCRIPTIONAL PROMOTER FOR THE SEROTONIN 5HT2 RECEPTOR GENE.

Mark Heller,* Bryan Roth, and Roland Ciaranello. Nancy Pritzker Laboratory, Department of Psychiatry, Stanford School of Medicine, Stanford University, Stanford, CA, 94305.

Expression of the 5HT2 receptor and its corresponding mRNA is developmentally regulated during ontogeny in the rat brain. We previously showed that the 5HT2 receptor and its mRNA increased 8-fold and 13-fold, respectively, between embryonic day 17 and early postnatal peak levels, before declining to adult expression levels (Roth et al, *Dev. Brain Res.*, 58:51-58, 1991). To further study the molecular regulation of the rat 5HT2 receptor transcription unit, we have isolated overlapping genomic clones which include the transcribed gene, its putative transcriptional promoter, and sequences 5' of the promoter region which might contain *cis* regulatory elements. The organization of these clones was confirmed by Southern analysis of rat genomic DNA. cDNA proximal to the transcriptional start was obtained by (i) PCR amplification of cortex cDNA using primers derived from genomic sequences which were shown to hybridize to the 5HT2 receptor transcript on Northern blots, and (ii) RACE PCR amplification of 5' sequences of the transcript. A large intron is present in the 5'-untranslated region of the receptor gene.

We are beginning the functional assay of the 5HT2 transcriptional promoter and possible *cis* regulatory elements by fusions of these gene segments to reporter genes.

287.7

CHARACTERIZATION OF AN ANTIBODY FOR THE 5-HT_2 RECEPTOR.

D.A Morilak, B.Roth, R. Desai*, S. Garlow and R.D. Ciaranello. Nancy Pritzker Laboratory of Developmental & Molecular Neurobiology, Dept. Psychiatry & Behavioral Sciences, Stanford Univ. Sch.of Med., Stanford, CA 94305-5425.

We have raised an antibody to a unique region of the 5-HT_2 receptor, and have used it to immunocytochemically label the receptor protein expressed in a transfected cell line. Rabbits were immunized with a synthetic peptide antigen representing a sequence unique to the amino terminal region of the 5-HT_2 receptor protein, coupled by sulfo-MBS to keyhole limpet hemocyanin. Resulting antibodies were affinity purified against the synthetic peptide. To verify the specificity of the putative 5-HT_2 antibody, the full length cDNA coding for the receptor was cloned into plasmid pSVK3 and verified by restriction enzyme digestion. The DEAE-dextran technique was then used to transfect COS-7 cells, grown directly on poly-lysine-coated slides, with the pSVK3/5-HT_2 clone. Maximum expression of the receptor protein was observed at 60-72 hours, at which time the cells were fixed for 5 min in 4% paraformaldehyde. Following washes in PBS, cells were incubated on the slide with antibody diluted 1:50 to 1:200 in PBS containing 0.3% Triton X-100 and 5% Blotto, for 48 hr at 4 °C. Following incubation in a biotinylated second antibody (1:200, 90 min, 20 °C) and streptavidin-conjugated HRP (1:1500, 90 min, 20 °C), antibody was visualized by the peroxidase reaction, using H_2O_2 with DAB as the chromogen. Approximately 10% of the cells showed a very intense brown reaction product, concentrated along the cell membranes and around, but not in, the nuclei. Control studies, in which no label or only background labelling was observed, included use of pre-immune serum, non-transfected cells, or pre-incubation of the antibody with varying concentrations of the synthetic peptide.

287.8

REGULATION OF THE 5-HT2 RECEPTOR LINKED PHOSPHATIDYLINOSITOL BREAKDOWN IN RAT CORTICAL SLICES: EFFECTS OF CHRONIC PRETREATMENT WITH D-FENFLURAMINE OR P-CHLOROAMPHETAMINE.

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Serotonin (5-HT) acts via 5-HT2 receptors to stimulate phosphatidylinositol (PI) breakdown in the rat cerebral cortex. We studied the effects of chronic pretreatment with serotoninergic drugs on the increases in PI breakdown caused by subsequently exposing cortical slices to 0.1mM 5-HT or to 1mM carbachol, a muscarinic agonist. Male rats were pretreated for 4 consecutive days and decapitated on day 7. Pretreatment with the 5-HT releasing agent and re-uptake blocker d-fenfluramine (10mg/kg) diminished the increase in 3H-IPl formation caused by 5-HT ($p<0.05$, t-test), but failed to modify the carbachol-induced increase. Unlike d-fenfluramine, the 5-HT neurotoxin p-chloroamphetamine (PCA)(5mg/kg) decreased the stimulation of 3H-IPl formation caused by both 5-HT and carbachol ($p<0.01$). Our data suggest that high levels of 5-HT in the synaptic cleft, as obtained by pretreatment with very large doses of d- fenfluramine, lead to down-regulation of the 5-HT2 receptor linked PI response. The mechanism by which PCA diminishes PI responses to the muscarinic agonist might involve a decrease in the number of structures (e.g. presynaptic 5-HT terminals) on which this agonist acts, or perhaps an enhancement in phospholipase C activity causing basal levels of PI turnover to be elevated.

287.9

NOVEL REGULATION OF 5-HT_{1C} RECEPTORS.

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The 5-HT_{1C} receptor shares many features with the 5-HT_2 receptor despite sites is also similar to that of 5-HT_2 sites, at which both agonists and antagonists induce down-regulation, we studied the effects of chronic treatment (10 mg/kg IP for 30 consecutive days) with six 5-$HT_{1C/2}$ drugs of different classes on [^{3}H]mesulergine specific binding (n=5-9). Spinal cord was chosen for its paucity of 5-HT_2 sites and we have previously shown that drug affinities at spinal 5-HT_{1C} sites are highly correlated with cerebral 5-HT_{1C} sites. Adult male Sprague-Dawley rats were sacrificed 24 hours after the last drug injection. Results from various vehicles were pooled because there were no significant differences (n=30): B_{max} = 3.7 ± 0.1 pmol/g, K_D = 2.6 ± 0.1 nM, n_H = 1.0 ± 0.1. The putative 5-$HT_{1C/2}$ agonists m-CPP (-43%) (±)DOI(-39%), and quipazine (-30%) significantly decreased the B_{max} of spinal 5-HT_{1C} sites compared to vehicle treatment. The putative 5-$HT_{1C/2}$ antagonist ritanserin and metergoline (non-selective) eliminated [^{3}H] mesulergine specific binding. The lack of significant changes in K_D or n_H argues against the presence of residual drug in the tissue. All of these drug effects were highly significant ($p<0.0001$, ANOVA). In contrast, (±)pindolol, with virtually no affinity for 5-HT_{1C} sites in vitro, did not alter receptor binding parameters. B_{max} values correlated with the affinities of the drugs at 5-HT_{1C} sites in vitro ($r=0.77$). These data demonstrate a pattern for 5-HT_{1C} sites shared with 5-HT_2 receptors of down-regulation by both 5-$HT_{1C/2}$ agonists and antagonists.

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287.10

[^{3}H]R-ZACOPRIDE BINDS WITH A HIGH AFFINITY TO A NEW SITE IN ADDITION TO 5-HT_3 RECEPTORS.

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5-HT_3 antagonists such as R,S-zacopride exert potent anxiolytic-like effects in relevant test in rats and monkeys, but, the involvement of 5-HT_3 receptors in these effects is open to question. Indeed, R-zacopride is 1000 times more potent than S-zacopride as a potential anxiolytic drug although its affinity for 5-HT_3 receptors is 10 times less than S-zacopride. This discrepancy led us to examine the recognition sites for [^{3}H]R- and [^{3}H]S-zacopride in membranes from the rat entorhinal cortex and NG 108-15 clonal cells. Both ligands label pharmacologically identified 5-HT_3 receptors but [^{3}H]R-zacopride bind to additional high affinity (10 nM) sites in the two membrane preparations. These sites are stereoselectively recognized with a high affinity by various zacopride derivatives (10-50 nM) but not by other benzamides. Prazosin and mianserin bind with a moderate affinity to these sites but 5-HT_3 ligands (S-zacopride, ondansetron, ICS 205-930 etc..) and other anxiolytic drugs (benzodiazepines, buspirone, ipsapirone...) are poorly recognized (10-100 µM). Interestingly, this new site has been identified in the kidney and in other peripheral tissues. The functional role of these new sites needs further investigation.

287.11

5HT3 RECEPTORS IN POSTMORTEM BRAINS OF NORMAL AND SCHIZOPHRENIC SUBJECTS. A. Abi-Dargham*, M. Laruelle, D. T. Wong, D. W. Robertson, D.R. Weinberger and J. E. Kleinman, Clinical Brain Disorders Branch, IRP, NIMH Neuroscience Center at St Elizabeths, Washington, D.C. 20032.

5HT3 receptor antagonists have been reported to modulate dopaminergic transmission in rodents. We studied the binding in human brain of [3H]LY278584 , which has been shown to label 5HT3 receptors in rat cortex (Wong D.T. et al., Eur. J. Pharmacol., 166: 107, 1989). Saturation experiments were performed with increasing concentrations of the labeled ligand (0.1 to 20 nM) and the nonspecific binding was defined with 1μM MDL72222. Scatchard analysis revealed a saturable binding site in the amygdala (Kd= 3.08 ± 0.66 nM, Bmax=7.02 ± 0.84 fm/mgP, n=4) as well as in other striatal and limbic areas such as the caudate, putamen, nucleus accumbens, hippocampus and entorhinal cortex. Specific binding in neocortical areas was negligible. Pharmacological characterization of [3H]LY278584 in the caudate and the amygdala was consistent with the pharmacology of 5HT3 receptors. We compared the [3H]LY278584 specific binding in the amygdala in postmortem brains of schizophrenic subjects (n=8) and matched normal controls (n=10) and found no significant differences in the Kd (4.82 ± 1.63 nM versus 3.01 ± 0.41 nM) or the Bmax (13.48 ± 3.08 fm/mgP versus 11.67 ± 1.2 fm/mgP) values between these two groups. 5HT3 receptors binding appear to be normal in the amygdala of schizophrenics, but other areas remain to be studied.

287.12

SENSORY PREJUNCTIONAL 5-HT_{1B} RECEPTORS MEDIATE BLOCKADE OF NEUROGENIC PLASMA EXTRAVASATION WITHIN RAT BUT NOT GUINEA PIG DURA MATER M.A. Moskowitz, T. Matsubara, M.G. Buzzi* Neurosurgery and Neurology, Mass Gen Hospital, Harvard Med Schl, Boston, MA 02114.

Neurogenic inflammation develops within the rodent dura mater after trigeminal ganglion stimulation (5Hz, 5ms, 1.0mA, 5min) by capsaicin-sensitive mechanisms and is presumably mediated by SP and NKA release. We describe the effects of pretreatment with 5-HT_1 receptor agonists on the leakage of ^{125}-albumin within dura mater. Extravasation in the rat was significantly reduced by 5-CT> >DHE>sumatriptan>8-OH-DPAT whereas 5-HT_2 and 5-HT_3 antagonists were inactive. The potency order is most consistent with a 5-HT_{1B}/5-HT_{1D} response among the known 5-HT_1 family of receptors. The drugs did not block SP, or NKA-induced extravasation, however,suggesting prejunctional localization. We recently tested CP-93,129 (140 nmol kg^{-1};threshold dose), a potent and selective 5-HT_{1B} receptor agonist possessing nmolar and μmolar affinities for the 5-HT_{1B}/5-HT_{1D} binding sites, respectively. CP-93,129 (140 nmol kg^{-1}) blocked extravasation within rat but 1400 nmol kg^{-1} did not in guinea pig. By contrast, sumatriptan, a drug exhibiting 30-fold higher affinity for 5-HT_{1D} than 5-HT_{1B} recognition sites, blocked extravasation in the guinea pig at 7 nmol kg^{-1}. Taken together, these data are consistent with the presence of 5-HT_{1B} heteroreceptors blocking neurogenic inflammation within rat, whereas the 5-HT_{1D} receptor may be more important in guinea pig.

Macor et. al. (1990) J Med Chem 33: 2087.

287.13

DIFFERENTIAL EFFECTS OF SEROTONERGIC (5-HT) AGONISTS ON NEURAL CONTROL OF MICTURITION AND SEXUAL FUNCTION. W.D. Steers, J.B. Tuttle, E. van Asselt and M. Albo* Univ. of Virginia School of Medicine, Charlottesville, VA 22908

Behavioral and electrophysiological methods were used to study the role of 5-HT receptors on bladder and erectile pathways in the male rat. 5-HT_{1A} (PAPP, 5MeODMT) and 5-HT_{1C} (MK212,TFMPP,mCPP) agonists did not alter voiding frequency (VF) in awake rats or affect micturition threshold (0.4 vs. 0.3cc) in anesthetized rats. 5-HT_{1B} agonist (ORG 6997) reduced VF. 5-HT_{1C} agonists abolished rhythmic reflex bladder contractions by inhibiting pelvic nerve firing. No 5-HT agonist produced firing in bladder nerves or affected pelvic nerve evoked bladder contractions. 5-HT_{1B} and 5-HT_{1C} agonists produced penile erections (2-5/30 min) in awake rats. These drugs also elicited efferent burst firing in cavernous nerves mediated by preganglionics in the pelvic nerve. Neural activity was abolished by 5-HT_1 (pindolol) and 5-$HT_{1C/2}$ (mianserine) antagonists but not by 5-HT_3 antagonist (ICS 205-930). 5-HT_{1A} agonists caused emission and ejaculation with firing in cavernous and vasal nerves via preganglionics in the hypogastric nerve. 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} agonists did not influence synaptic transmission in the pelvic ganglion. These data support the existence of functional diversity for 5-HT receptor subtypes and suggest clinical use of related agents for genitourinary disorders.

TRAUMA III

288.1

CEREBRAL EDEMA AFTER PERCUSSIVE TRAUMA IN MATURE AND IMMATURE RATS. P. D. Grundl*, K. V. Biagas*, P. M. Kochanek, J. K. Schiding*, and E. M. Nemoto. Depts. of Anesthesia/Critical Care Medicine and Pediatrics, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Studies suggest that children are more likely than adults to show early diffuse cerebral swelling after head trauma (1). Unlike hypoxia or ischemia, the response to cerebral trauma in an immature model is not well characterized. Hypothesis: Reproducible, age-related differences in the time course of traumatic edema formation can be demonstrated in an animal model.

Wistar rats, sexually immature (3.5-4.5 wks) and mature (>9 wks), were anesthetized and ventilated. Injury to the exposed right parietal cortex was produced by weight drop indexed to brain weight. Percent right brain water (%RBW) was measured at 2, 24, 48, and 168 h post-trauma by wet-dry weight. Within group comparisons to respective, normal controls and between group comparisons were made by t-test with Bonferroni correction. N=34 in each age group.

As anticipated, normal %RBW in immatures was higher when compared with mature rats (80.73 ± 0.16 vs. 79.31 ± 0.20, mean ± S.E.M., $p<0.01$). Survival was 100% in both groups. In immatures, %RBW was increased at 2 h post-trauma (81.29 ± 0.18, $p<0.05$) and remained elevated for 24 h after trauma (81.51 ± 0.27, $p<0.05$). In matures, however, %RBW was not elevated until 24 h post-trauma (80.06 ± 0.27, $p=0.05$). The magnitude of increase in %RBW vs. respective normals was similar in both groups 24 h post-trauma (0.75 ± 0.27 matures and 0.78 ± 0.27 immatures, p=NS). 7 d after trauma, %RBW did not differ significantly from normals in either group.

These results suggest enhanced early edema after traumatic brain injury in the immature. Future studies using this model may provide insight into the mechanisms of this edema formation.

1 Bruce DA, et al: J Neurosurg 1981; 54:170.

288.2

CARBACHOL-STIMULATED INOSITOL PHOSPHATE (IP) PRODUCTION FOLLOWING TRAUMATIC BRAIN INJURY (TBI). S.E. Robinson, G.S. Borrelli*, J.L. Ang*, J.R. Pascua*, K.P. McDowell,*and E.K. Enters. Department of Pharmacology & Toxicology, Medical College of Virginia, Richmond, VA 23298-0613.

Central muscarinic blockade has been found to reduce behavioral deficits observed following TBI (Lyeth et al, Brain Res.448: 88, 1988; Brain Res., Robinson et al, 511: 141,1990). In order to determine whether TBI alters the responsivity of the IP second messenger system to cholinergic agonists, carbachol-stimulated IP production was quantitated following TBI. Male Sprague-Dawley rats (270-320 g) were surgically prepared under Equithesin anesthesia 24 h prior to receiving moderate fluid percussion injury (2.0-2.4 atm) or sham-injury while under methoxyflurane anesthesia. 3 or 24 h later, methoxyflurane-anesthetized rats were decapitated, and the hippocampi removed, sliced into 225 μm cubes, and labelled for 90' with 1 μM [^{3}H]myo-inositol (14 Ci/mmol) in Krebs-Ringer bicarbonate buffer (KRBB) at 37°C under O_2/CO_2. After labeling, the tissue was incubated for 60' with carbachol (final conc.: 0, 20, 50, 200, 2000 μM) in Li^+KRBB. Lipids and IPs were extracted, and the IPs were separated from myo-inositol on Dowex 1x8 columns (formate form). Carbachol-stimulated IP production was significantly reduced 24 h and slightly, but not significantly, reduced 3 h after TBI, as compared to sham-injured rats. Thus, it appears that TBI leads to a delayed reduction in phosphatidylinositol hydrolysis in response to cholinergic stimulation. Whether this represents a decrease in receptor number or a change in the receptor coupling to this pathway must be elucidated. (Supported by NS24413 and NS7288)

288.3

INITIAL INTRAAXONAL ABNORMALITIES ASSOCIATED WITH TRAUMATIC BRAIN INJURY. A.B. Valadka*, A.A. Yaghmai*, J. Astruc, and J.T. Povlishock, Div. of Neurosurgery and Dept. of Anatomy, Med. Col. of VA., VA. Commonwealth Univ., Richmond, VA 23298.

Axonal injury has long been a consistent finding in head-injured animals and man. Laboratory studies do not suggest the immediate tearing of axons, but rather suggest that injury initiates a series of intraaxonal changes that lead to disconnection over a 6-24 h period. What is unknown is the initial intraaxonal event which triggers this reactive sequence. To critically assess this, anesthetized rats and cats were subjected to moderate fluid-percussion injury and allowed to survive for 15 min to 4 h. The animals were perfused with aldehydes and their brains blocked to include loci which, with prolonged survival, manifest reactive axonal change. These regions were immunocytochemically processed for the light and electron microscopic visualization of the 68 kD neurofilament (NF) subunit, which has been shown to undergo selective change with injury. Through this approach, traumatic brain injury never resulted in the direct disruption of the axoplasm, axolemma or related myelin. Within 15 min of injury, the axon displayed focal infolding and irregularity associated with detachment from the overlying myelin. By 1 h, these axons displayed regions of densely packed immunoreactive 68 kD NF. Over a 1-4 h course, these immunoreactive NF became disordered, no longer paralleling the axon's long axis. By 4 h such disordered NF were associated with an initial accumulation of organelles, setting the stage for focal axonal swelling and detachment. These studies suggest that direct tearing is not a factor in the genesis of traumatically induced axonal change. Rather, these findings support the concept that stretching triggers cytoskeletal change leading to progressive axonal failure. (Supported by NS 20193)

288.4

REGIONAL CEREBRAL BLOOD FLOW CHANGES AFTER TRAUMATIC BRAIN INJURY IN THE RAT. C.-L. Chou*, B.G. Lyeth, L.W. Jenkins, R.L. Hayes, and J.T. Povlishock. Depts. of Anatomy and Neurological Surgery, Med. Col. of VA., VA. Commonwealth Univ., Richmond, VA 23298.

Numerous investigators utilize rodent models of traumatic brain injury (TBI) to better understand the pathobiology involved. To date, various structural, functional, and behavioral changes have been described following relatively moderate TBI. Although most evidence points to the primary traumatically-related events in the genesis of these abnormalities, the possible contribution of related regional cerebral blood flow (CBF) change has not been fully explored. To address this issue, quantitative autoradiography using [^{14}C] iodoantipyrine was used to assess CBF in 26 rats subjected to moderate fluid-percussion brain injury. Regional blood flows were assessed at 1 and 6 h postinjury. Sampling for autoradiographic analysis included numerous regions along the rostral-caudal extent of the neuraxis, including neocortex, cingulate gyrus, caudate-putamen, all subsectors of the hippocampus and various brain stem nuclear groups. By 1 h postinjury, a generalized reduction in CBF was seen in all regions sampled. Statistically significant reductions were seen within the rostral neocortex and cingulate gyrus as well as the mid-neocortex and the CA3 and CA4 subsectors of the hippocampus. Importantly, these flow reductions never reached ischemic levels, and all were noted within anatomical loci in which no evidence of hemorrhage or overt structural damage were identified. At 6 h postinjury, all regional CBF values appeared to recover; yet, they did not attain control levels. The results of this investigation demonstrate that moderate TBI in rat results in some regional reduction in CBF following injury which tends to recover over time. Importantly, since these blood flows never reached ischemic levels, primary ischemia is most likely not responsible for the pathobiology associated with this level of TBI. (Supported by NS 20193)

288.5

Normal and Heat-Induced Patterns of Expression of Heme Oxygenase-1 (HSP32) in Rat Brain. J.F. Ewing*, M.D. Maines* and V.G. Laties, Department of Biophysics, University of Rochester Med. Ctr., Rochester, NY 14642

Heme oxygenase (HO) isoenzymes, HO-1 and HO-2, catalyze the rate limiting step in formation of bile pigments possessing potent antioxidant activity. Presently we have identified HO-1 as the only HO isoenzyme responsive to heat shock and defined hyperthermia as the only stimulus reported to date which causes an increase in brain immunoreactive HO-1 protein in vivo. Using an HO-1 specific polyclonal rat antibody and HO-1 specific cDNA probe we examined the expression of HO-1 immunoreactive protein and the level of HO-1 mRNA in normal rat brain and in brain 6 h following heat shock. Exposure of rats to 42^{0}C for 20 min caused a marked increase up to 30-fold in brain HO-1 1.8 Kb mRNA within 1 h post-treatment as determined by Northern blot hybridization. In normal brain, HO-1 protein was observed in select neuronal and non-neuronal cell populations in forebrain, diencephalon and cerebellum. Six hours following heat shock an intense increase in HO-1 protein was observed in glia throughout the brain, ependyma lining the ventricles of brain and Purkinje cells of cerebellum. The specificity of HO-1 antibody-antigen complexes immunochemically detected in 50 micron coronal brain sections using horseradish peroxidase was established by preadsorption of HO-1 antisera with an excess of HO-1 protein. This study was supported by NIH grants R3704391, ES03968, ES01247 and ES07026.

288.6

EFFECT OF CNQX AND HIGH CONCENTRATIONS OF DIHYDROPYRIDINES ON TRAUMATIC CORTICAL NEURONAL INJURY IN CULTURE. R.F. Regan and D.W. Choi. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

A traumatic insult to mixed murine neuronal and glial cortical cell cultures was delivered by multiply tearing the cell layer with a stylet. Injury was assessed by measurement of lactate dehydrogenase in the bathing media 20-24 hours later. Consistent with prior observations, neurons adjacent to a tear rapidly developed acute swelling and then went on to degenerate over the next several hours. About half of this cell death was blocked by the NMDA receptor antagonists MK-801 or dextrorphan. 10-100 μM CNQX plus 1 mM glycine was also neuroprotective in concentration-dependent fashion; at 100 μM CNQX, neuronal death was also reduced about half compared with untreated cultures. Nimodipine provided weaker and more variable neuroprotection, with approximately 20% reduction of injury at 10-30 μM. Similar results were noted with 100 μM nifedipine. Addition of nimodipine or nifedipine to MK-801 consistently reduced injury greater than MK-801 alone. Approximately 35% of neuronal death after trauma in the presence of 10 μM MK-801 was prevented by 30 μM nimodipine or 100 μM nifedipine. These results suggest that traumatic-induced neuronal death in this in vitro model may be mediated in part by excessive activation of non-NMDA receptors, and by mechanisms sensitive to high concentrations of dihydropyridines.

288.7

THE VERY LONG-TERM CONSEQUENCES OF SEVERE CHI ON IQ. R.F. Zec, J. Belman, J. Miller, D. Zellers, J. Matthews, D. Ferneau, S. Vicari, M. Kocis, S. Verhulst, and R. Robbs. SIU Sch. of Med., Springfield, IL 62794.

It is unclear from the published literature to what degree there may be permanent residual impairment in IQ after severe CHI. The subjects in the present study were 32 patients with severe TBI and 42 controls (age, ed., gender did not differ; t-tests). The M length of coma was 65.4 days and time since injury was 10.1 yrs. A comprehensive test series was administered including the WAIS-R. The TBI group displayed significantly poorer performance on every WAIS subtest (t-tests). The degree of impairment for the WAIS-R VIQ was -1.0 SD difference from the control group mean, PIQ was -1.2 SD, and FSIQ was -1.0 SD. Thirty-eight percent of the TBI patients were greater than 2 SD below the control group mean for PIQ and FSIQ (<76), while only 16% were below 2 SD for VIQ. The greatest percentage of impaired subjects (>2 SD) using raw scores were found on block design (59%), followed by digit symbol (53%), object assembly (50%), picture arrangement (41%), picture completion (28%), arithmetic (25%), vocabulary (22%), comprehension (19%), similarities (19%), digit span (6%) and information (6%). Our findings indicate that many years after sustaining a severe TBI, IQ impairment remains mildly impaired on average, although 38% of patients will have severely impaired PIQ & FSIQ.

288.8

LONGITUDINAL POWER SPECTRAL ANALYSIS (PSA) FOLLOWING OVERNIGHT SLEEP STUDIES IN MINOR HEAD INJURED (MHI) ADOLESCENTS. L.C. PARSONS, M.PERLIS*, T. KWAN*, M. KHALIFA*, and L.J. CROSBY*. University of Arizona, College of Nursing, Tucson, AZ 85721.

The objective of this study is to measure the PSA of overnight sleep/awake cycles of MHI adolescents within 72 hours, 6 weeks, and 12 weeks of injury. Four channels of electroencephalographic (EEG) data were down loaded from FM magnetic tape (IRIG standard) and converted from analog to digital format using metrabyte AD board (Dash-16). EEGs were sampled at 128 samples/sec. for each of the sleep recordings. The digitization process was governed by Stellate Cooperation's Rhythm software (Ver 7.10). PSA was performed by Rhythm software for the following bandwidths: Delta (.75-2.5), Theta (2.75-7.25), Alpha-1 (7.5-9.75), Alpha-2 (10.0-11.75), and Sigma (12.0-14.0). EEG data were submitted for PSA in the form of NREM and REM cycles. Prior to PSA analysis, all NREM and REM cycles were visually inspected for perspiration and electromyographic artifacts. Artifacts > 2 sec. were removed. Data were accumulated in terms of power in 4 sec. epochs. Multiple epochs were averaged to yield the average 4 sec. power spectral distribution for each NREM/REM cycle. Preliminary Findings: PSA analysis of 6 overnight sleep studies performed on 2 subjects within 72 hrs, 6 weeks, and 12 weeks of injury indicated significant changes (one-way ANOVA). The p values for temporal NREM derivations were Delta (.000), Alpha 1 (.001), Alpha 2 (.015), Sigma (.05), and Total Power (.01). Temporal REM derivations indicated p values were Delta (.035), Alpha 2 (.05), Sigma (.03) and Total Power (.04). Conclusion: Preliminary findings suggest that the PSA may serve as a sensitive tool for quantifying electrophysiologic changes in vulnerable brain regions such as the temporal lobes. This study is supported by the National Institute on Neurological Disorders and Stroke under grant number 1 R01 NS 24169-01A1.

288.9

ELECTRICAL FIELD MAPPING IN THE RAT SPINAL CORD. R.J. Hurlbert and C.H. Tator. 12-423 Playfair Neuroscience Unit, Univ. of Toronto, 399 Bathurst St., Toronto, CANADA M5T 2S8

Direct current (DC) fields have been shown to posses beneficial effects in the treatment of acute spinal cord injury in animal models. However, little is known about the distribution of these fields within the spinal cord. We report results of field mapping in 5 normal rats. Epidural disc electrodes were placed 10 mm apart under the lamina of C6 and T2. Stimulation consisted of a balanced 20 Hz sine wave, current limited by an on-line ammeter. Field strengths were measured with a lock-in differential amplifier and glass microelectrodes inserted directly into the spinal cord. Recordings were made from various positions and depths between the stimulating electrodes.

The relationship between stimulating current and field strength was linear; for a doubling of current there was a proportional doubling of measured voltage. The field strength was highest in close proximity to the stimulating electrodes; 14 μA of current produced voltages exceeding 1 mV/mm. At midpoint between the electrodes the measured field dropped to < 400 μV/mm in the spinal cord and < 200 μV/mm in the paraspinal musculature. These results characterize the field distribution resulting from this type of stimulation in the normal spinal cord, and will help to determine the optimal stimulation parameters necessary to promote recovery following spinal cord injury in the rat.

288.10

SPINAL CORD BLOOD FLOW (SCBF) CHANGES FOLLOWING GRADED CONTUSIVE INJURIES IN THE RAT THORACIC CORD. A. Martinez-Arizala, D. H. Hesse*, A. Marcillo*, The Miami Project, University of Miami School of Medicine, Miami, FL 33136.

In support of ischemia as a secondary mediator of injury in spinal cord trauma, significant reductions in gray matter SCBF have been described after spinal cord injury (SCI). Changes in white matter blood flow have been less consistent as both hyperemia and ischemia have been reported. This study was designed to establish a correlation between severity of SCI and changes in white matter SCBF. Anesthetized female S-D rats underwent a T8 laminectomy and graded spinal SCI by the weight drop method (10 g weight dropped 2.5, 5.0, 7.5, or 10 cm). SCBF was monitored pre- and for one hour post-injury with laser doppler flowmetry by placing the laser probe on the dorsal columns, adjacent to the site of impact. Neurologic function (Tarlov score, inclined plane, sensory score) was assessed at 2, 7, 14, 21, and 28 days post-injury. SCBF decreased within a few minutes following SCI in all but the mild (2.5 cm) injury group, where some animals exhibited a transient hyperemia. All animals in this group had partial recovery of SCBF by one hour (the end of the observation period), but in animals with more severe injuries ($\geq$5.0 cm), SCBF remained significantly reduced. At one hour post-injury, SCBF changes from baseline for each group were (mean $\pm$sem): 2.5 cm: -36.3 $\pm$8.2%, 5.0 cm: -64.8 $\pm$11.9%, 7.5 cm: -70.2 $\pm$ 8.4%, and 10 cm: -76.8 $\pm$4.3%. Greater neurologic deficit was also present at one month in more severe lesions as reflected in the mean Tarlov scores (0 to 5, where 0=plegic and 5=normal): 2.5 cm group: 3.0, 5.0 cm group : 2.6, 7.5 cm group: 1.8, and the 10 cm group: 1.1. In summary, we find that after SCI, the magnitude of neurologic deficit and degree of post-traumatic ischemia increase as the severity of the injury increases. However, it must be emphasized that ischemic levels for the injured spinal cord white matter have not been defined.

288.11

SPINAL CORD INJURY PRODUCED BY DORSAL SPINAL VENOUS OCCLUSION IN THE RAT PW Madsen and A Martinez-Arazila, The Miami Project to Cure Paralysis, Univ. of Miami Sch of Med, Miami, FL

Although mechanical disruption of the vascular system occurs with traumatic spinal cord injury, the effect of the resultant impairment of the venous drainage is undefined. The restriction of venous outflow can contribute to the development of tissue edema and delay its resolution. Histological examination of rodent spinal cord following the occlusion of the dorsal spinal vein demonstrated edema and hemorrhagic infarction of the dorsal columns (Martinez et al. Neurosci. Abst. 1990). To investigate the neurological deficits associated with this lesion, 17 anesthetized Sprague-Dawley rats were subjected to T7-T9 laminectomy to expose the dorsal venous system of the spinal cord. In all animals the vein was isolated, focally coagulated with a micro-bipolar cautery and sectioned at the rostral and caudal extent of the laminectomy. Eight animals undergoing this selective cauterization (SO) and an additional nine animals in which the major branches of the dorsal vein were also coagulated (EO), were neurologically evaluated for a one month period postoperatively. Motor function was assessed using a modified Tarlov scoring scale (0-5) while sensory function was assessed grading the response to hindlimb paw pinch. EO animals manifest a marked motor deficit 48 hrs post-injury (2.0 mean Tarlov score), which became maximal (1.3) at 7 days, and then slowly improved over the next three weeks (3.5). The SO animals demonstrated a similar temporal profile with significantly less motor impairment: 3.6 at 48 hrs, 2.9 at 7 days, and 4.5 at 1 month. The sensory scores demonstrated a similar pattern. The results of this preliminary study suggest that the degree of venous obstruction is directly associated with the development of neurological deficits and may contribute to the pathophysiology of spinal cord injury.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS III

289.1

SYNAPTIC RECEPTOR HETEROGENEITY AT INDIVIDUAL RETINAL SYNAPTIC BOUTONS Charles L. Zucker[1] and Berndt Ehinger[2]. Eye Research Institute and Harvard Medical School[1], Boston, Massachusetts, and Department of Ophthalmology, University of Lund, Sweden[2].

It is now well established that neurons often contain more than one neuroactive substance, typically a conventional neurotransmitter such as acetylcholine, GABA or glycine and one or more neuropeptides. There is also evidence that a class of retinal amacrine cells contains both acetylcholine and GABA, which are considered excitatory and inhibitory, respectively. It remains unexplained how these neurons utilize their multiple neuroactive substances. In the present study, we have examined the ultrastructural localization of glycine receptors in the turtle retina using a monoclonal antibody directed to the intracellular domain of the strychnine sensitive glycine receptor. We have found that glycine receptors are only localized to 56% of the synapses made by presumed "glycinergic" amacrine cells. The remaining synapses made by these same boutons show no evidence of glycine receptors, even though they may be located within 0.5 µm of a glycinergic synapse. These data indicate that only a portion of the postsynaptic sites contacted by the glycine-containing neurons are able to respond to glycine. They also suggest that a neuron containing multiple neuroactive substances can selectively affect postsynaptic elements. In light of our findings, we suggest that it is less useful to classify a neuron in its entirety as glycinergic than to define individual synapses as glycinergic. The same argument can be made for all types of neurons and such a "synergic" hypothesis may provide a functional explanation for the rapidly growing number of neurotransmitter colocalizations being reported throughout the central nervous system.

Supported by NIH grant EY07552 to CLZ and grants from the Crafoord Foundation, the T and R Söderberg Foundation, the Swedish Institute and the Swedish Medical Research Council (project 14X-2321).

289.2

SORTING OF SODIUM CHANNELS AND GABA RECEPTORS IN POLARIZED CELLS. K.J. Angelides, B. Wible*, J. Velazquez*, and E-H. Joe*, Departments of Molecular Physiology and Biophysics and Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Neurons are characterized by a cell surface organization in which proteins are segregated and maintained in discrete domains. Fluorescence imaging has shown that GABA receptors are distributed in high density on cell bodies and dendrites and sodium channels (NaChs) are localized to axons. In neurons it is not known how specific cell surface domains are created, but recent work with viral proteins expressed in neurons and epithelial cells suggests similar mechanisms for protein sorting.

In order to gain insight into the subunit requirements and signals that target GABAR to dendrites and cell bodies and NaChs to axons, we examined the sorting of specific combinations of GABAR α and β subunit subtypes and rat brain NaChs after transfection of their cDNAs into polarized epithelial cells. Digital and confocal microscopy with NaCh specific antibodies and fluorescent neurotoxins show that NaChs are targeted to the apical membrane and distributed in patches. In addition, both ankyrin and spectrin, cytoskeletal proteins that associate with NaChs, accumulate with NaCh patches. Using GABAR subunit subtype-specific antibodies, defined receptor assemblies composed of α- and β-subunit subtypes are specifically routed to either apical or basolateral membranes. The results indicate that a signal contained in the NaCh primary sequence is sufficient to target NaChs to a specific membrane domain and to induce NaCh-ankyrin assembly, while the assembly of specific subunit subtypes may determine the differential targeting of GABAR to either dendrites or cell bodies. NIH and NMSS supported.

289.3

STRUCTURAL AND PRECLINICAL STUDIES WITH THE RADIOIODINATED CHOLINERGIC NEURON MARKER (-)-5-IODOBENZOVESAMICOL (5-IBVM). Y-W. Jung*, M.E. Van Dort*, D.L. Gildersleeve*, D.M. Wieland, D.E. Kuhl. University of Michigan Medical Center, Ann Arbor, MI 48109.

Radioiodinated derivatives of benzovesamicol are promising mapping agents for central cholinergic neurons. Structural studies have shown radioiodine incorporation in position 5 or 6 of benzovesamicol does not lower *in vivo* neuronal selectivity; iodine substitution in the 8 position, however, destroys selectivity. Based on rodent screening studies, 5-[^{123}I]IBVM has been chosen for further preclinical testing. Dosimetry analysis in rats with 5-[^{125}I]IBVM shows the intestinal tract to be the critical organ. Nearly 60% of the injected dose is excreted in 24 h, 95% in the feces. Radiation dose to the unblocked thyroid is low. The LD_{50} of 5-IBVM in rabbits is 120-180 μg/kg (University of New York at Buffalo Toxicology Research Center). A no-carrier-added synthesis of 5-[^{123}I]IBVM was accomplished in 94% yield by chloramine-T/radioiodide reaction with the respective (±)-5-tributyltin analog followed by chiral-HPLC purification on a Chiracel OD column; specific activity was greater than 20,000 Ci/mmol. Tomographic brain imaging of 5-[^{123}I]IBVM distribution and kinetics in Alzheimer's disease patients will be undertaken.

289.4

PET IMAGING AND QUANTITATION OF THE CHOLINERGIC PRESYNAPSE WITH FLUORINE-18 ANALOGS OF VESAMICOL. G.A. Rogers, L. Eriksson,* M. Ingvar, S.M. Parsons, S. Stone-Elander* and L. Widen. Neuroscience Res. Inst., UCSB and Karolinska Inst. and Hosp., Stockholm.

The in vivo pharmacological properties of three fluorine-18 analogs of vesamicol were examined in both rat and monkey. In rat, 4-fluoromethylvesamicol (FMV) is rapidly transported across the blood brain barrier and binds with high affinity (k_{diss} = 0.008 min^{-1}) to a saturable site (the vesamicol receptor, VR) and with low affinity (k_{diss}= 0.12 min^{-1}) to a nonsaturable site (probably the vesamicol binding protein, VBP). A second analog that was designed to bind more specifically to VR, fluoroacetyl-4-aminobenzovesamicol, was metabolically labile (time-dependent rebound of radioactivity in plasma). However, a structural modification that apparently blocks metabolism produced an analog with increased affinity for VR and diminished binding to VBP.

In both rat and monkey, a prior injection of vesamicol prevents the binding of [^{18}F]FMV to the VR. Reconstructed images of monkey brain taken ca 1 hr postinjection of [^{18}F]FMV show good contrast with high specific activity in areas expected to be rich in cholinergic neurons. A prior injection of vesamicol greatly reduces tracer binding in these areas. Our results suggest that fluorinated analogs of vesamicol will be useful tools in imaging and quantitating cholinergic neurons in vivo.

289.5

SPECT IMAGING OF THE BENZODIAZEPINE RECEPTOR: COMPARISON OF RECEPTOR DENSITY AND RADIOLIGAND DISTRIBUTION. E. Sybirska, M. Al-Tikriti, S. Zoghbi*, R.M. Baldwin*, R.B. Innis, Depts. Psychiatry & Radiology, VA Med. Ctr. and Yale Univ., West Haven, CT 06516.

^{123}I-Ro16-0154 is a SPECT (single photon emission computed tomography) probe for the central benzodiazepine (BZ) receptor. The purpose of this study was to compare the distribution of radioligand in brain following the i.v. administration of ^{123}I-Ro16-0154 measured with *ex vivo* autoradiography with the distribution of receptors measured with *in vitro* autoradiography using ^{125}I-Ro16-0154.

^{123}I-Ro16-0154 was administered i.v. to six monkeys, and transaxial images were monitored with SPECT until maximal brain uptake was reached, approximately 100 min post injection. Animals were then euthanized with an overdose of pentobarbital. The planes of the SPECT images were marked on the underlying brain. Tissue blocks 5 mm thick were cut in a mold and then frozen. Cryostat sections 20 μm thick were apposed to film for *ex vivo* autoradiography. After decay of ^{123}I for at least 1 week, sections were incubated with ^{125}I-Ro16-0154 using standard *in vitro* receptor autoradiographic techniques. Digitized *ex vivo* and *in vitro* autoradiograms were analyzed relative to calibrated autoradiographic standards.

SPECT images showed high levels of activity in neocortical areas, with greatest activity in the occipital lobe. *Ex vivo* autoradiograms provided a detailed distribution of radioactivity in tissue sections at the level of the light microscope. Tissue radioactivity measured in a gamma counter was highly correlated in 12 regions with that measured in the *ex vivo* autoradiograms (r=0.93). The distribution of BZ receptors determined by *in vitro* autoradiography was highly correlated in 16 regions with that determined by *ex vivo* autoradiography (r=0.89), although several regions including LGN, pallidus, and putamen showed consistent discrepancies.

These studies demonstrate the feasibility of quantitative *ex vivo* autoradiographic measurements of SPECT radioligands.

289.6

PRESENCE OF A- AND B-TYPE CCK BINDING SITES IN RAT VAGUS NERVE. E.S. Corp, M. Curcio* and G.P. Smith. Bourne Laboratory, New York Hospital-Cornell University Medical Center, White Plains, NY 10605.

Cholecystokinin (CCK) binds with high affinity (approx. 1 nM) to axonally transported receptor binding sites in afferent fibers of rat vagus nerve. Based on the relative binding potencies of competing agonists, these sites have been characterized as A-type. To analyze subtype composition further, we used two selective CCK antagonists, devazepide (A-type) and L365,260 (gastrin/B-type) in competition for vagal sites occupied by ^{125}I-CCK (50 pM). Cervical vagus nerves (n=5) ligated 24 h. prior to extraction were cryostat-cut, slide-mounted and run under equilibrium binding conditions. We employed in vitro quantitative autoradiography with computerized image analysis to characterize parameters of binding to the proximal accumulation of sites.

Analysis (LIGAND) of competition data resolved devazepide binding to a single class of sites with a K_i= 18.4 ± 6.6 nM and a Bmax=2.6 ± 0.27 fmol/mg. Surprisingly, L365,260 was also a potent competitor of vagal ^{125}I-CCK binding: K_i=37.1 ± 4.6 nM; Bmax=2.0 ± 0.5 fmol/mg. These data suggest that afferent vagal fibers contain comparable densities of A-type and gastrin or B-type receptors.

Moran et al. (1990) have shown that vagal afferents project to the brainstem nucleus of the solitary tract (NTS). Hill and Woodruff (1990) report, and we confirm, a medial-lateral heterogeneous distribution of A-type and gastrin/B-type receptors in the NTS. Medially, in the commissural NTS, devazepide competes with high affinity for ^{125}I-CCK-occupied sites (K_i= 13.1 ± 4.0 nM) and L365,260 is relatively impotent (K_i>100nM). Laterally, L365,260 competes potently (K_i=21.8 ± 6.5) and devazepide is impotent (K_i>1000). Taken together these studies suggest a topographical distribution of A-type and gastrin or B-type receptors associated with vagal afferents projecting to the NTS.

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289.7

RTA Gene Expression in the Rat Cerebellum. Drew D. D'Angelo, Jeffrey K. Harrison, Pamela Woodring, Scott C. Baraban, Ruth L. Stornetta and Kevin R. Lynch. Department of Pharmacology, University of Virginia School of Medicine. Charlottesville, VA 22908 USA.

RTA is a 343 amino acid protein that is a member of the seven transmembrane helix superfamily that includes the guanine nucleotide binding protein (G-Protein) interactive receptors. It is most similar to the *mas* oncogene product followed by the complement C5a receptor; the ligand for RTA is not known at present. RTA cDNAs were isolated initially by screening a rat thoracic aorta cDNA library using an oligonucleotide complementary to a conserved region of a M_1 muscarinic acetylcholine receptor. A 2.4 kb RTA RNA species accumulates in tissues that are predominantly smooth muscle (e.g. aorta, uterus, stomach, intestines, vas deferens) or skeletal muscle (e.g. tongue). Several fibroblastic cell lines express the RTA gene also. CNS RTA gene expression is restricted to the cerebellum, where in addition to the 2.4 kb RNA species, there are 3.0 kb and 6.0 kb RNAs. An alternative form of RTA cDNA, which contained a different 5' non-coding region, was isolated from a cerebellar cDNA library. Analysis of the rat RTA gene showed that the point of divergence between the aorta and cerebellum cDNAs is located at an intron/exon boundary. Further analysis of the RTA gene structured showed the existence of multiple introns. The appearance of different forms of RTA RNAs in the cerebellum raises interesting possibilities including the differential expression of alternative RTA RNAs in different cell types. The expression of different forms of RTA RNA in the context of the rat cerebellum are being examined by *in situ* hybridization.

290.1

SEROTONIN INHIBITION OF ASCORBATE-STIMULATED LIPID PEROXIDATION IN PREFRONTAL CORTEX:DIFFERENCES BETWEEN ALZHEIMER'S DISEASE AND CONTROL. Anne C. Andorn and Bryan A. Ballot*, Dept. of Psychiat. and Hum. Behav., St. Louis Univ. Schl. of Med. and St. Louis VAMC, St. Louis Mo. 63125.

Unstimulated lipid peroxidation (LP) is increased, and 0.1 mM ascorbate-stimulated LP is decreased in Alzheimer's disease (AD) prefrontal cortex (PFC) as compared to age-matched (AM) controls. We now report that serotonin (5-HT) inhibits ascorbate-stimulated LP in young non-AD, AM non-AD and AD prefrontal cortices. However, 5-HT is less potent as an inhibitor of ascorbate-stimulated LP in AD than in either AM or young non-AD PFC. In the presence of 10 μM 5-HT, ascorbate stimulated LP is 45.5±10.8% (N=5) of the ascorbate-stimulated LP in the absence of 5-HT in young PFC; 30.3±13.3% (N=3) in AM PFC but 66.0±8.7% (N=3) in AD PFC. There is statistically no significant difference between young and AM non-AD, but there is a significant difference ($p < 0.05$) between AM and AD as determined by Student's t-test. 5-HT itself does not affect unstimulated LP (not shown). We have observed that ketanserin increases ascorbate-stimulated LP in non-AD PFC, with 10μM ketanserin stimulating between 151-224% (N=2) of the ascorbate-stimulated LP in young PFC. These observations suggest that antagonists and agonists may have opposite interactions with ascorbate stimulated LP in human PFC. Current investigations should ellucidate whether this might be a receptor mediated phenomenon.

290.2

BRAIN CELL MEMBRANE ABNORMALITIES IN ALZHEIMER'S DISEASE ARE DISTINCT FROM OTHER NEURODEGENERATIVE DISEASES. R. Nitsch*, J.K. Blusztajn, R.J. Wurtman & J.H. Growdon. Dept. of Neurology, Mass. General Hospital, Boston, MA 02114, Dept. of Pathology, Boston University, Boston, MA 02118, and Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Nerve cell degeneration in Alzheimer's disease (AD) is associated with abnormalities in membrane phospholipid (PL) metabolism: Glycerophosphocholine (GPC), a water soluble catabolite of membrane PL, is increased in AD brain. To determine if this alteration is specific to AD, we compared levels of GPC in frontal cortex from AD, Huntington's disease (HD), Parkinson's disease (PD), and Down's syndrome (DS) patients with control subjects using HPLC. GPC was increased 2x in AD cortex but was unchanged in HD, PD and DS. These results indicate that increased GPC reflects an abnormality of brain cell PL metabolism that is specific for AD. Accelerated membrane PL turnover may contribute to amyloid deposition by exposing the intramembranous domain of the amyloid precursor protein (APP) to proteolytic cleavage. The formation of amyloidogenic peptides in DS is not related to this metabolic PL abnormality, but probably reflects the increased gene dosage of APP on chromosomes 21.

290.3

COMPARISON OF NMR AND HPLC TECHNIQUES FOR THE ESTIMATION OF BRAIN METABOLITES IN POSTMORTEM ALZHEIMER'S DISEASE BRAIN. W.E. Klunk, K. Panchalingam, R.J. McClure, D. McKeag, A. Heist, G. Branthoover, and J.W. Pettegrew. Lab. of Neurophysics, Univ. of Pittsburgh, WPIC, Pittsburgh, PA 15261

In vitro and *in vivo* NMR methods have been used to provide relative quantification of brain metabolites in a variety of CNS disease states, including Alzheimer's disease. The noninvasive nature of NMR and its potential for clinical use makes it likely that this technique will gain even more extensive use in the future. More traditionally, metabolites such as amino acids and phosphomonoesters have been measured by HPLC. Since NMR also is being applied to measure levels of these same metabolites in brain, it is important to directly compare the two techniques to determine if they produce equivalent results. We have measured mixtures of phosphoserine, phosphoethanolamine, and glycerophosphoethanolamine by both HPLC of FMOC derivatives and ^{31}P NMR. Additionally, we have compared the relative amounts of these compounds in perchloric acid (PCA) extracts of Alzheimer's brain by both techniques. Alzheimer's brain PCA extracts and mixtures of glutamate, glutamine, GABA, aspartate, and taurine also have been compared by HPLC and ^{1}H NMR. In general the two techniques produced similar results. Specific advantages of each technique will be discussed. For example, NMR can measure some additional compounds without different methods of derivatization. These include phosphocholine, glycerophosphocholine and N-acetylaspartate (NAA).

290.4

^{1}H NMR *IN VITRO* STUDY OF AMINO ACIDS IN ALZHEIMER'S DISEASE BRAIN. R.J. McClure, K. Panchalingam, W.E. Klunk, and J.W. Pettegrew. Laboratory of Neurophysics, University of Pittsburgh, WPIC, Pittsburgh, PA 15261

The relative mole % of glutamate, aspartate, GABA, taurine, and N-acetyl-L-aspartate (NAA) in Alzheimer's disease (AD) brain have been evaluated by *in vitro* proton NMR at 500 MHz. Perchloric acid extracts of 9 brains with autopsy-verified AD and 5 age-matched control brains have been studied. We find an **increase** in mole % glutamate, glutamine, and taurine, **little change** in aspartate and GABA, and a **decrease** in NAA in AD as compared to controls. Senile plaque counts of the immediately adjacent histological sections are used to indicate the progression of the AD samples. We find that the decrease in the mole % of the putative neuronal marker, NAA, significantly and inversely correlates with increasing numbers of SP in AD. This finding is consistent with the profound loss of neurons in AD. The mole % of glutamate, glutamine, and taurine increase with increasing numbers of SP. The decrease in NAA and the increase in glutamate in AD suggests an increase of excitatory neurotransmitter per neuron as AD progresses. This may result in excitotoxic damage to the remaining neurons.

290.5

INCREASED COTRANSLATIONAL ASSOCIATION OF HEAT SHOCK 70 PROTEINS WITH NASCENT POLYPEPTIDES IN ALZHEIMER'S DISEASE W. Wallace, J. Sugar*, N. Perez*, G. Johnson, L. Bierer, V. Haroutunian, and C. Merril; Dept. Psychiatry and Center for Neurobiology, Mt. Sinai School of Medicine, New York and Laboratory of Biochemical Genetics, NIMH, Washington

The cellular disruptions that lead to the production of the abnormal proteins present in the AD brain are unknown. We have investigated potential cotranslational disruptions. Chaperonins are proteins that associate with polypeptides as they are being synthesized and mediate their initial processing. AD cortical tissues contained elevated levels of two heat shock proteins which act as chaperonins. The cytoplasmic chaperonin, hsp 72/73 was measured by immunoblots (259±142 vs 128±56; n=8/group; $p<0.05$), while in the endoplasmic reticulum, binding protein (aka grp 78), was measured on two dimensional gels (1.9-fold increase). The elevation showed specificity to the disease in that cerebellar tissues did not exhibit this increase (302±48 vs 275±66; n=4/group). AD polysomes were found to synthesize significantly higher levels of hsp 72/73 indicating that they are induced in the AD tissues. Immunoprecipitation of the newly synthesized hsp 72/73 resulted in the coprecipitation of numerous other nascent polypeptides. This binding indicates a long term association of the hsp with newly synthesized proteins, which may account for the presence of the abnormal proteins in the AD brain.

290.6

SERUM PROTEINS IN NEUROFIBRILLARY PATHOLOGY OF ALZHEIMER'S DISEASE (AD): ANTITHROMBIN III-LIKE IMMUNOREACTIVITY. R. N. Kalaria and S. N. Kroon*. Dept. of Neurology, Case Western Reserve Univ. Sch. of Medicine, Cleveland, OH 44106.

We and others have previously shown the presence of amyloid P component, a serum α_1-glycoprotein, in amyloid deposits of AD and other neurodegenerative disorders. Here, we used immunoblotting and immunocytochemical methods to investigate the presence of antithrombin III (AT III), a serum protein of the *serpin* family, in the pathological lesions of AD. Both mono- and polyclonal antibodies to AT III, obtained from various commercial sources, recognized protein(s) of *Mr* ~58 kD on immunoblots of cerebral microvascular proteins. This was verified by electrophoresis of authentic AT III protein run in parallel. Using the ABC immunocytochemical method at the light microscope level, at least three antibodies labelled neurites associated with plaques and neurofibrillary tangles (NFT) in neocortex and hippocampus of all the AD cases examined. The distribution and intensity of staining in NFT were similar to that of thioflavin S staining in serial sections. However, most of the antibodies stained intracortical vessels and capillaries in both AD and aging controls. Our results suggest the presence of AT III-like immunoreactivity or homologous epitopes in the neurofibrillary pathology of AD. Like other amyloid-associated factors e.g. α-ACT, protease nexin I, AT III may play a role in the pathogenesis of NFT and cerebral amyloidosis.

290.7

INCREASED PHOSPHORYLATION OF ELONGATION FACTOR 2 IS RESPONSIBLE FOR REDUCED POLYSOME ACTIVITY IN ALZHEIMER'S DISEASE BRAINS. G. Johnson[1], C.Merril[1], L.Bierer[2], V. Haroutunian[2], J.Sugar[2], and W.Wallace[2]. [1]Lab. of Biochemical Genetics, NIMH, Washington, D.C. and [2]Dept. of Psychiatry/Center for Neurobiology, Mt. Sinai School of Medicine, New York.

We have previously observed that polysomes prepared from Alzheimer's disease (AD)-afflicted brain tissues exhibit reduced ability to synthesize proteins in an in vitro translation system. No differences were found in either levels or translational activities of mRNA or ribosomes purified from AD polysomes. This result indicates that the reduced polysomal activity may be due to associated soluble factors. Based upon a reduced sensitivity of AD polysomes to the protein synthesis inhibitor, emetine, we proposed that the translocation step of elongation is disrupted. Elongation factor 2 (EF2) mediates this translocation in a phosphorylation-dependent manner. Calcium/calmodulin kinase III-mediated phosphorylation of EF2 results in the inhibition of elongation.

We investigated the phosphorylation state of EF2 in AD brain homogenates. EF2 was identified on two-dimensional (2D) gels of brain homogenates with factor-specific antibody. EF2 exhibits four distinct charge variant polypeptides reflecting its phosphorylation state. The phosphorylation state was directly measured as the distribution of the four polypeptides on silver stained 2D gels. The ratio of the phosphorylated (acidic) forms of EF2 to unphosphorylated forms was significantly greater in AD homogenates as compared to controls (1.11+0.37, n=10 vs 0.70+0.19, n=8; p<.001), indicating an increased phosphorylation of the AD EF2. Therefore, decreased AD polysomal function is due, at least in part, to increased phosphorylation of EF2.

290.8

CATION ENTRY/EFFLUX RATIO IN ALZHEIMER'S DISEASE ERYTHROCYTES IS CORRELATED WITH THE DEGREE OF DEMENTIA. J. W. Pettegrew, K. Panchalingam, G. Branthoover*, A. Heist*, Laboratory of Neurophysics, Dept. of Psychiatry, U. of Pittsburgh, Pittsburgh, Pa 15261

Alteration in Alzheimer's Disease (AD) erythrocyte membrane molecular dynamics and cation transport were studied by magnetic resonance spectroscopy techniques. Na^+ and Li^+ transport and relaxation parameters were measured. Correlation between the degree of dementia and membrane alteration was investigated in 7 mildly to moderately demented (Blessed = 1.5 to 13, Mattis 92 to 125) males and females (ages 65 and older) with probable AD (NINCDS-ADRDA criteria). Linear regression analysis showed negative correlation (p=0.004, r=0.8) between degree of dementia and cation entry/efflux ratio as measured by Li^+ transport. No association of Na^+ or Li^+ relaxation times with degree of dementia was observed. Comparison of AD patients with age matched controls does not indicate any disease dependent changes in the relaxation times. A shortening of the entry/efflux ratio would suggest either fast entry or slower efflux. This could be due to an alteration in plasma membrane or membrane associated cytoskeleton. The result could potentially explain why Li^+ has enhanced neurotoxicity in AD patients while is an effective treatment for mania and a prophylactic treatment for depression.

290.9

EVIDENCE FOR ALTERED THIAMINE NEUROCHEMISTRY IN ALZHEIMERS DISEASE. M. Héroux, A.-M. Besnard and R.F. Butterworth, Lab. of Neurochemistry, André-Viallet Clin. Res. Center, Hôpital St-Luc (Univ. of Montreal), Montreal, Que., Canada H2X 3J4.

Previous studies reveal decreased activities of thiamine-dependent enzymes in autopsied brain tissue from patients with Alzheimer's Disease. In the present study, thiamine-dependent enzymes were measured in autopsied temporal and frontal cortex samples obtained from 8 patients with neuropathologically confirmed Alzheimer's Disease and from an equal number of age-matched controls free from neurological or psychiatric diseases. Significant reductions were observed of pyruvate dehydrogenase (decreased by 70%, p<0.01), α-ketoglutarate dehydrogenase (decreased by 71%, p<0.01) and transketolase (decreased by 52%, p<0.01). Activities of the presynaptic cholinergic marker enzyme choline acetyltransferase were reduced by 42% (p<0.01); activities of the non-thiamine-dependent enzyme glutamate dehydrogenase were within normal limits. Analysis of thiamine esters by HPLC revealed reductions of 46% (p<0.01) of thiamine pyrophosphate (the enzyme cofactor form of thiamine) in Alzheimer brain tissue. These results suggest that abnormalities of thiamine neurochemistry could be implicated in the pathogenetic mechanisms in Alzheimer's Disease (Supported by MRC Canada)

290.10

SEROLOGICAL ALPHA-1-ANTICHYMOTRYPSIN MAY NOT BE A MARKER FOR ALZHEIMER'S DISEASE PATHOLOGY. K. Brugge*, R. Katzman, L. A. Hansen*, R. Terry*, and T. Saitoh. Univ. of California, San Diego, Dept. of Neurosciences, 0624, School of Medicine, La Jolla, CA 92093, U.S.A.

Alpha-1-antichymotrypsin (ACT) is a serine protease inhibitor which is markedly elevated in the serum and cerebral spinal fluid of patients with Alzheimer's disease (AD). Patients with Down's syndrome (DS) are known to develop neuropathological changes of AD by age 40 years and many become demented. Thus, in the present study, we obtained plasma ACT levels from DS subjects and non-DS controls and serum ACT levels from postmortem confirmed AD and control subjects. Plasma ACT levels between the DS (388.0±17.5 mg/dl) and age-matched, mentally retarded control (385.7±37.3 mg/dl) and healthy control (341.7±22.3 mg/dl) groups were not found to be significantly different. Furthermore, we failed to observe a positive correlation of ACT levels with age in the DS group, in spite of the age-dependent premature increase in neuropathological changes of AD that are known to occur in DS. A positive correlation between serum ACT levels and the density of plaques or tangles, neuropathological hallmarks of AD, in brains of AD patients also failed to exist. Thus, our results suggest that ACT levels may not serve as a marker for the development of AD in DS or parallel the development of the neuropathological hallmarks of AD.

290.11

PROPERTIES OF DEPHOSPHORYLATED τ_{PHF} PROTEINS. S. G. Greenberg, L. I. Binder and P. Davies*. Pathology Dept., Albert Einstein College of Medicine, Bronx, New York 10461.

This study examines the possibility that the distinct properties of τ_{PHF} results from modifications of normal τ_s. Hydrofluoric acid (HF) treatment resulted in the loss of SDS-insoluble and soluble PHF by electron microscopy. Therefore, modifications may serve a critical role in determining the structural integrity of PHF. HF-treated τ_{PHF} proteins are also heat and acid stable, soluble in MES buffers and display the same MW, pI, and immunochemical properties as normal τ. Alkaline phosphatase treatment of disassociated PHF resulted in similar, although less extensive changes in the MW and pI of τ_{PHF}. Therefore, the predominant effect of HF is likely to result from dephosphorylation.

Although dephosphorylated τ_{PHF} displays the same biochemical properties as τ_s, the relative abundance of τ isoforms differs in HF-treated PHF and τ_s samples. Therefore, a selective incorporation or exclusion of specific τ isoforms may also contribute to the formation of PHF. Comparison of dephosphorylated τ obtained from PHF or from a baculovirus vector expressing a human τ sequence indicates that the least abundant τPHF isoform is the 4 repeat form of τ. While Alz 50 reactivity was not selectively altered by the dephosphorylation conditions tested, the τ-1 epitope could be exposed under conditions that did not significantly change the MW of τ_{PHF} and PHF-1 reactivity was only significantly reduced under conditions which caused a distinct shift in the MW and pI of τPHF. Therefore, the PHF-1 epitope and not the τ-1 epitope may be associated with the aberrant MW and pI of τ_{PHF}. Although PHF-1 does not exhibit strong reactivity with normal τ_s isolated from human adults, PHF-1 recognizes phosphorylated human adult τ expressed from a baculovirus vector (generous gift of Ken Kosik). Therefore, the high specificity of PHF-1 in human adults for τ_{PHF} appears to be created by phosphorylation of normal τ_s.

290.12

BRAIN ALPHA-KETOGLUTARATE DEHYDROGENASE ACTIVITY IN ALZHEIMER'S DISEASE. Stephen J. Kish and Frank Mastrogiacomo*. Clarke Institute of Psychiatry and Departments of Psychiatry and Pharmacology, University of Toronto; Toronto, Canada

Circumstantial biochemical evidence has suggested that a failure of one or more energy metabolizing enzymes may be involved in the brain neurodegenerative processes in Alzheimer's disease (AD). Gibson and coworkers (Arch Neurol 45:836, 1988) reported markedly reduced (-75 to -100%) activity of alpha-ketodehydrogenase (KGD) in both degenerated and morphologically normal areas of postmortem brain of AD patients. A failure of this enzyme could lead to neuronal cell death by a variety of mechanisms including excitotoxic damage. We measured the activity of KGD in autopsied frontal and temporal cortex from 8 neuropathologically confirmed AD cases and 6 neurologically normal matched controls. Although enzyme activities in the AD frontal and temporal cortices were reduced, on average, by 57% and 40%, respectively, as compared with the controls, the differences were not statistically significant (p>0.05) due to a large overlap between the AD and control values with 3 of the 8 AD patients having distinctly normal control levels. We conclude that although decreased KGD activity may, in principle, contribute to the brain neurodegeneration in some AD patients, it is not a constant feature of this human brain disorder. (Supported by U.S. NIH NS26034.)

291.1

DIRECTION SENSITIVITY OF Aδ FIBER MECHANORECEPTORS. M.B. MacIver & D.L. Tanelian. Pain Research Laboratory, Department of Anesthesia, Stanford University School of Medicine, CA 94305.

Structure-function relationships of pain fiber mechanosensitive nerve endings were investigated to determine a physiological basis for directional sensitivity in trigeminal ganglion sensory afferents. Corneas were isolated from albino rabbits and maintained *in vitro* at constant temperature (35°C) and intraocular pressure (18 mmHg). Fluorescence microscopy was used to visualize and digitally reconstruct individual nerve endings. Combined microstimulation and electro-physiological recording of single isolated fibers were used to map terminal field excitability patterns for each nerve ending. Fiber type was determined on the basis of stimulus/response modality and conduction velocity (Aδ fibers > 2 M/s). Aδ mechanosensitive endings travelled parallel to the epithelial surface at a depth of 15 to 45 μm for distances of 0.1 to 1.5 mm. Mechanical stimuli which moved parallel to the long axis of these endings produced 5 to 10 fold more action potentials than did stimuli applied perpendicular to this axis. C fiber endings, in contrast, terminated as finger-like projections which were perpendicular to, and approached to within 2 μm of, the epithelial surface. C fibers were not mechanosensitive. Both Aδ and C fiber endings could be electrically activated and their excitability maps closely followed the underlying anatomy. This study is the first to demonstrate distinct terminal patterns for Aδ *vs.* C fiber nociceptors, and to demonstrate that directional sensitivity of mechanoreceptors results from their unique elongated "leash-like" free nerve endings. Supported by NIH grant NS28646-01 A1.

291.2

FAST CONDUCTING (GROUP II) SENSORY NERVE FIBERS IN THE CAT'S KNEE JOINT WITH CHEMORECEPTIVE PROPERTIES K. Meßlinger*, S.K. Hong*, M. Pawlak*, K. Schepelmann*, R.F. Schmidt Inst. of Physiology, University of Würzburg, D-8700 Würzburg, Germany

Articular group II sensory nerve fibers are known to innervate (rapidly and slowly adapting) mechanoreceptive corpuscular nerve endings. These mechanosensors are usually insensitive to chemical stimuli which typically excite slowly conducting (polymodal) afferents. To examine the response properties of articular afferents, recordings were made from filaments of the medial articular nerve in α-chloralose anesthetized cats using mechanical stimulation (passive movements of the knee) and chemical stimuli, applied i.a. as a bolus close to the knee joint. In this study, we searched for fast conducting fibers which were either excited or sensitized by the inflammatory mediators bradykinin and prostaglandin E_2 and I_2. A total of 36 afferents with conduction velocities of 20.7 up to 61 m/s were tested. Twelve of 28 units responded to bradykinin in a concentration of 0.26-26 μg/0.3 ml bolus. Six of 20 units, tested with PGE_2 or PGI_2 in a concentration of 0.3-30 μg/bolus, were excited and 8 of 15 units were sensitized to movements (decrease of mechanical threshold). Units tested with both bradykinin and prostaglandins showed differential sensitivity to these compounds. The chemosensitive properties were restricted to more or less slowly adapting units, while rapidly adapting mechanosensors were not chemosensitive. The results suggest that some articular group II sensory receptors, which may correspond to Ruffini-like endings without a perineurial capsule, behave like polymodal sensors similar to slowly conducting nerve fibers. Taken together, it seems probable that specific group II sensors are activated in acute arthritic processes, although their role in nociception remains unclear.

291.3

MICRONEUROGRAPHIC AND PSYCHOPHYSICAL STUDIES OF MECHANICAL PAIN AND HYPERALGESIA USING A NEW STIMULATION TECHNIQUE. M. Koltzenburg* and H.O. Handwerker, Institut für Physiologie, D-8520 Erlangen, F.R.G.

We have developed a new technique for the evaluation of mechanical pain and hyperalgesia. Light metal cylinders (300 mg; 6 mm diameter) were guided and accelerated in a barrel. On impact against the skin they elicit a brief sensation of pain. In 19 psychophysical experiments using magnitude estimation techniques monotonically increasing stimulus response functions were obtained for different acceleration velocities (7.5-22.5 m/s; r=.84) with a mean pain threshold of 11.6±0.5 m/s (mean±SEM). Using repetitive painful stimulation (9 impacts, 1 Hz) there was a gradual build-up of pain intensity indicating that temporal summation is important for mechanical pain. This stimulus train also resulted in the reliable development of mechanical hyperalgesia as shown by the elevated pain ratings of a constant test stimulus. Hyperalgesia developed within 1-3 minutes and was maintained for up to 20 minutes. Using percutaneous microneurography single primary afferents were recorded from the superficial radial nerve of conscious humans. Low threshold large diameter afferents responded with few impulses and could not encode stimulus intensity. Some slowly adapting afferents became unresponsive for several minutes following a strong impact stimulus. Nociceptive C-fibers were also activated, often at stimulus intensities that were not rated painful by the subjects. However, in contrast to myelinated low threshold mechanoreceptors, C-fibres showed graded responses to suprathreshold stimuli. (Supported by DFG & Marohn Stiftung)

291.4

CENTRAL NERVOUS SYSTEM LESIONS AND DEAFFERENTATION BY C5-T2 GANGLIONECTOMIES IN RATS. J.Ovelmen-Levitt, E. Rossitch,Jr., S-N Hwang*, M. Abdulhak*, M.Levitt and B.S.Nashold,Jr. Div. Neurosurgery, Duke U. Med. Ctr., Durham, N.C. 27710.

A deafferentation syndrome (DS) has been produced in rats by C5-T2 ganglionectomies that is expressed by scratching of partially deafferented areas and/or biting of anesthetic limb regions. The projection to the brainstem from the spinal cord is bilateral in the rat, with a higher percentage to the midbrain than to the sensory thalamus. In addition, the spinothalamic tract has been found to project to and/or make collateral synapses at locations in the brainstem including the midbrain central gray, the parabrachial region, and the intralaminar as well as the ventrobasal thalamus. We have studied the effect of lesions in these regions on the onset, progression and extent of DS after deafferention. All surgery was performed under aseptic conditions after anesthesia with I.P. Ketamine (90 mg/kg). The brainstem lesions were made using a thermocouple electrode with a Radionics Lesion Generator. The spinal cord lesions were made using a micromechanical technique. Simultaneous lesions of the dorsal horn significantly reduced DS, as did pre- or post-ganglionectomy lesions of the parabrachial region and the medial thalamus when compared to ganglionectomy only controls. Midbrain central gray lesions did not have this effect, however, and could augment DS. The results validate the rat ganglionectomy model of neural injury and indicate that the spinal cord dorsal horn is important in the generation of this type of pain. They also suggest that certain CNS structures are differentially involved in the expression of dysesthetic sensation following neural injury.

291.5

THE CONTRIBUTION OF G_s AND cAMP TO DECREASED MECHANICAL NOCICEPTIVE THRESHOLDS IN STREPTOZOTOCIN-DIABETIC RATS. S.C. Ahlgren and J.D. Levine*, *Depts. of Anatomy, Medicine and Oral Surgery, and Division of Neurosciences, UCSF, Box 0452, San Francisco, CA 94143.*

The purpose of this study was to examine the contribution of stimulatory GTP-binding proteins (G_s) and of cAMP to the lowered mechanical threshold in the primary afferent nociceptor in streptozotocin-diabetic (STZ-D) rats. The effect of the intradermal (id) injection of test agents on mechanical nociceptive threshold in the hairy skin of the hindpaw of adult male Sprague-Dawley rats, diabetic for 21 to 35 days, was monitored by the Randall-Selitto paw-withdrawal test.

For normal rats, the action of prostaglandin E_2 (PGE_2) and of A_2-type purinergic agonists, both direct-acting hyperalgesic agents, is mediated via activation of G_s. In STZ-D rats, hyperalgesia induced by PGE_2 (100 ng, id) was attenuated by GDP_β-S (1 μg) (PGE_2= -41.9 ± 5.0%, n=6; PGE_2 + GDP_β-S = -6.4 ± 6.0%, n=6; p<0.05). However, GDP_β-S alone had no significant effect on nociceptive thresholds in the diabetic rat (-7.48 ± 4.1%, n=6; p>0.05).

While phosphodiesterase (PD; 0.1 unit/2.5 μl, id) did not effect nociceptive threshold in normal rats (-2.8 ± 1.9%; p>0.05, n=12), it attenuated the hyperalgesia produced by the intradermal injection of PGE_2 (co-injected with indomethacin (10 μg); PGE_2 = -39.1 ± 5.8%, n=6; PGE_2 + PD = -19.6 ± 6.3, n=6; p<0.05), confirming a contribution of cAMP to prostaglandin hyperalgesia. In STZ rats, PD produced a 17.0 ± 5.1% increase in nociceptive threshold compared to vehicle in STZ rats (n=28; p<0.01).

The membrane permeable cAMP analogue, 8-bromo cAMP, which acts on nociceptors to produce hyperalgesia in non-diabetic rats, was more potent in inducing hyperalgesia in the STZ rat, demonstrating a shift to the left in the dose response curve by an order of magnitude compared to normal rats. These results support a contribution of both increased cAMP levels and increased cAMP responsiveness to the hyperalgesia observed in the STZ-diabetic rat. *Supported by NIH grant NS21647*

291.6

STUDIES WITH PHASIC AND TONIC NOXIOUS STIMULI SUGGEST THAT BOTH CHOLERA- AND PERTUSSIS TOXIN-SENSITIVE MECHANISMS MEDIATE MORPHINE ANTINOCICEPTION IN THE RAT SPINAL CORD. H. Wheeler-Aceto* and A. Cowan. Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

A common feature of opioid receptors is their ability to modulate effector function via PTX-sensitive G-proteins. Using noxious stimuli of different intensities and durations, we have previously shown that i.th. PTX antagonizes morphine (M) antinociception against a high intensity phasic endpoint (50°C water tail-dip reflex). A reduction in the potency of M against intermediate intensity (early phase formalin) and, to a lesser extent, low intensity (late phase formalin) stimuli also occurs but, in these cases, maximum efficacy is still achieved [Eur. J. Pharmac. 195: 411, 1991]. However, accumulating evidence suggests that opioid receptors are not exclusively coupled to PTX-sensitive G-proteins. The present study was therefore undertaken to assess the contribution of CTX-senstive mechanisms to the antinociceptive profile of M. Male S.D. rats (70-90 g) were pretreated i.th. with CTX (0.5 μg) or saline (5 μl) at -24 h. M or saline (n=5-10 rats per dose) was given s.c. 20 min before formalin. Critically, since all three endpoints were recorded in the same rat, dose-response profiles against each endpoint were obtained simultaneously. In controls, M was fully efficacious against each stimulus. After CTX, there was a 3x *decrease* in the A50 of M in the tail-dip test; this is in contrast to PTX which caused a >12x *increase* in A50. However, in early phase formalin, both CTX and PTX caused an increase in A50 of 5x and 11x, respectively. In both cases, unlike the effect of PTX in the tail-dip test, maximum efficacy was still achieved. A smaller shift in antinociceptive potency (increases of 1.5x and 4x with CTX and PTX, respectively) was observed in the late phase of formalin. These data, in conjunction with our previous findings, suggest that *(i)* M acts at spinal sites coupled to both PTX- and CTX-sensitive G-proteins, *(ii)* high intensity phasic stimuli are down-regulated by PTX-sensitive transducers and transmission of these signals can be enhanced by a CTX-sensitive mechanism, *(iii)* the effect of M depends on a balance of stimulatory and inhibitory actions which can be shifted by pretreatment with PTX or CTX, and *(iv)* the down-regulation of intermediate and low intensity stimuli is complex and may involve both PTX- and CTX-sensitive mechanisms. (DA 03945 and DA 07237 from NIDA).

291.7

ON THE ROLE OF NK-1 AND NK-2 TACHYKININ RECEPTORS IN MEDIATION OF SPINAL CORD REFLEX EXCITABILITY

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We have studied the effects of intrathecal (i.t.) Spantide II (SII, non-selective tachykinin antagonist), CP-96,345 (NK-1 antagonist) and Men10207 (NK-2 antagonist) on the spinal nociceptive flexor reflex (FR) and facilitation of the FR by i.t. substance P (SP), neurokinin A (NKA) and conditioning stimulation (CS, 1 Hz, 20) of C-afferents in cutaneous and muscle nerves.

I.t. CP-96,345 antagonizes FR facilitation induced by i.t. SP, but not NKA, whereas Men10207 antagonizes NKA-induced FR facilitation without influencing SP's effect. SII blocks the effect of both tachykinins. CS of cutaneous and muscle nerve facilitates the FR for a brief or prolonged period, respectively. Both types of facilitation were blocked by SII. Men10207 antagonized muscle, but not cutaneous, nerve CS-induced FR facilitation whereas CP-96,345 blocked the latter. I.t. CP-96,345 does not depress the FR whereas SII and Men10207 do so at high doses.

It is suggested that NK-1 and NK-2 tachykinin receptors have a distinct role in the mediation of spinal cord reflex excitability and nociception.

291.8

CENTRAL SENSITIZATION AFTER C-FIBER ACTIVATION IS MEDIATED BY NMDA AND SUBSTANCE P RECEPTORS

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A brief conditioning stimulus (CS) train (20 s, 1 Hz) to C-afferents facilitates spinal flexor reflex excitability for minutes. Evidence has been presented that NMDA (Woolf and Thompson, Pain, 1991, 44, 293-299) and tachykinin (Wiesenfeld-Hallin et al., Brain Res., 1990, 526, 284-290) receptors may have a role in central sensitization. We tested the interaction of the nonpeptide NK-1 antagonist CP-96,345 and the NMDA antagonist MK-801 on sural nerve CS-induced flexor reflex facilitation in decerebrate, spinalized, unanesthetized rats.

CP-96,345 administered I.v. at 1 and 3 mg/kg significantly attenuated the prolonged facilitation of the flexor reflex for up to 3 h. MK-801 (0.5 mg/kg) had a similar effect. When subthreshold doses of both drugs were coadministered (0.1 mg/kg MK-801 plus 0.3 mg/kg CP-96,345), the central sensitizing effect of the sural CS was totally blocked for 1-2 h.

Prolonged reflex facilitation after a brief C-fiber CS may be due to cumulative depolarization of dorsal horn interneurons. The NK-1 agonist substance P produces prolonged depolarization in the dorsal horn. Glutamate and substance P, which coexist in primary afferent terminal vesicles, may be coreleased and interact synergistically to induce central sensitization.

291.9

GABA-B RECEPTORS MUDULATE THE SENSITIVITY OF WIDE DYNAMIC RANGE NEURONS IN RAT SPINAL DORSAL HORN TO INNOCUOUS MECHANICAL STIMULI

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Transient spinal ischemia in rats is induced by a photochemical reaction, leading to platelet aggregation, when a dye, Erythrosin B, injected I.v., is activated by an argon laser irradiating an exposed vertebra. The ischemia leads to the development of hypersensitivity (allodynia) to innocuous mechanical stimuli in the dermatomes of the irradiated segments (Hao et al., Pain, in press). The activity of single wide dynamic range (WDR) neurons in allodynic rats was recorded to examine the neural mechanisms underlying this sensory abnormality.

The responses of WDR cells to electric shocks or mechanical stimulation with von Frey hairs in their receptive fields was recorded in normal and allodynic rats. In normal rats electrical stimulation evoked a biphasic response corresponding to A and C fiber input. The mechanical threshold in normal WDR cells was 13.8 g. In the majority of allodynic rats the neural response to electrical stimulation was a single prolonged burst and the mechanical threshold was 2.1 g. The response characteristics of the cells in allodynic rats were normalized by systemic GABA-B agonist baclofen (0.1 mg/kg), but not GABA-A agonist muscimol (1 mg/kg).

Transient spinal ischemia leads to a dysfunction of GABAergic inhibitory mechanisms in the dorsal horn, which normally may control sensory input from myelinated afferents.

291.10

CHRONIC PAIN-RELATED SYNDROME IN RATS AFTER ISCHEMIC SPINAL CORD LESION: AN ANIMAL MODEL FOR PAIN IN PATIENTS WITH SPINAL CORD INJURY.

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Spinal ischemia in rats is induced by a photochemical reaction, leading to platelet aggregation, when a dye, Erythrosin B, injected I.v., is activated by an argon laser irradiating an exposed vertebra. Various degrees of neurological deficits and morphological damage may be observed after this photochemical lesion, which is correlated with the duration of laser irradiation.

We report here that a chronic pain-like syndrome develops within weeks in some of the irradiated rats. This syndrome includes mechanical allodynia, which is expressed as a clearly painful reaction to light pressure applied to skin at or near the dermatome of the injured spinal segments, and self-mutilation of the hindpaws (autotomy). The allodynia in the majority of rats is not relieved by clonidine, baclofen, muscimol, guanethidine or carbamazepine and is only relieved by morphine at a sedative dose. In contrast, low, non-sedative doses of the local anesthetic tocainide have a good effect when applied systemically.

We suggest that the present finding may represent an animal model for chronic central pain after spinal cord injury or stroke.

291.11

INTRACELLULAR RECORDINGS FROM DORSAL HORN NEURONS IN THE FLUOROCARBON-PERFUSED RAT.

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The cellular properties of spinal neurons are likely to contribute significantly to the processing of nociceptive and non-nociceptive input from somatic and visceral sensory receptors. Their investigation, however, has been hampered by the difficulties of obtaining long-term stable intracellular recordings and manipulating the extracellular environment in intact, *in vivo* preparations. Consequently, we have developed a fluorocarbon-perfused, whole rat preparation, which combines the advantages of *in vitro* preparations (intracellular recording and solution alteration) and *in vivo* preparations (adult, normothermic and intact CNS/periphery), in order to study the role of cellular properties in sensory processing.

Adult rats were perfused through the ascending aorta using non-pulsatile flow of a fluorocarbon-based artificial blood which contained pentobarbital and pancuronium and was maintained at 37°C. Intracellular recordings were obtained from the lumbar dorsal horn using 50-100 mohm 3 M KCl filled micropipettes. Viability of the nervous system, as assessed by the presence of a normal EEG, was maintained for up to 12 hours.

To date, we have obtained 8 intracellular recordings from dorsal horn neurons that responded to cutaneous stimulation of the hindlimb; all neurons exhibited pronounced synaptic potentials often accompanied by spontaneous activity. Receptive fields were similar to those seen with extracellular recording *in vivo*, including both noxious and non-noxious input. Based on micropipette depth, recordings were obtained from both superficial and deeper lamina. These results demonstrate the usefulness of the fluorocarbon-perfused preparation for the intracellular recording of dorsal horn neurons.

Supported by NIH Grant NS19912.

291.12

LOCATIONS OF AXONS OF PHYSIOLOGICALLY CHARACTERIZED SPINOTHALAMIC TRACT (STT) NEURONS IN RATS.

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Recent anatomical studies have described a projection of STT axons through the contralateral dorsal lateral funiculus. We extended these findings using microantidromic mapping techniques. This allowed determination of both the positions of individual STT axons and the response characteristics of the recorded neurons. We recorded from 41 neurons in the cervical enlargement that were antidromically activated (mean threshold = 16 uA) from the contralateral posterior thalamus. Recording points were in the superficial dorsal horn (SDH; n=17) and nucleus proprius/lateral reticulated area (NP/LRA; n=23). Approximately 96% of the neurons tested (n=24) responded preferentially or exclusively to noxious mechanical stimuli and 80% tested (n=15) responded to noxious heat. The position of each STT axon was located at one (n=16) or more (n=25) levels of the cervical cord white matter using a second antidromic stimulating microelectrode (mean =9.3 uA). The axons of neurons recorded in the SDH were located primarily in the contralateral dorsal lateral funiculus in segments C3-5. In contrast, axons of neurons in NP/LRA were found primarily in the contralateral ventral lateral funiculus in these segments. However, at C2 the axons of both SDH and NP/LRA neurons were found scattered throughout the dorsal and ventral lateral funiculus. These results confirm that many STT axons ascend in the dorsal lateral funiculus. They also indicate that nociceptive STT axons are segregated in mid-cervical cord according to the location of their cell bodies but often shift locations and become intermingled throughout the lateral funiculus at upper cervical levels. Supported by NS25932 and DA07234.

292.1

EFFECTS OF BRIGHT LIGHT ON HUMAN NOCTURNAL PERFORMANCE, MOOD AND SERUM MELATONIN LEVELS

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We examined the effects of nocturnal exposure to bright light on human behavior, pineal function, oral temperature and cardiovascular status in 23 healthy male volunteers. On three separate occasions, subjects were admitted to a clinical research center in groups of 2 to 4 for a 13.5-hour session (1630 - 0800 h). Each subject sat at an individual work station that was maintained throughout the night at an illumination of approximately 300, 1500, or 3000 lux. (Three hundred lux is a typical indoor level of illumination). On admission, a catheter with a heparin lock was established in a forearm vein for withdrawal of blood samples. Throughout the night subjects were required to complete interactive computer tasks designed to measure auditory and visual reaction time and vigilance, and a questionnaire to assess mood state. The order and timing of test administration were held constant across test nights. Temperature, blood pressure, heart rate, and sleepiness were assessed every hour. Blood samples were taken at 1900, 2100, 2300 and at hourly intervals thereafter until 0800. Serum was assayed for melatonin concentration by radioimmunoassay. The normal nighttime secretion of melatonin was largely suppressed by the brightest light, partially suppressed by the moderate intensity and appeared to be intact at 300 lux. Mood state was also significantly affected by exposure to bright light. It appeared that bright and moderate light exposure throughout the night increased depression without affecting alertness. However, only subtle variations in performance were noted as a function of light exposure.

292.2

DYNAMIC ANALYSIS OF CARDIAC AND RESPIRATORY COUPLING DURING SLEEP IN INFANTS. S.L. Raetz, A. Garfinkel, V.L. Schechtman, D.O. Walter*, and R.M. Harper. Brain Research Institute and Dept of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024-1763.

During maturation, physiologic systems couple and decouple, therefore measures of the degree of coupling between systems provide useful indices of development. Traditionally, linear measures (correlation, coherence) are used to assess coupling. We developed two nonlinear measures of association between physiologic variables: (1) mutual information, applied to the cross-plot of two variables, and (2) statistical dependency of changes in one variable on changes in the other. When two variables are plotted on an x-y axis, mutual information is a measure of the extent which the value of one variable reduces uncertainty about the value of the other. When differences in one variable are plotted versus differences in another, chi-square procedures can assess the dependency of changes in one variable on changes in another. We assessed the degree of coupling between cardiac rate and respiration in seven infants who subsequently succumbed to sudden infant death syndrome (SIDS) and seven age- and sex-matched controls. We found that coupling changes with maturational age, and that the extent of cardiorespiratory coupling is significantly greater in this group of control infants than in the group of infants who subsequently succumbed to SIDS. Supported by HD22695

292.3

TIME-OF-NIGHT EFFECT ON HEART RATE VARIATION IN HUMAN NEONATES. V.L. Schechtman and R.M. Harper. The Brain Research Institute and the Dept. of Anatomy & Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024.

Circadian patterns have been observed in infants as early as the first few postnatal days. We hypothesized that, in each sleep-waking state, heart rate variation in 3 distinct frequency bands would show consistent changes across a night in 1-week old infants. Twelve-hour nighttime recordings of EEG, EOG, ECG, digastric EMG, respiratory movements, and expired CO_2 were obtained from 25 normal full-term infants at 1 week postnatal age. The extents of 3 types of heart rate variation were determined for all epochs of quiet sleep, rapid eye movement sleep, and waking during each 4-hour period of the night. In sleep states, the extent of all three types of heart rate variation decreased over the course of the night; however, 2 of the 3 did not show this time-of-night effect in waking. Heart rate variation at the respiratory frequency showed a time-of-night effect in quiet sleep only, resulting in a significant sleep state effect on the extent of respiratory-related heart rate variation during the evening that disappeared later in the night. Results indicate that time-of-night effects on heart rate variation are not constant across physiological states in neonates, and heart rate variation during waking is particularly unresponsive to circadian influences.

Supported by NIH Grant HD22695. Data acquisition and sleep state classification were performed under the direction of Drs. J. Hodgman & T. Hoppenbrouwers under NIH Contract HD22777.

292.4

EFFECTS OF CORTICOTROPIN-RELEASING FACTOR ON SLEEP AND RESPIRATION DURING SLEEP IN HEALTHY SUBJECTS. K. Mann*, J. Röschke, M. Nink*#, H. Lehnert#, U. Krause*#, J. Aldenhoff, J. Beyer*# and O. Benkert*. Dept. of Psychiatry and Dept. of Endocrinology#, University of Mainz, D-6500 Mainz, Germany.

In a single-blind placebo controlled design the effects of corticotropin-releasing factor (CRF) on sleep EEG and respiration parameters during sleep were investigated in up to now 8 healthy male volunteers aged 24 to 30 years. After an adaptation night either 50 ug ovine CRF or placebo was administered intravenously as a bolus in two successive nights every hour from 0.00 h to 6.00 h. Polysomnography was performed between 23.00 h and 7.00 h, respiration was studied by thermistor measuring of nasal and oral air flow, thoracic wall movements and blood oxygen saturation.

Total sleep time and number of awakenings were not altered by CRF. Also, subjective perception of sleep quality was approximately identical following CRF and placebo administration. Sleep analysis according to the criteria of Rechtschaffen and Kales revealed only slight alterations in sleep architecture. REM sleep as well as slow wave sleep, particularly stage IV, showed a tendency to decrease under CRF, whereas light sleep, particularly stage I, tended to increase. Immediately after an injection of CRF mostly a stimulation of respiration could be observed with an increase of tidal volume over a time interval of a few minutes, whereas blood oxygen saturation was not markedly altered.

292.5

PLASMA NOREPINEPHRINE MANIPULATION BY DIETARY SODIUM FAILS TO MODIFY SLEEP PATTERNS IN HEALTHY OLDER MEN. M.V. Vitiello*, R.C. Veith*, D.D. Ralph* and P.N. Prinz. Psychiatry and Medicine, University of Washington, Seattle and American Lake VAMC, Seattle, WA 98195.

We have shown that elevated nighttime plasma norepinephrine (NE) levels following a low sodium diet were associated with disturbed sleep in healthy young men, indicating that increased sympathetic nervous activity (SNS) may adversely affect sleep. To extend this finding we studied the sleep and nighttime NE levels of 7 healthy older men (61.7± 1.6) after four days of low (500), normal (2000) and high (5000mg/day) sodium diets presented in counterbalanced order. Sleep ratings and NE assays were made blind to condition. NE was significantly elevated ($p<.02$) in the low relative to the normal condition. No change in NE was seen in the high condition. No significant differences were observed in any measure of sleep or wakefulness across conditions. Although most measures of wakefulness (total wake time, number of wakes, etc.) were higher in the low compared to the normal condition, variability of these measures in the low condition was also higher obscuring possible differences in this small sample. These results fail to extend our findings of the relationship between elevated SNS activity and sleep disturbance to a healthy aged male sample. It is unclear whether this relationship does not exist in the elderly or is masked by the increased variability in physiological measures often seen in this population and specifically present in the low sodium condition.

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292.6

CIRCADIAN PHASE SHIFTS AND BENZODIAZEPINE-INDUCED LOCOMOTOR HYPERACTIVITY IN SIGHTED AND BLINDED HAMSTERS. T.A. Houpt*, Z. Boulos, M.C.Moore-Ede. Dept. Physiology, Harvard Medical School, and Inst. for Circadian Physiology, 677 Beacon St., Boston, MA 02215.

The benzodiazepines (BZD) may cause phase shifts in hamsters either by transiently attenuating light input to the circadian system, or indirectly by inducing locomotor hyperactivity with feedback effects on the circadian pacemaker. However, there have been few quantitative studies correlating BZD-induced hyperactivity with phase shifts under different lighting conditions. We compared the hyperactivity induced by diazepam (DZP) and triazolam (TRZ) in sighted and blinded hamsters.

Male golden hamsters were housed in running-wheel-equipped tub cages under LL (~200 lux) or after optic enucleation. Individuals were placed in a large tub cage (45cm x 38cm) at either CT5 or CT17. Infrared photocells divided the cage into 10cm x 13cm rectangles and mean distance (m) per 5 min. was reconstructed from the beam breakages. After 1 hr baseline, the hamsters received either 25mg/kg DZP or 0.5mg TRZ i.p., both in 0.25ml DMSO, and activity was recorded for 2 hrs. Six injections were given at each time for each drug and lighting condition. Both DZP and TRZ induced marked hyperactivity compared to baseline or vehicle (6 m/5 min), reaching a plateau ~1 hr post-injection. In LL, DZP induced similar levels of activity (CT6: 24 m/5 min, CT18: 26 m/5 min) and large phase shifts (CT6: 95 min adv., CT18: 171 min del.) at both time points. In blinded hamsters, DZP induced comparable advances at CT6 but delays at CT18 were greatly attenuated (16 min. del.), despite activity levels similar to those in LL. TRZ induced high levels of activity that were not different in LL and in blinded hamsters (CT6: 32 m/5 min, CT18: 43 m/5 min). However, TRZ induced larger phase shifts in LL (CT6: 191 min adv., CT18: 28 min del.) than in blinded hamsters (CT6: 95 min adv., CT18: 4 min del.).

Since optic enucleation attenuated BZD-induced phase shifts without reducing hyperactivity, we suggest that ambient light perception is necessary for maximal BZD phase shifts, particularly during subjective night. The light-modulating mechanism of BZD phase shifts may be independent of activity induction.

292.7

DIURNAL RHYTHM OF CORE BODY TEMPERATURE IS PHASE-ADVANCED IN RATS WITH A GENETICALLY UPREGULATED CHOLINERGIC SYSTEM PJ Shiromani, H Klemfuss, S Lucero* and DH Overstreet *Dept Psychiatry, San Diego VA Medical Center & UCSD, La Jolla, CA 92093*

We examined the diurnal rhythm of core body temperature in a strain of rats with an upregulated central muscarinic receptor system. The Flinders Sensitive Line (FSL) were derived by selectively breeding rats for sensitivity to cholinergic agonists and, compared to randomly bred rats, FSL rats have increased density of muscarinic receptors in various brain regions. Previously, we showed that FSL rats have increased REM sleep *(Neuropsychopharmacology 1:127-133, 1988)* and this is in agreement with the cholinergic hypothesis of REM sleep generation. We now report that FSL rats have a phase-advanced temperature rhythm.

Six each male FSL and control rats were implanted with a Mini-Mitter transmitter in the abdominal cavity under Nembutal anesthesia and continuous temperature recordings were made for 30 days. Temperature data collected during the last two weeks of the experiment were analyzed. Under LD 12:12, FSL rats reached peak temperature 2 hrs and 37 minutes earlier compared to control rats. However, the 24 hr mean temperature and amplitude of the temperature rhythm were not different between the groups.

Carbachol, a cholinergic agonist has been shown to phase-shift circadian rhythms. Therefore, the increased muscarinic activity in the FSL rats might be contributing to the phase-advanced temperature rhythm. These findings are also relevant to depression because some patients with some types of depression show phase advances in a number of circadian rhythms, including temperature. These patients also have short REM sleep latency. Our finding of a phase advance in a rodent model with a known upregulated muscarinic receptor system is compatible with both the phase advance and the muscarinic overdrive theories of depression. These findings also further validate the usefulness of the FSL rats in the study of depression.

292.8

OSCILLATORY PROPERTIES OF BASAL FOREBRAIN NEURONS IN GUINEA PIG BRAIN SLICES. M. Mühlethaler, A. Khateb, M. Serafin, B.E. Jones and A. Alonso, *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and *Montreal Neurological Institute, McGill University, Canada H3A 2B4*

Although electrophysiological studies of basal forebrain neurons (BFn) have been performed in both in vivo and in vitro preparations, they have revealed little concerning the intrinsic properties of the cell population and particularly of the specific cholinergic and GABAergic neurons that are distributed through the region and project to the cerebral cortex. In the present study, neurons were recorded in guinea pig brain slices through the horizontal limb of the diagonal band and substantia innominata, where choline acetyl transferase (ChAT)- and glutamic acid decarboxylase (GAD)-immunoreactive neurons were localized. The recorded cells were filled with biocytin for subsequent morphological and histochemical study. A majority of the recorded BFn were characterized by the presence of a large afterhyperpolarization (AHP) and a transient rectification due to the presence of a strong A-current. In addition, they exhibited strongly voltage-dependent low threshold calcium spikes. In the hyperpolarized condition, this latter property was a major determinant in the manifestation of slow oscillatory activity. These results indicate that a class of BFn may behave in a manner similar to thalamic or some cortical pyramidal cells by displaying two modes of firing: a tonic mode, when the cells are at or above their resting potential, and an oscillatory mode when the cells are hyperpolarized. Although the chemical identity of the neurons is not yet determined, our data indicate that certain BFn that are functionally as well as anatomically linked to other rhythmic prone systems of the thalamus and limbic and neocortex, also have the intrinsic ability to oscillate. (Supported by the Swiss NSF and Canadian MRC)

292.9

PROTEIN DIFFERENCES IN TAU MUTANT HAMSTERS: CANDIDATE CLOCK PROTEINS. J.E.Joy[1], G.S.Johnson[1], C.R.Merril[1] and M.Menaker[2]. [1]Lab.of Biochemical Genetics, NIMH Neurosciences Center, Washington D.C., 20032, [2]Dept. Biology, University of Virginia, Charlottesville, VA 22901.

In the tau mutant hamster, the period of the circadian rhythm is shortened from about 24 hours to about 22 hours in heterozygotes and to about 20 hours in homozygotes. The inheritance pattern of the mutation suggests that it has occurred at a single locus. That a single gene is involved in regulating period suggests that its product(s) is close to the mechanism of the circadian pacemaker, and may even be a component of it. We used two-dimensional gel electrophoresis to look for protein differences among the different phenotypes that may provide clues to the molecular mechanisms of the pacemaker.

We found two sets of proteins that differ among the different phenotypes in gels from both SCN and cortical tissue. "Clock Spot 1" (~33 kD; pI 6.5) was found in all gels from wild type and heterozygous animals, but not in gels from homozygous mutant animals. The protein immediately below this was altered in the heterozygote gels. "Clock Spot 2" (~30 kD; pI 4.8) appeared as a chain of spots, which showed a striking difference in pattern between gels from wild type animals and those from mutant animals. These proteins should be useful as new tools to explore the biochemistry of circadian pacemakers.

292.10

THYROPARATHYROIDECTOMY (TPX) SHORTENS THE FREE-RUNNING CIRCADIAN ACTIVITY PERIODS OF BLINDED MALE AND FEMALE RATS. D.L. McEachron, Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104

Previous experiments have demonstrated that sighted TPX male rats display significantly shorter free-running activity periods in constant dim red light when compared with sham-operated (Sham) controls (Schull,et.al., 1988). Intact sighted female rats displayed significantly shorter activity rhythms when compared to intact sighted males, and TPX surgery did not significantly change their free-running periods (Schull,et.al., 1989). In both experiments, the masking effects of light appeared enhanced in TPX animals. The objective of this experiment was to determine: 1. if TPX- induced period changes were mediated by altered light sensitivity; and 2.if sex differences in the effects of TPX surgery could be confirmed.

Forty-eight blinded Sprague-Dawley rats were divided into 4 groups: male Sham; male TPX; female Sham; and female TPX. Each rat was individually housed in a metal cage with an attached running wheel. Cages were housed in groups of 6 in light-tight cabinets. Animals were allowed to free-run for 8 weeks, the last 6 of which were used for period estimation. Periods were calculated by raters using computer-generated actograms, and the averages of 2 such estimates were used in the statistical analysis. Analysis showed that TPX rats displayed significantly shorter activity periods ($F=8.35$, $p<0.01$) but that there was no sexual dimorphism in activity periods ($F=0.008$, ns) nor interaction of sex with thyroid state ($F=0.282$, ns). Evidently, TPX surgery does alter central mechanisms involved in circadian activity rhythms but these mechanisms are not sexually dimorphic.

292.11

PHOTOPERIOD-DEPENDENT DIFFERENCES IN THE FEEDING EFFECTS OF GROWTH HORMONE-RELEASING FACTOR (GRF) ADMINISTERED INTO THE SUPRACHIASMATIC NUCLEUS/MEDIAL PREOPTIC AREA (SCN/MPOA) OF THE HAMSTER F.J. Vaccarino, P. Sovran, M.R.Ralph, Dept. of Psychology, University of Toronto, Toronto, Ont., M5S 1A1

Previous studies from our laboratory have demonstrated that intra-SCN/MPOA injections of GRF stimulate feeding in rats. This effect is found during the light, but not dark photophase. Unlike rats, hamsters feed throughout the light-dark cycle, although they tend to hoard only during the active dark phase. The present study examined the feeding characteristics of GRF in hamsters.

Male golden hamsters with intra-SCN/MPOA cannulae implants were entrained to a 14:10 light:dark cycle and tested with GRF (1.0 picomole) in the dark and light photoperiods. Food intake and related behaviors were monitored for 90 min following injection.

GRF increased food intake and time spent eating in the light, but not dark, photoperiod. There were no significant effects on other behaviors. Together with work indicating that GRF phase advances activity rhythms in the hamster, these results suggest that GRF plays a role in circadian regulation of food intake. Supported by NSERC grants 35036 to F.J.V. and 43304 to M.R.R.

292.12

GRF: A POSSIBLE NEUROCHEMICAL CONNECTION BETWEEN FEEDING AND CIRCADIAN RHYTHMICITY. M. R. Ralph, P. Sovran and F.J. Vaccarino. Dept. of Psychology, Univ. Toronto, Toronto, M5S 1A1, Canada.

Daily rhythms of ingestive behavior are under the influence of a central circadian pacemaker. In turn, schedules of restricted feeding opportunities are effective in entraining freerunning circadian rhythms in rodents. However, neural substrates for this effect have not been identified. We have hypothesized a role for GRF (growth hormone releasing factor) for a number of reasons: (1) GRF increases feeding behavior in rats and hamsters; (2) the effect of GRF depends on the time of day; and (3) the suprachiasmatic nucleus (SCN) is a site of this GRF action.

We tested the hypothesis by asking whether central injection of GRF through cannulae aimed at the SCN would induce either permanent or transient changes in the phase of freerunning locomotor rhythms in hamsters. GRF (1.0 pmole in 1.0μl saline) induced permanent phase shifts of the locomotor rhythm. Magnitude and direction were circadian phase dependent, and the resulting PRC was similar in shape to other non-photic curves: eg. large phase advances were predominant in the early subjective day. The results suggest a role for GRF in mediating effects of restricted feeding opportunities on the clock. GRF may be involved in a positive feedback loop connecting a specific rhythmic behavior with the control of rhythm generation. Supported by NSERC grants 43304 to MRR and 35036 to FV.

293.1

AGONISTIC BEHAVIOR IN JUVENILE AMERICAN LOBSTERS. R. Huber* and E.A. Kravitz. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

A quantitative study of agonistic behavior in juvenile American lobsters (*Homarus americanus*) demonstrates that fighting proceeds according to strict rules of conduct. Detailed analysis of encounters in ten pairs of behaviorally naive individuals, who were closely matched in size and molt state, indicated that six common agonistic behavioral patterns were displayed by all individuals in a highly stereotypical manner. Furthermore, the data revealed a distinct temporal sequence to these behavioral patterns during a fight. Upon first contact the opponents exhibited extensive threat displays, followed by periods of restrained contact and ritualized fighting. If no winner emerged by this point, the fight culminated in a brief period of unrestrained combat. Claws are dangerous weapons in these fights; therefore the observed orderliness of the encounters may be interpreted in evolutionary terms as effectively reducing the potential for damage. The presence of a rigidly structured behavioral system in this species should prove to be a considerable advantage in attempts to relate fighting behavior to the action of neuromodulatory substances like serotonin and octopamine. Preliminary experiments, relating individual differences in fighting behavior to the action, concentration or metabolism of amines, will be presented (Supported by Harvard and NINDS).

293.2

DIFFERENTIAL INDOLAMINE AND CATECHOLAMINE ACTIVATION FOLLOWING AGGRESSIVE INTERACTION IN MALE LIZARDS. C.H. Summers* and N. Greenberg. Calif. State Univ. San Marcos, CA 92096 and Graduate Program in Ethology, University of Tennessee, Knoxville, TN 37996.

Reproductively active male *Anolis carolinensis*, were paired, allowed to fight, and set up dominant/subordinate relationships. While combatants cohabited, the losers of the fights displayed darker color, selection of lower sites, and lower body posture. At one hour, one day, one week, and one month after the fight both dominant and subordinate individuals were sacrificed. Each member of a pair was killed at the same time and animals kept isolated were sacrificed as controls. Brains were rapidly removed and dissected on ice, frozen at -70°C, and then hindbrains were analyzed for indolamines and catecholamines by coulochem electrode array HPLC. Rapid activation of the serotonergic system of losing male lizards is evident from depleted 5-HT levels, increased 5-HIAA levels and 5-HIAA/5-HT ratio. Substrate 5-HTP also increases in losing males, suggesting an activation of production as well as turnover. Significant 5-HTP and 5-HIAA/5-HT increases for subordinate males, compared with levels in control and dominant males, were greatest at one hour and diminished with time. This pattern of indolamine activation is consistent with stress-induced psychogenic stimulation of the serotonergic system and with prolonged activation of stress mediation in subordinate animals.

In contrast with serotonin, dopamine systems were progressively activated as time of interaction increased, in both dominant and subordinate males. Early noradrenergic activation, indicated by normetanephrine/norepinephrine ratio, also decreases with time in losing males, except that the systems are again active after one month of cohabitation of paired males. Patterns of MHPG, epinephrine, and metanephrine activity are significantly different from those of NE/normetanephrine. Differences in measures of adrenergic and noradrenergic activation indicate a siginificant role for central epinephrine in the lizard's accomodation of social stress.

293.3

ALPHA2-ADRENOCEPTORS IN THE BRAINS OF CHRONICALLY STRESSED TREE SHREWS. G. Flügge, O. Jöhren, and E. Fuchs. German Primate Center, 3400 Göttingen, FRG.

Male tree shrews (*Tupaia belangeri*) defend their territories vigorously against intruders. When a male lives in the same cage together with another male a persistent dominant-subordinate relationship will be established within a short time. Thus, tree shrews provide a useful model for studying the physiological consequences of chronic psychosocial stress. Dominant (DOM) and subordinate (SUB) individuals can be distinguished by physiological parameters such as body weight, urinary catecholamine concentration and by behavioral parameters. In DOM animals, the activity of the sympathico-adrenomedullary system is clearly increased.

Since we are interested in the central nervous effects of persistent stress we quantified alpha$_2$-adrenoceptors in the brains of 8 DOM and 8 SUB by in vitro-autoradiography with the antagonist ^{3}H-rauwolscine (^{3}H-RAU). Significant differences in numbers of ^{3}H-RAU binding sites were detected in two medullary nuclei: In the nucleus of the solitary tract (SOL) and in the dorsal motor nucleus of the vagus (X). Since SOL and X are both involved in the transfer of peripheral signals like those from the baroreceptors to the brain, the difference in adrenoceptor numbers may be related to changes in blood pressure which can be observed in DOM during confrontation with the male conspecific.

293.4

THE EFFECTS OF NORADRENERGIC NEUROTOXIN, DSP-4, ON WATER MAZE TASK DIFFER BETWEEN YOUNG AND AGED RATS.

J. Sirviö, P. Riekkinen Jr., A. Valjakka* and P.J. Riekkinen. Dept. of Neurology, Univ. of Kuopio, P.O.Box 1627, Kuopio, Finland.

The present study investigated the role of central noradrenergic system in spatial learning/memory. The effects of noradrenergic neurotoxin, DSP-4, on the acquisition of water maze task were investigated in young and aged rats. Young (4-month-old) and aged (22-month-old) male Wistar rats were treated with DSP-4 (N-(2-chloroethyl)-N-ethyl-2bromobenzylamine) 50 mg/kg (ip) or saline. Ten days after treatment, rats were trained in a water maze task for 4 days (3 trials/day) using a hidden platform procedure. DSP-4 treatment impaired the acquisition of water maze task (expressed using escape distance) in aged rats ($F(1,235)=10.1$, $p<.005$), but it slightly improved in young rats ($F(1,252)=7.3$, $p<.01$). Previously, it has been shown that even almost complete depletions of forebrain noradrenaline do not impair place navigation in normal adult rats. On the other hand, DSP-4 treatment may aggravate muscarinic receptor blockade (scopolamine) induced place navigation impairment. Because spatial navigation deficit may be related, at least partly, to the dysfunction of basal forebrain cholinergic system in aged rats, DSP-4 treatment induced spatial learning/memory deficit in aged rats may be due to aggravation of cholinergic dysfunction in these rats. It is also possible that DSP-4 treatment impaired the use of some learning strategies other than navigation by distal cues which might be important for aged rats in the solving of water maze task.

293.5

CONDITIONED NOREPINEPHRINE RESPONSE IN THE OLFACTORY BULB OF 7 DAY-OLD RAT PUPS. C.L. Kirstein, F.B. Weihmuller, J.F. Marshall and M. Leon. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Young rat pups learn to prefer an odor which has been paired with tactile stimulation. This tactile stimulation has been shown to activate noradrenergic locus coeruleus neurons, which project heavily to the olfactory bulb. NE input is present and functional in the olfactory bulb as early as the first postnatal week. Moreover, noradrenergic antagonists can block this early learning.

To determine whether tactile stimulation increases NE overflow in the bulbs of naive pups we used *in vivo* microdialysis to monitor extracellular NE. Tactile stimulation to awake 7 day-old pups increased NE, while odor alone had no effect on NE overflow.

To study the subsequent NE response to a learned odor, we trained rat pups from postnatal days 1-6 (P1-6), with odor and stroking presented in either a paired (forward) or unpaired (backward) fashion. On P7, odor presentation in forward-trained pups increased NE overflow, while the backward stimulus experience did not elicit this conditioned response. These results suggest that conditioned neurotransmitter responses may play a role in the expression of the neural and behavioral changes accompanying early olfactory learning.

This work was supported by ONR grant N00014-89-J-1960 to ML and NINDS grant NS22698 to JM.

293.6

EFFECTS OF ISOLATION REARING ON INDICES OF DOPAMINE FUNCTION IN THE RAT. F.S. Hall, L.S. Wilkinson*, D.A. Kendall*1, C.A. Marsden1 and T.W. Robbins. Experimental Psychology, University of Cambridge, Cambridge CB2 3EB. 1Physiology and Pharmacology, University of Nottingham, Nottingham NG7 2UH. U.K.

Rats reared in isolation exhibit a variety of behavioural disturbances. In particular, isolates are spontaneously hyperactive, display increased responding in reward-related situations and show deficits in the expression of schedule-induced behaviours. The present study examined the effects of isolation rearing on pre- and postsynaptic indices of dopamine function in the nucleus accumbens (NAC). In the first experiment male weanlings (21 days postnatal) were divided into isolates and social controls. After 90 days the animals were anaesthetised with halothane and extracellular dopamine monitored using *in-vivo* microdialysis. The dose-response curve for d-amphetamine (dose range 0.5-4mg/kg s.c.) was shifted to the left. In the second experiment weanlings were isolated as above and then NAC tissue taken for dopamine receptor linked adenylate cyclase activity measurements. Isolation rearing had no effect on the D1 response but the inhibitory action of D2 receptors on the D1 response was attenuated. These data suggest that isolation rearing can have effects on both pre- and postsynaptic dopamine mechanisms in NAC by increasing dopamine release and down-regulating D2 receptors, respectively.

293.7

THE RAT PUP ULTRASONIC CALL PREDICTS ANXIOLYTIC AND ANXIOGENIC EFFECTS OF 5-HT LIGANDS. J.T. Winslow and T.R. Insel. Lab. Clin. Sci., NIMH, Poolesville, MD. 20837

A modulatory role for serotonin has been described for the development and expression of the ultrasonic call of infant rat pups during brief maternal separations. Serotonin reuptake inhibitors selectively reduced the rate of calling following acute administration to 10 day old pups and a serotonin neurotoxin (MDMA) systematically disrupted the development of ultrasonic vocalizations. Acute administration of $5HT_{1A}$ agonists buspirone and 8-0H-DPAT ((±)-8-hydroxy-2-(di-N-propylamino)tetralin) reduced the rate of calling at doses which did not affect motor activity or core body temperature. Administration of purported $5HT_{1B}$ receptor agonists, CGS12066B and TFMPP increased the rate of calling depending on receptor specificity. d,l-Propranolol, a $5HT_1$ receptor antagonist, blocked the effects of both 8-OH-DPAT and TFMPP. m-CPP and DOI, drugs with putative actions at $5HT_{1C}$ and $5HT_2$ receptor sites both decreased calling but differed according to their effects on motor activity. Ritanserin, a $5HT_2$ and $5HT_{1C}$ antagonist, produced a dose related increase in call rate. A dose of ritanserin with no apparent intrinsic effects effectively antagonized DOI but potentiated the effects of m-CPP. These data confirm a role for serotonin in the expression of rat pup separation calls and further demonstrate that 5HT may increase or decrease calling depending on which receptor subtype is affected. This rat pup separation test appears to have great potential for elucidating the brain mechanisms mediating the development of anxiety-related behavior.

293.8

EFFECTS OF YOHIMBINE ON REGIONAL CEREBRAL BLOOD FLOW IN PANIC DISORDER. S. W. Woods, P. B. Hoffer*, C. J. McDougle, J. H. Krystal, G. R. Heninger, D. S. Charney. Depts. of Psychiatry and Diagnostic Radiology, Yale U. Sch. of Med., New Haven, CT 06508.

Previous work has shown that behavioral and plasma biochemical responses to the alpha-2 adrenergic antagonist yohimbine (YOH) are abnormal in psychiatric patients (PTS) with panic disorder (PD). The present study aimed to evaluate regional cerebral blood flow (rCBF) responses to YOH in PD and healthy subjects (HS) using single photon emission computed tomography (SPECT). METHOD: Thirteen drug-free PTS (5M, 8F, age 37±9 yrs) and 11 HS (2M, 9F, 37±15 yrs) underwent IV infusion of YOH 0.4 mg/kg or saline placebo (PLA) in balanced sequence on separate days. The rCBF tracer Tc-99m HMPAO was injected 15 minutes after YOH or PLA, followed by 40 min SPECT acquisitions (Strichman 810X Brain Imager, Medfield, MA). Data from image analysis blind to diagnosis and drug conditions were expressed as the percent change [(YOH - PLA)/PLA x 100%] in the region to supratentorial whole brain rCBF ratio. RESULTS: As expected, YOH-induced, PLA-corrected visual analog rating scale increases in anxiety were greater in PTS than in HS (34±26 vs 9±14 mm, $p<.01$). Preliminary analyses suggest that YOH significantly reduced frontal rCBF ratios bilaterally in PTS compared to HS (right, -3.9±1.0 vs -0.5±2.2 %, $p<.001$; left, -1.5±2.5 vs 0.4±1.4 %, $p<.05$). YOH tended to increase cerebellar ratios in both PTS and HS. DISCUSSION: The data are consistent with studies showing that frontal cortical rCBF and neuronal activity decrease with noradrenergic neuron stimulation and suggest roles for altered noradrenergic regulation of frontal cortical neuronal or vascular function in the pathophysiology of PD.

293.9

CIRCANNUAL CHANGES IN 5-HT FUNCTION: AN HYPOTHESIS REGARDING FLUOXETINE-INDUCED SUICIDALITY. T.D. Brewerton. Institute of Psychiatry, Medical Univ. of S.C., Charleston, SC 29425.

Although suicides occur significantly more frequently during the spring season (especially May), the pathophysiology of this phenomenon is unknown. Since serotonin (5-HT) has a major role in suicide/affective illness, it may be relevant that many parameters of 5-HT function in humans appear to exhibit seasonal changes and include hypothalamic 5-HT content, CSF 5-HIAA levels, Vmax of platelet 5-HT uptake and IMI binding, plasma L-TRP levels, and peak delta PRL responses to m-CPP and L-TRP. Taken together, these data suggest that spring is associated with the most rapid rate of increasing 5-HT turnover, which in turn may be associated with the observed spring increase in suicides. Although speculative, new onset suicidality reported to follow artificial enhancement of 5-HT function with fluoxetine (Am J Psych 147:207, 1990) and other antidepressants may mimic these seasonal changes thereby leading to increases in suicidal behavior. The clinical variables which may predispose certain patients toward such 5-HT hypersensitivity will be discussed and include gender, rate of dose increases, polypharmacy, and presence of suicidal ideation.

293.10

IN VIVO HIPPOCAMPAL NOREPINEPHRINE RELEASE AND LEARNED HELPLESSNESS. F. Petty, G.L. Kramer*, L.A. Speece*, and T. Phillips*. Dept. Psychiat., VA Medical Center, University of Texas Southwestern Medical School, Dallas, TX 75216.

A role for norepinephrine (NE) in the behavioral depression caused by inescapable stress has long been postulated (Weiss, 1981, Brain Res. Rev. 3:167). Since then, hippocampal NE was found necessary for development and maintenance of learned helplessness (LH) and for its reversal by antidepressant drugs (Soubrie et al., 1987, Brain Res. 437:323). We have previously shown micro-injection of NE into HPC to prevent LH. We have applied *in vivo* microdialysis to measuring extracellular NE in HPC in LH. Rats were perfused before inescapable tailshock stress, 1 day after stress, and after shuttlebox testing for LH (Day 3). Also high K+ release was performed after the basal release on Day 3. Unstressed controls demonstrated a gradual decrease in basal HPC NE release during the 3 experimental days, presumably due to gliosis. Rats receiving tailshock stress maintained basal release at prestress levels. On Day 3, after testing, there was a significant difference between control, and stressed rats with high escape latencies (LH) in basal release. There was a significant positive correlation between stress-induced NE release and latency on testing, suggesting that greater NE release accompanies the development of LH. K+ stimulated NE release was lower in both stressed groups (LH and non-LH) than control, suggesting stress had depleted intraneuronal NE stores,. Taken together, these findings support an important role for NE in HPC in the development of LH.

293.11

PLASMA PROLACTIN AND HOMOVANILLIC ACID AS MARKERS FOR PSYCHOPATHOLOGY AND DYSKINESIA DURING MAINTENANCE HALOPERIDOL TREATMENT IN MALE SCHIZOPHRENICS. J.W. Newcomer,* S.J. Riney,* S. Vinogradov,* J.G. Csernansky. Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

The use of plasma prolactin concentration (PRL) and plasma homovanillic acid concentration (HVA) as indicators of central dopaminergic activity has been well investigated during acute neuroleptic treatment. We now report clinical symptom and side effect ratings, along with PRL and HVA, in 24 male schizophrenics during maintenance haloperidol treatment; 14 also underwent a placebo-controlled, double-blind 50% dose decrease. At baseline, an inverse correlation was confirmed between PRL and both dyskinesia (r_S=-0.600; $p<0.005$, one-tailed) and "thought disorder" (r_S=-0.343; $p<0.05$, one-tailed). Although positive and negative symptoms were not related, PRL was also correlated with negative symptoms (r_S=0.428; $p<0.05$, two-tailed). No relationship was found between HVA and any rated symptom at baseline. Twelve patients received an apomorphine challenge (0.02 mg/kg) at baseline, and a significant inverse correlation was also found between dyskinesia and apomorphine-induced decreases in PRL. In the patients who underwent a dose decrease, no relationship was found between PRL or HVA and any clinical variable. These data suggest that PRL, but not HVA, may be a useful marker of central dopamine function during maintenance neuroleptic treatment.

294.1

VENTRAL ORIGIN OF A2B5-IMMUNOREACTIVE GLIAL PRECURSOR CELLS IN THE DEVELOPING RAT SPINAL CORD. Juin Fok-Seang* and Robert H. Miller, Department of Neurosciences, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

Cultures of newborn rat spinal cord contain two distinct populations of migratory A2B5-immunoreactive glial precursor cells. One population, which resembles optic nerve O-2A progenitor cells, gives rise to oligodendrocytes and possibly to type-2 astrocytes, while the other, which has been called MARPs (Migratory Astrocyte-Restricted Precursors), gives rise to distinct classes of astrocytes and not to oligodendrocytes. These two precursor populations also differ in their response to certain mitogenic and differentiation factors in tissue culture.

Explant and dissociated cell cultures in combination with immunocytochemical analysis were used to show that at early developmental stages, A2B5+ glial precursors were initially restricted to ventral regions of the spinal cord. Thus, at embryonic day 14 (E14), only ventral and not dorsal spinal cord regions subsequently gave rise to oligodendrocytes and MARP progeny. By E18, A2B5+ glial precursors were present in both the ventral and dorsal regions. The appearance of A2B5+ glial precursors in dorsal regions coincided with the acquisition by dorsal spinal cord of the capacity to give rise to oligodendrocytes and MARP progeny.

These observations suggest that both O-2A progenitor cells, which give rise to oligodendrocytes, and MARPs which give rise to distinct classes of astrocytes, initially develop in ventral spinal cord regions and subsequently colonize dorsal regions by a ventral-to-dorsal migration.

294.2

MIGRATION PATTERNS OF RAT SUBVENTRICULAR ZONE CELLS DURING POSTNATAL GLIOGENESIS AS REVEALED BY RETROVIRAL MEDIATED GENE TRANSFER. Steven W. Levison and James E. Goldman. Dept. of Pathology and Center for Neurobiology and Behavior, Columbia University, New York, NY 10032.

To study the migration patterns of glial progenitors, the BAG retrovirus was injected unilaterally into the anterior subventricular zone (SVZ) of P2-3 rats. At 2 days post injection (dpi) greater than 70% of the infected cells (X-gal+) were within the SVZ. Few labeled cells were observed along the needle track. The labeled SVZ cells were approximately 10 μm in diameter with a single process. During the next 3 weeks labeled cells appeared in overlying white matter, cortex, and adjacent striatum. Labeled cells were not observed in the SVZ after 21 dpi. The apparent direction of migration was medial to lateral since migration along the rostral caudal axis was generally restricted to a few millimeters from the injection site. Labeled cells were rarely observed in the other hemisphere. Radial arrays of labeled cells were common as were clusters. Discrete clustering suggests that glial precursors can divide once they reach their final destinations. Based on morphological criteria, the large majority of labeled cells in white matter were oligodendrocytes. Myelinating oligodendrocytes first appeared 14 dpi. In grey matter satellite oligodendrocytes were observed as well another population of neuroglia. These cells had round perikarya and several processes, often emerging as thick trunks, that branched extensively a distance from their origin. Many of these cells had processes contacting blood vessels. Experiments are in progress to characterize these cells further. Thus in early postnatal development SVZ generates several populations of neuroglia.
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294.3

CLONAL ANALYSIS OF GLIOGENESIS IN THE NEONATAL RAT CEREBRAL CORTEX. K.W. McDermott*[1], E. Breding*[1,2] and M.B. Luskin[1,2]. Departments of Anatomy & Cell Biology[1] and Pediatrics[2], Emory University School of Medicine, Atlanta, GA 30322.

Previous studies have revealed that neuronal, astroglial and oligodendrocytic lineages have diverged in the developing rat cerebral cortex as early as the onset of cortical neurogenesis. However, it is presently unclear whether glial progenitor cells in the postnatal developing cerebral cortex are bipotential. We have now undertaken an ultrastructural analysis to investigate whether separate progenitor cells give rise to astrocytes and oligodendrocytes throughout gliogenesis.

A retroviral lineage tracer containing the reporter gene β-galactosidase (*lacZ*) from E. coli was injected into the cerebral ventricles of rat pups on postnatal day 1. Animals were perfused 1 to 2 weeks later and their brains were serially sectioned at 100 μm. Sections were stained with X-gal to reveal lacZ-(+) cells and subsequently processed for electron microscopy. Discrete groups of stained cells (clones) were identified from camera lucida drawings of lacZ-(+) cells. The size of such groups varied between 2 and 30 cells. According to ultrastructural criteria, some clones appeared to be composed of a single phenotype. For example, some clones in the subventricular zone were composed entirely of immature glia. Other clones contained cells at different stages of differentiation appearing to be of a single phenotype. Further studies with cell-type specific markers should definitively determine the phenotypes of clonally related cells.

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294.4

GLIAL CELL DEVELOPMENT AND REGIONAL SPECIALIZATION IN XENOPUS SPINAL CORD. C.E.Maier and R.H.Miller Dept. of Neuroscience, Case Western Reserve Univ. Cleveland OH 44106

Glial cell development has been examined in the spinal cord of developing Xenopus using antibodies specific for astrocytes and oligodendrocytes. At Nieuwkoop and Faber stages 44/45, labeling of transverse and longitudinal frozen sections of spinal cord with anti-GFAP antibodies demonstrated the presence of radially oriented astrocytes that spanned the entire width of the developing cord. Though radial astrocytes were found in both dorsal and ventral regions at these early stages, the earliest immunoreactive oligodendrocytes were restricted to the ventral white matter. At later stages (54/55) immunoreactive oligodrocytes were seen ventrally and focally in developing dorsal columns but not laterally. The distribution of oligodendrocytes and myelin was confirmed at this stage by ultrastructural analysis. In the adult, oligodendrocyte labeling was seen throughout the spinal cord white matter. Anti-GFAP labeling of astrocytes remained radially oriented and continuous from the central canal to the pial surface. The labeling of astrocytes by an anti-radial glia antibody, however, was restricted to the white matter, suggesting a regional specialization for astrocytes. These observation imply that: 1) astrocytes develop before oligodendrocytes, 2) during development oligodendrocytes appear in a ventral to dorsal gradient, 3) during development myelination is not associated with changes in radially oriented astrocyte labeling. These observations suggest that, in Xenopus, oligodendrocytes are not derived from GFAP positive radial glial cells.

294.5

GLIOGENESIS AND GLIAL MIGRATION DURING EMBRYONIC FORMATION OF THE INSECT ENTERIC NERVOUS SYSTEM. P.E. Copenhaver. Cell Biology & Anatomy L215, Oregon Health Sciences University, Portland, OR 97201

The formation of the enteric nervous system (ENS) in the moth, Manduca sexta involves two distinct programs of neurogenesis: cells of the anterior enteric ganglia are derived from neuronal precursors that emerge from three neurogenic zones in the foregut epithelium, while the neurons of the enteric plexus (which spans the foregut-midgut boundary) are derived from a separate neurogenic placode. In both regions, neuronal differentiation is accompanied by the proliferation of a distinct population of non-neuronal glial cells that contribute to the structural integrity of the enteric ganglia and nerves. We now report that the neurons and glia of the insect ENS share a common developmental origin. Specifically, the neurogenic zones of the anterior enteric ganglia also produce a limited number of glial progenitor cells. Unlike the neuronal precursors from these zones, the glial precursors remain mitotically active during neuronal cell migration and ganglion formation. Labelling the precursors with a thymidine analogue and injecting individual glial progenitors with lineage-tracing dyes has shown that each glial precursor produces a population of cells that becomes progressively distributed along a particular domain of the ENS. Glial progeny become integrated into the enteric ganglia and also disperse along the migratory pathways of the placode-derived neurons. Thus glial migration follows neuronal migration and contributes to the differentiation of the enteric plexus, as well. We are currently investigating the regulatory interactions between neuronal and glial progeny of the ENS with respect to the timing and extent of glial proliferation and migration during development. Supported by NSF # BNS 9010538 and by the Medical Research Foundation of Oregon.

294.6

DEVELOPMENT OF SATELLITE GLIA IN THE RAT SUPERIOR CERVICAL GANGLION. A.K. Hall and S.C. Landis Dept. Neurosciences, Case Western Reserve Univ. School of Medicine, Cleveland, OH 44106.

Both neurons and glia in peripheral ganglia arise from the neural crest, but little is known about the subsequent maturation of satellite glia in these ganglia. Morphological, immunocytochemical and culture techniques were used to examine the division and migration of glial satellite precursors in the developing rat superior cervical ganglion (SCG). Numerous clusters of non-neuronal cells were present transiently in the ganglion at embryonic day 18. The clustered cells appear to be generated from individual precursor cells with a similar morphology which are observed at E16. At E18, the clustered cells possessed a characteristic appearance and were morphologically distinct from neurons, as well as from endothelial cells and fibroblasts in the ganglion. An antibody to tyrosine hydroxylase (TH) was used to identify the adrenergic neurons in the E18 ganglion, and propidium iodide (PI) counterstaining to visualize all cell nuclei. The E18 ganglion contained doubly stained neurons as well as clustered cells stained with PI which lacked detectable TH immunoreactivity, consistent with their identification as non-neuronal cells. To assess the possibility that cell clusters arose from cell division, BrdU was administered to pregnant mothers between E16 and E18, and cells in the ganglia examined at E18. Numerous non-neuronal cells divided during this period, and could compose clusters, but few neurons were labelled. An antibody against the low affinity NGF receptor, MAb 217c, was used to identify ganglionic glia. Over half of the 217c immunoreactive glia incorporated BrdU in vivo during this labelling period, consistent with a high level of glial division. The non-neuronal cells were no longer in clusters at birth, but instead were associated with neuronal cell bodies and processes. These findings support the notion that, between E16 and E18, after the peak of neurogenesis, glial precursors divide rapidly to form clusters, and their daughter cells subsequently migrate thoughout the ganglion to associate with nerve cell bodies and processes.

294.7

DEVELOPMENT OF GFAP-POSITIVE CELLS IN THE CA1 REGION OF THE RAT HIPPOCAMPUS. B. E. Nixdorf, D. Albrecht*, U. Heinemann*, Department of Neurophysiology, University of Cologne, 5000 Cologne 41, FRG.

Astrocytes are thought to play a major role in the maintenance of ion homeostasis. Our electrophysiological experiments have shown that the ceiling levels of extracellular potassium concentration ($[K^+]_o$) induced by extensive neuronal activity is higher in young animals than in adults (Albrecht and Heinemann, Dev. Brain Res. 48:316, 1989). These differences between young and adult animals may be due to a limited maturation of the potassium buffering via glia cells in young animals. Therefore, we have studied the development of GFAP-positive cells in different laminae of the hippocampus of the rat in animals of 8, 16, 24 days and in adults. The data of a total of 271 slices were compared in a one way analysis of variance using SYSTAT. Our results have shown that in very young animals (8d) the glial cell population is very small and therefore, may be unable to buffer the high $[K^+]$ from the extracellular space. The quantitative analysis on the number of GFAP-positive cells reveals a different temporal pattern for the development in the different laminae. The adult value of GFAP-positive cells is reached in Stratum oriens at 16 days, in S. radiatum and S. moleculare at 24 days. However, the high $[K^+]_o$ can also be observed in the age groups of 16 and 24 days where the number of glial cells had reached adult values. The discrepancy between electrophysiology and GFAP ICC may be due to a different time course in the structural and functional maturation of the glial network. Investigations on the connectivity (e.g. via gap junctions) in the glial cell population may give insight to the functional status of the glial network. Supported by the Sander Foundation.

294.8

SURVIVAL OF MICROGLIA IN CULTURES DEPENDS ON AUTOCRINE SECRETION OF CSF-1 INDUCED BY LPS. C. Hao, I. Ahmed and S. Fedoroff. Department of Anatomy, College of Medicine, University of Saskatchewan, Saskatoon, Sask. Canada S7N OWO

Survival, differentiation and function of normal microglia depend on CSF-1 paracrine secretion by astroglia. Microglia themselves normally do not secrete CSF-1, but have receptors for CSF-1 (Hao et al., J. Neurosci. Res. 27:364-323, 1990). To determine whether the secretion of CSF-1 in microglia can be induced, we stimulated microglia of C_3H/HeJ mice in cultures with bacterial wall lipopolysaccharides (LPS) at a concentration of 10 µg/ml for 24 h. The microglia released approximately 100 U of CSF-1/ml, i.e., 4-5 times less than astroglia secrete. To determine whether the LPS stimulation of microglia persists, we set up three sets of microglia cultures. The first were grown in regular growth medium to which CSF-1 was added and the second set had only regular growth medium. The microglia in the third set of cultures were stimulated with 10 µg/ml LPS for 24 hours, the cultures were washed, and regular medium, without CSF-1, was added. The cultures in the first set (with CSF-1) grew and proliferated throughout the experimental period of 3 weeks. Cells in the second set (grown without CSF-1), all died within 8-10 days and the cells in the third set (grown without CSF-1) survived 3 weeks in cultures similar to those of the first set. These results indicate that LPS induces autocrine secretion of CSF-1 in microglia and that the amount of CSF-1 produced is sufficient for their survival but not proliferation.

The authors are members of the Canadian Centre of Excellence in Neural Regeneration and Functional Recovery.

294.9

CULTURED MICROGLIAL CELLS AND A SUBPOPULATION OF BONE MARROW DERIVED MACROPHAGES HAVE A DISTINCT PATTERN OF MEMBRANE CHANNELS DIFFERENT FROM OTHER BODY MACROPHAGES. D.Hoppe, K.Gottmann*, R.Banati, G.Kreutzberg and H.Kettenmann. *Max-Planck-Institut für Psychiatrie, Am Klopferspitz 18A, 8033 Planegg-Martinsried, Germany.

Microglia are a population of resident macrophages ubiquitously distributed in the central nervous system. Sharing a number of antigenic and morphological charactaristics with macrophages from other tissues they cannot be readily distinguished from body macrophages. The patch-clamp technique, however, revealed that microglia express a distinct, different pattern of ion channels. While body macrophages normally have voltage activated outward and inward currents, cultured microglia lack the outward current and express only an inwardly rectifying K^+ current. The reversal potential in different K^+ gradients and the sensitivity of the current to Ba^{2+}, TEA and 4-AP indicates that this current is K^+ selective. In single channel recordings a 30 pS K^+ selective channel was observed which is similar to the classical inward rectifier K^+ channel. Since the origin of microglia is still under discussion we studied cells of the mononuclear phagocyte system, the stem cells of the macrophages. A subpopulation of bone marrow derived macrophage-like cells indeed expresses an inward rectifier K channel similarly to microglia. These results suggest that, firstly, microglial cells form a unique cell population and secondly, may originate from a stem cell population of bone marrow.

294.10

EXPRESSION OF AN INTERMEDIATE FILAMENT-RELATED ANTIGEN IN A SUBGROUP OF CULTURED TYPE-1 ASTROCYTES AND IN ASTROCYTES OF POSTNATAL AND ADULT SPINAL CORD OF RATS. H.-Y. Yang, N. Lieska*, D. Johnson-Seaton*, D. Shao*, V. Kriho* and G.D. Pappas. Dept. of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL 60612.

Intermediate filament (IF) cytoskeleton is characterized by both IF structural proteins and IF-associated proteins (IFAPs). There is substantial evidence showing that the expression of various IF structural proteins is intimately related to astrocyte development (e.g., vimentin and GFAP in immature and mature astrocytes, respectively). Recently, we have described the differentiation-related expression of IFAP-70/280kD, a vimentin-associated IFAP originally identified in BHK-21 cells, in radial glia and in a subgroup of cultured type-1 astrocytes of rats (Soc. Neurosci. Abstr., 16 (1):352).

In the present study, a monoclonal antibody (3-16C5) which reacts with the astrocytic IF cytoskeleton was produced by the hybridoma technique using glial IF-enriched preparations as antigens. The IF preparations were isolated from primary astrocyte cultures obtained from neonatal rat brain. The immunoreactivity of 3-16C5 was studied in normal and colchicine-treated primary cultures of neonatal rat astrocytes by double-labeling immunofluorescence microscopy using 3-16C5 and GFAP. The astrocytes were identified by the GFAP antibody. The results revealed that the 3-16C5 antigen is present in a subgroup of non-stellate (type-1) astrocytes, but not in the stellate (type-2) ones. In addition, the co-distribution of this antigen with GFAP-containing IF cytoskeleton, both in normal and colchicine-treated astrocytes, indicates that it is a glial IF-related antigen.

The ontogeny of the 3-16C5 antigen was also investigated by immunofluorescence microscopy in spinal cord of rats from embryo day 19 (E19) to postnatal day 12 (P12), as well as adult. The 3-16C5 immunoreactivity is not present in prenatal spinal cord, and is first detected in the peripheral region of newborn rat spinal cord. It is present only in the astrocytes in white matter of the rat spinal cord by P12 and in both white and gray matter of the adult spinal cord. This development-related expression of 3-16C5 antigen suggests a dynamic role in astrocyte maturation and the potential for use of this IF-related antigen as a differentiation marker of astrocytes, both in vitro and in vivo. (Supported by NIH grant NS26396.)

294.11

DISTRIBUTION AND CHARACTERIZATION OF MICROGLIA IN THE NORMAL HUMAN FETAL BRAIN. L.A. Mattiace, C. Brosnan* and D.W. Dickson. Dept of Pathology, Albert Einstein College of Medicine, Bx, NY 10461.

Fetal brain tissue obtained from 17 week to 24 week abortuses from HIV seronegative mothers, were examined in serial sections, histochemically, with the lectin RCA-1, and immunocytochemically, with antibodies that detect microglia. These markers included FcgRI, EBM/11, KP1 and LN-3. FcgRI appeared to be the most consistent marker for all forms of microglia, immunolabeling the most highly ramified cells, in addition to the less differentiated cells, which include round and amoeboid microglia. In comparison, macrophage markers EBM/11 and KP1, which recognize CD 68, immunolabeled round cells and amoeboid microglia in the subventricular zone, and to a lesser extent microglia with more ramified processes throughout the developing cortical layers and subcortical grey and white matter. LN-3, an antibody to the major histocompatibility complex class II antigen (HLA-DR), was unexpectedly expressed on microglia in the fetus. Morphologically, LN-3-immunoreactive microglia appeared to be either round and more rarely amoeboid cells with few processes. Their distribution was restricted to the subventricular zone and adjacent areas. The absence of HLA-DR on highly ramified cells, which are considered a more differentiated form of microglia, suggests that the phenotypic expression of HLA-DR may be lost with maturation. The characterization of normal microglia and their genesis may have implications for the understanding of the pathogenesis and pathophysiology of AIDS, since microglia are the main cell type that is productively infected by HIV-1 in the CNS, and since HIV-1 gp120 shares homology with HLA-DR, a marker of microglial activation. Supported by USPHS grant MH 47667.

294.12

THE DEVELOPMENT OF CUTANEOUS PEPTIDE-CONTAINING NERVES IN MAN. J.M.Polak, M.Sundaresan, *G.Moscoso, G.Terenghi. Histochemistry Department, RPMS, Hammersmith Hospital and *Department Morbid Anatomy, King's College School of Medicine, London, UK.

Classical neuroanatomical studies have investigated the skin innervation during foetal development, but little information is available on the development of cutaneous sensory and autonomic neuropeptide-containing nerves. There is evidence that in the foetus the reflex arc is functional at 8 weeks, which is consistent with the detection of sensory neuropeptides CGRP and SP in spinal cord at 10 weeks gestation. In this study we have assessed the developmental pattern and distribution of CGRP, VIP, SP and NPY-immunoreactive nerves, as well as those of the neuronal marker PGP, in various areas of foetal skin. At 6 weeks gestation, thick and club-shaped PGP-immunoreactive nerves were first seen in all skin areas, mainly distributed in the parallel portion of the subepidermal plexus. Only at later ages (10-12 weeks), thin twig-like nerves extend from this plexus to penetrate the epidermis. At 9 weeks, more PGP-immunoreactive nerves were seen in skin of the palm than in the dorsum. Sweat glands were first noted in axillary skin at 17 weeks, accompanied by few PGP-immunoreactive nerves. Occasional small CGRP-immunoreactive fibres were first noticed in the dermis at 7 weeks, but it was at 17 weeks that this neuropeptide is unequivocally present in axillary dermis. Sparse VIP-, SP- and NPY-immunoreactive fibres are not found until 16-17 weeks gestation, when they are seen in the dermis and around small blood vessels. Developing cutaneous innervation shows immunoreactivity for sensory neuropeptides soon after neuronal protein markers are detectable, at a gestational age consistent with the reported reflex movements of the foetus. The results of this study show a close relationship between morphological and functional development of the peripheral nervous system which might compare to the neuronal regeneration processes observed in adult skin.

295.1

REMODELING OF SYNAPTIC EXTRACELLULAR MATRIX DURING NERVE TERMINAL SPROUTING IN LIVING FROG NEUROMUSCULAR JUNCTIONS. L. Chen * & C. P. Ko. Dept of Biol. Sci., Univ. Southern California, Los Angeles, CA 90089.

Remodeling of synaptic extracellular matrix (ECM) and its dynamic relationship with nerve terminal plasticity have been demonstrated in normal frog neuromuscular junctions (NMJs) *in vivo* (Chen et al., J Neuros. 1991, In Press). This work has led to a hypothesis that extension of synaptic ECM may precede nerve terminal growth during synaptic remodeling. To test this hypothesis, the dynamic relationship between synaptic ECM and nerve terminal during active growth of NMJs was examined in muscles in which nerve sprouting was induced. Sartorius muscles were double-stained with rhodamine conjugated peanut agglutinin (PNA) and 4-Di-2-Asp to label synaptic ECM and nerve terminals respectively. The identified NMJs were observed *in vivo* with a SIT camera attached to a fluorescent microscope. Nerve sprouting was then induced by placing segments of the contralateral sciatic nerve on the muscle surface. Two to three months later, the same NMJs were restained and re-viewed as above. There were signs of extensive sprouting and growth in more than 50% of NMJs. Among these NMJs, about two thirds showed correlated extension of synaptic ECM and nerve terminals, while almost all of the rest had PNA staining longer than the terminal staining by at least 10 μm. All of the extended, PNA-stained regions were devoid of cholinesterase staining which makes it unlikely that these regions had been occupied by nerve terminals that had retracted. Thus, these findings are consistent with the hypothesis that synaptic ECM recognized by PNA leads the nerve terminal outgrowth.

295.2

EXOGENOUS CGRP PARTIALLY PREVENTS THE dTC INDUCED INCREASE IN INTRAMUSCULAR NERVE BRANCHING IN EMBRYONIC CHICKEN. Kubke, M.F. and Landmesser, L.T. Dept Physiol & Neurobiol., Univ. Connecticut, Storrs, CT 06269

Blocking neuromuscular activity in chicken embryos during the cell death period by in ovo treatment with d-tubocurarine (dTC), results in an increase in intramuscular nerve branching and a parallel increase in the number of synapses formed (Dahm & Landmesser '88,'91). Activity blockade in adult muscle also results in nerve sprouting at the neuromuscular junction. Calcitonin Gene-Related Peptide (CGRP) was previously shown to be present in motoneuronal cell bodies and nerve terminals during the periods of muscle nerve ingrowth and synaptogenesis (New & Mudge,'86). It was also shown to prevent tetrodotoxin induced neuronal sprouting in adult rat muscle (Tsujimoto & Kuno,'88). To test whether CGRP would prevent the inactivity induced increase in nerve branching in embryonic chick muscle, CGRP was applied to the chorioallantoic membrane at a dose of 50 ug/egg/day, simultaneously with 2mg/egg/day of dTC. This treatment resulted in a decrease in the amount of intramuscular nerve branching within the mixed (fast/slow) illiofibularis muscle when compared to treatment with dTC alone. The effects of CGRP were more pronounced in the fast than in the slow region of this muscle. These results suggest that CGRP may normally play a role during the formation of the intramuscular nerve branching pattern in embryonic development.
Supported by NIH grant 19640.

295.3

DIFFERENTIAL INDUCTION OF SCG-10 AND GAP-43 mRNA IN THE CONTRALATERAL CORTEX FOLLOWING UNILATERAL STRIATAL DEAFFERENTATION. H-W. Cheng, N. Mori and T.H. McNeill. Andrus Gerontology Center, Univeristy of Southern California, Los Angles, CA. 90089

The present study was undertaken to identify molecular markers that characterize axonal outgrowth during reactive synaptogenesis in the striatum following a unilateral lesion of the corticostriatal pathway. Previously, we have reported that homologous axons from the contralateral cortex are induced to sprout paraterminal axons that reinnervate deafferented striatal cells; however, the genomic events that regulate neurite outgrowth after striatal deafferentation are unclear. In order to address this issue, we used northern blot analysis to assess changes in mRNA prevalence for growth associated protein (GAP-43), SCG-10 and P-19 in the contralateral cortex after a unilateral corticostriatal lesion. In addition, the time course for changes in mRNA prevalence was correlated with data from our previous morphological studies.

We found that both SCG-10 and P-19 mRNA in the contralateral cortex were increased by 13 fold at 3 days postlesion, followed by a gradual decline in message that remained elevated above intact controls at 27 days postlesion. This increase in SCG-10 mRNA could be blocked by cutting the corpus callosum. In contrast, the prevalence of GAP-43 mRNA did not significantly changed from intact controls at any time after the lesion. These findings suggest that SCG-10 and P-19 are involved in the cellular events that characterized synaptic remodeling of the striatum following neuronal deafferentation. In addition, we hypothesize that during reactive synaptogenesis that SCG-10 and GAP-43 mRNA may differentially regulated in the brain based on the type of axonal outgrowth required at the lesion site (i.e. paraterminal vs collateral sprouting). Supported by PHS grants AG 00300, AG 09793 and the National Parkinson Foundation.

295.4

UNILATERAL ENTORHINAL CORTEX/FIMBRIA FORNIX LESION (EC/FFL) INDUCES GAP-43 mRNA IN THE CONTRALATERAL HIPPOCAMPUS. McNeill, T.H., Cheng, H.-W., Day, J.R. and C.E. Finch. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Unilateral perforant path transection eliminates over 80% of the presynaptic input to the ipsilateral molecular layer of the dentate gyrus. Under these conditions, surviving axons (septohippocampal and C/A projections) are induced to sprout and form new synaptic contacts with deafferented target neurons. In addition, GAP-43 immunostaining in the ipsilateral hippocampus is markedly increased in the inner molecular layer of the dentate gyrus coincident with the time when C/A fibers begin to sprout axon collaterals. However, the increase in GAP-43 immunoreactivity has not been found to be associated with an increase in GAP-43 mRNA in the cells which project to this area.

In order to address this issue the present study was undertaken to determine if an increase in GAP-43 mRNA in commissural projection neurons to the dentate gyrus could be induced by altering the severity and/or neurotransmitter composition of the deafferentation lesion. This was accomplished by combining a unilateral entorhinal cortex lesion with a septohippocampal path transection (EC/FFL). Northern blot analysis of total RNA from the contralateral hippocampus revealed that there was an initial 2-fold decrease in GAP-43 mRNA at 3 and 6 days after the lesion, followed by a 3-fold increase in message at 14-days postlesion compared to unlesioned controls. In situ hybridization found that the increase in GAP-43 message was restricted to the CA4 and CA3 commissural projection neurons of the contralateral hippocampus. These results suggest GAP-43 mRNA may be differentially regulated in the hippocampus based on the severity and/or neurotransmitter composition of the deafferentation lesion. Supported by PHS grants AG 00300, AG 09793, AG 07909.

295.5

SYMMETRICAL B50/GAP43-IMMUNOREACTIVITY IN THE CAT SPINAL CORD CAUDAL TO A PARTIAL HEMISECTION. W. Nacimiento, A. Mautes*, A.C. Nacimiento, J. Noth, A.B. Oestreicher* and W.H. Gispen. Dept. of Neurology, Alfried Krupp-Hospital, 4300 Essen, F.R.G., Neurosurgical Research Laboratory, Saarland University Medical School, 6650 Homburg, F.R.G., and Division of Molecular Biology, Rudolf Magnus Institute, 3584 CH Utrecht, Netherlands

The neuron-specific phosphoprotein B50/GAP43 is involved in axonal outgrowth and synaptic plasticity during both ontogenesis and regeneration.

Sprouting of dorsal root axons (DRA) in the mature cat spinal cord caudal to a partial hemisection was evaluated by densitometric comparison of the B50/GAP43-immunoreactivity (IR) on both sides of the affected cord. The lesions were performed at the low thoracic level sparing the dorsal column. Thus, supraspinal descending projections to lumbosacral segments were removed leaving the ascending collaterals of DRA intact. The distribution pattern and density of B50/GAP43-IR was symmetrical in all segments below the hemisection at 2, 3, and 8 weeks postoperatively. The present result indicates that under the experimental conditions used functional plasticity in response to removal of descending pathways is not mediated by sprouting DRA reinnervating partially denervated spinal gray areas in adult cats.

295.6

GAP-43 AND SBA IMMUNOLABELING OF AXOTOMIZED FINE PRIMARY AFFERENTS. R.E. Coggeshall[1], M.L. Reynolds[2], C.L. Woolf[2], A.A. Cameron[1] and C.M. Pover[1], [1]The Marine Biomedical Institute, Dept. of Anatomy and Neurosci. and Dept. of Physio. & Biophy., The Univ. of Texas Medical Branch, Galveston, TX 77550 and [2]The Dept of Anatomy and Developmental Biology, Univ. College, London.

Peripheral nerve transection clearly results in changes in central primary afferent axons. Many of these changes result from such things as ganglion cell loss or interruption of axonal transport and are thus degenerative in nature. Regenerative changes are less well studied. Here we report the presence of growth cones immunolabeled by growth associated protein (GAP-43), the possibility that presynaptic terminals of fine primary afferent axons are withdrawing from their presynaptic targets and an areal increase in the distribution of reactivity to the lectin soybean agglutinin (SBA) in the superficial dorsal horn two weeks after sciatic nerve transection in adult rats. These changes are interpreted as regenerative in nature. If so, the indication is that there is regenerative sprouting of fine sensory axons following peripheral axotomy. Supported by the MRC, Wellcome Trust, Bristol Myers-Squibb Pain Research grants and NIH grants NS10161 and NS11255.

295.7

CELLULAR AND MOLECULAR LOCI OF THE SELECTION OF TRANSMITTER RESPONSES DURING INNERVATION OF AN IDENTIFIED LEECH NEURON.

S. Catarsi*, S. Ching* and P. Drapeau, McGill Univ. Centre for Research in Neuroscience, Montreal, Quebec, Canada H3G 1A4.

Application of 5-HT activates both a Cl and a cationic conductance in the pressure-sensitive (P) neuron. However, the serotonergic Retzius (R) neuron evokes only a synaptic Cl response.

We cultured R and P cells so that their processes overlapped. Localized (~25 μm) application of 5-HT elicited depolarizing responses in untouched P cell bodies, neurites and growth cones. In contrast, the response of P cell neurites contacted by R cells appeared to be greatly reduced.

Cation channels in excised inside-out membrane patches from single P cells were activated in the presence of protein kinase C (PKC). In contrast, channels recorded from contacted P cells were insensitive to 5-HT and phorbol esters. These results suggest that the cation channels are no longer activated by PKC at sites of neurite overlap. The lack of a synaptic cationic response in vivo may be a consequence of the localized contact between processes during development.

Supported by the MPI of Italy and the MRC, FRSQ, AND FCAR of Canada.

295.8

CELL-SPECIFIC SURFACE COMPONENT SELECTS THE RESPONSE TO TRANSMITTER DURING SYNAPSE FORMATION BETWEEN IDENTIFIED LEECH NEURONS.

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When serotonergic Retzius (R) neurons of the leech contact pressure sensitive (P) neurons in culture, they selectively eliminate the cationic response to 5-HT and reform the Cl-dependent synapse seen in vivo. P cells were paired in culture with other identified neurons. Contact with sensory neurons (T,P, or N), non-serotonergic synaptic partners (AE and AP), or with serotonergic non-synaptic neurons (VL and DL) failed to alter the P cell responses to 5-HT. In contrast to standard segmental R cells, R cells from the two sex ganglia were also ineffective in selecting P cell responses to 5-HT. Treatment of standard R cells with trypsin or exposure to wheat germ agglutinin, but not to other lectins, blocked the loss of the cationic response in the P cell. We conclude that the selection of 5-HT responses in the P cell may be mediated by surface glycoproteins specifically expressed on the presynaptic (standard) R neurons. Contact with the presynaptic partner clears the non-synaptic response to transmitter as an early functional event during neuronal recognition.

Supported by the MRC, FRSQ and FCAR of Canada.

295.9

UNEQUAL REGENERATION OF TWO AXONS OF AN IDENTIFIED MOLLUSCAN NEURON.

P. J. Kruk, R. L. Ridgway and A. G. M. Bulloch. Dept. of Medical Physiology, Univ. of Calgary, Calgary, Alta., Canada, T2N 4N1.

The success of axonal regeneration of an identified buccal ganglion neuron (B4) of the pulmonate pond snail *Helisoma trivolvis* depends on the site of axotomy. This neuron has two axons, one in the ipsilateral and one in the contralateral esophageal trunks (ETs), which together innervate the paired salivary glands. We have previously found (Kruk and Bulloch, Soc. Neurosci. Abstr. 14: 582, 1988) that complete removal of the ipsilateral axon results in failure of regeneration. This axon does regenerate, however, if a proximal stump is retained and/or when the contralateral axon is given a simultaneous distal crush. These results gave rise to two hypotheses: (1) a proximal axon stump is necessary for regeneration, or (2) an inhibitory factor is retrogradely transported from targets via intact axon(s), but does not enter, nor influence regeneration of, proximal axon stumps.

To resolve these two hypotheses, we attempted to completely remove both axons of neuron B4 by crushing *in situ* the buccal commissure and ipsilateral ET. The morphology of the neuron was examined 8-10 days post-lesion by Lucifer Yellow dye-filling. No sprouting should occur if hypothesis #1 was true; alternatively, the cell should regenerate both axons. Neither situation was the case, however. **Regeneration was profuse, but confined mostly to the contralateral axon**; a result which may be due in part to incomplete removal of the contralateral axon. Much weaker regeneration was observed after close axotomy of the contralateral axon alone. As both axotomy procedures produce contralateral axon stumps of similar length (the ipsilateral axon intact or absent) the main difference between the two is a loss of contact with the target. Our results thus appear to support hypothesis #2, although final resolution will require more precise removal of the contralateral axon. **Supported by AHFMR and MRC (Canada).**

295.10

MUSCARINIC RECEPTOR SUBTYPES AND OTHER CHOLINERGIC MARKERS ARE DIFFERENTLY AFFECTED IN RAT DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESIONS.

I. Aubert, J. Poirier, A. Baccichet and R. Quirion. Douglas Hospital Research Center and McGill University, Department of Neurology & Neurosurgery, Montreal, Quebec, Canada H4H 1R3.

It has been shown that lesions of the entorhinal cortex in rat promote the sprouting of cholinergic septal fibers in the dentate gyrus of the hippocampus. In our model, lesions of the entorhinal cortex in adult Fisher-344 rats were performed and the status of various cholinergic markers was assessed using receptor autoradiography, histological and enzymatic techniques in controls and at several times following the lesions (2, 6, 8, 14, 30 and 64 days post-lesion, DPL).

As reported by other groups, acetylcholinesterase (AChE) staining was significantly increased in the ipsilateral dentate gyrus (DG) from 6 to 64 DPL. However, muscarinic-M2 binding sites, labelled with [^{3}H]AF-DX 384, appeared to be increased significantly only at 14 DPL. In contrast, choline acetyltransferase (ChAT) activity and [^{3}H]pirenzepine/muscarinic-M1 receptors remained unchanged throughout the time course.

These findings suggest that AChE staining may not be fully adequate as a marker of cholinergic sprouting in this model. Moreover, the different effects of the lesions on putative pre (M2 and ChAT) and post (M1) synaptic markers reveal the complexity of this sprouting model in regard to the reorganization of the cholinergic synapse. Supported by the Alzheimer Society of Canada, the American Health Assistance Foundation and MRCC.

295.11

PROTEIN KINASE C BINDING SITES DURING NEURONAL PLASTICITY FOLLOWING ENTORHINAL CORTEX LESIONING IN RAT.

J. Poirier, A.R. Parent, A. Baccichet and R.Quirion. Douglas Hospital Research Centre, Verdun, Quebec, Canada. H4H 1R3.

Entorhinal cortex lesioning (ECL) produces a loss of more than 80% of the synapses in the outer molecular layer of the hippocampus. The loss of synapses, however, is transient. Beginning a few days after denervation, new synapses are formed, virtually replacing the lost inputs within two months. These new synapses originate from the cholinergic septal neurons, glutamatergic commissural-associational pyramidal cells of the CA3/Hilus areas and, to a lesser extent, from neurons of the contralateral entorhinal cortex. We hypothesize that the protein kinase C (PKC) has a role to play in the process of reinnervation that follows deafferentation. PKC is considered to be an important second messenger coupled to different neurotransmitters and it induces cellular proliferation. By autoradiography analysis on ECL rat brain sections labelled with tritiated phorbol 12,13-dibutyrate, we have evaluated the density of PKC binding sites. The PKC site density was significantly increased (> 25%) between 4 and 6 days post-lesion in molecular layer, in granular layer of dentate gyrus and in hilus. Similar increases of the PKC were observed in both the ipsilateral and contralateral side of the lesion. This results are consistent with the known location of the entorhinal cortex projections. Ten days after lesions, the values return to control levels. The profile of PKC binding sites in the hippocampus correlates quite well with the distribution of reactive astrocytes in this animal model. This phenomena could conceivably be involved the process of terminal sprouting. Supported by Alzheimer Society of Canada and the American Health Assistance Foundation.

295.12

ALTERED CHOLESTEROL METABOLISM DURING REACTIVE SYNAPTOGENESIS IN THE DEAFFERENTED HIPPOCAMPUS

A. Baccichet, D. Dea and J. Poirier. McGill Centre for Studies in Aging, 1650 Cedar Ave., Montreal, Canada, H3G 1A4 and the Douglas Hospital Research Centre, 6875 LaSalle Blvd., Verdun, Canada, H4H 1R3.

The hippocampus can be induced by deafferentation to selectively reorganize its neuronal inputs. In a widely studied rat model, entorhinal cortex lesioning (ECL) induces sprouting of glutamatergic commissural/associational fibers and cholinergic septal projections in the molecular layer of the dentate gyrus. Recent evidences were given for altered synthesis of the apolipoprotein E (apo E) mRNA in the hippocampus in response to ECL. The increase synthesis was shown to coincide with the acute phase of terminal proliferation, reactive synaptogenesis and protein synthesis. Apo E is a protein that facilitates cholesterol movement both in and out of cells. We have further documented the cascade of events leading to cholesterol up-take by regenerating neurons. Using a fluorescent Dil-LDL binding assay on frozen brain sections, we found that the apo E receptor binding activity (also called LDL receptor) is significantly increased (>2 fold) in the granule cell neurons in response to ECL. In contrast, the activity of the hydroxymethylglutaryl-CoA reductase (a rate limiting enzyme in cholesterol synthesis) was shown to be significantly reduced in the hippocampus during the acute phase of reactive synaptogenesis and apo E synthesis. These results are compatible with a receptor mediated down-regulation of the neuronal cholesterol synthesis in response to acute cholesterol uptake during the synaptogenesis phase. Supported by the American Health Assistance Foundation, a scholarship from the Medical Research Council of Canada and a studentship from the Alzheimer's Society of Montreal.

295.13

GLUCOCORTICOID MODULATION OF APOLIPOPROTEIN E AND LDL RECEPTOR EXPRESSION IN THE HIPPOCAMPUS DURING REACTIVE SYNAPTOGENESIS . D. Dea, A. Baccichet and J. Poirier, . McGill Centre for Studies in Aging, 1650 Cedar Ave., Montreal, Canada, H3G 1A4 and the Douglas Hospital Research Centre, 6875 LaSalle Blvd., Verdun, Canada, H4H 1R3.

Partial deafferentation in the hippocampus following entorhinal cortex lesion (ECL) induces a sequence of compensatory events in both the remaining afferent fibres and denervated dendrites, resulting in a reorganization of the circuitry in the denervated area. It is known to trigger complex sprouting responses in the denervated molecular layer of the dentate gyrus from cholinergic as well as glutamatergic surviving afferent systems. It was recently demonstrated that the apolipoprotein E (apo E), a protein that facilitates cholesterol movement both in and out of cells, shows a particularly close temporal relationship with the early phase of the reinnervation process in the hippocampus of rat with ECL. In order to further examine the role played by the cholesterol metabolism during reactive synaptogenesis, corticosterone (10 mg/Kg/days for 6 days) was administered (s.c.) to rats with ECL and the mRNA prevalence for the apo E and its receptor (the LDL receptor) was determined by Northern blot and PCR quantification. Northern blot hybridization of a rat apo E probes on pooled samples showed that glucocorticoid potentiates the apo E mRNA induction caused by the deafferentation process. In contrast, the hippocampal mRNA prevalence of the LDL receptor which is increased by more than 100% at 6 days post-lesion, was significantly reduced (-60%) in the corticosterone-treated animals. These results further confirm the key role played by the apo E-LDL receptor pathway during reactive synaptogenesis and suggest a possible biochemical mechanism by which glucocorticoids can block compensatory sprouting and reactive synaptogenesis in adult rats. Supported by the American Health Assistance Foundation and a Scholarship from the Medical Research Council of Canada.

295.14

ULTRASTRUCTURE OF AChE-POSITIVE FIBERS AFTER EXCITOTOXIC DAMAGE IN THE MOUSE HIPPOCAMPUS. RC Green, SM Hersch, HD Rees, RAE Bakay Depts. of Neurology and Neurosurgery, Emory University School of Medicine, Atlanta, GA 30322

Hippocampal acetylcholinesterase (AChE) fiber reorganization has been described in human diseases such as temporal lobe epilepsy and Alzheimer's disease. Previous light microscopic studies of AChE histochemistry after intraventricular injections of kainic acid (KA) in mice revealed increased staining over areas of cell loss in the hippocampal stratum oriens and stratum pyramidale, primarily in the CA3 region (Green et al. *Soc Neurosci Abstr* 16:947,1990). Since AChE staining was enhanced in an area where target neurons were lost without obvious deafferentation, we studied the region of increased staining with electron microscopy.

Male C57BL/6J mice, aged 2-3 months, received intracerebroventricular injections of KA (0.3 µg/side) or saline. After two weeks, the mice were perfused and brain sections were stained for AChE using a modified Karnovsky-Roots technique which gives detailed staining of fibers (Mesulam et al., *Ann Neurol* 22:683, 1987), followed by osmium fixation and embedding in Epon. In saline-injected control mice, AChE reaction product was principally extracellular in association with unmyelinated axons and axon terminals, and was intracellular within dendrites. In KA-treated animals, AChE reaction product was similarly distributed and neuronal degeneration with gliosis was observed. There was no association between reaction product and degenerating processes. In some animals with severe neuronal degeneration, there appeared to be an increased amount of AChE reaction product in stratum oriens.

295.15

5,7-DIHYDROXYTRYPTAMINE (5,7-dHT) CAUSES SPROUTING IN THE ADULT SNAIL CNS. M.W. Baker and R.P. Croll, Dept. Physiology. & Biophysics. Dalhousie Univ. Halifax, N.S. Canada.

The effects of in vivo 5,7-dHT adminstration on neuronal morphology was investigated in the snail Achatina fulica by axonal backfilling of the cerebrobuccal connective with nickel-lysine. Backfilling 21 days following a single injection of 5,7-dHT (equivalent to 300 mg/kg, in 1 cc saline with 0.5 mg L-ascorbic acid as an antioxidant) revealed supernumerary staining of fibers in different pathways and staining of novel cell bodies in the cerebral and buccal ganglia, when compared to untreated animals. Control injections using the carrier solution alone, revealed normal labeling. However, injections of the carrier with a high concentration of L-ascorbic acid (10 mg), yielded supernumerary labeling in some of the same fiber tracts as seen with 5,7-dHT treatment. The 5-HT synthesis blocker, PCPA, was also tested for effects on sprouting. One injection of 0.5 mg of PCPA per day for 3 consecutive days was shown to induce a similar supernumerary labeling of fibers and cell bodies. Evidence suggests that some of the same novel neurons were labeled with PCPA and 5,7-dHT treatments. The extent of depletion of 5-HT and the specificity of the effects are currently being investigated.

295.16

SUPPRESSION OF REACTIVE COMPENSATORY CHANGES IN THE BRAIN BY MORPHINE. A. Gorio, M.L. Malosio*, B. Tenconi*, M.L. Donadoni*, P. Mantegazza* and A.M. Di Giulio. Dept. of Medical Pharmacology, Univ. of Milano, v. Vanvitelli 32, 20129 Milano, Italy.

We are reporting that perinatal exposure to low doses of morphine, supplied in drinking water to pregnant rat and, after weaning, directly to the offspring, can impair compensatory events triggered by a neurochemical lesion. Neonatally administered 6-hydroxydopamine (6-OHDA) causes a permanent depletion of noradrenaline innervation of the cerebral cortex. By means of HPLC and immunocytochemistry we have observed that the denervated cortical territories are occupied by serotonin, dopamine and met-enkefalin neurons. In morphine exposed rats these compensatory events are absent. The inhibitory effect of opiates on CNS plasticity is detectable also in the mesencephalon, where a hyperinnervation by the short noradrenergic collaterals is normally observed following 6-OHDA neonatal injection. We have investigated the possible molecular mechanisms underlying these processes. The transcripts of several proteins thought to be involved in neuritogenesis, like GAP-43 and Go, or in receptor signal transduction, like Gi, Gs and Go, were assayed.

295.17

SYNAPTIC PLASTICITY IN LAMINA II OF THE DEAFFERENTED SPINAL CORD: QUANTITATIVE EM-IMMUNOCYTOCHEMICAL STUDIES. B. Zhang, M.E. Goldberger, W.P. Battisti and M. Murray, Dept. of Anat. & Neurobiol., Med. Coll. of Penn., Philadelphia, PA 19129.

Light microscopic studies show that projections to the dorsal horn change after complete deafferentation. We used quantitative EM-immunocytochemistry to study the changes in synaptic terminals in lamina II of the L5 dorsal horn resulting from unilateral lumbosacral deafferentation L1-S2 in adult rats. Animals survived 10 days (acute), and 60 days (chronic); the contralateral side served as control. We examined the total synaptic population and those terminals containing SP and 5HT. In controls, SP is found in simple and complex terminals, and 5HT is present in simple terminals. Complex terminals in lamina II were eliminated by deafferentation, but the number of simple terminals was greater than normal in deafferented lamina II in both acute and chronic groups. Post-embedding immunocytochemical labeling indicated that the percentage of simple terminals containing SP decreased by 30% in the acute group but recovered to a normal percentage in the chronic group. These results indicate that terminals lost after rhizotomy are replaced by terminals from intact systems and that intrinsic SP systems contribute to the synaptic replacement.

Supported by NIH grant No. NS24707.

296.1

HIGH-RESOLUTION IMAGING OF GROWTH CONE BEHAVIOR *IN VITRO* ON PATTERNED SUBSTRATES

A. Soekamo, B. Lom & P. E. Hockberger. Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611.

We have studied the behavior of rat neuroblastoma cells (NIE-115) growing on photolithographically patterned substrates (Kleinfeld et al., J. Neurosci. 8: 4098, 1988) using high-resolution digital imaging microscopy. The use of a homogeneous population of cells in a controlled and defined environment allows observation of behaviors that are poorly understood, e.g., guidance, turning, sampling, and decision making. Using time-lapse recording we have observed that cells migrating on ethylenediamine (EDA) lines, 10μm in width, exhibited ruffling and filopodial extension similar to what is seen on unpatterned substrates. While attachment and extension occured preferentially on EDA, cells occassionally did the same on the non-preferred (hexadecane) substrate. At junctions between lines, cells sampled their choices and made 'decisions' to either turn, go straight ahead, or retract. One of the most remarkable and consistent events involved nuclear translocation to the junction where it often remained. We also observed violent bouts of cellular blebbing both on and off EDA regions, which was sometimes followed by a return to normal cell activity. When this type of blebbing occurred at the growth cone, it was followed by retraction. Fluorescent images of patterned cells that were double-labelled for actin (rhodamine-phalloidin) and tubulin (FITC-antibody) showed compartmentalization of these cytoskeletal proteins. Actin filaments were restricted to the distal region of the growth cone, often radially arrayed, whereas tubulin was seen along the neurites and proximal growth cone. This arrangement was seen in cells on and off lines, indicating that these substrates do not differentially affect cytoskeletal organization. Actin filaments and tubulin were also concentrated in the hyaline membranes of blebbing cells.

This research was funded in part by a Biomedical Engineering Grant from the Whitaker Foundation.

296.2

PATTERNS OF NEURONAL ADHESION ON SUBSTRATES THAT DO AND DO NOT PROMOTE NEURITE OUTGROWTH.

J. Drazba, M.I.Weiner and C.L. Smith. Laboratory of Neurobiology and Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Neuronal process outgrowth in vitro is dependent upon the substrate. In order to better understand the cellular mechanisms underlying the observed differences between substrates, we have used reflection (IRM) and differential interference videomicroscopy to study adhesion and neurite outgrowth of chick sympathetic ganglion neurons grown on polyornithine and laminin substrates. Sympathetic neurons grown on polyornithine-coated glass coverslips form a lamellipodium and filopodia around their entire circumferences which adhere closely to the substrate as seen by IRM. The filopodia rarely transform into neurites. However, if a filopodium contacts another cell it lifts off of the substrate, thickens and transforms into a neurite. Filopodia that contact polyornithine coated latex beads also lift off the substrate and transform into neurites as described in another abstract (C. Smith). Neurons grown on a laminin substrate form a lamellipodium and filopodia around their entire circumferences, but they adhere closely to the substrate only in discreet patches. Filopodia typically adhere more closely at their tips than along their shafts. Multiple filopodia around the circumference of individual neurons thicken and transform into neurites, thus making the neurons multipolar. These observations suggest that transformation of a filopodium into a neurite requires that it be tightly adherent at its tip but not tightly adherent along its length. We are in the process of examining other substrates to further characterize the correlation between patterns of adhesion and process outgrowth.

296.3

RELATIONSHIP OF RETROGRADE MOVEMENTS OF NEURITE SURFACES TO RETROGRADE MOVEMENTS OF CYTOPLASM.

C.L. Smith. Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

We are using videomicroscopy to study interactions of chick sympathetic ganglion neurons with beads coated with different substrate molecules in order to understand the cellular mechanisms involved in neurite outgrowth. Sympathetic neurons grown on polyornithine-coated glass coverslips form filopodia around their entire circumferences but rarely form neurites. However, their filopodia can be stimulated to transform into neurites by contact with laminin or polyornithine-coated 10 μm latex beads. A filopodium that contacts a coated bead first straightens and then lifts off the substrate. Then a lamellipodium moves anterogradely toward the tip of the filopodium followed closely by a bolus of cytoplasm containing actin, microtubules and neurofilaments. As the bolus moves, the thickened region of the filopodium sprouts new filopodia, thereby resembling a growth cone. However, when the bolus reaches the bead, it dislodges the bead which then moves retrogradely to the cell body. The bolus accompanies the bead as it moves. The rate at which the bead is transported and the bolus of cytoplasm receeds (7.1±3.5 μm/min) is comparable to the rate at which smaller beads are transported retrogradely along the surfaces of filopodia (8.6±1.7μm/min) in separate experiments. This correlation between retrograde bead and cytoplasmic movement suggests that the mechanisms responsible for moving cytoplasm within developing neurites are linked to the mechansims that move molecules on the cell surface.

296.4

DIRECTIONAL, MORPHOLOGICAL, AND ELONGATION RATE CHANGES OF GROWTH CONES AS THEY ENCOUNTER LAMININ-FIBRONECTIN BORDERS.

T. M. Gomez and P. C. Letourneau. The University of Minnesota, Minneapolis, Minnesota 55455.

Previous studies have shown that when presented with a parallel array of alternating laminin (LMN) and fibronectin (FN) substrata, neurites elongate preferentially on LMN (Vielmetter et al., Exp Brain Res 81:283-287, 1990). Since both FN and LMN support neurite outgrowth of chick sensory neurons in culture, we examined closely the detailed behavior of the growth cones (GC) of these neurons as they encountered FN-LMN borders.

Using a silicon matrix consisting of an array of parallel channels, patterns of alternating LMN and FN substrata were bound to a glass coverslip. A small percentage of the total LMN applied to the coverslip was rhodamine conjugated in order to determine its distribution, homogeneity and concentration. FN distribution was localized by immunofluorescent staining. Dissociated dorsal-root ganglia neurons from E10 chicks were plated onto the patterned substrata and were examined using time-lapse video microscopy.

After 24 hours in culture, neurites were largely restricted to the LMN lanes, however outgrowth could be found on FN. We analyzed GC morphology and changes in rate, as well as direction of neurite outgrowth, as GCs contacted the FN-LMN borders. GCs crossing from LMN onto FN often collapsed and/or were deflected back onto the LMN substratum. Conversely, GCs on FN that encountered LMN rarely remained on FN. Further, these GCs were often seen to spread into lamellipodial structures, orient toward LMN and increase their rate of outgrowth. GCs that were migrating parallel to the substrata borders predominately did so in association with LMN.

These data suggest that if present along an axon pathway, LMN and FN could alter the direction and rate of neurite outgrowth. Further, our results imply that selection of one matrix molecule over another may involve alterations in growth associated intracellular mechanisms, leading to changes in membrane and cytoskeletal dynamics.

296.5

MODULATION OF GROWTH CONE MORPHOLOGY BY SUBSTRATE-BOUND ADHESION MOLECULES.

H.R. Payne, S.M. Burden and V. Lemmon. Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Neuronal growth cones mediate axon growth by providing a motile structure that can interpret and respond to environmental cues. As a result, growth cones can choose among different pathways to grow to their appropriate targets. The molecules that encode the guidance information along alternative pathways are not known, but these cues are thought to be responsible for the position-specific morphological complexity of growth cones. We have investigated substrate-based influences on growth cone morphology in vitro. We used scanning electron microscopy to analyze the morphologies of chick retinal ganglion cell growth cones on substrate-bound molecules. L1/8D9, N-cadherin, and laminin each induced distinctive morphological characteristics in growth cones. Growth cones elaborated lamellipodial structures in response to the cell adhesion molecules L1/8D9 and N-cadherin whereas laminin supported filopodial growth cones with small veils. The growth cones on L1/8D9 attained the largest sizes and extended more filopodia. Filopodial associations between adjacent growth cones and neurites were frequent on L1/8D9 but were uncommon on laminin or N-cadherin. These data establish that substrates normally present in the developing optic pathway can influence the details of growth cone morphology in vitro. Our observations are consistent with a hypothesis that variations in growth cone morphology in vivo are due to alterations in substrate-bound guidance cues along developing axonal pathways.

296.6

A NOVEL SUBMEMBRANOUS MOLECULE ENRICHED IN GROWTH CONE SHAFTS

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A group of three molecules (app. molecular weights of 150, 130 and 120 kD in western blot analysis of chicken neural tissue) was initially defined by two different monoclonal antibodies. One of the antibodies crossreacts with the N-CAM180-specific, cytoskeleton-associated intracellular domain of the Neural Cell Adhesion Molecule. Several cDNA clones (isolated from a chicken retina gt11 expression library employing the two monoclonal antibodies) show no homology to any known sequences.The three molecules (which might represent splice forms of one molecule) are differentially regulated during the development of the chick retinotectal system with a expression maximum in the phase of axonal outgrowth and target finding. The molecules are intracellular and appear to be distributed in a fine filamentous submembranous meshwork, indicating that the molecules might be components of the cortical cytoskeleton or the inner surface of the cell membrane, possibly linkerproteins. In retina single cell culture, antibody staining is most intense in a subpopulation of growth cone shafts, in vivo the molecules are predominantly expressed by postmitotic, postmigratory cells, so one might speculate that the molecules play a role in growth cone stabilisation.

296.7

Transfection of "Sense" and "Anti-Sense" Tau cDNA into PC12 Cells: Tau Regulation of Net Microtubule Assembly and Neurite Outgrowth. B. Esmaeli-Azad* and S.C. Feinstein, Neuroscience Research Institute and Department of Biological Sciences, University of California, Santa Barbara CA 93106.

The tau family of proteins are known to induce tubulin polymerization and suppress microtubule dynamics *in vitro* and to promote microtubule stabilization and bundling *in vivo*. Employing the NGF mediated induction of neurite outgrowth in PC12 cells, we and our colleagues had previously observed that the NGF mediated (i) induction of tau, (ii) accumulation of microtubule mass and (iii) induction of neurite outgrowth all proceeded in concert. These correlative data led to the model that tau is a fundamental and limiting factor promoting *in vivo* microtubule accumulation, which in turn promotes neurite outgrowth.

One experimental paradigm allowing direct examination of this correlative model is to alter the normal pattern of tau expression during neurite outgrowth and then examine the biochemical and morphological consequences. In the present study, we have isolated stable PC12 "sense" and "anti-sense" tau cDNA transfectants, which over-express and under-express tau protein, respectively. NGF induced microtubule accumulation and neurite outgrowth are both dramatically affected by the level of active tau protein. Anti-sense tau cells are deficient in NGF mediated microtubule mass accumulation, and project neurites poorly. Sense-orientation tau cells accumulate microtubule mass and project neurites more rapidly than wild type cells. Interestingly, newly synthesized tau in non-NGF treated, over-expressing cells is non-cytoskeletally associated. Following NGF administration, this tau becomes associated with the cytoskeleton. The NGF mediated "activation" of tau may represent a post-translational modification event. Alternatively, tau may be in excess over its cognate cytoskeletal structures/substrates required for tau function prior to NGF administration. This work provides direct evidence that tau functions *in vivo* to promote net microtubule assembly and neurite outgrowth.

296.8

TAU-HSV1 RESTORES NORMAL POLARITY TO TAU ANTISENSE TREATED NEURONS [1]G.Lee*, [1]S.Rook*, [1]G.Hall and [2]A.Geller. [1]Brigham & Women's Hosp. and [2]Dana Farber Cancer Institute, Boston, MA 02115.

Cerebellar neurons in dissociated culture exhibit polarity as defined by the acquisition of a single neurite whose length exceeds that of other neurites. Antisense experiments (Caceres and Kosik, 1990) have shown that this polarization process requires tau protein, a microtubule associated protein which locates primarily to the axon in vivo. We have used this system to study the structure and function of tau protein in neuronal cells. Antisense treatment is used to block endogenous tau expression and neurite elongation, then herpes simplex virus (HSV1) vectors are used to re-introduce tau protein into neurons. We have made a panel of 6 HSV1 vectors - two encode full length isoforms of tau protein, two encode tau fragments which co-localize with microtubules in vivo and two encode tau fragments which do not co-localize with microtubules in vivo. Virus and antisense are added to the culture media after plating; cells are fixed 2 days later and lengths of processes measured. We find that cells infected with vectors expressing full length tau protein overcome the effects of tau antisense; these cells regain polarity. By using vectors expressing tau fragments, we will assess whether any specific domains of tau protein are required for this activity. Since the elongation of an axon-like process during the acquisition of polarity is anticipated to require the stabilization of microtubules, tau fragments which lack in vivo microtubule binding activity are not expected to work. Our panel of vectors will determine if any domains, in addition to the microtubule binding domain, are required for activity.

296.9

AXOTOMY ACCELERATES SCb TRANSPORT IN MOTOR NEURONS WITHIN 3-7 DAYS. J.M. Jacob and I.G. McQuarrie, Neural Regen. Ctr., Cleveland VA Med. Ctr., Cleve., OH 44106.

In sciatic motor neurons of the rat, increases in the outgrowth rate of newly-formed (daughter) axons reflect an increased rate of SCb transport in both parent and daughter axons (McQuarrie and Jacob, J. Comp. Neurol. 305:139, 1991; Jacob and McQuarrie, J. Neurobiol., Sept. 1991). The increased SCb rate, which is produced by the initial or conditioning axotomy, can be assayed indirectly by measuring the outgrowth rate after a subsequent testing axotomy. Using this assay, and a variety of intervals between conditioning and testing axotomies, we have examined the time-course of SCb acceleration. With conditioning intervals of 0 and 3 d, outgrowth rates were the same as in sham-conditioned controls, whereas, with conditioning intervals of 7, 14, and 21 d, outgrowth rates were greater ($p<0.01$ at each interval). This time-course reflects the cell body reaction to axotomy in motor neurons: a catabolic phase, lasting 3-4 d, followed by an anabolic phase (Grafstein and McQuarrie, in Neuronal Plasticity (ed. C.W. Cotman), Raven Press, New York, 1978). We conclude that the SCb rate may be regulated, in part, by the magnitude of synthesis of SCb (cytoskeletal) proteins in the nerve cell body.

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296.10

INHIBITION OF AXONAL GROWTH BY BREFELDIN A IN CULTURES OF EMBRYONIC RAT HIPPOCAMPAL NEURONS. M.I. Jareb and G.A. Banker, Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

In culture, embryonic rat hippocampal neurons initially develop several short, minor processes. After a delay of 12 to 24 hours, one of these begins to elongate rapidly, becoming the cell's axon. We have examined the effects of brefeldin A (BFA), which specifically inhibits the translocation of vesicles through the Golgi complex, on the initiation and elongation of axons. To ascertain the effects of BFA on axonal growth we followed individual cells by video microscopy for up to 12 hours. Within 1 hour after adding BFA (1 μg/ml), axonal elongation was completely inhibited. After several hours axons began to retract, and in some cases this continued until the axon had shortened to the length of a minor process. When BFA was added earlier in development, when cells had only minor processes, axons failed to develop. Minor processes themselves were unaffected by BFA. It did not inhibit their elongation or cause them to retract. The effects of BFA were completely reversible. Within 1 hour after removing the drug, axonal elongation resumed at a normal rate. The effects of cycloheximide, an inhibitor of protein synthesis, were quite different. In the presence of cycloheximide (10 μg/ml) axonal elongation did not stop abruptly; instead axons became thinner, but continued to elongate for 4 to 6 hours. These results suggest that continuous vesicle transport from the Golgi complex is essential for the elongation and maintenance of axons. This requirement appears to be specific to axons.

296.11

REGULATION OF SPINAL NEURITE GROWTH CONE MORPHOLOGY BY TARGET TISSUE. T. Somasekhar* and E.D. Pollack, Inst. Study of Developmental Disabilities, Dept. of Biol. Sciences and Committee on Neuroscience, Univ. of Illinois at Chicago, IL 60680.

Since the growth cone plays a critical role in target-directed axon extension during development, we sought to determine if target tissue has an influence on neuronal growth cone morphology *in vitro*. Spinal cord explants from *Xenopus* tadpoles were cultured either alone (control) or in co-culture with mesenchymal limb buds on poly-dl-lysine or collagen coated cover glasses. Growth cones were examined at light, scanning, and transmission electron microscope levels. In contrast to control cultures, the majority of growth cones in target tissue-containing cultures were larger with elaborate morphologies characterized by broad lamellipodia and more filopodia. While filopodial branching was seen in control cultures as well as in cord+target cultures, branching of neurites at the growth cone was more common in the latter. The distribution of filopodia was restricted mostly to the neurite tips in cord+target cultures, whereas in control cultures filopodia tended to be more diffusely distributed over neurite shafts as well. Interestingly, within the cord+target cultures, there were variations in growth cone morphologies depending on their proximity to the target tissue. Growth cones that were closest to, or oriented towards, the target had the elaborate morphological configuration; growth cones that were farther from the target or that extended in the opposite direction of the target had the simpler morphology of the control cords. TEM observations revealed that most of the growth cones of cord-target cultures were rich in filamentous material and membrane bound organelles in contrast to those from control cultures. These results revealed profound target-mediated influences on the morphology of neuronal growth cones and provide additional evidence for a target-originated, graded growth influence on extending neurites.

296.12

AXONAL MORPHOLOGY IN THE DEVELOPING CHICK OPTIC NERVE. S.M. Fraley, W.M. Ross and S.A. Dunlop, Department of Psychology, University of Western Australia.

The morphology and trajectories of individual axons in the developing chick optic nerve were examined after anterograde labelling with horseradish peroxidase (HRP) *in vitro*. Small groups of axons were labelled by "stabbing" the retina close to the optic disk with an HRP-coated insect pin. After transport, nerves were sectioned longitudinally on a vibratome, reacted with cobalt-enhanced DAB and viewed using Nomarski optics. We examined tissue at E9-11 and E19-21, that is, at times when axon numbers were at their peak or at mature values respectively (*Rager & Rager, 1978, Exp. Brain Res. 33, 65-78*). At E9-11, axons had a very fine calibre and many had complex axonal spines projecting from the main shaft. Spine length varied from less than 1 um to over several microns. Axons tipped by growth cones were a prominent feature. Growth cones varied from round with a diameter of approximately 5um to elongate and up to 30 um long; all growth cones were characterised by prominent filopodia which were of a similar calibre compared to the main axon shaft. Whereas most axons were aligned with their axes parallel to the length of the nerve, other axons at all levels had aberrant trajectories with kinks or loops, or took sinuous routes across the nerve. At E19-21, axonal calibre varied considerably; axonal spines were rarely seen and growth cones were absent. Axons with aberrant trajectories were prominent only at three levels. These levels were within the length of the optic disk, the junction between the disk and the optic nerve and as axons entered discrete fascicles within the chiasm. We conclude that developing optic axons in the chick undergo considerable remodelling to reach their mature form.

296.13

GROWTH CONES OF DESCENDING SEROTONERGIC AXONS IN XENOPUS SPINAL CORD. R.H.Nordlander and S-X.Liu. Depts. Oral Biol., Anat., Ohio State Univ., Columbus OH 43210.

Serotonergic axons are among the earliest descending brainstem axons to enter the developing Xenopus spinal cord. They enter the cord after longitudinal pathways have already been initiated by local spinal neurons. In this study we examined growth cones of descending serotonin-immunoreactive (5HT-I) axons in Xenopus embryos and larvae (st 25-40). There are no intrinsic 5HT-I spinal neurons at these stages. Our primary focus was the patterns of growth cone shape and distribution for these fibers as viewed in wholemounts and sections of the spinal cord.

Growth cones are generally small (4 x 16 um) and vary from simple fusiform to branched filiform shapes. We observed that growth cones at leading (caudal) positions tend to be more elaborate than those that follow, confirming for an identified neuron class what we had earlier observed as a general phenomenon among descending spinal axons (J Comp Neurol, 263:485-496,'87). Growth cones and axons of 5HT-I neurons are distributed in the ventral half of the lateral marginal zone. There is no clear evidence that the early 5HT-I axons serve as guides for those that follow. Immunoreactive growth cones do not consistently contact other 5HT-I axons and the axons themselves are dispersed among their non-reactive fellows.

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296.14

THE FIRST AXONS IN THE MOUSE CNS. L. S. Ross and S. S. Easter, Jr. Dept. Zool. Biomed. Sci. Ohio U., Athens, OH 45701; Dept. Biology, U. Michigan, Ann Arbor, MI 48109; and INSERM U. 106, Paris, France.

With an antibody to neuron-specific beta tubulin (provided by Dr. A. Frankfurter) and DiI, we visualized axons in wholemounted fixed neural tubes from E8.5-E12.5 embryos. (Convention: at noon on the day after a night's mating, the age is E0.5). The development of individual littermates varied substantially; this description applies to "average" embryos of a given age.

At E8.5, before neural tube closure, the neural folds contain labeled cells (<10 per side) at the boundary of the presumptive neural tube and skin, at the rostrocaudal level of the junction of the presumptive fore- and midbrain. These are the only labeled cells in the embryo. By E9.0, the neural tube has closed, and the labeled cells are more numerous (>20 per side). They flank the dorsal midline in the presumptive midbrain. Their growth-cone tipped axons course caudally and ventrally in the ipsilateral alar plate, the pioneers of what later stages (examined immunocytochemically and with DiI) show is the descending tract of the mesencephalic nucleus of the trigeminal nerve.

At E9.5, two bilaterally symmetrical ventral longitudinal tracts begin to form. One originates from a cell cluster adjacent to the ventral midline at the transverse cephalic flexure, near the junction of the presumptive fore- and midbrain. Its axons course caudally in the ipsilateral basal plate, close to the midline. By E10.0, the leaders extend past the otic vesicle. This tract is the forerunner of the medial longitudinal fasciculus, and the first cells that produce axons in it probably contribute to the interstitial nucleus of Cajal. The second ventral longitudinal tract originates from a cell cluster at the base of the optic stalk. Its axons course caudally through the ipsilateral presumptive diencephalon, and reach the cephalic flexure by E10.0, but by E12.5, have not advanced more caudally. This is the tract of the postoptic commissure, which is joined later by the retinofugal axons. Unlike the other two tracts, it can not now be identified with an adult structure.

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296.15

DEVELOPMENT OF CALLOSAL AXON ARBORS IN HAMSTERS. C.Hedin-Pereira*, S. Jhaveri, R.Lent. Dept. Brain and Cog.Sci., M.I.T., Cambridge, MA 02139; Inst. Biofisica, UFRJ, 21941 Rio de Janeiro, Brazil.

We have used DiI (for younger animals) and biocytin labeling to visualize individual callosal axon arbors. Crystals of DiI were placed in the parietal or occipital cortex of aldehyde-fixed hamster brains (E15-P6; where E16=P0) and the tissue analyzed 1-3 months later with fluorescence microscopy. Older pups (P4-P30) had biocytin injected in the cortex and were sacrificed 24-48h later. Brain sections were treated with an avidin-biotin-HRP complex and DAB.

Parietal axons, bearing simple growth cones, first cross the midline on E15. Over the next few days, increasing numbers of axons pass under the cortical plate of the homotopic target regions and continue to grow laterally, without branching, in the white matter. Simple collateral sprouts, tipped by a tiny bead, appear on callosal axons around P4. On the following days lateral branches are emitted from the pially directed sprouts but arbors are still primitive. By P6, although the sprouts have reached the upper cortical laminae, signs of a more complex arbor are seen predominantly in the infragranular layers. Subsequently, by P9, elaborate, lamina-specific arborization becomes focussed in both the infra- and supra-granular layers. Occipital callosal axons lag behind parietal fibers by about 2 days.

Thus, in contrast to thalamocortical afferents which enter the cortical plate upon arriving at target regions (Catalano et al., PNAS, 1991), the later-arriving callosal axons show a distinct waiting period. Callosal axon morphogenesis is a relatively slow process, occuring over at least 10 days of postnatal life.

Support: NIH grant EY005504; CNPq, Brazil.

296.16

SPECIFICITY AND MODE OF GROWTH OF DEVELOPING CORTICOSPINAL AXONS INTO THEIR SPINAL GRAY TERMINAL FIELDS. L. López-Mascaraque and D.D.M. O'Leary Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

Layer 5 is the source of cortical inputs to the brainstem and spinal cord. Layer 5 neurons contact their brainstem targets by extending collateral branches from primary axons that form the corticospinal projection; the branches form days after the axons grow past their targets (O'Leary & Terashima Neuron 1:901 '88; Soc NS Abs 15:875 '89). Here, we address the mode by which layer 5 axons invade their terminal fields in the spinal gray, and the topographic specificity of this process. Corticospinal axons labeled with DiI injected into the forelimb and hindlimb regions of sensorimotor cortex (SMC) were examined in P1, P4, P6, P8 and P11 rats. In mature rats, forelimb axons terminate at cervical levels, hindlimb axons at lumbar levels. To correlate the somatotopic map in SMC with corticospinal projections, the injected cortex was processed for AChE. Early on P1, labeled axons extending down the dorsal funiculus (DF) reach the cervical enlargement. By P4, they attain lumbar levels. By P6, axons reach mid-levels of the lumbar enlargement, and by P8 extend beyond it. Axons labeled from forelimb and hindlimb regions extend similar distances. The spinal gray is invaded by collaterals that branch at right angles from primary axons that have already grown past, and form in a rostral to caudal progression. Only occasionally do primary axons deviate from the DF and grow into the spinal gray. Branches are first seen on P4; the few labeled are found at cervical levels. By P6, branches increase in number and are found mainly in the cervical enlargement, and some at thoracic levels. Most collaterals are simple; many do not extend out of the DF. At P8, collaterals are more complex and are found throughout cervical to lumbar levels, but their number decreases caudally. The timing and patterning of collateral extension is similar for forelimb and hindlimb axons. By P11, collaterals are preferentially found at the correct spinal level. These findings indicate that (1) corticospinal axons initially overshoot their appropriate spinal level; (2) corticospinal axons innervate the spinal gray as they do the brainstem, by the delayed extension of collaterals from the primary axon; (3) the initial extension of collaterals into the spinal gray is topographically inaccurate. Supported by the American Paralysis Assoc.

296.17

DEVELOPMENT AND DISTRIBUTION OF PERIPHERAL AND CENTRAL NEURONS LABELED BY THE MONOCLONAL ANTIBODY LAN 3-6 IN LEECH. K.K. Briggs, K.M. Johansen and J. Johansen. Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011.

The lan 3-6 antibody recognizes an epitope which is expressed by central and peripheral neurons in hirudinid leeches. The epitope appears to be a reliable marker for the same group of peripheral neurons in all the leech species examined; however, the distribution by central neurons vary considerably between species. In *Haemopis* approximately 20 different CNS neurons are labeled by the antibody whereas 6 small unidentified neurons are recognized in *Hirudo* and only the two pairs of pressure neurons are stained in *Macrobdella*. This pattern of expression is consistent and reproducible within each species. The functional reason for this diversity in expression of the epitope between central neurons in different species is unknown. However, in order to explore these questions we are in the process of analyzing the antigen(s) biochemically. Since the epitope loses its immunoreactivity after SDS-treatment and thus cannot be identified on SDS Western blots we have assayed dot blots after non-ionic detergent extraction. Under these conditions the antigen retains its immunoreactivity and it can be shown that the antibody binding is abolished by treatment with protease K suggesting that the antigen is a protein. Immunoprecipitation, native gels and IEF-techniques are being applied to further characterize the molecular nature of the antigen. We are also using the antibody as a marker for analyzing axon fasciculation of the peripheral neurons.

Supported by NIH grant NS 28857, an Iowa State Biotechnology grant, and an Iowa State University grant.

297.1

DISTRIBUTION OF LAMININ A, B1 AND B2 CHAINS IN THE DEVELOPING MOUSE HINDBRAIN. S.A. Moody and M. Fisher. Dept. Anatomy & Cell Biology, Univ. Virginia School of Medicine, Charlottesville, VA 22908.

Laminin is a large, extracellular matrix glycoprotein that usually consists of three distinct polypeptide chains, A, B1 and B2. Using antisera that specifically recognize each of these chains, and one that recognizes mature basement membrane laminin (EHS), we studied the immunohistochemical distribution of these polypeptides in the hindbrain of the mouse during periods when the longitudinal fascicles of axons are forming and when the postmitotic trigeminal (V) motoneurons migrate. At E9 and 10 the lateral mesenchyme through which V peripheral nerves grow is more intensely stained with all four antisera than other mesenchymal regions. The chain-specific antisera all stained the medial mesenchyme surrounding the notochord very intensely at E10-12, whereas EHS laminin antisera stained this region beginning at E11. At none of the days studied did we detect mature laminin in the parenchyma of the hindbrain. However, at E9 B2 staining was ubiquitous and B1 staining was in cells scattered throughout the neuroepithelium. At E10 all chains were detected in the marginal zone, which contains numerous growing axons and migrating neurons. Staining with B1 was heaviest; staining with B2 did not include the most medial fascicles, and staining with A was the faintest. At E11 B1 and B2 chains additionally were found in commissural axonal paths and the V motor root, whereas A chain was found in a secondary migratory path in the intermediate zone. By E12 B1 and B2 staining in the marginal zone was virtually gone, but staining in the longitudinal axons of the raphe was intense. These results demonstrate that laminin is present in the along neuronal migratory pathways as well as axonal tracts, and is heterogeneous in its chain composition. Perhaps microheterogeneity in the ECM regulates its widespread roles. Supported by NS23158 (SAM) and NS25350 (MF).

297.2

AN INTEGRIN LAMININ RECEPTOR INVOLVED IN NEURITE OUTGROWTH. Ivan de Curtis and Louis F. Reichardt, Dept. of Physiology and HHMI, University of California, San Francisco, CA 94143-0724.

Chick embryo neural retinal cells show extensive neurite outgrowth when cultured on laminin. Cell attachemnt and neurite outgrowth on laminin diminish between embryonic day 6 and 12, when the production of retinal ganglion cells has ceased, and the majority of their axons have entered the target tissue, the tectum. The monoclonal antibody CSAT, against the chick integrin β1 subunit, completely abolishes neurite outgrowth on laminin, and immunoprecipitation of metabolically labeled retinal neurons from day 6 and day 12 embryos shows different profiles of α integrin subunits coprecipitated with β1. The integrin α6β1 is a prominent laminin receptor expressed in the retina. We have cloned the chick α6 subunit and we have studied the expression of this protein in retinal cells. We show that in retinal ganglion cells the loss of the ability to extend neurites on laminin is correlated with a dramatic decrease of both α6 mRNA and polypeptide, suggesting that changes in gene expression account for the developmental regulation of the interactions of these neurons with laminin. In other retinal neurons the level of α6 mRNA and polypeptide remain high, while function is lost, suggesting that integrin function is regulated at the posttranslational level in these cells. Recently we have obtained a polyclonal antibody against a fusion protein containing 70 kD of the amino-terminal extracellular portion of the chick α6 integrin. This antibody reduces dramatically neurite outgrowth by retinal neurons on laminin, providing more direct evidence for the role of this laminin receptor in the retina.

297.3

LAMININ/PROTEOGLYCAN IMMUNOREACTIVITY AND DEVELOPMENTAL LOSS OF REGENERATIVE SPECIFICITY. M.L. Meeker and P.B. Farel. Dept. of Physiol. Univ. N. Carolina Sch. Med., Chapel Hill, NC 27599.

Regenerating lumbar motoneuron axons can specifically reinnervate their hindlimb targets during the first third of larval (tadpole) life in the bullfrog, after which time regenerative specificity is lost. We hypothesize that non-neural structures develop in the hindlimb which prevent regenerating axons from responding to guidance cues. The most likely structure is the Schwann cell basal lamina which surrounds all axons of the peripheral nerve.

Ultrastructural appearance of the basal lamina coincides with the loss of regenerative specificity, consistent with our hypothesis. We have analyzed the immunocytochemical distribution of two major constituents of the basal lamina, laminin and heparan sulfate proteoglycan (HSPG, antibody provided by M.J. Anderson), in relation to neurofilament protein (NF) in tadpole hindlimbs at stages when regenerative specificity is present and after it is lost. Controls for nonspecific staining included omission of the primary antibody and the substitution of a non-immune serum or irrelevant antibody. Even at the earliest stages examined, where no basal lamina is seen ultrastructurally, both laminin and HSPG immunofluorescence are present in regions of NF staining. These observations indicate that the molecular components of the basal lamina are present prior to their organization into a discrete structure. This finding reinforces the need for morphological studies in order to interpret correctly the results of immunocytochemical analyses.

297.4

DISTRIBUTION OF TENASCIN ALONG THE DEVELOPING VISUAL PATHWAY IN CHICK AND MOUSE. R.G. Perez, A. Smetanka*, W. Halfter and M.H. Hankin. Dept. Neurobiology, Anatomy & Cell Science, Schl. of Medicine, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Tenascin (**TN**) is an extracellular matrix protein reported to be a non-permissive substrate for cell migration and neurite outgrowth *in vitro*. We have studied the distribution of TN in developing chicks and mice to determine whether its expression is temporally and spatially related to regions of the visual pathway where the outgrowth of retinal axons is confined to stereotyped patterns. Such crucial "decision" areas include the optic disc, the optic chiasm and tract, and the optic tectum (superior colliculus (**SC**) in mammals) - the principal termination site for optic axons.

In the chick between E4-8, high concentrations of TN were found at the optic disc within the retina, and at the supraoptic commissure. In the optic tectum, TN was expressed prior to neurite ingrowth in a rostral to caudal gradient in the optic fiber layer.

In the SC of the normal mouse, TN was seen at E15 in a narrow band 20-50 μm below the pia. By E16, the staining intensity increased and extended from the pial margin to a depth of approximately 100 μm. By E18, TN expression had decreased in the 100 μm below the pia, but increased in deeper regions of the SC.

These findings show that TN is distributed in regions of the developing visual pathway where optic axons make major decisions regarding directional outgrowth or terminal arborization. The data are consistent with the idea that TN is a non-permissive substrate, and may provide a "stop" signal for retinal axons in the tectum/SC.

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297.5

A GROWTH CONE COLLAPSING ACTIVITY FROM ADRENAL MEMBRANES THAT AFFECTS RETINAL GANGLION GROWTH CONES MORE THAN DORSAL ROOT GANGLION GROWTH CONES. J. L. Baird* and J. A. Raper. Dept. of Anatomy, Univ. of Penn. Sch of Med., Philadelphia PA 19104.

Factors that repulse growth cone advancement may influence pathway choice. When neuronal explants from peripheral and central nervous tissues are cocultured, growth cones will collapse when they encounter neurites of unlike origin. Growth cone collapse can also be induced when plasma membrane extracts are applied to neuronal explants. Membrane extracts of the rat pheochromocytoma cell line PC12 have previously been shown to collapse chick retinal better than DRG growth cones in culture.

We have found that plasma membrane extracts of neonatal bovine adrenals contain a growth cone collapsing activity that, like PC12 extracts, is more active towards retinal as compared to DRG growth cones. This activity is inhibited by low concentrations of several irreversible serine proteinase inhibitors, such as 10μM p-nitrophenyl p'-guanidobenzoate, suggesting that it is a serine proteinase. Retinal growth cones respond to the adrenal membrane extract within 15 minutes of application. When the adrenal membrane extract is washed out of the medium, growth cone collapse is rapidly reversed and treated explants return to control values within 1 hour. Thus the growth cone collapsing activity from neonatal bovine adrenal membranes exhibits several features that are consistent with an activity that may play a role in growth cone guidance. These include differential effectiveness, rapid onset, and termination of action.

297.6

THE BARRIER FUNCTION OF TECTAL MIDLINE GLIAL CELLS AND ITS ASSOCIATION WITH PROTEOGLYCAN DISTRIBUTION. D.-Y. Wu, J. Silver, G.E. Schneider, and S. Jhaveri. Dept. of Brain & Cogn. Sci., M.I.T., Cambridge, MA 02139 and Dept. of Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106.

A group of radial glia, present along the midline of the superior colliculus (SC), may serve as a barrier in the establishment and maintenance of laterality in the retinotectal projection (Wu et al.,'90, SN abstr.). Keratan sulfate proteoglycan (KSPG), a molecule which inhibits axon growth, has also been detected in this midline region (Snow et al., Dev.Biol.,'90). We have examined the growth of retinal axons which abnormally cross the tectal midline after selectively severing the glial processes. The distribution of KSPG was also studied in the SC during normal development and in the cases with lesions.

KSPG is first detected at the tectal midline on E13 just prior to the entry of retinal axons in the SC. Its expression continues at least into early postnatal life. In animals with the midline glia severed, the apical processes degenerate within 12 h, and by 72 h retinal axons have crossed the midline. The crossing is observed only in regions where the glial processes have degenerated. Correlated with this, KSPG immunostaining also disappears from the same regions, whereas the molecule persists along the basal, non-degenerated regions of the glia.

We conclude that 1) the degeneration of apical processes of radial glia in the superior colliculus precedes abnormal growth of retinofugal axons across the midline; 2) KSPG is co-localized with the tectal midline glial processes and 3) KSPG is a likely contributor to the barrier function of the glial cells at the SC midline.

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297.7

A MOVING GRADIENT OF CHONDROITIN SULFATE PROTEOGLYCAN MAY BE A CONTROLLING FACTOR DURING NEURONAL PATTERN FORMATION IN THE DEVELOPING MAMMALIAN RETINA. P. Brittis, D. Canning, and J. Silver, Dept. of Neuroscience, Case Western Reserve Univ., Cleve. OH 44106

We have developed a rat retinal organ culture model to elucidate the roles that different proteoglycans play during neuronal pattern formation. On E12.5, chondroitin sulfate proteoglycan (CSPG) was detected in the marginal zone over the entire vitreal surface of the retina. On E13, the neuroepithelium nearest to the optic fissure loses CSPG. With further development through E16.5, CSPG disappears from the retina in a moving wave that progresses outwardly in all directions. Differentiating ganglion cells and axons, identified with anti-beta tubulin antibodies, are found first in the reduced CSPG region near the fissure. Thereafter, ganglion cells appear sequentially toward the periphery but always in a region of diminished CSPG staining. The moving boundary between high and low CSPG levels is not sharp but, rather, appears as a gradient that dissipates gradually over 75um. Ganglion cells without axons have been found in the distal regions of the gradient. Axons are initiated from endfeet processes near the mid-point of the gradient. As axons emerge they turn away from the high CSPG zone and grow toward the fissure amid other ECM molecules.

Retinal perturbation using chondroitinase resulted in polarity reversals of the ganglion cell bodies as well as gross axon guidance abnormalities with ectopic axons travelling toward the pupil and/or in the wrong level of the retina. Our data suggest that CSPG, and possibly other sulfated proteoglycans, may play several roles during retinal development. First, CSPG may suppress ganglion cell differentiation and axonogenesis and second, by offering an unfavorable substratum, may guide the growth cone away from sites where it is transiently in abundance.

297.8

A ROLE FOR PROTEOGLYCANS IN THE GUIDANCE OF A SUBSET OF PIONEER AXONS IN CULTURED EMBRYOS OF THE COCKROACH. **L.S. Wang and J.L. Denburg. Biology Department, University of Iowa, Iowa City, IA 52242.**

Many of the interactions that proteoglycans make with molecules involved in the development of the nervous system are mediated by their glycosaminoglycan (GAG) side chains. The addition of free GAGs should compete for the proteoglycan binding site, inhibit these interactions and perturb development. It is demonstrated that GAGs perturb axon guidance in cockroach embryos cultured in vitro during the period of growth of several pioneer axons. The specificity of this phenomenon is evident from the observation that of all the GAGs tested only heparin and heparan sulfate produced perturbation. In addition, of the 6 axon tracts being pioneered during the culture period the GAGs alter only 2 of them, the median fiber tract in the CNS and the Til axons in the leg. In the perturbed median fiber tract the pioneer axons no longer grow along the midline. The perturbed Til pioneer axons defasciculate and follow various alternate paths. These results indicate that subsets of neurons may be characterized by their heparan sulfate proteoglycans which in some pioneer axons are involved in selective fasciculation while in others are involved in axon pathfinding.

Supported by NIH grant NS 15350.

297.9

MOLECULAR GRADIENTS ALONG THE PROXIMAL-DISTAL AXIS OF EMBRYONIC INSECT LEGS: POSSIBLE GUIDANCE CUES OF PIONEER AXON GROWTH. J. L. Denburg, B. A. Norbeck* and Y. Feng*. Biology Department, University of Iowa, Iowa City, IA 52242.

The existence of gradients of environmental cues has been proposed to explain the proximal growth of pioneer axons in embryonic legs of insects. Hybridoma techniques have been used to produce 3 monoclonal antibodies that bind to components associated with the basal lamina/extracellular matrix that are distributed in a gradient along the proximal-distal axis of cockroach legs at the time of pioneer axon growth. Two of these, PROD-1 and PROD-2, label the proximal parts of the leg more intensely than the distal ones. The other, DIP-1 has the inverse pattern of binding with the distal parts of the leg labeled more intensely. The gradient distribution of these antigens only exists just before and during the period of growth of the pioneer axons. Western blot analysis has identified the PROD-1 antigens as 4 major proteins of 112, 74, 69, 59 kd, and the PROD-2 antigen as a 225 kd protein. The spatial and temporal distribution of these molecules makes them good candidates for environmental guidance cues of pioneer axon growth.

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297.10

CONNECTIN: A SURFACE PROTEIN EXPRESSED ON A SUBSET OF MOTONEURON AXONS AND THE TARGET MUSCLES THEY INNERVATE IN DROSOPHILA. A. Nose* and C.S. Goodman, Howard Hughes Medical Inst, Dept of Molecular and Cell Biology, U. of California, Berkeley, CA 94720

Each hemisegment of the Drosophila embryo contains 31 or fewer identifiable muscle fibers, each of which is innervated in a highly stereytypic fashion by a small number of specific motoneurons. We have screened over 11,000 enhancer trap lines and have isolated 6-8 lines which express β-galactosidase in small subsets of muscle fibers prior to and during innervation, and are thus good candidates for target recognition molecules which guide motoneuron growth cones.

One of these lines, F400, expresses β-gal in the nuclei of four lateral and one ventral muscle fiber, and also in a small subset of CNS neurons. We cloned and sequenced the F400 cDNA and found that it contains a signal peptide and eight 24-amino-acid leucine-rich repeats (LRR); many proteins with LRR repeats have been implicated in adhesion and/or recognition. In Drosophila, LRR repeats are also found in Toll, chaoptin and slit (interestingly, Toll is one of our other lines). We next generated antibodies against the F400 protein. The protein is expressed on the surface of the same muscle fibers that express β-gal, and on the surface of the motoneuron axons and growth cones which innervate these muscles. It is also expressed on several cells (probably glia) along the peripheral pathway of these motoneuron axons.

These results strongly support the notion that F400 plays a role as a recognition molecule in neuro-muscular connectivity and thus we call this molecule connectin. We are currently conducting a genetic analysis of the function of connectin to test this hypothesis.

297.11

MOLECULAR CHARACTERIZATION OF EMBRYONIC GLIA AND THEIR ROLE DURING GROWTH CONE GUIDANCE IN DROSOPHILA. V.J. Auld, C. Klämbt*, R. Tsoo*, and C.S. Goodman, Howard Hughes Medical Inst, Dept of Molecular and Cell Biology, U. of California, Berkeley, CA 94720

The growth cones that pioneer many of the major CNS axon tracts and nerve roots in the Drosophila embryo extend towards and along specifically positioned glial cells. When these glial cells are eliminated selectively by either laser (in grasshopper) or genetic (in Drosophila) ablation, specific pathways do not form or form abnormally. In order to obtain new molecular lineage markers that selectively label different subsets of glia, and most important, to identify new genes expressed in these glia that might function in the formation of the early axon pathways, we conducted a large scale enhancer trap screen. Over 11,000 enhancer trap lines were screened (representing over 6,000 different genes or at least 1/5 of the genome), and of these, 54 express β-galactosidase (β-gal) in subsets of glia. These 54 lines represent 46 independent sites of insertion: 22 are expressed in midline glia, 10 in nerve root and peripheral glia, and 14 in longitudinal glia. Of particular interest are the four glial cells positioned just outside the CNS (called "exit" glia) that specifically mark the point at which the intersegmental and segmental nerve roots come together before they split into several peripheral axon pathways. Detailed analysis of exit and peripheral glia lines show that some genes are likely to be expressed in only two or a small group of these cells per segment. Several lines are under further molecular genetic analysis to determine the nature and function of their gene products, and the potential role of these genes in establishing the pattern of early axon pathways.

297.12

COMMISSURELESS, A MUTATION IN DROSOPHILA THAT SPECIFICALLY DISRUPTS GROWTH CONE GUIDANCE TOWARDS THE MIDLINE. M.A. Seeger, G. Tear*, D. Ferres-Marco*, and C.S. Goodman. HHMI, Dept of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

To understand the mechanisms by which commissural and longitudinal axon pathways are established in the Drosophila embryonic CNS, we have initiated an F2 genetic screen for mutations that disrupt the formation of these pathways. Our goal is to saturate the Drosophila genome (by screening ~20,000 independent lines with roughly 2-3 lethal hits per chromosome) for mutations that produce specific defects in the major CNS pathways. Mutant phenotypes are identified by staining embryos with MAb BP102 which recognizes all CNS axons.

A relatively discrete number of phenotypic classes have emerged from our initial analysis of >5,000 lines, the most dramatic being a CNS lacking all commissural tracts but with normal longitudinal tracts, peripheral nerve roots, muscles, sensory organs, and body organization. We have isolated two independent mutations in a single gene that produce this phenotype, which we name *commissureless*. Neurons, such as RP1, that normally project their axons across the midline fail to do so in *commissureless* mutant embryos; instead they make an ipsilateral projection that is normal in all aspects except for their failure to cross the midline. The projection of commissural axons across the midline is a property common to arthropods and vertebrates. We suspect that the *commissureless* gene product represents either the signal or the receptor for the guidance of growth cones towards the midline. To test this hypothesis, we have begun a molecular analysis of *commissureless*.

297.13

ROLES OF SECOND MESSENGERS IN AXON INITIATION AND GUIDANCE OF Til PIONEER NEURONS IN THE GRASSHOPPER. K. L. Lankford, Dept. of Molecular & Cell Biology, Univ. California, Berkeley CA 94720.

During normal embryonic development in the grasshopper limb, Til pioneer axons navigate along a stereotyped route to the CNS that includes an initial proximal directed outgrowth and two sharp turns in the region of the Tr/Cx segment boundary. To assess the possible roles of different second messenger systems in specific steering events during pioneer growth cone navigation, I exposed cultured grasshopper embryos to a variety of second messenger agonists, antagonists, and analogues at different developmental stages and for variable lengths of time and examined the resulting Til pathways. Although many treatments affected the fasciculation of the Til axon pairs, axon diameter, or rate of neurite outgrowth, the steering itself was extremely resistant to manipulation. One notable exception was an apparent delay or inhibition of axon initiation caused by very early calcium manipulations, and a misdirection of initial outgrowth in some neurons exposed to transient calcium elevations. A second exception was an increased frequency of anomalous turns and Tr/Cx boundary crossing caused by exposure to cGMP analogues. These effects were very age specific.

297.14

RNA SYNTHESIS DEPENDENCE OF GROWTH CONE PATHWAY CHOICE DURING EMBRYONIC DEVELOPMENT. R. von Bernhardi and M. J. Bastiani, Dept. of Biology, University of Utah, Salt Lake City UT 84112.

The role of transcription in the ability of growth cones to make pathway choices has been largely unexplored. Actinomycin-D was used to examine if synthesis of new mRNA was necessary for 1) neurite outgrowth of pioneer neurons, 2) neurite outgrowth along an established axonal pathway, and 3) change in the direction of outgrowth when the neuron reaches a "pathway choice" point. Grasshopper embryos of 32-33% development were cultured for 24 to 48 hrs in media containing 0.01-0.05 μg/ml of Actinomycin-D (control embryos are cultured in absence of transcription blockers). At this stage the aCC, pCC and Q1 neurons have just initiated axonogenesis . After the culture period, the embryos were fixed, the cells were labeled by iontophoresis with 0.5% Di-I in ethanol, and the extension of their axons was evaluated with standard epi-fluorescence or confocal microscopy.

Inhibition of transcription at a stage when the growth cone approaches its choice point specifically blocks its ability to change the direction of extension. Of 64 aCC axons evaluated, 86% were unable to choose their correct pathway. There is no blocking affect upon either neurite outgrowth of pioneer neurons (Q1, n= 34) or axons that grow along a single pathway without changing their direction of extension (pCC, n= 106). Our results suggest that the extending axon is able to grow for a period of time in the absence of transcription. However, neurons require new mRNA in order to change their direction of growth at a pathway choice point.

Supported by NIH grant NS25378 and the McKnight Foundation.

297.15

A ROLE OF POLYSIALIC ACID IN SPECIFIC AXONAL GUIDANCE. J. Tang*, L.T. Landmesser and U. Rutishauser. Dept. Physiology & Neurobiology , University. of Connecticut, Storrs, CT 06269 and Depts. Genetics & Neuroscience, Case Western Reserve University. School of Medicine, Cleveland, OH 44106.

Axon-axon interactions during early motoneuron axon outgrowth and pathway formation are mediated by a variety of cell adhesion molecules. Polysialic acid (PSA) , a post-translational modification of NCAM, can regulate cell-cell interactions mediated not only by NCAM, but by other cell-cell ligands as well. To test whether modulation of PSA levels was involved in motoneuron axonal pathfinding, we determined the temporal and spatial pattern of PSA expression during motoneuron outgrowth using Mab 5A5, which recognizes highly sialylated NCAM. PSA expression was increased on motoneurons only at stage 23 as their growth cones converged at the plexus region (a region where axons segregate into motoneuron pool-specific groups) and was found to differ dramatically between motoneurons projecting to different targets; specifically PSA was higher on motoneurons that project to dorsal thigh, and lower on those that project to ventral thigh and shank. When PSA was removed by injecting a PSA specific endosialidase into the limb bud during different stages of axon outgrowth, retrograde HRP labeling of motoneuron pools indicated that projection errors had occurred on both the antero-posterior and dorsal-ventral axes. These results indicate that PSA may modulate the interactions of these growing motoneuron axons with guidance molecules, and thereby contribute in a permissive and/or instructive manner to axonal pathway selection.

Supported by NIH grants NS 19640, NS 25557 and HD 18369.

297.16

POLYSIALIC ACID PROMOTES GROWTH OF AXONS ON NEURONAL MEMBRANES BY MODULATING L1 FUNCTION H. Zhang*, R.H. Miller, and U. Rutishauser. Dept. of Neuroscience and Genetics, Case Western Reserve Univ., Clevelend, OH 44106

During development, growing axons reach their targets by interacting selectively with both neuronal or non-neuronal surfaces in their environment. A variety of cell adhesion molecules, such as NCAM, N-cadherin, L1 and integrins appear to promote axonal outgrowth, and regulation of the expression of these adhesion molecules may contribute to formation of specific axonal pathways. We have proposed previously that cell-cell interactions can also be regulated by the presence of an unusual cell surface carbohydrate, called polysialic acid (PSA). PSA, which is attached to NCAM, is strongly expressed by growing axons but is largely lost from the surface of mature fibers.

In the present studies, the influence of PSA on axonal outgrowth has been examined *in vitro* using two substrates, a monolayer of cortical astrocytes and a carpet of neuronal membrane vesicles. Effect of PSA on outgrowth of single embryonic retinal axons on these substrates were studied using a PSA-specific endoneuroaminidase (endo-N) while the molecular basis of this effect was analysed by antibodies that block the function of adhesion molecules. Enzymatic removal of PSA from retinal axons and tectal membrane substrate was found to decrease embryonic retinal axon elongation. This decrease was reversed by antibody against L1 but not by antibodies against NCAM, N-cadherin, or integrins. In contrast, growth of axons on astrocytes was not affected by endo-N. These results suggest that presence of PSA produces optimal retinal axon outgrowth on the neuronal substrate by attenuation of L1-mediated interactions, and that this mechanism of regulation is related to axon-axon but not axon-glial interaction.

297.17

REGIONAL DISTRIBUTION OF NEURAL CELL ADHESION MOLECULES (NCAMs) IN RODENT HIPPOCAMPUS. P. J. Miller*, W-W. Chung, C.F. Lagenaur, and S.T. DeKosky, Depts. of Neurobiology, Anatomy & Cell Science, Psychiatry, and Neurology, Univ. of Pittsburgh Sch. of Med. and Western Psychiatric Inst. & Clinic, Pittsburgh, PA 15213.

Cell surface adhesion molecules NCAM and L1 are implicated in CNS cell migration and axon outgrowth in *in vitro* and *in vivo* developmental studies. The distribution of these two molecules is quite distinct in CNS development, suggesting that NCAMs and L1 subserve different roles in process outgrowth and tissue organization. A variety of NCAM isoforms are known, and individual NCAMs undergo post-translational modification. These changes may mediate development of specific neural cell contacts and circuitry. We evaluated immunohistochemical staining of antibodies to total NCAM, embryonic NCAM, and L1 in rat and mouse hippocampus. Staining patterns in the two species were similar. A distinctive pattern of staining was found, corresponding to the known anatomy of the structure. Total NCAM staining was intense in the hilus and inner molecular layer (ML) of the dentate gyrus with less marked staining in the dentate outer ML. The mossy fiber tract (MFT) was well demarcated. There was abundant staining the stratum radiatum (SR) and stratum oriens (SO) of CA1. Stratum lacunosum-moleculare (L-M) had very little staining. L1 staining was more evenly distributed throughout the hippocampus and dentate, with little hilar staining, no MFT staining, and even staining through the SR, SO, and L-M. Embryonic NCAM had little CA1 staining but strong hilar and MFT staining; thus, this "embryonic" molecule continues to be expressed in limited regions in the adult. These distinctive patterns suggest that the distribution of the NCAM and L1 molecular species have clearcut structural and functional roles in the laminated circuitry of the hippocampus.

297.18

cDNA CLONING OF THE AXON-ASSOCIATED ADHESION MOLECULE AXONIN-1. R.A.Zuellig[1]; C.Rader[1]; A.Schroeder[1]; M.Kalousek[1]; F.v.Bohlen[1]; A.Fritz[2]; E.Stoeckli[1]; E.Hafen[2]; H.U.Affolter[3]; P.Sonderegger[1]. [1]Biochemisches Institut, [2]Zoologisches Institut, and [3]Institut für Hirnforschung, Universität Zürich, CH-8057 Zürich, Switzerland.

Axonin-1 is an axon-associated cell adhesion molecule (AxCAM) occurring both as a membrane-bound and secreted form in the chicken embryo. Recent studies have revealed that it is a potent promoter of neurite outgrowth, when presented as a substratum to cultured neurons. Here we report the molecular cloning and nucleotide sequence determination of axonin-1. About 50% of the amino acid sequence of axonin-1 was determined by Edman degradation of randomly selected peptides generated by enzymatic cleavage. The amino acid sequence of most of the peptides showed homology to F11. The axonin-1 peptides were aligned to F11 and primers for PCR were selected. mRNA from retina of 12-day-old chicken embryos was isolated and first strand cDNA was produced . With PCR a fragment of 560 bp was amplified. It was confirmed to represent a segment of axonin-1 by nucleotide sequencing. Screening of an oligo(dT)-primed cDNA library from 14-day-old embryonic chick brain resulted in the isolation of two different clones with a 2.4 kb and a 4.0 kb cDNA insert, respectively. The nucleotide sequence of both clones was determined. The shorter cDNA overlapped entirely with the 3' part of the longer cDNA. The longer cDNA contained an open reading frame of 3108 nucleotides, 132 noncoding nucleotides at the 5' end, and 727 noncoding nucleotides at the 3' end. The open reading frame codes for a polypeptide of 1036 amino acids including a putative hydrophobic N-terminal signal sequence of 23 amino acids and a C-terminal hydrophobic sequence of about 25 amino acids which may be cleaved at the time of connecting the GPI-anchor. As in TAG-1 of the rat and F11 of the chicken, the predicted primary structure comprises six immunoglobulin-like domains, followed by a short collagen-like segment of 8 amino acids, followed by four fibronectin type III repeats. The following overall sequence identity between axonin-1 and other neural CAMs was found: TAG-1 74.5%; F11 52.7%; F3 51.4%; Ng-CAM 29.2%; L1 27.6%; N-CAM 19.6%. Thus, among chicken AxCAMs, the closest relative of axonin-1 is F11. In view of the high degree of sequence similarity to TAG-1 of the rat, it appears likely that axonin-1 and TAG-1 are species homologues.

297.19

EXPRESSION OF NEURAL CELL ADHESION MOLECULE AXONIN-1 IN DEVELOPING FIBER TRACTS OF THE MOUSE NERVOUS SYSTEM. D.P.Wolfer, A.Beatty*, P.Sonderegger, E.T.Stoeckli and H.P.Lipp. Institutes of Anatomy and Biochemistry, University of Zürich, Zürich CH-8057, Switzerland.

The neural cell adhesion molecule axonin-1 of the chick occurs in two forms, one axonally secreted, one phosphoinositol-anchored to the axonal membrane. It shares 75% of the amino acid sequence with TAG-1 of the rat. *In vitro* it stimulates axonal growth and affects neurite fasciculation. We have used an antiserum raised against axonin-1 purified from chick embryos to study the expression of axonin-1 in the developing nervous system of the mouse.

Starting at embryonic day 8, we find immunoreactivity mainly localized to developing tracts formed by long axons. Axonin-1 first appears in outgrowing peripheral nerves, in ascending tracts and commissural fibres in the spinal cord. Later it can be seen in corpus callosum and anterior commissure, fimbria and alveus hippocampi, fornix, internal capsule, fasciculus retroflexus, as well as in the olfactory and optic tract. At early stages of axon outgrowth, corresponding neuronal somata may be identified (e.g. in motor neurons or projection neurons of the cortex), often, however, somata are unstained. Staining in terminal zones, as seen in cortex, hippocampus or olfactory nuclei, is usually less intense than in related fiber tracts. In the cerebellum, axonin-1 is found within forming parallel fibres and in neurons descending into the granular cell layer. At 3 weeks of age most of the immunoreactivity has disappeared, except for weak neuropil staining in the hippocampus and olfactory bulb.

The tract-related patterns of axonin-1 expression during development are consistent with a role in axoaxonal interactions in developing fiber tracts, as postulated from experiments in culture. *Supp. by SNF Grant 31-027737.89.*

297.20

REGULATION OF NEURONAL DEVELOPMENT BY SURFACE PROTEIN PHOSPHORYLATION. M.V. Hogan*, Z. Pawlowska* and Y.H. Ehrlich. CSI/IBR Center for Developmental Neuroscience, City University of New York, Staten Island, NY 10301

Ecto-protein kinase (ePK) utilizes extracellular ATP to phosphorylate proteins localized at the outer surface of the neuronal plasma membrane. To determine the role of ePK in neuronal development we studied the phosphorylation of surface proteins in CNS neurons cultured from the telencephalon of 7-day chick embryos, and in cloned PC12 cells induced to differentiate with nerve growth factor (NGF). Enzymatic assays were carried out with cells attached to 48-well plates incubated for 10 mins with extracellular [γ-^{32}P] ATP (0.1μM; 15μCi). Phosphorylated proteins were identified by autoradiography of slab gels. In CNS neurons, two surface phosphoproteins (M.W. of 11.7K and 13K) were found to be exclusive substrates of ePK activity. Their extracellular phosphorylation is developmentally regulated and peaks at the onset of rapid neuritogenesis. Stimulators of neurite extension (5nM gangliosides) increased this surface phosphorylation whereas preincubation of the cells with neuraminidase (0.1U) inhibited this activity. In PC12 cells, NGF (50ng/ml) stimulated ePK activity whereas a known inhibitor of NGF-stimulated protein kinase, 6-thioguanine (6-TG), inhibited surface protein phosphorylation in PC12 cells. 6-TG also potently inhibited the phosphorylation of surface proteins by ePK in primary CNS neurons. These results suggest a significant role for ePK in neuronal development. Supported by the AFOSR.

297.21

DIFUSSIBLE FACTORS FROM INNERVATED TARGET REGIONS INFLUENCE OPTIC AXON OUTGROWTH *IN VITRO*. M.H. Hankin. Dept. Neurobiology, Anatomy & Cell Science, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Studies *in vivo* have demonstrated "homing" of optic axons toward the superior colliculus (SC), and a variety of substances have been shown to have trophic effects on retinal ganglion cells and their neurites in tissue culture. Although these studies suggest that target-derived diffusible factors influence optic axons, previous studies using explants of retina and pre-innervation targets have been inconclusive regarding such trophic (or tropic) influences. Recent studies using retinal transplants, however, raise the possibility that optic axons respond to target-derived factors only after the first optic axons have reached the SC (reviewed in Hankin & Lund (1991) *TINS*).

To address this issue, retinae (E16/17) were co-explanted in hydrated collagen gels with SC (E16/17) - a developmental stage after the initial innervation by retinal axons. Cortical co-explants were used as non-target control tissue. During the first 3 days after explanting, very litte outgrowth was seen from retinal explants under any of the conditions. By day 4, however, optic axons in the presence of innervated target and competing non-target regions grew preferentially (although not exclusively) toward targets. Between 4-6 days, individual retinal neurites could be seen to make sharp turns towards innervated targets, but apparently avoided non-target explants.

These data show that target regions can exert trophic effects on optic axons, and they support the hypothesis that the initial optic input to the SC initiates the release of diffusible factors that influence the outgrowth of optic axons.

Supported by NIH grant NS26777.

DEVELOPMENT OF NEUROTRANSMITTER SYSTEMS

298.1

LABETALOL-INDUCED DIRECT AND LONG-LASTING NEUROCHEMICAL CHANGES AFTER POSTNATAL EXPOSURE IN RATS. E.Erdtsieck-Ernste*, M.Feenstra*, M.Botterblom*, G.Boer* (spon:ENA) Neth. Inst. for Brain Research, Amsterdam, The Netherlands.

Interference with the noradrenergic system during brain development was hypothesized to result in an altered functioning of this system in adulthood. Rat pups were injected from postnatal day (PN) 1-10 with the α1- and β-adrenergic antagonist labetalol (2x10 mg/kg SC) or received one injection on PN 10. Labetalol levels and monoamine metabolism were determined with HPLC 90 min after last injection and β-receptors with [125I]CYP 20 h after the treatment in several brain areas. One injection on PN 10 resulted in brain labetalol levels of 2.1 μg/g, PN 1-10 treatment in 3.3 μg/g. Acute as well as chronic labetalol exposure increased NA metabolism (MHPG/NA +20-100%), suggesting that adaptation did not develop. Minor direct effects on 5-HT or DA metabolism were seen. After chronic treatment brain β1- but not β2-adrenoceptors, were up-regulated (30%). Lasting effects, which were measured on PN 60, could not be detected for NA and DA metabolism or for β-adrenoceptors. 5-HT metabolism, however, was strongly increased (20-70%) in adulthood. In conclusion, chronic labetalol exposure during rat brain development did not cause long-lasting changes in β-receptor number or NA metabolism, but was critical for the setting of 5-HT metabolism later in life.

298.2

SEROTONIN-GATED CONDUCTANCES OF AN IDENTIFIED NEURON FROM THE SNAIL *HELISOMA TRIVOLVIS* . C. J. Price and J. J. Goldberg. Dept. of Zool., U. of Alberta, Edmonton, Alta., Canada T6G 2E9.

Serotonin (5-HT) influences the regeneration and development of identified neuron B19 from the snail *Helisoma trivolvis*. This action of 5-HT involves the influx of calcium through voltage-gated calcium channels. The specific mechanism through which 5-HT exerts its effects on B19 membrane potential, however, is unknown. To examine this, tight-seal whole-cell recordings were performed on B19s isolated in cell culture. Bath application of 25 μM 5-HT evoked a robust depolarization (5-20 mV) that persisted as long as 5-HT was present. In voltage clamp, 5-HT induced an inward shift in the holding current level that ranged between 30 and 100 pA (Vhold: -70 mV). To understand the ionic conductances associated with this inward current, a ramp depolarization protocol coupled with ion substitution was used. The ramp protocol involved stepping the membrane potential by 2 mV every 2 s, from -110 mV to -50 mV. The net 5-HT - induced current was calculated by subtracting the the ramp-generated current before 5-HT addition from that generated in the presence of 5-HT. When extracellular Na^+ was replaced by choline, the 5-HT - induced current was outward at potentials negative to the calculated K^+ equilibrium potential (E_K), and inward at potentials positive to E_K, suggesting that serotonin closes a K^+ conductance that is normally open at rest. When K-Asp was substituted by N-methylglucamine in the pipette, and extracellular Na^+ was normal, another 5-HT - induced current was revealed. While this current was inward throughout the voltage ramp, its amplitude decreased at more positive voltages. Extrapolation of this curve indicated a reversal potential quite positive to 0 mV, suggesting Na^+ selectivity. Therefore, the depolarizing effect of 5-HT on neuron B19 involves both the closure of a K^+ conductance and the opening of a Na^+ conductance.

Supported by NSERC Canada.

298.3

ORGANIZATION OF PROJECTIONS FROM RAPHE NUCLEI TO SENSORY CORTICES IN PERINATAL RATS? M.J. Leslie, N. L. Chiaia and C.A. Bennett-Clarke. Dept. of Anatomy, Medical College of Ohio, Toledo, OH, 43699.

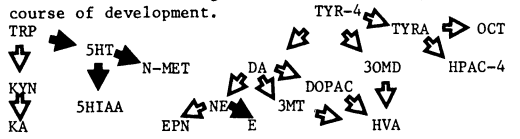
We have shown that the dense and precisely patterned serotonin (5-HT) immunoreactivity (IR) present transiently in the developing somatosensory (S-I) and visual (area 17) cortices arises from nucleus raphe dorsalis (NRD) and perhaps also the median raphe nucleus (MRN). We undertook this experiment to determine whether the patterned 5-HT-IR seen in the sensory cortices of perinatal rats arises from topographically organized projections from NRD and MRN. Neonatal rats (P-5) received injections of rhodamine (RB) and fluorescein (GB) labelled beads into the right parietal cortex, diamidino yellow (DY) into the left parietal cortex, and true blue (TB) into the left occipital cortex. Only cases demonstrating retrogradely filled cells in appropriate portions of the ventrobasal and dorsal lateral geniculate nuclei were analyzed. An average of 90 NRD cells and 42 MRN cells were labelled after parietal cortical injections. The respective values were 92 and 60 after occipital cortical injections. Ten percent of retrogradely filled cells from paired parietal cortical injections contained both GB and RB, with 78% of these located in NRD and 22% in MRN. Six percent of cells retrogradely labelled from paired injections of parietal and occipital cortex contained both DY and TB, 84% of these were located in NRD and 16% were in MRN. Only 2 bilaterally projecting cells were noted. In several cases, brainstem sections were also immunocytochemically stained for 5-HT; 61% of the back-filled cells were immunoreactive. Patterns of retrograde labelling for these neurons did not differ significantly from those reported above. These results indicate that the dense and patterned 5-HT-IR in developing sensory cortices is not the result of topographically organized raphe-cortical projections. DE07734, DE08971

298.4

DYNAMICS OF NEUROTRANSMITTER METABOLISM IN THE CENTRAL NERVOUS SYSTEM OF FROGS. NAOKUNI TAKEDA & HIDEO TAKAOKA * Department of Biotechnology, COSMO Research Institute, Satte, Saitama, 340-01, JAPAN.

The dynamics of neurotransmitters including biogenic amines, neuroactive amino acids and acetylcholine were examined with the progress of embryonic development and metamorphosis. These compounds were analysed by three dimension coulometric HPLC system (ESA Inc., USA) and HPLC system with ECD using enzyme column.

The main species were Rana japonica, Bufo bufo japonica and Xenopus laevis. In Rana, the main metabolic pathways in monoamines during embryonic development (⇨) and metamorphosis(➡)are shown below. With the development of hind limb, E, 5HT, 5HIAA and N-methylserotonin (N-MET) detected. In Xenopus, 5HT-MEL pathway was found in larvae. In adults, N-MET, EPN and OCT disappeared. Acetylcholine and amino acids also changed characteristically during the course of development.



These results will be discussed from the view points of ontogeny and phylogeny.

298.5

EMBRYONIC GLIAL CELLS EXPRESS 5-HT RECEPTORS AND EXHIBIT REGIONAL DIFFERENCES IN NEURONOTROPHIC ACTIVITY IN RESPONSE TO 5-HT. J. Liu*, J. Raymond*, H. Tamir, D. Millhorn and J.M. Lauder. Univ. N.C. Sch. Med., Chapel Hill, NC 27599-7090

In the embryonic rat brain, 5-HT neurons appear to influence the early differentiation of target cells such as dopamine (DA) neurons of the substantia nigra (SN). Since astrocytes can promote growth of DA neurons in a regionally specific manner, express 5-HT receptors, and neuronal growth factors in response to 5-HT agonists, we have asked whether 5-HT neurons may regulate prenatal neurogenesis by mediating region-specific interactions of neurons with glia. In support of this hypothesis we have found different effects of glia from E14 raphe (RN) and SN on the growth and survival of 5-HT and DA neurons, in response to 5-HT. 5-HT1A and 5-HT1C receptor mRNAs were localized in RN and SN glia using combined in situ hybridization (oligonucleotide probes) and GFAP immunocytochemistry. Immunoreactivities for 5-HT1A and 5-HT1C/2 were also found in these cells suggesting that receptors are being synthesized. These results suggest that embryonic glia may produce region-specific neuronotrophic activities following activation of 5-HT receptors.

298.6

FIRST DESCRIPTION OF THE CENTRAL CATECHOLAMINE (CA) SYSTEMS IN 6-8 WEEK-OLD HUMAN EMBRYOS. **Zečević N., Verney C.*, Milošević A. and B.Berger*. Inst.for biol.res. Belgrade 11000, Yugoslavia and *INSERM, U106, Paris,France.**

Dopamine and noradrenaline systems were identified with antibodies raised against tyrosine-hydroxylase (TH) and dopamine-B-hydroxylase (DBH) in human embryos of 6-8 gestational weeks (g.w.) and 9-11 g.w. fetuses. In 6 week-old embryos, only a few DBH-IR cels and fibers were observed in the myelencephalon and pons, while the diencephalon only contained labeled fibers. In contrast, numerous TH-like reactive (TH-IR) neuronal cell bodies and fibers were seen in the myelencephalon, pons (locus coeruleus), ventral mesencephalon (prospective ventral tegmental area and substantia nigra) and ventral diencephalon (hypothalamus). TH-IR fibers reached the intermediate zone of the latero- and rostroventral telencephalon only from 8 g.w. on, following the gradient of cortical plate development. At 11 g.w., TH positive axons had penetrated the subplate layer of the telencephalon running rostrocaudalwards, but did not reach the most posterior areas. Very few DBH-IR axons were present in the same areas and layers. Presently 6 g.w. is the earliest age of CA systems identification in the human CNS.

298.7

PRENATAL ADRENERGIC RECEPTOR BINDING SITES IN RAT CEREBRAL CORTEX **X.-K.Gao,A.J.Friedhoff,J.C.Miller, and K.A.Bonnet, Millhauser Lab.,Dept.of Psychiatry,New York Univ.Sch.of Med.,New York, NY 10016**

Prenatal adrenergic alpha-1, alpha-2, and beta receptor binding sites in fetal rat cerebral cortex are detected respectively by [3H]-prazosin, [3H]-paraaminoclonidine (3H-PAC) and [3H]-dihydroalprenolol (3H-DHA). Some degree of specificity of ligand binding sites can be detected early in gestation. Specifity of [3H]-prazosin binding sites appears on GD15, but does not exhibit good kinetic properties until GD20. Specificity of [3H]-PAC binding sites is seen early in GD13 and exhibits mature kinetic properties by GD14. The density of binding sites increases two-fold by GD20. Specificity of [3H]-DHA binding sites also appears early in GD12-13, and has some degree of kinetic properites by GD15-16 and has relatively mature kinetic properties after GD16. These different receptor subtypes have different maturation courses from the protoreceptor to the mature receptor.

298.8

COUPLING BETWEEN NEURONES OF THE DEVELOPING RAT LOCUS COERULEUS REVEALED BY INTRACELLULAR INJECTION OF BIOCYTIN. M.J.Christie and H.F. Jelinek*. Department of Pharmacology, The University of Sydney, N.S.W. 2006 AUSTRALIA.

Simultaneous intracellular recordings from pairs of locus coeruleus neurones in neonatal rat brain slices revealed synchronous, rhythmic oscillations of membrane potential (rats less than 27 days old) and electrotonic coupling between 40% of pairs of neurones (rats less than 10 days old; Christie et al., J.Neurosci., 9: 3584, 1989). In the present study, slices from rats 1 to 20 days old were stained with avidin-HRP (Vector Labs.) only if the first neurone impaled was maintained for longer than 10 min with an electrode (30 - 50 Mohm) containing biocytin (2%, Sigma). In slices from rats 1 to 10 days old, multiple stained neurones (3.7 ± 0.6 neurones/slice) were observed in 12 of 17 slices studied. Contacts between stained neurones were observed at varying distances along dendrites. In rats older than 10 days multiple stained neurones were observed in 2 of 14 slices studied (2 neurones/slice). The presence of multiple stained neurones was not correlated with the frequency of rhythmic oscillations, cell input resistance, or the shape of electrotonic potentials. These results suggest that low resistance pathways between locus coeruleus neurones are common in brain slices from rats less than 10 days old, consistent with electrotonic coupling studies. However, this coupling cannot fully explain synchronous rhythmic activity, because the latter persists until at least the 27th postnatal day.

298.9

ONTOGENY OF SENSITIVITY TO NEUROMODULATORS IN RAT NEOCORTICAL NEURONS. Lorenzon, N. M. and Foehring, R. C., Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38103-4901.

We tested the hypothesis that rat neocortical neurons from developing (PND 6-28) and adult rats are differentially affected by noradrenergic (NE) and muscarinic agonists. Intracellular recordings were obtained from an in vitro slice preparation.

Action potential width decreased and the rate of action potential repolarization increased over the first 2-4 postnatal weeks. Input resistance also decreased during development. We observed the same AHP components (fast, medium, and slow) in adult and immature neurons. However, in several of the 1-2 week old rats, an extremely long slow AHP (> 4 s) was observed after repetitive firing which was not seen in the adult rats.

In adult neurons, 10-100 μM NE or muscarinic agonists reversibly reduced the amplitude of the medium and slow AHPs after repetitive firing. This led to an increase in the f-I slope and reduced spike frequency adaptation. Neurons at all ages responded to NE and muscarinic agonists and similar reductions in the medium and slow AHPs were observed at all ages tested (PND 6-28). Both NE and muscarinic agonists eliminated the late slow AHP that was seen in some of the immature neurons resulting in AHPs which resembled those of the adult neurons.

Most neurons tested were regular-spiking pyramidal cells located in layers II-V (confirmed from biocytin filled cells). We also sampled burst-firing and fast-spiking neurons from animals two weeks to adult in age. Supported by NINDS grant #R29NS27180.

298.10

POSTNATAL DEVELOPMENT OF THE PROENKEPHALIN SYSTEM IN THE RAT HIPPOCAMPUS: DETECTION BY IN SITU HYBRIDIZATION AND IMMUNOCYTOCHEMISTRY. D. D. Song and R. E. Harlan. Dept. Anatomy, Tulane University School of Medicine, New Orleans, LA, 70112.

Immunocytochemistry (ICC) and in situ hybridization (ISH) was performed on sections through the hippocampus on embryonic day 14 (E14), E16, E18, E20, postnatal day 0 (P0), P5, P10 and P15. Antisera to synenkephalin (syn-enk) and to methionine enkephalin (met-enk) and a PPE cDNA were used. PPE mRNA, syn-enk and met-enk first became detectable by P5 in the hippocampus. Cell bodies expressing PPE were located predominantly in the molecular layer (ML) of hippocampal fields CA1 and CA3 and in the hilar margin of the granular layer (GL) of the dentate gyrus (DG). A few labeled cell bodies were also detected in the oriens (OL) and pyramidal cell (PCL) layers of the hippocampus and the polymorph layer (PL) of the DG. Scattered immunoreactive fibers were detected in the medial margin of the fimbria, the ML of the DG, and the ML and OL of fields CA1 and CA3. By P10 there was a general increase in the numbers of PPE labeled cell bodies and immunoreative fibers in the above regions. Additionally, cells expressing PPE were found in the PCL and OL of fields CA3 and CA1 and the ML of the DG. A few scattered immunoreactive fibers in the PCL of CA1 and CA3 and in the PL of the DG also became detectable at this age. However, there was a sharp decline in the number of PPE expressing cells in the GL of the DG. At P15 no remarkable changes from P10 were detected. No significant differences were found in the results obtained by the syn-enk antiserum over that of the met-enk antiserum. No cells were detected by ICC with either antisera, despite heavy labeling of cell bodies for PPE by ISH. These results indicate expression of the enkephalinergic system is postnatal in the rat hippocampus. Supported by NS 24148.

298.11

DEVELOPMENT OF NEUROPEPTIDE EXPRESSION IN RAT SYMPATHETIC GANGLIA. S. Tyrrell, J. S. DeJonge and S. C. Landis. Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

Many neurons contain one or more neuropeptides which can modulate neuronal transmission. Although neuropeptide distribution and colocalization in the mature autonomic nervous system are well characterized, their patterns of expression and the factors which regulate them in the developing nervous system are incompletely understood. Using immunocytochemistry, we have examined the development of several neuropeptides in two sympathetic ganglia, the stellate and superior cervical ganglia (SCG), which lie in the thoracic and cervical regions respectively and innervate overlapping but distinct target tissues. Neurons in the embryonic stellate ganglia contained immunoreactivity (-IR) for neuropeptide Y (NPY), vasoactive intestinal peptide (VIP) or leucine-enkephalin (L-Enk). In addition, calcitonin gene related peptide (CGRP) was found in a subset of VIP-IR neurons. With the exception of L-Enk, all these peptides were present in neurons of the adult stellate ganglion. In the SCG, NPY, L-Enk and a small number of VIP-IR neurons were present in both developing and adult ganglia. Substance P-IR was not detected in neurons from either ganglion. There were significant alterations in the proportion of neurons possessing immunoreactivity for the different peptides. NPY-IR was first seen on E15 and the percentage of NPY-IR neurons steadily increased until adult levels (approximately 50%) were reached at E19. In contrast, the number of VIP-IR neurons decreased between E14 and E18. Furthermore, while L-Enk-IR neurons were present in both SCG and stellate ganglia early in development, L-Enk-IR neurons are found in adult SCG but not adult stellate neurons. These data indicate that there are changes in neuropeptide expression in sympathetic neurons during the period when they become post-mitotic, become innervated and begin to contact their targets.

298.12

Characterization of neurons differentiating in cultures of mammalian neural crest cells. Matsumoto, S.G.* and J.F. Brons Dept. Anat. SD OHSU Portland, OR 97201

Collectively the cells of the neural crest give rise to all of the neurons of the sympathetic, parasympathetic and enteric systems and the sensory neurons of the dorsal root ganglia as well as many of the cranial sensory neurons. These neurons possess different classes of voltage-sensitive and ligand-gated channels, neuro-transmitters and have specific growth factor requirements. Neurons differentiating in cultures of neural crest cells are heterogeneous in their morphology, transmitter expression and growth factor (eg. NGF, CNTF) dependence. There is evidence for NGF/CNTF-dependant and -independant neurons. The neurons display little variation in their physiological traits. For example, when crest cells are grown alone the neurons that differentiate are devoid of neurotransmitter receptors commanly found on crest-derived neurons *in vivo*. Thus there is no evidence for receptors for ACh, 5-HT, NE, DA, ATP or glutamate. Both nicotinic and muscarinic-mediated ACh responses are detected however, when the crest cells are co-cultured with ganglionic non-neuronal cells dissociated from adult sympathetic ganglia. Experiments are in progress to further investigate the possible role of target cells and ganglionic non-neuronal cells in inducing neuro-transmitter receptors.

This study was supported by NIH grant R29NS25644 (SGM)

298.13

DIFFERENTIATION OF PROLACTIN AND GROWTH HORMONE SECRETING CELLS IN THE RAT ANTERIOR PITUITARY. S.-W. Kim, A.E. Morris*, L.A. Chasin+ & S.A. DeRiemer. Dept. of Biological Sciences, Columbia Univ., NY NY 10027.

The growth hormone secreting somatotrophs (ST) and prolactin secreting mammotrophs (MT) differentiate from a common precursor cell. There exists a third cell type in the pituitary which secretes both prolactin and growth hormone called a somatomammotroph (SMT). It has been proposed that these cells are intermediate in the development of some, if not most, STs and MTs. We are addressing this question by characterizing the functional properties of these three cell types and their developmental regulation. We have developed a procedure for producing enriched fractions of each type of cell using discontinuous and continuous Percoll gradient separation. Cells were identified by immunofluorescence using antibodies to prolactin (PRL) and growth hormone (GH) or with a sequential reverse hemolytic plaque assay. At day P5, 46%±16 of the total cells in the pituitary are recovered in the ST-SMT-MT fraction. A similar percentage is found in the adult (45±16). The ratio of SM:SMT:MT cells, however, changes from 5:84:12 at day P5 to 60:1:39 in the adult. When the GH positive cells are further separated they distribute into three density classes (1.062, 1.069 and 1.075). Binding of the G-protein (G_i) activator cholera toxin has been used to distinguish ST and MT cells. We have compared GH secretion by the two denser classes in the presence and absence of the other G-protein regulating toxin, Pertussis Toxin (PTX). The mean and the distribution of basal plaque sizes were the same in both cell types. Pertussis toxin had both inhibitory and stimulatory effects on both cell classes, but the relative magnitudes differed. In the 1.075 cells the predominant effect was inhibition while secretion was enhanced in the lighter fraction. We are continuing to characterize the properties of SM, SMT and MT cells. In addition, we are correlating the changes in the differentiation state of these cell populations with the expression of transcription factors known to regulate the GH and PRL genes. Supported by NSF grant #DCB-86-15840.

298.14

Neonatal exposure to GABA-transaminase inhibitor: Effects on behavior and neurochemistry in rat. T. Taira, E.R. Korpi & T. Porkka-Heiskanen. Univ. of Helsinki, Dept. Physiology and Research Laboratories, Alko Ltd, 00170 Helsinki, Finland.

Effects of GABA-transaminase inhibitor ethanolamine O-sulphate (EOS) (200mg/kg/d IP during postnatal days 3-21) were studied in rats. EOS increased the amount of REM-sleep (152% of control, $p<0.01$) on postnatal day 7. At the age of 1-4 months the EOS rats showed reduced activity in the open-field test (60% of control $p<0.01$) and increased voluntary alcohol intake (161% of control, $p<0.05$). Maximal GABA-stimulated [^{3}H] flunitrazepam binding was decreased (72% of control, $p<0.05$) in cerebral cortex and EC_{50} value for stimulation increased (203% of control, $p<0.05$) in hippocampus of the EOS rats. No changes were seen in the hypothalamic monoamine concentrations.

The results suggests that during the EOS treatment the central GABAergic mechanisms may be permanently desensitized by elevated GABA concentrations. On the other hand, after the EOS treatment there may prevail enhanced GABAergic tone, which could explain the down-regulation of the coupling between the GABA and benzodiazepine binding sites.

298.15

THE EFFECTS OF Ca^{2+} ON HISTAMINE RELEASE IN THE CEREBRAL CORTEX OF SPRAGUE DAWLEY RATS.

B.Washington, K.Y. Nguyen, S.Williams-Scott, M.O. Smith, T.J. Robinson and R.F. Ochillo. Labs. of Pharmacology and Toxicology, Biomedical Research Center, Xavier University of Louisiana, New Orleans, LA 70125.

There is growing evidence that calcium ions (Ca^{2+}) are involved in the release of histamine in the brain. Also, histamine has been postulated to be a neurotransmitter in the central nervous system (CNS). The purpose of this investigation was to use a technique, combining microdialysis and high performance liquid chromatography (HPLC), to investigate the effects of Ca^{2+} on the release of histamine in the cerbral cortex of conscious freely-moving Sprague Dawley rats using diltiazem, a specific Ca^{2+} channel blocker, as a tool. This method involves implanting a dialysis probe stereotaxically into the cerebral cortex. Three to 5 days following surgery, the cerebral cortex was perfused with an isotonic artificial cerebrospinal fluid (ACSF) or ACSF containing diltiazem (0.5µg/µl) at a flow rate of 1.2 µl/min for 120 minutes. The analysis of the perfusate for histamine was done using a modified HPLC procedure published previously (Washington et al., 1991). The results showed that diltiazem reduced control levels of histamine from 92 ± 7.39 pmols/5 µl to 50 ± 4.1 pmols/5 µl in perfusate within 75 minutes. This study adds additional evidence that the release of histamine in the cerebral cortex is calcium dependent. (Supported by RCMI grants #SRCR, 1 G12 RR050750 & #RR08008 from NIH).

298.16

CHANGES IN THE ELECTRICAL PROPERTIES OF CHICK CILIARY GANGLION NEURONS DURING DEVELOPMENT

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Electrically mature neurons of the chick ciliary ganglion (stages 40-44) are known to express TTX-sensitive sodium currents (I_{Na}), high threshold (L-type) calcium currents (I_{Ca}), several calcium dependent potassium currents [$I_{K(Ca)}$] and a group of voltage dependent potassium currents (I_A, I_{DR}). In order to study the development of the electrical properties of these neurons, whole cell recordings were made at embryonic stages 26, 30, 35 and 40 from acutely dissociated cells. This ensured that the developmental changes observed occured *in ovo*. Stage 26 is a time at which the cells have just undergone terminal mitosis. By stage 40 the cells have formed synapses with their target tissue and naturally occurring cell death is nearly complete. Densities of various ionic currents were determined from the ratio of peak current to the cell surface area. Neurons at stage 26 were found to express I_{Na}, I_{Ca} and I_{DR} . The potassium currents I_A and $I_{K(Ca)}$ when present, were barely detectable. Nevertheless these cells could be made to fire regenerative spikes under current clamp conditions. Most of the currents were found to increase in density between stages 26 and 40. However the increase in $I_{K(Ca)}$ and I_A was especially dramatic between stages 30 and 35. This coincides with the time when these neurons form synapses with their target tissues. In contrast, no significant change was observed in I_{DR} density during embryonic development.

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DEVELOPMENT: VOLTAGE-GATED CHANNELS

299.1

SLOW POSTSYNAPTIC K^+ CURRENT OF *APLYSIA* LUQ NEURONS IN CULTURE.

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We have studied synaptic mechanism of co-cultured neurons dissociated from the abdominal ganglion of *Aplysia californica*. Puffing acetylcholine (Ach) or FMRFamide on left upper quadrant (LUQ) cells *in vivo* was found to elicit slow IPSP response, possibly involving 'S' like K^+ current mediated by a G protein (Brenzina, J. Physiol., 407, 1988). We found neuron L10, L12 or L13 made specific synapses onto an LUQ cell in culture, exhibiting similar slow IPSP of the postsynaptic LUQ cell by repetitive firing of the presynaptic neuron.

Tissue culture procedures were used as described by Schacher et. al. (J.Neurosci., 3, 1983), and standard two-electrode voltage-clamp techniques were used.

The reversal potential of the slow postsynaptic current shifted with altered extracellular K^+ concentration. The K^+ current was partially blocked by extracellular application of 4-aminopyridine, but not blocked by tetraethylammonium and apamin. Since bath application of Ach or FMRFamide partially desensitized the K^+ current, a presynaptic cell is thought to release both Ach and FMRFamide simultaneously. Pressure injection of GTP-r-S into the postsynaptic cell occluded the K^+ current, eliciting outward current. These results suggested that the K^+ current had similar characteristics to that found *in vivo*.

299.2

VOLTAGE SENSITIVE CALCIUM CHANNELS DEMONSTRATE A PRECISE NEURODEVELOPMENT PATTERN IN MOUSE CORTEX.

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The development of voltage sensitive calcium channel (VSCC) types shows a critical period in Swiss Webster mouse neurodevelopment (Grover et.al., 1990, Litzinger et.al., 1990). In mouse whole brain omega conotoxin (*w*-CgTx), believed to mark the presynaptic N type VSCC, and PN-200, a 1,4-dihydropyridine which marks L type VSCC's, have shown a rapid period of increase of 40 - 50% in postnatal days 10 - 15. This critical period parallels that observed by Himwich in 1962, and corresponds to dendritic sprouting, increase in cortical width and weight and maturation of EEG potentials.

Previous experiments in our lab have shown that regional binding differences between days 11 - 14 are due predominately to cortex binding site increase. The purpose of this study was to further define the specific days of developmental increase of *w*-CgTx binding. Our most recent data shows 40% increase during a precise 48 hour period - postnatal days 11 and 12. This narrowed cortical period suggests a developmental time frame to begin to look for potential maturational factors for central nervous system VSCC development. This work was sponsored by NICHD K08 00886-02.

299.3

WHOLE CELL RECORDING OF VOLTAGE ACTIVATED CURRENTS IN EMBRYONIC *XENOPUS* SPINAL NEURONS DURING DEVELOPMENT *IN VIVO*.

M.G. Desarmenien and N.C. Spitzer. Department of Biology and Center for Molecular Genetics. UCSD, La Jolla CA 92093.

Analysis of the differentiation of excitability has been facilitated by studies of *Xenopus* spinal neurons developing *in vitro*. Although action potentials are similar to those recorded *in vivo*, currents and perturbation experiments in culture have not been compared with recordings *in situ*. We have applied the whole cell patch-clamp technique in a freshly isolated spinal cord preparation to study the mechanisms of development of voltage-activated currents in neurons in their natural environment. Interneurons in the dorsal anterior cord were recorded under direct visual control. Sodium current served both as a neuronal marker and to demonstrate adequate control of membrane potential.

As predicted by previous studies performed on primary cultures, inward calcium and sodium currents predominate in young cells. The density of outward potassium currents increases during the first embryonic day, and they eventually largely overcome calcium currents. These findings are in agreement with the observation that action potentials are long lasting and Ca/Na-dependent in young *Xenopus* spinal neurons whilst they are short and predominantly Na-dependent in mature cells. The development of the delayed rectifier potassium current (I_{K_V}) is completed during the first day; the current density increases more than 3 fold and the rate of activation is accelerated by more than 2 fold.

In culture, the acceleration of the rate of rise of I_{K_V} is dependent on calcium influx triggered by action potentials during a critical period and on the activation of the calcium- and phospholipid-dependent protein kinase (PKC). Intracellular application of protein kinases and phosphatases during recording from young and mature neurons can now be used to study the metabolic pathways controlling the development of voltage-dependent currents *in vivo*.

Supported by the CNRS (MGD) and NS25916 (NCS).

299.4

EXCITATORY AMINO ACID DISTRIBUTION DURING DEVELOPMENT OF ELECTRORECEPTORS IN EIGENMANNIA (GYMNOTIFORMES).

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Weakly electric, South American glassknife fish of the genus Eigenmannia were bred under laboratory conditions. To study the uptake of excitatory amino acids, 1-3µl solutions of tritiated Glutamate, Taurine, GABA, Aspartate, Glycine and ß-Alanine (activities: 1µCi/µl) were injected subcutaneously into animals of various ages. After survival times of 1-9 hours, fish were processed for autoradiography. Glutamate, Taurine and Aspartate were strongly concentrated in the supporting cell layer of tuberous electroreceptor organs (TO), whereas no uptake was observed for GABA, Glycine and ß-Alanine. Aspartate was taken up by ampullary organs (AO) even after shorter survival times, whereas Glutamate and Taurine-uptake was only observed after longer survival times (>4hrs), suggesting a slower uptake mechanism in AO. Pre-injection of the Glutamate-uptake blockers Ibotene, AP-4, AP-5, CNQX and MK 801 [10^{-4}M] inhibited Glutamate-uptake, indicating the existence of NMDA and quisqualate controlled receptors. In contrast to ß-Alanine, GES [10^{-2}mg/ml] blocked Taurine- uptake. Since AP-4, AP-5, Ibotene and GES did not block the uptake of Glutamate and Taurine in primordial electroreceptors, it is suggested that the uptake mechanism is not yet specific at this early stage.

299.5

MECHANISM OF NA CHANNEL INDUCTION BY NGF AND FGF IN PC12 CELLS. G. D'Arcangelo, D. Shepherd*, P. Brehm, S. Halegoua* and G. Mandel*. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) elaborate a sympathetic neuron-like phenotype in PC12 cells, which includes neurite outgrowth and the establishment of a Na^+-based action potential. NGF-treatment results in an increase in the density of functional Na channels which correlates with the induction of Na channel type II-specific mRNA. We show here that the addition of NGF and bFGF to PC12 cells leads to the induction of at least three Na channel mRNAs of different sizes. Two of the three transcripts encode the previously identified rat brain type II Na channel : levels of these mRNAs increase three to five fold within 24 hrs and remain at this level over the next several days. The third and largest transcript, to be further characterized, is regulated with a distinct time course: this mRNA is rapidly induced five to seven fold within 5 hrs of NGF treatment and declines to two to three fold over the next several days. We have been investigating whether induction of Na channel mRNA is regulated by signal transduction pathways mediating other aspects of NGF and bFGF action. We have found that the non-receptor tyrosine kinase $pp60^{c\text{-}src}$ as well as the GTP binding protein $p21^{c\text{-}ras}$ mediate NGF- and bFGF-induced neurite outgrowth in PC12 cells. Both A-kinase and C-kinase mediate at least some NGF actions. The A-kinase, but not $p21^{c\text{-}ras}$ has been suggested to mediate the NGF induction of Na channels measured electrophysiologically (Kalman *et al*, 1990, Neuron 2, 355). However, a role for cAMP in this NGF response has been refuted (Pollock *et al*, 1990, J. Neurosci 10, 2626). To resolve the mechanism of Na channel induction by neuronal growth factors at the molecular level, we have created unique, permanent PC12 sublines expressing inducible oncogenic forms of either Src or Ras proteins, an inducible dominant inhibitory form of Ras, or expressing high, blocking levels of the A-kinase inhibitor protein. The kinetics of induction of Na channel mRNA and Na channel activity by growth factors, oncogenes or by cAMP elevation in these cell lines will be presented. This work was supported by NIH grants to G.M. and S.H.

299.6

cAMP-DEPENDENT PROTEIN KINASE IS REQUIRED FOR INDUCTION OF FUNCTIONAL SODIUM CHANNELS BY NGF David D. Ginty[1], John A. Wagner[1], and Robert A. Maue[2], [1]Dept of Biological Chemistry and Molecular Pharmacology, Harvard Medical School and Dana Farber Cancer Institute, Boston, MA 02115 ; [2]Dept of Physiology, Dartmouth Medical School, Hanover, NH 03756.

The molecular mechanisms underlying the biological effects of nerve growth factor (NGF) on the electrical membrane properties of neuronal cells are not well understood. To determine whether cAMP-dependent protein kinase (A kinase) activity is necessary for the NGF-mediated induction of voltage-dependent sodium (Na) channels in rat pheochromocytoma (PC12) cells, whole-cell patch clamp recordings and Northern Blot hybridization studies were used to analyze Na channel expression in a variety of A kinase-deficient PC12 subclones we have created. AB-11 and 123.7 cells (Ginty et al., JBC in press), stably transfected with genes encoding inactive mutant forms of the type I regulatory subunit of A kinase (courtesy of S. McKnight, Univ. of Wash.; JBC (1987) 262:13111), as well as A126-1B2 cells, a chemically mutagenized subclone (Mol. Cell. Biol. 5(1985):1984), all responded to NGF treatment, as evidenced by increased neurite outgrowth, cell size, changes in gene expression (EGR-1, fos, GAP-43, ODC), and protein phosphorylation. In normal PC12 cells, the percentage of cells exhibiting large Na currents, the magnitude of the Na currents, and the Na current density all increased ~ 5-fold in response to NGF. In contrast, there were no increases in the expression of functional Na channels in the AB-11 and 123.7 cells treated with NGF, and only small increases in the NGF-treated A126-1B2 cells. Northern Blot analysis of mRNA from control and NGF-treated cells revealed 3- to 5-fold increases in the steady state levels of Na channel mRNA in both A kinase-deficient cells (AB-11 and 123.7) and in normal PC12 cells. The results indicate that A kinase activity is necessary for the NGF-mediated induction of Na channel expression at a postranscriptional level. In addition, when coupled with the biochemical differences between the A1261-B2 cells and the AB-11 and 123.7 cells, the results may also suggest differential roles for the two isozymes of A kinase in mediating Na channel expression and the biological actions of NGF during neuronal differentiation.

299.7

EXPRESSION OF M-RNA FOR A NONALPHA NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT IN THE DEVELOPING MOUSE BRAIN. T.R. Podleski and A. Wo*. Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

We are studying the regulation of the synthesis of the receptor subunits for the nicotinic acetylcholine receptor in the developing mouse brain. We have cloned the nonalpha2 subunit from a c-DNA library of a 20 day old mouse brain. We have isolated, and sequenced the mouse c-DNA. This c-DNA is about 2kb in length; it is missing portion of the 5' end, and it is approximately 500bp longer on the 3' end compared to the rat. It is 96% identical to the rat at the amino acid level. We have labelled this c-DNA with ^{35}S d-CTP, and have used it for *in situ* hybridization studies on mouse brains, beginning on prenatal day 13, and extending through to the adult. In the youngest brains studied, prenatal day 13, positive hybridization was obtained throughout the intermediate zone (IZ) with no binding above background in the ventricular zone. All cells in the IZ appeared to be labelled to approximately the same extent. At prenatal day 15 to 19, labelling to the IZ persisted, and positive hybridization was observed in virtually all cells in the cortical plate (CP) and in the regions between the IZ and CP. Therefore, a gene for this receptor subunit is expressed very early in development, probably soon after the cells undergo their last mitotic cycle. Acetylcholine has been suggested to play a general morphogenic role in the development of the nervous system (Kostovic and Rakic, J. Neurosci. 4:25 [1984]), but similar results to those we have described have been obtained with somatostatin receptors (Gonzalez *et al.*, J. Comp. Neurol. 305:177 [1991]). This work was supported by a grant from the Cornell Biotechnology Program which is sponsored by the New York State Science and Technology Foundation, a consortium of industries, the U.S. Army Research Office and the National Science Foundation.

299.8

DEVELOPMENT AND AGING OF SODIUM-DEPENDENT EXCITATORY AMINO ACID TRANSPORT BINDING SITES. J. J. Mitchell, L. Skipper*, M. Sapper* and K. J. Anderson. Departments of Physiological Sciences and Neuroscience, University of Florida, Gainesville, FL 32610.

High affinity transport of excitatory amino acids such as L-glutamate is necessary for the termination of its excitatory signal and the prevention of excitotoxicity. The removal of glutamate from the synaptic cleft is carried out by primarily by a sodium-dependent system that can be assessed by D-[^{3}H]aspartate autoradiography. Furthermore, there appears to be at least two distinct D-[^{3}H]aspartate recognition sites designated Type I (primarily forebrain) and Type II (primarily cerebellar). In this study we have examined the development and aging of these binding sites in rat brain. Briefly, assays were conducted using 6µm-thick, horizontal sections of rat brain. D-[^{3}H]Aspartate binding (100 nM in 50 mM Tris-acetate with 300 mM NaCl, pH 7.4) was carried out in delipidated sections at 0-2°C. Following rinsing in ice-cold buffer, sections were air dried and exposed to [^{3}H]-sensitive film for 3-4 weeks. Sodium-dependent D-[^{3}H]aspartate binding sites were first observed in the neonate at approximately postnatal day 8 and the density of these sites reached peak values and equivalence with adults at postnatal day 21. No differences were observed between the development of type I (primarily forebrain) and type II (primarily cerebellar) sites. Once transporter levels reached adult levels, they appeared to remain stable even in aged (24-26 month-old) rats. The ontogeny of transporter sites contrasts with EAA receptors which can be detected at postnatal day 1 and show a developmental "overshoot" with respect to adult levels (Insel et al., *Neurosci.* 35:31-51). This suggests that the the developmental differences between receptors and transporters may play a role in neonatal sensitivity to excitotoxicity. Supported by AG-08843 (KJA).

299.9

CO-CULTURE WITH STRIATED MUSCLE CONFERS DIHYDROPYRIDINE SENSITIVITY TO EVOKED RELEASE OF ^{3}H-ACH FROM EMBRYONIC CILIARY GANGLION NEURONS. D.B. Gray, C. Rossi*, J. L. Bruses*, and G. Pilar. Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269.

The intact neuromuscular junctions between chick ciliary ganglion neurons and their peripheral muscle targets have been used to analyze the pharmacology of the Ca^{++} dependency of evoked ^{3}H-ACh release (Gray et al., J. Neurosci., 10:2687,1990). In the following experiments this analysis has been extended to tissue culture where manipulation of neuron-target relationships is much more convenient.

In dissociated cell culture for 24 hours, st. 40 CG neurons and their processes display K^+ evoked ^{3}H-ACh release which is not sensitive to DHPs. In control samples, incubation in 55 mM K^+ Tyrodes resulted in 102% increase in ^{3}H-ACH over baseline. With addition of 10 uM nifedipine, 55 mM K^+ caused a 106% increase in label over baseline. However, if st. 40 choroid neurons are co-cultured with embryonic myotubes for the same 24 hr period, DHP antagonists can abolish evoked release from these cultures. In the presence of 10 uM nifedipine, ^{3}H-ACh release evoked by 55 mM K^+ is acually 10% less than baseline release. This is similar to Ca^{++} dependent ACh release at the intact st. 40 ganglionic terminals which are also in contact with their muscle targets in vivo.

Although it is clear that transmitter release is coupled to different Ca^{++} channels in each culture condition, it is not yet clear whether the presence of muscle induces expression of new (L type ?) Ca^{++} channels in neurons or whether formation of more extensive neuronal processes and synaptic endings in co-cultures result in clustering or selective segregation of channels already present in cells. Supported by NIH grant # 5R01 NS 10338.

299.10

CONSEQUENCES OF CEREBELLAR SYNAPTIC CIRCUITRY CHANGES ON $GABA_A$/BZ RECEPTOR α_1 SUBUNIT GENE EXPRESSION IN PURKINJE CELLS. D. Zdilar, A. Rotter and A. Frostholm, Dept. of Pharmacology, Ohio State University, Columbus, OH 43210.

Expression of $GABA_A$/benzodiazepine receptor α_1 subunit mRNA in Purkinje cells of developing normal and adult weaver (*wv/wv*), staggerer (*sg/sg*) and reeler (*rl/rl*) mutant mice were studied by in situ hybridization with a riboprobe, transcribed from a cDNA clone provided by Dr. A.J. Tobin (Neuron, 3:745-753, 1989). In the normal developing mouse cerebellum, diffuse labeling was present in the Purkinje cell layer from birth. Purkinje cells acquired their characteristic punctate appearance only later, between P5 and 7. In the 60 day old *wv/wv* cerebellum, in which most granule cells are lost by approximately day 30, the clusters of high grain density characteristic of Purkinje cells were still present. In the *sg/sg* mutant cerebellum, a defect in Purkinje cells results in the failure of synapse formation with granule cells. Although Purkinje cells were fewer in number and were scattered throughout the cerebellar cortex, they remained densely labeled. In the *rl/rl* mutant, Purkinje cells fail to migrate to their final destination remaining in cellular masses beneath the cerebellar cortex. The heterotopic Purkinje cells also contained high levels of α_1 subunit mRNA from birth, despite their lack of excitatory and inhibitory afferent input. These studies suggest that α_1 subunit gene expression in Purkinje cells precedes synapse formation and remains stable despite the loss of both afferent inhibitory (basket and stellate cells) and excitatory (parallel fiber) input.

299.11

ONTOGENY OF $GABA_A$/BZ RECEPTOR β_2 AND β_3 SUBUNIT GENE EXPRESSION IN MOUSE CEREBELLUM. V. Luntz-Leybman, D. Zdilar, A. Frostholm and A. Rotter. Department of Pharmacology, The Ohio State University, Columbus, OH 43210.

[^{35}S]-oligo and cRNA probes were used to study the developmental expression of β_2 and β_3 subunit mRNAs in the mouse cerebellum by in situ hybridization. The signal for β_2 subunit mRNA hybridization was detectable at birth and was most concentrated in the molecular/Purkinje cell layer. The deep cerebellar nuclei were also labeled, but less intensely. This pattern persisted up to postnatal day (P) 9 when additional labeling became visible in the granule cell layer. By P11, the granule cell layer was densely labeled, the intensity approaching that of the adult. At P20, the highest labeling was present in the granule and Purkinje cell layers; the molecular layer and white matter were unlabeled. Hybridization signal was absent from the external germinal layer (egl) throughout its existence.

Whereas the β_2 hybridization signal was completely absent in the egl, the β_3 signal was observed over the premigratory cells of the external germinal layer and in the internal granular layer at birth. The deep cerebellar nuclei were also densely labeled. Low grain density was present in the molecular layer. By P7-9, the external germinal and internal granule cell layers were labeled at equal density. Between P11 and P13, the hybridization signal over the external germinal layer became gradualy reduced as granule cells completed their migration into the granule cell layer. The adult distribution was reached by approximately P15; the highest grain density was present over the granule and Purkinje cell layers, and lower signal in the molecular layer was associated with basket and stellate cells.

These studies suggest that the expression of β_2 and β_3 subunit genes occurs during, or prior to, cell migration, and before granule cells form afferent and efferent synaptic connections.

DEVELOPMENT: LIGAND-GATED CHANNELS

300.1

$GABA_A$ RECEPTOR ACTIVATION CAUSES ELEVATION OF INTRACELLULAR CALCIUM IN EMBRYONIC RAT SPINAL CORD CELLS. M.K. Walton, A.E. Schaffner, and J.L. Barker. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

$GABA_A$ receptor activation has previously been shown to produce depolarization in neurons dissociated from rat spinal cord embryos. The intracellular calcium level response to externally applied muscimol was examined in dissociated embryonic rat spinal cord cells using computerized digital imaging microscopy and the calcium sensitive fluorescent dye fura-2. Cervical regions of spinal cords were removed from rat embryos and divided into dorsal and ventral portions. The tissue was enzymatically dissociated into single cell suspensions, placed onto culture dishes and allowed to adhere. Recordings were made both within hours of dissociation and after 1 day in culture. The intracellular calcium response due to activation of functional $GABA_A$ receptors was probed for by adding muscimol (2.5 uM) to the dish perfusate.

Muscimol raised intracellular calcium levels by a factor of 2-3 in cells showing a response. Calcium elevations occurred in a larger fraction of cells from ventral portions of the spinal cord than from the dorsal region. Dishes that had been in culture for 24 hours showed a greater fraction of cells producing calcium rises than cells recorded within hours of dissociation, and this was true for both dorsal and ventral cells. The response was effectively blocked by bicuculline as well as by addition of cobalt or cadmium to the perfusate, indicating that calcium entry may be through voltage sensitive calcium channels. These results indicate that $GABA_A$ receptor activation can lead to calcium elevation in embryonic neurons, and this may have a role in spinal cord development.

300.2

PARALLEL EXPRESSION OF $GABA_A$ RECEPTOR AND GABA IN EMBRYONIC RAT SUBCORTICAL BRAIN. W. Ma, T. Behar, A. Schaffner, S. Smith and J. Barker. Lab of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

The temporal relationship between the expression of $GABA_A$ receptor and transmitter GABA was investigated in the diencephalon, mesencephalon and rhombencephalon of E11-E21 rat embryos. The functional appearance of $GABA_A$ receptors was recorded in acutely dissociated cell suspensions by flow cytometric analysis of membrane potential responses to GABA and mucimol using the voltage-sensitive fluorescent dye oxonol. GABA was demonstrated immunocytochemically in 10μm-thick sections through the subcortical regions. At each age, embryos used for $GABA_A$ receptor examination and GABA immunostaining were taken from the same mother. At E12 functional Na^+ channels appeared in a minority of subcortical neurons that did not respond to GABA or muscimol, whereas a few GABA-immunoreactive (IR) fibers were seen coursing through these regions. By E13 membrane potential responses to supramicromolar GABA and muscimol became detectable in a minority of cells, while some GABA-IR cell bodies were visible in the pontine flexure region, developing substantia nigra, zona incerta and hypothalamus. Well developed GABA-IR fiber bundles passed through the marginal zone from the myelencephalon to the diencephalon. GABA and muscimol consistently depolarized progressively more subcortical cells over E14-21. The responses were blocked by bicuculline or picrotoxin. During this period GABA immunoreactivity increased dramatically in cells and axons throughout subcortical brain regions. Our results show that in subcortical regions functional $GABA_A$ receptors and GABA develop in parallel, suggesting that GABA might mediate important signals during the development.

300.3

DEPOLARIZING RESPONSES TO 3αOH-REDUCED PREGNANE STEROID METABOLITES AND GABA OCCUR IN THE MAJORITY OF EMBRYONIC RAT CORTICAL CELLS. S.V. Smith* and J.L. Barker. Lab. of Neurophysiology, NINDS, Bethesda, MD 20892.

Rat cortical cells from embryonic (E) day 15 to postnatal day 2 were enzymatically dissociated with papain and run through a FACStar Plus flow cytometer. The effects of steroids and the $GABA_A$ receptor ligands, GABA and muscimol, were studied using oxonol, a fluorescent, voltage-sensitive dye. The majority of the cortical cells recorded during this period were polarized according to a K^+ ion gradient. By E15 3αOH-reduced steroid metabolites and GABA ligands consistently depolarized cells with progressively more cells responding at later developmental ages. Curiously, 3βOH-reduced steroids hyperpolarized cells and antagonized 3αOH-reduced agonist responses. The depolarizing responses to steroids and $GABA_A$ agonists were blocked by bicuculline and picrotoxin, both of which often hyperpolarized cells, and not affected by Na^+-free conditions. EC_{50} values for the depolarizing responses were ~0.2 μM (steroids), ~0.4 μM (muscimol), and 1 μM (GABA). The widespread cellular distribution of depolarizing $GABA_A$ receptors (75-80% of all cells) sensitive to submicromolar steroids and GABA suggests important roles for GABA and steroid metabolites during embryongensis of the rat cortex.

300.4

ELECTRICAL AND CHEMICAL EXCITABILITY DEVELOP ALONG ANATOMICAL GRADIENTS IN THE EMBRYONIC RAT SPINAL CORD. A. E. Schaffner, S. V. Smith* and J. L. Barker. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Spinal cords from embryonic (E) and early postnatal (PN) rat pups were divided into 6 regions- cervical dorsal (CD), and ventral (CV), thoracic dorsal (TD) and ventral (TV) and lumbosacral dorsal (LSD) and ventral (LSV), dissociated in papain and incubated with a fluorescent, voltage-sensitive oxonol dye. Changes in fluorescence (FL) intensity were monitored in a FACStar Plus flow cytometer. At E13 consistent, TTX-sensitive, depolarizing responses to veratridine were recorded in C>T>LS cells. Responses to muscimol, kainate and the progesterone metabolites 5α-pregnan-3α-ol-20-one and 5β-pregnan-3α-ol-20-one were negligible. Over E14-E16 depolarizing responses to veratridine, muscimol, kainate and the steroid metabolites were recorded in progressively more V than D and C>T>LS cells. Interestingly, these gradients in excitability parallel the gradient of terminal cell division in the cord, the first neurons to withdraw from the mitotic cycle being motoneurons in the ventral horn of C regions. Dose-response curves to muscimol in CV and LSV at E15 had similar slopes with an EC_{50} of~ 200 nM. Since more cells respond in CV, this suggests that differences are due to numbers of responding cells and not efficacy of the ligand. At E19 the gradients had reversed such that D>V but there was no longer a clear C to LS gradient. By PN3, D>V and LS>C and T. A low forward angle light scatter, high FL (progenitor?) population appeared at E19 and did not express these forms of excitability.

300.5

NOISE ANALYSIS OF CULTURED EMBRYONIC RAT HIPPOCAMPAL NEURONS REVEALS SPONTANEOUS Cl^- ION CHANNEL ACTIVITY.

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Whole-cell voltage clamp recordings with CsCl-filled pipettes were performed on E17-21 hippocampal neurons cultured for 1-15 days. In many of these cells the baseline current noise was voltage-sensitive, becoming minimal at 0 mV, which corresponds to the reversal potential of Cl^- according to the Nernst equation. Spectral analysis of the baseline noise revealed that a multi-component Lorentzian equation fit better than the expected 1/f for a random process. The application of antagonists at $GABA_A$ receptors (bicuculline, picrotoxin) decreased the spontaneous noise and evoked detectable changes in membrane current, which became null at 0 mV. The resulting spectra approximated a 1/f function. Surprisingly, similar results were obtained with the $GABA_B$ agonist (-)baclofen and the steroid 5β-Pregnane-3β-OL-20-ONE. Under these conditions current changes were also elicited that reversed polarity at 0 mV. The results suggest that under these recording conditions many embryonic hippocampal neurons exhibit spontaneously active Cl^- channels that may be differentiating $GABA_A$ receptors.

300.6

CHARACTERIZATION AND DEVELOPMENTAL FUNCTION OF DEPOLARIZING RESPONSES TO GABA IN ROHON-BEARD CELLS.

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GABA, which typically acts as an inhibitory neurotransmitter, causes depolarization when applied to Rohon-Beard (RB) cells in the embryonic *Xenopus* spinal cord. We are using whole cell voltage clamp techniques to study the currents underlying this unusual response to GABA in RB cells. Reversal potentials for Na^+, K^+ and Cl^-, calculated on the basis of internal and external recording solutions, are +65 mV, -90 mV and -61 mV, respectively.

Most RB cells respond to 250 ms puffer applications of 100 μM GABA with an inward current when held at - 80 mV. 40% of all RB cells have GABA currents which reverse at - 38 ± 4 mV. This reversal potential is indicative of a current carried by more than one type of ion. Another 20% of the RB cells have responses to puffer applications of GABA that reverse at - 63 ± 5 mV, close to the calculated equilibrium potential for Cl^-. The final 40% of RB cells respond to the application of GABA with either a small inward current or a small outward current when held at - 80 mV. These currents reverse at - 80 ± 5 mV and have current-voltage relationships with negative slopes. This is consistent with the involvement of a K^+ current that is blocked by GABA. Ion substitution experiments show that K^+ is involved in two of these response types; the one that reverses at -38 mV and the one that reverses at - 80 mV and that Na^+ is involved only in the GABA response that reverses at - 38 mV.

Neurotransmitter-induced depolarization inhibits neurite outgrowth in several systems. GABA has a depolarizing effect in RB cells and might be expected to inhibit neurite outgrowth. In preliminary studies we find no difference in the lengths of neurites in spinal cord cells from early neural plate stage embryos grown in culture in either the presence or absence of 100 μM GABA for varying lengths of time.

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300.7

POSTNATAL DEVELOPEMENT OF GABA-B RECEPTORS IN RAT HIPPOCAMPUS

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An earlier study from this laboratory has shown that γ-aminobutyric acid (GABA) acting on GABA-A receptors has a depolarizing effect until postnatal day 5, and a mixed hyperpolarizing-depolarizing action, as in adult, afterwards (Ben-Ari & al, J. Physiol. 416, 303-325, 1989). We have now examinated the development of GABA-B mediated synaptic events and evoked currents.

Intracellular recordings were made from immature CA3 rat hippocampal neurons (P6-P10) in the in vitro slice preparation. Functional postsynaptic GABA-B receptors are present in P6 neurons and afterwards since i>stimulation of the hilar region evoked, at resting potential (-57±7 mV), a fast and slow ipsp. The slow ipsp reversed polarity at -90±4 mV, and was blocked by phaclofen (1mM); ii> bath application of phaclofen increased the frequency of spontaneous large hyperpolarizing potentials from 0.05±0.01 to 0.2±0.08 Hz; iii> bath application of baclofen (10 μM) induced an outward current which reversed polarity near -100 mV.

We conclude that functional postsynaptic GABA-B receptors are present in immature hippocampal neurons starting from P6. Experiments are currently performed to determine wether GABA-B mediated events are present at a earlier stage of developement.

300.8

NMDA REGULATES THE EXPRESSION OF THE ENKEPHALIN PHENOTYPE IN DEVELOPING SPINAL CORD CULTURES.

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In developing embryonic spinal cord dorsal root ganglia (SC-DRG) cultures, spontaneous electrical activity regulates the establishment of the enkephalin phenotype during neurodifferentiation in a calcium-dependent manner (J. Neurosci. Res.,28:140, 1991). Since a large proportion of the ongoing synaptic activity is mediated by excitatory amino acids in the central nervous system, we have tested different agonists of the glutamate receptor for their effects on enkephalin biosynthesis in differentiating mouse SC-DRG cultures. Cells were treated on day 10 postplating with N-methyl-D-aspartate (NMDA), quisqualate, kainic acid or 2-amino-5-phosphonovaleric acid (AP5) (10^{-6} to 10^{-4} M) either with or without blocking the spontaneous electrical activity with 1 μM tetrodotoxin (TTX). Enkephalin transcripts were analyzed by Northern blot hybridization using a complementary DNA probe. In electrically active cultures, NMDA treatment increased the expression of mRNAenkephalin in a concentration-dependent manner, showing maximum effect at 1 μM. Treatment with 1 μM NMDA doubled mRNA transcripts after 12 hrs and reached the maximum effect after 48 hrs of treatment however showing little increase between 24 and 48 hr of treatment. Co-treatment of electrically active cultures with NMDA (1 μM) and the competitive antagonist of the NMDA receptor, AP5 (10 μM) caused no increase in enkephalin transcripts. None of the concentrations of NMDA tested showed any effect on enkephalin expression when electrical activity was blocked by TTX. Furthermore, pretreatment of the cells with 1 μM NMDA did not prevent TTX-induced down-regulation of the enkephalin transcripts. Application of 1 μM quisqualate or kainic acid, agonists of the glutamate receptor of the non-NMDA subtype, failed to increase enkephalin transcripts. Our results indicate that the selective stimulation of NMDA glutamate receptor positively influences the establishment of the enkephalin phenotype probably through intracellular calcium as second messenger.

300.9

GLUTAMATERGIC PROPERTIES OF DEVELOPING NEURONS IN RABBIT RETINA.

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Glutamate functions as an important neurotransmitter in the adult retina; in addition, it may also influence neuronal development and synaptogenesis. Glutamate immunoreactivity is expressed at birth by neurons which are immature and have few if any synapses. Furthermore glutamate uptake sites are not preferentially localized on cells which are immunoreactive to glutamate. Metabolism of ^{3}H-glutamate through its conversion to ^{3}H-glutamine and the first expression of avid uptake by glial cells do not occur to any significant extent until day 9 thus potentiating a mechanism for maintaining a high extracellular concentration of glutamate. The concentration of endogenous glutamate present in effluents released from neonatal retinas (35μM) is significantly higher than that collected from adult retinas (15μM). The concentration is doubled after exposure to potassium depolarization in neonates but not adults. These results suggest that, in the neonate, glutamate is not restricted to synaptic compartments although it is highly localized in maturing glutamatergic neurons. Secondly, in the absence of the rapid buffering capacity of glial cells, extracellular concentrations of glutamate in the neonate are relatively high and they may increase more dramatically during periods of stimulation. Supported by EYO-1655-15.

300.10

INFLUENCE OF RETINOIC ACID-INDUCED DIFFERENTIATION ON NMDA RECEPTORS IN CULTURED NEURO-2A CELLS.

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Retinoids have a variety of potent effects on cell growth and differentiation. Neuroblastoma cell cultures can be induced to differentiate (i.e., to sprout neurites) by retinoic acid. In the present study, the influence of retinoic acid on the expression of NMDA receptors by Neuro-2A mouse neuroblastoma cells was investigated. Neuro-2A cells were exposed to retinoic acid (1 μM) for 7 days. NMDA receptors were characterized using [^{3}H]MK-801 as a probe. [^{3}H]MK-801 binding was measured in the presence of 100 μM glutamate and 100 μM glycine. The number of NMDA receptors decreased by approximately 50% after treatment with retinoic acid (Bmax, control = 765 ± 93 fmol/mg protein; Bmax, retinoic acid-treated = 375 ± 51 fmol/mg protein; $p< 0.01$). NMDA stimulated ^{45}Ca uptake into Neuro-2A cells by about 50%. This stimulated uptake was blocked by MK-801 ($IC50 \approx 3$ μM) as well as a number of other NMDA receptor antagonists and ion channel blockers. The magnitude of the NMDA-stimulated ^{45}Ca uptake was depressed ≈ 50% following treatment with retinoic acid, closely paralleling the decrease in receptor number. Exposure of Neuro-2A cells to NMDA (10 μM) resulted in a rapid ($t_{1/2} \approx 10$ sec) desensitization of NMDA-stimulated ^{45}Ca uptake. This rate of desensitization was markedly slowed following treatment with retinoic acid. The decrease in NMDA receptors following treatment with retinoic acid is consistent with results with muscarinic receptors in neuroblastoma cells (Baumgartner and Aronstam, *FASEB J.* 5:A856, 1991), and suggests that a decrease in the expression of neurotransmitter receptors is a common feature of neuronal differentiation. (Supported by USPHS grant AA-07698).

300.11

POSTNATAL DEVELOPMENT OF SYNAPTIC EXCITATION AND INHIBITION IN RAT NEOCORTEX. E.C. Burgard and J.J. Hablitz. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL. 35294.

Whole cell patch clamp recordings were obtained from neocortical brain slices prepared from rat pups 3-12 days of age. Synaptic potentials were evoked in layer II-III pyramidal cells by stimulation in layer IV-V. At a membrane potential of -60 mV, excitatory postsynaptic potentials (EPSPs) could be evoked at all ages. In 3-7 day old rats, EPSPs were typically 10-20 mV in amplitude and 300-450 msec in duration. EPSPs generally had a latency-to-peak of 50-90 msec; some neurons exhibited multiple-component EPSPs consisting of an early (8-10 msec) and late response. Late EPSPs increased in amplitude with depolarization and decreased when stimulation frequency was changed from 0.03-1 Hz. In addition, the NMDA receptor antagonists AP5 and AP7 (10μM) reversibly depressed the amplitude of the late component by approximately 40-60% in this age group. No evidence of chloride-dependent inhibitory postsynaptic potentials (IPSPs) was observed until day 7. Late potassium-dependent IPSPs were routinely observed by day 11-12.

These results demonstrate that an NMDA receptor-mediated component of the EPSP is present early in postnatal development, and that development of synaptic excitation precedes that of inhibition in the neocortex (NS22373).

300.12

SINGLE CHANNEL CURRENTS ACTIVATED BY PHYSOSTIGMINE AT JUNCTIONAL NICOTINIC ACETYLCHOLINE RECEPTOR (AChR) OF MAMMALIAN AND AMPHIBIAN. M.M. Fróes-Ferrão, R. Rozental and E.X. Albuquerque. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ 21941, Brazil and Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Medicine, Baltimore, MD 21201, USA.

Shaw *et al.* (*Mol. Pharmacol.* **28**:527, 1985) showed that physostigmine (Phy), a reversible cholinesterase inhibitor, has agonistic effects on the AChR in the frog *Rana pipiens*. Recently, Okonjo *et al.* (*Neuron*, in press) suggested that Phy induced cation flux into AChR-rich *Torpedo* membrane vesicles, even in the presence of α-bungarotoxin, a competitive antagonist of AChR. In light of these findings we decided to evaluate the agonist property of Phy on peripheral AChR present in different animal species. Single fibers were dissociated from interosseal muscle of the frog *Leptodactylus ocellatus* and flexor digitorum brevis muscle of adult Wistar rat. Cell-attached patch-clamp technique was used to record single channel currents generated by the activation of AChR. All records were obtained at 10°C in frog and 27°C in rat. ACh (0.1 - 0.4 μM) induced single channel openings with several conductance classes (45/40, 30, 18 and < 18 pS) in the frog and only one conductance class of 55 pS in the rat. Lifetimes were voltage dependent and increased with hyperpolarization. Phy did not induce single channel currents (10 μM-100 μM) in isolated muscles of adult rat. However, in the frog, Phy (10 μM) activated channel openings with at least three conductance classes. Our data suggest an important molecular difference between the AChRs located at the end-plate regions of adult rats and frogs. Support: DAMD-17-88-C-8119, FINEP, UFRJ/UMAB Mol. Pharmacol. Training Program and CNPq.

300.13

DENERVATION *IN VITRO*: COMPARATIVE STUDIES OF THE MULTIPLE CONDUCTANCE CLASSES OF NICOTINIC RECEPTORS IN *RANA PIPIENS* AND *LEPTODACTYLUS OCELLATUS*. R. Rozental and M.M. Fróes-Ferrão. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ 21941, Brazil & Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Our previous work showed the appearance of altered conductance states of junctional nicotinic receptors (AChR) of the frog *Rana pipiens* after muscle denervation *in vitro* and suggested that this phenomenon could be due to post-translational modifications (*Soc. Neurosci. Abs.* **15**:298, 1989). To test whether the AChR subconductance classes also appeared in another species, the patch-clamp technique (cell-attached configuration, 10°C) was applied to single muscle fibers of the frogs *Rana pipiens* (*Rp*) and *Leptodactylus ocellatus* (*Lo*). Single fibers from interosseal muscles of *Rp* and *Lo* were kept at 4°C for a period of up to 14 days and treated with 10 μg/ml cycloheximide (a protein synthesis inhibitor) and 0.1 mM phenylmethylsulfonylfluoride (a protease inhibitor). After acute dissociation of *Rp* fibers, ACh (0.4 μM) induced single channel openings with 40 pS conductance and voltage-dependent lifetimes. On the 2nd-3rd day after dissociation in Rp, we observed a second conductance state (30, 18 or < 18 pS) and by the 7th day several conductance classes were expressed (40, 30, 18 and < 18 pS). In contrast, immediately after acute dissociation of fibers from *Lo*, all above conductance classes were induced by ACh. The channel lifetimes of the lower conductance states were shorter than the 40-pS population for both *Rp* and *Lo* and all currents were blocked by α-bungarotoxin (5 μg/ml). Chronic treatment of the fibers with cycloheximide did not prevent the appearance of the multiple conductance classes in Rp. Our data show a different time course for the conductance changes in AChR in *Lo* compared to *Rp* frogs. Support: U.S. Army Med. Res. & Devel. Comm. Contr. DAMD-17-88-C-8119, USPHS NS25296, FINEP/UMAB Mol. Pharmacol. Training Program and CNPq.

300.14

A POSSIBLE INTERACTION BETWEEN THE ACCUMULATION AND EXPRESSION OF ACETYLCHOLINE RECEPTORS IN CULTURED MAMMALIAN MYOTUBES. C.G. Carlson and Y. Feng*. Dept. Physiology, Univ. N. Dakota, School of Medicine, Grand Forks, ND 58202.

We have examined the expression of embryonic low conductance-long burst duration acetylcholine receptors (LL-AChRs) and high conductance-short burst duration acetylcholine receptors (HS-AChRs) in myotube cultures derived from embryonic mouse muscle (Day 16-19 embryos, C57Bl6J or C57Bl10/Sn.J, DMEM-HAMS F12, 10% Fetal Bovine Serum, penicillin-streptomycin, laminin substrate). Cell-attached patch clamp recordings ($5x10^{-7}$ M AChCl in 0 Mg^{2+} Ringer solution) obtained prior to day 15 in culture exhibited only LL-AChRs. Between days 15 and 29, approximately 80 to 90% of recordings exhibited both receptor classes with an average percent HS-AChRs of 12.8%. LL-AChR accumulation preceded and slightly overlapped the period of HS-AChR expression. Individual culture runs that had higher LL-AChR densities (minimum channels per patch) prior to day 15 also had greater percent HS-AChR values after day 15. In two experiments, surface AChRs were blocked by alpha bungarotoxin (5μg/ml, 2 hrs) on day 15. In one case, receptor density was rather low and only LL-AChRs were observed following receptor blockade (days 18-27). In a second case, receptor density was higher and both classes were observed following receptor blockade (days 18 to 29). These results are consistent with the hypothesis that factors associated with the expression and accumulation of LL-AChRs are responsible for activating the expression of HS-AChRs (UND-Epscor 90, NDBHE-NSF).

GROWTH FACTORS AND TROPHIC AGENTS III

301.1

GABAERGIC NEURONS OF THE BASAL FOREBRAIN MAGNOCELLULAR COMPLEX (BFMC): EFFECTS OF NEUROTROPHIC FACTORS AND PATHOLOGY IN ALZHEIMER'S DISEASE (AD). G.K. Gouras, V.E. Koliatsos and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Balto., MD 21205.

Previously, we have described that GABAergic neurons of the BFMC labeled by glutamic acid decarboxylase (GAD) *in situ* hybridization degenerate after fimbria-fornix transection. These GABAergic neurons do not express the nerve growth factor (NGF) receptor nor do they respond to NGF in control animals or following axotomy. Employing *in situ* hybridization with digoxigenin-labeled riboprobes antisense to rat GAD, we report that degenerating GABAergic neurons are also refractory to NT-3, ciliary neurotrophic factor, and the pluripotent basic fibroblast growth factor. Effects of brain-derived neurotrophic factor on these neurons will be discussed. Digoxigenin-labeled human GAD antisense riboprobes have allowed us to visualize GABAergic neurons of the BFMC in human tissue. Preliminary findings suggest that there is a loss of these GAD-hybridizing neurons in AD. Further research is necessary for the identification of factors that might influence this very important transmitter-specific subpopulation of the BFMC.

301.2

HIGHLY SELECTIVE EFFECTS OF NERVE GROWTH FACTOR (NGF), BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF), AND NEUROTROPHIN-3 (NT-3) ON NEURONS OF THE BASAL NUCLEUS COMPLEX (BNC). D.L. Price, V.E. Koliatsos, G.K. Gouras, L.E. Burton, J.W. Winslow and K. Nikolics. Neuropathol. Lab., The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205; Genentech, Inc., S. San Francisco, CA 94080.

Cholinergic neurons of the BNC respond to NGF. Recently, molecular genetics have revealed that NGF belongs to a polypeptide gene family (neurotrophins), the other identified members of which include BDNF and NT-3. Because all three neurotrophins are enriched in hippocampus, we sought to examine the specificity and selectivity of the effects of these peptides on injured cholinergic and GABAergic BNC neurons that project through the fornix in the rat. Following a complete transection of the fimbria-fornix, recombinant human (rh) NGF as well as rhBDNF prevented the shrinkage and loss of phenotype of cholinergic neurons in the medial septal nucleus (MSN). rhNGF had no effects on GABAergic neurons. The influences of BDNF on GABAergic neurons and of rhNT-3 on cholinergic and GABAergic neurons of the MSN will be presented. Our data indicate that some neurotrophins act upon MSN neurons and that specific neurotrophins influence certain neurons of the MSN but do not have effects on other neurons. Thus, neurotrophins exert highly selective trophic effects on the septohippocampal circuit.

301.3

SEXUALLY DIMORPHIC VS. NONDIMORPHIC SPINAL MOTOR NEURONS: TWO PERIPHERAL SYSTEMS WITH DISTINCT TROPHIC PROFILES THAT MAY RESPOND TO DIFFERENT TROPHIC FACTORS.

V.E. Koliatsos, R.E. Clatterbuck, N.Y. Ip, G.D. Yancopoulos and D.L. Price. Neuropathol. Lab., The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205; Regeneron Pharmaceuticals, Tarrytown, N.Y. 10591.

Although most motor neurons express the nerve growth factor receptor (NGF-R) following axonal injury, some lumbar motor neurons in the male express NGF-R under normal conditions. These cells comprise the equivalent of Onuf's nucleus, as shown by the colocalization, in their perikarya, of HRP-WGA or Fluoro-Gold transported from perineal muscles and immunoreactivity for NGF-R (192-IgG). To examine potential factors active on conventional motor neurons and neurons of Onuf's nucleus, we performed Northern blotting for neurotrophin and ciliary neurotrophic factor (CNTF) mRNA on the muscles gastrocnemious and bulbospongiosus of two-week male rats. Although CNTF was very abundant in the gastrocnemious, CNTF mRNA was not detected in the bulbospongiosus. NGF and BDNF were very low in both muscles, whereas NT-3 was highly expressed, especially in the bulbospongiosus. The above results suggest that the presence of the low-affinity NGF-R in Onuf's nucleus is associated with a possible role of neurotrophins on the differentiation and, possibly, survival of these sexually dimorphic neurons.

301.4

EFFECTS OF TROPHIC FACTORS ON ANTERIOR THALAMIC NEURONS.

R.E. Clatterbuck, V.E. Koliatsos and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Anterior thalamic nuclei show abnormalities in several neurological disorders, including Alzheimer's disease. Previously, we reported that peripheral nerve grafts exert both trophic and tropic effects on axotomized anterior thalamic neurons. The present investigation was designed to test the hypothesis that previously identified neurotrophic factors, known to be abundant in these grafts, might be responsible for the observed effects. Such factors include nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), and basic fibroblast growth factor. After making unilateral aspiration cingulectomies in adult male Sprague-Dawley rats, we placed cannulas attached to mini osmotic pumps into the ventricle for the delivery of trophic factors or vehicle. Following two weeks of treatment, animals were sacrificed, and tissues were processed for morphometric analysis. Although NGF did not prevent retrograde degeneration of anterior thalamic neurons, CNTF appeared to have some protective effect on these thalamic neurons. Currently, we are examining this possible trophic interaction further as well as testing other trophic factors in this model.

301.5

COMPARISON OF THE TRANSFORMING GROWTH FACTOR-β1 (TGFβ1) mRNA RESPONSE IN THE HIPPOCAMPUS AFTER PERFORANT PATH TRANSECTION AND KAINIC ACID INDUCED LIMBIC SEIZURES.

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Three TGFβ genes have been cloned and sequenced from numerous mammalian sources. These genes belong to a multigene family and share extensive homology with each other but appear to be regulated differentially. Using a cRNA probe directed against the coding region of TFGβ1, we showed that a 2.5kb mRNA increased in the ipsilateral hippocampus after partial deafferentation by an electrolytic entorhinal cortex lesion (ECL) (Nichols et al.,1991 J.Neurosci.Res.28:134-139). A DNA oligomeric probe that is specific for TGFβ1 was synthesized and used to confirm that this mRNA species was: (1) TGFβ1 and (2) not an alternately spliced TGFβ1 mRNA of same size (as found in porcine tissues). TGFβ1 mRNA was also examined in other rat brain lesion models involving neurodegeneration/regeneration. TGFβ1 mRNA levels increased in the ipsilateral hippocampus and entorhinal cortex after perforant path transection in a time course similar to the electrolytic ECL response. TGFβ1 mRNA levels increased rapidly in the hippocampus after the onset of limbic seizures induced by systemic kainic acid injection. It has been shown that TGFβ1 modulates the production of extracellular matrix molecules and receptors, proteases and protease inhibitors, cellular migration and growth factor activities in the periphery. Therefore, TGFβ1 may influence similar mechanisms during reactive synaptogenesis in the hippocampus. (Supported by AG07909 to CEF)

301.6

BIOLOGICAL ACTIVITY OF THE SECRETED FORM OF AMYLOID β/A4 PROTEIN PRECURSORS: III THE GROWTH REGULATORY EFFECT ON NEUROBLASTOMA CELLS.

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We previously demonstrated that secreted forms of amyloid β/A4 protein precursor (APP) have growth regulatory activity on fibroblasts (Cell 58:615-622, 1989) and modulate adhesion of neuron-like cultured cells to substrata (Neuron 3:689-694, 1989). In the current study, we further analyzed the biological functions of two secreted APP isoforms, one without the protease inhibitor domain (APP-695) and the other with protease inhibitor domain (APP-751). Conditioned media from cell clones overproducing APP-695 (Amy5), APP-751 (Amy6) and control- transfectant (293) were applied to cultured neuroblastoma Lan-5 cells. Amy6 at 20% reduced Lan-5 growth by 30%, although 293 and Amy5 had no effect. At the same concentration, both Amy5 and Amy6, but not 293, restored the growth of A-1 fibroblast, the cell line that secretes very low amount of APP and depends on exogenous APP in the medium for normal growth. Essentially the same results were obtained with secreted APP-695 and APP-751 produced by recombinant baculovirus expression system. The difference between APP-695 and APP-751 was also seen in the signal transduction systems affected by these proteins. By immunoblotting technique, only APP-695 was shown to reduce PKC(α) level in prolonged cultures of Lan-5 or fibroblast. Since the ratio of APP-751/APP-695 is reported to be changed in AD brains (Science 248:854-857, 1990), the differential effects of two forms of secreted APP may be involved in the neuronal degeneration in AD.

301.7

EFFECTS OF TGF-α AND TGF-β ON VENTRAL MESENCEPHALIC DOPAMINERGIC CULTURES.

T. Alexi, T.L. Denton, and F. Hefti. Andrus Gerontology Center, U.S.C., Los Angeles, CA 90089.

Transforming growth factor-α (TGF-α) is a known mitogen for several cell types, it is expressed in the brain (including the striatum, Wilcox and Derynck, J. Neurosci. 8:1901,1988) and has been proposed as a trophic agent for dopaminergic cells. We therefore characterized trophic actions of TGF-α on rat fetal dopaminergic neurons in culture. E15 ventral mesencephalic dissociated neurons were grown in the presence of TGF-α for 5-8 days. TGF-α elevated dopamine uptake in a variable manner by 25-50%. There was a parallel increase in the total number of cells and protein content, reflecting a general growth promoting action of TGF-α and suggesting that the effect on dopaminergic neurons is mediated by non-dopaminergic cells. Preliminary evidence shows that the TGF-α induced elevation in dopamine uptake is blocked by neutralizing antisera to TGF-β, a mitogen structurally unrelated to TGF-α and thought to participate in the regulation of neurotrophic factor expression. The trophic actions of TGF-α on dopaminergic neurons will be further characterized by testing whether it affects their survival, neurite extension, and expression of tyrosine hydroxylase. In order to characterize the specificity of TGF-α, its actions will be compared with those of EGF, its structural and functional analog. Furthermore, we will explore the possibility that TGF-α's actions are mediated by non-dopaminergic cells and TGF-β.

301.8

Localization of Transforming Growth Factor alpha Precursor and Epidermal Growth Factor in Forebrain Regions of the Vervet Monkey (*Cercopithews Aethiops*).

J. Callaway, R. Kinyamo*, I. Opole*, J. Kimani*, S.E. Loughlin and J.H. Fallon. Department of Anatomy and Neurobiology, Univ. of California Irvine, Irvine, Calif. 92717 and Department of Human Anatomy, Univ. of Nairobi, Kenya.

Transforming growth factor alpha (TGFα) and the structurally related epidermal growth factor (EGF) have been immunocytochemically localized in the rodent brain, (Fallon et. al. '84, '87, '90; Loughlin et. al. '89). In the present study, TGFα precursor and EGF-like immunoreactivity were localized in the primate brain. Adult Vervet monkey brains (Univ. of Nairobi, Kenya) were processed for immunoperoxidase and immunofluorescence (double labeling). As in the rat brain, TGFα-LI was localized in a subpopulation of glial fibrillary acidic protein immunoreactive astrocytes. In the forebrain, TGFα-LI was observed in astrocytes of the corpus collosum, external capsule, anterior commissure, dorsal striatum and globus pallidus. In addition, EGF-LI was localized in axonal-like processes in the globus pallidus. These data support previous findings in the rat suggesting that TGFα and EGF are potential candidates for neurotrophic function in these regions of the mammalian brain. Supported by the National Parkinson Foundation, NS 15321, NS 26761 and a Bud Corbin Neuroscience Award.

301.9

PLATELET-DERIVED GROWTH FACTOR RECEPTOR EXPRESSION IN RAT NEURONAL AND ASTROGLIAL CULTURES. J.B. Hutchins and M.D. Ard. Dept. of Anatomy, Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

Platelet-derived growth factor (PDGF) is known to play a role in the differentiation of glial cell precursors into oligodendrocytes or type-2 astrocytes. It is not yet clear which cell types in the developing brain synthesize and release PDGF, or which cells respond to the presence of this growth factor via surface receptors. Three isoforms of PDGF exist (AA, AB or BB dimeric forms) and there are thought to be at least three receptor types ($\alpha\alpha$, $\alpha\beta$, and $\beta\beta$, with various binding specificities). We have used an antibody to the β subunit of the PDGF receptor (anti-PDGF-Rβ) to study the cell types which express this protein *in vitro*.

Neurons were cultured from 15-day embryonic rat cerebral cortex, either as explants or as dissociated cells. Astrocytes were prepared from newborn rats by standard methods. Following maintenance of cells or explants *in vitro* for two to four days, the tissue was fixed and processed for PDGF-Rβ-like immunofluorescence. Some tissue was double-labeled with antibodies to either glial fibrillary acidic protein or phosphorylated neurofilaments. This combination of labels allowed us to identify PDGF-Rβ^+ cells as either astrocytes or neurons.

Neurites express both PDGF-Rβ and phosphorylated neurofilament in isolated culture, in a co-culture with astrocytes, or in cortical explants. PDGF-Rβ^+ processes extend for relatively long distances in these cultures. As expected, astrocytes have moderate but detectable levels of PDGF-Rβ in culture. The levels of PDGF-Rβ are much higher on embryonic astrocytes than on those cultured from newborn rats, consistent with the findings of others that the response of these cells to PDGF declines with maturity.

PDGF may influence the differentiation and development of neurons as well as glial cell precursors. These observations, combined with earlier studies demonstrating the possible release of PDGF by neurons, further support a fundamental role for PDGF in neuronal as well as glial development. Supported by BRSG RR05386 (to U.Miss., J.B.H. and M.D.A.).

301.10

COORDINATE EXPRESSION OF STRIATAL TGFß-1 AND FIBRONECTIN mRNA FOLLOWING CORTICAL DEAFFERENTATION. G.M. Pasinetti, N.R. Nichols, M. Gordon, D.G. Morgan and C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, University Southern California, Los Angeles, CA 90089-0191

Striatal deafferentation by frontal cortex ablation in adult rat induced fibronectin mRNA 3 days postlesioning, while TGFß-1 mRNA increased 10 days postlesioning as assessed by northern blot hybridization. By in situ hybridization, the apparent elevation of fibronectin mRNA signal at 3 days postlesioning was localized to cells lying on the border of the wound cavity rostral to the deafferented striatum. TGFß-1 mRNA signal on the wound cavity surface was also elevated 3 days postlesioning. In striatum, the in situ hybridization assay of fibronectin mRNA and TGFß-1 mRNA showed an identical schedule of change with an overlapping peak of elevation 10 days postlesioning. The elevation of striatal fibronectin and TGFß-1 mRNA recovered toward control levels by 27 days postlesioning, as assessed by northern blot and in situ hybridization assay. By in situ hybridization combined to immunocytochemistry on the same tissue section, fibronectin mRNA was localized to glial fibrillary acidic protein (GFAP) immunostained astrocytes; TGFß-1 mRNA appears to be colocalized with microglia immunostained by OX-42 antiserum which specifically recognize the microglia CR3 complement receptor. This study indicates the in vivo coordinated expression and differential cellular localization of fibronectin and TGFß-1 mRNA following brain injury. Supported by National Parkinson Foundation to GMP and NIH grant AG-7909 to CEF.

301.11

REGULATION OF TRANSFORMING GROWTH FACTOR-β1 mRNA IN BRAIN: IMPLICATIONS FOR TISSUE REPAIR AND PLASTICITY. N.R. Nichols, M. Lampert-Etchells*, N.J. Laping, T.E. Morgan*, C.M. Pasinetti, J.D. Rittenberg* and C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles CA 90089-0191.

Transforming growth factor-β1 (TGF-β1) is a growth regulatory peptide with well-known roles in differentiation and wound repair in peripheral tissues. Recently, we cloned TGF-β1 from hippocampus of 3 mo old rats as a mRNA that was decreased in response to glucocorticoid treatment. We also showed by RNA blot hybridization that TGF-β1 mRNA increased 5-fold in the deafferented hippocampus and striatum after entorhinal cortex lesion and decortication, respectively. In situ hybridization studies show that sites of increased TGF-β1 mRNA in response to both lesions include the edge of the wound cavity as well as the deafferented area. In addition, hippocampal TGF-β1 mRNA is elevated in 15 and 24 mo rats compared with 6 mo control male Fisher 344 rats. These data indicate that TGF-β1 might be involved in neural plasticity. If these changes in mRNA levels result in changes in active peptide, then TGF-β1 could play an important role in brain injury, aging and neuroendocrine adaptation, particularly in view of the similarities between tissue repair and disease processes regulated by TGF-β peptides in peripheral tissues. (Supported by PHS #AG-07909 and The Brookdale Foundation)

301.12

TGF-ß AS A POTENTIAL REGULATOR OF SCHWANN CELL PROLIFERATION. J.B. Davis*, A.J. Shores*†, A.G. Watts, P. Stroobant*†. The Salk Institute for Biological Studies, San Diego, CA 92186; †Ludwig Institute for Cancer Research, London W1P 8BT, UK.

In order to respond to PDGFs or FGFs rat Schwann cells require cooperation from other factors which are able to induce expression of the requisite polypeptide growth factor receptor. Using DNA synthesis studies, normal Schwann cells and a Schwann cell line, the cooperative effects of TGF-ß have been studied and a role for TGF-ß in an autocrine loop demonstrated.

TGF-ß supports a Schwann cell response to PDGF-AB, PDGF-BB, acidic-FGF or basic-FGF, factors otherwise inactive, and to serum platelet-derived factors. The requirement for TGF-ß is saturable and the effect dose dependent. These effects are similar to the reported cooperation between polypeptide growth factors and forskolin, suggesting a similar mechanism involving receptor regulation.

A rat Schwann cell line, made during these studies, is able to proliferate in response to platelet-derived factors alone and secretes a soluble factor that is mitogenic for normal Schwann cells. The mitogenic effect is blocked by an anti-TGF-ß antibody, suggesting that paracrine or autocrine secretion of TGF-ß may enable Schwann cells to respond to other growth factors during development or tumourigenesis.

This work was supported by the Ludwig Institute for Cancer Research.

301.13

TRANSFORMING GROWTH FACTOR β MODULATES NGF EFFECTS ON CULTURED SENSORY NEURONS. A.Chalazonitis(1), J.Kalberg(1), *D.R.Twardzik(4), *R.S.Morrison(3) and J.A.Kessler(1,2). Depts of Neurosci.(1) and Neurol.(2) Albert Einstein Coll. Med., New York, NY 10461; R.S.Dow Neurol. Sci.Inst.(3) Portland, OR 97209 and Bristol Myers Squibb Pharm. Res.Inst.(4) Seattle, WA 98121.

TGFβ exerts potent growth stimulatory and differentiating effects on a wide variety of non-neuronal cells during embryogenesis and in wound healing. Two dimeric forms of TGFβ (β1 and β2) were tested for their neuronotrophic actions on rat neonatal dorsal root ganglion (DRG) neurons in dissociated culture. TGFβ1 and β2 (10 ng/ml) significantly increased neuronal survival (2 fold) and the level of the neuropeptide substance P (SP) (3 fold) per culture. Maximal effects occurred at 1 ng/ml for TGFβ1 and 10 ng/ml for β2. Treatment with both factors was not additive. Co-treatment of DRG cultures with a 2.5S NGF antibody prevented the neuronotrophic effects of TGFβ, implicating a role for NGF. TGFβ did not significantly alter endogenous expression of NGFmRNA in DRG cultures nor content of NGF in the conditioned medium. However, TGFβ1 (5ng/ml) potentiated neuronal survival induced by exogenous (1-100 ng/ml) NGF. These data suggest a modulatory role for TGFβ in the actions of NGF on sensory neurons. Supported by NIH grants NS 26766 (AC) and NS 20013 and NS 20778 (JAK).

301.14

The development of sympathetic innervation in footpads of Tabby mutant mice. M. Rao and S.C. Landis. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH. 44106

Tabby is an X-linked mutation in mice that causes a number of abnormalities in the development of epidermal derivatives (Falconer, 1952), including the absence of sweat glands (Sofaer, '69; Blecher et al., '90). Despite the lack of sweat gland ducts and secretory tubules in adult male hemizygous Tabby mice, the epidermis and underlying connective tissue which contains blood vessels and nerve fibers appears normal. We have examined the sensory and sympathetic innervation of footpads in Tabby and control mice using catecholamine histofluoresence and immunocytochemistry. The distribution and number of Substance P and CGRP-labelled sensory fibers in Tabby appeared similar to those of control mice. In addition, arteries and arterioles were innervated by noradrenergic sympathetic fibers in both control and Tabby mice. In contrast to control mice, however, no VIP and choline esterase positive fibers were evident in Tabby footpads. This observation suggests that the absence of sweat glands is accompanied by a corresponding lack of sweat gland innervation. The absence of gland innervation in adult Tabby mice could be due either to a failure of sympathetic axon growth to the footpads or the subsequent failure of the glandless pads to support an initially normal innervation. To distinguish between these possibilities, we examined the sympathetic innervation of footpads in young Tabby mice. At 10 days, in both Tabby and control mice, catecholaminergic fibers were present. In footpads of normal mice, the fibers are associated with developing sweat glands and blood vessels. In footpads of Tabby mice, some fibers are associated with blood vessels while others ramify in the connective tissue and beneath the epidermis. These observations suggest that despite the absence of their normal target, sympathetic fibers initially project appropriately to the footpads but are subsequently lost. Supported by NS23678 and the AHA.

301.15

ACETYLCHOLINE PROMOTES THE DEVELOPMENT AND MAINTENANCE OF SECRETORY FUNCTION IN RAT SWEAT GLANDS. M. P. Grant and S. C. Landis Depts. of Pharmacology and Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

Previous studies have suggested that the sympathetic cholinergic innervation of rat sweat glands plays a critical role in regulating the development and maintenance of secretory gland function. To learn more about the nature of the molecular signal(s) responsible, we disrupted muscarinic cholinergic transmission in developing postnatal rat pups and adult rats by administering atropine, and replaced muscarinic transmission in acutely denervated sweat glands by administering pilocarpine.

Treatment of neonatal rat pups for 7 days (P11-P18) with l-atropine (20 mg/kg/day) using Alzet minipumps resulted in the failured development of sweat gland secretory function. Secretory function was absent for 4 days following the withdrawal of atropine and then developed over the next 5 days, reaching control levels on P27. Atropine treatment did not impair development of appropriate gland morphology. In adult animals, a similar treatment protocol produced a loss of secretory function for 3 days; function recovered to control levels by day 7. This was not due to the presence of residual atropine since both the pupillary light response, and acetylcholine-mediated decrease in blood pressure recovered within 24 hrs; further, direct testing of the serum for atropine indicated residual atropine was not present 1 day following the treatment protocol. In addition, we treated adult rats whose sweat glands were denervated by sciatic nerve section with pilocarpine, a muscarinic agonist. A seven day treatment of pilocarpine begun immediately after denervation resulted in a 60% preservation of gland function; untreated animals lost all function within 3-5 days. These results suggest that acetylcholine itself released from sympathetic nerve terminals is required for the normal development and maintenance of secretory function in sweat glands. Supported by NINDS 23678.

301.16

TRANSFORMING GROWTH FACTOR-β1 INCREASES GLIAL FIBRILLARY ACIDIC PROTEIN mRNA IN THE HIPPOCAMPUS OF YOUNG ADULT MALE RATS 24 HOURS AFTER AN INTRA-VENTRICULAR INFUSION. N. J. Laping, T. E. Morgan*, N. R. Nichols, and C. E. Finch. Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

Transforming growth factor-β1 (TGF-β1) is a cytokine implicated in many cellular responses involving both differentiation and responses to lesion. Functions of this factor in peripheral tissues include chemotaxis of fibroblasts and macrophages, as well as the regulation of proteases and modification of extracellular matrix proteins. Recently, our laboratory showed that TGF-β1 mRNA is increased in the hippocampus after an entorhinal cortex lesion (ECL) which deafferents the hippocampus without causing cell death in the hippocampus (Nichols et al., J. Nsc. Res. 28:134-139, 1991). To examine the potential role of TGF-β1 in regulating or mediating the astrocyte responses to ECL, TGF-β1 was infused into the lateral ventricle in young adult male Fisher 344 rats. The rats were infused with 2 μl vehicle or vehicle containing either 15 ng or 100 ng TGF-β1. Astrocyte mRNA levels of glial fibrillary acidic protein (GFAP) and sulfated glycoprotein-2 (SGP-2) were determined in the hippocampus and entorhinal cortex 24 hours after the infusion by RNA blot hybridization analysis. Hippocampal GFAP mRNA levels increased 40% in rats infused with 100 ng of TGF-β1 vs. vehicle, with indications of dose response. The entorhinal cortex showed similar trends. In contrast, another astrocyte marker, SGP-2 mRNA was not affected by TGF-β1 24 hours after the infusion in either tissue examined. These results implicate TGF-β1 as an intermediate signal that activates certain astrocyte functions in response to deafferentation. Activation of other cell-cell interactions which might involve SGP-2 might be delayed or require longer exposure to TGF-β1. (Supported by AG-07909 to CEF and AG-05528 NRSA fellowship to NJL).

301.17

PROTEASE NEXIN I (PNI) INCREASES THE SURVIVAL OF SPINAL CORD NEURONS. B.W. Festoff and D.E. Brenneman, Neurobiology Res. Lab., KC VA and Univ. Kansas Med. Centers, Kansas City, MO 64128 and KS 66103; Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, Maryland 20892

Neuron-glia interactions have important roles in regulating the development of the nervous system. Neuropeptides released from neurons can stimulate receptors on glia that result in the release of substances that, in turn, affect neuronal maturation and survival. Our goal is to identify these neurally evoked, glia-derived proteins. Previous studies have indicated that low concentrations of vasoactive intestinal peptide (VIP) increase the survival of neurons grown in dissociated spinal cord cultures (PNAS 83: 1159, 1986) and that this action is mediated through a nonneuronal cell type (J. Cell Biol. 104: 1603, 1987). VIP has been shown to increase the release of protease nexin I (PNI) from rat cortical astrocyte cultures (Neurosci. Abs. 16:909, 1990). We studied whether PNI, a 43-47 kD serine protease inhibitor (Serpin) that stimulates neurite outgrowth, has an additional effect on neuronal survival. Purified PNI was added to dissociated spinal cord cultures that were co-treated with tetrodotoxin (TTX), to prevent the release of endogenous VIP. After a five day test period, a 70-75% increase in neuronal survival was observed in cultures treated with 1 nM PNI as compared to those treated with TTX alone. Concentrations of PNI $\geq$ 100 nM were not effective. Similarly, spinal cord cultures treated with leupeptin, a tripeptide protease inhibitor, also increased neuronal survival within a narrow range of concentrations (10 nM); whereas, ammonium chloride, a lysosomal protease inhibitor, did not increase neuronal survival. Thus, PNI may participate in VIP-mediated regulation of neuronal maturation and survival possibly by preventing the degradation of extracellular growth-promoting substances which determine the structure of the developing nervous system.
Supported by DVA and American Health Assistance Foundation.

301.18

PURIFICATION OF ACTIVITY DEPENDENT NEUROTROPHIC FACTOR (ADNF) D.E. Brenneman, R. Barth*, D. Warren*, A. Davidson and I. Gozes. Lab. of Developmental Neurobiol., NICHD, Bethesda, MD 20892, Dept. of Pathol. Chem., Tel Aviv Univ., Tel Aviv, Israel.

The survival of developing spinal cord neurons has been shown (PNAS 83: 1159, 1986) to be dependent on glial-derived substance(s) that can be released by vasoactive intestinal peptide (VIP). The survival-promoting activity present in the conditioned medium of VIP-stimulated astroglia cultures (J.Cell Biol. 104:1603, 1987) can be detected in test cultures made from dissociated spinal cord which have been electrically blocked with tetrodotoxin, a condition which decreases the release of the endogenous secretagogue, VIP. An activity-dependent neurotrophic factor (ADNF) has been purified from conditioned medium of rat cortical astrocytes stimulated for three hours with 0.1 nM VIP. Sequential chromatographic separations by anion exchange, molecular seiving and reverse phase were utilized to obtain an electrophoretically (SDS-PAGE and IEF) pure peptide that increased the survival of spinal cord neurons effectively at concentrations of 10^{-14} M during a five day test period. The isolated neurotrophic substance has an apparent molecular weight of 7300 Daltons and a basic pI. A 3000-fold increase in specific activity was achieved from the isolation procedures. On the basis of total amino acid composition and other physical characteristics, ADNF does not appear to be any of the already identified neurotrophic growth factors. ADNF was heat- and trypsin-sensitive and did not bind to heparin or Con A affinity columns. The glia-derived ADNF may play a significant role in the mechanism of neurotrophism elicited by VIP in CNS cultures.

301.19

INTERLEUKIN-1ß PROMOTES LONG-TERM SURVIVAL OF CULTURED RAT HIPPOCAMPAL NEURONS. J.W. Francis, T.H. Oh and G.J. Markelonis. Dept. Anatomy, Univ Maryland Sch Med., Baltimore, MD 21201.

Interleukin-1 (IL-1) is known to have potent effects on various CNS cells in vivo and in vitro. Since several studies have shown the presence of IL-1ß and its mRNA in rat hippocampus, we examined the effects of IL-1ß on dissociated rat hippocampal neurons. Cultures enriched in neurons were prepared from E18 rat hippocampus by treating the cultures with an antimitotic. Control cultures survived for one to two weeks and then gradually degenerated. These cultures were comprised of 90-95% neurons as determined by neurofilament immunopositivity. However, addition of IL-1ß increased neuronal survival two-fold over control cultures. While basic fibroblast growth factor also increased long-term survival, IL-2 and epidermal growth factor showed no effect. The survival promoting effect of IL-1ß was dose-dependent. Although IL-1ß treatment also increased the number of astrocytes, the proportion of astrocytes in these cultures was actually slightly decreased relative to controls. Furthermore, when the antimitotic was added to cultures at the time of initial plating, the survival-promoting effect of IL-1ß was observed in the absence of increased [^{3}H]-thymidine incorporation. Based upon these initial results, it appears that the survival enhancing effect of IL-1ß upon neurons may be independent of its effect on astrocytes. (Supported by NIH grant NS 15013).

301.20

EXPRESSION OF MURINE INTERFERON-α/β IN THE CENTRAL NERVOUS SYSTEM. B. Tedeschi, F.J. Liuzzi and C.W. Morgan. Dept. of Anatomy & Neurobiology, East. Va. Med. Sch., Norfolk, VA 23501.

Previous studies have shown that cultured astrocytes (but not neurons), isolated from neonate mouse cerebral cortex, can be induced to produce interferon-α/β (muIFN-α/β) when exposed to a polyribonucleotide stimulus (Tedeschi et al., J. Cell Biol. 102:2244, 1986). In the present study, we examined (1) the *in vivo* expression of muIFN-α/β in the CNS and (2) the *in vitro* induction of IFN-α/β from a neural cell line by growth factors.

1-day and adult mouse brain/optic nerves were either frozen or paraffin-embedded and immunohistochemically examined for the presence of IFN-α/β. Results showed that neural cells in both neonate and adult optic nerve expressed IFN-α/β. The cell type(s) expressing IFN-α/β in optic nerve has not yet been identified while the expression of IFN-α/β by other brain regions will be described.

Since these data showed that IFN-α/β is expressed in normal CNS, we investigated whether growth factors might be an inducing stimulus for IFN-α/β. Growth-arrested C6 glioma cells were grown in the presence of EGF (a growth factor for C6 glioma), polyribonucleotides, or serum-free media. Results showed that both EGF and polyribonucleotides induced the expression of immunoreactive IFN-α/β, while cells grown in serum-free media did not express detectable immunoreactive IFN-α/β. These results suggest that growth factors can serve as an inducing stimulus for the expression of neural IFN-α/β.

301.21

EFFECTS OF PLATELET DERIVED GROWTH FACTOR ON THE DEVELOPING RAT CENTRAL NERVOUS SYSTEM. MB Giacobini*[1], A Smits*[2], K Funa*[2], B Westermark*[3], L Olson[1]. [1]Dept of Histology & Neurobiology, Karolinska Institutet, Stockholm, Sweden, [2]Ludwig Institute for Cancer Research, Biomedical Center, Uppsala, Sweden and [3]Dept of Pathology, University Hospital, Uppsala Sweden

The two forms of platelet derived growth factor, PDGF AA and PDGF BB, and one of their receptors have recently been found to be expressed in the central nervous system (CNS). These discoveries have promted us to investigate a possible functional role of PDGF in the developing CNS. Growth and survival of grafts from different regions of the developing rat CNS were followed by using the *in vivo* method of intraocular transplantation. Grafts were incubated in either PDGF AA (100ng/ml), PDGF BB (100 ng/ml) or vehicle solution alone (0,5 mg/ml HSA) at the time of transplantation and 5 µl injections of the same solution was administered to the anterior chamber of the eye on day 5, 10 and 15 postgrafting. Both PDGF AA and PDGF BB significantly increased the volume of transplanted E16 parietal cortex grafts when compared to grafts treated with vehicle alone. PDGF BB also seemed to sustain growth of transplanted E18 hippocampal tissue whereas PDGF AA showed a volume decrease when compared to PDGF BB and vehicle alone treated grafts. Histochemical and immunohistochemical studies were carried out on cryostat sectioned grafts to look at effects on glial and neuronal populations and vascularization, among others. An increased gliosis was seen in both PDGF AA and PDGF BB treated cortical grafts whereas all the hippocampal grafts, regardless of treatment, maintained a normal cytoarchitecture. These studies suggest that administration of exogenous PDGF can enhance survival and/or stimulate proliferation of cells in fetal brain tissue grafts, and that the effects of the different PDGF family members are region-specific. Furthermore, the two PDGF family members, AA and BB, are probably acting on different cell populations in the developing rat central nervous system.

301.22

TYPE I ASTROCYTES MEDIATE THE REGION SPECIFIC SUPPORT CELL INCREASE IN SUBSTANTIA NIGRA DOPAMINERGIC NEURON SURVIVAL. E. K. O'Malley, B.-A. Sieber, I. B. Black, and C. F. Dreyfus. Dept. Neuroscience & Cell Biology, R.W.J. Med. Sch, UMDNJ, Piscataway, NJ 08854.

Previous work in our laboratory has demonstrated that substantia nigra (SN) support cells selectively increase SN dopamine (DA) neuron survival in primary cell culture. Moreover, increased DA cell survival was elicited specifically by nigral support cells; glia from other brain regions exerted lesser effects. We now present evidence that Type I astrocytes, the principal components of SN support cell monolayers, mediate the enhanced DA survival.

Initially, we established the identity of the predominant glial subtypes present in SN support cell cultures. Pooled tissue from postnatal day 1 rat SN was dissociated and cells were grown to confluence (7-9 days in vitro (DIV)). Fixed monolayers were immunostained with antibodies against GFAP (an astrocyte specific marker), MBP (an oligodendrocyte cell marker), or A2B5 (identifying O-2A progenitors and Type II astrocytes). GFAP+ cells constituted more than 82% of monolayers, suggesting that astrocytes were the predominant subgroup comprising support cell monolayers. Further, direct comparison of GFAP+ (Type I & Type II astrocytes) and A2B5+ (Type II astrocytes) cell number indicated that the population consisted primarily of Type I astrocytes. Greater than 98% of cells were stained with these antibodies.

To definitively characterize the glial subtype that augments survival, individual cultures of each glial subpopulation were established (McCarthy and DeVellis,1980). At 2 DIV, enriched populations of Type I or Type II astrocytes, or oligodendrocytes, were tested for ability to elicit DA neuron survival. Embryonic day 16 rat SN dissociates were added and DA cell number was assessed with antibody against tyrosine hydroxylase (TH), the DA biosynthetic enzyme. After 2 days in coculture, the Type I astrocyte group exhibited greater than a 2-fold increase in TH cell number, while oligodendrocyte and Type II astrocyte cocultures did not differ from control. Our observations suggest that the Type I astrocyte subtype specifically elicits enhanced DA neuron survival in the SN. (Support:NINDS,NICHD,March of Dimes,JDF,McKnight)

301.23

CHARACTERIZATION OF A FACTOR WITH NEUROTROPHIC PROPERTIES ON TYROSINE HYDROXYLASE-POSITIVE VENTRAL MESENCEPHALIC NEURONS. B.A. Maguire, T.J. Collier, and J.E. Springer. Dept of Neurology, Hahnemann University, Philadelphia, PA, 19102 and Dept. of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

We have previously shown that conditioned medium (CM) from peripheral nerve or a Schwann cell line contain a protein(s), designated as dopaminergic neurotrophic factor (DNTF), that exhibits neurotrophic effects on ventral mesencephalic neurons immunoreactive for tyrosine hydroxylase (TH). Recent studies have indicated that one of these molecules may be related to brain-derived neurotrophic factor or BDNF. We used protein purification strategies to isolate and characterize fractions from Schwann cell CM that exhibit neurotrophic properties on TH positive ventral mesencephalic neurons. In addition, we tested various fractions of Schwann cell CM in explant cultures of chick retinal ganglion cells, which are thought to be responsive to BDNF. A Schwann cell line was grown in a stirring culture flask containing OPTIMEM plus 1% fetal bovine serum. Schwann cell CM was precipitated in 70% ammonium sulfate, dialyzed against 0.1M phosphate buffer, and eluted from carboxy-methyl cellulose with sodium chloride (NaCl). Protein peaks were collected, dialyzed, and tested using measures of cell number and neurite outgrowth in embryonic day 15 (E15) rat ventral mesencephalic neurons. In addition, embryonic day 6 (E6) chick retinal ganglion cells were cultured to test for the presence of BDNF-like activity in different eluted fractions of Schwann cell CM. An increase in the number of TH-positive ventral mesencephalic neurons was observed following treatment with Schwann cell CM fractions eluted with 0.5M NaCl. A similar procedure is used to isolate BDNF, although we have subsequently determined that the molecular weight of DNTF is different from BDNF. Chick retinal ganglion cells did not respond to any concentrations of fractions previously shown to exhibit DNTF-like bioactivity. Although DNTF can be isolated using techniques similar for BDNF, we have shown that the molecular weight and biological activity of DNTF is not similar to BDNF. Supported by PHS grants AG-08969 (JES) and AG-08133 (TJC).

301.24

MODULATION OF IMMEDIATE-EARLY GENE EXPRESSION BY GM1 GANGLIOSIDE. D. Krajnc[1], A-M. Duchemin[1], N.H. Neff[2,3] and M. Hadjiconstantinou[1,2,3]. Depts. of Pharmacology[1] and of Psychiatry[2] and The Neuroscience Program[3]. The Ohio State Univ. Col. of Med., Columbus, OH 43210 USA

We have previously shown that brief hypoxia in neonatal rats induces transient neurochemical changes and permanent neurochemical pathology. Moreover, we showed that treatment with GM1 ganglioside corrects both the long- and short-term hypoxia-induced deficits.

Immediate-early genes, that act as transcriptional modulators, are induced following acute insults to the brain, such as injury, ischemia and seizures. We now report that transient hypoxia, O_2 8% balance N_2 for 3 hr, induces the expression of *c-fos* and *NGFI-A* mRNA in the hippocampus and frontal cortex of 7 day old rats. The time-course for the hypoxia-induced expression of the mRNAs for these genes is similar, with a maximal increase of about 8-10-fold over control values, occurring within 1-2 hr after exposure and return to normal within about 3 hr. Treatment with GM1, 50 mg/kg i.p. daily, starting on day 5 and one dose immediately after the insult, appearers to enhance expression as well as induce earlier expression of the genes.

301.25

GM1 GANGLIOSIDE CORRECTS THE LOSS OF DA UPTAKE ACTIVITY IN EMBRYONIC MESENCEPHALIC CULTURES TREATED WITH MPP$^+$. A. Dalia[1*], N.H. Neff[1,3] and M. Hadjiconstantinou[1,2,3]. Depts. of Pharmacology[1] and Psychiatry[2] and the Neuroscience Program[3]. The Ohio State Univ. Col. of Med., Columbus, OH 43210.

We have reported that treatment with GM1 partially restores the biochemistry, pharmacology, morphology and behavior of MPTP-treated mice. The mechanism(s) for this activity of the compound is unknown. It has been reported that MPP$^+$, the neurotoxic metabolite of MPTP, decreases dopamine (DA) uptake activity in embryonic mesencephalic cultures. We have used embryonic mesencephalic cultures to investigate the action of GM1. Cultures were treated with MPP$^+$, 3 µM, for 24 hr, then GM1 was added to the medium and DA uptake assayed 12 days later. GM1 restored DA uptake activity in a dose-dependent manner, with about 500 nM GM1 returning transport activity to control values. Pretreatment or cotreatment with GM1 did not prevent the MPP$^+$-induced neurotoxicity. As has been reported, GM1 increased the DA uptake activity in control untreated cultures in a dose-dependent manner as well. For both MPTP-treated and untreated cultures the EC_{50} for GM1 was about 200 nM.

301.26

PLATELET-DERIVED GROWTH FACTOR INCREASES INTRACELLULAR CALCIUM LEVELS IN CULTURED NODOSE SENSORY NEURONS. G. Hajduczok, R.V. Sharma*, R.C. Bhalla*, and F.M. Abboud*. Depts. of Internal Medicine, Anatomy, and Cardiovascular Ctr., Univ. of Iowa Coll. of Med., Iowa City, IA 52242.

Accumulating evidence indicates that neurons are dependent on specific trophic factors for their growth and maintenance. Platelet-derived growth factor (PDGF) is a crucial factor for oligodendrocyte differentiation. We tested the hypothesis that PDGF has neurotrophic actions on visceral sensory neurons in vitro. Cytosolic free Ca^{++} concentration ($[Ca^{++}]_i$) was measured in cultured adult rat nodose ganglion neurons using fura-2 as the fluorescent Ca^{++} indicator and a microscopic digital image analysis system. In 13 of 21 neurons (62%), addition of PDGF (BB-homodimer;10ng/ml) on average resulted in a 6-fold rise in $[Ca^{++}]_i$ above basal levels. The results are shown below (*; significantly different from Control; $p<0.05$).

		PDGF	
	Control	Peak Response	Absolute Change
$[Ca^{++}]_i$ (nM)	70 ± 6	472 ± 100*	357 ± 103*

PDGF mobilized $[Ca^{++}]_i$ promptly within 10-20 sec and reached maximum levels within 2 min. These results suggest that in addition to its known mitogenic effects on nonneuronal cells, PDGF may have important neurotrophic activity in nodose sensory neurons mediated via Ca^{++} transduction pathways.

302.1

SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE EXPRESSION IN THE HUMAN MESENCEPHALON : IMPLICATION FOR PARKINSON'S DISEASE. P. Damier*, P. Zhang*, E. Hirsch, I. Ceballos[(1)]*, P.M. Sinet[(1)]*, Y. Agid, F. Javoy-Agid. INSERM U 289, Hôp. Salpêtrière, 75013 PARIS, [(1)]* URA CNRS 1335, Lab. de Biochimie Génétique, Hôp. Necker 75015 PARIS

Parkinson's disease (PD) is characterized by a massive loss in dopaminergic neurons in the midbrain, primarily the melanized neurons of the substantia nigra pars compacta. Although the cause of neuronal death remains unknown, oxygen free radicals have been implicated as a potential cytotoxic mechanism.

Examination of toxic species defence system in human midbrain post mortem showed : 1) the levels of expression of the gene of CuZn superoxide dismutase (SOD) (the enzyme catalysing conversion of superoxide radicals to hydrogen peroxide) were high in the neuromelanin-containing neurons, as revealed by in situ hybridization analysis at cellular levels. This indicates a neuronal localization of SOD mRNA. The high levels of transcripts in these neurons suggest biochemical pathways leading to oxygen species formation are particularly active. 2) Glutathione peroxidase (GPx), the enzyme scavenging hydroxygen peroxide was exclusively detected in glial cells by immunocytochemistry. The density of GPX positive cells was high in dopaminergic cell regions preserved in PD (central grey substance), low in the substantia nigra pars compacta dramatically affected. The density of GPX positive cells in dopaminergic cell subgroups in controls, was negatively correlated to estimated neuronal loss in PD. Dopamine neurons surrounded by GPX positive glial cells may be better protected against oxidative stress. Melanized dopaminergic neurons represent a subset of dopaminergic cells with respect to their oxygen defence system. A critical role of glial cells in neuronal protection against free radicals is suggested.

302.2

SEVERAL MECHANISMS CAUSE NEURONAL LOSS IN THE SUBICULUM IN ALZHEIMER DISEASE AND AGING P. Cras*, M. Kawai*, M. Stanik*, P. L. Richey*, S. G. Younkin, G. Perry. Div. of Neuropathology, Inst. of Pathology, Case Western Reserve Univ., Cleveland, OH 44106.

Development of neurofibrillary tangles (NFT) and neuronal loss are important pathologic events in Alzheimer disease. An unresolved question, however, is how many neurons die after developing NFT and what happens to the extracellular NFT (E-NFT) that remain. Alz-50, a monoclonal antibody to a modified form of τ exclusively stains intracellular NFT (I-NFT), while basic fibroblast growth factor (bFGF) binding is a sensitive assay for E-NFT. If all the neurons would follow the same path, develop I-NFT and all the E-NFT would remain indefinitely after the perikaryon disappears, the sum of I-NFT, E-NFT and the number of normal neurons would have to add up to a constant number. We determined the densities of SMI-32 (a marker of neuronal cytoplasm)-positive, Alz-50-negative pyramidal neurons (normal neurons), Alz-50-positive neurons (I-NFT) and of bFGF-positive E-NFT in the subiculum of 16 Alzheimer disease and 5 aged non- demented patients. Densities were calculated using the Abercrombie formula, but cerebral atrophy was not corrected for. We could divide our patients into 4 groups: group I consisted of 3 non-demented aged patients with normal subicular pyramidal neurons with a density ~250/mm^2; group II consisted of 4 patients (2 of whom were not demented) with significant neuronal loss, but only a few Alz-50 positive I-NFT and no E-NFT; group III consisted of 11 Alzheimer patients with varying numbers of I-NFT, E-NFT and normal pyramidal neurons; group IV consistied of 3 Alzheimer patients with a very high number of E-NFT and only a few I-NFT and pyramidal neurons left. In groups II and III, the sum of I-, E-NFT and normal neurons was well below the normal number of pyramidal neurons. In group IV, the sum of densities was ~250 mm^2 and therefore approached the number of normal pyramidal neurons found in the non-demented patients. These findings suggest: 1. In Alzheimer disease and aged patients not all neurons die by developing NFT. 2. A small group of Alzheimer patients has a high propensity to develop subicular NFT and all of these probably remain indefinitely as E-NFT after neuronal loss.This study was supported by a Fogarty International Fellowship to Drs. Cras and Kawai and by NIH grants K04-AG00415, AG-007552, AG09287.

302.3

ISCHEMIA SENSITIVE AND ISCHEMIA RESISTANT PRIMARY CULTURES OF CEREBELLAR GRANULE CELLS. W. Code, L. Peng*, L. Hertz*, A. Shuaib and B.H.J. Juurlink. Depts. Anesthesiology, Pharmacology, Medicine and Anatomy, University of Saskatchewan, Saskatoon, SK, Canada S7N 0W0.

Brain hypoxia and ischemia cause neuronal damage via a number of mechanisms, including, ATP depletion, free radical formation and excitotoxicity leading to receptor activated accumulation of calcium. It is difficult to sort out the contributions to neuronal damage of each of these. We describe here culture préparations that should facilitate a more ready dissection of the these mechanisms. Cerebellar neurons were prepared from 7-day-old mouse pups and cultured in the presence of serum at either a normal (NK: 5.4 mM) or elevated (HK: 25.4 mM) potassium concentration. Both culture types are viable for a period of three weeks. Cultures grown in the presence of HK developed a calcium-dependent glutamate release during superfusion with 50 mM potassium of ≈10 nmol/min/mg protein which is much larger than that seen in corresponding cultures grown in chemically defined medium. This response was absent in cultures grown in the NK medium although the voltage dependent uptake of calcium was not diminished. The ultrastructure of the cell bodies and the neuropil of cells grown in HK closely resembled those of cerebellar granule cells in vivo. Although cell bodies and axonal profiles including synaptic vesicles looked normal, dendritic development was poor with NK. Thirty min of hypoxia or ischemia caused death of neurons grown with HK; this death was prevented by the NMDA receptor blocker MK801. In contrast, approximately 6 hr of ischemia and more than 6 hr of hypoxia were required to cause death of the neurons grown with NK. Several factors might account for these differences in resistance to ischemia-hypoxia: 1) the release of glutamate under ischemia-hypoxia conditions may be less in NK cultures, or 2) glutamate release to the medium may be more toxic to HK cultures that are known to express NMDA receptors, than to NK cultures expressing quisqualate receptors (Cox et al., 1990. Neuron, 4: 941). The two conditions described for culturing granule cell neurons should enable us to dissect the contributions of excitoxicity to neuronal death from that of free radical formation and ATP depletion.

302.4

POST-ISCHEMIC SURGE IN CORTICOSTEROIDS AGGRAVATES ISCHEMIC DAMAGE TO GERBIL CA_1 PYRAMIDAL CELLS. G.D. Miller* and J.N. Davis, VA Neurology Laboratory and Duke University, Durham, NC

Glucocorticoids regulate CA_1 pyramidal cell damage after transient forebrain ischemia. To determine if normal glucocorticoid secretion contributes to damage after 5 minutes of carotid artery occlusion, we studied 4 groups of gerbils: 1) adrenalectomized 4 days before ischemia and then steroid replaced (10 mg cortisol / 3 mg corticosterone or 2 mg cortisol daily, n=9), 2) sham adrenalectomized (n=9), 3) no adrenal surgery, sham carotid occlusion (n=4), or 4) no adrenal surgery and carotid occlusion. The groups without adrenal surgery both showed an increase in plasma glucocorticoids at the time of anesthesia with halothane and continued to show elevated levels plasma levels for at least two hours after reperfusion. There was no statistically significant difference in body temperatures during or after ischemia between the adrenalectomized animals treated with glucocorticoids and the other groups. The animals were sacrificed 72 hours after reperfusion and CA_1 pyramidal cells were counted. Neurons were lost to the same extent in sham adrenalectomized animals (0.64 ± 0.10 neurons / 10 μ CA_1 length) and non-adrenalectomized animals subjected to ischemia (0.67 ± 0.10). Adrenalectomized animals with steroid replacement had significantly more CA_1 neurons than the non-adrenalectomized, ischemic animals (1.27 ± 0.20, $p<0.01$) but not as many as animals subjected to sham carotid occlusion (2.63 ± 0.19). Previous experiments have shown that steroid administration after ischemia worsens hippocampal damage. These data show that even the approximate doubling of plasma glucocorticoid levels during and after carotid occlusion significantly worsens hippocampal damage. (Supported by NS 06233)

302.5

INCREASE IN PROTEIN SYNTHESIS FOLLOWING TRANSIENT "ISCHEMIA" IN CA1 OF RAT HIPPOCAMPUS.
C. Charriaut-Marlangue*, M. Khrestchatisky and Y. Ben-Ari. INSERM U29, 123 bld de Port-Royal, 75014 Paris. France.

CA1 pyramidal cells of the hippocampus are particularly vulnerable to brief episodes of ischemia in vivo, exhibiting a prolonged inhibition of protein synthesis and a delayed morphological deterioration. In the present study, CA1 slices were maintened in vitro in a Krebs-bicarbonate solution at 32°C and gassed with 95 % O_2/5 % CO_2. After 2 hours equilibration, slices were then submitted to 3 to 10 min ischemia in glucose-free Krebs gassed with 95 % N_2/5 % CO_2, then transferred to normal Krebs for 30 min recovery in presence of ^{35}S-Met (10 µCi/ml media). We have compared the effects of 3-4 min to 5-10 min anoxia since the former but not the latter produced a reversible block of synaptic transmission (Hasboun D., Papas S., Crepel V. and Ben-Ari Y., unpublished results). A brief anoxia (<4') increased significantly (to 30 % of control levels, Student's t-test $p<0.005$) ^{35}S-Met incorporation into proteins. By two-dimensional gel analysis, the increase in protein synthesis concerned particularly a few proteins namely 20 kDa (pHi = 4.6); 30 kDa (pHi = 5); 38 kDa (pHi = 5.5) 68 kDa (pHi = 5.2) and 70 kDa (pHi = 5.9). In contrast a longer anoxia (>4') decreased ^{35}S-Met incorporation (to 20-25 % of control levels).

When slices were incubated with MK-801, a non-competitive antagonist of NMDA receptor-channel complex, 15 min prior and during a 5 min ischemia episode, protein synthesis rates were essentially identical to control rates. Although the functional consequences of increased expression of some proteins remain unknown, these results reflect the importance of protein synthesis on the anoxia cell damage.

302.6

THE (1S,3S)-ISOMER OF 1-METHYL1-1,2,3,4-TETRAHYDRO-β-CARBOLINE-3-CARBOXYLIC ACID, A BREAKDOWN PRODUCT OF PEAK 97 IMPLICATED IN THE L-TRYPTOPHAN EOSINOPHILIA MYALGIA SYNDROME (L-TRP-EMS), STEREOSPECIFICALLY PREVENTS NEURONAL DEATH IN SPINAL CORD CULTURES. E.M. Sternberg, S.W. Page*, Mark Smith and D.E. Brenneman. Clinical Neuroendocrinology Branch, NIMH; Center for Food Safety and Applied Nutrition, FDA and Lab. of Dev. Neurobio., NICHD, Bethesda MD 20892 and Washington D.C. 20204.

L-TRP-EMS, an inflammatory syndrome characterized by eosinophilia, perimyositis, fasciitis, neuropathies including Guillain-Barre syndrome, occurred in epidemic proportions in the United States in 1989, and was related to ingestion of contaminated batches of L-tryptophan (L-TRP). One high performance liquid chromatography peak (Peak 97), which is most highly statistically associated with human L-TRP-EMS, has been identified as 1,1^1-ethylidenebis(L-tryptophan) (EBT). At pH 2, or conditions present in gastric secretions, EBT decomposes to a diastereoisomeric mixture of (-)-(1S,3S)- and (-)-(1R,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (1S- and 1R-β-C). We evaluated the effects of synthetic EBT, the diastereoisomeric mixture, and pure 1R-β-C and 1S-β-C isomers (1 to 300 µM) on neuronal cell survival in dissociated spinal cord cultures derived from fetal mice. After a 5 day test period, 100 µM of the 1S-β-C, but not 1R-β-C or EBT, prevented the 30% cell death which normally occurs in these cultures. The dose response characteristics of this effect falls within the dose effect range for amino acids in this assay system. The stereospecificity suggests these effects may be receptor mediated.

302.7

Deprenyl Rescues MPTP-Exposed Nigrostriatal Neurons Independently of Blocking the Conversion of MPTP to MPP+. W. G. Tatton, C.E. Greenwood and P.T. Salo. Center for Research in Neurodegenerative Diseases and the Depts. Physiol. and Nutr.Sci., University of Toronto, Toronto, Canada M5S 1A8

Deprenyl has been hypothesized to slow the death of dopaminergic nigrostriatal neurons (DNSns) based on its slowing of the progression of Parkinsons Disease (PD). Previous workers co-administered MPTP and deprenyl to show that deprenyl blocks the astroglial conversion of MPTP to the active radical, MPP+, thereby protecting the DNSns from the toxin. To determine whether deprenyl protects DNSns independently of the above mechanism, 8 week old C57BL mice were treated with 30 mg/kg MPTP daily for 5 days. 72 hours after the last MPTP dose (day 8), the mice received L-deprenyl, either 0.25 or 10 mg/kg, or saline, i.p.3 times per week until day 25. Serial sections through the entire substantia nigra compacta (SNc) were alternately Nissl stained or immunoreacted for tyrosine hydroxylase (TH). Counts relative to the cross sectional areas of the Nissl and TH+ SNc somata were made for each entire nucleus. A proportion of the mice had Fluoro-Gold (FG; 8 nl) injected into one striatum 1 week before the MPTP treatment to unambiguously identify nigrostriatal somata. Collectively the immunocytochemistry , Nissl staining and FG identification showed that MPTP-exposed DNSns died gradually until day 25 with average losses of 18, 26, 35, 38, 39 and 37% at days 5, 10, 15, 25, 40 and 65 respectively. Whole nuclear counts at day 25 for animals only treated with saline were 3014 +/-304 (mean +/- SEM) and were 1872 +/- 187 (38% loss) for MPTP followed by saline. Counts at day 25 for animals treated with MPTP followed by deprenyl were 2586 +/- 161(14% loss) and 2535 +/- 169 (16% loss) for the 10 mg/kg and 0.25 mg/kg doses respectively. Since MPTP and MPP+ are completely cleared from the murine brain by 48 hours after an injection (Lau et al., Life Sciences 43, 1459-1464, 1988), these results show rescue of dying DNSns by deprenyl that is independent of the blockage of the conversion of MPTP to MPP+ and is similar to the rescue that can be effected by treatment with growth factors or gangliosides. (MRC & Parkinson Found. of Canada.)

302.8

EFFECTS OF LONG-TERM ALCOHOL CONSUMPTION IN THE HYPOTHALAMUS OF THE RAT. M.M. Paula-Barbosa, N. Sousa*, A. Mendes*, A. Cadete-Leite* and M.D. Madeira*. Dept. of Anatomy, Porto Medical School, Porto 4200, Portugal.

Chronic alcohol consumption (CAC) induces marked changes in blood hormonal levels. Notwithstanding, studies of the effects of CAC upon the structure of the hypothalamus are non-existent. To address this issue we analysed the following 6 groups of 6 male and 6 female rats: 1) alcohol-treated (A) with a 20% ethanol solution for 18 months, 2) respective pair-fed controls (C) and 3) withdrawn (W) animals following 12 months of CAC. The volume of the suprachiasmatic (SCN), supraoptic (SON), and paraventricular (PVN) nuclei was determined in celloidin sections and corrected for the shrinkage. The cross-sectional area of the neurons was calculated in semithin sections. The volume of the SON and of the magnocellular division of PVN was 30% larger in (A) than in (C). The nuclear volumes of (W) did not differ from those of (C). We found that in (A) the increase in size of magnocellular neurons reached 40%. The volumes of SCN and of the parvocellular division of PVN were reduced in (A) and (W). The sexual differences observed between controls remained among the experimental groups. We suggest that the widely known alcohol induced degenerative changes do not reflect on the volume of the magnocellular nuclei. This might be due to changes of hydric metabolism, which is altered after CAC leading to a continuous stimulation of the surviving cells. (Project PMCT/C/SAU/33/90 - JNICT).

302.9

ABSENCE OF C-FOS INDUCTION IN NEONATAL RAT BRAIN FOLLOWING SEIZURE. S.S.Schreiber, G.Tocco, I.Najm, and M.Baudry. Dept. of Neurology and Neurosciences Program, Univ. of Southern Calif., Los Angeles, CA 90033.

Activation of the proto-oncogene, c-fos, by a variety of extracellular stimuli has been implicated in the regulation of long-term adaptive changes within the mammalian central nervous system (CNS). We have been studying the pattern and time course of expression of c-fos following systemic administration of the excitatory neurotoxin, kainic acid (KA). In adult rats, KA induces a well-characterized seizure syndrome that results in irreversible morphologic changes and neuronal death in selectively vulnerable cell populations. In contrast, a behaviorally distinct seizure pattern occurs in neonatal rats characterized by prolonged seizure activity without subsequent neuronal death. We used *in situ* hybridization to localize and quantify c-fos messenger RNA (mRNA) in brain sections following KA treatment. There was no evidence of c-fos induction in hippocampus or other cortical structures 1 hour following seizure onset in rat pups less than 13 days old. In contrast, after postnatal day 13 elevated levels of c-fos mRNA were observed in the hippocampus and piriform cortex. Following seizure activity the level of c-fos mRNA gradually increased to reach adult values by 25 days of age. The increase in cortical structures was delayed by 5-6 days as compared to that seen in hippocampal structures. These results suggest that stimulus transcription coupling during early postnatal life may be different than in the adult. More importantly, the results suggest that the expression of c-fos may be an important determinant of neuronal survival. Finally, c-fos induction cannot be considered a reliable index of neuronal activation.

(Supported by NIH NS01337 to SSS and NS18427 to MB)

302.10

MDMA, A SUGGESTED 5-HT NEUROTOXIN, INCREASES CALCIUM INFLUX IN RAT BRAIN SYNAPTOSOMES. W.K. Park and E.C. Azmitia. Dept. of Biology, New York University, Washington Square East, NYC, NY 10003.

Excessive Ca^{++}- entry into neurons is one of the initial steps in cell death. 3,4-methylenedioxymethamphetamine (MDMA), a drug of abuse, produces neuronal degeneration by a mechanism involving 5-hydroxytryptamine (5-HT) release. The effect of MDMA is blocked by the calcium channel blocker nimodipine (Azmitia et al. Brain Res. 510:97,1990), suggesting that MDMA-induced degeneration may be linked to excessive calcium influx, probably through an interaction with 5-HT receptors. In the present study, we test if MDMA or 5-HT can stimulate calcium uptake using CNS synaptosomes. A micro-assay uptake system was modified to study the basal and stimulated (68.5 mMK^+) uptake of ^{45}Ca (Triggle, D.J. Trends Pharmac. Sci.5:4, 1984). Rat brain P_2 fractions are prepared for ^{45}Ca uptake by CNS synaptosomes using 96 well plates. Total uptake volume is 150 ul: 10 ul of ^{45}Ca (NEN, S.A. 16mCi/mg), 20 ul of tissue suspension, 10 ul of drug and 120 ul of buffers. The uptake is quenched by buffer containing 12 mM EGTA after 1s. K^+-stimulated ^{45}Ca-uptake is approximately 100% higher than that of basal conditions (4.5mM K^+). MDMA and 5-HT produced significant stimulation (40% of controls) in ^{45}Ca uptake in both conditions. Interestingly, MDMA-induced calcium uptake is blocked when synaptosomes are pretreated with the 5-HT uptake blocker fluoxetine. Current studies are aimed at understanding the possible involvement of the serotonergic system in the regulation of CNS calcium channels (NIDA contract, 271-87-8144).

302.11

RESPONSE OF MDCK AND "IMMORTALIZED" SEPTAL CELLS TO IONOMYCIN-INDUCED RISES IN INTRACELLULAR CALCIUM. A.M. Danks, R. VanBuskirk*, R.L. Isaacson, Dept. of Psychology & Biology, SUNY-Binghamton, Binghamton, NY, 13902-6000.

Increases in intracellular calcium were induced in SN56 B5 G4 (septal x neuroblastoma, derived from 21-day old mice) and MDCK (canine kidney) cells by incubation with 0.01 to 100 uM concentrations of the calcium ionophore ionomycin. The cells' response was monitored in situ using fluorescent probes in a multiple-endpoint assay system with the Cytofluor 2300 fluorescent plate reader (Millipore). Lysosomal integrity (neutral red), mitochondrial activity (rhodamine 123), intracellular Ca^{2+} (Fluo3-AM), intracellular pH (cfda-AM), and plasma membrane integrity (calcein-AM) were examined after 15 min incubation with the ionophore.

Fluo 3 indicated that $[Ca^{2+}]_i$ increased in response to increasing concentrations of ionomycin. In MDCK cells, intracellular pH and mitochondrial activity decreased in response to low concentrations of ionomycin, but increased in response to higher concentrations. Largest effects were seen on lysosomal integrity, where there was greater than 50% dissipation of neutral red. Plasma membrane was not compromised at any of these concentrations of the ionophore. Septal cells showed greater buffering capacity of ionomycin-induced rises in intracellular Ca^{2+}, as these indicators remained unchanged after 15 min incubation with the ionophore. Time-course studies are currently underway to examine when changes occur in these cells.

302.12

NEURONAL PLASTICITY AFTER SELECTIVE HIPPOCAMPAL DAMAGE; DETECTION BY CALCINEURIN IMMUNOHISTOCHEMISTRY. Y.Yamasaki*,H.Onodera*,K.Adachi*#,H.Shozuhara*,Y.Matsuo* and K.Kogure, Dept.of Neurology, Tohoku Univ.Sch.of Med., Sendai,980,Japan #; Sec.of Biology, Synphar Lab.Inc.,Edmonton,Canada

We observed regional alteration of immunoreactivity for calcineurin subunits (A and B) after selective hippocampal damage (CA1:transient ischemia, CA3:kainic acid injection) in rats. In control rats, the dentritic field of the CA1, CA3 and dentate gyrus exhibited high immunoreactivity (C-IR) of both subunits. After selective pyramidal cells damage in the CA1 and CA3 subfields, C-IR in the dentritic fields of the CA1 and CA3 was reduced, respectively. Interestingly, both CA1 and CA3 damages induced marked enhancement of C-IR (A and B subunits) in the CA3 stratum lucidum, where mossy fiber terminates, 4 to 100 days after lesioning. These increasing patterns for A and B subunits were different particularly after CA3 damage.

These results suggest that both calcineurin subunits are located predominantly on the CA1 and CA3 pyramidal cells and that enhanced C-IR of mossy fibers reflect plastic responses of dentate granule cells after hippocampal pyramidal cell depletion.

302.13

KAINIC ACID INDUCES PRODUCTION OF AMYLOID PRECURSOR PROTEIN IN RAT NEURONS. K. Shigematsu, T. Ishii and P.L. McGeer. Kinsmen Lab. for Neurol. Res., University of British Columbia, Vancouver, B.C., Canada, V6T 1Z3.

Wistar rats received unilateral intrastriatal injections of either 1 μg of kainate or saline and were sacrificed 1 day to 3 months thereafter. Brains were stained immunohistochemically with antibodies to various segments of amyloid precursor protein (APP) (R17:597-620, R36:528-540, R37:681-695, Ishii et al, Neuuropathol App Neurobiol 15:135-147). Immunoexpression of APP (R36 and R37) was observed in neurites and neurons 1 day after the kainate injections. The neurites were often thick and distorted with swollen varicosities. After 4 days immunoreactivity began to appear in macrophage/microglial cells and in the neuropil. This immunoreactivity increased for a further 1-2 weeks and then began to decline but was still present after 2-3 months. By double immunostaining it was demonstrated that the phagocytic cells loaded with APP immunoreactivity were Ia, LCA (OX1), C3bi receptor (OX42), and ED1 (macrophage) positive, but GFAP negative. No amyloid fibrils were detectable, and immunostaining for beta-amyloid protein (R17, 597-620) was consistently negative. These results suggest that APP production is upregulated in damaged neurons and accumulates in degenerating axons. In the rat, phagocytosis of APP by reactive microglia/macrophages does not result in amyloid production under the experimental conditions employed.

302.14

"Striatal D_1 and D_2 Receptor-containing Neurons in Culture: Susceptibility to Kainic Acid:

E.R. Mesco, J.A. Joseph, G.S. Roth* Gerontology Research Center, NIA/NIH Baltimore, MD. 21224 USA

Previous work in this laboratory showed a selective sensitivity of striatal D_2 receptor neurons to kainic acid (KA) in mature (6 mo.) Wistar rats. The receptor binding (B_{max}) for the D_2 receptor in the KA-treated rats resembled the binding seen in old (24 mo.) rats. In order to see if this phenomenon exists in cultured cells, which could provide a very accessible model for subsequent mechanistic studies, we examined striatal cultures grown for up to 30 days and labeled with either [^{3}H]-spiperone, for D_2 receptors, or with [^{3}H]-SCH23390, for D_1 recep- tors. Analysis of the cells was done using a digital imaging system (RAS). The cultured cells were examined for receptor binding, for cell size, and for susceptibility to KA. The D_2 cells showed an increased mortality over D_1 cells in the presence of KA [receptor x KA, $F(1,172)= 14.5$, $p < .002$], similar to in vivo, with larger D_2 cells showing the highest loss [mean cell area: -KA = 169.6 ± 5.0 μm^2; +KA = 155.5 ± 2.7 μm^2; $F(1,358) = 5.1$, $p < 0.03$].

302.15

The Rate of Cell Growth Determines Susceptibility to the Mitochondrial Toxin MPP^+.

A. Roghani, Y.-J. Liu*, R.H. Edwards. Department of Neurology, UCLA School of Medicine, LA, CA 90024.

Cells vary markedly in their vulnerability to the neurotoxin MPP^+. In PC12 cells, uptake of the drug through the plasma membrane catecholamine transporter appears required for toxicity. However, we find that CHO fibroblasts, without such transport activity, show even greater sensitivity to the toxin. Further, contact-inhibited confluent cultures show much greater vulnerability than rapidly growing subconfluent cultures. The effects seen under both conditions of growth occur with a similar half-maximal dose, suggesting that the differences in toxicity do not derive from differences in drug handling. These results suggest that the rate of cell growth constitutes a major determinant of susceptibility to a mitochondrial toxin.

In light of the modulation of toxicity by changes in growth rate, we have treated the NGF-responsive PC12 cell line with the toxin in the presence and absence of NGF. In these cells, NGF induces terminal neuronal differentiation to a postmitotic state. In accordance with the finding in CHO cells, NGF also markedly potentiates the toxicity of MPP^+ in PC12 cells.

302.16

APOPTOSIS IN A MOTOR NEURON-LIKE CELL LINE INDUCED BY SERUM DEPRIVATION OR 5-AZACYTIDINE.

N.R. Cashman, A. Abdollah*, I.T. Shaw*, J. Nalbantoglu*, A. Beaudet. Montréal Neurological Institute, Montréal, Québec, CANADA, H3A 2B4.

Selected neuronal growth factors may serve to suppress an apoptotic "cell death program" important in developmental matching of neuron number to target field. We have developed a model of apoptosis induced by growth factor deprivation in the motor neuron-like hybrid cell line NSC34 (Cashman *et al*, Soc Neurosci Abs 1987). NSC34 apoptosis, induced by replacement of 10% fetal calf serum (FCS) with 1% bovine serum albumin (BSA) in base DMEM, is accompanied by generation of "nucleosome ladders" on ethidium bromide-stained DNA gels, possibly due to activation of endogenous nucleases. Nucleosome ladders were also induced in DMEM 10% FCS with 3 μM 5-azacytidine, an inhibitor of maintenance methylase which reduces CpG methylation of proliferating cells and allows expression of previously quiescent genes. Ultrastructural features of BSA-induced NSC34 apoptosis include heterochromatinization, prominent nuclear indentation, and free membrane-bound nuclear fragments. Cell death, quantitated by propidium iodide exclusion on cytofluorometry, was inhibited to 50% at 3 days by 0.5 μM cycloheximide, an inhibitor of protein translation. We conclude that NSC34 cells, on serum deprivation or CpG demethylation, undergo cell death which exhibits molecular and morphologic features of apoptosis, and which requires expression of a cycloheximide-sensitive cell death gene cascade. The data are consistent with our hypothesis that developmentally silenced cell death genes, ectopically expressed in aging, could be responsible for selected neurodegenerative syndromes (Schwob *et al*, Ann Neurol 1990). NSC34 cells provide a convenient model to study the cell and molecular biology of apoptosis.

PATTERN FORMATION, COMPARTMENTS AND BOUNDARIES II

303.1

METALLOPROTEINASE IMMUNOREACTIVITY IN RAT BRAIN.

G.Nilaver, J.P.Alexander*, E.M.Shannon* and T.S.Acott*. Depts. of Neurology, Ophthalmology and Biochemistry, Oregon Health Sciences University, Portland, OR 97201.

Metalloproteinase (MP) activity has been implicated in remodelling of the extracellular matrix and in certain disease states (rheumatoid arthritis & tumor metastases). In the CNS, MPs are associated with neurite outgrowth. Growth cone extension is also blocked by inhibiting MP activity. We examined the distribution of transin [stromelysin (STR)] immunoreactivity in neonatal rats, adult rats, and rats bearing brain grafts. STR specific antibodies were used in immunohistochemical and Western (WoB) analyses. Staining in adult brain was confined to astrocytes and axon terminal profiles outlining neuronal somata. While astrocytic staining was present in most brain regions, axosomatic profiles were most intense in hypothalamus (preoptic, supraoptic, peri & paraventricular areas), amygdala, habenula and thalamus (periventricular & centromedian nuclei). Staining in neonate brain was diffuse and host brain around implant showed intense reactivity. A 30 and 138 kDa band were detected in neonatal and adult brain by WoB (in addition to the 57 & 45 kDa bands). The 30 kDa band was dominant in neonate with the 138 kDa band predominating in adult. Transin and type IV collagenase activities were detected in conditioned media from cultured mouse astrocytes, implying production in glial cells. The significance of the axosomatic staining is unknown. The expression of transin in brain has implications for CNS development and neurodegenerative diseases.

303.2

TOOTH PULP (TP) DEAFFERENTATION: CHANGES IN FUNCTIONAL PROPERTIES OF TRIGEMINAL (V) BRAINSTEM NEURONES IN RATS.

J.W. Hu, C. Kwan* and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto, Canada M5G 1G6.

This study was undertaken to determine if TP deafferentation can lead to functional changes in neurones in the V spinal tract nucleus of rats. Single neurone activity was recorded in V subnucleus oralis of adult anaesthetized rats 7-15 days after removal of the coronal TP of the mandibular molars. Compared with control animals, animals deafferented for 7-15 days showed no significant difference (Fisher's test; $p > 0.05$) in incidences of low threshold (LTM) neurones (92% in deafferented rats Vs 97% in controls), LTM neurones with rapidly adapting (81% Vs 87%) Vs slowly adapting (19% Vs 13%) properties, and neurones showing habituating tap sensitivity (1% Vs 1%); mean latencies to electrical stimulation of the mechanoreceptive field (RF) were also not significantly different. Deafferented animals did however show significantly increased incidences of LTM neurones with a RF involving 2 or 3 V divisions (28% Vs 9%), maxillary and mandibular divisions (17% Vs 3%) and periodontal input from both divisions (8% Vs 2%), and with spontaneous activity (16% Vs 8%). These findings show many similarities with TP deafferentation effects on oralis neurones in the cat and indicate that the rat can be used as a model for TP-induced V brainstem neuroplasticity. Supported by NIH grant DE04786.

303.3

REGIONAL DIFFERENCES IN CELL PROLIFERATION AND NEURITE GROWTH IN THE MAMMALIAN TELENCEPHALIC VESICLE. T.F. Coburn and A.-S. LaMantia, Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710.

We have sought evidence of cellular differentiation in the mammalian telencephalic vesicle prior to its division into the rudiments of the olfactory bulb, hippocampus, basal ganglia, basal forebrain and neocortex. At this stage (midgestation in mice or birth in the brazilian opossum) most cells in the vesicular epithelium are still actively dividing; few are post-mitotic. We analyzed the distribution of cells in S-phase of the cell cycle labelled after a brief pulse of bromo-deoxyuridine (BrDU) in mice and opossums at these ages. There was a high density of labelled cells at the dorsal anterior pole of the vesicle and at several foci along the lateral surface. A low density of labelled cells was seen at the mid-ventral region of the medial wall and the posterior pole. Further characterization of the epithelium showed that immunoreactivity for GAP-43, MAP-2, a synaptic vesicle antigen SVP-48, and a novel antigen found in growing neurites, EA1B1, was limited to regions with higher densities of BrDU-labelled cells. For each marker, immunoreactivity was observed around cells at the vesicular surface, suggesting the early appearance of a marginal zone in regions with high rates of cell proliferation. Anti-GAP-43 showed the sharpest boundaries between regions with high and low rates of cell proliferation; cells in both the marginal area and the underlying columnar epithelium were immunoreactive. Electron microscopic examination of the marginal regions revealed immature neurites and vesicle-filled profiles among otherwise undifferentiated neuroblasts; however, no neuritic processes were seen in the underlying columnar epithelium. These results indicate appreciable cellular and molecular differentiation in the telencephalic vesicle that may prefigure its division into the major regions of the forebrain.

303.4

GRAFTING ANALYSIS OF THE MATERNAL EFFECT MUTANT JERKY OF XENOPUS LAEVIS. R. Tompkins, Q. Le*, D. Reinschmidt*. Cell and Molecular Biology Dept., Tulane Univ., New Orleans, LA 70118

A mutant allele, jerky, which causes a lethal maternal effect targeting anterior neural development is described in X. laevis. All offspring of homozygous females exhibit abnormal swimming behavior and die during late embryonic stages. Chimerae consisting of a normal anterior half, including the brain and anterior spinal cord, and a maternally affected posterior half, developed normally whereas the reciprocal chimerae died of the maternal effect. Grafts of normal heads including the brain to posterior maternally affected bodies incorporating the anterior spinal cord resulted in normal anterior neural development but mutant swimming behavior and death. Orthotopic grafts of eyes from maternally affected embryos to normal hosts developed normally. The unusual localized lethal maternal effect on anterior neural development is exceedingly fine grained. The chimerae revealed autonomous expression of behavioral effects and lethality in the anterior spinal cord, autonomous retarded development and lethality in head transplants, yet only secondary or dependent effects on eye development.

Supported by a Department of Defense grant administered by the Defense Nuclear Agency and the Tulane Center for Bioenvironmental Research.

303.5

ANALYSIS OF CEREBELLAR CIRCUITS IN ENGRAILED-2 DEFICIENT TRANSGENIC MICE. K. Herrup, H. Zanjani, K. Millin*, J. Rossant*& A.L. Joyner*. E.K. Shriver Center, Waltham, MA. and Univ. of Toronto, Toronto, ONT

One of the mouse homologs of the *Drosophila engrailed* gene (*En-2*) has been mutated by homologous recombination in ES cells and introduced into the germ line through ES cell chimeras. Homozygous mutants survive but have unusual cerebellar foliation. Quantitative analysis of several different cell types reveals a reduction in Purkinje cell number of almost 40% in the mutant. This deficit, however, is not uniform. The Crus I folium of the hemisphere is missing, and the size of the paraflocculus is reduced. In the vermis, all folia are present, but certain folia (*e.g.* declive and uvula) show reductions in Purkinje cell number while others (*e.g.* culmen and tuber vermis) show little or no change. The number of granule cells is also reduced, by around 40%, but the density of granule cells is unchanged. In no region examined does the thickness of the granule cell layer differ from normal values, nor does the density of cells in the Purkinje cell layer change. This latter observation is in contrast to known neurological mutants where a late postnatal loss of Purkinje cells occurs. This suggests that the cell number deficits exist from early stages of postnatal cerebellar development. Preliminary cell counts of the neurons of the inferior olive also reveal a decrease in cell number. Breeding experiments with *reeler* and *weaver* mutants in progress as is a developmental profile of both the morphological abnormalities and the pattern of *En-2* expression.

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303.6

A NOVEL ROLE FOR SYNAPTIC COMPETITION IN THE DEVELOPMENT OF CORTICAL LAMINATION. Z.F. Mainen,* B.J. Claiborne,† and T.H. Brown. Dept. of Psychology, Yale University, New Haven, CT 06520 and †Division of Life Sciences, Univ. of Texas, San Antonio, NW Loop 1604, 78285.

Numerous studies have supported the hypothesis that activity-dependent synaptic competition partly mediates the formation of neuronal connection patterns in the visual cortex (reviewed in C. Shatz, 1990, *Neuron*, 5, 745-756). Using computer simulations, we have investigated the possibility that similar competitive mechanisms may act *within* single neurons to sharpen the spatial lamination of different sets of afferent fibers.

Standard compartmental techniques were used to model the electrotonic structure of hippocampal CA1 pyramidal neurons based on data from anatomical reconstructions and physiological recordings (see Brown, T.H., Zador, A.M., Mainen, Z.F., and Claiborne, B.J., 1991, in M. Baudry & J. Davis, eds., *LTP: A Debate of Current Issues*, MIT Press). Individual lamina with different distributions of afferents were chosen and stimulated such that synaptic activity within a lamina was more strongly correlated than that between laminae. The initial distributions of afferents were subject to modification based on a Hebbian rule with potentiation and depression terms linked to subsynaptic voltages.

Our results suggest that synaptic competition may act to eliminate afferents with less "favorable" locations—those located among afferents with whose activity they are not correlated or those located in regions that have less dense synaptic contacts. Thus, activity-dependent processes may help to fine tune a less precise distribution of afferents specified by other processes earlier in development. These processes could be important to the development and maintenance of lamination in the hippocampus as well as other laminated structures such as neocortex. (Supported by ONR and DARPA grants to T.H.B. and NSF to B.J.C.)

303.7

THE DEVELOPMENT OF CRUDE AND REFINED MAPS IN THE RAT SI CORTEX. L. Zhang and N. G. F. Cooper. Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163

Patterns of thalamocortical projections from the ventrobasal complex of the thalamus to SI cortex were examined with the carbocyanine dye, DiI, in aldehyde-fixed neonatal rats. Patterns of neurons in layer IV of SI cortex were also examined in cresyl violet stained sections. Patterns of molecular expression evident as "boundaries" within layer IV of SI cortex were observed in sections labeled with the monoclonal antibody to the HNK-1 epitope. Thalamocortical axon projections are organized into several patches in the cerebral cortex by late P1 (day of birth). These patches, together, represent the complete body surface in the cortex. Five rows of distinctive axonal arbors, representing individual mystacial vibrissae of the contralateral face, are clearly present in the face subfield by late P2. However, distinctive axonal arbors within lower lip, forelimb and hindlimb subfields become evident, sequentially, between P3 and P5. Layer IV neurons are similarly aggregated into several dense cellular patches by P3. These patches indicate the presence of subfields which become more distinct by P4. Barrels are first detected in the snout and posterior medial barrel subfield on P4. Barrels in other subfields become clear, sequentially, between P5 and P7. Subfields, labeled with anti-HNK-1 antibodies were evident prior to the appearance of barrel boundaries. These data indicate that the development of cortical representation of the body surface proceeds from the development of a crude anatomical map to one that is shaped and refined further with increasing postnatal age. supported by NIH:NEI EY02708.

303.8

QUANTITATIVE ANALYSIS OF SPARED INFRAORBITAL (IO) PRIMARY AFFERENTS IN PARTIALLY DENERVATED SUBNUCLEUS INTERPOLARIS (SpVi). S.S. Stansel, M.F. Jacquin and W.E. Renehan Dept. of Anatomical Sciences and Neurobiology, University of Louisville; Dept. of Anatomy and Neurobiology, St. Louis Univ. School of Medicine and Div. of Gastroenterology, Henry Ford Hospital, Detroit, MI.

We have previously shown that complete transection of the IO nerve at birth results in expansion of the central terminal arbors of vibrissa-sensitive primary afferents reinnervating the periphery (Renehan et.al., JCN 289:493-508, 1989). In the present study we have partially denervated the trigeminal brainstem nuclear complex by cauterizing the mystacial vibrissae of rows A, C and E. This paradigm permits a more effective evaluation of the role of competition in arbor development. Thirty physiologically characterized afferents supplying the spared B and D row vibrissae were labeled by intraaxonal injection of HRP. Central terminal arbors in SpVi were compared with previously obtained normative data (Jacquin et.al., JCN 267:107-130, 1988), revealing: 1) a significant (two-tailed, independent t-test, $p<0.001$) increase in the area (13512 ± 7052 vs. 6130 ± 2060 um^2) perimeter (657 ± 292 vs. 379 ± 87 um) and circularity (form factor: 0.74 ± 0.14 vs. 0.55 ± 0.11) of the central terminal arbors as well as a marginally significant ($p<0.05$) decrease in the number of boutons per collateral (83 ± 13 vs. 124 ± 28). The results of this investigation suggest that 1) spared IO primary afferents undergo arbor expansion but not sprouting following partial denervation of the trigeminal brainstem nuclear complex at birth, and 2) arbor dimensions are constrained by terminals of adjacent vibrissae. Supported by DE07734 and DE07662.

303.9

SEX-DEPENDENT ASYMMETRY IN BARREL CORTEX ORGANIZATION DURING POSTNATAL DEVELOPMENT. A.L. Roca, J.E. Crandall, D. Butler*, R.C. Whorf, & S.A. Tobet. Depts. of Biochem. & Develop. Neurobiol., EK Shriver Ctr., Waltham MA 02254 & Prog. Neurosci., Harvard Med. Sch., Boston, MA 02115.

Layer IV of rodent somatosensory cortex contains discretely patterned morphological units characterized as barrels which correspond to physiologically functional units. In rats, Nissl-stained barrels become increasingly difficult to distinguish after postnatal day 20 (P20; Rice, 1985). We investigated sex-dependent barrel patterns in rat cortex in 3 independent experiments. Barrel field areas were estimated from 2-D composites of flattened tangential Nissl-stained sections. An asymmetry index (AI) was calculated: (R-L)/(0.5(R+L)), with R the right side area and L the left side area. In P20 Long-Evans rats, the barrel field in females was significantly larger on the right (AI= +0.31 $\pm$ 0.08); in males, on the left (AI= -0.33 $\pm$ 0.15). In P20 Sprague-Dawley rats, females tended toward lateral asymmetry (AI= +0.32 $\pm$ 0.23) whereas males did not (AI= +0.06 $\pm$ 0.06). Another experiment using Long-Evans rats confirmed that the barrel field in females (AI= 0.45 $\pm$ 0.14) was significantly different from males (AI= -0.20 + 0.11) in the direction and magnitude of asymmetry. No significant sex differences in symmetry were found at P10 or P30, although the absolute degree of asymmetry increased between P20 and P30. We hypothesize that sex-dependent asymmetries result from lateralized, developmental differences in cellular reorganization. Ongoing experiments test this using cytochrome oxidase histochemistry to demarcate barrel boundaries in the same sections that Nissl stains determine cellular patterns.

303.10

EXPRESSION OF MOLECULES WITH THE HNK-1 EPITOPE IN BARRELFIELD AND NON-BARRELFIELD CEREBRAL CORTEX OF POSTNATAL RATS. N. G. F. Cooper, and L. Zhang. Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163

We have used the monoclonal antibody to the HNK-1 epitope to determine how many molecules bearing this epitope are present in the rat cerebrum and also to determine if there is any variability in the expression of such molecules with respect to space and time. Postnatal day 7 and 26 rat pups (3 experimental groups from different litters) were anesthetized with Ketaset. Cerebral cortex was removed and 100 micron tangential vibratome sections of the fresh tissue were examined under a dissecting microscope for the presence of barrels. Barrelfield cortex (BFLD) and more posteriorly located areas in non-barrelfield cortex (N-BFLD) were dissected out and homogenized separately in tris-buffered saline. The tissue extract was solubilized and separated by PAGE. Western blots were incubated with anti-HNK-1 antibody, followed by secondary antibody coupled to peroxidase. Molecules bearing the HNK epitope were visualized following peroxidase chemistry. Anti-HNK-1 antibody was omitted from incubations of control blots which were otherwise similarly treated. Four novel pieces of information can be discerned from these blots: (1) approximately 10 molecules are labeled by the antibody, with major glycoproteins having relative molecular weights of 240K, 200K, 175K, 150K and 145K; (2) there is not a reduction in the number of molecules between P7 and P26. This is not consistent with the loss of boundaries observed with tissue immunocytochemistry between these ages; (3) the presence of an additional glycoprotein 175K in the P26 animals is seen in both BFLD and N-BFLD; (4) reduction in the amount of a glycoprotein 145K in BFLD relative to N-BFLD is seen at both P7 and P26. These data lead us to suggest that several cell adhesion molecules bearing the HNK epitope could be involved in barrel formation. Glycoprotein 145K maybe down regulated in barrel hollows during barrel formation. This could be due to the invasion of thalamocortical afferents which may inhibit glia from producing cell adhesion molecules. Supported by NIH:NEI EY02708.

303.11

THE EXPRESSION OF 3-FUCOSYL-N-ACETYL-LACTOSAMINE (FAL) ON GLIA IN RELATIONSHIP TO NEURONAL LAYER DEVELOPMENT. J. Mavity-Hudson[1], J.Witty[1] and V.A.Casagrande[1,2], Depts.of Cell Biology[1] and Psychology[2], Vanderbilt University, Nashville, TN 37215-2175. Recent studies from our laboratory have suggested that glial cells may play an active role in the Lateral Geniculate Nucleus (LGN) cell layer development (Hutchins and Casagrande, PNAS, 1988 and JCN, 1990). In this study we examined the possibility that specific glial associated cell adhesion molecules (CAMs) may be involved in this process. Evidence suggests that FAL is a carbohydrate epitope on a glial CAM (Niedieck and Loehler, Acta Neuro-Path., 1987) that may be developmentally regulated in the LGN (Mai and Schoenlau, Soc for Neurosci. Abst., 1990; Mavity-Hudson et al. Invest. Opthalmol. Vis. Sci. Suppl., 1991). We examined the expression of an antibody to FAL (Leu-M1) and to Glial Fibrillary Acid Protein (GFAP) in developing ferret LGN from birth (P0) to adult. At P0 no cell layers are present and GFAP and FAL appear mainly in radial glia. As Interlaminar Zones (ILZs) first appear (P7-P14) both antibodies show darkest staining in these zones. Staining is also somewhat heavier in the A layers than in the C layers. At P21 ILZ staining with FAL is still distinct; GFAP staining of astrocytes is uniform from this stage on. In adults FAL shows a mirror image pattern to that exhibited in development, with light staining confined to the layers and absent from the ILZs. Taken together with previous results showing that FAL is developmentally regulated in the visual system, these results suggest that LGN layer formation may involve the differential expression of CAMs on subsets of astrocytes.
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303.12

CC1 GLYCOCONJUGATES DEFINE A DEVELOPMENTALLY REGULATED ROSTROCAUDAL GRADIENT IN THE ACCESSORY OLFACTORY SYSTEM AND IN SYMPATHETIC GANGLIA IN RATS. G. Deutsch*, D. Gattey*, C.M. Story*, J.E. Crandall and G.A. Schwarting. Developmental Neurobiology Dept. EK Shriver Ctr, Waltham, MA 02254; and Program in Neuroscience, Harvard Medical School, Boston, MA

The CC1 monoclonal antibody reacts with beta-N-acetylgalactosamine terminal glycolipids which are expressed selectively on cells of sympathoadrenal lineage and on sensory neurons in the vomeronasal organ (VNO). In the accessory olfactory system, CC1-immunoreactivity (CC1ir) was demonstrated throughout the VNO and vomeronasal nerves from E15 to PO. Postnatally CC1ir was spatially restricted in the VNO to centrally located cell bodies. In the accessory olfactory bulb, where vomeronasal axons terminate, CC1ir became restricted postnatally to the rostral half of the nerve and glomerular layers in the target area.

In cells of the developing sympathoadrenal lineage, CC1ir was detected in the superior cervical ganglion (SCG) and stellate ganglion perinatally. At E15-17, most cells in the SCG were CC1ir. Between E18 and P3, CC1ir became restricted to groups of cell bodies predominantly in the rostral half of the SCG. CC1ir was no longer detectable in the SCG after P5. In the stellate ganglion at E15-18, CC1ir was concentrated in cells located in the rostral half and along the perimeter. These studies raise the possibility that CC1ir glycoconjugates participate in a specific chemical guidance system for subsets of peripheral axons. Alternatively, CC1ir glycoconjugates may play a role in establishing selective target regions for innervation by CNS neurons.

303.13

GLYCOCONJUGATES IN A DEVELOPING OLFACTORY SYSTEM. C.E. Krull, D.B. Morton, and L.P. Tolbert, ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

During the development of the olfactory (antennal) system of the moth *Manduca sexta*, glial cells may play an intermediary role in the influence of olfactory axons on their target neurons in the antennal lobe (Tolbert & Oland, 1989). Glia envelop developing olfactory glomeruli and appear to be necessary for induction of glomeruli by the olfactory axons. To begin to explore the molecular basis for these intercellular interactions, we have examined the distribution of glycosylated molecules, implicated in many cell-cell associations (Lander, 1989). We have examined the patterns of binding of an antibody to the J1/tenascin molecule (gift of Dr. M. Schachner) and of a panel of lectins in antennal lobes at various stages of normal development and in antennal lobes deprived of olfactory axons throughout development. We find J1/tenascin immunoreactivity associated with glial cells, just as it is associated with the glial boundaries between barrels in the developing mouse somatosensory cortex (Steindler et al., 1989). While other lectins show no specific staining patterns, the lectin peanut agglutinin (PNA) binds to the target neurons of the antennal lobe. PNA specifically labels the fine terminal branches of the neurons as they grow and form synapses. Both PNA and anti-J1/tenascin binding appear even before afferent axons from the antenna arrive in the lobe, and also are present in unafferented antennal lobes. Western-blot analysis of the PNA binding in normal antennal lobes has revealed multiple protein bands underlying the histological staining pattern; two bands appear during development at potentially interesting stages. Experiments are under way to explore whether the molecules we are labeling play important roles in the development of glomeruli. (Supported by NS20040 to LPT.)

303.14

DEVELOPMENTAL EXPRESSION OF MEMBERS OF THE ANNEXIN FAMILY OF PROTEINS IN THE DEVELOPING CNS OF THE MOUSE. D. Goldowitz, K. Hamre, H. Horn*, N. Ahn* and K.P. Chepenik* University of Tennessee College of Medicine, Memphis, Howard Hughes Medical Institute, Seattle, and Jefferson Medical College, Philadelphia.

This study was carried out to examine the role that the annexins, a family of proteins that have calcium/phospholipid binding activity, play in the development of the murine CNS. Antibodies specific for 5 members of this family (lipocortinI, lipocortinII, endonexin, endonexinII, and 67K calelectrin) were used for immunocytochemical localization.

Of the 5 annexins studied, lipocortinI & II and endonexin had the most interesting temporal and spatial patterns of expression. LipocortinI immunoreactivity was first noted on embryonic day 11 (E11) in the floorplate of rostral spinal cord. The staining extended anteriorly through the brainstem as a densely immunopositive midbrain raphe. LipocortinII immunoreativity was noted at E10; staining was present throughout the floor plate of the spinal cord and was co-extensive with lipocortinI staining along the midbrain raphe. Endonexin-positive staining was present at E9 in the floor plate of the spinal cord. Staining was also evident in the roof plate at E10, in regions of postmitotic cells, and in the developing CNS vasculature. Not only was endonexin immunoreactivity expressed earliest, but was the most extensive along the rostral-to-caudal axis. Furthermore, dense endonexin immunopositivity in the adjacent notochord preceded staining in the floor plate by 24 hours.

These findings indicate that members of the annexin family have specific roles in the early development of the mammalian CNS.

303.15

SPECIFIC AXONAL PATHWAYS EXPRESS A DEVELOPMENTALLY REGULATED ANTIGEN RECOGNIZED BY A MONOCLONAL ANTIBODY. J.D. Peduzzi, A.M. Stewart and E.E. Geisert, Jr. Department of Physiological Optics, School of Optometry (J.D.P.) and Department of Cell Biology, Neurobiology Research Center (A.M.S. & E.E.G.), University of Alabama at Birmingham, Birmingham, AL 35294.

Within the developing CNS, putative axonal pathways can express a variety of proteins that appear to be involved in the guidance of growing axons. We have identified a mAb designated 11-59 that labels the plasma membranes of a subpopulation of cultured living astrocytes and appears to interfere with neuron-astrocyte interactions in culture. This antibody also labels discrete regions of the developing and mature rat brain. At E17, 11-59 binds to fibers in specific pathways of the brain including the internal capsule and fasciculus retroflexus. Heavy labeling is also observed in the median raphe glial structure. Labeling in the neocortex is present deep to the cortical plate. In the adult, there appears to be a generalized increase in the expression of the 11-59 antigen. However, localized concentrations are observed in the supragranular layers of neocortex, CA1, substantia nigra, basal ganglia and granular cell layer of cerebellum. The pattern of 11-59 labeling in the developing and adult brain appears to differ from other known developmental markers such as NCAM, TAG-1 and S-100. Taken together, these data suggest that the 11-59 antigen may play an important role in the development of specific pathways within the CNS. Supported by The Whitehall Foundation, Inc.

303.16

EXPERIMENTAL EVIDENCE THAT AN INHIBITORY BOUNDARY DELAYS NEURAL CREST MIGRATION. C. Lasky,* R.A. Oakley, D. Dehnbostel and K.W. Tosney. Biology Dept. and Neuroscience Prog., Univ. of Michigan, Ann Arbor, 48109.

Last year we reported that neural crest cells delay their migration into the dorsolateral path between the ectoderm and the dermamyotome: they enter 24 hours after the first neural crest cells have invaded the anterior sclerotome, during the period when the dorsolateral path is losing peanut agglutinin (PNA)-binding sites (Schroeter et al., 1990, Neurosci. Abs. 16: 313). This year we report experimental evidence that the early dorsolateral path does inhibit neural crest migration.

We deleted the dermamyotome before neural crest cells left the neural tube and later identified neural crest cells with HNK1 (kind gift of Carol Erickson) or diI-label in serial frozen sections. We found that when the dermamyotome is absent, PNA-binding activity is greatly diminished in the dorsolateral path and neural crest cells enter this path precociously. We conclude that the early dorsolateral path normally inhibits neural crest migration. In addition, since neural crest cells enter the path without delay when PNA-binding activity is prematurely diminished by surgery, we conclude that inhibitory activity and PNA-binding sites are expressed coordinately. Since PNA-binding sites are also expressed in the inhibitory posterior sclerotome before neural crest cells encounter it, and since PNA-binding sites typify all tissues shown to inhibit the outgrowth of motor and sensory axons, we suggest that molecules that bind PNA or co-regulated molecules are likely to restrict the advance of both neural crest cells and axons.

Supported by NIH grant NS-21308.

303.17

NON-SEGMENTAL PATTERN FORMATION IN THE VERTEBRATE SPINAL CORD. A.Chen and R.D.Heathcote. Department of Biological Sciences, University of Wisconsin, Box 413, Milwaukee, WI, 53201.

Early in development, a population of interneurons differentiated in a distinct pattern within the central nervous system. These neurons extended from the base of the brain to the tip of the tail in larvae of the frog *Xenopus laevis*. Throughout this domain, the number and spacing of cells was similar. The iterative spatial pattern differed from that known for other central neurons and appeared to be generated independently from the segmental somites.

The population of neurons was identified by the presence of catecholamines within their cell bodies. Shortly after hatching (2.3 days or stage 39), catecholamine-containing cells were visible following treatment with glyoxylic acid. Cells containing immunoreactivity to tyrosine hydroxylase, the enzyme needed to synthesize catecholamines, were present in the same location and pattern within the spinal cord. The neurons bordered the central canal and their location was limited to 2 columns centered around the midline within the narrow sheet of floor plate cells. At 1 week of development, the spacing between catecholamine-containing neurons was variable, with cells apparently located at random points along the spinal cord. However, the cells maintained a mean distance of approximately 21 μm. The average distance maintained between cells within a single column was roughly twice this value. This pattern is what would be expected of cells that differentiated in a density-dependent manner. In this situation, the field over which the differentiation occurred was restricted to the essentially 1 dimensional array of floor plate cells. Perhaps this simple pattern is unusual because it consists of interneurons. Motor and sensory neurons project to the periphery and are patterned by the mesodermally-derived segments, but the catecholamine-containing interneurons appear to be patterned by a mechanism operating entirely within the central nervous system.

303.18

RETINOIC ACID REGULATES EXPRESSION OF HOX 1.6 PROMOTER-LAC Z TRANSGENE IN DEVELOPING MOUSE HINDBRAIN. M. Zhang, M.T. Gendron-Maguire*[1], D.A. Lucas*, A. Baron*[2], T. Gridley*[1] and J.F. Grippo*. Dept. Toxicology and Pathology, Hoffmann-La Roche, Nutley, NJ 07110, [1]Dept. Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, NJ 07110, [2]Institute for Immunology, Hoffmann-La Roche, Basel, Switzerland.

A fragment of mouse homeobox gene Hox 1.6 is fused with a reporter gene lac Z and used to generate transgenic mice via pronuclear injection. This construct contains 6.3 kb of Hox 1.6 genomic sequence upstream of the transcription initiation site, 0.7 kb of the first exon fused in-frame with lac Z and a SV40 polyadenylation signal. The transgene is prominently expressed in a restricted region of the developing mouse hindbrain at days 7.5 - 10.5 pc, exhibiting a more anterior boundary of expression than endogenous Hox 1.6. During this time a lower level of expression is seen in the somites. When the embryos are exposed to retinoic acid at 7.5 pc, expression of the transgene in the hindbrain is repressed, while that in somites is not affected.

303.19

A ROLE FOR *ENGRAILED* GENES IN ZEBRAFISH MUSCLE PATTERNING. M. Westerfield, J. Wegner*, M. Akimenko*, M. Ekker*, J. Eisen, M. Halpern. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Homeoproteins, like *engrailed (eng)*, are DNA-binding proteins which regulate cell fate and pattern formation in *Drosophila*. To better understand their functions during vertebrate development, we have examined the expression of *eng*-like genes in zebrafish embryos.

In zebrafish, *eng* proteins are expressed by a variety of cells including a small subset of axial muscle cells, called muscle pioneers (MPs). MPs express *eng* before differentiation of the muscle, and patterning depends upon these cells; ablation of the MPs disrupts subsequent organization of the muscle. DNA, RNA and protein analyses and the sequences of cloned cDNAs demonstrate that zebrafish have at least two *eng* genes that share common features with mouse and *Drosophila* homologues. We have studied ectopic expression of one of these genes, *eng-2*, in transgenic embryos and find that its expression in non-pioneer muscle cells is insufficient to change them into MPs.

The MPs and *eng* expression in the muscle are both absent in *ntl-1* mutants, which lack notochords, and after notochord ablation in wild-type embryos. This suggests that the notochord and the MPs are developmentally related. One possibility is that the notochord induces *eng* expression in precursors of the MPs which, in turn, pattern the axial muscles. Supported by NIH HD22486 and NS21132.

303.20

MORPHOLOGICAL EVIDENCE OF SEGMENTATION IN THE DEVELOPING VERTEBRATE RETINA. D. Nordquist and S. C. McLoon, Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

It is now well established that the development of the anterior-posterior axis of the vertebrate CNS involves a genetic and morphological segmentation of the neural tube. However, little attention has been given to whether similar mechanisms of segmentation may play a role in the development of the retina, a derivative of neural tube. We have found morphological evidence in embryos of chicken and a teleost fish, Esox lucius, that is suggestive of retinal segmentation. Shortly after the infolding of the optic vesicle to form an optic cup, a dorsal "fissure" was observed in the gross morphology and in histological sections of both species. This structure was most striking in the teleost fish, where it appeared to demarcate nasal and temporal compartments in the retina. In addition to the dorsal structure, two indentations were also noted in the nasal and temporal sides of the retina, indicating a possible division between dorsal and ventral retina. The stage of development at which this morphology first appeared is curiously similar to that at which nasal-temporal and dorsal-ventral retinal polarity is specified. It is possible that the morphological pattern observed in the early retina may reflect underlying patterns of genetic determination.

303.21

DISTANCES BETWEEN "REELER" AND NEARBY LOCI ON MOUSE CHROMOSOME 5. A.M. Goffinet. Dept. Physiology, FNDP Sch. Med., B-5000 Namur, Belgium.

Reeler (rl) is a recessive mutation of the mouse leading to widespread anomalies of the central nervous system. The reeler locus is in the paracentromeric region of chromosome 5, in the vicinity of the P-glycoprotein locus (Pgy-1) and the engrailed-2 gene (En-2), and proximal to the T31H translocation breakpoint (Goffinet and Dernoncourt, Mammalian Genome, 1991).

In this study, genetic distances were estimated between reeler, Pgy-1, the sorcin locus (Sor) and En-2, as well as between reeler and T31H. Recombination frequencies between reeler, Pgy-1, Sor and En-2 were measured using a N2 backcross panel (127 mice) between rl/rl-BALB/c and C57 mice. The distance estimates are: 7.9 ± 2.4 cM between En-2 and reeler (10 recombinants); 7.1 ± 2.3 cM between Pgy-1/Sor and reeler (9 recombinants); and 15.0 ± 3.2 cM between Pgy-1/Sor and En-2 (19 recombinants). No recombination was found between Pgy-1 and Sor. Analysis of 32 N2 backcrosses between rl/rl-BALB/c and mice with the T31H translocation showed no recombination, so that T31H is located less than 3 cM from reeler.

These observations suggest the following gene order: **Cen - Sor/Pgy-1 - rl - T31H - En-2** - other loci.

MOLECULAR AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT II

304.1

An Extended Family of Protein-Tyrosine Kinase Genes Differentially Expressed in the Vertebrate Nervous System. C. Lai* and G. Lemke. Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

We have used PCR to identify 13 novel protein-tyrosine kinase genes(tyro-1 to -13), six of which (tyro-1 to-6) are preferentially expressed in the developing vertebrate nervous system. The tyro-2 and tyro-9 genes encode kinase domains that exhibit strong amino acid sequence similarity to the equivalent regions of the receptors for EGF and FGF, respectively, and may encode novel receptors for these or related polypeptide ligands. The tyro-1 to -6 genes are all expressed during central nervous system neurogenesis and exhibit distinct and highly regionalized patterns of expression in the adult brain. Together with recent studies from other laboratories, which demonstrate that the transmembrane protein-tyrosine kinase, *trk*, is required for the high-affinity recognition of nerve growth factor, these data are consistent with the hypothesis that protein-tyrosine kinases play a central role in neural development. (Supported by grants from the N.I.H. and the National Multiple Sclerosis Society)

304.2

EXPRESSION AND CHARACTERIZATION OF STY, A MEMBER OF THE MULTIFUNCTIONAL PROTEIN KINASE FAMILY. M.P. MYERS*, G.M. DOBREA, M.B. MURPHY* AND G.E. LANDRETH. Alzheimer Research Laboratory. Departments of Neurology and Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

A novel class of protein kinases has recently been identified which phosphorylate proteins on serine, threonine, and tyrosine residues. One member of this class, termed STY (or CLK) is closely relate to the cdc2/cdc28 family of kinases (Howell et. al. *MCB* **11**, 568-572 (1991)). STY kinase was expressed as a trpE fusion protein and was shown to undergo autophosphorylation on serine and tyrosine residues. STY phosphorylated myelin basic protein exclusively on serine residues, whereas MAP2 and enolase were phosphorylated on both serine and tyrosine residues. STY is expressed at high levels in the rat brain. *In situ* hybridization studies using a 600nt cRNA probe revealed that STY was expressed throughout the adult rat brain. STY mRNA levels were greatest in association with fiber tracts including the corpus callosum, anterior commisure and cerebellar white tracts, suggesting expression of this gene within oligodendroglia.

304.3

LAR GENE: A TYROSINE PHOSPHATASE MEMBER OF THE N-CAM FAMILY IS EXPRESSED IN THE NERVOUS SYSTEM. F.M. Longo[1], J.P. Barnes*[1] and J.A. Martignetti*[2]. Dept of Neurology[1], UCSF Sch. of Med., San Francisco, CA 94143 and Fishberg Center for Neurobiology[2], Mt. Sinai Med. Ctr., NY, NY 10029.

The Leukocyte common Antigen-Related (LAR) gene is a member of a novel class of cell growth-regulating receptors known as the tyrosine phosphatases (Science 251:744, 1991). Human LAR is expressed as 8 kb or shorter transcripts *in vitro* in non-neural cell lines (Streuli M. et al, J Exp Med168:1523, 1988). Extracellular domains of LAR have 16-33% sequence similarity to N-CAM, a molecule which promotes neurite outgrowth. While screening a Sprague-Dawley rat cDNA brain library for NGF receptor-like genes, we found a 1.8 kb insert with approximately 90% sequence similarity to human LAR and which corresponds to the 3' end of the full-length human transcript. Poly (A) RNA was isolated from rat cortex, cerebellum, brainstem, heart, liver, cultured neurons, astrocytes and PC12 cells. Northern blots probed with a riboprobe derived from our 1.8 kb insert and washed under high stringency demonstrate predominant 8 kb and 5 kb transcripts in neural-derived tissue and 5 kb transcripts in heart and liver. The relative abundance of different-sized transcripts expressed in specific brain regions varies during development. These studies suggest that LAR is expressed *in vitro* and *in vivo* in neuronal and non-neuronal cells and that its expression is developmentally regulated. These observations along with LAR's extracellular sequence similarity to N-CAM and its association with tyrosine phosphatase activity lead to the hypothesis that the LAR molecule may constitute a novel mechanism regulating neural development. (Supported by United Cerebral Palsy and a travel award from the American Paralysis Association)

304.4

ND-1: A HELIX-LOOP-HELIX PROTEIN PROMINENTLY EXPRESSED IN BRAIN [1]M. Armanini, [2]Y. Li*, [1]G. R. Laramee*, [2]E. Peralta*, [1]J. W. Winslow, and [1]H. S. Phillips, [1]Dept. Dev. Biol., Genentech, S.S.F., CA 94080 and [2]Dept. Biochem. & Molec. Biol., Harvard U, Cambridge, MA 02138

Certain aspects of differentiation are controlled by a class of transcriptional regulation factors which share a structural motif known as the helix-loop-helix (HLH) domain. We report here the distribution of a recently cloned HLH protein, ND-1, in the developing and differentiated mammalian CNS (Winslow *et al*, in prep.). Northern blot analyses revealed that among cell lines of neuronal and glial lineage, ND-1 expression was limited to lines of neuronal character. *In situ* hybridization revealed that ND-1 is prominently expressed in telencephalon and cerebellar cortex of postnatal rat brain. Comparisons with the distribution of MASH and Id, potential dimerization partners of ND-1, revealed that MASH is expressed in many of the same regions shown to express ND-1, albeit at apparently much lower abundance, while n-Id is largely excluded from regions of ND-1 expression. Although the general pattern of ND-1 and MASH expression were similar, some areas were seen to hybridize almost exclusively for ND-1 (amygdala, layers 4-6 of neocortex), while other regions (layer 2 of entorhinal and piriform cortex) displayed significant hybridization signals for MASH as well as ND-1. Comparisons of ND-1 and MASH expression in developing postnatal brain revealed that hybridization for ND-1 declines from P1 to P60, while hybridization for MASH in most sites maintains a low, but constant, level. These results suggest that ND-1 may play a role in neuronal differentiation and/or maintenance of neuronal plasticity in the adult brain.

304.5

DEVELOPMENTAL REGULATION OF IMMEDIATE EARLY GENES IN RAT BRAIN. R.V. Bhat, B.A. Christy*, Y. Nakabeppu*, A.J. Cole, J.M. Baraban and P.F. Worley. Depts of Neuroscience, Neurology, and Howard Hughes Med. Inst., Johns Hopkins University School of Medicine, Baltimore, MD 21205-2185.

We have examined the regulation of several immediate early genes (IEGs) during postnatal development in rat brain. Northern analysis of unstimulated forebrain RNA indicates that mRNA levels of c-*fos*, *fos*-B, *zif268*, *Krox20* and *nur77* are low between postnatal days 1 and 10 but increase markedly between days 12 and 21 and thereafter variably decline in adult brain. Gel shift assays indicate a robust age dependent increase in AP-1 and *zif268* binding activities that parallels mRNA levels. Peak developmental IEG expression occurs during a period of exuberant excitatory synapse formation and may be regulated by natural synaptic activity. Manipulations that reduce excitatory synaptic activity result in rapid reductions of IEG expression supporting this concept. Restriction analysis of the *zif268* promoter region (2.5 Kb 5' to message) fails to detect changes in methylation during postnatal development suggesting that developmental changes in expression are not due to altered DNA methylation. Moreover, certain stimuli that induce IEG responses in adult brain can induce their expression in brain as early as postnatal day 1. These data suggest that IEGs are "available" for activation prior to normal developmental increases and are poised to respond to appropriate stimuli.

304.6

LOCALIZATION OF GAP JUNCTION mRNAs DURING DEVELOPMENT OF THE RODENT CNS. D.J. Belliveau and C.C.G. Naus. Dept. of Anatomy, University of Western Ontario, London, Ontario, Canada, N6A 5C1

We have previously described the developmental expression of gap junction mRNA and protein utilizing Northern and Western analysis. We extended this study to examine the cellular localization of connexin32 (cx32) and connexin43 (cx43) mRNA by using in situ hybridization to follow the pattern of expression during development of the rodent CNS. The brains of three Sprague Dawley rats were used at each postnatal time point studied and in situ hybridization was performed on cryosections using an ^{35}S labelled riboprobe. The first time point examined, postnatal day 3 (P3) showed very little hybridization with cx32, that which occurred was found in fibre tract areas of the brain stem. At P10 the signal increased significantly with hybridization to cells of various fibre tracts of the brainstem. The forebrain regions showed signal only in the corpus callosum and hippocampal regions. Hybridization signal was seen in the white matter of the cerebellum by P15. The forebrain showed signal in the corpus callosum, cortical layers, hippocampus and dentate gyrus. The signal in fore- and hindbrain regions increases through P20 and began to decline by P32. The signal distribution for cx43 differed from cx32, being more dispersed throughout regions of the fore- and hindbrain. Cx43 positive cells were present at P3, the number increasing through to P32. The strongest signal was found in the leptomeninges. The differential expression of cx32 mRNA during development suggests a cell specificity, probably being localized to oligodendrocytes and neurons as evidenced by fibre tract and discrete neuronal nuclei labelling. The hybridization pattern of cx43 is consistent previously reported astrocytic localization due to the more general distribution of signal. Supported by the Medical Research Council of Canada.

304.7

DEVELOPMENTAL REGULATION OF RAB3A IN THE RAT BRAIN. B. Tavitian, K.L. Moya, A. Zahraoui*, O. Stettler* and A. Tavitian*. INSERM U334, C.E.A., S.F.H.J., Orsay and INSERM U248, Paris, France.

RAB3A is a small G protein member of the *ras* superfamily that is expressed preferentially in the brain and implicated in exocytosis. Immunoblot analysis of total brain homogenates using a monospecific antiserum showed that relative levels of RAB3A increase markedly during brain development. The lowest levels were observed in the late embryonic brain and remained low on the day of birth (P0) and postnatal day 4 (P4). At P7 relative levels began to increase and by P14, RAB3A had attained the high levels seen in adult brain.

Localization of RAB3A by immunohistochemistry revealed striking changes in its anatomical distribution. In general, the early stages of development were marked by dense staining of neurons with abbreviated processes. An intermediate pattern in which both cell bodies with more elaborate neurites and a diffuse neuropil staining followed. By the end of the first month, an adult-like distribution of RAB3A was seen with dense staining in the neuropil and virtually none in cell bodies.

For example, in the neonatal cortex we found numerous RAB3A-positive cells against a background that was virtually devoid of staining. These cells had an elongated morphology, were radially oriented and formed a band in the presumptive layer IV. By P14 the cortical neuropil was moderately stained and few immunoreactive cells could be observed.

Whereas in the adult hippocampus, the pyramidal cell layer was not stained for RAB3A, this layer showed considerable immunoreactivity from embryonic day 18 (E18) through P14. While at earlier ages the terminal zones of the hippocampus were not marked by the antibody, starting at P14, the strata oriens and radiatum assumed a marked labeling that by P21 was indistinguishable from the adult pattern.

These results show that RAB3A undergoes a striking shift in its cellular localization during brain maturation. The protein is present within cell bodies at early stages of development, and then shifts to the neuropil coincident with the elaboration of terminals and formation of synaptic contacts. Furthermore, this shift coincides with the marked increase of RAB3A in the brain.

304.8

MOLECULAR CLONING AND EXPRESSION OF SC1, A NOVEL ADHESION MOLECULE EXPRESSED ON EARLY MOTONEURONS. H. Tanaka, T. Matsui*, A. Agata*, M. Tomura*, A. Lee*, H. S. Phillips, and D. L. Shelton. Gunma U. Sch. Med., Maebashi 371, Japan. and Genentech, Inc., South San Francisco, CA. 94080.

SC1 is an integral membrane protein, first identified by generation of a monoclonal antibody using chick spinal cord membranes as immunogen. Among the neurons of early spinal cord, SC1 is unique to motorneurons, although SC1 is expressed in other tissues and neurons of the peripheral and central nervous systems at other developmental stages. SC1 was purified by affinity chromatography, and N-terminal sequence and polyclonal sera were obtained. cDNA clones were isolated by screening an E4 chick embryo cDNA library, positive clones being verified by sequence analysis. The predicted amino acid sequence of SC1 consists of a signal sequence, five immunoglobin-like repeats, a single transmembrane domain, and a short cytoplasmic tail. Its sequence is most similar to MUC18, a marker of melanoma progression. Northern analysis demonstrates two transcripts, of 4.5 and 3.9 kb. *In situ* hybridization shows a pattern similar to that obtained using immunocytochemistry with either the mono- or polyclonal antibodies. Transfection of SC1 into mammalian cells leads to expression of immunoreactive protein at the cell surface and an increase in adhesion to other cells expressing SC1. SC1 expression *in vivo* may play a role in lateral motor column formation or axon growth or fasciculation.

304.9

DIFFERENTIAL EXPRESSION OF PLASMA MEMBRANE CA2+-ATPASE mRNAS IN DEVELOPING RAT BRAIN AND IN RAT BRAIN SUBREGIONS. P. Brandt and R.L. Neve. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

In rat brain at least seven mRNAs encode the plasma membrane Ca^{2+}-ATPases. Because Ca^{2+} regulation is crucial to cell function, especially in excitable cells, any alteration in Ca^{2+}-ATPase regulation may be important. We have used PCR amplification of reverse transcribed mRNA to examine the patterns of mRNA expression for the Ca^{2+}-ATPases (termed PMCA mRNAs) in developing rat brain and in rat brain subregions. Of the PMCA1 mRNAs, PMCA1b was initially expressed at embryonic day (E) 10, whereas PMCA1b and c appeared at E14. There was a slight increase in PMCA1a between postnatal (P) 2 and P18, while PMCA1b expression steadily declined and PMCA1c remained fairly constant. There was little expression of any PMCA2 mRNA until E18, at which point PMCA2a and two PMCA2-related transcripts appeared. The levels of these PMCA2 mRNAs were relatively invariant from P2 to P8. PMCA3b mRNA was expressed from E10 to P28, increasingly steadily from E10 to P2, and then remaining constant through P28. The PMCA3a transcript appeared at E14 at low levels and steadily increased in abundance until P3, then remained constant through P28. Examination of the developmental expression of PMCA mRNAs in specific brain subregions revealed some isoform variability from region to region. In situ RNA hybridization with isoform-specific probes is presently under way.

304.10

EMBRYONIC GAD STOP CODON MESSAGE DISAPPEARS COINCIDENT WITH THE EMERGENCE OF GABA IMMUNOREACTIVITY IN DEVELOPING RAT CNS.
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During embryogenesis, the gene encoding the enzyme glutamic acid decarboxylase (GAD) is spliced to include an exon containing a premature stop codon (ES), which lies upstream of the sequence encoding the enzyme's co-factor binding domain. While a translated product of the embryonic transcript is predicted to be enzymatically inactive, it has not yet been reported. In these studies, we used the GAD product, GABA, as an index of functional enzyme activity. Sections of embryonic rat brain and spinal cord were probed for GAD messages using in situ hybridization and ^{35}S-labeled synthetic oligonucleotides that correspond to either the exon containing the ES codon or to the functional, full-length enzyme. GAD protein and GABA expression were analyzed in adjacent tissue sections by immunocytochemistry. GAD+ cells were present in regions that expressed messages for the ES codon and/or full length enzyme. GABA+ cells were not detected in regions of the CNS that expressed the ES message. Disappearance of the stop codon coincided with the emergence of full length message and GABA+ cells. Full length GAD message and GABA+ cells first appeared in the brainstem. In the spinal cord, message for full length GAD appeared along a rostral-to-caudal and ventral-to-dorsal gradient during development, preceding the appearance of GABA. Coincident emergence of full length GAD message and GABA immunoreativity suggests that the translated product of the ES transcript is enzymatically inactive.

305.1

ANALYSIS OF GENE EXPRESSION IN THE RAT SOMATOSENSORY CORTEX DURING DEVELOPMENT. M. Hennegriff, M. M. Heeb*, R. Neve and H. Killackey. Department of Psychobiology, University of California, Irvine, CA 92717.

It has recently been suggested that the ingrowth of thalamocortical afferents into rat neocortex correlates temporally with differentiation of each cortical layer from the cortical plate (Catalano, Robertson and Killackey, 1991). The present study is an initial attempt to determine whether changes in gene expression are also correlated with these events. We are using RNA blot analysis coupled with in situ hybridization to examine the temporal and spatial expression of the mRNAs encoding synapsin Ia and Ib in rat brain from embryonic day E16 to adulthood. Previous Northern blot analysis revealed that synapsin I is encoded by two mRNAs (Haas and DeGennaro, 1988). We currently show that the 3.4 kb mRNA appears in rat brain as early as E16 and displays sharp increases on P2, P8 and P21, after which the level of the mRNA drops to adult levels. The 4.5 kb mRNA shows a more restricted pattern of temporal expression. It is present at P2, with increased levels at P8, after which it falls to adult levels by P15. It is not present at E16. In situ hybridization studies, utilizing an mRNA probe that detects both synapsin I mRNAs, indicates that synapsin I expression is highly localized during the development of the embryonic and postnatal rat brain.

305.2

EARLY CORTICAL REGIONALIZATION DETECTED BY AN AREA-SPECIFIC MONOCLONAL ANTIBODY.
Y. Arimatsu, M. Miyamoto* I. Nihonmatsu*, and K. Hirata*. Lab. of Neuromorphology, Mitsubishi Kasei Inst. of Life Sci., Machida-shi, Tokyo 194, Japan.

The timing and mechanism of the areal specification in the cerebral cortex remain to be mostly unknown. We have begun to examine this process using a monoclonal antibody, designated PC3.1, that binds selectively to a neuronal subpopulation located in lateral but not dorsomedial cortical areas in the adult rat. Here we address when instructions for PC3.1 expression are given in the limited cortical region. When cultured in vitro, significant percentages of neurons derived from lateral portion of the cortical primordium at E14, E15, E16 and P4 expressed PC3.1-immunoreactivity, while virtually none (E16, P4) or very small numbers (E14, E15) of PC3.1-positive neurons were found in the culture of dorsal cortical cells. Double labeling experiments combining PC3.1-immunohistochemistry and [^{3}H]thymidine-autoradiography showed that presumptive PC3.1-immunopositive neurons undergo their final mitosis around E15. These results support the assumption that the capacity to produce PC3.1-immunopositive neurons is restricted to some extent within the lateral cortical primordium before the final mitosis of presumptive PC3.1 neurons and that the dorsolateral cortical regionality is established by the end of neurogenesis.

305.3

DEVELOPMENT OF RAT THALAMUS AND CEREBRAL CORTEX AFTER EMBRYONIC INTERRUPTION OF THEIR CONNECTIONS
Zoltán Molnár*[1], Kathleen Yee[2], Raymond Lund[2] and Colin Blakemore[1]
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We are interested in the various interactions between thalamus and cerebral cortex during the establishment of their specific interconnections. Without removing any part of the cortex or thalamus the developing connections between them were mechanically disrupted with a cut in one hemisphere near the internal capsule, made during intrauterine surgery at various stages (embryonic day 16,18 or 20).

Thalamocortical projections were examined by application of carbocyanine dyes (DiI, DiA, DiAsp) to various thalamic nuclei on each side in paraformaldehyde-fixed brains. In newborn and postnatal (P) two-day-old rats, there was a drastic reduction in thalamic innervation of the cortex on the lesioned side posterior to the cut. Despite the isolation of a large region of cortex from thalamic input, its basic laminar organisation was intact, with cell densities similar to those on the non-lesioned side and in normal controls of the same age. The thalamus was not obviously smaller on the lesioned side. Dyes were applied to the cortex at symmetrical points in the lesioned and normal hemispheres at P0 and P2. Anterior to the cut, corresponding groups of cells were back-labelled in the thalamus, symmetrically positioned and very similar in appearance on the two sides. But for cortical sites posterior to the cut, very few thalamic cells were labelled on the lesioned side. In addition, descending corticofugal axons often ended in neuromas near the cut. Application of dye to isolated regions of the cortex also back-labelled large numbers of neurons in the subplate with substantial local projections in the white matter. Labelled subplate cells were not seen on the non-lesioned side or in matched control animals.

The innervation of cortex by thalamic axons may, then, influence the distribution of the axons of subplate cells within the cortical plate. It is not needed for the emergence of the fundamental six-layered structure of the cortex, though it may play a role in determining regional cytoarchitectural differentiation.

305.4

DEVELOPMENT OF ACETYLCHOLINESTERASE-POSITIVE THALAMOCORTICAL AFFERENTS TO EMBRYONIC RAT NEOCORTEX. J.A. De Carlos, B.L. Schlaggar, and D.D.M. O'Leary Molecular Neurobiology Laboratory, The Salk Institute, La Jolla CA 92037.

We examined the growth of ventrobasal thalamocortical (VB) afferents to presumptive somatosensory cortex (SI) using AChE histochemistry, a transient marker of VB neurons and their axons (Kristt JCN '79; Neurosci '89), and DiI in fixed tissue. At E15 (E0 is day of insemination) AChE labels, but is not restricted to, VB and VB afferents in the internal capsule. At E16, AChE reaction product in VB is more intense than at E15, but AChE-positive afferents have yet to emerge from the internal capsule into the cortical mantle. However, DiI labeling shows that thalamic afferents already reach the cortex on E16. Thus, early expression of AChE by developing VB afferents is limited to more proximal portions of the axons. By E17, though, entire axons, including growth cones, contain AChE. AChE-positive growth cones, with morphologies ranging from simple to complex, travel tangentially in the subplate (layer 6b) and upper intermediate zone. The presence of AChE in growth cones is, to our knowledge, a novel finding. AChE histochemistry and DiI, placed in VB, appear to label the same population of thalamocortical axons. Axons labeled by these methods have common dispositions, branching behavior, and timecourse of growth into the cortical plate (CP). On E18, VB afferents begin to invade the CP by sending, in a directed manner, radially oriented collaterals into its deepest part. Ingrowing VB afferents branch profusely beneath, but rarely enter the dense undifferentiated CP. These fibers preferentially invade a distinct region of the developing CP, clearly delineating presumptive SI by E21, in the absence of any indication of cytoarchitectonic borders that demarcate this area. This regionally specific ingrowth of VB afferents is the first overt indication of a parcellation of the undifferentiated cortex into areas. The finding that the growth cones of VB axons contain AChE as they invade the developing CP is consistent with a significant function for AChE in the subsequent differentiation of primary sensory areas of cortex.
Support: NIH FIC fellowship F05 TW04401 and NINDS grant P01 NS17763.

305.5

THALAMOCORTICAL AXONS PREFERENTIALLY EXTEND ALONG A CHONDROITIN SULFATE PROTEOGLYCAN ENRICHED PATHWAY COINCIDENT WITH THE NEOCORTICAL SUBPLATE AND DISTINCT FROM THE EFFERENT PATH A.R. Bicknese, A.M. Sheppard, D.D.M. O'Leary' and A.L. Pearlman Depts. of Cell Biology, Neurology and Pediatrics, Washington Univ. Sch. of Med, St. Louis, MO 63110, and 'Molecular Neurobiology Lab, Salk Inst., La Jolla, CA 92037

The first postmigratory cells in the neocortex form the preplate zone [PPZ], which is split by arriving cortical plate neurons into the marginal zone [MZ] and subplate [SP]. Previously, we have shown that immunolabeling of both fibronectin and the glycosaminoglycan chains of chondroitin sulfate proteoglycans (CSPG) is associated with cells of the PPZ, MZ and SP, but is sparse in the cortical plate (Sheppard et al., Soc. Neurosci. Abstr. 16:315). Here we examine the prospect that specific ECM components may delineate the pathways taken by the growth cones of cortical efferent or afferent axons. We used the fluorescent tracer DiI to define the trajectory of early axons in the neocortex of embryonic mice and compared it to the distribution of CSPG defined by immunolabeling. As early as embryonic day (E) 12, prior to cortical plate formation, efferent axons of preplate neurons leave the CSPG-rich PPZ and extend tangentially through the upper intermediate zone (IZ) which contains much less CSPG immunolabeling. Neurons of the early (E13-15) cortical plate extend axons that cross the CSPG-rich subplate, then turn abruptly to run in the upper IZ. In contrast, thalamocortical axons, which arrive on E14-15, travel long distances within the CSPG-rich subplate, above the path taken by the efferent axons from preplate/subplate and cortical plate neurons. Our findings demonstrate a molecular distinction between afferent and efferent pathways in the developing neocortex; early afferents preferentially travel in the CSPG-rich subplate, while efferents cross it to follow a pathway that contains much less. In neither case does the CSPG labeled by the antibody we used form a barrier to axonal extension.

305.6

THE EARLY DEVELOPMENT OF VISUAL CORTICOTHALAMIC AND THALAMOCORTICAL PROJECTIONS IN THE GOLDEN HAMSTER. B. Miller, L. Chou,* and B. L. Finlay. Department of Psychology, Cornell University, Ithaca, New York, 14853.

We examined the development of corticothalamic and thalamocortical projections with particular attention to the possible roles of these systems in axonal pathfinding and cortical area specification. DiI or DiA was placed in the presumptive visual cortex or dorsal thalamus of fixed brains of E12 to P5 hamster pups. After two months the brains were sectioned with a vibratome at 75-150um and examined with fluorescent microscopy.

In the hamster, visual cortex is produced between E10 and P1 (birth is E16), and the lateral geniculate between E9.5 and E12.5. At E14 cortical fibers are partly through the internal capsule, but have not yet reached the thalamus. In contrast, thalamic fibers have grown to visual cortex by this age. The first visual cortical axons reach posterior thalamus at one day after birth and are in very small numbers. These first axons come from subplate (SP) and layer 6 cells. By P3 axons of layer 5 cells reach the posterior thalamus, and they greatly outnumber the layer 6 and SP cells. The number of layer 6 and SP cells projecting to the thalamus does not increase at P5, while layer 5 projections do increase in number. Double injections of DiI and DiA show that the corticofugal and thalamocortical pathways are physically separate during their development. The corticofugal axons travel deep in the intermediate zone to the thalamic axons.

Thus, cortical fibers do not reach the thalamus until 3 days after thalamic fibers reach visual cortex. Also, these pathways are physically separated during development and have no opportunity to interact except at their respective targets.
Supported by NIH R01 NS19245 and NIMH 5 T32 GM07469.

305.7

TEMPORAL & SPATIAL RELATION BETWEEN CORTICAL NEURON MORPHOGENESIS, GENICULOCORTICAL AXON INGROWTH AND SYNAPTOGENESIS IN RAT VISUAL CORTEX: A WGA-HRP, DiI - E.M. STUDY. G.H. Kageyama, M.A. Bielstein*, R. Khuu*, J. Yu & R.T. Robertson. Dept. Anat. & Neurobiol., Univ. of Calif., Irvine, CA 92717.

In order to study the temporal relation between the morphogenesis of cortical neurons and the ingrowth geniculocortical (GC) axons, several related studies were undertaken in pre-fixed (4% paraformaldehyde) neonatal rat brains: (1) DiI , placed in the LGN or optic radiation for 1-2 weeks at 37°C was used as an anterograde GC axon tracer, and compared with previous results using transneuronal WGA-HRP (Kageyama and Robertson,'90), (2) DiI, placed in the cortical layers or subcortical white matter in or adjacent to visual cortex was used as a selective Golgi-like stain to examine the dendritic maturation of superficial and deep cortical neurons, respectively, and (3) DiI fluorescence labeled GC axons were then photo-oxidized with DAB in order to study the developing laminar pattern of GC axon morphogenesis at the light microscope level and GC synaptogenesis at the E.M. level. At all ages studied, the DiI labeled geniculocortical axon arborizations matched closely the temporal and spatial pattern of geniculocortical projection as shown previously using transneuronal WGA-HRP. DiI-labeled GC axons formed axodendritic and axosomatic synapses throughout the developing terminal field. An outward (deep to superficial) wave of basal dendrogenesis also accompanied the sequential pattern of GC axon ingrowth and synaptogenesis. The results demonstrate that developing GC terminals form synapses earlier and more extensively than previously thought, at a time and place where they may influence the elaboration of dendrites (especially in layer IV) as each laminar cohort of cortical neurons begins to differentiate at the base of the cell dense CP. It is possible that GC axons may play an important role in cortical histogenesis.
Supported by NSF 87-08515 and NIH NS 25674.

305.8

DEVELOPMENT OF GENICULOCORTICAL PROJECTIONS: DiI AND ACHE STUDIES IN RATS. K.A. Gallardo*, G.H. Kageyama, M.A. Bielstein*, J. Yu and R.T. Robertson, Depts. of Anatomy and Neurobiology & Physical Medicine and Rehabilitation, Univ. California, Irvine, CA 92717.

We have been studying the development of geniculocortical axonal projections to primary visual cortex in the rat, with particular attention paid to the time of ingrowth of geniculocortical axons in relationship to the differentiation of cortical neurons (Kageyama and Robertson, 1990). In the present studies, we investigated development of individual and populations of fibers in visual cortex using DiI fluorescent microscopy and acetylcholinesterase (AChE) histochemistry. Experiments used Sprague-Dawley rats from late fetal stages through postnatal age P-10. Aldehyde fixed brains received placements of DiI into the lateral geniculate body and were then stored at 37oC for 1-2 weeks. Other brains were prepared for AChE histochemistry using the Tago et al. (1986) technique.

AChE and DiI techniques both resulted in patterns of distinctly labeled axons in visual cortex. In pups younger than P-1, most DiI labeled geniculocortical axons were found oriented tangentially in the cortical subplate zone. Fewer labeled axons could be seen leaving the subplate zone to course radially or obliquely through the deeper cortical layers. At P-2, when layer IV can first be identified, some DiI labeled axons have reached layer IV, and the projection to layer IV is robust by P-4. Some labeled axons course through layer IV to reach the pial surface. AChE stained axons show areal and laminar patterns similar to the DiI labeling, with a dense plexus in visual cortex in layer IV with some axons reaching layer I. However, fewer fibers appear stained with AChE than with DiI. Neonatal enucleation results in reduced numbers of AChE labeled axons in layer IV of visual cortex. These data demonstrate an early ingrowth of geniculocortical axons into visual cortex and these techniques allow morphological characterization of individual axons.
Supported by NSF grant 87-08515.

305.9

CROSSED CORTICORUBRAL AXONS IN NEWBORN KITTENS AND HEMISPHERECTOMIZED KITTENS ARE COLLATERALS OF UNCROSSED AXONS. F. Murakami, Y. Kobayashi*, T. Uratani* and A. Tamada*. Dept. of Biophysical Engineering, Fac. of Engineering Science, Osaka University, Toyonaka, Osaka 560, Japan.

Crossed corticorubral projections occur in early postnatal development and after early hemispherectomy in kittens, although they are virtually absent in adults. The present study was performed to determine whether crossed corticorubral axons are collaterals of uncrossed ones. Fluorescence labeled latex microspheres were injected bilaterally into the red nucleus (RN) of normal (3 weeks postnatal) and hemispherectomized (lesioned at 3 weeks postnatal and were allowed to survive for 3 - 7 weeks) kittens, red ones on one side and green ones on the other, through a glass micropipette. The RN was activated orthodromically from the interpositus nucleus and field potentials were recorded by the micropipette to locate its tip. Survival ranged from 4 - 7 days, and after removal the RN and the cerebral cortices were sectioned at 50 µm and observed with a fluorescence microscope.

Both in normal and lesioned kittens the tracers were well restricted to the RN in most of the animals and many corticorubral cells were labeled in layer V of the sensorimotor cortex, mainly in area 4, ipsilateral to the injection site. Labeling also occurred on the contralateral side, though the number of labeled cells were fewer. A major part of the contralaterally labeled cells were also labeled ipsilaterally in normal kittens, suggesting that a single cortical cell innervate the RN bilaterally. Bilateral cells were also found in lesioned kittens. Bilaterally or contralaterally projecting cells were intermixed among ipsilaterally projecting cells. These results are consistent with the view that bilateral cells which existed at the time of lesion participate in the establishment of lesion-induced bilateral innervation.

305.10

AXONAL BRANCHING IN THE DEVELOPING HAMSTER CORPUS CALLOSUM. H.J. Kadhim*, P. G. Bhide, W.C. West*, D.O.Frost. Dept. of Neurology, Massachusetts General Hospital, Charlestown, MA 02129

Single axons of the developing corpus callosum (CC) were labeled in hamster pups by placing crystals of the fluorescent tracer DiI unilaterally at multiple cortical sites in paraformaldehyde fixed brains. After 3-6 months, the brains were cut horizontally and the DiI was photoconverted to a DAB reaction product. Labeled axons were then studied using a light microscope linked to a computer or a drawing tube and, in some cases, subsequently examined by electron microscopy.

Many immature callosal axons branch transiently within the corpus callosum. Branching is prominent between the day of birth (P0) and postnatal day 3 (P3). Branching is reduced by P6-P8 and is completely absent by P11. Callosal axons branch both before and after crossing the midline. Axonal branching occurs across the full extent of the CC. On P0-P3, collaterals contained within single 100 µm thick sections are 5-90 µm long, (n=390; mean=15.1 µm; SD=12.7 µm), but other collaterals extended up to 136 µm before being cut at the surface of a section, and thus, were even longer. Occasionally, axons in the CC had multiple first order branches or higher order branches.

In cats and monkeys, the developing CC transiently contains more axons than the mature CC. It has been reported that in cats but not monkeys, the developmental overproduction of callosal axons occurs, at least in part by a transient excess of cortical neurons that send an axon through the CC. Our results show that transient branching of callosal axons can also contribute to their apparent overproduction. We are now examining cats and monkeys to see if transient branching contributes to the apparent overproduction of callosal axons in those species.
Support: NIH EY03465; Milton Fund; The Medical Foundation.

305.11

INDUCTION OF CEREBROCORTICAL HETEROTOPIAS BY PUNCTURE WOUNDS TO THE NEONATAL RODENT NEOCORTEX. G.D. Rosen, L.V. Stone*, J.M. Richman*, G.F. Sherman, and A.M.Galaburda. Beth Israel Hospital and Harvard Med. School, Boston, MA 02215.

Cerebrocortical heterotopias have been seen in the molecular layer of dyslexic humans (Galaburda et al., *Ann Neurol.* **18**:222-33, 1985) and in immune-disordered mice (Sherman et al., *Acta Neuropath.* **74**:239-42, 1987). These ectopias can be seen as early as E14 in the NZB mouse and are associated with breaks in the external glial limiting membrane and with disturbances in the radial glial fibers beneath the ectopias (Sherman et al., *Soc. Neurosci Abs.*, **16**: 1152, 1990). The present study attempted to induce cerebrocortical molecular layer ectopias by mechanically damaging the external glial limiting membrane by puncture wound.

Rat and mice pups were anesthetized by hypothermia at P0 and a puncture wound induced by manual placement of a 25 gauge needle through the skull and into the underlying developing cortical plate. Animals were sacrificed from 2 hours to 30 days later by transcardial perfusion with saline followed by 4% paraformaldehyde. Sections were Thionin-stained for Nissl substance and adjacent series immunohistochemically stained for radial glial fibers with Rat-401 (obtained from S. Hockfield) or vimentin.

Puncture wounds resulted in the formation of molecular layer ectopias in both rats and mice. These ectopias ranged in size from small (<10 cells) to large (>50 cells). Here too, immunohistochemical staining revealed disturbance of the external glial limiting membrane as well as changes in the disposition of underlying radial glial fibers. These results support the hypothesis that a disturbance of the external glial limiting membrane is the primary factor in the formation of molecular layer ectopias.
(This work was supported, in part, by NIH Grant 20806).

305.12

THE DEVELOPMENT OF NEURONAL ECTOPIAS IN FETAL NEW ZEALAND BLACK MICE. G.F. Sherman, L.V. Stone*, G.D. Rosen, and, A.M. Galaburda. Department of Neurology, Harvard Medical School, and Beth Israel Hospital, Boston, MA 02215.

Previous studies have shown that the New Zealand Black mouse strain (NZB), which spontaneously develops severe autoimmune disease, has ectopic collections of neurons in layer I of the cortex with underlying distortion of the cortical layers (Sherman et al., Acta Neuropathol., 74:239-242, 1985). These ectopias have been seen on the day of birth, and it is expected that they originate during the prenatal period after the beginning of neuronal migration to the cortex on day (E)13. Therefore in the present study we examined the fetal NZB brain from E14 to E19 with a Nissl cell stain for evidence of ectopias and with a radial glial antibody for evidence of a disturbance of the glial fiber patterns.

NZB mice were time-mated (E1 was the day a vaginal plug was found). The pregnant females were anesthetized on E14-E19, the fetuses removed and the heads immersion fixed in 4% paraformaldehyde for 4 hours. The specimens were placed into 10% buffered sucrose overnight, followed by 30% buffered sucrose for 24 hours, after which they were frozen using dry ice and cut coronally at 30 µm on a sliding microtome. Every fifth section was stained with thionin, and some adjacent series were stained for radial glial fibers using Rat-401 antibody (provided by S. Hockfield).

Nine of the 34 brains examined thus far had large ectopias that were similar in appearance to those seen in adulthood. Fully formed ectopias were detected as early as E14. The ectopias consisted of extrusions of cells from the underlying cortical plate into layer I and the overlying meninges. Under most of the ectopias there was a narrow channel perpendicular to the pial surface involving the entire width of the cortical plate . This channel had a decreased cell density, contained some distorted cells, and often a blood vessel was seen lying within this area.

As in previous work in the newborn NZB, there was aberrant organization of radial glial fibers focally in the area of the ectopias. The most striking aspect was the increased density of the fibers under the ectopias. In addition, the external limiting membrane was interrupted in the area of the ectopia and it appeared that neurons had migrated into the space created by the interruption. (Supported in part by NIH grant HD 20806.)

305.13

DEVELOPMENTAL SCULPTING OF THE TANGENTIAL DISTRIBUTIONS OF CORTICAL PROJECTION NEURONS OCCURS EVEN IN THE ABSENCE OF CNS MYELIN: OBSERVATIONS IN *JIMPY* MUTANT MICE. B. Stanfield. Lab. of Neurophysiology, NIMH, NIH Animal Center, Poolesville, MD 20837.

The developmental restriction of the tangential distributions of cortical projection neurons through collateral elimination occurs fairly late in cortical development and, in at least some systems, at around the same time as the relevant axons become myelinated. Observations from several studies suggest that neuronal activity may be involved in the determination of which collaterals are eliminated and which are maintained. Since myelination clearly contributes to the functional maturation of developing axonal systems, the possibility that myelination may be causally related to collateral elimination would seem to merit serious consideration. I have therefore examined the distributions of neocortical commissural and corticospinal neurons in dysmyelinated *jimpy* mice, and in unaffected littermate controls, during the first and third postnatal weeks. *Jimpy* is an X-linked, juvenile-lethal mutation in which the lack of oligodendrocyte differentiation, together with frank oligodendrocyte loss, results in the virtual absence of myelin from the developing and mature CNS.

First, it was confirmed that the relevant projection neurons are widely distributed in early postnatal mice. For this I injected 2% fast blue into either the cerebral cortex on one side, or into the pyramidal tract at its decussation, on postnatal day 2 or 3. The mice were killed 1 or 2 days later. In these early postnatal mice both callosally projecting and corticospinal neurons are distributed essentially throughout the tangential extent of the neocortex. Next, I made similar injections, only in 16 to 19 day old *jimpy* mice and littermate controls. These mice were killed after 1 to 2 days. By this age, both callosally projecting and corticospinal neurons are spread discontinuously through the *jimpy* neocortex, and the distributions of these cortical projection neurons in *jimpy* are indistinguishable from those seen in the cortex of the control mice. Thus the congenital absence of CNS myelin in *jimpy* does not affect the normal developmental sculpting through collateral elimination of the distributions of these cortical projection neurons.

305.14

MYELINATION OF THE CEREBRAL COMMISSURES OF THE HAMSTER, AS REVEALED BY THE RIP MONOCLONAL ANTIBODY. R. Lent, S. Jhaveri & B. Friedman. Instituto de Biofísica, UFRJ, Brazil; M.I.T. Cambridge, Ma. 02139; Regeneron, Tarrytown, NY.

Myelination of the cerebral commissures of the hamster was studied by immunostaining with a monoclonal antibody specific for myelin and oligodendrocytes (Rip). Cells expressing the Rip antigen were first observed in the anterior commissure (AC) on P6 (day of birth = P1), and in the corpus callosum (CC) and the hippocampal commissure (HC) on P8. By P8, myelinated fibers appeared around immunopositive oligodendrocytes within the posterior limb of the AC, and also ventrally at the rostral half of the CC. On P12, all the commissures had myelinated fibers throughout their extent, but in the CC and in the HC, a gradient had formed with a higher density of myelinated fibers rostrally. On P15 and P22, the pattern of myelination approached that of the adult. In the context of other developmental events, myelination of the CC and of the AC is a late event, occurring predominantly after stabilization of the axon number, either at the end of the progressive accretion of axons, as in the AC, or after the selective elimination of callosal projections.

305.15

MAP2-IMMUNOREACTIVE NEURITES OF PREPLATE NEURONS IN REELER MUTANT EMBRYONIC CORTEX. J.E. Crandall and V.S. Caviness, Jr. Develop. Neurobiol., E.K. Shriver Ctr, Waltham, Dept. Neurol., Mass. Gen. Hosp. and Neuroscience Program, Harvard Med. Sch., Boston, MA.

We have used antisera to MAP2 to study neuritic development of preplate neurons in normal and reeler cortex. At this developmental age, the cortical plate is just beginning to form in the mid-lateral region of the cerebral wall, whereas dorsal and medial cortical regions are still in a relatively undifferentiated stage consisting of only a primordial plexiform zone (PPZ). Total neuritic length of PPZ neurons is similar in the two genotypes on embryonic day 13. However, there are major differences in the organization of the neurites. In the normal PPZ, MAP2-positive neurites are distributed predominantly parallel to the pial surface. In the reeler PPZ, many neurites extend radially from cell somata toward the ventricular zone. Polar plots of neurite distribution were generated to quantify neuritic orientation using the Eutectics Neuron Trace program. The difference in orientation of the neuritic trees between normal and reeler preplate neurons was reflected in a statistically significant genotype by angle sector interaction. The mean number of neurite branches per branch order was significantly greater for reeler neurons only for higher order branches. The organizational anomaly of neurites from reeler preplate neurons occurs despite apparent positional similarity to normal developing cortical neurons and is prior to the major epoch of neuronal migration. We hypothesize that this network of neurites in the mutant PPZ forms a disoriented mesh beyond which successive cohorts of migrating neurons may be incapable of navigating.

305.16

DEPLETION OF LAYER IV HAS NO EFFECT ON SURVIVAL OF SUBPLATE NEURONS. T.U. Woo and B.L. Finlay. Department of Psychology, Cornell University, Ithaca, New York 14853.

In the cat, thalamocortical axons make temporary functional synaptic contacts with subplate neurons during development before establishing permanent connections with layer IV neurons. After thalamic invasion of the cortex, most of the subplate neurons die. In the golden hamster, normally 50 to 80% of subplate neurons die by adulthood. Perhaps the excessive loss of subplate cells results from loss of their thalamic afferents to layer IV. We thus tested if depletion of layer IV might rescue part of the subplate neurons by maintaining some of the temporary thalamocortical connections with the subplate, depleting the number of neurons in layer IV by injecting methylazoxymethanol (MAM), a mitotic inhibitor, during the time of their generation.

MAM (20mg/Kg) was injected into pregnant hamsters on embryonic day 14 (E14) when the major population of layer IV neurons are being generated. Animals were examined prior to and after the cell death period. Layer IV was reduced in thickness compared to control although it was not completely deleted. Reduction in thickness and cell denstiy of layer II and III was also observed. The absolute number of cells in the subplate was not changed significantly by the manipulation (normal adult=207,000±31,600; experimental=181,000±7,000)

Our results suggest that loss of competition of subplate neurons for thalamic afferents is not a cause of cell death.

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305.17

DEVELOPMENT OF OVER REPRESENTED AREAS IN SOMATIC MAPS. D. R. Riddle, A.-S Lamantia, A. Richards* and D. Purves. Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Since the discovery of cortical maps it has been recognized that central representations are not proportional to the dimensions of the body; body parts involved in fine discrimination or movements are over represented relative to other structures. This observation raises the question of whether early somatic maps are configured as in the adult, or whether over representation arises by disproportionate growth of the appropriate cortical regions. To address this issue, we have asked whether the sensory representation of the rodent face (an over represented feature) grows to the same extent as the cortex as a whole. Neonatal (4-5 days old) and adult (10-12 weeks old) mice were perfused with fixative and the brains removed. The cerebral cortices were flattened, cut tangentially, and stained for cytochrome oxidase. The cortical barrels representing the anterior face (excluding the lower jaw region, which is poorly defined in mice) were counted, and the area of the facial barrel field was compared to that of the flattened cortex as a whole. In neonatal animals the average area of the hemispheric cortex was $43.0 \pm 1.3 mm^2$ (mean±SEM, N=17 cortices from 12 animals); the facial representation (about 80 barrels) measured $2.0 \pm 0.1 mm^2$. In adult animals the mean cortical area was $76.4 \pm 2.8 mm^2$ (N=12 cortices from 9 animals), and the facial representation was $6.0 \pm 0.2 mm^2$. Thus, the map of the face increased from 4.7% of the total cortical area in the neonate to 7.9% at adulthood. These findings are consistent with previous measurements of the subset of barrels that represent the mystacial vibrissae (Rice and Van der Loos, 1977), and suggest that over represented areas in cortical maps grow relatively more postnatally than other cortical regions.

305.18

DEVELOPMENTAL REGULATION OF BRAIN DERIVED NEUROTROPHIC FACTOR mRNA IN NEURONS OF FETAL AND ADULT MONKEY PREFRONTAL CORTEX. G.W. Huntley, D.L. Benson, P.J. Isackson and E.G. Jones. Depts. of Anatomy and Neurobiology and Biological Chemistry, University of California, Irvine, CA 92717.

Brain Derived Neurotrophic Factor (BDNF) is thought to be a target-produced, CNS-derived neurotrophic factor with homologies to nerve growth factor that is thought to be produced by target cells of responsive neurons. In situ hybridization with cRNA probes was used to examine the expression and distribution of BDNF mRNA in a series of prefrontal cortices taken from six fetal (E110-E155) and two adult rhesus monkeys (Macaca mulatta). In adult prefrontal cortex, cells labeled by hybridization of the probe were located throughout all areas of the prefrontal cortex with their greatest density in the deeper half of layer III and layer VI. In the developmental series, no specific labeling was present at the youngest age examined (E110), and few, sparsely hybridized cells were present by E121. By E130 and thereafter, intensely hybridized cells were present and the areal and laminar distribution was similar to that seen in the adult. These data demonstrate that developing and adult neocortical cells express BDNF and suggest that expression of this neurotrophic factor may be important in the establishment or maintainence of functional interactions between prefrontal cortical cells and the widely distributed sets of cells projecting to them. Supported by USPHS grants numbers NS 21377, MH 44188, NS 26748, EY 07193 and AG 00538.

306.1

IN VIVO AND IN VITRO STUDIES OF THE DEVELOPMENT OF CORTICAL CONNECTIONS. D.Carić*, S.Rennie* and D.J.Price. Dept. of Physiology, Univ. Med. Sch., Teviot Place, Edinburgh EH8 9AG, U.K.

Geniculocortical fibres start to grow towards the cortical plate during the final third of gestation, as their cortical target cells are being born. Corticocortical development occurs later, largely postnatally.

Embryonic explants of murine lateral geniculate nucleus (LGN) were cultured alone or with slices of occipital cortex. Outgrowth from the LGN was seen only if cortex was present, and was more prolific the closer the explants. Cortex may secrete a neurotrophic substance stimulating outgrowth from the LGN.

We have tested the possibility that the presence of the geniculocortical pathway at birth is required to guide the development of corticocortical connections by lesioning the LGN in neonatal kittens. The results suggest that the loss of geniculocortical inputs prevents the normal maturation of corticocortical pathways.

These findings suggest that geniculocortical projections are stimulated to grow by the neurotrophic influence of their targets, and corticocortical pathways develop within the framework of the geniculocortical pathway.

306.2

LAMINAR SPECIFICITY OF EXTRINSIC VISUAL CORTICAL CONNECTIVITY STUDIED IN COCULTURE PREPARATIONS. N. Yamamoto, K. Yamada*, T. Kurotani* and K. Toyama. Department of Physiology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602, Japan.

The previous study demonstrated that laminar specific neural connections were established between cocultured lateral geniculate nucleus (LGN) and visual cortex (VC) (Yamamoto et al., *Science* 245:192, 1989). We further studied the mechanisms responsible for the formation of extrinsic cortical connectivity by using VC-VC, VC-superior colliculus (SC) and somatosensory cortex (SMC)-LGN cocultures in addition to the VC-LGN coculture. The subcortical blocks and cortical slices were dissected from fetal (ED15-17) and neonatal (PD1-2) rats respectively and cultured on the collagen-coated membrane for 2-3 weeks. Retrograde labeling with a fluorescent dye (DiI) showed that most of the cortical efferent cells were located in layers 2/3 in VC-VC, in layer 5 in VC-SC and in layer 6 in VC-LGN as well as in SMC-LGN cocultures. Anterograde labeling with DiI and current source density analysis indicated that in SMC-LGN or VC-LGN cocultures the thalamic fibers established synaptic connections in and around layer 4 of the cortices while in VC-VC cocultures the cortical fibers formed synaptic connections in layers 2/3 and 5. These results suggest that interactions between the neuron and its target are sufficient to produce the laminar specific neural connectivity, although other factors may be required for the formation of the modality specificity.

306.3

A THERMODYNAMIC MODEL FOR SELF-ORGANIZATION PREDICTS PATTERNS OF SYNAPTIC CONNECTIONS FROM THE LGN TO THE VISUAL CORTEX FOR MONKEYS AND CATS. S. Tanaka. Fundamental Research Laboratories, NEC Corporation, Tsukuba, Ibaraki 305, Japan.

A thermodynamic formulation previously proposed on the basis of a local Hebbian learning mechanism and synaptic competition for the limited amount of available postsynaptic trophic factors is applied to the development of the afferent projection from the LGN to the visual cortex. For model (A) in which ocularity, retinotopy and either on-center or off-center pathway are considered, computer simulations give rise to striped ocular dominance patterns and isotropic receptive fields without orientation selectivity. For model (B) in which both on-center and off-center pathways are considered in addition to ocularity and retinotopy, simulations yield irregular beaded ocular dominance patterns and simple-cell-like receptive fields. The on-center and off-center terminals also segregate superposing the ocular dominance patterns. Furthermore, for model (B), iso-orientation domains can be reproduced according to the orientation of individual receptive fields. Comparing simulated and experimental results, it is found that the ocular dominance patterns and receptive fields for models (A) and (B) are consistent with those observed in monkeys and cats.

306.4

GENICULO-CORTICAL REROUTING FOLLOWING NEONATAL MONOCULAR ENUCLEATION IN THE GOLDEN HAMSTER. A.J.Trevelyan* and I.D.Thompson*, (SPON: Brain Research Association). University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

The effect of early eye removal on the development of the geniculo-cortical pathway was studied in golden hamsters. Small, discrete injections (150nl) of rhodamine- and green-latex microspheres (Katz and Iarovici, 1990, Neurosci., 34:511-520) were made in visual cortex of adult hamsters, and the resultant backlabelling of the dorsal lateral geniculate nucleus (dLGN) examined. In normal adults, the projection has a precise topographic order. This is also true of the projection contralateral to the remaining eye in animals monocularly enucleated at birth. However, on the side ipsilateral to the remaining eye, visual cortex appears to receive two convergent projections from the deafferented dLGN, one mirroring the other. A very lateral cortical injection labels cells in two discrete regions of the dLGN. As the injections are made progressively more medially, the two foci of labelled cells converge until eventually just one patch of labelling is seen. The borders of area 17 and the dLGN were confirmed histologically. Despite this dual geniculate input to lateral area 17, electrophysiological recording has shown that cells here each have a single receptive field originating from the temporal retina of the remaining eye.

By postnatal day 12 the geniculo-cortical pathway can not be altered in this way. In animals enucleated at this stage, the projections on both sides appear normal.

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306.5

SHORT-TERM SYNAPTIC PLASTICITY IN THE DEVELOPING RAT VISUAL CORTEX. A. Ramoa and M. Sur. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139, and Biophysical Institute, Federal University of Rio de Janeiro, 21941, Brazil.

Short-term, stimulus-dependent changes in synaptic efficacy may contribute to information processing and to the formation or rearrangement of connections in the visual cortex during development. Therefore, we have examined the physiological properties of short-term synaptic plasticity in the developing visual cortex.

Intracellular recordings were made in slices of visual cortex using potassium acetate microelectrodes. Bipolar electrodes were used to deliver pairs of electrical stimuli (0.05 msec duration, 0.1 mA, 0.25-0.5 Hz) to the underlying white matter. At postnatal day (P) 20-22, paired stimulation at short interstimulus intervals (<200 msec) caused the second synaptic response to be depressed (8 of 12 cells) or facilitated (2 cells) relative to the first response, even though the first response had decayed to baseline before the second stimulus. Depression was reduced by phaclofen but not by bicuculline, suggesting the involvement of GABA-b receptors. Depression was not observed with electrodes containing cesium acetate, consistent with a role for postsynaptic potassium-dependent mechanisms. At earlier ages (P13-16), the second response was either only potentiated when elicited within 100 msec of the first epsp (3 of 8 cells), or else was independent of the first response (5 cells), consistent with less intracortical inhibition at early ages. Potentiation may require NMDA receptor activation, since it was reduced by APV.

These results indicate that temporal interactions between inputs can lead to short-term changes in synaptic transmission in the developing visual cortex; such physiological modifications may be a crucial substrate for function as well as normal development and plasticity in the cortex.

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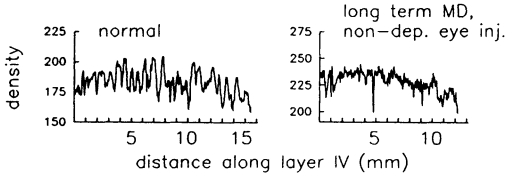
306.6

OCULAR DOMINANCE PATCHES: DEVELOPMENT AND DEPRIVATION EFFECTS VIEWED WITH TRANSNEURONAL WGA-HRP. Ying Tseng, Dane Copeland*, Kenneth Tovar*, and Barbara Gordon, Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

We have used WGA-HRP, rather than [^{3}H]proline, as a transneuronal marker to study the development of ocular dominance (OD) patches and the effects of monocular deprivation (MD) on these patches. Staining in layer IV of the cat visual cortex was first seen in animals injected at 29 days of age, but at this age staining was weak and uniform. OD patches first appeared in animals injected at 5 weeks of age, but at this age large regions were still unsegregated. OD patches appeared adult-like by about 9 weeks. Both short term MD (14 days beginning at 35 days of age) and long term MD (3 mos beginning at eye opening) produced expansion of the patches served by the nondeprived eye. In fact, after long term deprivation, injection of the nondeprived eye produced almost continuous label (see figure). Contraction of the patches served by the deprived eye was present but less obvious.



Sample density tracings through layer IV ipsilateral to injected eye.

306.7

ALTERED VISUAL TOPOGRAPHY IN AREA 17 OF THE PIGMENTED RABBIT FOLLOWING NEONATAL MONOCULAR ENUCLEATION. R.J. Clarke,[1] B.W. Datskovsky,[1] A.M. Grigonis[2] and E.H. Murphy.[1] [1]Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129, and [2]Department of Anatomy, Hahnemann University, Philadelphia, PA 19102-1192.

In the rabbit 90% of retinal fibers decussate at the chiasm. The ipsilateral retino-geniculo-striate pathway, representing 15-20° of the ipsilateral visual field, terminates in a region 2 mm wide at the area 17/18 border. This region is binocular and also contains the cells and terminals of the visual callosal projection. In rabbits monocularly enucleated at birth (ME), the callosal zone ipsilateral to the intact eye extends as much as 4 mm medial to the 17/18 border, but the callosal zone contralateral to the intact eye is limited to the normal 2 mm. Unless cortical visual topography is modified, these callosal projections must link cortical areas with disparate visual fields. In order to determine whether the topography of the ipsilateral visual projection is modified in the cortex of ME rabbits, we mapped multiunit cortical receptive fields. In ME rabbits (n=7), the ipsilateral visual field representation extended 3.5 mm medially from the 17/18 border, compared with 2 mm in normal rabbits (n=13). Visual topography was normal in the lateral 2 mm, but in the anomalous medial region, receptive fields were highly clustered in the extreme nasal visual field. In one ME rabbit, in which the cortex contralateral to the intact eye was ablated 3 hr prior to recording, the ipsilateral visual field representation also extended 3.5 mm medially from the 17/18 border. This indicates that the expansion of the area in cortex representing the ipsilateral visual field in ME rabbits is not dependent on callosal input and must result from modification of the retino-geniculo-cortical projection. The results suggest that altered visual topography in ME rabbit cortex may influence the abnormal development of callosal projections. Supported by NINDS grant NS26989.

306.8

EXUBERANT CALLOSAL CELL DISTRIBUTION IN THE STRIATE CORTEX OF THE RABBIT FOLLOWING CORTICAL EPILEPTIC ACTIVITY DURING DEVELOPMENT. A.M. Grigonis,[1] B. Ju*[2] and E.H. Murphy.[2] [1]Department of Anatomy, Hahnemann University, Philadelphia, PA 19102-1192, and [2]Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Cells which connect the two visual cortices via the corpus callosum are distributed throughout most of area 17 in the neonatal rabbit but are restricted, in the adult, to the area 17/18 border. Modification or elimination of activity in the retino-geniculo-cortical pathway during development through monocular enucleation, dark rearing, and intraocular injection of TTX, results in the maintenance of an immature, exuberant callosal cell distribution, whereas synchronous activity produced by strobe rearing results in a callosal cell zone which is more restricted than normal. In the present study we examined the effects of synchronous cortical activity on the development of visual callosal projections. Beads (Innovative Research of America), 1.5 mm diameter, which release an epileptogenic agent continuously for 3 weeks (1.5 mg penicillin), were placed on the surface of the posterior neocortex of 5 newborn rabbits (aged 2-5 days postnatal). In 2 control animals, beads containing only matrix were used. Rabbits were raised until adult, at which time multiple injections of HRP (Boehringer, 20% in H_2O) were made throughout one visual cortex. Animals were perfused 24 hours later and the brains were cut and reacted with TMB. The callosal cell distribution was normal in control animals. However, in the cortex which had penicillin released during development, the tangential extent of the callosal cell zone was twice as large as the normal distribution. The callosal cell zone was equally expanded in the cortex contralateral to the bead implant, indicating spread of epileptic activity to the opposite hemisphere. The results indicate that synchronous epileptic cortical activity, which is not correlated with visual input, prevents the normal process of retraction of early exuberant callosal projections. Supported by NINDS-NS26989.

306.9

ACTIVITY-DEPENDENT DEVELOPMENT OF ORIENTATION SPECIFICITY IN FERRET PRIMARY VISUAL CORTEX B. Chapman and M.P. Stryker Dept. of Physiology, UCSF, San Francisco, CA 94143-0444.

We have studied the orientation selectivity of neurons in primary visual cortex in normal ferrets of different ages and in ferrets deprived of vision or of visual cortical activity during development. In normal animals, when the earliest visual cortical responses could be recorded at postnatal day 23, only a very few cells showed any orientation bias. By week 7 cortical responses had matured to an adult-like state, with approximately 50% of cells showing clear orientation preference.

To determine whether the development of orientation-selective responses occurs through an activity-dependent mechanism, we silenced cortical neuronal activity during the time that orientation selectivity normally matures by infusing tetrodotoxin (TTX) into area 17 from postnatal week 4 through week 7. Four days after the infusion ended, neurons within the TTX-treated area were found to respond vigorously to visual stimulation, but lacked orientation selectivity. Neurons in the opposite (untreated) cortex and neurons in NaCl-treated control cortex showed a normal, adult-like distribution of orientation selectivity.

To test whether the development of orientation selectivity depends on visually driven activity, or whether spontaneous neural activity is sufficient, ferrets were visually deprived by binocular lid suture beginning before the time of natural eye-opening. In these animals, unlike in TTX-treated animals, some cells showed clear orientation specificity, but a larger fraction of cells lacked specificity than in control animals. Thus the development of any orientation specificity in ferret visual cortex appears to require neuronal activity, and normal development requires visually driven activity.

306.10

SPATIAL FREQUENCY TUNING OF CELLS IN AREA 17 OF ARTIFICIALLY INDUCED BILATERAL ESOTROPIA IN CATS. S. Quessy*, L. Richer*, M. Ptito, F. Lepore, J.-P. Guillemot. Dept. of Psychology and Kinesiology, Univ. of Montréal and Univ. of Québec, Montréal H3C 3P8, Québec.

Unilateral esotropia induced early in life results in amblyopia of the deviated eye and changes in the spatial frequency tuning of cells driven through this eye in visual cortex of cats. These functional changes are attributed to competitive interactions between the good and esotropic eye, the former generally gaining some functional and structural advantages over the latter. In order to modify the nature of this interaction, cats were made strabismic in both eyes and the spatial frequency characteristics of cells in area 17 were assessed. Strabismus was induced by cutting the lateral rectus muscle in each eye at about 21 days of age. Recording in visual cortex was carried out when the animals were at least 12 months of age. Micropipettes recorded extra-cellular activity, which was displayed and analyzed in conventional manner. Preparatory surgery to recording was carried out using Halothane-N_2O anesthesia and single cell activity was measured under paralysis, local anesthesia and N_2O. Stimulation consisted of sinusoidal gratings, swept at optimal orientation, direction and contrast, and whose spatial frequency could be varied in discrete steps. Results indicated that cells in the visual cortex responded well to the gratings. When compared to normal cats, however, a number of differences were observed: substantially more cells were monocularly driven; the spatial frequencie ranges of both narrow-band and low-band pass cells were higher in the normal animals; the half-widths of the narrow-band pass cells were larger in the latter. These results are confronted to behavioral acuity measures and discussed in terms of the relative deleterious effects of unilateral vs bilateral deviation of the eyes.

306.11

SHORT-TERM CONTOUR DEPRIVATION RESULTS IN INCREASES IN VISUAL CORTEX METABOLIC ACTIVITY IN RESPONSE TO FLASHING DIFFUSE LIGHT IN RATS. A.C. Gafka and R.M. Cooper. Behavioral Neuroscience Research Group, Department of Psychology, University of Calgary, Calgary, Alberta, Canada. T2N 1N4.

Previous 2-deoxyglucose (2-DG) work has demonstrated that, contrary to the null response in adult animals, visual cortex metabolic activity is increased by flashing diffuse light in neonatal rats (eye-opening at postnatal day [PND] 14 to PND 28). By PND 35, visual cortex shows little or no response to flashing diffuse light, indicating that adult status of visual cortical neurons is reached by this time. To test whether changes that occurred with development were the result of maturational processes or were experience-dependent, rats were either monocularly or binocularly lid-sutured prior to eye-opening to prevent contour experience. At PND 35 (following 21 days of contour deprivation) rats were injected with 2-DG, and monocularly exposed to 4 Hz flashing diffuse light (contour-deprived eye covered with a translucent mask, the other eye by an opaque occluder). Comparison of the two cortical hemispheres (stimulated vs occluded) indicated that regardless of whether the animal had received monocular or binocular lid-suture, the stimulated hemisphere demonstrated greater 2-DG uptake than the nonstimulated hemisphere. Combined with previous findings that contour deprived rats respond cortically to steady diffuse light (normally reared rats, at any age, do not), the present results argue that the increase in cortical metabolic activity by the deprived hemisphere is the result of the type of visual experience received during development. Thus, it appears that limiting visual experience to diffuse light may act to modify cortical neuronal response properties to match the type of visual experience.

306.12

CALLOSAL TRANSFER OF THE INTEROCULAR COMPETITION DURING DEVELOPMENT IN VISUAL CORTEX CELLS OF CATS. U. Yinon and A. Milgram*. Physiol. Lab., Goldschleger Eye Res. Inst., Tel-Aviv Univ. Fac. Med., Sheba Med. CTR, Tel-Hashomer, 52621, Israel.

Sagittal transection of the direct contralateral pathways at the optic chiasm and monocular deprivation (OCMD) were carried out in 9 kittens (age:6 weeks). In 3 of them reversal of the eye closure (OCMDREV) was carried out (age:10 weeks). Other 6 kittens served as chiasm transected (OC) controls. Unit recording was carried out in visual cortex areas 17,18 boundary. While in the OC cats equal proportions of cells reacted via the corpus callosum (indirectly contralaterally driven) to stimulation of each eye, in the OCMD cats the distribution was asymmetric; 12% of the cells had reacted contralaterally to the open eye and only 1.2% contralaterally to the deprived eye. In the OCMDREV cats 3.4% and 3.2% of the cells reacted contralaterally to the previously open and closed eye, respectively. Thus, the invasion of indirect contralateral input from the open eye via the callosum to the "weak" hemisphere (ipsilaterally to the deprived eye) is obstructed by the reversal of the deprivation effect. This indicates that an interocular competition takes place interhemispherically and it is bidirectionally mediated by the corpus callosum.

306.13

CORTICAL RESPONSIVENESS IN SPLIT-CHIASM STRABISMIC CATS M. Di Stefano*[1], G. Tassinari*[2] (Spon.: European Brain and Behaviour Society) [1]Dept. of Physiol. and Biochem., Univ. of Pisa [2]Inst. of Physiology, Univ. of Verona, Italy.

Behavioural studies of the visual fields in cats raised with unilateral convergent strabismus provide controversial results. Kalil (1977) and Ikeda and Jacobson (1977) reported that in esotropic cats there is an almost complete suppression of the nasal field of the deviated eye, whereas Berman and Murphy (1981) found that the esotropic eye retains a normal visual field. In partial agreement with the former finding, electrophysiological data revealed a response deficit through the esotropic eye in the ipsilateral area 17 (Kalil et al., 1984). Since visual information from the nasal field of each eye is conveyed to the ipsilateral cortex by the temporal hemiretina, behavioural and electrophysiological deficits might be accounted for by a failure of the temporal pathway from the deviated eye to drive cortical cells. To study this retinocortical pathway in isolation, we examined the cortical responsiveness of adult esotropic cats in which the optic chiasm had been sectioned by a trans-buccal approach. Recordings from single units were performed in areas 17 and PMLS of the two sides, which were fed by the normal or the deviated eye through their temporal projections only. Preliminary results indicate that in both 17 and PMLS the temporal projections from the deviated eye were as effective as the projections from the normal eye in driving cells on the respective side. Response properties and RF sizes of 17 and PMLS neurons did not differ in the two hemispheres. At variance with otherwise intact esotropic cats (Kalil et al., 1984), in both striate and extrastriate cortices ipsilateral to the deviated eye a large sample of binocular cells could be recorded. Due to the chiasmatic section, binocular cells in these areas must be driven by the contralateral eye through a commissural pathway. Some correspondence appeared to be preserved in their monocular RFs. It seems that chiasmatic section removes some influence exerted by the crossed projections from the normal eye on the direct projections from the deviated eye.

306.14

DEATH OF NEURONS IN AREA PMLS FOLLOWING ABLATION OF AREAS 17 AND 18 IN THE NEWBORN CAT. B.R. Payne, C. Conners* and P. Cornwell*. Dept. of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118.

The long term morphological consequences of removal of areas 17 & 18 from neonatal cats was assessed in cortical area PMLS. Measurements of laminar thickness revealed a significant thinning of layers III, V & VI and a significant loss of neurons in layers III & VI. The loss was greater in layer III than in layer VI. Following excision of the same areas in adult cats, there was no detectable thinning of any layer except layer V. No neurons were lost from any layer. Pathway tracing studies in adult cats show that the projections from areas 17 & 18 terminate most heavily in layers I, III & V in area PMLS, and that layers III & VI contain the largest number of neurons which project back to these same areas. In kittens, the laminar distribution of cells in area PMLS that project to areas 17 & 18 is similar to that in the adult, but the laminar terminations of the reciprocal projections from these same areas to area PMLS is relatively poorly differentiated. Thus, in the lesion part of the study, layer III of area PMLS contained neurons that were deprived of their target cells and/or deafferented by removal of areas 17 & 18, whereas neurons in layer VI were only target deprived, and neurons in layer V were partially deafferented. We conclude that following the lesion in the neonate, deafferentation in addition to target deprivation resulted in greater loss of neurons than target deprivation alone, and deafferentation alone resulted in no significant cell loss in layer V. There was no significant loss of neurons in layers II & IV; and neither of these layers forms a major connection with the ablated region. (Supported by MH44647.)

TRANSPLANTATION: ANIMAL MODELS OF PARKINSON'S DISEASE II

307.1

HUMAN FETAL TRANSPLANTS FOR PARKINSON'S DISEASE. C.R. Freed, R.E. Breeze, S.A. Schneck, C. Kruse, and N.L. Rosenberg, Depts. of Med., Surg., and Neurol., Univ. Colo. Med. Ctr., and Colo. Neurol. Institute, Denver, CO 80262.

We have previously reported successful transplantation of human fetal tissue in a patient with Parkinson's disease (Arch. Neurology 47: 505, 1990). We have now extended these experiments to three patients. Candidate patients have had Parkinson's disease for 10 to 20 years. Two of the three patients showed severe "on-off" phenomena while a third had a flat response to L-dopa. All patients had computers and video cameras in their homes for four months to one year prior to surgery for frequent measurements of motor performance. Single embryos of 7 or 8 weeks gestation were used for each patient. Two patients received implants of fetal tissue via 10 or 12 needle passes into caudate and putamen on one side of brain. The third patient was implanted bilaterally in putamen using 7 needle passes on each side of brain. One of the patients was immunosuppressed with cyclosporine and prednisone for one year. The two patients with the "on-off" phenomenon showed improvement beginning one to two months after surgery despite the fact that neither was immunosuppressed. The patient with less response to L-dopa had no response to transplant. These results suggest that patients with severe Parkinson's disease and the "on-off" phenomenon may be the best candidates for transplant with human fetal mesencephalic dopamine cells.

307.2

FIRST WEEKS OF DEVELOPMENT OF CENTRAL CATECHOLAMINERGIC (CA) NEURONS IN THE HUMAN EMBRYO, J. Cadusseau*, R. Jény*, M. Hammami* and M. Peschanski. Groupe Recherche Sce Méd. Nuc., CHU H. Mondor 94010 Créteil, HI Esquirol, 94410 St Maurice, France.

Intrastriatal transplantation of CA neurons dissected from human embryos is presently being carried out in several countries in the search for a therapy for parkinsonism. Brundin et al. (Exp. Br. Res. '88) have defined the general framework of this dissection by studying human-to-rat xenografts. In this study, we further define this protocol by analyzing the development of central CA neurons in human embryos ranging in age from 5 to 10 weeks of gestation.

Embryos were obtained from regular aspiration abortions under ultrasound guidance, according to a protocol accepted by the National Ethical Committee (12/3/90). They were immediately fixed with aldehydes then cut on a cryostat. Sections were immunostained with antibodies raised against either tyrosine hydroxylase or dopamine.

CA neurons appear at the mesencephalic flexure in 5 wk+5 d (14 mm) embryos. CA fibers are already present during the 6th week in large areas of the CNS, including the spinal cord and the rostralmost portions of the diencephalon. After 7 weeks of age, CA neurons are widespread in most of the tegmental primordium at the level of the mesencephalic flexure. They also extend in a more caudal area.

Most mesencephalic CA neurons start differentiation and axogenesis well before the end of the 6-8 week period that was presumed to be optimal for transplantation. 5+ to 7 wk-old embryos may therefore be preferable. The distribution of CA neurons in the tegmental primordium suggests that dissection should include more than the ventral half and should extend caudally. (supp by Réseau INSERM).

307.3

EFFECT OF HUMAN FETAL VENTRAL MESENCEPHALIC XENOGRAFTS ON D_1 AND D_2 DOPAMINE RECEPTOR BINDING IN THE UNILATERALLY 6-HYDROXYDOPAMINE (6-OHDA)-LESIONED RAT NEOSTRIATUM. C.E. Adams, I. Stromberg, C. Van Horne, B.J. Hoffer and S.J. Boyson. Depts. of Neurology and Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262

Neostriatal D_1 and D_2 dopamine receptor binding was examined in two groups of rats using quantitative autoradiography. Animals received either a unilateral 6-OHDA lesion of the medial forebrain bundle alone (6-OHDA control group) or a unilateral 6-OHDA lesion followed by an intraventricular transplant of human fetal ventral mesencephalic tissue (transplant group). D_1 receptors were labeled with ^{3}H-SCH-23390 (1.2 nM) while D_2 receptors were labeled with ^{3}H-spiperone (1.4 nM). In the 6-OHDA control group, D_2 receptor density was increased (5%) while D_1 receptor density was decreased (11%) within the lesioned as compared to the intact neostriatum. D_2 receptor density within the lesioned striatum was normalized when the transplant was present for 8 but not for 4 months. However, none of the transplants normalized the decrease in D_1 receptor density observed after unilateral 6-OHDA lesions. This study indicates that fetal cell transplants may only partially correct neostriatal dopamine receptor deficits associated with Parkinson's disease.

307.4

TARGET SPECIFIC AND LONG-DISTANCE OUTGROWTH FROM HUMAN FETAL MESENCEPHALIC GRAFTS. Ingrid Strömberg[1], Marc Bygdeman[2], Erik Sundström[3] and Per Almqvist[3]. Depts. of [1]Histol. & Neurobiol., [2]Obstetrics & Gynecol., and [3]Geriatrics, Karolinska Intitute, 104 01 Stockholm, Sweden.

Human fetal ventral mesencephalic tissue was grafted to unilaterally dopamine-depleted, immunosuppressed rats. Tissue was grafted as solid pieces and placed either in the lateral ventricle or in the cingulate cortex. The grafts and newly formed nerve fibers were visualized by immunohistochemistry using antibodies against TH, human specific Thy-1 and neurofilament (hNF). All rats showed reduced numbers apomorphine-induced rotations, beginning two months postgrafting. TH-positive nerve fibers reinnervated the entire striatum both when the graft was placed in the ventricle and in cortex. When the graft was placed in the cingulate cortex, fiber bundles penetrated corpus callosum to reach striatum. Thy-1-positivity was found only in the lesioned striatum, indicating that the graft reinnervated only the lesioned side. Nerve fibers from the graft placed in cortex, formed a bundle that followed the medial side of the ventricle and reinnervated the ventral limbic areas, where TH-positivity was overlapping with hNF- and Thy-1-immunoreactivity. The intact side of the ventral limbic areas were totally Thy-1-negative. When the graft was placed in the ventricle, cell migration had occured from the graft into host striatum. No cell migration was seen into other areas. Septum was sparsely reinnervated while globus pallidus was totally devoid of TH-positive nerve fibers. In conclusion, fetal human mesencephalic grafts innervate only areas that are normally target for dopaminergic neurons if they previously are dopamine denervated. Ongoing studies with injections of MPTP to rats with intraventricular human grafts are performed.

307.5

FUNCTIONAL COMPARISON OF FRESH VERSUS CULTURED HUMAN DOPAMINE (DA) NEURONS FOLLOWING TRANSPLANTATION.
B.D. Boss, Michael Lee*, M.E.C. Hancock*, and D.H. Spector*. Somatix Therapy Corp., Alameda, CA 94501.USA.

In an effort to test the relative effectiveness of our cultured embryonic human DA neurons, we compared the functional effects of transplanted fresh, uncultured human DA neurons to cultured human DA neurons. Donor tissue was obtained from the ventral mesencephalon of stage 15-16 embryos, mechanically dissociated, and transplanted (45,000 cells/rat) or cultured for 15 days prior to transplant (1,2, or 3 wells/rat). For culture, cells were seeded at 15,000 cells/well and grown as aggregates in serum-supplemented media for 5 days, then switched to a serum-free formulation (N2) for the remaining 10 days. Transplants were made into the DA-denervated striata of host rats, and functional recovery was tested by amphetamine-induced rotations scheduled at 4-wk. intervals for 24 weeks post-grafting. Preliminary results indicate cultured tissue is at least as effective as fresh tissue grafts and may be more viable. Immunohistochemical analysis of the transplanted cells corroborates the observed behavioral results.

307.6

EMBRYONIC MESENCEPHALIC AND STRIATAL CO-GRAFTS IN MPTP-TREATED MONKEYS.
J.R. Sladek Jr., T.J. Collier, J.D. Elsworth+, J.R. Taylor+, R.H. Roth+ and D.E. Redmond Jr.+ Dept. Neurobiology and Anatomy, Univ. Rochester School of Medicine, Rochester, NY 14642 and +Depts. Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06510.

Studies in rodents indicate that intrastriatal co-grafts of dopamine (DA) neurons of the embryonic ventral mesencephalon and their embryonic target tissue, the striatum, enhances the development of the grafted DA neurons and their innervation of the surrounding host striatum (Brundin et al., Devel. Brain Res. 24:77, 1986; Yurek et al., Exper. Neurol. 109:191, 1990). As part of our continuing program studying the efficacy of DA grafts in MPTP-treated monkeys, we have performed DA neuron-striatal co-grafts to determine whether this co-grafting procedure can augment graft survival and integration with the host brain. Two MPTP-treated African Green monkeys exhibiting mild parkinsonian symptoms served as subjects for co-grafting. Each monkey received block implants of embryonic ventral mesencephalon at each of three rostral-caudal sites in the caudate nucleus of one hemisphere, and mixed grafts of ventral mesencephalon and striatum from the same embryonic donor at three sites in the caudate nucleus of the other hemisphere. Monkeys survived for 8 months following grafting, and then were prepared for combined regional "punch" biochemistry and tyrosine-hydroxylase immunocytochemistry to assess the biochemical and morphological status of the grafts and the surrounding host striatum. Comparisons of measures derived from DA grafts alone and DA-striatal co-grafts will be presented. Supported by NS24032.

307.7

FETAL DOPAMINE CELL IMPLANTS IN MONKEYS WITH UNILATERAL MPTP LESIONS.
C.J. Hutt*, E.H. Kriek, M.L. Reite, M. Yamamoto*, Y. Kuroyanagi*, L.N. Heddleston*, G. Davila*, and C.R. Freed, Depts. of Med., Pharm., Surgery, Ob/Gyn, and Psych., Univ. Colo. Sch. of Med., Denver, CO 80262.

To test the value of fetal dopamine cell grafts in the treatment of Parkinson's disease, Bonnet monkeys were made Parkinsonian with a unilateral carotid infusion of MPTP.HCl (0.8 mg/kg). Monkeys were trained to reach for a food reward in a computer timed test and were tested for circling with apomorphine (APO) 0.15 mg/kg. Lesioning reduced the ability to reach for a food reward in the arm contralateral to the lesion. Lesioned animals showed spontaneous circling ipsilateral to the side of the lesion while APO led to circling contralateral to the lesion. Mesencephalic tissue from 18-45 mm crown rump length fetus was transplanted into the caudate and putamen on the lesioned side of brain via 5 needle injections. Half of the animals were immunosuppressed with cyclosporine 15 mg/kg/day p.o. Within 12 weeks after transplant, animals recovered the ability to spontaneously circle contralateral to the side of lesion while contralateral circling induced by APO slowed. At sacrifice, fetal dopamine cells were identified by tyrosine hydroxylase immunocytochemical staining. Graft growth was best in animals receiving tissue from 18-20 mm crown-rump-length fetuses. Preliminary evidence indicates that animals immunosuppressed with cyclosporine A had graft results similar to animals not receiving immunosuppressive treatment.

307.8

GRAFTING SURGERY PROTECTS AGAINST 6-HYDROXYDOPAMINE-INDUCED DOPAMINERGIC TOXICITY IN RATS: BEHAVIORAL AND MORPHOLOGICAL EVIDENCE.
M. LEVIVIER, S. PRZEDBORSKI[¶§], V. KOSTIC§, V. JACKSON-LEWIS§, D.M. GASH+, S. FAHN§, J.L.CADET§. Dpts of Neurosurgery and ¶Neurology, Univ. Libre de Bruxelles, B-1070 Brussels, Belgium; Dpt of Neurology, Columbia Univ., New York, NY 10032.;+Dpt of Neurobiology and Anatomy, Univ. of Rochester, Rochester, NY 14642.

Administration of the neurotoxin 6-hydroxydopamine (6-OHDA) to rat brain causes biochemical and neuroanatomical changes to the nigrostriatal dopaminergic pathway similar to those observed in Parkinson's disease (PD). Although the cause of PD remains unknown, it has been hypothesized that the neurodegenerative changes seen in PD might result from exposure to a neurotoxin. Therefore, strategies for limiting neurotoxin-induced dopaminergic damages, like those caused by 6-OHDA, may be of both clinical and basic interest. Accordingly, we tested the ability of both fetal neural (striatum) and fetal non-neural (liver) tissue implants to protect the rat striatum against the toxic effects of a subsequent intrastriatal injection of 6-OHDA. Non-grafted rats (lesion only) showed amphetamine-induced rotational behavior and a decrease in striatal [^{3}H]mazindol-labeled dopamine uptake sites after 6-OHDA injection. In contrast, the animals grafted with striatum or liver showed no behavioral or biochemical changes. Interestingly, sham-transplanted animals were also protected against the 6-OHDA-induced toxicity. These results suggest that the resistance of the dopaminergic system against 6-OHDA neurotoxicity observed in grafted and sham-transplanted animals is likely to be related to the surgical procedure itself. This observation points to the possible role for surgery-related events in the clinical improvements described in PD patients who underwent intracerebral transplantation.

307.9

βAPP AND GAP–43 GENE EXPRESSION IN NORMAL AND WEAVER MICE AND IN INTRASTRIATAL DOPAMINE NEURON GRAFTS.
C. Sola, G. Mengod, W.C. Low, B. Ghetti, J.M. Palacios & L.C. Triarhou.* Preclin. Res., Sandoz Pharma AG, 4002 Basel, Switzerland, Dept. Neurosurg., Univ. Minnesota, Minneapolis, MN 55455, and Dept. Pathol. (Neuropathol.) & Med. Neurobiol. Pgm, Indiana Univ., Indianapolis, IN 46202.

The cellular localization of β–amyloid protein precursor (βAPP) and growth–associated phosphoprotein GAP–43 RNA transcripts was studied by *in situ* hybridization in normal and weaver (*wv*) mice, which lose midbrain dopamine (DA) neurons, and in ventral mesencephalic grafts placed into the *wv* striatum. Both βAPP and GAP–43 genes are localized on chromosome Mmu 16, on which the *wv* locus has been assigned as well. Transcripts encoding GAP–43 and isoforms βAPP_{695}, βAPP_{714} and βAPP_{751} were present in normal SN and progressively reduced in *wv* SN, being correlated with DA neuron loss. Non–affected *wv* brain areas did not show changes. Graft survival was documented by rotation tests and TyrOHase immunocytochemistry. GAP–43, βAPP_{695}, βAPP_{714} and βAPP_{751} transcripts were present in the grafts; the βAPP_{770} species–normally seen in striatum and not SN–was not expressed in the grafts but was present in the host striatum. These findings *(i)* provide evidence for βAPP and GAP–43 gene expression by SN DA cells, *(ii)* indicate that the *wv* gene defect on Mmu 16 does not influence the expression of the closely linked βAPP gene, *(iii)* offer a correlate of structural protein gene transcription by grafted mesencephalic DA cells. (Supported in part by USPHS R29–NS29283 and RO1–NS14426).

307.10

IMPLANTED SYNTHETIC DOPAMINE (DA)-CONTAINING MICROSPHERES STIMULATE GROWTH OF DA FIBERS IN RATS WITH EXPERIMENTAL HEMI-PARKINSONISM.
A. McRae, S. Hjorth, A. Dahlström, L. Dillan, D. Mason* and T. Tice*, Depts. of Histology and Pharmacology, Univ. of Göteborg, Box 33031, S-400 33 Göteborg, SWEDEN, and Southern Research Institute, Birmingham, AL 35205, USA.*

Biocompatible poly (*DL*-lactide *co*-glycolide) injectable microspheres represents a novel means for controlled-release delivery of DA to target brain regions to substitute for subnormal levels of the endogenous transmitter. Male Sprague-Dawley rats were unilaterally lesioned in the MFB using 6-OH-DA (8 µg/4 µl; pentobarbital anesthesia). The rats were tested on a weekly basis until a stable contralateral rotational response was established to 0.1 mg/kg SC of the DA agonist apomorphine (APO). They were then stereotaxically implanted under ether anesthesia with 3 µl of a DA microsphere suspension, or a suspension of empty microspheres, in 2 sites in the denervated striatum. DA-microsphere implanted rats (n=13) and empty microsphere implanted rats (n=4) were subsequently challenged weekly for 8 weeks with APO and their contralateral rotatory behavior was recorded. Another group of DA microsphere-implanted rats (n=5) received no APO challenge during the 8-week period. Upon conclusion of the studies, immunocytochemical examination unexpectedly revealed growth of DA immunoreactive fibers in the striatum of DA microsphere implanted rats whether challenged or not with APO. No growth was noted in empty microsphere-implanted rats. Furthermore, functional recovery appeared related to the degree of DA fibers in the denervated striatum. These results suggest that implantation of DA microspheres may promote fiber growth and extended recovery of surviving dopaminergic neurons and therefore could be of therapeutic usefulness in Parkinson's disease.

308.1

AGING IMPAIRS ESTROGENIC SUPPRESSION OF HYPOTHALAMIC PROOPIOMELANOCORTIN (POMC) mRNA IN THE MOUSE. K. Karelus* and J.F. Nelson. Dept. of Physiology, University of Texas Health Science Center, San Antonio, TX 78284.

The objective of this study was to determine whether altered responsiveness of hypothalamic POMC mRNA to estradiol (E2) is part of the age-related loss of neuroendocrine sensitivity to estrogen in the mouse. Young (4 mo), middle-aged (13 mo), and old (25 mo) C57BL/6J mice were ovariectomized, implanted 2 weeks later with Silastic capsules containing E2 or cholesterol (CHOL), and sacrificed 3 days later. POMC mRNA was measured by solution hybridization/RNase protection, using a cRNA probe complementary to a transcribed portion of the mouse POMC gene. POMC mRNA in middle-aged and old CHOL-treated mice was 36% lower than in young animals. E2 treatment reduced POMC mRNA levels by 44% in young mice, but failed to suppress POMC mRNA in middle-aged and old animals. Polyadenylated RNA content was unaffected by either age or E2, indicating that the changes in POMC mRNA were not attributable to an overall change in hypothalamic messenger RNA content. These results confirm earlier evidence that levels of POMC mRNA are reduced in hypothalami of aging rodents, and indicate that the ability of E2 to suppress hypothalamic POMC mRNA is lost by middle-age in mice. In view of evidence that estrogenic suppression of hypothalamic POMC mRNA and beta-endorphin facilitates the preovulatory luteinizing hormone surge, the loss of estrogenic suppression of POMC mRNA may contribute to the reduction in the E2-dependent luteinizing hormone surge of middle-aged mice.

308.2

REGIONAL CHANGES IN GLIAL FIBRILLARY ACIDIC PROTEIN mRNA ACROSS THE ESTROUS CYCLE AND AGE. S.G. Kohama, C.E. Finch and T.H. McNeill. Andrus Gerontology Center and the Department of Biological Sciences. University of Southern California, Los Angeles, CA 90089-0191.

Anatomical studies have shown increased glial activity with age in the female rodent hypothalamus as a consequence of exposure to estradiol (E2). However, previous studies in our laboratory have shown that E2 treatment for 12 weeks failed to increase arcuate nucleus glial fibrillary acidic protein (GFAP) levels, measured by in situ hybridization (ISH) or immunohistochemistry (IHC), when compared to intact or ovariectomized controls. Similarly, aging alone did not increase arcuate nucleus levels of GFAP, although generalized increases were observed in both the gray and white matter. Therefore, we examined astrocyte responsiveness in the hypothalamus of young mice as a function of normal physiological stimulation, the estrous cycle. Young C57BL/6J mice exhibiting 4-day cycles were anesthetized and perfused in the late morning to generate a pool of brains representative of each cycle day. An additional group was collected on proestrous night. GFAP ISH revealed fluctuations of mRNA in the arcuate nucleus of the hypothalamus with the area of hybridization increasing on the day of proestrous, peaking proestrous night and decreasing thereafter. In summary, because ARC GFAP mRNA fluctuates during the estrous cycle this suggests a role for astrocytes in regional plasticity that may occur around proestrous.

Support for this study was from Grant AG-7909 and AG00300.

308.3

TYPE II GLUCOCORTICOID INCREASES CALCIUM CURRENTS IN HIPPOCAMPUS BY A CYCLOHEXIMIDE-SENSITIVE MECHANISM. D.S. Kerr, L.W. Campbell, O. Thibault and P.W. Landfield. Dept. Pharmacol., Univ. Kentucky Col. of Med., Lexington, KY, 40536-0084.

Previous studies have shown that adrenalectomy (ADX) decreases the calcium(Ca)-dependent, K-mediated afterhyperpolarization (AHP) in hippocampal slice neurons, whereas Type II glucocorticoid (GC) activation can restore or increase the AHP (Jöel and de Kloet, 1989; Kerr et al, 1989, Science, Vol. 245). The GC-dependent component of the AHP is greater with aging, and ADX also can reduce Ca spikes (Kerr et al, 1989), which are larger with aging (Pitler and Landfield, 1990, Brain Res.).

However, it is uncertain whether GCs can influence Ca spikes or currents directly (as opposed to effects on K) or if the effect is mediated by protein synthesis. The present studies investigated these questions using current clamp and single electrode voltage clamp (SEVC) analyses of Ca currents in hippocampal slices from animals that had been surgically or pharmacologically (with metyrapone) adrenalectomized. Approximately half of the ADX slices were exposed to RU 28362 (Roussel-UCLAF), a potent Type II GC receptor agonist, for 1-2 hr. In some experiments, slices were co-incubated with cycloheximide (CXM), to inhibit protein synthesis.

RU 28362 (RU) dramatically increased Ca spike width. In SEVC, TTX, TEA and Cs-treated CA1 pyramidal neurons, exposed to RU exhibited larger voltage-activated Ca currents. CXM blocked the actions of RU. Thus, the activation of Type II GC receptors increases inward Ca current through protein synthetic mechanisms. This effect may provide a link between the glucocorticoid and Ca hypotheses of brain aging. (e.g., Kerr et al, 1989).

308.4

CHRONIC EXPOSURE TO ELEVATED CORTICOSTERONE ALTERS SPATIAL MEMORY IN MID-AGE, BUT NOT YOUNG RATS. S.R. Bodnoff, S. Sharma & M.J. Meaney. Douglas Hospital Research Center, Montreal, Canada, H4H 1R3.

Chronic exposure to elevated, stress-like levels of corticosterone (CORT) results in the loss of neurons in the hippocampus (Sapolsky et al., J. Neurosci,1985), a brain region of considerable importance for learning and memory. In previous experiments from this lab, young (3-4 months) rats were treated for 3 months with CORT and spatial memory was assessed in the Morris water maze, a task sensitive to hippocampal damage. We observed rather unimpressive impairments in the CORT-treated rats. Here, we attempted to re-examine the effects of CORT by treating mid-aged rats. We implanted young and mid-aged (11-12 months) Long-Evans rats with fused pellets of CORT that produce plasma CORT levels of 20-25 ug/dl. Spatial memory was assessed using the maze procedure described by Dekker et al. (Neurosci Abst, 1990, p. 479). After 3 months of treatment, the mid-aged CORT-treated rats showed significant spatial memory impairments compared with age-matched cholesterol-treated rats and young rats of both treatment conditions. While the groups did not differ during the first 4 days, impairments became obvious during Days 5-7, and by Day 8, all groups attained the same level of performance. These data will be discussed in the context of hippocampal and cortical neuron loss and their relevance for the study of aging.

308.5

AGE DEPENDENT INCREASE OF POSTERIOR PITUITARY VASOPRESSIN IN RATS. Alan G. Robinson, Mark D. Fitzsimmons, and Michelle M. Roberts, Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15261.

The amount of vasopressin stored in the posterior pituitary is often used as an index of chronic hormone secretion. With hypernatremia, pituitary vasopressin decreases and with recovery it increases. During recovery from prolonged hypernatremia, we found that pituitary vasopressin content increased above starting baseline. We therefore studied pituitary vasopressin content over time. In Sprague-Dawley Rats (SDR), studied over 3 weeks, posterior pituitary vasopressin increased parallel with weight: weight, 301 ± 7 g to 487 ± 5 g; pituitary AVP, 1,139 ± 55 ng to 2,650 ± 206 ng. Fischer Rats (FR) showed less weight gain but similar changes in pituitary content: weight, 206 ± 3 g to 297 ± 5 g; pituitary vasopressin, 1,202 ± 96 ng to 2,429 ± 351 ng. Because we thought the vasopressin increase was due to the increasing size of the animals, weight was maintained constant for both SDR and FR by restricting feeding, 304 ± 7 g to 303 ± 7 g and 236 ± 3 g to 245 ± 5 g, respectively. Pituitary content still increased: 1,139 ± 55 to 2,406 ± 135 ng; and, 1,202 ± 96 to 1,959 ± 168 ng, respectively. Vasopressin mRNA in the supraoptic nuclei did not increase over the same 3 weeks: 8.1 ± 1.8 to 8.5 ± 1.1, arbitrary units. Studies at 6 and 9 months have demonstrated continued increase in pituitary vasopressin but again, no increase in vasopressin MRNA. When secretion of vasopressin was inhibited by chronic hyponatremia in Sprague-Dawley Rats (sodium less than 110 mEq/L) the vasopressin increase over 3 weeks was completely inhibited: 1,254 ± 122 ng to 1,022 ± 140 ng. Thus, while synthesis can be turned off by chronic severe hyponatremia, we conclude that on-going synthesis in excess of release in basal conditions is responsible for the formation and continued accumulation of vasopressin in the posterior pituitary with aging.

308.6

VASOPRESSIN AND OXYTOCIN CELL NUMBERS IN THE HUMAN HYPOTHALAMUS DURING AGING AND IN ALZHEIMER'S DISEASE. E. Goudsmit and D.F. Swaab*. Netherlands Inst. for Brain Res., Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

In order to clarify age-related changes in the human hypothalamo-neurohypophyseal system, total cell numbers and numbers of immunocytochemically identified vasopressin (VP) and oxytocin (OT) cells were determined in the supraoptic (SON) and paraventricular nucleus (PVN) in a series of human hypothalami ranging in age from 10 to 97 years including 10 Alzheimer's disease (AD) cases.

No changes in total cell numbers were observed in the human SON and PVN during the life-span or in AD. The number of OT cells in the PVN also remained unaltered during aging and in AD. In contrast, the number of VP-expressing cells in the PVN showed a gradual increase during aging, suggesting an increase in the rate of synthesis of this peptide. This increase was absent in AD patients. Animal studies suggested that an increase in VP synthesis in senescence might be caused by osmotic stimulation due to a decrease in renal sensitivity to VP.

In contrast to the SON and PVN, the suprachiasmatic nucleus (SCN) shows a marked cell loss in human senescence and even more so in AD, which might be related to a reduced input via the optic nerve. Therefore, differences in age-related cell loss between these hypothalamic nuclei might be related to differences in stimulatory input.

Human brain material was obtained from the Netherlands Brainbank in Amsterdam.

308.7

REPRODUCTIVE HISTORY AFFECTS AGING OF GNRH SYSTEM IN THE MALE RAT. J.W. Witkin. Dept. Anat. & Cell Biol., Columbia Univ. New York, NY 10032

Gonadotropin releasing hormone (GnRH) neurons receive an increased synaptic input with age beginning as early as 10 months in the virgin male rat. By 20 months, there is a tenfold increase in the density of synapses onto these neurons. (Witkin, Neurosci. 232:1003,'87). The present study is an examination of this parameter in reproductively active aged male rats. GnRH neurons from three month old rats (N=6) were compared to those from 20 month old rats (N=6) that had been retired from breeding for 3-5 months. Tissue from the preoptic area was prepared for electron microscopic examination using a double label immunocytochemical protocol (Chen et al., J.Comp.Neurol. 232:534,'89) so that we could identify the input by the inhibitory transmitter, gamma amino butyric acid (GABA) to GnRH neurons. Sites of GABA immunoreactivity were visualized after incubation in GAD 6 (a mouse monoclonal antibody to glutamic acid decarboxylase, a gift of Gottlieb, Chang and Gottlieb, J.Neurosci.8:2123,'88) using DAB; sites of GnRH , after incubation in LR1 (a rabbit polyclonal, Benoit) using TMB stabilized with DAB. Photographic montages of GnRH neurons (recognized by the presence of TMB crystals) printed at a final magnification of 25,000X (a minimum of 5 neurons per animal) were measured using a morphometric program (R&M Biometrics). The percent of perikaryal membrane with postsynaptaic modification was calculated for each neuron. Similar measurements and calculations were made on random unidentified neurons (one from each section in which a GnRH neuron had been photographed). The density of total synaptic input to GnRH neurons from young and aged animals was not found to be different, using a nonparametric test (Mann-Whitney U, $p < 0.05$). There was also no difference in the density of input to unidentified neurons. However, a larger percentage of the synapses were GABAergic in both the general neuronal population and in GnRH neurons in the older animals, perhaps indicating a general aging phenomenon. The increase in synaptic input to GnRH neurons which was so striking in aging virgin male rats was not observed in the retired breeders. GnRH neurons in aged male rats that have been reproductively active are innervated to the same degree as those in young adult animals. These results suggest that the reproductive history of the animal affects the anatomical milieu of the preoptic area in the male rat and in particular, that of the GnRH neuron. NIH AG05366

CALCIUM CHANNELS: MOLECULAR BIOLOGY

309.1

Neuronal L-type calcium channels: molecular cloning of an α_1 subunit cDNA and localization in rat brain. Hyung-Lae Kim * ‖, Mark A. Smith#, Hyun Kim‖, and Hemin Chin ‖
‖ Laboratory of Molecular Biology, NINDS, #Clinical Neuroendocrinology Branch, NIMH, NIH, Bethesda, MD, 20892.

We have cloned and sequenced a cDNA (prBCCA1) encoding an α_1 subunit of voltage-sensitive L-type calcium channels (dihydropyridine receptor) of rat brain. The cDNA (8,608 bp) encodes a protein of 2,108 amino acids with a Mr of 236,919. Northern blot analysis revealed that prBCC A1 was transcribed from a ~12 kb mRNA species present in rat brain. The neuroanatomic distribution of prBCCA1 mRNA in rat brain was examined by *in situ* hybridization histochemistry. The highest concentrations of the calcium channel α_1 subunit were found in the olfactory bulb, granular layer of the dentate gyrus, suprachiasmatic nucleus of the hypothalamus, and pituitary and pineal glands. The distribution of the α_1 subunit protein was mapped immunohistochemically with rabbit polyclonal antibodies raised against synthetic peptides containing amino acid sequences specific for the calcium channel α_1 subunit. Brain areas showing strong hybridization signals with the α_1 subunit antisense RNA probes also had high immunoreactivity levels. Thus, regional distribution of the α_1 subunit of L-type calcium channels in rat brain suggest that this type of calcium channel plays an important role in excitation-secretion coupling functions.

309.2

Bacterial expression of L-type Ca^{2+} channel α_1 subunit fusion proteins and generation of domain-specific antibodies. Hyun Kim, Hyung-Lae Kim*, and Hemin Chin. Lab of Molecular Biology, NINDS, NIH, Bethesda, MD, 20892.

Calcium entry through voltage-sensitive Ca^{2+} channels mediates several physiologic functions, including muscle contraction and neurotransmitter and hormone release in muscle, secretory and neuronal cells. We have recently cloned a rat brain cDNA (prBCCA1) encoding an α_1 subunit of L-type Ca^{2+} channel, and have shown that this channel is predominantly localized in neuroendocrine tissues. Although overall structural features are well conserved, the deduced primary structure of prBCCA1 indicated that amino and carboxy termini as well as cytoplasmic loops are significantly different from those of the α_1 subunits of skeletal and cardiac L-type Ca^{2+} channels. To investigate the gene products of three types of Ca^{2+} channel α_1 subunits cloned so far, we prepared antisera against various regions of the Ca^{2+} channel α_1 subunit. Bacterial expression plasmids (pET and pGEX) were used to construct recombinant plasmids that encode the fusion proteins containing the unique and homologous regions of the Ca^{2+} channel α_1 subunit. The fusion proteins were expressed in *E. coli* and used to produce rabbit polyclonal antisera. ELISA and Western blot analysis showed that the antisera were highly specific for the fusion proteins. Further functional characterization of the antisera is in progress.

309.3

CLONING AND EXPRESSION OF A NOVEL HUMAN NEURONAL CALCIUM CHANNEL. D.H. Feldman, M.E. Williams*, A.F. McCue*, R. Brenner*, G. Velicelebi, S.B. Ellis*, M.M. Harpold*. SIBIA Inc., P.O. Box 85200, San Diego , CA 92186.

We have cloned genes encoding subunits of voltage-dependent calcium channels (VDCCs) expressed in human brain and IMR-32 human neuroblastoma cDNA libraries. We report here the cloning and expression of a novel human α_1 subunit gene that encodes a 2161 amino acid protein, with 77.3% similarity to the α_1 subunit of rabbit skeletal muscle [Tanabe *et al* Nature 328:313 (1987), Ellis *et al* Science 241:1661 (1988)] and 80.7% similarity to the rabbit cardiac α_1 subunit [Mikami *et al* Nature 340:230 (1989)]. The predicted a.a. sequence suggests an overall topography shared with other VDCC α_1 genes. We also report α_2 and ß subunit genes expressed in human brain that encode proteins of 1091 and 478 amino acids respectively, with 97.2% and 98.3% similarity to the homologous rabbit skeletal muscle genes [Ellis *et al* 88; Ruth *et al* Science 245:1115].

The mRNAs transcribed *in vitro* from the α_1, α_2, and ß genes were injected in various combinations into *Xenopus* oocytes which were then studied under voltage clamp to detect barium currents. Co-injection of α_1, α_2, and ß transcripts elicited currents that first activated near -30 mV, reached a peak near 0 mV, inactivated slowly, and were weakly sensitive to holding potential. Bay K 8644 (1 μM) augmented the currents and dramatically prolonged the tail currents. Nifedipine (5 μM) blocked the current in a holding potential dependent manner. The currents were reversibly blocked by 50 μM Cd^{2+} and by 15 μM ω-conotoxin. Whereas neither α_1 transcripts alone nor α_1 and α_2 together elicited currents, α_1 and ß transcripts together elicited DHP-sensitive currents similar to, albeit smaller than, those elicited by α_1, α_2, and ß in combination.

Thus, we have identified a neuronal L-type VDCC α_1 gene that diverges significantly from previously cloned muscle L-type VDCC α_1 genes. The ß subunit appears to serve an obligatory function, whereas the α_2 subunit appears to serve an accessory role that enhances expression of the channel.

309.4

mRNA FOR THE CARDIAC CALCIUM CHANNEL IS EXPRESSED DURING DEVELOPMENT OF SKELETAL MUSCLE. N.Chaudhari and K.G.Beam. Dept. of Physiology, Colorado State University, Fort Collins, CO 80523.

Voltage-gated (L-type) calcium channels in skeletal and cardiac muscle cells are intimately involved in coupling nerve-induced excitation to contraction. Skeletal and cardiac muscle each possess L-type calcium channels with distinct functional properties and which are encoded by separate genes.

We have found that mRNA similar (in size, hybridization characteristics and sequence) to the cardiac calcium channel mRNA is detectable during early skeletal myogenesis. The cardiac-like mRNA is found at high concentration in early skeletal myotubes and precedes the expression of mRNA encoding the skeletal calcium channel. As skeletal myotubes mature, the concentration of the cardiac calcium channel mRNA decreases rapidly. The cardiac-like mRNA present in skeletal cells is probably transcribed from the same gene as the mRNA in heart cells, as evidenced by similarly sized protection fragments in RNase protection assays. The cardiac calcium channel mRNA in fetal skeletal muscle is not derived solely from vascular tissue since a similar mRNA is detectable also in tissue cultures of skeletal myotubes. Certain established lines of fibroblasts (e.g. 3T3) also express this cardiac calcium channel mRNA, in keeping with reports of cardiac-type calcium current in such cells (Chen et al.,1988). At present, we have no evidence whether the mRNA is translated and yields a functional channel in immature skeletal myotubes. Supported by NIH grant GM42652.

309.5

IN SITU LOCALIZATION OF CALCIUM CHANNEL mRNA.

ΨW.J. Tomlinson, ΦS.R.Vincent, and ΨT.P. Snutch. Division of Neurological Sciences, ΦDept of Psychiatry, and ΨBiotechnology Laboratory, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Previously, we have cloned a family of Ca^{2+} channel α_1-subunits from rat brain. Utilizing Northern blot and PCR analysis we have determined the regional distribution of distinct Ca^{2+} channel α_1-subunit isoforms in the rat CNS and in nonneuronal tissues.

To examine the cellular distribution of Ca^{2+} channel expression, we have performed in situ hybridization on rat brain sections using antisense oligonucleotide probes radiolabelled with [^{35}S] dATP. The oligonucleotides were directed to distinct coding regions of each of the rat brain class A and class C Ca^{2+} channel cDNAs. Specificity of the probes was determined by using variable hybridization and post-hybridization stringencies and control oligonucleotide probes. Autoradiography revealed that the class A and class C Ca^{2+} channel mRNAs overlap somewhat in their distribution, both being highly expressed in the cerebellar cortex. However, the C type Ca^{2+} channel transcripts showed a much broader distribution, being highly expressed in the hippocampus, hypothalmus, piriform cortex, neocortex, olfactory bulb, locus ceruleus, pontine nuclei and the sensory ganglia. These results indicate that the class A and class C type Ca^{2+} channels are differentially expressed in the nervous system.

309.6

FUNCTIONAL EXPRESSION OF A RAT BRAIN CALCIUM CHANNEL IN XENOPUS OOCYTES

J.P. Leonard, T.V. Starr[1], B.P. Schmidt, M. Pragnell[2], S.B. Ellis*[3], M. Williams*[3], M.M. Harpold*[3], K.P. Campbell[2], and T.P. Snutch[1]. Dept. of Biology, Univ. of Illinois at Chicago, Il, 60680; [1]Biotechnology Laboratory, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5; [2] H.H.M.I., Univ. of Iowa, Iowa City IA, 52242; [3]S.I.B.I.A., La Jolla CA, 92037.

The rat brain Class A calcium channel α_1 subunit encodes a 2,212 amino acid protein that is 33% homologous to muscle DHP-sensitive channels. Upon microinjection into *Xenopus* oocytes this principle pore-forming unit only produces functional calcium channels when co-expressed with the neuronal β and α_2 subunits. Under standard two-electrode voltage clamp in Ba saline the channels expressed from the cloned cDNAs remarkably resemble currents from total rat brain mRNA. The I_{Ba} is activated at high voltages (-30 to -20 mV), peaks at +10 to +20 mV, and is still inward at +50 mV. The current inactivates substantially but incompletely during a 1 sec depolarization. Positive changes in holding potential caused inactivation of I_{Ba} (50% at -20 mV). The pharmacological profile of the channel is also similar to that seen by expression of total rat brain mRNA. The I_{Ba} is insensitive to 10 μM nifedipine, or 1μM TTX, but is blocked by low μM Cd^{2+}. Conotoxin GVIA and *Agelenopsis aperta* venom sensitivity are currently under investigation. (Supported by the MRC of Canada, H.H.M.I., and the NIH)

CALCIUM CHANNELS: PHOSPHORYLATION

310.1

CYCLIC AMP MODULATION OF L-TYPE CALCIUM CURRENTS IN NON-DISSOCIATED HIPPOCAMPAL NEURONS.

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There is increasing evidence that cAMP can counteract "rundown" and calcium (Ca)-dependent inactivation of dihydropyridine (DHP)-sensitive Ca channels in molluscan neurons and other excitable tissues (e.g., Armstrong and Eckert, 1987, PNAS). However, it is unclear whether DHP-sensitive, or L-type, Ca currents in the mammalian CNS can be modulated by cAMP.

We, and others, have described DHP-sensitive currents in adult hippocampal slice neurons, using single electrode voltage clamp (SEVC). Because the cells are not disrupted or dissociated, Ca currents exhibit little "rundown" and remain viable for many hours. However, they are highly sensitive to Ca-dependent inactivation (Pitler and Landfield, 1987, Brain Res.; Campbell et al, 1990, Soc. Neurosci. Abstr.).

In the present studies, we impaled TTX and TEA-treated CA1 pyramidal neurons with pipettes filled with 1-10 mM dibutyryl cAMP, and 2 M CsCl (pH 7.1) to study inward Ca currents with SEVC.

Cells (n=16) loaded with db cAMP exhibited significantly stronger L-type currents in comparison to control cells (n=16), both during a depolarizing step and during the long post-activation "tail-like" currents that we have described previously (cf. references above). The amplitude increases were 25-30%, but were highly significant ($p < .005$). Input resistance did not differ between control and db cAMP cells. This effect may be mediated by reducing Ca-dependent inactivation, as in non-CNS cells. (Supported by AG04542).

310.2

cAMP-DEPENDENT PHOSPHORYLATION OF RYANODINE RECEPTOR OF RAT BRAIN.

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Ryanodine receptor was solubilized from rat brain microsomal membranes with CHAPS. A monoclonal antibody Ry-1 against cardiac ryanodine receptor immunoprecipitated more than 85% of the solubilized receptor. By immunoblotting with Ry-1, a large polypeptide having the same mobility in SDS-PAGE as that of cardiac ryanodine receptor was detected in the brain microsomal proteins. The brain ryanodine receptor, which was immunoprecipitated by Ry-1, was phosphorylated by cAMP-dependent protein kinase. The ryanodine receptor was expressed in cultured rat brain neurons and the phosphorylation of the receptor was markedly increased by a treatment with Bt_2-cAMP. The number of neurons showing the caffeine-induced Ca^{2+} transient was increased by the treatment. These results suggest that the Ca channel activity of brain ryanodine receptor is regulated by cAMP-dependent phosphorylation.

310.3

BIOCHEMICAL CHARACTERIZATION OF L-TYPE CALCIUM CHANNELS IN AtT-20 CELLS.

G.A. Stafford* and G.A. Weiland. Dept. Pharmacology, Cornell Univ., Ithaca, NY 14853.

Membrane binding studies have demonstrated a single population of high affinity dihydropyridine (DHP) binding sites in AtT-20/D16v-F2 (mouse corticotrophic pituitary tumor) cells. We have shown that in intact cells, the binding is dependent upon membrane potential. Functional studies correlate the binding with calcium influx, suggesting that these binding sites represent L-type voltage-gated calcium channels.

This class of calcium channels has been most extensively studied in skeletal and cardiac muscle, where it has been shown that phosphorylation of the channel protein regulates function. We have found that the channel in AtT-20 cells shares at least some epitopes of the L-type channel from skeletal muscle. Monoclonal antibodies directed against the α1 subunit of the rabbit skeletal muscle calcium channel specifically immunoprecipitate DHP binding from pre-labelled, solubilized AtT-20 cell membranes. Western blots indicate that the precipitated protein has a molecular weight of approximately 100 K, significantly smaller than its muscle counterpart. In cells loaded with ^{32}P-orthophosphate, the 100 KDa protein is phosphorylated in the basal state. Preliminary results indicate that activation of cAMP-dependent kinase increases the incorporation of ^{32}P. Continuing experiments will use back-phosphorylation to attempt to quantify the increase. Correlation will be made with alterations in function of the channel. (Supported by Cornell Biotechnology Program.)

310.4

OKADAIC ACID AND HEPARIN HELP SUSTAIN THE ACTIVITY OF L-TYPE CALCIUM CHANNELS IN PLANAR LIPID BILAYERS.

C. Townsend*, Y. Wang*, K. Fecho*, P.A. Koplas, & R.L. Rosenberg. Pharmacology and Neurobiology, Univ. of N. Carolina at Chapel Hill, NC

The activity of L-type Ca channels "runs down" in dialysed cells, excised patches, and planar lipid bilayers. Activated $G_{S\alpha}$ reduces the rate of Ca channel rundown, possibly due to a direct G-protein/channel interaction (Yatani et al., 1987, Science 238; 1288). We have been studying the activity of L-type Ca channels incorporated from cardiac sarcolemma into planar lipid bilayers in the presence of the DHP agonist (+)202-791. In the absence of $G_{S\alpha}$, Ca channels disappeared in less than 3 min. Intracellular $G_{S\alpha}$ caused channel activity to persist, with a mean "lifespan" of ~15 min (n>50). Channel lifespan was also dramatically improved by other additions in the absence of $G_{S\alpha}$. Treatment of the sarcolemma with okadaic acid, a potent phosphatase inhibitor, caused sustained channel activity in 14/19 experiments, with lifespans of 10-120 min. Incubation of the membranes with ATPγS, presumably leading to poorly-reversible thiophosphorylation, caused channel activity to be sustained for >10 min (4/5 experiments). Intracellular additions of heparin also inhibited channel rundown in 9/13 experiments. In the presence of $G_{S\alpha}$, the addition of heparin after channel incorporation caused an increase in channel activity above that seen with $G_{S\alpha}$ alone (20/32 experiments). This increase is unlikely to result from phosphorylation, because of the absence of added ATP. These results (1) support the idea that a dephosphorylation of the Ca channel is a mechanism for the rundown process, (2) indicate that heparin increases channel activity, but probably not only by acting as a phosphatase inhibitor, and (3) suggest that $G_{S\alpha}$ might improve channel lifespan by modulating G-protein-sensitive phosphatases or interfering with phosphatase/channel interactions.

310.5

FACILITATION OF LONG-LASTING (TYPE II) Ca CHANNEL CURRENTS BY A COGNITIVE ENHANCER DM-9384 REQUIRES A PHOSPHORYLATION PROCESS IN NG108-15 CELLS. M. Yoshii[1], S. Watabe[2], Y. L. Murashima[1]* and Y. Nakamoto[1]* [1]Dept. of Neurophysiology, Psychiatric Res. Inst. of Tokyo, Tokyo 156, Japan and [2]Exploratory Res. Lab.II, Daiichi Pharmaceutical Co.,Ltd., Tokyo 134, Japan.

A cognitive enhancer DM-9384 (a pyrrolidone derivative) causes an increase in the transmitter release in the brain (Watabe et al., Soc. Neurosci. Abstr. 15, 601, 1989; 16, 137, 1990). To examine a possibility that the drug might potentiate Ca channels responsible for the transmitter release, we have recorded Ca channel currents from neuroblastoma x glioma hybrid (NG108-15) cells using the whole-cell clamp technique. It has recently been found that DM-9384 markedly increases long-lasting (type II) Ca channel currents in a manner similar to the effect of dibutyryl cAMP (Yoshii and Watabe, Biophys. J. 59, 82a, 1991). In the present study, we have further examined whether cAMP-dependent mechanisms are involved in the DM-9384 action. Leu-enkephalin (50 nM), which activates an inhibitory G protein, caused a time- and voltage-dependent reduction in the type II current, which was antagonized by 1 μM DM-9384 as well as by 1 mM dibutyryl cAMP. H-7(100 μM), a protein kinase inhibitor, prevented the facilitatory effects of DM-9384 and dibutyryl cAMP. It is concluded that the facilitatory action of DM-9384 requires a phosphorylation process, which is probably mediated by cAMP.

310.6

PHOSPHORYLATION OF AN α1-LIKE SUBUNIT OF AN ω-CONOTOXIN-SENSITIVE BRAIN CALCIUM CHANNEL BY cAMP-DEPENDENT PROTEIN KINASE AND PROTEIN KINASE C. Michael K. Ahlijanian, Jörg Striessnig and William A. Catterall. Dept. of Pharmacology, SJ-30, University of Washington, Seattle, WA 98195.

Voltage-dependent calcium channels are responsible for rapid increases in intracellular calcium during depolarization in neurons. Electrophysiological evidence suggests that neuronal N- and L-type calcium channel function is regulated by phosphorylation, however, direct phosphorylation of subunits of neuronal calcium channels has yet to be demonstrated. We have used antibodies which recognize the α2δ and α1 subunits of skeletal muscle L-type calcium channels to investigate the subunit components and phosphorylation of ω-conotoxin (ω-CgTx)-sensitive N-type calcium channels from rabbit brain. Photolabeling of the N-type channel with a photoreactive derivative of ^{125}I-ω-CgTx results in the identification of a single polypeptide of 240 kDa. MANC-1, a monoclonal antibody recognizing α2δ subunits of L-type calcium channels from skeletal muscle, immunoprecipitates the ω-CgTx-labeled 240 kDa polypeptide and approximately 6% of the digitonin-solubilized ^{125}I-ω-CgTx-labeled N-type channels. MANC-1 also immunoprecipitates a phosphoprotein of 240 kDa which comigrates with ^{125}I-ω-CgTx-labeled N-type calcium channels, but not with L-type calcium channels, in sucrose gradients. Both cAMP-dependent protein kinase and protein kinase C are effective in the phosphorylation of this polypeptide. Similar to the α1 subunits of skeletal muscle L-type calcium channels, the immunoprecipitation of the 240 kDa phosphoprotein by MANC-1 is prevented by the detergent Triton X-100. Anti-CP(1382-1400), an anti-peptide antibody against a highly conserved segment of the α1 subunits of calcium channels, immunoprecipitates the 240 kDa phosphopeptide in Triton X-100. The 240 kDa protein is phosphorylated to a stoichiometry of approximately 1 mole of phosphate per mole of ω-CgTx-binding N-type calcium channels by both cAMP-dependent protein kinase and protein kinase C. The results suggest that the 240 kDa polypeptide is an α1-like subunit of an ω-CgTx-sensitive N-type calcium channel. The N-type calcium channels containing this subunit are phosphorylated by cAMP-dependent protein kinase and protein kinase C and contain noncovalently associated α1-like and α2δ-like subunits as part of their oligomeric structure.

310.7

MODIFICATION OF CALCIUM CURRENT BY HYPEROSMOTIC MEDIA IN CONTROL AND PHORBOL ESTER TREATED NEURONS. K.J. Loechner, R.J. Knox, J.A. Connor, L.K. Kaczmarek. Dept. of Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510, and Dept. Neurosciences, Roche Institute of Molec. Biol., Nutley, NJ 07110.

Alterations in extracellular osmolality have been shown to affect calcium-dependent secretion. We have reported previously that hyperosmotic media (H-M) (artificial sea water made hyperosmotic with mannitol, sucrose, NaCl, or choline chloride) inhibit KCl-induced release of peptides and terminate ongoing electrical activity (afterdischarge) in the bag cell neurons (BCNs) of Aplysia. We have now investigated the effects of H-M on the electrical properties of isolated BCNs. We have found that H-M inhibit action potentials elicited by depolarizing current injection in these neurons. This is associated with an attenuation of voltage-dependent calcium current measured using single microelectrode voltage clamp with either barium or calcium as the charge carrier. Furthermore, in BCNs loaded with fura-2, H-M inhibit the rise in intracellular calcium levels that normally occurs in response to depolarizing current injection. Basal levels of calcium, however, are not altered. Interestingly, the inhibition of the rise in intracellular calcium can be partially overcome by pre-treatment of the BCNs with the phorbol ester, TPA, which has been shown to recruit a new population of calcium channel in the plasma membrane (Strong et al., 1987). It appears, therefore, that H-M inhibit a portion of the voltage-dependent calcium channels in BCNs, an effect that may underlie the ability of H-M to inhibit afterdischarges and secretion.

310.8

OKADAIC ACID PROLONGS K-INDUCED INCREASES IN $[CA]_i$ IN LEECH RETZIUS CELLS. A.L. Kleinhaus, K.M. Lerea & R.J. Zeman. Dept.Cell Bio.& Anat., NYMC, Valhalla, NY 10595

The Retzius cell (R) of the leech is a multifunction neuron implicated in the control of essential behaviors. During excitation its membrane admits Ca through divalent cation channels. Apart from the existence of a Na/Ca exchange mechanism, there is no information regarding the processes involved in the control of $[Ca]_i$ in this neuron. Recent evidence suggests that protein phosphatases may influence Ca currents in other neurons. Therefore, we examined the effect of okadaic acid (OA), an inhibitor of type 1 and 2A protein phosphatases, on Ca transients induced by KCl depolarization in R cells. The average $[Ca]_i$ was calculated by ratioing of fura-2 fluorescence excited at 340 and 380 nm. Following exposure to 120 mM KCl (substituting for NaCl) $[Ca]_i$ rose from 64.7± 6.0 nM to 122.0 ± 13.9 nM. Recovery towards basal levels occurred rapidly after washout in normal (4 mM KCl) solution. In contrast, in the presence of OA (10^{-6}M) recovery was significantly slower. The time constant of recovery was 1.79 min. in the control as compared to 7.50 min. in the presence of OA ($P<0.02$). Assays of OA-sensitive phosphatases demonstrated the presence of approximately equal activities of PP1 and PP2A in lysates of leech ganglia.

These results suggest a role for PP1 and/or PP2A in the regulation of $[Ca]_i$ in leech neurons.

POTASSIUM CHANNELS: MOLECULAR BIOLOGY I

311.1

TEMPERATURE-SENSITIVE, LONG-LASTING PLATEAU POTENTIALS IN CULTURED NEURONS FROM THE *DROSOPHILA* MUTANT *SEIZURE*^{TS}. M.Saito and C.-F. Wu, Dept. of Biology, Univ.of Iowa, Iowa City, IA 52242

At non-permissive temperatures (>38°C), *seizure* flies undergo hyperactive behavior followed by paralysis. Previous toxin-binding studies show altered Kd and Bmax for STX binding in *sei*^{ts} head extracts, suggesting abnormal Na^+ channel properties (Jackson et al., 1984). However, neural activity in the adult cervical giant fiber path way was found to be elevated in *sei*^{ts} at 40°C (Elkins and Ganetzky, 1990). Recently voltage-clamp studies performed at room temperature detected no striking differences in cultured *sei*^{ts} neurons, except for a reduction in Na^+ current density in one of the alleles, *sei*^{ts1} (O'Dowd and Aldrich, 1988). However, intracellular recording of action potentials in *sei*^{ts} at different temperatures have not yet been examined. We have carried out such studies by using "giant" *Drosophila* neurons derived from cell-division arrested embryonic neuroblasts. These large neurons allow both current- and voltage-clamp recordings performed on the same cell (Wu et al,. 1990, Saito and Wu, 1991). Cells generating all-or-none action potentials were studied under current clamp. When temperature was raised to >35°C, action potentials in neurons from both wild-type and *sei*^{ts2} became sustained depolarization as long as current injection was maintained. However, this plateau potential in *sei*^{ts2} outlasted the depolarizing current pulse (800 ms) for several seconds. This response pattern could be seen in wild-type neurons when treated with the K^+ channel blocker, TEA. This temperature-induced phenotype could be explained by an enhancement in Na^+ current, a reduction in K^+ currents, or both. Further voltage-clamp analyses is underway to identify the current component(s) affected by the *sei* mutation.

311.2

EFFECTS OF FOUR POTASSIUM CURRENT MUTATIONS ON ONGOING ACTIVITY IN A *DROSOPHILA* NEURON. L. M. Hurley and J.Palka. Department of Zoology, University of Washington, Seattle, WA 98195.

A number of mutations affect potassium (K^+) currents in *Drosophila melanogaster*. Different mutations affect different currents, and the genes coding for them may also be expressed differentially in tissues. We examined the effects of four mutations affecting K^+ currents on ongoing neuronal activity associated with the posterior scutellar bristle, a mechanosensory structure located on the thorax of the fly.

A broken off glass capillary microelectrode filled with a receptor lymph-approximating high K^+ saline was used both to maintain electrical contact with the neuron and to deflect the bristle. The mutations investigated were two alleles of *Shaker* (*Sh*), a deficiency (*Df*$Sh^{B55\text{-}W32}$) and *Shaker* ^{KS133} (Sh^{KS133}), *ether-a-go-go* ($eag^{X\text{-}6}$), *slowpoke* (slo^1), and *Hyperkinetic* (Hk^1). Canton S (*C-S*) was used as the wild-type control.

Sh flies showed no significant differences in ongoing activity (spikes/sec.) from wild type. The average spike rate for *C-S* was 3.7±.541 spikes/sec. (n=19), for *Df*$Sh^{B55\text{-}W32}$ 0.98±.384 spikes/sec.(n=10), and for Sh^{KS133} 3.87±.353 spikes/sec. (n=14). $eag^{X\text{-}6}$, slo^1, and Hk^1 flies, however, all showed approximately four times as much ongoing activity as wild-type flies. The average spike rate for $eag^{X\text{-}6}$ was 15.4±1.067 spikes/sec (n=10), for slo^1 17.5±1.300 spikes/sec. (n=10), and for Hk^1 22.1±1.861 spikes/sec. (n=9). These results indicate that the currents affected by *eag*, *slo*, and *Hk* all play a role in determining the ongoing activity of this mechanosensory neuron, while those affected by *Sh* may not.

This research was supported by an NSF Graduate Fellowship (LMH) and by NSF Research Grant BNS-9011028 (JP).

311.3

Mutations in the S5-S6 Segment of K_V3 Affect Charybdotoxin Blockade and the Rate of Slow Inactivation. C. Oliva, R. Wiedmann, C. Bennett, K. Folander, R. Swanson, and J. Smith Merck Sharp and Dohme Research Labs, West Point PA 19486

Despite extensive sequence homology, *Shaker* H4 and a mammalian homologue, K_V3, display marked differences in their sensitivities to blockade by charybdotoxin (CTX). K_V3 is blocked with an IC_{50} of ~1 nM; *Shaker* H4 is relatively insensitive (IC_{50} ~300 nM). The putative pore-forming domain of these proteins (the S5-S6 region) differ by 10 of 37 amino acids and substitution of this entire region in K_V3 by the corresponding sequence from *Shaker* converts the channel to a toxin insensitive form (Oliva *et al.* 1991). A chimera containing changes at the 4 most distal residues that differ between the two channels (residues 401, and 404-406 in K_V3) displayed wt CTX sensitivity. Individual point mutations at the 6 remaining residues that differ have, therefore, been constructed in attempts to define the CTX binding site in more detail. The G377F mutant of K_V3 decreased the sensitivity of the channel to CTX by > 2 orders of magnitude. Interestingly, the corresponding mutation in H4, F425G, converts the *Shaker* channel from the wt (toxin-insensitive) form to a channel that is blocked with an IC_{50} = 1 nM. None of the other point mutants in this domain of K_V3 had any effects on CTX affinity.

When expressed in *Xenopus* oocytes, K_V3, activates rapidly and inactivates very slowly (τ_i= 751 +/- 40 ms). Some of the mutations in the pore forming domain had selective effects on the rate of slow inactivation. G377F eliminates slow inactivation, resulting in a noninactivating, toxin-insensitive current. D372G and D373S increase the rate of slow inactivation ~2X (τ_i= 440 +/- 44 ms) without changing CTX sensitivity. The chimeric channel (containing the H401T, T404G, I405V, and G406W mutations) also displays no inactivation.

311.4

MUTATIONAL ANALYSIS EXAMINING THE INTERNAL MOUTH OF THE *SHAKER* POTASSIUM CHANNEL. G.A. Lopez, E.Y. Isacoff*, Y.N. Jan & L.Y. Jan. Howard Hughes Medical Institute and Departments of Physiology and Biochemistry, Univ. of California, San Francisco, San Francisco, CA 94143

In order to identify regions of the protein which may contribute to the internal mouth and pore lining structure of the *Shaker* potassium channel, we have made multiple point mutations in several of the proposed transmembrane and intracellular regions of the protein. The approach taken in this study is to examine the potential function served by those residues that are highly conserved. Mutations were restricted to the fifth and sixth proposed transmembrane domains S5 and S6 since these domains are the most conserved among the various potassium channels cloned to date. Sequence comparison reveals that many of the residues in the S5 and S6 domain are 100% conserved in all cloned potassium channels. Mutated channels were expressed in *Xenopus* oocytes and analyzed using the two-electrode voltage clamp technique. Mutant channels were then further characterized at the single-channel level using inside-out patches in order to determine if the single-channel conductance and/or selectivity profile of the channel had been altered.

311.5

ION REPULSION IN CLONED VOLTAGE GATED K^+ CHANNELS C.F.Newland[*1], J.P.Adelman[*2], B.L.Tempel[3], E.W.McCleskey[4], W.Almers[1]. [1]Dept. Physiol & Biophys, and [3]Dept. Pharmacol & Medicine U. Washington, Seattle WA 98195. [2]Vollum Institute,Oregon Health Sciences U.,Portland OR 97201. [4]Dept of Cell Biol & Physiol, Washington Univ, St Louis MO 63110.

Both internal and external tetraethylammonium (TEA^+) block voltage-gated K^+ channels by acting at two functionally and molecularly distinct sites. We have tested two predictions of models explaining ion permeation, namely that ion channels can be simultaneously occupied by multiple ions, and that these ions repel each other. Currents were measured by patch-clamp in *Xenopus* oocytes injected with specific mRNA for RBK1, RBK2 and *Shaker* B. In *Shaker* B and RBK1, both TEA^+ sites lie within the aqueous pore because block by internal and external TEA^+ is voltage-dependent and antagonized by K^+ on the opposite side of the membrane. External TEA^+ antagonized block by internal TEA^+ and internal TEA^+ antagonized block by external TEA^+. For example, with 1.5 mM external TEA^+, 1.04 mM internal TEA^+ was required to block half the remaining current of RBK1, while without external TEA^+, 0.31 mM internal TEA^+ was sufficient. The antagonism was less than predicted for competition (*i.e.* K_d = 1.76mM for the above), hence both sites may be simultaneously occupied by TEA^+, and external and internal TEA^+ reduce each others affinity about tenfold. The strong antagonism suggests that the two sites are in close proximity. For RBK2, a clone 1000 times less sensitive to external TEA^+, 100mM external TEA^+ was ineffective in reducing block by internal TEA^+, hence TEA^+ must bind to antagonize block. External TEA^+ also antagonized block by another cationic blocker, virtually abolishing the inactivation induced by internal Ba^{2+}. This suggests an electrostatic contribution to the antagonism.

311.6

POTASSIUM CHANNELS CLONED FROM NEUROBLASTOMA CELLS DISPLAY SLOWLY INACTIVATING OUTWARD CURRENTS IN *XENOPUS* OOCYTES. Y. Ito, S. Yokoyama & H. Higashida. Dep. of Biophys., Neuroinformation Res. Ins., Kanazawa Univ. Sch. of Med., Kanazawa 920, Japan.

Oocytes injected with specific mRNA for NGK1 and NGK2 display outward K^+ currents (FEBS Lett. 259, 37, 1989). In previous reports in the literature it has not been possible to make direct physiological correlations between the currents expressed in oocytes and those from particular cell types. However, in this case NGK1 and NGK2 have been isolated from NG108-15 cells, enabling a direct comparison of the currents generated by the channels of these genes when expressed in oocytes with the native K^+ current in NG108-15 cells. The half activation potential of the NGK2 current (+29 mV) was more positive than that for the NGK1 (-8 mV). External tetraethylammonium chloride (TEA) was 1000 times more potent on the NGK2 current than the NGK1 current. The NGK2 currents showed faster inactivation during a 5 s depolarising pulse than did the NGK1 currents. These findings suggest that both NGK1 and NGK2 proteins form homomultimeric K^+ channels. Furthermore, NGK2 channels may be responsible for the native transient outward current with slow inactivation and underlying action potential repolarisation in NG108-15 hybrid cells, which was described by Robbins and Sim (Pflüger Archiv 416, 130,1990).

311.7

PEPTIDE TOXIN BLOCK OF K^+ CHANNELS EXPRESSED IN FIBROBLAST CELLS STABLY TRANSFECTED WITH THE NGK1 GENE: COMPARISON WITH CONVENTIONAL K^+ CHANNEL ANTAGONISTS. T.R. Werkman[1], T. Kawamura[*2], S. Yokoyama[*2], H. Higashida[2] and M.A. Rogawski[1]. [1]Neuronal Excitability Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892, and [2]Neuroinformation Research Institute, Kanazawa University, Japan.

Voltage-dependent K^+ currents with delayed rectifying properties were recorded in CL1023 fibroblast cells stably transfected with a K^+ channel gene (NGK1) from the NG108-15 neuroblastoma-glioma hybrid cell line (Kawamura *et al., Soc. Neurosci. Abst.* **16**: 670, 1990). NGK1 is identical to the rat brain K^+ channel protein BK2 and nearly identical to another rat brain K^+ channel protein RCK5. The blocking effects of three peptide toxins—charybdotoxin (CTX), dendrotoxin (DTX) and mast cell degranulating (MCD) peptide—were compared with the conventional K^+ channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA) using discontinuous whole-cell voltage clamp. All three toxins produced a concentration-dependent block of the K^+ current with IC_{50}'s in the nanomolar range (CTX, 2 nM; DTX, 3 nM; MCD peptide, 180 nM). The toxins were inactive when added to the patch electrode solution, indicating that they block from the outside of the channel. 4-AP was active either from the outside (IC_{50}, 0.3 mM) or the inside (1 mM), whereas TEA was nearly inactive when applied extracellularly but substantially reduced the current from the inside (5 mM). The blocking effect of 4-AP was strongly diminished at depolarized potentials, whereas the block induced by the toxins showed only a weak voltage dependence. We conclude that the peptide toxins are potent antagonists of the NGK1 delayed rectifier K^+ channel expressed in mammalian cells (as is the case in *Xenopus* oocytes; Stühmer *et al.*, EMBO J. **8**: 3244, 1989) and that they act by binding to an extracellular site.

311.8

LOCALIZATION IN RAT BRAIN OF mRNA FOR THE K_V4 POTASSIUM CHANNEL BY *IN SITU* HYBRIDIZATION HISTOCHEMISTRY. T.M. Perney, J. Marshall*, K.A. Martin, S. Hockfield and L.K. Kaczmarek. Dept.of Pharmacology and Sect. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Recently, it has been demonstrated that two different mammalian *Shaw*-type K^+ channels, K_V4 and NGK2, can arise by alternative splicing of transcripts from the same gene (Luneau et al., PNAS, in press). We have demonstrated that both splice variants are present in rat brain by RNAase protection assay, but that K_V4 mRNA was the more abundant message. To examine the distribution of these two mRNAs in rat brain, *in situ* hybridization histochemistry was performed using K_V4 or NGK2 specific oligonucleotide probes. No specific NGK2 hybridization was detected. In contrast, a heterogeneous expression of K_V4 mRNA was observed throughout the brain. Highest K_V4 mRNA levels were expressed in the cerebellum. High levels of hybridization were also detected in the globus pallidus, subthalamus, and substantia nigra reticulata. Many thalamic nuclei, but in particular the reticular thalamic nucleus, hybridized well to K_V4 specific probes. A subpopulation of cells in the cortex and hippocampus, which by their distribution and number may represent interneurons, were also found to contain high levels of K_V4 mRNA. In the brainstem, many nuclei including the inferior colliculus and the cochlear and vestibular nuclei also express K_V4 mRNA. Low or undetectable levels of K_V4 mRNA were found in the caudate-putamen, olfactory tubercle, amygdala, and hypothalamus. Interestingly, most of the neurons that hybridize with K_V4 specific probes have been characterized electrophysiologically to have narrow action potentials and display high frequency firing rates with little or no spike adaptation. It seems likely that the expression of the delayed rectifier-like K_V4 current in these neurons would allow for rapid repolarization following an action potential and thereby contribute to their firing patterns.

311.9

A COMPARISON OF MACROSCOPIC KINETIC PROPERTIES IN THREE CLONED AND EXPRESSED RAT BRAIN POTASSIUM CHANNELS. L.D. Chabala[1] & R.G. Sorensen[2], Depts. of Medicine[1,2] & Pharmacology[2], Jefferson Medical College, Phila., PA 19107.

We further characterized the macroscopic kinetic properties of three cloned rat brain potassium channels (Kv1, Kv2, & Kv3; Swanson et al., 1990, Neuron 4:929) with identical S4, S5, and S6 regions, nearly identical S4-S5 and H5 linker regions, but different S5-S6 loops. The expressed currents were studied with a two-electrode voltage clamp following injections of cRNA in Xenopus oocytes. [cRNA] was adjusted so that currents were usually less than 5 μA, and series resistance compensation was employed. The currents are quite distinct and differ both with respect to activation and inactivation properties. Currents induced by cRNA from Kv1 show rapid voltage-dependent activation (positive to 0 mV) followed by only modest (<10%) inactivation during a 1 s pulse (+50 mV); those from Kv2 have slower activation rates and show less inactivation; while those from Kv3 are somewhat intermediate with respect to activation but show much stronger inactivation, which is about 40% complete in 1 s at +50 mV (at 24°). Each clone also shows different conductance-voltage relations and prepulse-induced inactivation properties. Steady-state gating parameters were estimated from the prepulse-inactivation experiments using the assumption that the kinetic transitions after channel opening are voltage independent.

311.10

NEUROTOXIN SENSITIVITY IN THREE CLONED AND EXPRESSED RAT BRAIN POTASSIUM CHANNELS. R.G. Sorensen[1] & L.D. Chabala[2], Depts. of Medicine[1,2] & Pharmacology[1], Jefferson Medical College, Phila., PA 19107.

We studied neurotoxin sensitivity in three cloned rat brain potassium channels (Kv1, Kv2, & Kv3; Swanson et al., 1990, Neuron 4:929). We measured IC_{50} values of each clone expressed in Xenopus oocytes to five dendrotoxin (DaTX) homologues and to β-bungarotoxin (β-BuTX, the holotoxin) using a two-electrode voltage clamp. We find that both Kv2 and Kv3 clones are sensitive to some of the toxins. Kv3 clones show IC_{50} values of about 20 nM (at +40 mV) for α-DaTX and β-BuTX, and the inhibition is voltage dependent with tighter binding at positive potentials. Kv2 clones are also sensitive to those toxins, but the affinities seem to be somewhat reduced. This may be the first voltage-clamp evidence that some CNS potassium channels have receptor sites for phospholipase A_2 toxins. Other groups, however, have reported that Kv3 (cf. RCK3 or RGK5) channels are not sensitive to α-DaTX and that Kv2 (cf. RCK2) channels are not sensitive to β-BuTX. The reasons for these differences are not clear. One possibility is that the vitelline envelope represents a diffusion barrier to the bulky polypeptide neurotoxins, and it may be necessary to strip, tear, or digest it for quantitative studies. We are further characterizing the toxin binding site(s) by preparing chimeric channels and by using site-directed mutagenesis.

311.11

MOLECULAR CLONING OF TWO HUMAN BRAIN K^+ CHANNELS. F. Soler*, D. Aryee* and R. Joho, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

Voltage-gated K^+ channels are essential for a variety of electrical events in the mammalian brain. They terminate the action potential, repolarize the neuron, set its resting potential, and regulate neurotransmitter release from the presynaptic terminal. This versatility and importance may be the reason for the impressive diversity and number of K^+ channels in the brain. We have screened a human cortex cDNA library using a mixture of cDNA probes from different subfamilies of voltage-gated K^+ channels that have been cloned and characterized in our laboratory. We have assembled two full length clones of 3.8 kb and 2.8 kb. One encode the human homolog of *drk1* and the other a new human homolog of the *rck* family. The deduced amino acid sequence of the rat *drk1* and its human homolog is 95% identical with most of the amino acid substitutions occurring in the putative C-terminal tail. Expression of the isolated clones in the *Xenopus* oocytes system is now in progress.

311.12

A *SHAW* SUBFAMILY K^+ CHANNEL EXPRESSED IN HUMAN HEART. A. Butler*, D. P. McCobb, and L. Salkoff, Dept. of Anatomy and Neurobiology, and Dept. of Genetics, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Four voltage-gated potassium channel gene subfamilies defined by the *Drosophila* K^+ channel genes *Shaker, Shal, Shab,* and *Shaw* are conserved in mammals. In mammals, *Shaker, Shal* and *Shaw* are represented as multigene subfamilies, while *Shab* may be present only as a single gene. In the mammalian heart, recent reports indicate that at least four *Shaker* and one *Shal* subfamily genes are expressed (Roberds & Tamkun, 1991). We now report that a third subfamily, *Shaw*, is represented in the heart. We have isolated a cDNA (*HHShaw1*) from a human fetal heart cDNA library which has high sequence homology to Raw3, a *Shaw* subfamily member cloned from rat brain by Schroter et al., (1991). Unlike previously described *Shaw* subfamily members, Raw3 produces a transient (A-type) potassium current when expressed in *Xenopus* oocytes. (One *Shaker* subfamily gene and the *Shal* gene probably also produce transient currents.) We are expressing *HHShaw1* in oocytes to determine whether a similar transient current is produced. If so, there may be transient K^+ currents representing three potassium channel subfamilies expressed in heart. (Supported by NIH RO1NS24785-01 and a research grant from Monsanto-Searle.)

311.13

cDNA ANALYSIS OF A LEECH *SHAKER* K-CHANNEL HOMOLOG. K.M. Johansen, A. Wei†, L. Salkoff†, and J. Johansen, Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011 and †Department of Anatomy & Neurobiology, Washington University School of Medicine 63110.

We have cloned the *Shaker* K-channel homolog from leech with the polymerase chain reaction. Using degenerate oligonucleotides synthesized based on sequences conserved between *Drosophila* and mammals, we amplified and subcloned the core region encompassing transmembrane domains S1 through S5 from genomic DNA. We have used the genomic clone to screen a cDNA library and have isolated a cDNA clone extending from S1 through the poly A tail. We are currently primer extending and screening to isolate the 5' cDNA sequences. Analysis of both genomic and cDNA sequences reveal some very striking features. For example, while all the other characterized *Shaker* K-channels contain 7 positively charged amino acids in the S4 domain, the leech *Shaker* homolog contains only 5. Homology between leech *Shaker* and *Drosophila Shaker* within the currently analyzed domains is 58%, whereas homology with the other K-channel families *Shab, Shal,* and *Shaw* is only 28, 31, and 32%, respectively. Although several uniquely characteristic features of *Shaker* homologs are conserved within the leech sequence, the much higher degree of divergence (*Drosophila*-mouse *Shaker* homologs are 85% homologous in this region) will allow us to identify essential amino acids and motifs likely to be important for the structure-function relationship of the channel molecule. The structure-function of the leech *Shaker* K-channel will be further characterized by oocyte expression studies. In addition, by *in situ* hybridization and by using antibodies made to the cloned *Shaker* channel protein we plan to correlate and compare the expression, distribution, and subcellular localization of the *Shaker* K-channel among functionally identified neurons involved in known behavioral networks. Supported by an Iowa State Biotechnology grant and an Iowa State University grant.

311.14

HETEROLOGOUS EXPRESSION OF MODULATABLE CALCIUM-DEPENDENT POTASSIUM CHANNELS FROM RAT BRAIN. P.H. Reinhart, F. M. Schmalz* and I. B. Levitan, Dept. of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

We have shown previously that rat brain cortex plasma membrane vesicles contain at least four distinct calcium-dependent potassium channels that can be reconstituted in planar lipid bilayers (Reinhart *et al*, Neuron 2(1989)1031-1041). Two of these channels exhibit large single channel conductance (maxi-K channels). Both of these maxi-K channels can be modulated by cyclic AMP-dependent protein phosphorylation, and one is also modulated by a protein kinase activity closely associated with the reconstituted channel. We have now expressed potassium currents in *Xenopus* oocytes by injection of messenger RNA from the brains of adult and 21 day old rats. A portion of this current is blocked by BAPTA-AM and low concentrations of tetraethylammonium, indicating that it is a calcium-dependent potassium current. Size-selection of messenger RNA from 21 day old rat cortex demonstrates that most of the message coding for calcium-dependent potassium current is larger than 5.5 kilobases. Patch clamp analysis has demonstrated the presence of single maxi-K channels in the plasma membrane of oocytes injected with size-selected brain messenger RNA. The expression and cloning of members of the family of calcium-dependent potassium channels should allow the elucidation of structural features involved in channel modulation. Supported by NS17910 to I.B.L.

311.15

SYNTHETIC N-TERMINAL PEPTIDES OF DIFFERENT SHAKER K CHANNEL VARIANTS INDUCE INACTIVATION IN NONINACTIVATING MUTANT CHANNELS. R.D. Murrell-Lagnado and R.W. Aldrich. HHMI, Mol. and Cel. Physiology, Stanford University Sch. of Med., Stanford, CA 94305

Fast inactivation in *Shaker* potassium channels involves the interaction of the NH_2-terminal cytoplasmic domain with a region of the open channel. A "ball and chain" model for inactivation has been proposed for the ShB alternatively spliced variant whereby the first 20 residues form a structural domain that acts like a ball tethered to the rest of the channel protein which is able to bind within the channel pore thereby blocking ion flux. The *Shaker* variants C,D and H37 also inactivate even though their NH_2-termini show no sequence homology with the ShB NH_2-terminus. To determine whether the mechanism of fast inactivation is similar for all *Shaker* variants we applied synthetic peptides with the sequence of the amino terminal domains of ShB,C,D and H37, to the internal side of noninactivating mutant channels expressed in oocytes and in transiently transfected Cos7 cells. These peptides produced a time-dependent block of macroscopic currents in a concentration-dependent manner. The rate and steady-state level of block produced by the peptides were consistent with what one would predict from the time course of fast inactivation of the corresponding variants. To investigate the importance of structural features in the ball for inactivation point mutations were made in the ShB peptide. The two negatively charged residues at positions 12 and 13 were neutralised (ShB:E12QD13Q) and the positive charges at positions 16,17,18 and 19 were also neutralised. ShB:E12Q,D13Q had a higher binding affinity than the wild-type ShB peptide whereas neutralising the positive charges caused a decrease in binding affinity, suggesting that the inactivation receptor has a negatively charged region with which the positive charges interact electrostatically.

311.16

INDUCTION OF SUB-CONDUCTANCE STATES IN A RAT BRAIN CALCIUM-DEPENDENT POTASSIUM CHANNEL BY A "BALL" PEPTIDE DERIVED FROM THE *SHAKER* POTASSIUM CHANNEL . S. K. Chung*[1], W.N. Zagotta[2], R.W. Aldrich[2] and I.B. Levitan[1]. [1]Dept. of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, MA 02254 and [2]Dept. of Molecular and Cellular Physiology, School of Medicine, Stanford University, Stanford, CA 94305.

The amino terminal 20 amino acid residues of the *Shaker* potassium channel from *Drosophila* interact with the open channel to cause inactivation. Application of a synthetic peptide comprising these 20 amino acids, termed the *Shaker* "ball", to the intracellular side of non-inactivating mutant channels restores inactivation. We tested the actions of the ball on another potassium channel, a noninactivating large conductance calcium-dependent potassium channel reconstituted into lipid bilayers from rat brain plasma membrane vesicles. Two effects were observed. The ball causes blocks of channel activity lasting several hundred msec. In addition the ball induces the appearance of multiple sub-conductance states, lasting 10 msec - 10 sec. This latter effect is not mimicked by a single amino acid mutant ball that also fails to restore inactivation in *Shaker*. Thus the *Shaker* ball binds to some site on the calcium-dependent potassium channel that mediates the appearance of sub-conductance states. This site resembles the ball receptor in *Shaker* because it binds native but not mutant ball. Supported by NS17910 to I.B.L.

311.17

SHAKER INACTIVATING PEPTIDE ALTERS MAXI K_{Ca} SINGLE CHANNEL GATING. L. Toro*, E. Stefani & R. Latorre*[f]. Dept. Molec. Physiol. & Biophys, BCM, Houston, TX 77030, [f]Dept. Biol. Univ. Chile, & CECS Santiago 9, Chile.

In shaker A-type K channels the segment defined by the first 20 aminoacids of the amino terminus has been recognized to be an essential structural component of the inactivation gate (the "ball" in the ball-and chain model) (Hoshi et al., Science 250:533, 1990). Given the great similarities among the ion conduction domain of different K channels, it was interesting to ask if the "ball" receptor was present in the inner mouth of Ca^{2+}-activated K (K_{Ca}) channels as well. K_{Ca} channels from coronary smooth muscle incorporated into lipid bilayers were studied (270 pS in 250 KCl). Internally (10-100 μM) but not externally (80 μM) applied inactivating peptide dramatically altered the electrical behavior of K_{Ca} channels. The most salient features of the peptide effect were: *1)* Induction of long-lived closed events. Their mean time was dose-dependent (1.5 s at 40 μM and 3.5 s at 80 μM, 20 mV) as well as the mean burst time (12 s at 40 μM and 5 s at 80 μM, 20 mV). *2)* Reduction of the open probability during a burst (P_O) in a dose-dependent manner (P_O control = 0.9; P_O 40 μM peptide = 0.6; P_O 80 μM peptide = 0.3, at 20 mV). *3)* Stabilization of a number of conductance substates (up to eight). *4)* Internal TEA (30-60 mM) inhibited the peptide action in a competitive manner. These results suggest that the "ball" inactivating peptide is able to interact with the internal mouth of the maxi K_{Ca} channel in the same concentration range where it is able to functionally reconstitute the inactivation process in non-inactivating Shaker A-type channels. Authors thank Dr. R. Aldrich for the "ball" peptide. Supported by NIH, HD-25616 (E.S.), GM-35981 (R.L) and Grant-in-Aid 900963 AHA-National Center (L.T.)

POTASSIUM CHANNELS: PHYSIOLOGY AND REGULATION II

312.1

KINETICS AND VOLTAGE DEPENDENCE OF SODIUM-DEPENDENT POTASSIUM CHANNELS IN RAT OLFACTORY BULB NEURONS. T. M. Egan, J. Kupper* and I. B. Levitan. Dept. of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Among the multiple classes of potassium channels are those whose gating is regulated by intracellular ions, including calcium and sodium. We have recorded the activity of sodium-activated potassium channels (K_{Na}) from olfactory bulb neurons in culture, and in *in vitro* slices. In inside-out membrane patches exposed to symmetrical 150 mM [K^+], K_{Na} has a mean E_{rev} of 0 mV, a mean conductance of 172 pS, and a linear current-voltage relationship over the V_m range of -100 to 0 mV. At positive V_m, single channel currents show inward rectification, the degree of which is determined by $[Na^+]_i$. Channel gating is voltage-dependent, so that the channel is more likely to be in the open state at positive V_m. Because of this voltage-dependence, the K_{Na} channel may be more sensitive to $[Na^+]_i$ then has previously been suggested. The channel opens in bursts. Within a burst the channel alternates between the fully open state, multiple substates, and the fully closed state. Between bursts, the channel sometimes enters a very long-lived inactive state which can last for up to several minutes. These findings suggest that K_{Na} may be subject to modulation by intracellular factors yet to be identified. Supported by NS17910 to I.B.L.

312.2

A SODIUM-DEPENDENT POTASSIUM CURRENT IN SPINAL NEURONS OF THE *XENOPUS* EMBRYO. N. Dale. Department of Zoology, University of Bristol, Bristol, BS8 1UG, U.K.

The potassium currents of neurons acutely isolated from the spinal cord of the stage 37/38 *Xenopus* embryo have been studied using whole cell recordings. About half of the total outward current was blocked by substitution of extracellular Na^+ with N-methyl-glucamine or lysine chloride. Using Li^+ as a substitute for Na^+ caused a lesser reduction in the outward current. The Na^+-sensitive current activated in a voltage dependent manner and was sustained. Its reversal potential was close to E_k and depended on external K^+, suggesting that the current was carried by K^+. The amplitude of the Na^+-dependent K^+ current was progressively reduced as the neuron was depolarized to command potentials above E_{Na}. Activation of the Na^+-dependent K^+ current therefore requires the presence of internal Na^+. While the neurons do not possess a sustained voltage-dependent Na^+ current, they do have a steady state leakage current that is reduced by removal of external Na^+. Presumably lowering external Na^+ allows pumps to reduce the intracellular levels of Na^+ adjacent to the membrane and hence activation of the Na^+-sensitive current. The Na^+-dependent K^+ current possessed by *Xenopus* embryo spinal neurons appears to differ from those previously described in other vertebrate neurons which are transient, lack voltage sensitivity, and are not activated by Li^+.

312.3

CHARACTERIZATION OF A DELAYED RECTIFIER POTASSIUM CURRENT IN MUSCLE CELLS ISOLATED FROM THE FLATWORM SCHISTOSOMA MANSONI. T.A. Day*, R.A. Pax and J.L. Bennett*. Departments of Zoology and Pharmacology & Toxicology, Michigan State University, East Lansing, MI, 48824.

Whole-cell voltage clamp techniques have been applied to individual dispersed muscle fibers of the parasite Schistosoma mansoni (Phylum Platyhelminthes). The predomininant current activated upon depolarization of these fibers is a large outward K^+ flux. The current activates when the voltage is pulsed from a resting potential of -40 mV to > -15 mV, displays very slow inactivation, and is blocked by high extracellular concentrations of Ba^{2+}. In outside-out patches of membrane pulled from these muscles, the unitary conductance of the channels is 20-30 pS. On the basis of these characteristics this current is similar to delayed rectifier currents which are widespread in the animal kingdom. With the delayed rectifier current suppressed by extracellular Ba^{2+}, other voltage-gated currents also appear to be present.

312.4

GALANIN- AND BETHANECHOL-INDUCED POTASSIUM CURRENTS IN MUDPUPPY PARASYMPATHETIC NEURONS. L.A. Merriam and R.L. Parsons. Dept. of Anat. & Neuro., Univ. of Vermont Coll. Med., Burlington, VT 05405.

Galanin and bethanechol hyperpolarize parasympathetic postganglionic neurons in the mudpuppy cardiac ganglion by activating a membrane potassium conductance. In the present study, whole cell voltage clamp recordings of galanin- and bethanechol-induced currents have been compared in dissociated mudpuppy cardiac neurons maintained in a solution containing 12.5mM external potassium. In cells voltage clamped to -60mV, brief galanin applications (0.5 to 1 sec) initiated an inward current which lasted for tens to hundreds of seconds. In contrast, similar duration applications of bethanechol initiated inward currents which were shorter with the duration determined by the duration of the agonist application. The time- and voltage-dependence of agonist-induced inward currents were determined using 300 msec hyperpolarizing voltage steps from a V_H of -40mV. The shape of the I-V relationship was similar for the currents activated by either agonist. The reversal potential for the currents activated by either agonist was approx. -44mV. The inward currents activated by either agonist were time dependent with the time course generally fit as the sum of two exponentials. In summary, both galanin and bethanechol activate a time-dependent potassium current which is kinetically different from the background membrane currents recorded during hyperpolarizing voltage clamp steps in the absence of agonist. Supported by NIH Grants NS-23978 and NS-25973.

312.5

K^+ CHANNEL OPENERS INHIBIT VOLTAGE-ACTIVATED OUTWARD CURRENTS IN DORSAL ROOT GANGLION CELLS. D.G. Owen. Wyeth Research (UK) Ltd, Huntercombe Lane South, Taplow, BERKS, SL6 0PH, UK.

In addition to their established activity in a variety of peripheral tissues, K^+ channel openers (KCO), such as cromakalim, activate ATP-sensitive K^+ channels and reportedly can also enhance voltage-activated K^+ currents in central neurones (*eg* hippocampal cells). Accordingly, I have examined the effects of a number of KCOs, including the novel KCO, WAY-120491, on voltage-activated K^+ currents in rat sensory neurones.

Dorsal root ganglion cells were obtained from 1-3 day old rat pups and cultured according to established methods. Whole cell patch clamp recordings were made after 3-8 days in culture using a switching voltage clamp. In most cases cells were 'replated' 1-3 hours prior to use to reduce the extent of process outgrowth and thus facilitate adequate voltage clamping. Patch electrodes of 5-8MΩ were filled with (mM): 140 KGluconate, $2MgCl_2$, 5HEPES, 1.1EGTA/KOH, sucrose to 310mOsm, pH 7.2 and cells were bathed in (mM): 124NaCl, 2.5KCl, $4MgCl_2$, 5HEPES, 10glucose, 1μM TTX, sucrose to 320mOsm, pH 7.4. KCOs, excepting somatostatin (SS), were dissolved in DMSO or EtOH (final concentration ≤ 0.1%) and applied via microperfusion system to individual cells. WAY-120491 (10μM) blocked about 20% of the total outward current activated by voltage steps from -100mV to +60mV. Proportionally more block was evident at the end of depolarizing voltage steps (1s) than at the start, with a marked acceleration of the decay. WAY-124903, a structural analogue, blocked total outward current to a similar degree (*ca* 20% at 10μM), showing some selectivity for non-inactivating current (30% block) over transient outward current (15% block). Cromakalim (10μM) blocked relatively less outward current but, like WAY-120491, accelerated the decay with little effect on peak current . Inhibition of non-inactivating current from -30mV showed no such time-dependence, suggesting an open-channel type of block. SS which activates K_{ATP} channels in peripheral tissue also reduced outward current but only after relatively prolonged application (ca 4 min). Non-inactivating current was preferentially blocked.

312.6

ACTION POTENTIALS ACTIVATE AN INTERNODAL POTASSIUM CONDUCTANCE IN LIZARD MYELINATED AXONS. G.David*, J.N. Barrett and E.F. Barrett. Dept. of Physiology & Biophysics, Univ. of Miami Med. Sch., P.O Box 016430, Miami, Fla. 33101.

Action potentials and afterpotentials in motor axons innervating the ceratomandibularis muscle of lizards (Anolis sagrei) were recorded with microelectrodes inserted into axons (intra-axonal) and/or under the myelin sheath (peri-internodal). The contribution of internodal K currents to potentials across the internodal axolemma was studied in normal bath $[K]_o$ and in high peri-internodal $[K]_o$ achieved by ionophoresis from peri-internodal microelectrodes.

Peri-internodal recordings exhibited a negligible (<2 mV) resting potential but showed action potentials with peak amplitudes of up to 78 mV. High peri-internodal [K] (favoring K influx into the axon) resulted in: (1) a prolonged (hundreds of msec) negative afterpotential (PNP) recorded peri-internodally from the K-injected internode, (2) a simultaneous intra-axonal positive afterpotential accompanied by increased conductance of the internodal axolemma and (3) no significant changes in the axonal resting potential. PNPs could also be evoked by depolarizing the internodal axolemma in the presence of tetrodotoxin (10 uM). PNPs were blocked by tetraethylammonium (TEA, 10 mM), but not by 3,4-diaminopyridine (DAP). In normal peri-internodal [K] (favoring K efflux from the axon), a brief positive afterpotential, sensitive to TEA but not to DAP, was recorded in the peri-internodal region. These results indicate that depolarization of the internodal axolemma by an action potential activates an internodal K conductance. The activation of this conductance limits the peak amplitude of the passive depolarizing afterpotential, and thereby helps to prevent progressive depolarization of the axon during high frequency activity.

312.7

A PARADOX CONCERNING BLOCKADE OF THE DELAYED RECTIFIER CHANNEL (I_K) BY CESIUM IONS. J.R. Clay and V. Kowtha. NIH, Bethesda, MD 20892

A comparison of the effects of Cs_i^+ with the effects of Cs_e^+ on I_K in squid axons reveals a paradox. Cs_e^+ blocks inward current in a voltage-dependent manner resulting in an N-shaped current-voltage (IV) curve. Blockade is complete at ~ -150 mV with 100 mM Cs_e^+. A slight secondary increase in inward current occurs for V < -220 mV. The concentration dependence of Cs_e^+ block is consistent with a 2:1 stoichiometry, especially for V < -100 mV (Adelman and French, 1978: J Physiol **276**:13). Blockade of outward current by Cs_i^+ is also voltage dependent (N-shaped IV). However, the block is not complete at any potential, even with 300 mM Cs_i^+. A marked secondary increase in outward current occurs for V > 150 mV, which is blocked by TEA. Blockade of I_K by Cs_i^+ is consistent with a 1:1 stoichiometry. The electrical distance, s, of block with Cs_e^+ is ~ 0.9, whereas s ~ 0.5 for Cs_i^+. That is, Cs^+ can more readily pass through the channel from the inside than from the outside; 2 ions enter the channel from the outside, only 1 from the inside; and the blocker moves a considerable way through the electric field from either side. These results constitute a paradox.

312.8

NON-NEURONAL CELLS INFLUENCE THE EXPRESSION OF K CURRENTS ON CULTURED SCG NEURONS. S.McFarlane, E.Cooper. Dept. of Physiol., McGill Univ., Montréal, Qué. H3G 1Y6.

We have been investigating voltage-gated K currents on rat neonatal superior cervical ganglion(SCG) neurons to learn more about factors that influence K current expression. SCG neurons express 3 voltage-gated K currents: a non-inactivating current(IK); a rapidly inactivating A-current(IAf); and a slowly inactivating A-current(IAs). Using whole cell recording we have shown that the mean current densities change during post-natal development. On neurons(n=45) from day one animals(P1) IAf=27pA/pF, IAs=67pA/pF and IK=15pA/pF, whereas on P14 neurons (n=48) IAf increases to 64pA/pF, IAs decreases to 34pA/pF, and IK stays constant. In contrast, when P1 neurons develop in culture without other cell types, the mean current density of IK increases, while the densities of IAf and IAs decrease: by day 28, cultured neurons express IAs at low levels (9pA/pF, n=20), IK at higher levels (60pA/pF, n=20) and express no detectable IAf. These results suggest that some factor(s) required for the maintenance of A-current expression on SCG neurons is missing in culture. To test whether this factor is provided by the ganglionic non-neuronal cells, we grew SCG neurons for 4 weeks in the presence of ganglionic non-neuronal cells. In these conditions, SCG neurons express IAf(25.1pA/pF, n=40) and IAs(39.7pA/pF, n=41), and show no increase in IK (18.7pA/pF, n=41). These results show that a factor(s) provided by non-neuronal ganglionic cells is able to influence the expression of K currents. The nature of this factor is presently being investigated. (supported by MRC of Canada)

312.9

THE SLOWLY ACTIVATING, VOLTAGE-DEPENDENT POTASSIUM CURRENT (I_{sK}) EXPRESSED IN XENOPUS OOCYTES IS REGULATED BY OSMOTIC PRESSURE A. E. Busch, J. P. Adelman and R. A. North Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201

The potassium channel I_{sK} has been cloned from rat kidney, where it is expressed in the apical membrane of proximal tubule cells (Takumi et al., Science 242: 1042, 1988; Sugimoto et al., J. Membr. Biol. 113: 39, 1990). Kidney cells have been shown to regulate their volume in hypotonic shock by activation of potassium conductances (Ubl et al., Pfluegers Arch. 104: 223, 1988): therefore we studied the effects of changes in the osmolarity of the superfusion solution on I_{sK} expressed in Xenopus oocytes. Currents were evoked by depolarizing the oocyte from a holding potential of -80 mV, and measured by two-electrode voltage-clamp. A decrease in osmolarity from 220 mOsm to 160 mOsm caused an increase in I_{sK}: the current activated more rapidly and the threshold for current activation was shifted in a hyperpolarizing direction (to about -80 mV). The effects of hypotonic solution were mimicked by superfusion with the calcium ionophore A23187 (1 μM). Injection of BAPTA (2.5 nmoles/oocyte) reduced I_{sK} and prevented the effect of hypotonic solutions. The results suggest that hypotonic shock leads to an increase of an intracellular calcium and that this in turn increases I_{sK}.

312.10

THE EFFECTS OF PROTEIN MODIFYING AGENTS ON THE TRANSIENT OUTWARD K^+ CURRENT ($I_K(f)$) IN RAT PITUITARY MELANOTROPHS. J. Davidson*, T. Warren* and S.J. Kehl. Dept. of Physiology, University of British Columbia, Vancouver, B.C., V6T 1Z3

The $I_K(f)$ of melanotrophs is a voltage-gated K current which activates and inactivates rapidly. Since protein modifying agents have been shown selectively to remove inactivation both from a similar current in clonal pituitary cells and from Na channels in the squid axon, we examined the effects on $I_K(f)$ of internal or external applications of N-bromoacetamide (NBA), N-bromosuccinimide (NBS), chloramine-T, trypsin, papain and pronase. Whole-cell currents were recorded using conventional patch-clamp techniques. Internal trypsin (2 mg/ml), pronase (1 mg/ml), NBA (100 μM) or NBS (100 μM) did not alter $I_K(f)$. Internal chloramine-T (3.55 mM) or papain (1.25 mg/ml) caused a time-dependent decline of the peak amplitude of $I_K(f)$ but did not affect inactivation. External NBA (100 μM) rapidly and irreversibly reduced the amplitude of $I_K(f)$ but did not change its kinetics.

Protein modifying agents have no effect on or eliminate the $I_K(f)$ in melanotrophs.

312.11

QX-222 BLOCKS A FAST TRANSIENT POTASSIUM CURRENT IN NEOCORTICAL NEURONS. M. Andreasen* and J.J. Hablitz. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL. 35294.

The quaternary derivatives of lidocaine, such as QX-222, block voltage-dependent sodium currents thereby inhibiting the generation of action potentials. Studies have indicated that these compounds may also reduce potassium currents. Since the fast transient potassium current, known as I_A, has many properties similar to the fast voltage-dependent sodium current, we examined the effect of QX-222 on I_A.

The experiments were performed on neocortical neurons maintained in dissociated cell culture for 5-10 days. Whole-cell patch clamp techniques were used for recording. I_A was pharmacologically isolated by using an extracellular saline containing 1 μM TTX, 20 mM TEA and 200 μM $CdCl_2$. QX-222 (.1-2 mM) was added to the K-gluconate containing intracellular saline.

QX-222 reduced I_A in a dose-dependent manner; 2 mM QX-222 completely blocked I_A while 0.5 mM reduced it by 25-30 %. Depending on the concentration of QX-222 used, a steady-state reduction was obtained within 1 to 2 minutes after the membrane was broken. Concentrations of QX-222 that only partially blocked I_A had no apparent effect on steady-state activation or inactivation kinetics. These results indicate that QX-222 has a direct blocking effect on the channels generating I_A.(NS-18145)

312.12

TWO TRANSIENT VOLTAGE ACTIVATED POTASSIUM CURRENTS IN CULTURED RAT HIPPOCAMPAL CELLS. E. Ficker*, H. J. Luhmann, U. Heinemann. Department of Neurophysiology, Univ. of Cologne, 5000 Cologne 41, FRG.

Slowly inactivating K^+ currents have been proposed to determine the firing properties of neurons in the time scale of seconds (Storm, Nature 336, 379-381, 1988). We used the whole cell version of the patch clamp technique to extend our understanding of the A-current and to study in more detail a slowly inactivating potassium current in cultured rat hippocampal cells.

The two components are distinguished on the basis of voltage sensitivity and kinetics. The fast transient current decayed monoexponentially with a time constant of about 10 ms. The slow transient current decayed with two time constants in the order of 500 ms and of 3.4 ms. The reversal potential of the slow component shifted by 54 mV for a tenfold change in extracellular potassium concentration. The inactivation curve showed half maximal inactivation at -61 mV. The threshold for activation was determined between -40 and -30 mV. For the fast transient current half maximal inactivation occured at -81 mV , half maximal activation was reached at -19 mV. Studies on the removal of inactivation for the two currents revealed a time constant of 29 ms and of 107 ms for the fast and slow current.

Both currents were sensitive to 4AP. Most prominent was the ability of 4AP to speed up the inactivation process of both currents. TEA did not affect the fast transient current but reduced the slow transient in a dose dependent manner. In case of the slow transient also an acceleration in the time course of current decay by TEA could be observed. To our surprise, dendrotoxin applied in concentrations up to 600 nM was completely ineffective on both transient currents.

312.13

PROPERTIES OF THE TRANSIENT OUTWARD POTASSIUM CURRENT IN GH_3 CELLS. B. Robertson Electrophysiology Lab, Wyeth Research, Taplow, Maidenhead SL6 0PH , U.K .

Transient outward currents (TOCs) are found in a wide variety of cell types and it is envisaged that TOCs may be an important therapeutic target, since blockers of this potassium current are proving useful in the treatment of cardiovascular and perhaps also CNS disorders. The TOC in the GH_3 cell line provides a convenient model for physiological and pharmacological study (Oxford & Wagoner (1989) *J. Physiol.* **410**, 587).

TOCs were recorded using whole-cell voltage clamp techniques after precluding contaminating ionic conductances with blockers or by ion substitution. Experiments were performed at room temperature and drugs applied via a U tube rapid delivery system. TOC activates at potentials positive to -40mV and double-pulse protocol experiments show that steady-state half inactivation occurs at -40mV. The current activates more rapidly with increasing depolarization, and is best described by third power kinetics. Activation time constants are 3ms at -20mV, decreasing to 0.8ms at +100mV. The TOC typically inactivates with two exponentials, with mean τ's of 35ms (± 1.7, s.e.m.) and 164ms (± 8.3, s.e.m.) at +60mV. Deactivation of the current followed a single exponential. The TOC in GH_3 cells is similar to that seen in freshly islolated pituitary terminals (Thorn et al. *J.Physiol.* **432** 313)

TOC decay is slowed by extracellular application of N-bromoacetamide (100μM) and by including trypsin or papain in the internal solution. The current is blocked by 4-aminopyridine (1-5mM), but is unaffected by: toxin I (100nM), adenosine (1mM), pentobarbitone (100μM), baclofen (500μM), decanol (50μM) or ruthenium red (100μM). However, the TOC is inhibited by two compounds designed to treat cardiovascular disorders, albeit at very high concentrations. The antiarrythmic tedisamil (50-200μM) reduces peak current only a little, but markedly accelerates TOC decay. Sparteine (5mM), a natural precursor of tedisamil, also inhibits. The novel antihypertensive WAY 120491 (10-100μM) reduces TOC, principally by accelerating decay. The related compound cromakalim has no effect on TOC at 200μM.

312.14

EFFECT OF QUININE ON 'BASELINE CURRENT' IN BOVINE CHROMAFFIN CELL MEMBRANES. M.I. Glavinovic and J.M. Trifaro. Department of Anaesthesia Research and Physiology McGill University, Montreal, P.Q., Canada H3G 1Y6 and Department of Pharmacology, University of Ottawa, Ottawa, Ont., Canada K1H 8M5.

Cellular membranes are known to contain ion pumps - the transport systems that extrude or accumulate ions against an electro-chemical gradient. The molecular mechanism of active ion transport appears to involve a sequence of elementary steps (binding, dissociation and translocation) which are stochastic in nature. The baseline current in patch-clamp experiments, in addition to a contribution from the seal, can have a large contribution, blocked by quinine, which was suggested to be due to transport system (Christensen & Zeuthen, J Physiol., 387, 34P, 1987). This is an attempt to determine whether this current is due to a transport mechanism or due to a very small channel.

Both mean values and the variances of 'baseline current' are reduced after addition of quinine (0.5-1.5 mM) to the solution bathing the internal membrane. Power spectra of quinine-inhibited currents are white at low and high frequencies with transition intervals that typically occur at ≈ .5-1 kHz, as is expected for a transport mechanism or a pump (Lauger, Eur. Biophys. J., 11, 117-128, 1984) but not for a channel. Supported by MRC (Canada).

312.15

OUTWARD CURRENTS IN HEART MOTOR NEURONS OF THE MEDICINAL LEECH. C.A. Opdyke & R.L. Calabrese
Dept. of Biology, Emory University, Atlanta GA 30322

Heart motor neurons exist as bilateral pairs in ganglia 3 through 18 of the nerve cord of the medicinal leech. These motor neurons which innervate the hearts directly are rhythmically inhibited by heart interneurons which comprise the motor pattern generator for the heartbeat.

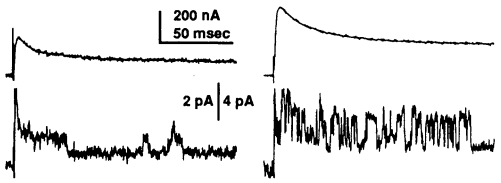
Heart motor neurons were voltage clamped using switching single electrode techniques to examine outward currents. We have found evidence for three distinct outward currents in the heart motor neurons: a fast transient current (I_A), a slowly inactivating current (I_K), and a calcium dependent outward current ($I_{K(Ca)}$). I_A and I_K were separated by their voltage sensitivity in external saline containing 0mM Na^+ and 0mM Ca^{2+} and 1.8mM Co^{2+}, and are in the process of being kinetically characterized. Depolarizing voltage steps from a holding potential of -80mV elicited both I_A and I_K currents whereas depolarizing voltage steps from -40 mV activates I_K only. I_A is activated at potentials positive to -45mV, I_K at potentials above -30mV. Steady state inactivation of I_A varies with holding potential and is nearly complete at -40mV. When the external saline contained 1.8mM Ca^{2+} and no Co^{2+} depolarizing voltage steps revealed a large increase in outward current that is presumably contributed by a calcium sensitive K^+ current. The calcium sensitive K^+ current is almost completely blocked by 25mM external TEA^+.

312.16

SIMULTANEOUS MACROSCOPIC AND SINGLE-CHANNEL CURRENTS IN LOBSTER STOMATOGASTRIC NEURONS.
B.R. Jones, M.C. Bieda* and D.K.Hartline, Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, Hawaii 96822

We used simultaneous two-microelectrode voltage clamp and cell-attached patch clamp to study the ion channels underlying macroscopic currents in intact, enzyme-cleaned neurons from the lobster, *Homarus americanus* (21°C). Intact ganglia were used for all experiments. Somata impaled with 3 M KCl-filled microelectrodes (3-15 MΩ) exhibited normal resting potentials (*ca.* -50 mV), action potentials, and macroscopic currents after enzyme treatment. Patch pipettes (2-10 MΩ) were filled with *Homarus* saline (seal resistance 10-40 GΩ).

Two types of channels were commonly observed. A low conductance channel (~7 pS), having a roughly linear I(V) relation, exhibited voltage-dependence characteristic of the fast, transient K+ current (I_A, left fig.). A larger channel (~30 pS), with an outwardly rectifying I(V) relation, showed a current reversal at -65 mV. In some cells this channel exhibited frequent spontaneous openings at rest (-50 mV). Preliminary evidence suggests that this channel may be associated with the transient, Ca++-dependent outward current that is activated by depolarization in these neurons (I_J, right fig.). (Figs: V_{hold}= -50 mV, V_{test}= 0 mV, I_A = difference current with and without V_{cond} = -100 mV). Supported by NSF BNS-8920698.



312.17

MODULATION OF CRAYFISH MOTONEURON EXCITABILITY AFTER A SINGLE ACTION POTENTIAL. M. Roux-Bruxelle, G. Czternasty and J. Bruner*. Lab. Neurobiol. Cell., Faculté des Sciences, 80039-AMIENS CEDEX, Lab. Neurobiol. Cell. Moléc., C.N.R.S., 91198-GIF/YVETTE, CEDEX, FRANCE

In the abdominal motor giant soma of the crayfish *Procambarus clarkii* a single Ca^{2+}-dependent action potential (AP) induced, for about 10 min, marked shortening of a subsequent AP. The present study aimed to determine the origin of such a long-lasting effect. Two microelectrode current or voltage clamp experiments were performed in modified crayfish saline containing 60 mM TEACl (substituted for NaCl), 1 mM 4-AP and 0.1 µM TTX. After a single AP the input resistance of the cell decreased from 0.6-0.8 MΩ to 0.1-0.2 MΩ and a hyperpolarization developed (about 10 mV, at a resting potential ≈ -72 mV). These effects became maximal 30 s to 1 min after an AP and recovered in 10-15 min. Similar changes were obtained after the membrane was clamped for 200 ms at 0 mV. Their magnitude and duration depended upon $[Ca^{2+}]_O$. Calcium current block by Cd^{2+} (1 mM) or replacement of Ca^{2+} by Ba^{2+} suppressed both effects. In voltage clamp experiments similar voltage pulses produced an outward current which lasted 10-15 min and reversed at about -80 mV, suggesting K^+ as a carrier. Such a long-lasting Ca^{2+}-dependent outward current would be responsible for the observed decreased excitability.

ACETYLCHOLINE: RELEASE

313.1

A Novel Technique For the Determination of Acetylcholine Release by Microdialysis in Electrically Stimulated Rat Striatum. U. Kischka*, S.A. Farber, J.K. Blusztajn[1] & R. J. Wurtman, Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139, [1] Dept. of Pathology, Boston Univ. School of Medicine Boston, MA 02118.

We have established a method for assessing the electrically-stimulated release of acetylcholine (ACh) from anesthetized rat striatal neurons in vivo. A teflon-coated tungsten wire (50 um) is attached to a standard microdialysis probe. This hybrid probe is used to stimulate electrically the neurons in the direct vicinity of the dialysis membrane. Maximal release of ACh during a 10 minute stimulation period (a dialysis flow rate of 2.0 ul/min) was found to be 600% (n=6, p<.01) greater than basal levels. Maximal stimulation (200 uA; 20-60 Hz; 0.5 msec duration) was often followed by a prolonged increase in basal ACh release, as well as an inhibition of subsequent, electrically-evoked release. By characterizing the electrical responses of these neurons we identified stimulation parameters (150 uA; 20 Hz; 0.2 msec duration) which induced a 158% increase (n=5, p<.01) in ACh release while minimally effecting basal levels and subsequent responsiveness to further stimulation . At pulse durations less than 0.1 msec we were unable to evoke release reliably, even using higher currents and stimulation frequencies. Supported by NIMH grant MH-28783.

313.2

CHOLINE ENHANCES SCOPOLAMINE-INDUCED RELEASE OF ACETYLCHOLINE IN THE DORSAL HIPPOCAMPUS OF CONSCIOUS FREELY MOVING RATS. D.A. Jackson, U. Kischka* and R.J. Wurtman. Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139

Peripheral administration of choline has been shown to elevate hippocampal tissue levels of choline and acetylcholine in the rat (Cohen and Wurtman,1976). In addition, recent findings have suggested that increasing available free choline may enhance brain acetylcholine release (Wecker,1989). In this study we used in vivo microdialysis to investigate whether peripheral administration of choline can enhance basal levels or evoked release of acetylcholine in the dorsal hippocampus of awake freely moving rats. Microdialysis probes were chronically implanted in the dorsal hippocampus and perfused with artificial CSF containing 10 µM of neostigmine. An intraperitoneal catheter was also chronically implanted for administration of choline and other drugs. The animals were allowed to recover for twenty-four hours prior to experimentation. Within 15 minutes of choline administration, choline dialysate levels were significantly elevated. However, there was no effect on basal acetylcholine levels. Administration of the muscarinic antagonist scopolamine (0.5 mg/kg,ip.) 60 minutes after vehicle injection caused a 5-8 fold elevation in acetylcholine release at thirty minutes post-injection. In animals pretreated with choline, the effect of scopolamine on acetylcholine release was further enhanced by 3-12 fold at thirty minutes post-injection. These results suggest that increasing free choline availabilty can enhance scopolamine-induced acetylcholine release in the dorsal hippocampus of conscious freely moving rats.
(Supported by NIMH grant MH-28783)

313.3

MUSCARINIC M2 NEGATIVE AUTORECEPTORS REGULATE ACETYLCHOLINE RELEASE IN CORTEX AND HIPPOCAMPUS: A MICRODIALYSIS STUDY IN FREELY MOVING RATS. J.W. Richard, A. Wilson, R. Quirion, Douglas Hospital Research Centre and Department of Psychiatry, McGill University, 6875 Blvd LaSalle,Verdun Quebec, Canada, H4H 9R3.

We have previously reported that atropine markedly stimulated the in vivo release of acetylcholine (Ach) in the rat cortex. In order to further investigate the pharmacological nature of the muscarinic autoreceptors involved, we studied the effects of M1 and M2 receptor antagonists on cortical and hippocampal Ach release using in vivo dialysis in behaving animals. 2-3 days after stereotaxic implantation of dialysis probes,male Sprague Dawley rats were dialyzed using Ungerstedt ringer buffer containing physostigmine and various muscarinic blockers. Ach levels were determined by GC/MS (PCI). In both cases, putatively selective M2 receptor antagonists such as AF-DX 116, AF-DX 384 and AQ-RA 741 markedly stimulated, in a dose dependent manner, Ach release. At 40 μM, AF-DX 384 and AQ-RA 741 were especially potent, increasing cortical release by 7 and 7.5 fold over basal level. Pirenzepine, a purported M1 antagonist, was less potent at equimolar concentrations. Interestingly, the combination with nicotine (5 mg/kg s.c.) further increased Ach release induced by M2 blockers (10 times over baseline). Thus, negative muscarinic autoreceptors are most likely of the M2 sub-type in rat cortex and hippocampus. Moreover, the simultaneous blockade of these receptors and the activation of nicotinic sites synergically act to greatly increase Ach release. Supported by the MRCC.

313.4

CONTROL OF THE BASAL FOREBRAIN CHOLINERGIC SYSTEM: A MICRODIALYSIS STUDY. Gary L. Wenk, Cheryl A. Harrington, and Paul E. Gold. Div. of Neural Systems, Memory & Aging, Univ. of AZ, Tucson, AZ 85724, and Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22903.

The basal forebrain cholinergic system (BFCS), includes the medial septal area and nucleus basalis magnocellularis, which project to the hippocampus and cortex, respectively. Drugs that alter the function of the BFCS also alter performance in tasks that require learning and memory. We investigated the neurochemical effects of pharmacological manipulation of BFCS activity. Various drugs and putative neurotransmitters were administered to rats. Extracelluar acetylcholine (ACH) in hippocampus and cortex was collected by microdialysis. ACH was analyzed by HPLC and electrochemistry. Among those drugs examined, scopolamine and glucose increased ACH release, enkephalin and barbiturates decreased ACH release and neurotensin had no significant effect. The pattern of results fits other, less direct, findings regarding control of ACH release by drugs and neuromodulators. Supported by the NSF (BNS 89-14941 & 90-12239).

313.5

SYSTEMIC CYSTEAMINE ADMINISTRATION INCREASES ^{3}H-ACETYLCHOLINE RELEASE IN RAT STRIATUM. M.A. Musgrave*, R.J. Boegman, J.V. Milligan. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Cysteamine (2-mercaptoethylamine) a natural product, constitutes part of the pantothanic acid moiety of Acetyl CoA. Studies utilizing radioimmunoassay and immunohistochemical techniques have shown that systemic administration of cysteamine selectively depletes somatostatin-like immunoreactivity in several brain regions including the striatum and cortex. The consequence of this loss in terms of neurotransmitter release has not been investigated. The purpose of this study was to determine the effect of cysteamine administration on ^{3}H-Acetylcholine (^{3}H-ACh) release stimulated by 25mM potassium (K^+). Rats received a subcutaneous injection of cysteamine (480 or 806 or 1050 mg/kg) four hours prior to sacrifice. In the cortex, no significant difference in K^+ stimulated ^{3}H-ACh release was observed between control and cysteamine treated rats at any dosage. However, in the striatum the highest dose of cysteamine resulted in a significant increase in K^+ stimulated ^{3}H-ACh release of 40% above control levels. Immunohistochemistry was used to verify cysteamine induced changes in somatostatin-like reactivity. The results of the study suggest a differential neuromodulatory effect of cysteamine in cortical versus striatal cholinergic neurons. (Supported by the Medical Research Council of Canada).

313.6

EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS ON CAUDATE ACETYLCHOLINE: BEHAVIORAL CORRELATES FOLLOWING AMPHETAMINE. S.M. Florin, D.S. Segal, R.Kuczenski. Dept. of Psychiatry, Univ. of California, San Diego, La Jolla, CA 92093-0603.

In vivo dialysis in rats was used to study the effects of systemically administered dopamine (DA) agonists and antagonists on behavior and extracellular acetylcholine (ACh) concentration in the caudate. Measurable ACh was obtained by intracaudate infusion of buffer (147mM NaCl,1.2mM $CaCl_2$,0.9mM $MgCl_2$,4.0mM KCl) containing 0.25 uM neostigmine bromide. Apomorphine (1.0 mg/kg) decreased, while haloperidol (1.0 mg/kg) increased ACh concentrations. A behaviorally transitional dose (1.75 mg/kg) of amphetamine (AMPH) had no significant effect on ACh. However, concentrations of the transmitter were positively correlated with the intensity of stereotyped behaviors. A higher dose of AMPH (5.0 mg/kg) significantly increased ACh concentrations. The divergent responses to the various DA agents suggest the absence of a simple relationship between ACh concentration and behavior. Furthermore, these results suggest other mechanisms in addition to DA receptor activation are involved in AMPH-induced effects on ACh. (Supported by DA04157 and DA01568).

313.7

PERTUSSIS TOXIN-SENSITIVE G-PROTEIN MEDIATES GALANIN'S INHIBITION OF SCOPOLAMINE EVOKED ACETYLCHOLINE RELEASE IN VIVO AND CARBACHOL STIMULATED PHOSPHOINOSITIDE TURNOVER IN RAT VENTRAL HIPPOCAMPUS. S. Consolo , R. Bertorelli*, P. Girotti*, C. La Porta*, T. Bartfai+, M. Parenti§, M. Zambelli*; Istituto "Mario Negri", Milan, Italy; §Dept. of Pharmacology, Univ. of Milan, Italy; +Dept. Biochemistry, Arrhenius Labs, Stockholm, Sweden.

The effects of intracerebroventricular (i.c.v.) injections of pertussis toxin were investigated on the inhibitory action of the 29-amino-acid peptide galanin on acetylcholine (ACh) release and phosphoinositide breakdown stimulated by muscarinic agents in rat ventral hippocampus. Pertussis toxin (0.6 ug, i.c.v., 96h) counteracted the in vitro inhibitory effect of galanin (3.1 nmol) on phosphoinositide breakdown stimulated by carbachol without altering the stimulatory action of the cholinergic agonist on signal transduction, in miniprisms from rat ventral hippocampus. Pertussis toxin also abolished the in vivo effect of galanin on scopolamine-stimulated ACh release in vivo but did not affect basal ACh release. The results indicate that pertussis toxin-sensitive G-protein(s) mediates the galanin receptor regulation of pre- and postsynaptic cholinergic functions in the ventral hippocampus.

313.8

INFUSION OF FENFLURAMINE INTO THE HIPPOCAMPUS OF RATS DECREASES POTASSIUM STIMULATED RELEASE OF EXTRACELLULAR ACETYLCHOLINE AS MEASURED BY IN VIVO MICRODIALYSIS. P. A. Shea, S. T. Ahlers, and A. C. Santucci[1]. Naval Medical Research Institute, Bethesda, MD 20889 and [1] Manhattanville College, Purchase, NY 10577.

In vitro experiments have shown that serotonin (5-HT) will inhibit potassium (K^+) evoked release of acetylcholine (ACh) in hippocampal tissue. In the present study dl-fenfluramine was used to examine the effects of serotonin release on both basal and K^+ stimulated extracellular levels of ACh and Choline (Ch) in the hippocampus using in vivo microdialysis. Male Long-Evans rats were implanted with a guide cannula into the dentate gyrus region of the hippocampus (AP: -5.0; L: + 2.6; V: - 2.0) one week prior to the beginning of the experiment. Dialysis was performed on awake, unrestrained rats using 2mm Carnegie Medicine microdialysis probes. The dialysis probe was perfused with artificial CSF at a rate of 2.6 μl/min; the esterase inhibitor neostigmine (10μM) was used in the perfusion medium in order to obtain measurable baseline levels of ACh. When 40mM of fenfluramine was infused through the microdialysis probe slight increases in the basal levels of extracellular ACh and Ch were observed. Infusion of 60mM K^+ without fenfluramine produced a 400% increase in extracellular ACh and virtually no change in Ch levels. When fenfluramine was infused a substantial decrease in K^+ elicited extracellular ACh release was observed. These results are consistent with a neuromodulatory role of serotonin on ACh release in the hippocampus.

313.9

EFFECTS OF FEEDING, DRINKING AND LEARNED TASTE AVERSION ON ACETYLCHOLINE RELEASE IN THE NUCLEUS ACCUMBENS OF FREELY-MOVING RATS. G. P. Mark, P. Rada*, J. B. Weinberg*, E. Pothos & B. G. Hoebel. Dept. of Psychology., Princeton Univ., Princeton, NJ 08544.

Microdialysis with a neostigmine Ringer (0.5 μM) was used to monitor extracellular acetylcholine (ACh) in the nucleus accumbens (NAC), striatum (STR) and hippocampus in 30 min intervals before, during and after free-feeding in 20 hr food-deprived rats. The effects on ACh in the NAC and STR were also observed in response to water intake in water-deprived animals. Feeding caused a 38% increase in extracellular ACh in the NAC ($P<.05$), 18% in the STR (n.s.), and no change in the hippocampus. Drinking caused statistically reliable 18-20% increases in both the NAC and STR ($P<.05$). To determine if ACh was released in the accumbens to promote feeding in response to deprivation or inhibit it in response to satiety, two additional experiments were performed. First, neostigmine (1mM) bilaterally infused into the NAC via reverse dialysis caused a reduction in food intake in food-deprived rats. Second, the conditioned taste aversion (CTA) paradigm was used to develop an aversion to a previously appetitive taste (2.5 mM saccharin). Intraoral infusions of the aversive taste stimulus resulted in a 40% increase in extracellular ACh. These results suggest a site specific increase in ACh release during feeding that may be related to the inhibition of food intake.

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313.10

ACETYLCHOLINE RELEASE IN THE NUCLEUS ACCUMBENS IS CORRELATED WITH OPIATE WITHDRAWAL SYMPTOMS. P. Rada*, E. Pothos, G. P. Mark & B. G. Hoebel, Department of Psychology, Princeton Univ., Princeton NJ 08544-1010.

Microdialysis with a neostigmine Ringer (0.5 μM) was used to measure changes in extracellular ACh in the nucleus accumbens (NAC) of freely moving rats during acute and chronic morphine treatment, and following naloxone-precipitated withdrawal with and without clonidine treatment. Basal recovery of ACh was not affected by chronic morphine treatment. Acute morphine (20 mg/kg, i.p.) caused a decrease in extracellular ACh ($F(6,42)=7.02$; $p<0.001$) which was not apparent after 7 days of exposure to this opiate. The effect of naloxone (20 mg/kg, i.p.) was identical to that of saline in the non-dependent state but naloxone caused an exaggerated increase in ACh levels after morphine dependence had been established ($F(6,48)=2.984$; $p<0.02$). The increase in ACh following naloxone-precipitated withdrawal was eliminated by pretreatment with clonidine (200 μg/kg, i.p.). To study the possibility that the changes in ACh levels were due to general activity or arousal, a different group of rats received cocaine (20 mg/kg, i.p.). After the administration of this drug, accumbens ACh decreased 28% ($F(6,66)=3.752$, $p<.005$). These results suggest involvement of the NAC cholinergic system in the aversive aspects of opiate withdrawal and therefore an important role in opiate addiction.

Supported by USPHS grant DA-03597.

313.11

IN VIVO CHARACTERIZATION OF THE EFFECT OF AMPHETAMINE ON ACETYLCHOLINE RELEASE IN THE FRONTAL CORTEX J. Day and H.C. Fibiger. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1Z3

Basal forebrain cholinergic neurons project diffusely to the cortex and have been proposed to be part of the reticular activating system. The present experiments sought to characterize the effects of the psychomotor stimulant *d*-amphetamine (AMPH) on ACh release in the frontal cortex using *in vivo* brain microdialysis. AMPH (2mg/kg) increased ACh release in the frontal cortex of rats to approximately 280% of baseline values. Local application of AMPH (10^{-3}M) in the frontal cortex did not affect ACh release; AMPH does not therefore appear to produce this effect locally in the cortex. The nonselective dopamine receptor agonist apomorphine (1mg/kg) increased dialysate concentrations of ACh to 220%. This suggests that AMPH's effect on cortical ACh release may be dopaminergically mediated. When given alone, the D_1 antagonist SCH23390 (0.3mg/kg) did not affect cortical ACh release. However, it completely blocked AMPH-induced increases in both cortical ACh release and locomotor activity. The D_2 antagonist haloperidol (0.15mg/kg) also did not itself affect cortical ACh. This neuroleptic only partially attenuated the effect of AMPH on cortical ACh (to 60% over baseline) while it completely blocked the drug's locomotor stimulant effect. These results suggest that in the awake, freely moving animal both D_1 and D_2 dopamine receptors mediate AMPH-induced increases in frontal cortical ACh release.

313.12

EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL ON ACETYLCHOLINE LEVELS IN THE PRIMATE HIPPOCAMPUS: AN IN VIVO MICRODIALYSIS STUDY. H. Ogura*, C. Chavoix, and T. Aigner. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

Delta-9-tetrahydrocannabinol (THC) is known to impair memory in monkeys and humans. One possible mechanism is that THC may affect acetylcholine (ACh), which is known to play a critical role in mnemonic processes. In vivo microdialysis was used to study the effects of THC on ACh in the rhesus monkey hippocampus, a structure rich in cholinergic innervation and thought to contribute to certain forms of memory. Magnetic resonance imaging was used to determine stereotactic coordinates for the target sites in the hippocampus. The monkeys (n=4) were anesthetized with isoflurane and 1-3 probes were then inserted into the dentate gyrus and perfused with Ringer's solution containing 10 μM neostigmine at a flow rate of 1 μl/min. Samples were collected every 15 min. ACh levels were measured by HPLC using isopropylhomocholine as an internal standard (detection limit: 100 fmol). THC (4, 6, & 8 mg/kg, i.m.) initially produced a significant rise (as much as 243%) in the extracellular ACh levels, with a peak effect 15-30 min after injection, which returned to baseline levels within 60 min. At that time, a second administration of the same dose had little or no effect on levels of ACh, suggesting that some form of acute tolerance had occurred.

Although the relationship of these findings to the THC-induced memory impairment remains to be determined, the microdialysis technique used here shows promise for studying compounds that affect cholinergic transmission in primates.

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313.13

EVOKED EFFLUX OF ENDOGENOUS ACETYLCHOLINE FROM RAT BRAIN SLICES: EFFECT OF INTERLEUKIN-2. D. Seto, U. -K. Hanisch*, J. -G. Chabot and R. Quirion. Douglas Hospital Research Centre, Departments of Psychiatry and Pharmacology, McGill University, Montreal, Quebec H4H 1R3 Canada.

It was shown earlier by our group that interleukin-2 (IL-2, 1-100 n*M*) inhibits the high-K^+ (25m*M*) evoked efflux of endogenous acetylcholine (ACh) using a model of static incubation. This effect was more pronounced in the rat hippocampus than in the cortex or striatum. Here we present data obtained in a time-course study using a model of superfusion of brain slices. Hippocampal slices were superfused for 60 minutes in normal Krebs buffer (NKB), followed by a first stimulation (S_1) with high-K^+ Krebs (25m*M*) buffer (HKB) for 30 minutes, another 20 to 30 minutes of NKB, a second stimulation (S_2) with HKB for 30 minutes, and finally, by a 20- to 30-minute exposure to NKB. IL-2 (1-100n*M*) was present during either S_1 or S_2 in experimental groups and the effects were compared to the response of controls untreated with IL-2. The superfusate was collected over the entire duration and analysed for ACh using a radioenzymatic assay. Results suggest that IL-2 present during S_2 reduced the evoked ACh efflux similarly as described for static incubation. Moreover, inhibition of evoked ACh efflux was observed not only during S_1, but also during S_2 in the group that have undergone IL-2 exposure during S_1, suggesting a long- lasting effect of IL-2 in this model.(This project is supported by the MRC of Canada and the FRSQ).

313.14

AMINO ALCOHOLS MODULATE THE RELEASE OF NEWLY SYNTHESIZED ACETYLCHOLINE IN RAT HIPPOCAMPAL SLICES. J.R.Bostwick, J.Sun and S.H.Appel. Dept. Neurology, Baylor Col. Med., Houston, TX 77030

A search for endogenous brain trophic factors which influence *in vitro* cholinergic development led to the discovery that phosphoethanolamine and ethanolamine enhance acetylcholine formation. Since the function of a cholinergic neuron is predicated on its ability to release ACh, we measured the effects of these and 36 other amino alcohols on the release of newly synthesized ACh in hippocampal slices. Ten compounds were found to significantly increase ACh release. A short (20 minute) exposure to the compounds followed by a wash and incubation with ^{3}H-choline resulted in an effect which persisted for at least 3 hours. This acute effect was not due to enhanced synthesis because the total ACh content, amount of ^{3}H-ACh formed or the specific activity of the total ^{3}H-ACh pool were not increased. To further confirm this finding, slices were incubated in ^{3}H-choline and allowed to synthesize ACh prior to any treatment. We measured ^{3}H-ACh subsequently released during brief depolarizations both before (S_1) and during (S_2) perfusion with test compounds. Active amino alcohols increased the ratio of the fractional ACh released (S_2/S_1). The action appeared to be unique to hippocampal cholinergic nerve terminals because amino alcohols did not modulate evoked release of ACh from cortical or striatal slices, dopamine from striatal slices or norepinephrine from hippocampal slices. Bay K 8644, a dihydropyridine calcium L-channel agonist, exhibited a release profile identical to amino alcohols. Bay K 8644 decreased the EC_{50} of amino alcohols without changing the maximal response, indicating that their actions converge through the same mechanism. Diltiazem, a benzothiazapine L-channel antagonist, blocked the amino alcohol induced effect whereas nifedipine, a dihydropyridine L-channel antagonist, did not. Thus, the modulatory action of amino alcohols appears to be mediated by a change in the mechanism which underlies the recruitment of ACh for release in the hippocampus and may involve an elevation of intra-terminal calcium concentrations through a specific activation of presynaptic L-channels. Supported by grant AG08664 from the NIA

314.1

THE HEAT-SHOCK RESPONSE PROTECTS NEURONS FROM CA++-MEDIATED GLUTAMATE TOXICITY IN VITRO. W.J. Koroshetz, G. Rordorf, J.V. Bonventre. Stroke Res. Ctr. Massachusetts Gen Hospital, Boston Ma. 02114.

Most cells synthesize a specific array of proteins after various stresses; heat stress has been especially well studied. In many cells this "heat-shock response" improves cellular tolerance to repeated stress. Expression of "heat-shock proteins" (HSPs) occurs in brain after ischemic or severe epileptic insults though a functional role of brain expression has not been shown. We demonstrate that the heat shock reponse can be induced by heating neuronal/glial cultures of rat cortex to 42.2 C^{o} for 20 minutes. This heat protocal increased the expression of mRNA for the 72kD HSP and increased the synthesis of proteins of approximately 72 and 83 kD, the approximate m.w.'s of the most highly conserved HSPs. Though less dense, these protein bands were present in non-heated cultures. However their density was much decreased by pretreating with glutamate antagonists.

Cultures heated to induce the heat-shock response demonstrated increased neuronal survival after exposure to toxic levels of glutamate in media with low [Cl-], a model of Ca++- dependent excitotoxicity. Consistent with a heat-induced expression of HSPs, this protective effect occurred only with a delay after the heating and was blocked by inhibitors of RNA and protein synthesis. The protective effect of heat-shock lasted 24 but not 48 hours. It was not the result of heat-induced glutamate release because heating in the presence of glutamate antagonist still conferred protection. These data indicate that glutamatergic processes induce HSP synthesis and that the heat-shock response protects neurons from Ca++ mediated excitotoxicity. HSPs may play a similar protective role in brain after ischemia or status epilepticus.

314.2

KAINIC ACID INJECTIONS INTO VENTROBASAL THALAMUS INDUCE THE EXPRESSION OF HEAT SHOCK PROTEIN IN THE CORTEX AND AMYGDALA OF THE RAT. M.F. Gonzalez, and E. Won. Department of Psychology, UCSD, La Jolla, CA 92093

Previous work has shown that systemic injections of kainic acid induce the expression of heat shock proteins of a 72 kD molecular weight (HSP72) in many structures of the rat's brain. In the present work the expression of HSP72-like immunoreactivity was examined following intrathalamic injections of 0.1 and 0.2 µg of KA. Sprague-Dawley rats were anesthetized and injections were aimed to the center of either the mediodorsal (MD) or the ventrobasal (VB) nuclear complex . Three days later the rats were sacrificed and their brains immunocytochemically reacted for the expression of HSP72 using commercially available HSP72 antibody (Amersham) and an ABC reagent kit (Vector Lab). Subjects injected in VB exhibited necrosis of the ipsilateral piriform cortex and basolateral amygdaloid complex. In the contralateral side, HSP72 positive neurons, mostly of a pyramidal type, were present in layers III, V and VI of perirhinal and parietal cortex and in the upper layers of hindlimb sensorimotor cortex. In the ipsilateral side, HSP72 positive neurons were present layer III of the parietal cortex above the ablated piriform cortex. The expression of HSP72 was more intense using the higher dose of kainic acid, but the regions affected were similar. Following kainic acid injections into the MD complex, many HSP72 positive non-pyramidal neurons were present in the deeper layers of parietal cortex, but he overall expression of HSP72 was less intense. These results suggest that thalamic nuclei participate in the induction of HSP72 following systemic injections of kainic acid, and that the level of receptors sensitive to the excitotoxin in different areas of thalamus influence the expression of the protein in distant brain sites.

314.3

OPENING OF NMDA-GATED IONIC CHANNELS STIMULATES cGMP FORMATION IN CA1 MINISLICES. K.L. Panizzon, R.A. Wallis, E. Csiszar* and C.G. Wasterlain. Dept. of Neurology, Sepulveda VAMC and UCLA Sch. of Medicine, Sepulveda, CA 91343.

Stimulation of second messengers and protein kinases plays an important role in the brain's adaptive responses to excitotoxic injuries encountered in seizures, ischemia, trauma and hypoglycemia. We examined the stimulation of cyclic GMP and cAMP formation, assayed by radioimmunoassay, by NMDA in superfused CA1 hippocampal minislices, in which CA3 and dentate gyrus were trimmed. Exposure to NMDA caused rapid, dose-dependent CA1 injury (60 min. recovery after 8 min. at 20 μM = 0 %). 20 μM NMDA raised cGMP concentration 2-fold after 3 min., 3-fold after 5 min. and nearly 4-fold after 10 min., without altering cAMP concentrations. However, 5 μM NMDA (8 min.) was not toxic but raised cGMP concentration by 53%. Both the cGMP increase and the neuronal injury induced by 8 min. exposure to 20 μM NMDA were completely blocked by 32 μM MK-801. These data suggest that the cGMP response to NMDA is mediated though opening of receptor-gated ionic channels and may play a role in neuronal injury.

Supported by the research service of the VA, the American Epilepsy Society and grant NS13515 from NINDS.

314.4

GLUTAMATE-INDUCED EXCITOTOXICITY RESULTS IN DECREASED CAM KINASE II ACTIVITY IN CULTURED RAT CORTICAL NEURONS. S.B. Churn, S. Sombati, W.C. Taft, *A.L. Willey and R.J. DeLorenzo. Department of Neurology, Medical College of Virginia, Richmond, VA 23298.

Exposure of neurons to excitotoxic levels of Glutamate results in extensive delayed neuronal cell death, 24 hr. later. The delayed cell death is thought to be a calcium dependent phenomenon (Choi et al. (1987) J. Neurosci. 7:369), however, the tertiary mechanisms whereby the glutamate-induced calcium increase causes delayed neuronal death are not understood. We utilized primary cortical neuronal culture to study the effects of glutamate toxicity on CaM kinase II activity. Cortical cells cultured for 14 days from embryonic (E15) rats were exposed to 500 μM Glutamate for 10 min. Following 60 min recovery, the cells were harvested and studied for CaM kinase II activity under standard conditions (Goldenring et al. (1983) J. Biol. Chem. 258:12632). The results show that glutamate exposure results in greater than 50% inhibition of CaM kinase II activity. The decrease in CaM kinase II activity occurred at a time prior to extensive cell loss and was not reversible with phosphatases shown to be active towards CaM kinase II autophosphorylation. In addition, inclusion of the NMDA antagonist MK-801 (20 μM) completely protected both CaM kinase II activity and neurons from the effects of 500 μM glutamate exposure, suggesting that the inhibition of CaM kinase II activity is due to activation of NMDA channels. The results are consistent with the hypothesis that CaM kinase II may be involved in the glutamate-induced neuronal excitotoxicity.

314.5

FAILURE OF A PROTEIN SYNTHESIS INHIBITOR TO MODIFY GLUTAMATE RECEPTOR MEDIATED NEUROTOXICITY IN VIVO C. Leppin*, F. Finiels-Marlier*, J.N. Crawley, P. Montpied and S.M. Paul. NSB, NIMH, Beth., MD 20892.

It has recently been reported that the protein synthesis inhibitor anisomycin administered before and after experimentally-induced CNS ischemia reduces the development of delayed neuronal death (DND) in hippocampal (CA1) neurons (Neurosci. Lett. 120:117, 1990). Given the postulated role of excitatory amino acids (EAA's) in mediating DND, we tested whether anisomycin, could modify DND induced by unilateral intra-caudate administration of excitotoxins. Neuronal survival was assessed 7-12 days later by comparing glutamatic acid decarboxylase activity in the ipsilateral vs. contralateral caudate. Excitotoxic lesions were induced by N-methyl-D-aspartate (NMDA;100, 150, 200 nmol), α-amino-3-hydroxy-5-methylisoxasole-4-proprionate (AMPA; 50 nmol) or kainate (5 nmol). Anisomycin (50 mg/kg i.p.) was given 1-2 hours before as well as 12 and 36 hrs after EEA administration. Intracaudate injection of NMDA, AMPA and kainate resulted in a marked reduction in GAD activity. Anisomycin administration did not alter DND following any of the three excitotoxins although there was a trend ($t[11] = 2.01$; $p<0.07$) towards a reduction in neurotoxicity induced by AMPA. These data should be interpreted cautiously, given the limited number of AMPA-treated animals. It appears that the synthesis of a "killer protein(s)" does not underlie neurotoxicity induced by NMDA or kainate acid.

314.6

GLUTAMATE NEUROTOXICITY IS MEDIATED BY NITRIC OXIDE. V.L. Dawson+, T.M. Dawson#, E.D. London+, D.S. Bredt#,, and S.H. Snyder#. +Neuropharmacology Lab., NIDA/ARC, Balto., MD 21224, #Dept. Neurosci., Pharmacol. and Mol. Sci., and Psychiatry, Johns Hopkins Med. Sch., Balto., MD 21205.

Several biological actions are mediated by nitric oxide (NO) including relaxation of blood vessels, formation of cGMP by glutamate receptor activation in cerebellar slices, and cytoxicity produced by activated macrophages. Nitric oxide synthase (NOS) activity accounts for nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase staining. NADPH diaphorase neurons are uniquely resistant to toxic or ischemic insult, therefore NO may play a role in neurotoxicity. N-methyl-D-aspartate (NMDA) neurotoxicity in primary cortical cultures, 24 hrs after a 5-minute exposure, is prevented by the NOS inhibitors N^{G}-nitro-L-arginine (EC_{50}=20µM) and N^{G}-monomethyl-L-arginine (EC50=170µM). This effect is competitively reversed by L-arginine. NO is formed from the conversion of L-arginine to citrulline by NOS. In arginine-free media the LD_{50} of NMDA neurotoxicity is shifted from 280 µM to 7.5 mM. Sodium nitroprusside (SNP) which spontaneously releases NO, produces dose-dependent cell death which parallels cGMP formation and NMDA cytotoxicity. Hemoglobin complexes NO and prevents the neurotoxicity of both NMDA and SNP. These data establish that NO mediates glutamate neurotoxicity.

314.7

NITRIC OXIDE SYNTHASE/NADPH DIAPHORASE NEURONS: ROLE IN NEUROTOXICITY. T.M. DAWSON[1], V.L. DAWSON[2], D.S. BREDT[1], M. FOTUHI[1], P.M. HWANG[1], G.R. UHL[1,2] AND S.H. SNYDER[1].
[1]Johns Hopkins University School of Medicine, Dept. of Neuroscience, Baltimore, MD, 21205; [2]Lab. Mol. Neurobiol, ARC, NIDA, Baltimore, MD 21224

Nitric oxide synthase (NOS), generates a novel neuronal messenger molecule, nitric oxide (NO), and is co-localized with neuronal NADPH diaphorase in brain and peripheral tissues. Transfection of human kidney cells with NOS cDNA elicits NADPH diaphorase staining, thus NOS catalytic activity accounts for neuronal diaphorase. We show that striatal and cortical somatostatin and NPY containing neurons co-localize with NOS/NADPH diaphorase staining neurons, neurons which are uniquely resistant to several neurodegenerative disorders and excitotoxins. Since NO can mediate glutamate neurotoxicity, we have evaluated NOS/NADPH diaphorase neurons as a potential source of cytotoxic NO. In neuronal cultures NOS/NADPH diaphorase neurons are resistant to NMDA neurotoxicity, but are exquisitely vulnerable to quisqualate or kainate neurotoxicity. Utilizing the susceptibility of NOS/NADPH diaphorase neurons to quisqualate we were able to kill >90% of these neurons while the remaining neurons stayed viable. Under these conditions subsequent NMDA neurotoxicity was attenuated. These data suggest that the NOS/NADPH diaphorase neurons are the source of NO and in situations of excess glutamate, they may become "killer neurons."

314.8

POTENTIATION OF QUINOLINATE-INDUCED HIPPOCAMPAL LESIONS BY AN INHIBITOR OF THE EDRF/NO PATHWAY. K. A. Haberny and C.U. Eccles, Dept. of Pharmacol. and Toxicol., Sch. of Pharmacy, University of Maryland, Baltimore, MD 21201.

An EDRF/NO generating system similar to that described in vascular tissue and other cell types has been identified in brain. The NO is formed via a calcium/calmodulin-dependentenzymatic conversion of l-arginine to l-citrulline (Bredt and Snyder, PNAS 87:682-685, 1990). Despite much speculation, no physiological function for an NO-generating system in the central nervous system has been demonstrated. We undertook the present study to determine whether the EDRF/NO pathway could play a role in modulating cytotoxicity produced in vivo by the excitotoxin quinolinic acid (QA).

Intracerebroventricular (ICV) administration of L-N^G-nitroarginine (NARG), a specific inhibitor of the EDRF/NO pathway, while not toxic when given alone, markedly potentiates the toxicity of quinolinic acid in the hippocampus when given 15 min prior to QA. Administration of QA only (225 nmoles ICV; N=10) was accompanied by mild (7/10) or, in some cases, severe (3/10) seizures and produced minimal to moderate damage in the pyramidal cell layer of the hippocampus. Administration of QA following pretreatment with NARG (120 nmoles; N=11) resulted in severe seizures (9/11) and death in some cases (3/11) as well as severe hippocampal damage in the surviving animals which was manifested as complete loss of the pyramidal cell layer. Administration of NARG only (N=4) produced no remarkable behavior signs and no evidence of hippocampal damage. In conclusion, the endogenous EDRF/NO system in rat brain appears to exert a protective influence in the case of cytotoxicity produced by QA. We have not yet determined whether this protection is mediated via vascular or neuronal mechanisms.

314.9

NORDIHYDROGUAIARETIC ACID POTENTIATES RAPIDLY TRIGGERED EXCITOTOXIC INJURY IN MURINE CORTICAL CELL CULTURES. K. Rose, L.L. Dugan, L. Bjerknes, and D.W. Choi Dept. of Neurology, Stanford Univ. Med. Sch., Stanford CA 94305.

We have previously found that nordihydroguaiaretic acid (NDGA) and other lipoxygenase inhibitors attenuate the slowly-triggered neuronal injury induced by 24 hr exposure to AMPA, kainate, NMDA, or quinolinate (Soc. Neurosci. Abstr. 16: 288, 1990). In contrast, NDGA potentiates rapidly-triggered excitotoxic injury.

The neuronal degeneration induced by brief (5-10 minute) exposure to 150 μM glutamate is significantly potentiated (20%) by the addition of 30μM NDGA. 45-Calcium influx studies with 100μM NMDA and 30μM NDGA suggest that this injury is not mediated by an increase in calcium through the NMDA receptor. Furthermore, NDGA addition produced a marked increase in the injury induced by combined oxygen and glucose deprivation. A similar increase in oxygen and glucose deprivation injury was also produced by 30μM baicalein.

While further studies will be needed to determine the basis of these observations, one possibility is a toxic buildup of arachidonic acid, due to inhibition of lipoxygenase pathway metabolism during intense NMDA receptor-mediated excitotoxic injury. Twenty-four hour exposure to exogenously administered arachidonic acid (100-200 μM) induces neuronal, and to a lesser extent, glial degeneration.

314.10

OUABAIN POTENTIATES GLUTAMATE TOXICITY IN NEURONAL CULTURES. Michael L. Brines and Richard J. Robbins, Neuroendocrine Program, Yale University School of Medicine, New Haven, CT 06510

Excitatory amino acids (EAA) kill sensitive neurons through ionic fluxes, resulting in increased intracellular calcium, and associated cellular swelling. The activation and effectiveness of homeostatic mechanisms to buffer these changes are largely unexamined. The sodium pump (Na,K-ATPase) is a family of proteins which maintain the transmembrane gradients necessary for regulation of many ions and small molecules. We hypothesized that this pump buffers ionic perturbations induced by EAA and that decreased activity would result in amplified toxicity. To test this idea, we used 10μM ouabain to inhibit specifically the high-affinity (α2 and α3) but not the low-affinity (α1) isoforms of the pump in monolayer cultures of rat telencephalic cells. This decreased total ouabain sensitive flux by ≈50% in ^{86}Rb studies. Cultures were exposed to agents for 5 min and the degree of toxicity assessed by measuring LDH released over 24 hr. Inactivation of the high-affinity pumps alone did not increase LDH release above control. Inhibition of both high- and low-affinity pumps, however, was very toxic to neurons. Exposure to 100μM glutamic acid (GLU) released only 5% of the total GLU sensitive neuronal LDH pool, but simultaneous exposure to 100μM GLU and 10μM ouabain increased this to 30% (n=18; $p<0.001$). Similar treatment of glial cells alone did not release LDH. Thus, in this paradigm inhibition of high affinity sodium pumps significantly increases the neurotoxicity of GLU. We conclude that high affinity sodium pumps are an important element in the overall homeostatic response of neurons to EAA.

314.11

PHYSIOLOGICAL RESPONSES OF TURTLE CORTICAL NEURONS TO PROLONGED N-METHYL-D-ASPARTATEEXPOSURE. Herman C. Sullivan and Arnold R. Kriegstein, Department of Neurology and Neurological Sciences, Stanford University Medical Center, Stanford, CA 94305

We have demonstrated in an *in vitro* slice model that turtle cortical neurons exposed to excitatory amino acids undergo delayed neuronal degeneration, although a prolonged exposure is necessary to elicit this phenomenon when compared to mammalian cortical neurons (Sullivan and Kriegstein, Soc.Neurosci.Abst. 16:1123). We have recorded from turtle cortical neurons using the whole cell, patch clamp method, during and after exposure (5-25 minutes) to 50 μM N-methyl-D-aspartate (NMDA).

Several sequential phases of physiological activity have been identified that correlate with receptor-ligand interaction: 1. increased synaptic activity, 2. massive increase in conductance, 3. depolarization, and after washout, 4. a prolonged and sustained hyperpolarization of 15-25 mV. The pattern of response is dependent on the length of exposure to NMDA. In those cells that undergo prolonged exposure of 15-25 minutes, the sustained after-hyperpolarization does not occur after washout; an intermediate exposure of 8-12 minutes results in a return to resting membrane potential followed by a variable phase of brief hyperpolarization and spontaneous depolarization. During brief exposure of less than 5 minutes, a sustained after-hyperpolarization occurs after washout but no spontaneous depolarization.

The after-hyperpolarization is characterized by an increase in resistance (decrease in conductance). This would occur if leak currents are turned off with or without an increase in ionic pump activity (Na-K, Na-Ca). Therefore, we postulate that depolarizing leak currents are impaired following exposure to the excitatory amino acid NMDA, and the abolition of this leak current may be an important factor in protecting neurons from cell death.

314.12

PHYSIOLOGICAL PROCESSES UNDERLYING INDUCTION OF THE EXTENDED NEURONAL DEPOLARIZATION ELICITED BY PROLONGED GLUTAMATE EXPOSURE. Douglas A. Coulter, Sompong Sombati, and Robert J. DeLorenzo Department of Neurology, Medical College of Virginia, Richmond, VA 23298.

We have previously shown that excitotoxic exposure to glutamate (GLU) elicits an extended neuronal depolarization (END) after washout of GLU, which lasts for hours (Sombati, et al. *Soc. Neurosci. Abstr.*, 1990). We employed whole-cell current-clamp recordings of 2-3 week old neonatal cultured hippocampal neurons to study processes involved in the induction of the END.

ENDs were induced following exposure to 500 μM GLU for 10 minutes in 100% of the neurons (7/7) recorded using an electrode solution in which calcium was unbuffered. ENDs, defined as a prolonged depolarization of at least 20 mV from rest, persisted for the duration of the recording with no sign of recovery, in some cases for over 4 hours. During the END, cells remained responsive to GLU, and retained electrical excitability, as evidenced by their ability to fire sodium spikes if repolarized by current-clamp. ENDs were never (0/6) elicited in neurons recorded with intracellular calcium strongly buffered by BAPTA (11 mM). This was true even with extended GLU exposure (20 min, n=2). In recordings where calcium was buffered less strongly (by 1.1 mM EGTA), ENDs were elicited in 60% of the experiments (15/25). Concurrent application of MK-801 (10-30 μM) with GLU blocked induction of the END in cells recorded without calcium buffer in the electrode solution (0/5).

We therefore conclude that excitotoxic activation of glutamate receptors consistantly produces an extended neuronal depolarization in hippocampal neurons in culture and that a rise in intracellular free calcium concentration, elicited by activation of excitatory amino acid receptors, is involved in initiation of this phenomenon. Supported by NIH-NINDS Javits award RO1-NS23350 and Epilepsy Program Project grant PO1-NS25630.

314.13

HIGH DENSITY HIPPOCAMPAL NEURON POPULATION IN CULTURE INCREASES VULNERABILITY TO GLUTAMATE EXCITOTOXICITY.

S. Sombati, D.A. Coulter, E.R. Jakoi* and R.J. DeLorenzo. Dept. of Neurology, Medical College of Virginia, Richmond, VA 23298.

Glutamate (GLU) excitotoxicity has been implicated in various neurodegenerative diseases. We were interested in whether neuronal population density may affect cellular vulnerability to GLU toxicity. We report here a correlation between neuronal plating density and susceptibility to GLU excitotoxicity. Dissociated hippocampal cells from 1 day old rat were grown at low and high density (LDC and HDC, 100,000 and 600,000 cells/dish) with added conditioned medium. Non-neuronal proliferation was inhibited one day after plating. Experiments were performed on 10 day old cultures.

Intracellular recordings from LDC neurons revealed discreet synaptic potentials and some sporadic activity while those from HDC neurons showed barrages of synaptic potentials, accompanied by rhythmic spike activity, indicating increased synaptic connections and activity in HDC vs LDC conditions. Neurons from both culture conditions were exposed to 500 μM GLU plus 10 μM glycine for 5 min. GLU caused membrane depolarization to -15 to -5 mV without marked receptor desensitization in either culture density. In separate experiments cell viability was assessed using trypan blue stain pre and post GLU treatment. Under these conditions 58.81 ± 4.26 % (n = 12, 4 experiments) of neurons from LDC survived while only 15.03 ± 1.78% (n = 8, 4 experiments) from HDC survived 24 hours post GLU treatment. Neurons in groups or clusters were more affected. These results suggest that the density of neuronal cultures affect endogeneous release of neurotransmitter, which may augment the excitotoxic effects of exogenously applied GLU.

Supported by NIH-NINDS Javits award RO1-NS23350 and Epilepsy Program Project grant PO1-NS25630.

314.14

RELATIONSHIP BETWEEN CHANGES IN INTRACELLULAR FREE CALCIUM ($[Ca^{2+}]_i$) AND NEUROTOXICITY.

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An increase in ($[Ca^{2+}]_i$) has been proposed to be the major cause of excitotoxic neuronal death. Using fura-2, we measured $[Ca^{2+}]_i$ changes in single fetal rat forebrain neurons exposed to various agonists at concentrations which produce little or no toxicity after 24 hr for 5 min.: 100μM glu (+1μM gly), 30μM NMDA (+1μM gly), 100μM kainate and 50mM K^+, under control and ischemic (agonist with 5mM KCN in glucose free HBSS) conditions. The $[Ca^{2+}]_i$ changes were chacterised in terms of rise in $[Ca^{2+}]_i$, level of $[Ca^{2+}]_i$ at the time agonist was washed out (plateau), the time taken by cells to recover by 90% of peak rise in $[Ca^{2+}]_i$ and area under the curve (AUC).

Parameter	Glu/gly	NMDA/gly	Kainate	K^+
Rise, nM	606 ± 53	242 ± 23	587 ± 28	462 ± 49
Plateau,nM	536 ± 56	173 ± 20	410 ± 59	179 ± 23
AUC,μM.sec	381 ± 68	52 ± 6	165 ± 30	62 ± 8
Recovery,min	17 ± 2	3 ± 0.5	6 ± 1	0.9 ± 0.1

Addition of KCN significantly increased the $[Ca^{2+}]_i$ changes induced by NMDA, kainate and K^+. However, only NMDA induced neurotoxicity was potentiated by KCN. Thus, in addition to increase in $[Ca^{2+}]_i$, excitotoxic cell death also depends on other factors linked to NMDA receptor activation.

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314.15

REPEATED NMDA RECEPTOR ACTIVATION INDUCES DISTINCT INTRACELLULAR CALCIUM CHANGES IN SUB-POPULATIONS OF STRIATAL NEURONS IN VITRO.

B.A. MacVicar, D. Hochman and S. Weiss. Neuroscience Research Group, University of Calgary, Calgary, AB, Canada.

We used imaging analysis of fura-2 fluorescence to examine changes in the concentrations of intracellular calcium ($[Ca^{2+}]_i$) in striatal neurons in primary culture. We examined > 700 NMDA-responsive striatal neurons (15-25 neurons/field) in separate coverslips from 8 independent culture preparations. Neurons were exposed to three pulses of NMDA (200μM; 2 min) separated by 7 min intervals. A subset (17%) of the NMDA-responsive neurons displayed uncontrolled increases in $[Ca^{2+}]_i$ (final $[Ca^{2+}]_i \geq$ peak response), usually after the second or third application. The rest of the NMDA-responsive neurons displayed either a persistent increase in $[Ca^{2+}]_i$ (33%; 150% of baseline $\leq$ final $[Ca^{2+}]_i <$ peak response) or no long term changes in $[Ca^{2+}]_i$ (50%). After 3 NMDA applications, OCa^{2+}/EGTA aCSF was superfused and this resulted in a significant reduction in only the persistent increases in $[Ca^{2+}]_i$ but not uncontrolled increases. In addition, while some persistent increases in $[Ca^{2+}]_i$ were attenuated in the presence of 1μM MK-801 or 100μM verapamil, uncontrolled increases were unaffected. This work indicates that the uncontrolled $[Ca^{2+}]_i$ increases may be principally due to increased intracellular Ca^{2+} release whereas the persistent increases may be due to Ca^{2+} influx.

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314.16

RELEASE OF GLUTAMATE AND TAURINE INDUCED BY ALTERED SODIUM GRADIENTS.

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Release of glutamate (Glu) and other transmitters may mediate neuronal damage produced by metabolic insults. Most of the oxidative metabolism of the CNS is used to maintain Na^+ gradients, suggesting release of transmitters during metabolic insults could involve altered Na^+ concentrations. Rat hippocampal slices were incubated in Hank's balanced salt solution with additional HEPES and glucose and amino acids were measured in supernatants and homogenates by HPLC. Monoclonal antibodies were used for immunocytochemical localization of Glu or taurine (Tau). Glu was released in a Ca-independent manner when intracellular Na^+ was increased by treatment with ouabain or monensin. Treatment with tetrodotoxin blocked the ouabain-induced release, suggesting the release was primarily from neurons. Na^+ replacement with N-methyl-D-glucamine decreased both intra- and extracellular Glu and resulted in little Glu-like immunoreactivity (IR) remaining in slices, suggesting increased catabolism of Glu. In contrast to Glu, Tau release was increased by Na^+ replacement. Hypotonic removal of NaCl resulted in release of both Glu and Tau and in strong Glu-like IR in glia while little Tau-like IR remained in slices. These results suggest that Glu and Tau may be released from neurons by reversal of their Na^+-amino acid cotransporters during Na^+ influx, while replacement of Na^+ depletes intracellular Glu and leads to decreased release of Glu. In contrast to Na^+ replacement, Na^+ removal results in increased release of Glu and in Glu accumulation in glia.

314.17

Protection Against Kainic Acid Toxicity With Lipophilic Antioxidants in Cerebellar Granule Cell Cultures.

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Previous evidence from this laboratory indicates that treatment with the antioxidant idebenone provides significant protection against neuronal degeneration induced by intrastriatal injections of kainic acid (KA). We now report that treatment of cerebellar granule cell cultures with lipophilic antioxidants significantly attenuates the neurotoxic effects of KA. Thirty min. exposures to KA produces an acute NaCl dependent neuronal swelling which leads to rapid lysis of approximately 50% of the neurons during the exposure period, followed by degeneration of the remaining neurons over the next 12 hours. However, if the cells are exposed to KA for 30 min. in the absence of extracellular Na^+, the acute swelling phase is alleviated and the cells degenerate more slowly over an 18 to 24 hour period. Using (-)Na^+ exposure conditions as a model for delayed KA mediated toxicity, we have found that addition of the lipophilic antioxidants idebenone and vitamin E (α-tocopherol) provides significant protection against KA toxicity. KA dose response curves were constructed in the presence and absence of either 3μM idebenone or 50μM vitamin E. Idebenone produces a rightward shift in the KA dose response curve with complete reversal of toxicity at low doses of KA (50-300μM). At maximal doses of KA (500-1000μM) idebenone reduced cytotoxicity by 50%. Vitamin E was less effective in preventing toxicity but provided from 20 to 40% protection. These data suggest that generation of free radicals may be involved in the process of KA mediated delayed neurotoxicity.

314.18

DISSOCIATION OF NITRIC OXIDE GENERATION AND KAINATE MEDIATED NEURONAL DEGENERATION IN CEREBELLAR GRANULE CELLS.

P.S. Puttfarcken, W.E. Lyons and J.T. Coyle. Dept. of Psychiatry. The Johns Hopkins School of Medicine, Baltimore, MD 21205.

The mechanism by which kainate (KA) kills neurons in response to persistent activation of its receptors remains poorly defined. Recent studies have demonstrated that stimulation of the KA receptor leads to formation of nitric oxide (NO) from L-arginine, and that subsequent activation of soluble guanylate cyclase by NO accounts for cGMP generation in slices of rat cerebellum. In light of growing evidence that KA-mediated neurotoxicity may involve oxidative stress as the cause of membrane disruption and that NO can lead to oxidative stress, we investigated the relationship between KA-mediated NO generation and neurotoxicity in rat cerebellar granule cell cultures. Neither NO nor cGMP appear to be directly involved in the mechanism(s) underlying KA mediated-toxicity. This conclusion is based on the fact that the neuronal damage elicited by KA was not L-arginine-dependent, and treatment with competitive NO synthase inhibitors did not provide protection against KA toxicity. However, we conclude that the generation of free radicals may be involved in the process of KA-elicited neuronal death in cultures of cerebellar granule cells (see accompanying abstract by Lyons et al., 1991). Protection provided by preincubation with MA(hbs), appeared to be due to the counterion, HBS, whose structure resembles lipophilic antioxidants.

314.19

PEROXIDE ALTERS [^{3}H]GLUTAMATE RELEASE BY CEREBRAL CORTICAL SYNAPTOSOMES. S.C. Gilman* and T.C. Pellmar. Physiology Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20889-5145.

Hydrogen peroxide (H_2O_2) is a by-product of oxidative metabolism normally maintained at low levels in the CNS. Certain conditions, such as aging, ischemia or radiation exposure, elevate H_2O_2 levels causing increased oxidative stress to the neuron and hydroxyl free radical generation. Previous studies in our laboratory have shown that H_2O_2 reduces synaptic potentials. In an effort to define the mechanisms of H_2O_2 damage, we examined its effect on [^{3}H]glutamate release by guinea pig cerebral cortical synaptosomes. Glutamate is a major excitatory neurotransmitter in the CNS, but can be neurotoxic in high concentrations, suggesting a role in H_2O_2 induced pathophysiology.

Basal and high [K^+]-stimulated [^{3}H]glutamate release were assessed. Basal release was Ca^{2+}-independent. Pretreatment with H_2O_2 substantially increased the basal release, while producing a 25% depression in high K^+-evoked release in the presence of Ca^{2+}. Evoked release in the absence of Ca^{2+} was unaffected. Subtraction of Ca^{2+}-independent curves from Ca^{2+}-dependent showed that H_2O_2 completely blocked Ca^{2+}-dependent high K^+-evoked release. We hypothesize that under non-depolarized conditions, H_2O_2 is affecting a high-affinity Na^+-dependent glutamate transporter, while under depolarized conditions, H_2O_2 is interfering with exocytosis.

314.20

EFFECTS OF A SYNTHETIC CATALYTIC SCAVENGER OF OXYGEN FREE RADICALS ON KAINIC ACID-INDUCED PATHOLOGY IN RAT LIMBIC SYSTEM. A. Bruce, I. Najm, B.Malfroy*,and M. Baudry. Neuroscience Program, USC, Los Angeles, CA; *Eukarion, Inc. Arlington, MA.

Oxygen free radicals have been implicated in many types of neuronal injury, such as ischemia and excitotoxicity. Transgenic mice expressing high levels of superoxide dismutase (SOD) exhibit reduced degrees of brain pathology following ischemia or MPTP treatment, suggesting that exogenously administered SOD could be used to protect neurons against free radicals. However, the large size and rapid clearance of recombinant SOD may account for the limited success of this approach. A small molecule consisting of a complex of desferrioxamine and manganese (DFn-Mn) has been shown to have catalytic SOD activity. We report our initial attempts to test this compound on neuronal damage. We selected the model of kainic acid (KA)-induced seizure activity which results in widespread neuronal damage in hippocampus and piriform cortex. Rats were first treated with DFn-Mn (40 mg/kg; i.v.) before receiving KA (10 mg/kg; i.p.), and were sacrificed 7 days after KA injection. The extent of neuronal damage was determined by quantifying the amount of binding of 3H-Ro5-4864, a ligand for the peripheral type benzodiazepine receptors, which has been shown to be a good marker of reactive gliosis. Pretreatment of rats with DFn-Mn almost totally suppressed the increase in 3H-Ro5-4864 binding elicited by KA treatment in hippocampus and piriform cortex. Other markers of neuronal damage (cell counts, spectrin breakdown) will be determined to further evaluate the protective capability of this catalytic scavenger.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY III

315.1

EXCITOTOXIC ACTIONS OF THE CYANIDE METABOLITE, 2-ICA (2-IMIDOTHIAZOLIDINE-4-CARBOXYLIC ACID). R.S. Bitner*, M.N. Patel, L. Cintron*, A. Kanthasamy*, G.E. Isom* and G.K.W. Yim, Dept. Pharmacology & Toxicology, School of Pharmacy, Purdue Univ., W. Lafayette, IN 47907.

Rosling (1989) suggested that the reaction product of cyanide with cystine, 2-ICA (2-Imidothiazolidine-4-Carbocylic Acid) might be an excitotoxin responsible for cassava-induced neurotoxicity (CIN). Hence 2-ICA was synthesized and examined for neuroexcitatory actions. After icv injection in mice, 2-ICA (0.4 -3.4 uM) caused ear scratching, wild running accompanied by clonic and tonic seizures that was blocked by PCP but not by diazepam. NMDA caused a similar excitatory syndrome that was also selectively blocked by PCP. In primary cultured hippocampal cells, 2-ICA (500 uM) and glutamate (200-500 uM) caused comparable increases in intracellular calcium levels. These results indicate that the possible role of cyanide-related excitotoxins in motoneuron diseases deserve further study. (Supported by NIH Grants ES04140, RR05586, & Marc fellowship to L.C.)

315.2

CHRONIC INTRASTRIATAL APPLICATION OF KYNURENINES PRODUCE A PATTERN OF NERVE CELL LOSS SIMILAR TO HUNTINGTON'S DISEASE. G.K. Rieke. Dept. Anat. & Cell Biol., Sch. of Med., Univ. of North Dakota, Grand Forks, ND 58202.

The kynurenines are neuroactive metabolites of tryptophan and are present within the brain. Chronic intrastriatal injection of L-kynurenic acid, 3-hydroxykynurenine or quinolinic acid selectively destroyed GABA-ergic neurons, while sparing NADPH-diaphorase positive cells. The spared neurons were located in the spongiose zone of the lesion. Each compound was delivered through an indwelling cannula coupled to an osmotic pump filled with buffered (pH 7.4-7.8) test compounds at concentrations 2-5 times the normal levels per gram wet weight rat forebrain. Whether 3-hydroxyanthranilic acid, xanthurenic acid or kynurenine are neurotoxic remains to be determined. If the kynurenines and their metabolic side products (viz., L-kynurenic acid, xanthurenic acid, anthranilic acid) prove to be neurotoxic, they may represent a chain of interrelated brain chemicals with a potential role in HD, as brain levels of some of these compounds are elevated in HD.

315.3

SYSTEMIC LOADING WITH KYNURENINE AND PROBENECID MARKEDLY INCREASES STRIATAL KYNURENIC ACID LEVELS AS MEASURED BY *IN VIVO* MICRODIALYSIS.

J.M. Miller, U. MacGarvey* and M.F. Beal. Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Kynurenic acid (KYA) is the only endogenous NMDA receptor antagonist currently identified in mammalian brain. To pharmacologically maximize brain concentrations of this compound peripheral injections of the combination of its immediate precursor, kynurenine (KYN) with probenecid, a known inhibitor of membrane transport were administered and KYA levels measured in striatal dialysates of freely moving rats.

Baseline KYA levels in striatal dialysates were found to be 1.6 ± 0.3 pmol/ml (mean ± SEM). Peripheral injections of KYN 150 mg/kg increased KYA levels to 30.9 ± 7.8 pmol/ml 2 hr after injection. Administration of the combination of KYN 150 mg/kg with probenecid 100, 150, 200 and 250 mg/kg resulted in significant increases compared with KYN alone at each dose. The largest KYA increases were with 200 mg/kg probenecid (945 ± 210 pmol/ml KYA 3 hr after injection) and no further increase was observed with 250 mg/kg. Probenecid alone at 200 mg/kg slowly increased KYA levels to 16.0 ± 5.2 pmol/ml 5 hr after injection. Using 200 mg/kg probenecid in combination with KYN 150, 300 and 450 mg/kg, striatal KYA levels were maximally increased to 2085 ± 391 pmol/ml 3 hr and 20 min after injection with the highest KYN dose. These data show that kynurenic acid concentrations in striatal dialysates can be modestly increased with kynurenine alone but increases of several orders of magnitude can be achieved with the combination of probenecid and kynurenine.

315.4

QUINOLINIC ACID INDUCED NEUROTOXICITY ANTAGONIZED BY INDOLE-2-CARBOXYLIC ACID C.M.WRAY*,R.J.BOEGMAN AND C.ROMERO-SIERRA, DEPT. PHARMACOLOGY AND TOXICOLOGY,QUEEN'S UNIVERSITY, KINGSTON, ONTARIO,K7L 3N6

It has been demonstrated in Xenopus oocytes that glycine may be absolutely required for NMDA activity. In cell culture, indole-2-carboxylic acid (I2CA) has been shown to competitively inhibit the potentiation by glycine at the NMDA receptor. The ability of I2CA to protect against the neurotoxicity induced by the NMDA receptor agonist QUIN when coinjected into the rat striatum was evaluated. When coinjected with 200 nmol/ul I2CA, QUIN(15nmol/ul), which is neurotoxic to the NADPH-diaphorase (NADPH-d) neurons while sparing the acetylcholinesterase (AChE) neurons resulted in approximately 50% cell survival of the NADPH-d neurons. 60 nmol/ul QUIN, which is neurotoxic to both NADPH-d and AChE neurons, resulted in significant protection of the AChE neurons as determined morphologically and from studies measuring choline acetyltransferase (ChAT) activity. There was no decrease in ChAT activity when I2CA was coinjected with QUIN. In contrast a 35% decrease in ChAT activity was obtained with QUIN alone. No sparing of the NADPH-d neurons was seen at this dose of QUIN. Our studies indicate that inhibiting the action of glycine on the NMDA receptor decreases the neurotoxicity induced by QUIN. Supported by the Medical Research Council of Canada and the Huntington's Society of Canada.

315.5

INCREASED STRIATAL AND PALLIDAL MET-ENKEPHALIN LEVELS AFTER MICROINJECTIONS OF QUINOLINATE. B.B. Ruzicka & K. Jhamandas, Dep't. Pharmacology & Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

In an attempt to develop a model of the striatopallidal enkephalin deficit associated with Huntington's disease, we examined the sensitivity of the enkephalinergic neurons to quinolinate (QUIN, 18-288 nmol), an excitatory amino acid (EAA) which activates the NMDA receptor. At specific times (2 hr - 14 days) after a single, unilateral QUIN-injection into the rat striatum, the levels of met-enkephalin-like immunoreactivity (ME-i.r.) in the striatum and globus pallidus were estimated by RIA. At 7 days postinjection, QUIN produced dose-related and bilateral elevations in the striatal and pallidal ME-i.r.. The maximal responses, representing 3- and 4-fold increases in the ME-i.r., respectively, occurred at a dose of 72 nmol. The peak pallidal response was attenuated by the coinjection of QUIN with kynurenate (KYN, 36 nmol), a non-selective EAA receptor antagonist, or CPP (1.8 nmol), a selective NMDA receptor antagonist. The injection of KYN into the striatum contralateral to the QUIN-injection attenuated the QUIN-induced response in both the contralateral and the ipsilateral pallidum, suggesting that the contralateral changes in ME-i.r. may involve the mobilization of an endogenous EAA. Experiments conducted with the QUIN-injected tissues revealed that the elevations in ME-i.r. were not associated with an increased basal or K+-evoked ME-i.r. release. These results suggest that at 7 days following the intrastriatal QUIN injection, the striatopallidal enkephalinergic neurons surviving the EAA exposure contain an enhanced level of ME-i.r., a response that may reflect neuronal adaptation to injury. (Supported by the MRC of Canada).

315.6

Chronic intrastriatal administration of high and low dose quinolinic acid using a novel device for dialytic delivery

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We employed a microdialysis probe mated to an Alzet 2002 osmotic minipump to administer a high (40 mM) or a low (4 mM) dose of the excitotoxin quinolinic acid (QA) over a 3 week period. In vitro experiments showed >93% delivery of QA via the dialysis apparatus. The effects of chronic unilateral QA delivery to the striatum were evaluated with behavioral tests, routine histology and histochemistry for NADPH diaphorase and cholinesterase containing neurons, and D1/D2 receptor autoradiography.

Chronic delivery of 40 mM QA produced near total striatal necrosis with some damage to adjacent regions. This dose of QA also produced a significant increase in ipsilateral rotation in response to a 3 mg/kg challenge of amphetamine. By contrast, with both young (<3 months) and old (18-24 months) rats, 4 mM QA did not produce significant lesions or changes in D1/D2 receptor binding. After receiving the low dose of QA, animals did not rotate in response to amphetamine, nor was there a change in nocturnal activity.

The dialysis device used appears to increase total area of drug delivery while eliminating nonspecific damage seen after pressure injection techniques. Further experiments using this device are now in progress using an intermediate dose of QA.

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315.7

TOPOGRAPHICAL EXPOSURE OF ENDOGENOUS GM1 AFTER GLUTAMATE TOXICITY: EXOGENOUS GM1 PROTECTIVE EFFECTS.

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Reports describing the neuroprotective effects of exogenous GM1 (monosialoganglioside) and their possible interaction with trophic factors suggests that the endogenous molecule may itself play a critical role in CNS injury and recovery processes. In order to begin to study these possible relationships we have begun a series of experiments which assess the topographical localization of GM1 during injury since membrane surface exposure of GM1 is indicative of membrane integrity. Cortical neuronal cultures (+14days) from fetal (15 day gestation) and postnatal day 0-1 were subjected to glutamate (0.5-10uM) for 30min with and without preincubation with GM1 (80uM for 90min). Following fixation the effects on the distribution of endogenous GM1 were studied using cholera toxin (ligand for GM1) and anti-toxin immunohistochemical analysis. No significant differences were seen in the distribution of GM1 in the cultures when comparing untreated and 1hr post-challenged cultures. In unchallenged cultures GM1 is localized continuously along the entire cellular plasma membrane surface. The highest intensity of staining is associated with synaptic structures (soma and fiber connections). In challenged cultures (24hrs) there is a significant loss of staining, indicating primarily a disappearance of cell processes. This staining is markedly preserved in cultures challenged with glutamate but pretreated with GM1. Dye exclusion studies and LDH measurements confirm these assessments.

This work was supported in part by a grant from the FIDIA Research Foundation.

315.8

ANTAGONISTS OF N-METHYL-D-ASPARTIC ACID (NMDA) DEMONSTRATE TOXIC EFFECTS IN ORGANOTYPIC ROLLERTUBE CULTURES OF SPINAL CORD (OTC-SC). D.M. Saroff*, Y. Nishida and J.R. Delfs. Lab. of Neurodegenerative and Aging Studies, Beth Israel Hosp., Boston, MA. 02215

We have previously shown the toxic effects of NMDA on ventral horn acetylcholinesterase-positive neurons (VHANs) could be attenuated by the competitive receptor antagonist DL-2-amino-5-phosphonovaleric acid (DL-AP5). (Delfs et al. New Adv. in Tox. and Epidem. 1990; Chap. 35:273-281) Besides partially protecting neurons from the effects of NMDA, addition of DL-AP5 was associated with a shift in the distribution of neuron sizes.

In this study, OTCs were used to examine the effects of NMDA antagonists, including DL-AP5, D-2-amino-5-phosphonovaleric acid (D-AP5), and 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP). These effects were quantified by performing morphometry on the neurons in OTCs stained for acetylcholinesterase activity. Each antagonist studied caused a depression in the number of VHANs. The racemic mixture of AP5 (DL-AP5) demonstrated a lesser toxic effect than did its Dextro component (D-AP5) or CPP. While demonstrating toxicity, each of the three antagonists was less toxic than NMDA. These results indicate that the decrease in neurons observed in cultures with NMDA plus an antagonist was not the result of partial excitotoxic blockade, rather that the antagonist was having a toxic effect of its own.

315.9

AGE RELATED SUSCEPTIBILITY OF VENTRAL HORN NEURONS TO N-METHYL-D-ASPARTATE (NMDA) IN ORGANOTYPIC EXPLANT CULTURES OF SPINAL CORD (OTC-SCs). Y. Nishida, D.M. Saroff* and J.R. Delfs. Lab. of Neurodegenerative and Aging Studies, Beth Israel Hosp., Boston, MA 02215.

To elucidate developmental changes in neuronal sensitivity to NMDA, we examined the effect of NMDA on ventral horn acetylcholinesterase (AChE)-positive neurons (VHANs) in OTC-SCs from neonatal rats. OTC-SCs were performed as previously described (Delfs, et al. Brain Res 1989;488:31-42). After 1, 2, and 3 weeks in vitro, each culture was incubated in control medium or 100 μM NMDA. After 72 hours, cultures were stained for AChE and analyzed by histochemical and morphometric evaluation.

Decreases in AChE staining were observed in NMDA treated groups as compared with control cultures. In both control and treatment group, mean area of VHANs increased while mean numbers of VHANs decreased over 3 weeks. That decrease was especially remarkable in the smaller size groups (neuronal area less than 500 μm^2). At each age in vitro, NMDA affected mainly smaller VHANs. Survival rates of VHANs (NMDA vs control) in each size group was not obviously different between age groups.

Our results show that VHANs are susceptible to NMDA neurotoxicity by the first week in vitro. The relatively selective decrease in neurons in the smaller size groups could be related to a differential susceptibility to NMDA.

315.10

HOMOCYSTEATE, KAINATE AND AMPA INDUCE SPINAL CORD LESIONS WHEN ADMINISTERED SUBCUTANEOUSLY TO INFANT RATS. G.J. Wang, Y.Q. Qin*, M.T. Price, J.W. Olney, Washington University Medical School, St. Louis MO, 63110

Experiments were undertaken to explore an unpublished observation (JWO, circa 1970) that sc administration of DL-homocysteic acid (DL-HCA) to infant rats damages the developing spinal cord. We found that DL-HCA induces acute massive degeneration of neurons in both ventral and dorsal portions of the spinal cord, predominantly at lumbar and lower thoracic levels. Sacral, upper thoracic and cervical levels were relatively spared. Dose response studies at various ages revealed a definite developmental time window when spinal neurons are particularly sensitive. Vulnerability to a fixed dose (150 mg/kg sc) was negligible from postnatal days 1 to 3 but then progressively increased to reach a peak at 7-9 days followed by a gradual decline to zero vulnerability by the 15th postnatal day. Administering the D and L isomers separately to 7 day old rat pups revealed the D isomer to be about twice as potent as the L isomer. In part, this may reflect the more efficient uptake/inactivation system for L-HCA. Also relevant may be our observation that in the isolated chick embryo retina, the D isomer activates both NMDA and non-NMDA receptors, whereas the L isomer activates only NMDA receptors. To explore the role of each receptor type, we administered AMPA and kainate, which are selective agonists for the two major subtypes of non-NMDA ionotrophic receptors, and found that each induces a spinal lesion. However, it was difficult to keep the pups alive long enough for a full lesion to develop because each of these agents induces respiratory failure at doses (2-4 mg/kg sc) required to induce spinal pathology. All three of the agonists studied, DL-HCA, AMPA and kainate, caused typical excitotoxic cytopathology which was accompanied by conspicuous bleeding at multiple foci within the spinal cord; however, there did not appear to be any correlation between locus or degree of bleeding and severity of spinal cord damage. Intermediolateral and ventral horn motor neurons were comparably sensitive to all three agents, whereas dorsal horn neurons were differentially more sensitive to DL-HCA than to AMPA or kainate. Supported by HD24237, DA05072, AG05681 and RSA MH 38894 (JWO).

315.11

COMPARATIVE EFFECTS OF EXCITOTOXINS ON BASAL FOREBRAIN CHOLINERGIC NEURONS. R.J. Boegman, J. Cockhill K. Jhamandas and R.J. Beninger. Dept. Pharmacology & Toxicology, Queen's University, Kingston, Canada K7L 3N6.

Large neurons of the nucleus basalis magnocellularis (nbm) provide a major cholinergic input to the cerebral cortex and amygdala. While excitotoxic lesions of cholinergic projections to the cortex have implicated it in memory, little emphasis has been placed on the projection to the amygdala. We compared the response of the cholinergic projections to the cortex and amygdala to excitotoxin-induced damage. Unilateral (0.5 μl) stereotoxic injections of quinolinate (Quin), quisqualate (Quis), ibotenate (Ibo), NMDA or AMPA into the nbm of rats. Five days later animals were sacrificed and choline acetyltransferase (ChAT) activity measured in the cortex and amygdala. Our dose response results show that Quis and AMPA are preferentially neurotoxic to cholinergic neurons projecting to the cortex with an IC_{50} for AMPA of 0.75 n moles. Quin was preferentially neurotoxic to the cholinergic neurons projecting to the amygdala with an IC_{50} of 18 n moles. In contrast Ibo and NMDA did not discriminate between the cortical and amygdala projection. Our results offer an explanation for the lack of correlation reported between cortical cholinergic deficit and behavioural impairment observed with different neurotoxins. Supplied by M.R.C./N.C.E.

315.12

RECEPTOR ABUSE-DEPENDENT ANTAGONISM (RADA): IN VITRO AND IN VIVO EVIDENCE FOR AN ANTIEXCITOTOXIC ACTION OF NATURAL AND SYNTHETIC GLYCOSPHINGOLIPIDS. H. Manev, G. Lombardi, S. Francioni, D.M. Armstrong, A. Guidotti and E. Costa. F.G.I.N., Georgetown Univ., Washington, DC 20007.

RADA is a therapeutic strategy of selectively targeting drugs to the site where homeostasis is destabilized by paroxysmal receptor stimulation (FASEB J., 1990, 4: 2789). RADA strategy avoids that the same receptor is blocked indiscriminately throughout the brain. In vitro, natural (GM1, GD1a, GD1b, GT1b), and synthetic (LIGA4, LIGA20,) glycosphingolipids protected rat cerebellar granule neurons in primary culture against glutamate-induced neuronal death (measured by MTT or fluorescein diacetate/propidium iodide staining). LIGA 4 and LIGA 20 acted faster and more persistently than natural gangliosides. The neuroprotection correlated with the ability of glycosphingolipids to prevent the protracted protein kinase C translocation and intraneuronal destabilization of Ca^{++} homeostasis. In vivo, unilateral thrombotic stroke of the sensorimotor cortex (lesion 3-4 mm in diameter) induced by injecting the rats intravenously with 80 mg/kg rose bengal, and focally illuminating the skull, caused a behavioral deficit, quantitated by a battery of tests (vertical grid, inclined plane, patch removal, computer-assessed open field behavior). Pretreatment (1 day) with GM1 or LIGA 4 (50 and 10 mg/kg,iv., respectively), followed by a subcutaneous post-treatment (4 days) resulted in behavioral recovery, giving the first evidence of the in vivo RADA profile of these drugs.

315.13

EFFECT OF KAINIC ACID ON BRAINSTEM RESPIRATORY CHEMOSENSITIVE ZONES. R.M. Douglas*, D.G. Bernard, Y. Pan, L.M. Sexcius*, J.A. Holloway, R.M. Millis*, and C.O. Trouth. Dept. of Physiol. & Biophysics, Coll. of Med., Howard University, Washington, D.C. 20059.

Central respiratory chemosensitivity has been attributed to specialized neurons located on the ventrolateral medullary surface (VMS). In this investigation, the response of rat VMS chemosensitive neurons to inspired CO_2 and topically applied acetylcholine (Ach) were examined before and after topical application of 1% Kainic Acid (KA). Inspired CO_2 and Ach increased medullary neuronal activity (MNA) as well as diaphragmatic activity (DA). KA, an excitotoxic amino acid, caused a marked and prolonged increase in both MNA and DA, followed by depression to extinction and respiratory arrest. This experiment shows that: 1) KA may have destroyed superficial chemosensitive VMS cell bodies. 2) Due to VMS neuronal destruction, it was not possible to effectively assess the effect of KA on the central chemosensory response. 3) The integrity of chemosensitive VMS neurons in the anesthetized rat appears essential to the maintenance of the central drive to respiration. **SUPPORT:- NIH-NIGMS Grant # S06GM08016 AND NIH Grant PH 55-T 32 GM-07800.**

315.14

EFFECT OF INTRACEREBROVENTRICULAR INFUSION OF EXCITATORY AMINO ACIDS ON NEUROPEPTIDES IN RAT BRAIN. H. Kaneda, E. Kawata*, Y. Komurasaki*, K. Sakai*, K. Maeda and K. Chihara*. Dept. of Psychiat., Kobe Univ. Sch. of Med., Kobe 650, Japan.

Recently roles of excitatory amino acids (EAAs) on neurodegenerative diseases have been investigated biochemically and histologically. "Axon-Sparing lesion" is the one of the characteristic changes induced by EAAs. On the other hand, there are many findings about alterations of neuropeptides in postmortem brains or cerebrospinal fluids with such diseases in clinical study. Then, in this study, we have examined effects of three excitotoxins, ibotenic acid (IA), kainic acid (KA) and quinolinic acid (QA) on somatostatin (SS), neuropeptide Y (NPY) and arginine-vasopressin (AVP) levels in the discrete regions of rat brain. Male Wistar rats were used (300-350 g). The excitotoxins were infused into the right lateral ventricle for 14 days using an osmotic minipump (Alzet 2002). Each dosis of excitotoxins was 500 nmol IA, 5000 nmol QA or 5 nmol KA for 14 days. After the infusion the brain was taken out and dissected with modulated Palkovits' punch technique. Neuropeptides were measured by radioimmunoassay.

IA infusion resulted in a significant decrease in SS levels in the anterior cortex (AC), septum (SE), amygdala (AM), hippocampus (HC) and posterior cortex (PC), compared to those in control animals which were infused the vehicle. NPY and AVP in AM and HC, respectively, were reduced following IA administration. On the other hand, these neuropeptide levels in some discrete regions of the brain were elevated by either KA or QA infusion.

This result suggests that there exists a difference in the effects on brain neuropeptide metabolism among these excitotoxins. Small amount of IA infusion into rat lateral ventricle for long term may produce closely resembling pathophisiology to Alzheimer's disease in human.

315.15

UNILATERAL FOCAL NEURONAL DEATH INDUCED BY INTRA-ARTERIAL INFUSION OF KAINATE IN THE RAT. K. Shiraishi and H. Shinozaki. The Tokyo Metro. Inst. Med. Sci., Tokyo 113, Japan.

Systemic administration of kainate to the rat induces characteristic neuronal damages in the CA1 area of the hippocampus, which are similar to the delayed neuronal death caused by transient global ischemia. We intended to induce unilateral focal neuronal death in the rat by intra-arterial infusion of kainate with the Harvard pump through a thin catheter introduced into the left common carotid artery without affecting cerebral blood flow. 72 hours after the infusion of kainate, mild hemiparesis and slight decrease in muscle tone developed in all rats without demonstrating generalized convulsions, and pathological examination demonstrated unilateral focal neuronal death in the cortex and the CA3 area of the hippocampus. Infusion doses of kainate were significantly lower than those of systemic administration, and there was no case of death. When a bolus of kainate was given, generalized convulsions and bilateral neuronal damages were observed, like systemic administration. It is of great interest that there is a considerable difference in anatomical distribution of neuronal damages induced by systemic and intra-arterial administrations. The present case would be expected to be a valuable model for inducing focal neuronal death under preserving cerebral blood flow in rats. Supported by Seijin-byo Institute Memorial Foundation.

315.16

LOSS OF VISION AND SUBSEQUENT RECOVERY AFTER EXPOSURE OF ADULT RAT RETINA TO N-METHYL-D-ASPARTATE (NMDA). R. Siliprandi, R. Canella*, J. Sautter*[1], K. McDonald*[1], G. Carmignoto and B. Sabel[1]. Fidia Research Laboratories, Abano Terme, Italy and [1]Inst. of Med. Psychology, Univ. of Munich, 8000 Munich, FRG.

We studied the effects of a single intraocular (i.o.) injection of varying doses of NMDA on visual performance of adult rats. Rats were trained for a week to orient towards visual stimuli (Duvdevani et al., Restor. Neurol. Neurosci. 2: 31, 1990), and then received a unilateral i.o. injection of NMDA (2, 20, 100 nmoles, each n=5) or PBS. Loss of vision was quantified by assessing the size of the visual field ipsilateral to the NMDA injection. Rats receiving 2 nmoles of NMDA did not show deficits compared to control-injected counterparts. However, when receiving 20 or 100 nmoles NMDA, they showed near-complete blindness followed by significant recovery of vision which was almost complete 10 days post-lesion. Anatomical correlates were obtained after a combined Nissl and retrograde HRP-stain, with the 20 nmoles group displaying a loss of about 80% of the retinal ganglion cells. Thus rats can significantly recover visual function, despite a severe cell loss. This system provides a standardized behavioral assessment of excitotoxicity applicable to the evaluation of drugs effective on CNS functional recovery.

315.17

HIGH EXTRACELLULAR POTASSIUM INDUCES MARKED CHANGES IN EXTRACELLULAR AMINO ACID LEVELS AND NEURONAL NECROSIS IN THE RAT AMYGDALA. D.G. Fujikawa, A.F. Alcaraz* and N.C. Phan*. Exp. Neurol. Lab., VA Med. Ctr., Sepulveda, CA 91343 and Dept. of Neurology and Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024.

We used in vivo microdialysis and high-performance liquid chromatography (HPLC) to determine the effects of high extracellular K^+ ($[K^+]_o$) on extracellular amino acid concentrations in the rat amygdala, and correlated this with a histological assessment of neuronal viability.

Bilateral guide cannulae were positioned to place microdialysis probes within the basolateral amygdaloid nuclei of adult Wistar rats (n=3). The next day the probes were inserted and perfused with Krebs-Ringer-bicarbonate (KRB) solution for 2 h. One side was switched to 100 mM KCl in modified KRB for 3 h, after which rats underwent brain perfusion-fixation for histological examination. The dialysate was analyzed by HPLC.

100 mM KCl induced sustained 3-h increases in aspartate (251-503%) and taurine (447-629%) and decreases in glutamine (down 82-88%) compared to the side perfused with KRB. Glutamate was elevated during the first 60 min of 100 mM KCl perfusion (365% at 30 min and 202% at 1 h) but subsequently declined. Glycine increased only at 2 h (367%). Serine levels did not change. The side perfused with 100 mM KCl showed extensive edema and necrotic neurons up to 500 μm from the probes. Cell loss (primarily neurons) was most pronounced in the first 100 μm. The side perfused with KRB showed minimal changes. Thus, large, prolonged increases in extracellular aspartate, glutamate and taurine are produced by high $[K^+]_o$ and are associated with neuronal necrosis.

315.18

HYPOXIC AND EXCITOTOXIC LESIONS IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES. N. Tønder and J. Zimmer. Pharma-Biotec, Inst. of Neurobiology, Univ. Aarhus, Denmark.

Slices of hippocampal tissue (350 μm) from 5 day old rats, were grown in culture using the roller-tube technique. After 4 weeks in vitro, cultures with a complete, organotypic appearance were selected for either hypoxia (½ - 4 hrs at 36°C in 3% O_2 and 5% CO_2) or addition of excitotoxins (kainic acid, 3 μM for 48 hrs; NMDA, 10 μM for 1 to 11 hrs; AMPA, 10 μM for 5 or 10 hrs), after which they were returned to normal medium for up to 14 days.

After 45 min hypoxia hippocampal CA1 and CA3c pyramidal cells and dentate granule cells degenerated, while CA3a,b pyramidal cells and somatostatinergic (SS) neurons in all areas appeared intact. Exposure to NMDA for 10 hrs resulted in a similar lesion. Cultures exposed to AMPA for 10 hrs showed additional degeneration of SS neurons. Kainic acid selectively damaged CA3 pyramidal cells.

The results demonstrate that hypoxic and excitotoxic lesions in organotypic hippoampal slice cultures, with some differences (granule cell degeneration), resemble the lesions in vivo. The method may, accordingly, be used for experimental analysis of cellular mechanisms, and testing of pharmacologically active substances, in relation to cerebral ischemia.

Supported by the Danish State Biotechnology Program, the Aarhus University Research Foundation and the Lundbeck Foundation.

315.19

INTRAVENTRICULAR INFUSION OF N-METHYL-D-ASPARTATE LEADS TO ACUTE HYPERTENSION, INCREASED VASCULAR PERMEABILITY AND NEURONAL INJURY. W.D. Dietrich, O.Alonso, R. Busto, M. Halley*, and M.Y.-T Globus. Dept. Neurology, Univ. Miami School Medicine, Miami, FL 33101.

N-methyl-D-aspartate (NMDA) receptor activation has been implicated in a variety of neurotoxic processes. This study determined whether excessive NMDA receptor activation would produce blood-brain barrier (BBB) dysfunction and neuronal injury in the acute experimental setting. NMDA (20 ug/min) or vehicle was infused over a 30 min period into the lateral ventricle of rats. At 15 min into the infusion period, the protein tracer horseradish peroxidase (HRP) was injected and perfusion-fixation conducted 15 min later. NMDA infusion (n=6) increased arterial blood pressure (mean 54 ± 5 mmHg). In NMDA infused rats, multifocal regions of HRP extravasation were observed bilaterally throughout the neuraxis. Sites of BBB disruption included the cerebral cortex, hippocampus, thalamus, septum, hypothalamus, and cerebellum. HRP was also detected bordering the lateral and third ventricles. Both swollen and dark-shrunken neuronal cell bodies and processes were seen at leaky sites. Pretreatment with MK-801 (2 mg/kg) attenuated the hypertensive and BBB response to NMDA. In experimental settings where extracellular levels of glutamate are elevated, excessive NMDA receptor activation may contribute to acute vascular and neuronal injury.

315.20

ABNORMAL GLUTAMATE TRANSPORT IN AMYOTROPHIC LATERAL SCLEROSIS: PATHOPHYSIOLOGICAL IMPLICATIONS. RW Kuncl and JD Rothstein. Dept of Neurology, Johns Hopkins Univ. Baltimore, MD 21205

A number of studies have suggested abnormal glutamate metabolism in ALS. Glutamate and glutamate analogs can induce neuronal degeneration *in vivo* and *in vitro*, and could be responsible for chronic human neuronal degenerations. Recently we demonstrated marked increases in CSF glutamate in ALS. Of the various explanations for these findings, it is possible that the pathophysiology of glutamate neurotoxicity in ALS could be excessive synaptic glutamate due to defective glutamate uptake. Therefore, we measured high affinity glutamate uptake in postmortem brain and spinal cord tissue from ALS and control patients. Uptake could be reliably measured up to 12 hours postmortem. Mean age and postmortem delay were similar for both groups. High affinity, sodium-dependent uptake was reliably measured in all specimens. In ALS cervical spinal, cord there was a 65% decrease in the V_{max} for glutamate uptake [0.34 ± 0.06 (SEM) nmoles/min/mg protein, n=8] compared to control [0.98 ± 0.01 nmoles/min/mg protein, n=8]. In some ALS spinal cord tissue, glutamate uptake was decreased as much as 90% compared to control. Motor, sensory, and visual cortex were also assayed. The mean V_{max} for glutamate uptake was significantly decreased 75% in ALS motor cortex compared to control motor cortex. Glutamate uptake in sensory cortex was slightly (but significantly) decreased, but not changed in occipital cortex. The affinity for glutamate was similar, both in ALS and control specimens, whether from spinal cord, motor cortex, sensory cortex, or occipital cortex. These changes were specific for glutamate, since GABA and phenylalanine transport in ALS cortex did not differ from control. These studies provide the first physiological evidence for dysfunction of glutamatergic systems in ALS and have important therapeutic implications.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY IV

316.1

THE ROLE OF GLIAL CELLS IN KAINATE-INDUCED NEURODEGENERATION. Y. Cheng, Q. Bu, P. Wixom and A.Y. Sun. Department of Pharmacology, University of Missouri, Columbia, MO 65212.

Excessive release of excitatory amino acids, such as glutamates can lead to the development of excitotoxicity. Recently, we have demonstrated that the action of glutamate at its presynaptic KA receptor provokes the calcium influx and the release of more glutamate. It is possible that glutamate released from terminals not only binds to postsynaptic receptors, but also feeds back onto presynaptic receptors and thus increases glutamate release. We have used kainate to study this amplification mechanism as caused by glutamate excitotoxicity. Since the glutamate uptake by glutamaterigic terminals and by their surrounding glial cells is the important mechanism for removing glutamate from continuing action at the postsynaptic receptor, the possible interaction of neuron and glial cells on the glutamate excitotoxicity was also investigated. Results indicate that kainate can induce the release of free fatty acids, which are potent inhibitors of both synaptosomal and glial uptake of glutamate. Thus, the neurotoxicity of kainate may involve the potentiation of glutamate acting on the postsynaptic receptor by stimulating glutamate release and inhibiting the high affinity uptake of this excitatory neurotransmitter.

316.2

Differential vulnerability of Ts16 neurons to excitotoxic damage. Paul J. Schwartz and Joseph T. Coyle. Depts. of Neuroscience and Psychiatry, The Johns Hopkins School of Medicine, Baltimore MD 21205

The homology between the 16th mouse and 21st human chromosomes has led to the use of mice trisomic for chromosome 16 (Ts16) as a model of human trisomy 21 (Down syndrome, DS). A consequence of DS is the appearance of pathological and behavioral changes associated with Alzheimer's disease. There are indications that a common pathway for many neurodegenerative disorders is the failure to regulate reactive oxygen species such as the superoxide anion and hydrogen peroxide. Since both the murine 16th and the human 21st chromosomes contain the gene for superoxide dismutase-1 (SOD-1), we have investigated potential differences in the vulnerability of neurons from Ts16 and euploid embryos. We have used both "acute" excitotoxic and long term survival studies to determine the direction and mechanisms of such differences.

Mature (14DIV+) cultures grown on glial monolayers were used for "acute" studies. Long (12h) exposures to kainic acid (KA) showed a subtle dose response shift (trisomic less vulnerable to KA), and initial studies indicate that NMDA affects Ts16 cells less than euploid cells.

Cultures prepared for long term survival were not plated on glial monolayer and drugs were added the day after plating. After 7 days, the trisomic neurons began an accelerated attrition until, at three weeks, the number of Ts16 neurons in untreated cultures was 50% that of euploid controls. This effect could be reversed entirely by 3 uM idebenone (a lipophilic antioxidant) and partially with 10 uM MK801. The control cultures also exhibited some attrition; while maximum survival with idebenone was the same for both trisomic and euploid cells, MK801 was much more efficacious in saving euploid neurons.

316.3

UPTAKE AND DISTRIBUTION OF THE EXCITOTOXIN L-BMAA IN RODENT BRAIN TISSUE. G.E. Kisby, V. Nottingham,* R. Kayton,* and P.S. Spencer. Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR 97201.

β-*N*-Methylamino-L-alanine (BMAA), a weak glutamate-like excitotoxic amino acid, is one of the many potentially toxic agents in cycad seed which is under study as a possible etiological factor for western Pacific ALS/P-D. Studies were initiated to determine if L-BMAA, unlike other excitotoxins, is taken up by CNS tissue *in vitro* and *in vivo*. Mouse cortical explants were treated with 1.6 mM BMAA and L-[-*N*-$^{14}CH_3$]-BMAA (sp. activity 265 μCi/mmol) and tissue levels determined by HPLC and LSC, respectively. BMAA uptake was time-dependent, an indication that the neurotoxicity of this compound may in part be due to its intracellular effects. Similarily, linear uptake of [^{3}H]-BMAA (sp. activity 4.4 mCi/mmol) into mouse brain synaptosomes was sodium, protein, and pH dependent. To determine the regional brain distribution of BMAA, [^{14}C]-BMAA (2.0 μCi) was injected in the right common carotid artery of adult male Sprague-Dawley rats (n = 6) and the radioactivity determined in brain (hippocampus, cortex, striatum, sub-cortex, midbrain, cerebellum) and spinal cord tissue. BMAA differentially distributed within the CNS of rats. These studies demonstrate that BMAA is rapidly taken up by CNS tissue. The intracellular fate of this foreign amino acid is presently under study and preliminary results indicate production of a toxic metabolite. Further study is critically needed before conclusions can be drawn about the potential role of this agent in western Pacific ALS/P-D. [Supported by a grant from AHAF and NS19611]

316.4

β-N-OXALYL-L-α-DIAMINOPROPIONIC ACID (β-L-ODAP) INCREASES GLUTAMINE SYNTHETASE ACTIVITY IN CULTURED RAT ASTROCYTES. S.E. Miller[1], P.B. Nunn[2]*, and R.J. Bridges[3]. Depts. of Psychobiology[1] and Neurology[3], Univ. of California, Irvine, CA 92717 and Dept. of Biochemistry[2], King's College, London WC2R 2LS England.

β-L-ODAP, an amino acid found in the seeds of *Lathyrus sativus*, is believed to be the causative agent of human lathyrism, a disease characterized by a permanent bilateral spastic paralysis. Studies have shown that β-L-ODAP is a potent non-NMDA agonist and is toxic to both neurons and glia. In the present study we have examined glutamine synthetase (GS) activity in astrocytes exposed to β-L-ODAP. This glial-specific enzyme catalyzes the conversion of glutamate and ammonia to glutamine, thus playing a critical role in regulating the levels of two potential CNS toxins.

Type I astrocytes were cultured from 4 day old rats and GS activity was quantitated on the basis of the L-γ-glutamyl hydroxymate transfer reaction. 24 hours after adding β-L-ODAP to the cultures (100 μM initial concentration), GS activity was increased to approximately 140-160% of control activity. This effect was stereospecific, as β-D-ODAP produced no increase. Studies in cell-free preparations with purified GS failed to show any direct activation of GS by β-L-ODAP. When homogenates of β-L-ODAP treated cells were combined with homogenates of untreated cells the resulting GS activity was an average of the two, suggesting that the increased GS activity was not due to the production of an activating factor which modified the existing enzyme. Importantly, this dose of β-L-ODAP did not appear to be gliotoxic as measured by LDH release, suggesting the increased GS activity was not dependent upon astrocyte degeneration. These results imply that the induction of GS activity by β-L-ODAP involves upregulation at the transcriptional or translational level, and may represent an early event in the astrocytic response to high levels of excitatory amino acids.

316.5

NEURONAL DEATH IN THE HIPPOCAMPUS INDUCED BY INTRACEREBRAL INJECTION WITH AN NMDA RECEPTOR AGONIST, L-CCG-IV BUT NOT WITH A METABOTROPIC RECEPTOR AGONIST, L-CCG-I.

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Neurons in the CA1 sector of the hippocampus are susceptible to die several days after cerebral ischemia known as delayed neuronal death (DND). The excitatory neurotoxicity by glutamate has been surmised in the pathogenesis of DND. Here we report that intracerebral injection with a potent NMDA receptor agonist, the (2R,3R,4S) isomer of α-(carboxycyclopropyl)glycine (L-CCG-IV) induced death of the CA1 neurons. Using adult rats under halothane anesthesia, we injected either L-CCG-IV or a metabotropic receptor agonist, the (2S,3S,4S) isomer (L-CCG-I) into the unilateral cortex, CA1 or CA3. In the contralateral side, we injected vehicle for controls. The dose was 50nmole in 1μl vehicle. After L-CCG-I injection and discontinuation of anesthesia, the rats remained calm for several hours. By contrast, L-CCG-IV injection caused clonic twitching of both legs for around one hour. All rats survived for one week and served for histological examinations. The vehicle injection did not cause any neuronal damage except for local gliosis along the needle track. The cerebral cortex, CA1 and CA3 sector injected with L-CCG-I did not show any change, either. However, when L-CCG-IV was injected into either CA1 or CA3 sector, we observed complete loss of neurons and marked gliosis in the CA1 sector. Neurons in the CA3 sector appeared intact but with slight local gliosis even when the agent was injected into the CA3 sector. There was no damage in the cortex. Thus, the pathogenesis of DND might be related with neuroexcitatory damage via the NMDA receptor.

316.6

GLUTAMATE-INDUCED SEIZURES IN ANOXIC AND NORMAL MICE: EFFECT OF NMDA AND AMPA ANTAGONISTS. H. Klitgaard*, U.B. Olsen* and E.B. Nielsen. CNS Division, Novo Nordisk A/S, DK-2880 Bagsvaerd, Denmark.

The mammalian cortex is exquisitely sensitive to anoxia. Recently, excessive release of glutamate has been postulated to be involved in neuronal cell death by generating a lethal extracellular concentration of glutamate. This process is known to be counteracted by NMDA and AMPA antagonists. Therefore, the present study investigated the protective effect of these compounds against glutamate-induced seizures in normal and anoxic mice.

Male NMRI mice (25±2 g) were exposed to 100% nitrogen for 30 sec. After 14-22 sec, the animals became unconscious and after about 45 sec, respiratory movements terminated. After 75 sec, artificial respiration was started resulting in normal respiration and consciousness after about 120 sec and 3-5 min, respectively. Glutamate-induced seizures were evoked in normal and anoxic mice, 30 min after nitrogen treatment, by i.c.v. infusion of 100 μg L-glutamate pr. min according to the i.c.v. infusion method of Steppuhn and Turski (Naunyn-Schmiedeberg's Arch. Pharmacol. 344, 1991). MK-801, CGP 39551 and NBQX were evaluated by co-infusion i.c.v. in a dose range of 0.03-1.0 μg/min.

In general, all three compounds were markedly more potent against glutamate-induced seizures in anoxic mice than in normal mice. In all experiments, NBQX was the most potent compound. Thus, in the anoxic mice, NBQX had an ED_{50} value of 0.08 μg/min against glutamate-induced seizures. This was about ten times lower than the ED_{50} values of MK-801 (0.82 μg/min) and CGP 39551 (0.69 μg/min). In conclusion, anoxia seems to potentiate the protective effect of NMDA and AMPA antagonists against glutamate-induced seizures. Of the compounds tested, the AMPA antagonist NBQX was markedly more potent than the NMDA antagonists MK-801 and CGP 39551.

316.7

A HIGH THROUGHPUT *IN VITRO* BIOASSAY FOR ANTAGONISM AT THE GLYCINE SITE OF THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR. Dwight L. Needels, Brenda J. McKinley*, Joseph A. Cipollina* and Andrew D. Williams*. Cell & Molecular Neurobiology and CNS Chemistry, Bristol-Myers Squibb Co., Wallingford, CT 06492.

The damage to the hippocampus by an ischemic/hypoxic event is thought to be mediated by overstimulation of the postsynaptic NMDA receptor complex and its regulatory glycine site caused by accumulation of glutamate or other excitotoxins. [For review: Choi and Rothman (1990) Ann. Rev. Neurosci. 13:171-182].

Cultured hippocampal neurons have been used for some time as an *in vitro* model for stroke. We describe here the adaptation of such cultures to a specific, high throughput screen for the evaluation of glycine antagonists. Hippocampal neurons were dissected from E18 rat and cultured under serum-free conditions with low endogenous glycine. On day 11 (after neurons had developed sensitivity to NMDA) cultures were exposed to a continuous toxic dose of NMDA in the presence of various concentrations of test drug. Neuronal survival was evaluated 24 hr later by cell counting, which is both labor and time intensive. As neurons die in response to NMDA they lyse, releasing the enzyme lactate dehydrogenase (LDH) which was assayed in parallel to cell counts using a high throughput 96 well plate procedure.

A series of 7-Cl-kynurenate analogs were synthesized and used to evaluate this bioassay for generation of structure/activity relationship (SAR) data for glycine antagonists. A parallel shift in the dose-response curve upon addition of 50 μM glycine is indicative of competitive antagonism. Cell count and LDH release data gave very similar results, but the LDH method was found to provide a more sensitive (and convenient) measure of protection from NMDA toxicity. This neuroprotective bioassay provides a consistently reliable method for evaluation of antagonists at the glycine site of the NMDA receptor.

316.8

GLUCOCORTICOID DOES NOT EXACERBATE EXCITOTOXIC DAMAGE IN THE IMMATURE RAT BRAIN. J.D.E. Barks, Dept of Pediatrics, Section of Newborn Services, Univ. of Michigan, Ann Arbor, MI 48109-0254.

Damage to mature neurons due to a variety of insults is exacerbated by glucocorticoid (GC) exposure. The purpose of this study was to determine whether administration of the synthetic GC dexamethasone (DEX) to infant rats increased the extent of damage due to intrastriatal injection of N-methyl-D-aspartate (NMDA). DEX 0.1 mg/kg (n=13) or vehicle (n=13) was administered i.p. on day 6 of life. Twenty four hours later NMDA 12.5 nmol was injected into the right posterior striatum (CPu) under ether anesthesia. Brains were removed on day 12 of life. Cross sectional areas of CPu, hippocampal formation (HIP), neocortex (CX), and hemisphere (HEMI) ipsi- and contralateral to NMDA injection were measured from Nissl stained sections with a microcomputer image analysis system. Left-right area differences [(L-R)/L] were calculated for all regions and treatment groups were compared using analysis of variance.

Lesions consisting of neuronal loss, gliosis and reduced cross-sectional area of the right CPu as well as overlying HIP and CX resulted in all animals. The reduction in regional cross sectional areas ipsilateral to NMDA injection was not significantly greater in animals pretreated with DEX compared to controls (reduction on right side, DEX vs. control: CPu 30% vs. 33%; HIP 47% vs. 45%; CX 26% vs. 20% ; HEMI 14% vs. 14%). Although DEX pretreatment protects the infant rat brain from hypoxic-ischemic damage (Pediatr. Res., in press, June 1991) this benefit does not extend to excitotoxic injury due to NMDA injection. Nevertheless, glucocorticoids do not appear to enhance the vulnerability of the immature brain to excitotoxic injury.

316.9

Effects of N-methyl-D-aspartic acid in neonatal monosodium glutamate treated rats. C. Ryan[1]*, C. Smythe[2]*, R. Brown[1,2], and M. Wilkinson[2]. Depts. Psychology[1] and Physiology & Biophysics[2], Dalhousie Univ., Halifax, N.S., Canada, B3H 4J1.

Neonatal treatment of rats with acute doses of monosodium glutamate (MSG) accelerates sexual maturation (MacDonald & Wilkinson, Neuroendocr., 1990, 521, 143-149). In the young rat, antagonists of the glutamate analogue N-metyl-D-aspartate (NMDA) exhibit anxiolytic action, while NMDA itself reportedly increases isolation stress in rat pups as measured by ultrasonic vocalization (UV) rate (Windsor et al., Eur. J. Pharmacol., 1990, 190, 11-21). We have investigated the disruptive effect of MSG on behavioral development by giving neonatally MSG treated pups a pharmacological challenge of NMDA. We were interested in whether earlier treatment with MSG would modify the pups response to an NMDA challenge.

At two days of age rat pups (Sprague Dawley) from two soruces (Charles River and Canadian Hybrid Farms) received a single s.c. dose of 2.0 mg/g MSG or saline vehicle. At ten days of age, these two groups were split, half of each receiving a single s.c. dose of NMDA (2.5 mg/kg) or saline. 30 minutes later, pups were assessed on a number of parameters including, ultrasonic vocalizations, core temperature, activity, movement, and negative geotaxis. Animals treated with NMDA showed signs of increased stress including hyperlocomotion, lowered core temperature, and stiff hyperextension of the tail. However, UV rates were not altered. With the exception of UV, NMDA increased behavioral stress responses, confirming its anxiogenic potential and this effect was modified by neonatal MSG treatment, particularly in the CHF strain.

316.10

MK-801 PREVENTS BODY AND ORGAN WEIGHT REDUCTIONS IN THE ADULT RAT RESULTING FROM NEONATAL ADMINISTRATION OF MONOSODIUM GLUTAMATE. J. Kleim*, M. Saari, K. Fisher*, R. Turner*, M. McIsaac*, & E. Bowen*. Neuroscience Research Unit, Nipissing University, North Bay, Ont. Canada.

On day 4 postpartum, half of 48 male rat pups were injected (s.c.) with 1mg/Kg of MK-801 and the other half with an equivalent volume of physiological saline. Thirty minutes later, half of each group were then injected with 4g/Kg of MSG (s.c.) and the other half with an equivalent volume of physiological saline, yielding 4 treatment groups: MK/MSG, MK/SAL, SAL/MSG and SAL/SAL. On day 70 postpartum, the animals were sacrificed via CO_2 asphyxiation and the carcasses immediately dissected. Results show that MK-801 blocks the reduction in brain, liver, testis and body weights associated with neonatal exposure to MSG. The possible adverse effects of MK-801 on organ development are discussed.

316.11

UBIQUINONE PROTECTS CEREBELLAR GRANULE CELLS AGAINST GLUTAMATE-INDUCED CYTOTOXICITY. A. Favit*, F. Nicoletti, U. Scapagnini* and P.L. Canonico. Institute of Pharmacology, University of Catania, 95100, Catania, Italy.

Ubiquinone (UBI) or coenzyme Q is an endogenous quinone with pharmacological actions probably related to its antioxidant properties. Here we report that UBI protects cultured cerebellar granule cells against glutamate-induced neurotoxicity. In control cultures at 8-9 DIV, a 20-min exposure to 100 μM glutamate induced neuronal degeneration, as reflected by the high percentage of cells labelled with propidium iodide (>90%) 24 hours after the exposure. Glutamate-induced neuronal death was dramatically reduced in cultures treated daily with UBI (1 μM) since the 1st day of maturation. In these cultures, glutamate failed to induce a "delayed" increase in the basal influx of $^{45}Ca^{2+}$, an established parameter of excitotoxicity. Similarly, repeated addition of UBI attenuated the age-dependent degeneration of granule cells that occurs after 10-12 days of maturation in culture, due to the toxic action of endogenous glutamate progressively released into the medium. These results suggest that UBI may be a useful drug in the therapy of acute and chronic degenerative diseases of the Central Nervous System.

316.12

OXIDATION OF THE NMDA RECEPTOR REDOX MODULATORY SITE BY PYRROLOQUINOLINE QUINONE (PQQ).

E. Aizenman, K.A. Hartnett, C. Zhong*, P.M. Gallop* & P.A. Rosenberg U. of Pittsburgh Sch. of Med., Pgh, PA 15261 and Children's Hosp. and Harvard Med. Sch., Boston, MA 02115.

The putative nutrient PQQ can efficiently mediate reduction and oxidation reactions in a variety of systems. Therefore, we investigated whether this compound could alter the function of the N-methyl-D-aspartate (NMDA) receptor via its redox modulatory site (Aizenman et al., *Neuron* 2:1257; 1989). In rat cortical neurons *in vitro* PQQ (50 μM) could reverse the enhancement of NMDA-induced whole-cell ionic currents produced by the reducing agent dithiothreitol (DTT; 2-4 mM), as well as depress native responses in a DTT-reversible fashion. PQQ mediated oxidation may occur via the generation of oxygen free radicals and we have observed that these species are effective at oxidizing the NMDA receptor redox site (Aizenman et al., *Neuron* 5:841; 1990). However, inclusion of 20 μg/ml each of superoxide dismutase and catalase did not prevent the oxidizing actions of PQQ. Finally, 50-200 μM PQQ was also observed to produce a significant degree of neuroprotection in an acute model of NMDA-mediated neurotoxicity in astrocyte-rich cultures of rat cerebral cortex. These results suggest a novel role for PQQ, PQQ-like substances, and for quinone containing proteins in the brain, and may represent a novel therapeutic approach for the amelioration of NMDA receptor-mediated neurotoxic injury.

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316.13

THE PUTATIVE ESSENTIAL NUTRIENT PQQ PROTECTS AGAINST NMDA NEUROTOXICITY IN ASTROCYTE-POOR CORTICAL CULTURES. P.A. Rosenberg, C. Zhong*, P.M. Gallop*, and E. Aizenman. Children's Hosp. & Harvard Med. Sch., Boston, MA 02115; Dept. Phys., U. Pittsburgh Med. Sch., Pgh, PA 15261.

We have examined the actions of the soluble redox cofactor pyrroloquinoline quinone (PQQ) upon NMDA neurotoxicity in astrocyte-poor rat cortical cultures in which drugs and enzymes in the medium have free access to dendrites. Exposure of cultures to the reducing agent dithiothreitol (DTT; 1-2 mM) increased the sensitivity of cortical neurons to a thirty minute exposure to 50-75 μM NMDA. Exposure to 50 μM PQQ protected neurons exposed to DTT against NMDA toxicity. This effect of PQQ could not be blocked by superoxide dismutase (SOD; 10 μg/ml) and catalase (CAT; 10 μg/ml). Exposure to PQQ following DTT, without extensive washing between these steps, resulted in delayed neurotoxicity independent of NMDA receptor activation which could be blocked by SOD and CAT. We conclude that one mechanism of action of PQQ involves direct interaction with the redox modulatory site. The possibility of an indirect action, e.g. via formation of oxygen-derived free radicals, has not been excluded, and would be expected in the presence of a suitable electron donor.

316.14

AMELIORATION OF GLUTAMATE TOXICITY BY KYNURENIC ACID: ^{31}P NMR SPECTROSCOPY STUDIES OF PERFUSED, LIVE RAT CEREBROCORTICAL SLICES. M.T. Espanol*, G.Y. Yang*, L. Litt, P.R. Weinstein, P. Chan and T.L. James*. Departments of Pharmaceutical Chemistry, Anesthesia, Neurosurgery, Radiology, and CVRI, University of California, San Francisco, CA 94143.

Glutamate (glu) is toxic to neurons in acute ischemia. Metabolic toxicity of 60 min exposures to glu was studied with ^{31}P NMR spectroscopy in live, normoxic cerebrocortical slices (350 μ) obtained from neonatal Sprague Dawley rats. Within 5 min after initiating exposures to glutamate concentrations above 1 mM, intracellular decreases were seen in pH, phosphocreatine (PCr), and adenosine triphosphate (ATP), while an increase was seen in inorganic phosphate (P_i). After discontinuation of glu $\leq$ 1 mM, ^{31}P metabolites recovered to control levels. In contrast, after 60 min exposure to $\geq$ 5 mM glu, no recovery was observed during or after 60 min reperfusion with normal medium. Partial recovery of ^{31}P metabolites was observed for brain slices exposed to 2 and 3 mM glu. Kynurenic acid (kynu), a glu receptor antagonist, was administered with the same glu protocols. Exposure of slices to both 3 mM glu and 1 mM kynu produced delays in the rates of decrease of pH, PCr, and ATP and a delay in the rate of increase of P_i. The results demonstrate that deleterious changes to the ^{31}P NMR energy profile of slices occurs with administration of glu, and that addition of a glu receptor antagonist has protective effects. The study directly links dysfunctions in brain energy metabolism with glu neurotoxicity, which is widely acknowledged to be associated with Ca^{++} influx and subsequent amplification of Ca^{++}-associated events.

316.15

NMDA NEUROTOXICITY AND ITS ENHANCEMENT BY DITHIOTHREITOL IS ANTAGONIZED BY CGS-19755.

Karen A. Hartnett, Hai N. Vo* and Elias Aizenman, Dept. of Physiology, Univ. of Pittsburgh Sch. Med. Pittsburgh, PA 15216.

The neuroprotective properties of the novel NMDA receptor antagonist CGS-19755 (*cis*-4-phsphonomethyl-2-piperidine carboxylic acid) were studied in cultures of rat cerebral cortex under normal and altered redox conditions (Aizenman et al., *Neuron* **2**: 1257, 1989). This antagonist was effective in preventing neurotoxicity induced by a prolonged (24-72 hr) exposure to 500 μM glutamate with an EC_{50} near 5 μM. Furthermore, 100 μM CGS-19755 was also effective in preventing delayed neuronal death produced by an acute (5 min) exposure to either glutamate (500 μM) or NMDA (500 μM). In contrast, toxicity induced by a prolonged exposure to kainate (500 μM) was not blocked by 100 μM of this antagonist. We observed that the reducing agent dithiothreitol (DTT; 500 μM)), but not oxidized DTT, could dramatically enhance the toxicity and electrophysiological responses produced by 50 μM NMDA, and that CGS-19755 (100 μM) was effective in blocking these effects. Any toxicity produced by higher concentrations of DTT alone were also antagonized by CGS-19755. These results indicate that CGS-19755 is an effective and specific neuroprotectant acting at the NMDA receptor, and that the enhancement in toxicity induced by DTT is mediated by an increase in activity at this receptor complex.

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316.16

ANIRACETAM PREVENTS GLUTAMATE-INDUCED NEUROTOXICITY.

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The effect of aniracetam, a novel nootropic drug, was studied on the neuronal damage induced by different glutamate receptor activation in a preparation of rat cerebellar granule cells. We observed that the rapid cell loss induced by 15 min application of 50 µM glutamate was significantly prevented by the addition of aniracetam at concentrations higher than 1 µM. Moreover, aniracetam counteracted the glutamate-mediated slow neurotoxicity reproduced by 24 h application of 20 µM AMPA or 60 µM kainate.

The maximal effect for neuroprotection was obtained with 7 µM aniracetam. Higher concentrations were less effective.

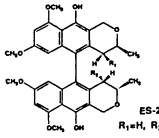
Finally, aniracetam greatly potentiated the protective effect elicited by quisqualate on both AMPA- and kainate-induced neuronal death. A role for aniracetam in potentiating the quisqualate metabotropic receptor transmission will be discussed.

316.17

THE DISCOVERY OF ES-242-1 FROM A MICROBIAL SOURCE AND ITS CHARACTERIZATION AS A NOVEL TYPE OF NMDA ANTAGONIST.

Y. Matsuda, S. Toki*, M. Nozawa*, M. Yoshida*, H. Sano, K. Ando*, and I. Kawamoto*. Kyowa Hakko Kogyo Co.,3-6-6 Asahimachi, Machida-shi, Tokyo 194. Japan.

We isolated ES-242-1, a novel bioxanthracene, from the culture broth of *Verticillium* sp. ES-242-1 competitively inhibited the binding of [^{3}H]MK-801 to NMDA receptor complex in rat brain synaptic membrane fractions with an IC_{50} =66nM. It also competitively inhibited the binding of [^{3}H] CGS19755 with an IC_{50}=1.1μM, while it did not inhibit the binding of [^{3}H]glycine, [^{3}H]kainate, [^{3}H]AMPA, or[^{3}H]3-PPP .These data show that HS-142-1 specifically interacts with both the glutamate recognition site and the channel domain . Using a histofluorescence staining method, we examined the protective effects of ES-242-1 on glutamate-induced neuronal death in primary cultures of hippocampal neurons prepared from fetal mice. ES-242-1 significantly blocked the glutamate-induced neurotoxicity to a similar extent as MK-801, but not nuerotoxicity induced by kainate or quisqualate. ES-242-1 is a new chemical existence with a novel mechanism of action at the NMDA receptor, and as such may provide a new tool for understanding the molecular pharmacology of this receptor and may possess neuroprotective properties useful in the treatment of disease involving glutamate toxicity.



ES-242-1 : R_1=H, R_2=$OCOCH_3$

316.18

Mg^{2+} AND ACUTE EXCITOTOXICITY ASSOCIATED WITH METABOLIC STRESS.

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Several laboratories have demonstrated the involvement of the NMDA receptor during metabolic stress. Data from our laboratory indicate that changes in the physiological state of the receptor rather than increases in extracellular glutamate or aspartate (EAAs) may be responsible for activation of the receptor such that the receptor could become activated by low endogenous or ambient levels of EAAs when energy stores are compromised. One hypothesis for the mechanism by which this may occur is by mitigation of the voltage dependent Mg^{2+} blockade of the NMDA channel caused by a deteriorating membrane potential (MP). Previous studies in which MP was titrated with increasing extracellular K^+ supported this proposal since gradually decreasing the MP caused NMDA-mediated acute excitotoxic cell swelling and GABA release in embryonic chick retina without any net increase in extracellular EAAs (JPET 253, 1991). The above hypothesis was further tested in the present study by examining the dose response characteristics for NMDA, glutamate and kainate in the presence or absence of Mg^{2+} and by examining the effects of 0 Mg^{2+} on pharmacologically induced metabolic inhibition. The studies showed that removal of Mg^{2+} greatly decreased the minimal concentration needed to produce acute excitotoxicity in retina; 25 versus 5 µM for NMDA and 300 versus 10 µM for glutamate in 1.2 and 0 mM Mg^{2+}, respectively, and shifted the EC_{50} for swelling induced GABA release to the left for both agonists. Mg^{2+} removal had no effect on kainate induced toxicity. Acute swelling and GABA release caused by pharmacological inhibition of metabolism was not potentiated in the absence of Mg^{2+} and was attenuated by high Mg^{2+} (20 mM) consistent with the proposal that the Mg^{2+} block is lifted during metabolic inhibition.

316.19

SELECTIVE DESTRUCTION OF THE THALAMIC RETICULAR NUCLEUS BY DOMOIC OR KAINIC ACID

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Domoic acid (DA), a potent analogue of the excitatory amino acid kainic acid (KA), produces memory and attentional deficits in humans who have ingested shell fish contaminated with this excitotoxin. The thalamic reticular nucleus (RT), by virtue of its location, connections and intrinsic properties, has been implicated as playing a major role in attentional processes. This study was designed to establish 1) whether the RT was vulnerable to DA toxicity and 2) whether complete and selective lesions of the RT could be produced with intrathalamic KA or DA injections.

Adult Long Evans rats anesthetized with chloryal hydrate were given stereotaxic injections (4 x 0.025µl each) of either 0.1% DA or 0.5% KA at the coordinates of their RT and allowed to survive for 7 days. RT lesions were characterized by almost total neuronal loss and dense microglial proliferation throughout the entire RT. In most cases neuronal loss was restricted entirely to the RT and relay neurons in adjacent regions of the VPL were spared. In a few cases where the injections were too large, too fast ,or too medial the lesions extended into the VPL or VPM.

Upon awakening from anesthesia the rats exhibited hyperactive-stereotyped paddeling movements that evolved into brief episodes of ballistic jumping followed by periods of hypoactivity with intermitent bursts of vigorous-awkward grooming and wet dog shakes. The rats were disoriented and nonresponsive to vibrissal stimulation. One day later rats were hypoactive with decreased whisking and exploratory behavior, and a diminished responsiveness to visual and tactile stimulation.

The death of RT neurons has been postulated to be the organic basis for some types of post traumatic and post ischemic attentional and sensory processing deficits. Selective RT lesions produced by either KA or DA may be an efficacious means with which to study the neurobehavioral role of the RT (Supported by NIH Grant 5-T32-MH 18902 {EBF}; NIH BRSG 2-507-RR05415-29, and R01 NS-28852 {DTR})

316.20

ANTAGONISM OF Ca^{+2}-DEPENDENT GLUTAMATE RELEASE BY SUBSTITUTED GUANIDINES.

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^{3}H-glutamate (GLU) release from rat brain synaptosomes was resolved with ~60 msec time resolution by rapid superfusion (c.f. Turner et al. [1989] Anal. Biochem. 178, 8). Three distinct components of GLU release stimulated by K^+-depolarization were observed: a rapidly decaying ("phasic") Ca-dependent component (decay constant <100 msec), a more persistent Ca-dependent component, and a Ca-independent component that may result from reversal of the Na-dependent GLU uptake system. Several substituted guanidines were found to selectively block Ca-dependent GLU release at 10 uM concentrations. The ability of these compounds to block K^+-stimulated synaptosomal ^{45}Ca uptake, correlated well with their ability to block the persistant (r^2=0.965) but not the phasic (r^2=0.364) component of Ca-dependent GLU release. CNS 1029 (10 uM) selectively blocked persistent vs phasic GLU release. Whole cell recordings from cultured neurons from rat parasympathetic ganglia during extracellular perfusion with CNS 1029 revealed inhibition of depolarization-activated high threshhold Ca currents, with a K_i of ~10 uM; in the same preparation, no significant effect of CNS 1029 on voltage-gated Na or K currents were observed. We hypothesize that blockade of GLU release in the synaptosomal preparation is due to antagonism of a subclass of Ca channels present in glutamatergic nerve terminals. These compounds are potential attentuators of glutamate excitotoxicity *in vivo*. Selective block of the persistent vs. the phasic component of GLU release may be a desirable characteristic for a neuroprotective agent.

317.1

LOCALIZATION OF GLUTAMATE RECEPTOR TYPES IN THE RAT BRAIN USING CHARACTERIZED ANTIPEPTIDE ANTIBODIES. R. S. Petralia and R. J. Wenthold, Lab. of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892.

Antibodies were made to synthetic peptides corresponding to the C-terminal segments of the glutamate receptor (GluR). These peptides were SHSSGMPLGATGL, EGYNVYGIESVKI, and RQSSGLAVIASDLP for GluR A, B/C, and D, respectively. Peptides were conjugated to BSA with glutaraldehyde and injected into rabbits. The antibodies were affinity purified using the peptides, either the entire peptide or shorter portions of it, covalently attached to agarose. Antibodies were characterized with respect to their immunoprecipitation of solubilized ^{3}H-AMPA binding activity and their staining patterns on western blots. Antibodies to GluR A, B/C, and D immunoprecipitated 28%, 56%, and 13% of solubilized ^{3}H-AMPA binding activity, respectively. On immunoblots of rat brain samples separated on SDS polyacrylamide gels, each antibody showed an immunoreactive band corresponding to the GluR subunit. Antibodies to GluR B/C often showed a poorly resolved dimer.

Immunocytochemistry was carried out on vibratome sections of rat brain fixed with 4% paraformaldehyde with or without 0.1% glutaraldehyde using avidin-biotin-peroxidase visualization. Highest labelling was found in the hippocampus, olfactory bulb and cerebellum. In contrast, labelling was moderate to low in many regions of the brainstem. Often, distinct differences in staining were evident among the 3 antibodies. For example, in the cerebellum, antibodies to A and D stained Bergmann glia, while the granule cell layer stained with antibodies to B/C and D.

Ultrastructural studies in the cerebrum and hippocampus revealed antibody labelling for all 3 receptor types localized in the postsynaptic densities, as well as in the cytoplasm of the dendritic processes. Typically, labelled synapses contained asymmetric densities and round vesicles. No presynaptic labelling was evident.

317.2

BIOCHEMICAL CHARACTERIZATION AND LOCALIZATION OF NON-NMDA GLUTAMATE RECEPTOR IN THE RAT BRAIN. C.D. Blackstone, S.J. Moss*, L.J. Martin, A.I. Levey, D.L. Price and R.L. Huganir, Dept. of Neuroscience, Howard Hughes Medical Inst., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

The structure and distribution of non-NMDA glutamate receptors in the rat brain were studied using subunit-specific antibodies for the receptor subunit GluR1. This subunit is found to be a 106 kD glycoprotein in human 293 cells transfected with the GluR1 cDNA as well as the rat brain. Immunoblotting of dissected brain regions shows an enrichment in the hippocampus and cerebellum. Immunocytochemical localization demonstrates a heterogeneous distribution on neurons within the CNS, closely matching the results of [^{3}H]AMPA autoradiography. Interestingly, in the cerebellum GluR1 is localized to the Bergmann glial processes. When GluR1 is solubilized with CHAPS, both high-affinity AMPA binding and GluR1 immunoreactivity comigrate at a M$_r$=610,000 by gel-exclusion chromatography and sucrose gradient density centrifugation. Purification of a non-NMDA glutamate receptor by domoic acid affinity chromatography reveals a single band at 106 kD on SDS-PAGE by silver staining. This protein band is recognized by the specific GluR1 antibodies. These data suggest that GluR1 is a subunit of an oligomeric AMPA-preferring glutamate receptor, most likely a pentamer of similarly-sized subunits, which has roles in neuronal and glial signaling.

317.3

LOCALISATION OF A RAT METABOTROPIC GLUTAMATE RECEPTOR mRNA IN RAT BRAIN BY IN SITU HYBRIDIZATION. Peter Kristensen*#, Eileen Mulvihill*§, Betty Haldeman*§, Teresa Gilbert*§ and Birgitte Guldhammer#. #CNS Division, Novo Nordisk, Måløv, Denmark. §ZymoGenetics Inc., Seattle, WA 98105.

A cDNA coding for a G-protein coupled (metabotropic) glutamate receptor was isolated by expression cloning in frog oocytes (Houamed et al.,. Science, in press (1991)). Two non-overlapping subclones of this cDNA was used to prepare RNA probes for in situ hybridization. 35-S labeled anti-sense and sense probes was employed on adjacent sections of rat brain. Expression was found in cerebellum, olfactory bulb, tenia tecta, hippocampus, diagonal band nucleus, caudate putamen, lateral septal nucleus, medial geniculate nucleus, reticular part of substantia nigra, the red nucleus and a number of brain stem and thalamic nuclei. Lower, but definite expression was seen in cerebral cortex.

317.4

LOCALIZATION OF GLU-A RECEPTOR mRNA IN THE SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA. Ma-Li Wong and Ariel Y. Deutch. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06510 and Psychiatry Service, Veterans Affairs Medical Center, West Haven, CT 06516.

Transmitter release from dopamine (DA) neurons innervating the striatal complex can occur secondary to an increase in the firing rate of DA neurons, or alternatively can occur via presynaptic (impulse-independent) regulation at the axon terminal. An example of the latter mode is the release of striatal DA in response to excitatory amino acids such as glutamate. In an effort to determine the localization of excitatory amino acid receptors in the striatal complex, we have examined the distribution of an AMPA family receptor (Glu A) mRNA. Oligonucleotide probes complementary to the Glu A receptor mRNA (flop) were used for *in situ* hybridization histochemistry. This Glu A mRNA was localized to a variety of CNS sites, including the ventral mesencephalon. Glu A receptor gene expression was observed throughout the pars compacta of the substantia nigra. In addition, Glu A mRNA was present in the ventral tegmental area, particularly within the dorsolateral (nuc. parabrachialis pigmentosus) region. Studies are under way to confirm that this receptor is expressed specifically in DA neurons. These data suggest that glutamate and other excitatory amino acids may presynaptically elicit DA release via interactions with a KA/quisqualate type excitatory amino acid. Supported by MH-45124, the Schizophrenia Res. Ctr. of the West Haven VAMC, and the Natl. Parkinson Foundation Center at Yale Univ.

317.5

GLUTAMATE RECEPTOR GENE EXPRESSION IN DEVELOPING RAT BRAIN: AN *IN SITU* HYBRIDIZATION STUDY. R.S. Zukin, M.Y.L. Bennett and D.E. Pellegrini-Giampietro. Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

Glutamate receptors are thought to play a major role in synaptic plasticity, neuronal survival and excitotoxicity in the developing brain. The present study examined the developmental pattern of GluR-1, -2, and -3 gene expression in rat brain. *In situ* hybridization revealed different spatial patterns throughout the brain for the related mRNAs at all ages examined, as well as different temporal patterns during development. In adult all mRNAs were expressed prominently in the pyramidal and granule layers of hippocampus and in the Purkinje cell layer of cerebellum, where detailed differences among probes were apparent at the cellular level. In neocortex, GluR-2 mRNA exhibited prominent lamination and regional differences which were less marked for GluR-1 and -3 mRNAs. In caudate-putamen GluR-2 mRNA was at high levels; but GluR-1 and -3 were not. At early ages transcripts were transiently elevated relative to adult levels. GluR-1 mRNA reached peak expression in cortex at P14 (225% of adult), striatum at P4 (255% of adult), hippocampus at P14 (195% of adult), and cerebellum at P21 (150% of adult). GluR-3 exhibited more modest peaks in neocortex and hippocampus. In contrast, GluR-2 message was at near adult levels at P4 and exhibited a peak only in cerebellum at P14 (168% of adult). GluR-1 and -3 homomeric channels are permeable to Ca^{2+}, heteromers with the GluR-2 subunit are not (McGurk et al., this meeting). Thus, cells in specific brain regions may contain glutamate receptors that differ significantly in their functional properties at different stages in development depending on whether they express GluR-2 mRNA.

317.6

GLUTAMATE RECEPTOR GENE EXPRESSION IN KINDLED RATS. S.L. Moshé, D.E. Pellegrini-Giampietro, E.F. Sperber, S.G. Xu, M.Y.L. Bennett and R.S. Zukin. Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Glutamate receptors have been implicated in the establishment of kindling, an animal model of chronic epilepsy. Glutamate receptor antagonists can retard the development and block the expression of kindling, and receptor binding studies indicate that both NMDA and non-NMDA type glutamate receptors are altered in the hippocampus following kindling. This study was conducted to determine whether the receptor changes are associated with alterations in non-NMDA glutamate receptor gene expression. *In situ* hybridization was used to examine the levels of GluR-1, -2 and -3 mRNAs (encoding channels activated by both kainate and AMPA) in amygdala kindled as compared to control rats. Kindling was produced with stimulations (1 sec train, 400 μA peak to peak, 60 Hz sine wave current) administered 3 times daily. Upon acquisition of three stage 5 kindled seizures, stimulations were terminated and rats were sacrificed 3 or 15 days later. Sections (20 μm) from the brains of kindled and control rats were hybridized at high stringency with ^{35}S-labeled riboprobes transcribed from GluR-1, -2, and -3 cDNAs (10^6cpm/section). Three days after development of kindling, the levels of all 3 mRNAs were increased in the granule cells of the dentate gyrus relative to levels in control rats. Conversely, after 15 days, labeling (especially for GluR-2) was decreased markedly in all regions studied, including hippocampus and neocortex. These results indicate that alterations in kainate/AMPA-type glutamate receptor expression may play a causal role in kindling.

317.7

GLUTAMATE RECEPTOR GENE EXPRESSION IN NEONATE AND ADULT RATS FOLLOWING FLUROTHYL- AND KAINATE-INDUCED SEIZURES. L.K. Friedman, D.E. Pellegrini-Giampietro, E.F. Sperber, M.V.L. Bennett, S.L. Moshé and R.S. Zukin. Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Glutamate receptors are thought to play a role in epilepsy. NMDA and non-NMDA receptor blockers possess anticonvulsant properties and can prevent seizure-induced brain damage. Moreover, seizures induced in adult animals by any of a number of agents result in brain damage resembling glutamate cytopathology and in glutamate receptor loss. An important question is whether these changes are associated with altered glutamate receptor gene expression. *In situ* hybridization was used to measure levels of GluR-1, -2 and -3 mRNAs (which encode channels activated by both kainate and AMPA) in status epilepticus induced by systemic administration of (i) flurothyl in 15 day old rat pups and by (ii) kainate in pups and adults. Brain sections from control and seizure-induced animals were hybridized with ^{35}S-labeled riboprobes transcribed from GluR-1, -2 and, -3 cDNAs. Morphological lesions and levels of mRNAs were monitored in film autoradiograms and emulsion-dipped sections. No morphological or mRNA changes were seen in brain sections from flurothyl- or kainate-treated neonates. Selective brain damage was observed in limbic regions of adult rats following kainate-induced seizures. In these animals all 3 receptor mRNAs were virtually absent in the CA3 field of the hippocampus, presumably due to cell loss. However, increased labeling (most prominent for GluR-3) was observed in granule cells of the dentate gyrus, which project to CA3. This increase in expression may be related to kainate-induced destruction of CA3 cells and may lead to synaptic reorganization. Thus, the different patterns of neurodegeneration in adults vs. neonates after seizure may lead to differences in glutamate receptor gene expression.

317.8

GLUTAMATE RECEPTOR GENE EXPRESSION IN NEURODEGENERATIVE DISEASES. D.E. Pellegrini-Giampietro, Bennet M.V.L. and R.S. Zukin. Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

Excitatory amino acid (EAA) systems are thought to play a major role in the pathogenesis of neurodegenerative diseases such as Huntington's disease (HD) and Alzheimer's disease (AD). Neurotoxins acting at EAA receptors produce lesions closely resembling those seen in these pathologies, and EAA receptor numbers are altered in autopsy tissue from HD and AD patients. *In situ* hybridization experiments were undertaken to examine the levels of GluR-1, -2, and -3 mRNAs (encoding channels activated by kainate and AMPA) in normal and diseased human brain. Sections (20 μm) of human autopsy material were hybridized under conditions of high stringency with ^{35}S-labeled GluR-1, -2, and -3 riboprobes transcribed from rat brain cDNA. Autoradiograms of human brain hippocampal sections hybridized with riboprobes for GluR-1,-2, and -3 mRNAs displayed selective labeling over dentate gyrus, pyramidal cells of Ammon's horn, subiculum, and entorhinal cortex. Striatal sections also displayed specific glutamate receptor mRNA hybridization. In emulsion dipped sections labeling was observed at the cellular level in dentate gyrus granule cells and in CA1 and CA3 pyramidal cells. Competition experiments indicated the virtual absence of cross-hybridization under these labeling conditions. Brain tissue from HD and AD patients is also being examined. To date, labeling was found to be significantly lower in AD hippocampal sections relative to control autopsy tissue from age- and sex-matched controls. These studies should provide new insights into mechanisms underlying these neurodegenerative diseases.

317.9

GLUTAMATE RECEPTOR GENE EXPRESSION IN DEVELOPING RAT BRAIN: A NORTHERN ANALYSIS STUDY. G.M. Durand, M.V.L. Bennett and R.S. Zukin. Dept. of Neuroscience, Albert Einstein College of Med., Bronx, NY 10461.

The recent cloning and sequencing of cDNAs encoding a family of non-N-methyl-D-aspartate (NMDA) glutamate receptors indicate that these belong to the large superfamily of ion channel forming receptors. We examined the developmental pattern of GluR-1, -2, and -3 gene expression in embryonic and neonatal rat brain by Northern analysis using full-length ^{32}P-labeled riboprobes under conditions of high stringency. Northern analysis with GluR-1 and -3 riboprobes revealed the presence of an abundant 5.2 kb RNA species and minor bands of ~ 3.2 and ~ 3.9 kb in poly(A)$^+$RNA isolated from all ages and regions examined. In contrast, hybridization of these same samples with the GluR-2 riboprobe revealed two RNA species, ~ 5.9 and ~ 3.9 kb, of about equal abundance. The ratio of the 5.9 to 3.9 kb species labeled by GluR-2 remained nearly constant in all tissues and all ages examined. In the developing animal the GluR-1 5.2 kb species was first detected in whole brain mRNA at embryonic day 16; highest expression (normalized to 18s rRNA) was observed at postnatal day 28 (P28) (133% adult level). GluR-1 mRNA reached peak expression at P28 in hippocampus (~ 200% of adult) and in cerebellum (~ 300% of adult). GluR-2 and GluR-3 mRNAs reached their peak expression (>200% of adult) at P28 in hippocampus and neocortex. These findings show differential regulation of GluR mRNAs during development with transient elevation of specific mRNAs in specific regions.

317.10

GLUTAMATE RECEPTOR BINDING IN THE FROG BRAIN. H.T. Cline, J. McDonald, M. Constantine-Paton. Dept Physiology & Biophysics, Univ. Iowa; Dept Neuroscience, U. Michigan; Dept Biology, Yale Univ.

We used receptor binding techniques to map the presence of Quis and NMDA type glutamate receptors in the brains of postmetamorphic and adult frogs. The highest Q binding was seen in telencephalon, pallium (the frog's version of the hippocampus), ventral hypothalamus, basal optic nucleus, preoptic area and cerebellum, all of which have 25-33 pmol/mg protein. The highest N binding was seen in telencephalon, cerebellum and preoptic area, which have 2.4 - 3.9 pmol/mg protein. We determined to ratio of N binding to Q binding for postmets and adults as a possible indicator of the relative degree of plasticity of the structure. In most brain regions (ie telencephalon, torus, optic nerve, layer 6 of optic tectum) the N/Q binding ratio is about 0.1 in both postmets and adults. However, in the superficial neuropil layer of the optic tectum, where NMDA receptor-mediated plasticity has been shown to occur, N binding is enriched relative to Q binding in postmets. Here the N/Q ratio is .256. In the adult the tectal neuropil and the lower rim cortex of the nucleus isthmi both share the highest N/Q ratios of .18. The deep layers of the tectum, which receive auditory input, display an elevated N/Q ratio of .18 in postmets. This value returns to about 0.1 in adults. Interestingly, significant Q binding is detected in several axon pathways, including the optic nerve (18.2 pmol/mg protein), tectal commisssure (5.6 pmol/mg protein) and tecto-bulbar commissure (19 pmol/mg protein). NMDA binding could not be detected in these regions. Supported by NIH EY05818 (HTC), EY06039 (MCP), MSTP Grant 5 T326M07863-07 (JM).

317.11

EXCITATORY AMINO ACID BINDING SITES IN THE SPINAL TRIGEMINAL NUCLEUS OF RAT AND HUMAN S.J. Tallaksen-Greene[1], A.B. Young[1], J.B. Penney[1], and A.J. Beitz[2] [1]Department of Neurology, University of Michigan, Ann Arbor, MI 48104-1687 and [2]Department of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

The spinal trigeminal nucleus (STN) is involved in the processing of orofacial sensory information. Previous studies suggest that excitatory amino acids (EAAs) are contained within trigeminal primary afferent neurons and may play an important transmitter role in the processing of nociceptive information. We have examined the distribution of excitatory amino acid binding sites in the STN of rat and human using *in vitro* autoradiography.

Within the subnucleus caudalis of both rat and human, the outer laminae (I and II) contained a high density of NMDA, ionotropic-quisqualate (AMPA), metabotropic-quisqualate and kainate binding sites. This region has been shown to receive a major input from nociceptive and thermal receptors. In contrast, the density of EAA receptors was generally very low within the inner magnocellular portion of nucleus caudalis (laminae III and IV), regions known to contain wide-dynamic range neurons and low-threshold mechanoreceptive neurons. Likewise, low densities of EAA binding sites were observed in rat STN subnuclei interpolaris and oralis. These findings support the hypothesis that excitatory amino acids are neurotransmitters involved in the processing of orofacial nociceptive information.

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317.12

DENSITY AND DISTRIBUTION OF GLUTAMATE RECEPTORS IN THE DEVELOPING HUMAN BRAIN: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. Haesung Lee* and Ben H. Choi. Ewha Women's University, Seoul, Korea and University of California, Irvine, CA 92717.

Developmental changes in glutamatergic innervation of the CNS would provide valuable information not only of normal maturation but also of age-dependent selective vulnerability of the CNS to various insults. Using in-vitro autoradiography, we have examined the status of the N-methyl-D-aspartate (NMDA), kainate (KA), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the brains of 18- and 26-week-old aborted human fetuses. Densities in outer and inner layers of 6 cortical regions including hippocampus, basal ganglia and thalamus were quantified (fmol/mg protein) using computer-assisted image analysis system. Regional heterogeneity and age-related changes in each receptor subtype were apparent. For example, AMPA densities were highest as compared to KA or NMDA at both ages. Highest labeling of all receptor subtypes was noted in the hippocampus at both ages. By 26 weeks, there was a significant increase in AMPA receptor densities in all regions, however, both NMDA and KA receptor densities were significantly lower as compared to those in 18-week-old fetus. In 18-week-old fetus, both KA and AMPA were prominently labeled in the basal ganglia, subthalamic nucleus and external lamina of the thalamus. NMDA densities, on the other hand, were prominent only in the ventral nuclei of the thalamus. In 26-week-old fetus, AMPA labeling was more in the outer cortical layers whereas NMDA and KA densities were significantly higher in the deeper cortical layers. These results provide essential baseline data for our understanding of the developmental plasticity as well as excitotoxic damage in the CNS. (Supported in part by NIH grant ES02928).

317.13

AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-MK 801 BINDING SITES IN THE RAT BRAIN. S. Kito, R. Miyoshi and T. Nomoto*. Division of Health Sciences, University of the Air, Chiba 260 and Department of Pharmacology, Tokyo Women's Medical College, Tokyo 162, Japan.

It has been advocated that the N-methyl-D-aspartate (NMDA) receptor complex consists of an agonist recognition site, a strychnine-insensitive glycine modulatory site and a cation channel. To detect the channel activity, MK 801 has become a useful tool. In the present study, in vitro autoradiographic studies of ^{3}H-MK 801 binding sites were done using the rat brain. ^{3}H-MK 801 binding were increased dose-dependently by L-glutamate, glycine and spermidine, when Triton X-100 treated sections were used. Distribution patterns of ^{3}H-MK 801 binding sites in the presence of either amino acids or spermidine were similar to those of binding sites for glycine and an NMDA antagonist, CPP. However, in the cerebellum, low but specific ^{3}H-MK 801 binding sites were detected when stimulated by either L-glutamate or glycine, although no specific binding sites were observed by adding spermidine. It has been reported that ^{3}H-spermidine binding sites are more richly contained in the cerebellum than the hippocampus and cerebral cortex. Since an enhancing effect of ^{3}H-MK 801 binding by spermidine was not found in the cerebellum, it was considered that spermidine binding sites in this brain area had no relation with the NMDA receptor complex.

317.14

GLUTAMATE RECEPTOR ACTIVATION IS EVALUATED IN VIVO BY POSITRON EMISSION TOMOGRAPHY

C. Ferrarese, R.S. Miletich, I. Linfante, R. Brooks, D. Kiesewetter, *A. Guidotti, *E. Costa, R.E. Carson, M. Fulham, K.C. Rice, B.R. de Costa, K. Hayashida, K. Borbely and G. Di Chiro. N.I.H., Bethesda, MD and *F.G.I.N., Georgetown University, Washington, DC.

The phencyclidine (PCP) derivative fluorothienylcycloexylpiperidine (FTCP) binds in vivo to the PCP site of the NMDA-selective glutamate receptor (Soc. Neurosci. Abst. 1990, 16:396.5). Using [18F] FTCP we performed eight PET experiments in four rhesus monkeys under curare immobilization. Heart rate, blood pressure, ECG, and EEG were recorded continuously. Serial arterial plasma samples were withdrawn to calculate the kinetics of drug disposal and metabolism. To date, PET and blood data were fully analyzed by the multiple-time graphical method and by compartmental analysis in two studies. In the control experiment, FTCP influx constants were greater in the cerebral cortex than in the cerebellum. N-methyl-D-aspartate, (NMDA) 150 mg/kg iv, induced prolonged EEG epileptic abnormalities and a parallel increase in FTCP influx in the temporal cortex. This suggests that activation of NMDA-selective glutamate receptors may be monitored in vivo by PET. This may be clinically useful in disorders involving glutamate receptor overstimulation, such as epilepsy, stroke, and neurodegenerative diseases.

317.15

PHOSPHOLIPASE A_2 PRETREATMENT MODULATES DIFFERENT SUBTYPES OF NON-NMDA RECEPTORS: AN AUTORADIOGRAPHIC STUDY. M.V. Catania*, J.B.Penney and A.B. Young Dept. of Neurology, U. of MI., Ann Arbor, MI 48109.

Increasing evidence suggests that phospholipase A_2 (PLA_2) is involved in mechanisms underlying synaptic plasticity. We used in vitro quantitative autoradiography to evaluate the effect of PLA_2 pretreatment on NMDA and non-NMDA glutamate receptors in rat brain. Slices were pretreated with various concentrations (0.06-600 U/mg prot.) of PLA_2 from porcine pancreas and bee venom at 37° for 20' in presence of $CaCl_2$ (25 mM). In all regions examined, the pretreatment with concentrations equal or greater than 0.6 U/mg prot. of phospholipase A_2 increased [^{3}H]AMPA binding by 30-40 %, without affecting NMDA sensitive [^{3}H]glutamate and [^{3}H]kainate binding, in accord with other previous results performed in homogenates (Massicotte and Baudry, Neurosci Lett ,118, 1990) . In addition, in the pretreated slices , [^{3}H]Glutamate binding, to the metabotropic receptors was significantly increased by 25-30 % in CA1 of hippocampus ($p<0.05$). A significant increase in non-NMDA, non-Kain,non-Quis [^{3}H] glutamate binding was also observed in the dentate gyrus and in CA1 of the hippocampus.

Our results support the hypothesis that modification of different non-NMDA receptor binding properties by the activation of PLA_2 , may play an important role in the mechanisms that regulate synaptic plasticity. *Supported by CNR-Italy n. 224.04.10 cod. 24.04.10 and USPHS NS19613.*

317.16

EXCITATORY AMINO ACID RECEPTORS IN CONTROL AND ALZHEIMER'S AMYGDALA. W.F. Maragos, S.W. Newman, R.W. Price, A.B. Young and J.B. Penney. Depts. of Neurology and Anatomy and Cell Biology, Univ. Michigan, Ann Arbor, MI 48105.

The basolateral (BL) subdivision of the amygdaloid complex has properties in common with the cerebral cortex. This limbic structure has strong reciprocal connections with entorhinal and subicular cortices, regions with marked pathological changes in Alzheimer's disease. Using quantitative autoradiography, we analyzed ligand binding to the NMDA, AMPA and kainate receptor subtypes in the amygdala obtained post-mortem. Nuclear boundaries were determined using cholinesterase and cresyl violet stained sections. Thioflavin-S staining was also performed. In control tissue, 3H-glutamate binding to the NMDA receptor was highest in the basal medial and lateral nuclei, with moderate binding in the basolateral, accessory basal and basal central nuclei. 3H-AMPA binding was highest in the basal medial nucleus with moderate binding in the accessory basal, basal lateral, basal central and lateral medial nuclei. Binding was lowest in the lateral division of the lateral nucleus. 3H-kainate binding displayed little regional variation in binding. In Alzheimer's disease tissue, reductions of NMDA and AMPA receptors were confined to several of the basal nuclei, sparing the lateral nuclei. It may be significant that several subdivisions of the basolateral complex that have the greatest number of both NMDA and AMPA receptors are the same nuclei that send and receive projections to the entorhinal and subicular cortices. Supported by USPHS Grant AG08671

317.17

IN VIVO [^{3}H]MK-801 BINDING: NMDA-ASSOCIATED ION CHANNEL STATUS. C.Arons[1]*, M.Thomas[2], T.McIntosh[2] and W.Shoemaker[1]. Dept. Psychiatry[1] and Dept. Surgery[2], UConn Health Center, Farmington, CT 06030.

In vitro binding studies have shown that binding of MK-801 (N-methyl-D-aspartate noncompetitive antagonist) is dependent on the configurational state of the NMDA receptor. MK-801 binding increases if the NMDA receptor associated ion channel is open. Activation of both the glutamate and glycine binding sites of the receptor complex potentiate channel opening. We examined [^{3}H]MK-801 binding using in vivo binding methods. A tracer dose of [^{3}H]MK-801 was injected into the tail vein of anesthetized rats. After a 25 minute labelling period, animals were decapitated, and brains dissected. Tissue was homogenized, filtered, and radioactivity counted. The highest concentrations of specifically bound [^{3}H]MK-801 were detected in hippocampus and cortex, less in thalamus and hypothalamus, and very little in cerebellum. MK-801 binding increased in some regions following administration of NMDA and decreased following administration of CGS-19755 (NMDA competitive antagonist). These results suggest that in the anesthetized animal, the NMDA receptor complex may be active (open channel) in some regions, while in other regions it may be inactive (channel closed).

317.18

METABOTROPIC GLUTAMATE RECEPTORS AND REGULATION OF NEURONAL PLASTICITY. F. Nicoletti, G. Casabona*, G. Aleppo*, P. Dell'Albani#*, C. Amico#*, E. Aronica*, V. Perciavalle♦, K.G. Reymann*• and D.F. Condorelli#. Institutes of Pharmacology, #Biochemistry and ♦Physiology, University of Catania, Italy; and *Institute for Neurobiology and Brain Research, University of Magdeburg, Germany.

Specific glutamate receptors coupled to polyphosphoinositide hydrolysis (metabotropic receptors) are present in neurons and astrocytes. Activation of metabotropic receptors in both cell types leads to a rapid and transient increase in the expression of immediate early genes, including zif/268, c-fos and c-jun. The activation of a coordinated transcriptional programm implicates a role for metabotropic receptors in the regulation of neuronal plasticity. The antagonist L-2-amino-3-phosphonopropionate (AP3), shorthens the duration of long-term potentiation (LTP) in the Schaffer collateral-CA1 synapsis in the hippocampus, suggesting that activation of metabotropic receptors contributes to the series of events enabling the late expression of LTP. We are currently studying the activation of the specific transcriptional programm that follows the induction of LTP in hippocampal slices incubated in the absence or presence of L- AP3.

317.19

NOOTROPIC DRUGS AS POSITIVE MODULATORS OF AMPA RECEPTORS. A.A. Genazzani*, A. Copani*, G. Aleppo*, P.L. Canonico and F. Nicoletti. Inst. Pharmacology, Univ. Catania, Italy.

Micromolar concentrations of piracetam, aniracetam, and oxiracetam enhanced AMPA-stimulated $^{45}Ca^{2+}$ influx in primary cultures of cerebellar granule cells. Nootropic drugs increased the efficacy, but not the potency, of AMPA and their action persisted in the presence of the voltage-sensitive calcium channel blocker, nifedipine. Oxiracetam and aniracetam had no effect on the stimulation of $^{45}Ca^{2+}$ influx induced by NMDA or kainate. Nootropics did not affect the stimulation of inositol phospholipid hydrolysis induced by quisqualate in cultured cerebellar neurons or by trans-ACPD in hippocampal slices, indicating that piracetam and its derivatives are devoid of activity on metabotropic receptors. To characterize further the mechanism of action of nootropics, we have studied [^{3}H]AMPA binding in membranes from rat cerebral cortex. All nootropic drugs increased the maximal density of low affinity [^{3}H]AMPA binding sites, without affecting the high affinity component of the binding. These results support the view (Ito et al., J. Physiol., 424, 1990) that nootropic drugs positively modulate AMPA receptors in neurons.

GABA RECEPTORS: FUNCTION III

318.1

SINGLE CHANNEL GATING KINETICS OF $GABA_A$ RECEPTORS ACTIVATED BY DESENSITIZING CONCENTRATIONS OF GABA. R.A. Gross and R.E. Twyman. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI.

When exposed to high agonist concentrations, ligand gated ion channels exhibit desensitization or a decline in evoked current over time to a smaller equilibrium value. During desensitization, bursts and clusters of channel openings can occur after irregular long closed periods. We studied processes involved in $GABA_A$ receptor regulation and desensitization by analyzing single channel equilibrium kinetic properties of GABA receptors exposed to high concentrations of GABA (10-1000 μM).

In 0.1-1.0 μM strychnine and symmetric chloride solutions, excised outside-out patches were obtained from mouse spinal cord neurons in culture and voltage clamped at -75 mV. Average open durations increased with concentration up to 75 μM GABA. Open duration time constants were concentration independent. However, several burst and cluster properties were concentration dependent over the entire range tested. At the highest GABA concentrations, open channel block was occasionally observed and there were excess numbers of isolated brief openings that were independent of bursts and clusters. Two brief intraburst closed duration time constants and their ratios were concentration independent.

These results suggest that the closing (open state) properties of the GABA receptor were saturable at low concentrations, but opening (burst and cluster) properties were affected by a concentration dependent desensitization process. This suggests that the kinetic processes involved with GABA binding and the regulation of open state gating may be different from those regulating GABA binding and desensitization of the GABA receptor.

This work was performed in the laboratory of Dr. Robert L. Macdonald.

318.2

DITHIOTHREITOL ENHANCES $GABA_A$ RECEPTOR CURRENT IN MOUSE NEURONS IN CULTURE. N.M. Porter+, R.E. Twyman+, and R.L. Macdonald+#. Depts. of Neurology+ and Physiology#, Univ. of Michigan, Ann Arbor, MI 48104

The predicted structure of the neuronal $GABA_A$ receptor places cysteine disulfide loops on extracellular domains of the receptor. Sulfhydral reducing agents, such as dithiothreitol (DTT), have been shown to enhance NMDA receptor currents in neurons. To determine the role of disulfide loops on GABA receptor function, we measured GABA receptor chloride currents in the presence of DTT or oxidized DTT (an inactive form) in mouse spinal neurons using the whole cell and excised patch clamp techniques.

Whole cell recordings were obtained and GABA (5 μM) + oxidized DTT (5 mM) or GABA + DTT (5 mM) were applied at regular intervals for 7-10 min. Peak GABA-evoked inward chloride current was reduced by 50% at 3 min in the presence of oxidized DTT. Replacement of oxidized DTT with DTT resulted in a recovery to within 86% of the initial GABA-evoked current. In excised outside-out patches, externally applied DTT enhanced GABA receptor channel opening frequency. The effects of DTT on the inside surface of the receptor also were examined. DTT included in the recording pipette reduced the time-dependent decrease in channel opening frequency typically observed upon repeated application of GABA to patches. This "run down" was not reduced when either oxidized DTT or DTNB (5-5-dithio-bis-2-nitrobenzoic acid, an oxidizing agent) were included in recording pipettes.

We conclude that DTT may affect GABA receptor function when exposed to either the inside or outside surface of the receptor. DTT enhanced current primarily via an increase in channel opening frequency. These results suggest that activity of the GABA receptor may be dependent on the redox state of the receptor and/or of a closely associated regulatory protein.

318.3

DESENSITIZATION OF GABA INDUCED CURRENT IN CULTURED RAT HIPPOCAMPAL NEURONS. D.J. Oh and M.A. Dichter. Dept. of Neurology, University of Pennsylvania School of Medicine and Graduate Hospital, Philadelphia, PA 19104.

Basic characteristics of desensitization were investigated in cultured rat hippocampal neurons (3 days- 4 weeks in vitro) using whole cell patch clamp techniques. 10 to 500 μM GABA was perfused for 30 or 60 seconds with 60 second intervals. Between applications neurons were washed with control bath solution.

Desensitization, evaluated by peak to plateau ratio (PPR) and time constants of current decay (τ), was dose-dependent and culture age-dependent. Higher concentrations of GABA induced larger and faster desensitization, which was more prominent in older cells. Membrane potential influenced both desensitization and resensitization. Desensitization decreased with membrane depolarization; PPR went from 6.3 to 1.4 and τ went from 4.6 to 26.8 seconds at -80 mV and +30 mV, respectively. Resensitization, studied with brief applications of 20 μM GABA at different membrane potentials after 200 μM GABA-induced desensitization at -80 mV, occurred faster at +30 mV than at -80 mV in older cells.

Low concentrations of GABA (1-2 μM) perfused for 2 to 60 seconds, which did not induce any current, had no effect on the maximal response nor desensitization produced by a subsequent application of 100 μM GABA. This finding suggests that GABA receptors were not desensitized without first being activated, as has been demonstrated for AMPA receptor/channels. This work was supported by NS 24927.

318.4

Current source-density (CSD) analysis of GABA-mediated monosynaptic responses in area CA1 of hippocampus and supragranular neocortex A.M. Borroni, N.A. Lambert and T.J. Teyler *Department of Neurobiology, NE Ohio Universities College of Medicine, Rootstown, OH 44272.*

Monosynaptic GABA-mediated population synaptic responses were recorded using a 16-channel multielectrode array in area CA1 of hippocampal and area Oc2 of visual cortical slices in the presence of DNQX and APV. The laminar distribution of inhibitory synaptic current evoked at several stimulation sites was analyzed. In area CA1, stimulation in any layer evoked maximal current sources horizontal to the stimulation site that were blocked by bicuculline-methiodide (BMI; 30μM). Stimulation in stratum pyramidale evoked larger BMI-sensitive current sources than did stimulation in other layers, consistent with dense GABAergic innervation of pyramidal cell bodies. BMI-sensitive sources in both preparations displayed use-dependent depression that was antagonized by the $GABA_B$ receptor antagonist CGP 35348 (0.8mM). In both preparations, in the presence of 4-aminopyridine (100μM), BMI-sensitive dendritic current sinks occurred spontaneously or could be evoked. The distribution of BMI-sensitive current sources and sinks was similar in both preparations. These results suggest that activation of dendritic $GABA_A$ receptors in areas CA1 of hippocampus and Oc2 of neocortex elicits outward chloride current that can be detected using extracellular recording.Supported by NS28698

318.5

ATYPICAL EFFECTS OF GABA ON FROG TECTAL CELLS IN VITRO. L. Sivilotti[1] and A. Nistri[2]. Dept. Pharmacology, Queen Mary and Westfield College, London E1 4NS, U.K.

GABA enhances excitatory neurotransmission evoked by optic nerve stimuli on neurones of the frog optic tectum. The present study, employing intracellular recording from deep tectal neurones of an *in vitro* slice preparation kept at 10°C, sought to clarify the mechanism of this unusual action of GABA. The membrane potential of these cells was -50 ± 2 mV (with either KCl or K-acetate microelectrodes). About 50% of cells were depolarized (6 ± 1 mV) by 1 mM GABA while the remaining ones were hyperpolarized (6 ± 1 mV). Regardless of their response polarity, cells displayed either decreases (20 ± 3%) or increases (23 ± 6%) in input resistance in the presence of GABA. Only in a minority of neurones these responses were associated with inhibition of spontaneous activity. In most cells the effects of GABA persisted in TTX (1 μM) medium. The peak mean amplitude of EPSPs elicited by electrical stimulation of afferent axons was reversibly enhanced from 8 ± 1 mV to 11 ± 2 mV by GABA; a prolongation of the EPSP decay time was also apparent. These data suggest that GABA facilitates excitatory neurotransmission by a combination of pre- and postsynaptic actions which include disinhibition coupled with postsynaptic changes in membrane potential and resistance.

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318.6

GABA CAUSES ELEVATION OF INTRACELLULAR CALCIUM ION CONCENTRATION IN SOME DORSAL HORN NEURONS. J. Wang*, D. B. Reichling, and A.B. MacDermott, Dept. Physiology & Cellular Biophysics and Center for Neurobiology and Behavior, Columbia Univ., New York, NY 10032.

In these experiments, we have examined the effects of GABA on the intracellular concentration of calcium ions ($[Ca^{2+}]_i$) in neurons isolated from the spinal cord dorsal horn of embryonic rats. Neurons were cultured on a glass coverslip and loaded with the calcium indicator dye, indo-1. GABA (100 uM) was rapidly applied for a period of 3 sec while indo-1 fluorescence was continuously monitored. In most neurons tested, $[Ca^{2+}]_i$ did not change from the normal resting level of approximately 50 nM during GABA application. Approximately 30% of neurons, however, responded to GABA with fast increases in $[Ca^{2+}]_i$ to concentrations between 5 and 50 nM above resting levels. The $GABA_A$ receptor subtype is involved in this action since the amplitude of $[Ca^{2+}]_i$ transients evoked by GABA was reduced by co-application of 50 uM bicuculline, and $[Ca^{2+}]_i$ transients could be evoked by 10 uM muscimol, a $GABA_A$ agonist. It has been reported that activation of the $GABA_A$ receptor can depolarize some types of neurons, and suggests the possibility that GABA might elevate $[Ca^{2+}]_i$ by activating voltage-gated calcium channels. This possibilty is now being tested using antagonists of voltage-gated calcium channels. In contrast to the relatively fast inhibitory postsynaptic potentials usually associated with GABA transmission, GABA-stimulated increases in $[Ca^{2+}]_i$ might cause relatively long-term changes in sensory transmission in the dorsal horn.

318.7

CHARACTERIZATION OF GABA-MEDIATED CHLORIDE INFLUX INTO CEREBELLAR MICROSACS - MODULATION BY BENZODIAZEPINES THROUGH THE TYPE I RECEPTOR. M.F. Davies, P.A. Maguire, N.F. Tsai* and G.H. Loew*. Molecular Research Institute, Palo Alto, CA 94304

In order to investigate benzodiazepine actions at one a single receptor subtype, modulation of GABA-mediated ^{36}Cl influx was studied in rat cerebellar microsacs, which we have shown to contain only the Type I benzodiazepine receptor. The sensitivity of the $GABA_A$ receptor complex to GABA and muscimol was similar, with IC_{50} of 7.9±1.4μM (n=10), reaching a maximal stimulation at 100 μM GABA or muscimol. The response to each ligand was biphasic, decreasing chloride flux at concentrations above 100 μM. Pentobarbital (20μM) produced an increase in the maximal response to GABA. Flunitrazepam (1μM) enhanced GABA stimulation of ^{36}Cl influx at GABA concentrations below the IC_{50} but had no effect at high concentrations. The inverse agonist, DMCM (1μM), shifted the GABA dose-response curve to the right. This system will be used to assess the modulation of the native $GABA_A$/BDZ Type I receptor complex by benzodiazepine receptor ligands with different chemical structures.

318.8

ANTAGONISM OF GLYCINE AND TAURINE INDUCED ION CHANNELS BY TAG IN CULTURED MOUSE SPINAL NEURONS. D.A. Mathers. Department of Physiology, University of British Columbia, Vancouver, B.C. V6T 1Z3 Canada.

We studied the blocking action of 6-(aminomethyl)-3-methyl-4H-1,2,4-benzothiadiazine 1,1-dioxide (TAG) on single chloride channels activated by glycine and taurine in mouse spinal cord cell membranes. This agent is regarded as a selective taurine antagonist at some central synapses. (Girard et al., J. Med. Chem. [1982] 25: 113-116). Neurons were dissociated from CD1 white embryos at day E12 and cultured at 37°C in Minimum Essential Medium with 10% horse serum for 2-3 weeks prior to use. Voltage-clamp recordings were made at 21-23°C in the outside-out patch configuration using a List EPC-7 amplifier (bandwidth DC-2 kHz). Patch electrodes contained a solution of composition (mM): 140 Tris-Cl, 3 NaCl, 1 $MgCl_2$, 11 EGTA, 10 HEPES, pH 7.2. The external membrane face was exposed to a solution containing (mM): 140 Tris-Cl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.2. At concentrations up to 200 μM, TAG did not alter the conductance or reversal potential of multi-conductance chloride channels activated by glycine or taurine. At concentrations above 50 μM, TAG reduced the probability, P of these channels being in the open state. 100 μM TAG reduced P by 94 ± 2.7 % (mean ± S.E.M., 5 patches) in the case of channels activated by 80 μM taurine. However, TAG was considerably less effective in antagonising channels activated by glycine. 200 μM TAG was required to produce 85 ± 6.1% reduction in P during channel activation by 20 μM glycine.

318.9

HETEROGENEITY OF $GABA_A$ RECEPTOR PHYSIOLOGY AND PHARMACOLOGY IN DORSAL ROOT GANGLION NEURONS FRESHLY ISOLATED FROM ADULT RATS. **G. WHITE. Neurogen Corporation, 35 Northeast Industrial Rd., Branford, CT 06405.**

Electrophysiological and pharmacological heterogeneity of $GABA_A$ receptor/ionophore function has been demonstrated using combinations of cloned $GABA_A$ subunit cDNAs in a variety of expression systems. A systematic demonstration of such heterogeneity within a group of neurons has yet to be reported. Because dorsal root ganglia (DRG) have a heterogeneous population of neurons as defined by voltage gated ion currents, sensitivity to pharmacological agents, presence of peptides, and antigenic expression, I examined this category of neuron for heterogeneity in properties of the $GABA_A$ receptor/ionophore complex. DRGs were dissected and neurons were isolated using 2/1.5/0.5 mg trypsin/collagenase/DNase (respectively) in 5 ml DMEM in a shaking water bath at 35c for 45-60 minutes. Electrophysiological responses were recorded using the whole-cell patch clamp technique. External solution contained (mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH 7.4) and 10 glucose. Recording electrode solution contained (mM): 130 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, and 10 HEPES (pH 7.4). Using the logistic equation, EC_{50}s for GABA ranged from 28 μM to >100 μM between neurons. Pseudo-Hill coefficients ranged from 1 to 2. In addition, a large degree of variability was found in current/voltage rectification. Finally, responses to a given benzodiazepine ligand varied in amplitude between neurons by up to 4 fold while the EC_{50} for that ligand remained constant and pseudo-Hill coefficients varied between 1 and 2 for individual ligands. As determined by PCR (C. Hartnett et al., this meeting), DRGs contain messenger RNA for the following $GABA_A$ subunits: α1, α2, α4, β2, β3, γ_{long}, but not α3, α6, β1, γ_{short} or δ. Thus the substrate exists for many different combinations of subunits in this category of neuron. This data suggests that DRG neurons do express diverse $GABA_A$ subunit combinations and that such combinations can alter molecular interactions at benzodiazepine receptors without greatly altering affinity.

318.10

DETECTION BY PCR OF $GABA_A$ RECEPTOR SUBTYPES EXPRESSED IN DORSAL ROOT GANGLIA OF THE ADULT RAT. C. Hartnett. Neurogen Corporation, Branford, CT. 06405

Molecular characterization of the $GABA_A$ receptor has demonstrated the existence of multiple subtypes, leading to the speculation that receptor subtype heterogeneity can be correlated with distinct clinical properties. To determine which subtypes are present in small numbers of cells, a modified PCR protocol was used to detect mRNA expressed in dorsal root ganglia of the adult rat. Using subtype specific oligonucleotides as PCR primers, the presence of the following subtypes has been demonstrated: α1, α2, α4, β2, β3, and the long form of γ2. The α3, α6, β1, δ, and the short form of the γ2 subtypes were not found. The PCR protocol included incorporation of digoxygenin-labelled dUTP in the amplified product. The labelled DNA can then be linked to an alkaline phosphatase conjugated antibody, and detected with a chemiluminescent substrate. This method allows detection of mRNAs with several-fold more sensitivity than ethidium bromide staining, without using radioisotopes. Based on the data presented here, it is possible to quickly detect the presence or absence of mRNAs coding for specific receptor subtypes in a small number of cells.

318.11

LOCALLY-APPLIED ETHANOL ATTENUATES NMDA-EVOKED NEURONAL ACTIVITY WHILE POTENTIATING INHIBITION BY GABA IN THE INFERIOR COLLICULUS. P.E. Simson, H. E. Criswell, and G. R. Breese. Dept. of Psychiatry, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC 27599.

Systemically administered ethanol (ETOH) inhibits NMDA-evoked activity in only a subpopulation of neurons in the medial septum (Simson et al., J. Pharmacol. Exp. Therap., 1991), a site where systemically-administered ETOH potentiates the behavioral and electrophysiological effects of GABA (Givens and Breese, J. Pharmacol. Exp. Therap., 1990). Another brain site where ETOH has behavioral effects is the cortex of the inferior colliculus (IC), where ETOH withdrawal is related to seizure production (McCown and Breese, Alcoholism, 1990). In the present study, the effects of electro-osmotically-applied ETOH on NMDA-evoked and GABA-inhibited activity of IC neurons were investigated. As with systemic administration of ETOH in the medial septum, locally-applied ETOH potently inhibited NMDA-evoked activity in only a portion of IC neurons tested (75%), while having no effect on NMDA-evoked activity in the remaining neurons. Local application of ETOH also potentiated, in a dose-dependent manner, the inhibitory effects of GABA on IC neuronal activity. In contrast, locally-applied ETOH failed to potentiate the inhibition by glycine of IC activity, as well as the inhibition by GABA in the lateral septum, a site where systemically-administered ETOH has no effect on spontaneous and GABA-inhibited activity. These findings suggest that ETOH has actions on specific brain areas, and that these differences may be related to actions on specific receptor subtypes. Supported by NS26595 and AA-08024.

318.12

SPECT IMAGING OF THE BENZODIAZEPINE RECEPTOR: FEASIBILITY OF *IN VIVO* POTENCY MEASUREMENTS FROM STEPWISE DISPLACEMENT CURVES. R.B. Innis, E. Sybirska, M. Al-Tikriti, S.S. Zoghbi*, R.M. Baldwin*, M. Laruelle, S.W. Woods, J. Seibyl, R. Malison, P.B. Hoffer*, D.S. Charney, and G.R. Heninger. Dept. Psychiatry, VA Medical Center and Yale Univ., West Haven, CT 06516.

Ro16-0154 is a high affinity, iodine-containing antagonist of the benzodiazepine (BZ) receptor. We have examined the pharmacological specificity of ^{123}I-Ro16-0154 as a BZ receptor probe with SPECT (single photon emission computed tomography) and show the feasibility of measuring potency of BZ agents from an *in vivo* stepwise displacement curve.

In a series of 28 SPECT studies, baboons were injected with 1-18 mCi ^{123}I-Ro16-0154 and scanned for 2-6 hours in the Strichman 810X. Radiolabel uptake was concentrated in cortical areas with highest density in occipital cortex. Maximum uptake was reached within 30-90 min and was relatively stable for the following 180 min. Repeated injections of increasing doses of each of five BZ drugs (Ro 16-0154, Ro 15-1788, clonazepam, alprazolam, and diazepam) yielded stepwise displacement curves, which were analyzed to measure the *in vivo* potencies of these agents. The relatively long half-life of ^{123}I and the stable biological uptake of the radiotracer allowed such potency estimations in just one experiment. The *in vivo* potencies of these five agents were highly correlated with their affinities for the BZ receptor determined with homogenate binding.

In conclusion, stepwise displacement by agents administered following the injection of the radioligand ^{123}I-Ro 16-0154 provided a reliable means of measuring the *in vivo* potencies of BZ receptor agents. This *in vivo* determination may better predict the clinical potency of BZ drugs than *in vitro* homogenate estimations, because the *in vivo* measure provides the summed effects of receptor affinity, plasma protein binding, penetration of the blood brain barrier, and metabolism of the agent.

318.13

GENERAL ANESTHETICS POTENTIATE GABA ACTIONS ON $GABA_A$ RECEPTORS EXPRESSED BY XENOPUS OOCYTES: LACK OF INVOLVEMENT OF INTRACELLULAR CALCIUM. L.-H. Lin and R. A. Harris. Dept. of Pharmacology, UCHSC, Denver, CO 80262; Veterans Admin. Med. Res. Serv., Denver, CO 80222.

Potentiation of $GABA_A$ receptor channel response may be a primary action of general anesthetics (GAs). Enhancement of $GABA_A$-induced synaptic inhibition by halothane was suggested to be mediated through the release of intraneuronal Ca^{2+} (Mody et al., Brain Res., 538:319, 1991). Effects of GAs on GABA receptor-gated Cl^- channels and the participation of intracellular Ca^{2+} were assessed in *Xenopus* oocytes microinjected with mouse cortex mRNA. GABA-activated Cl^- current was measured using 2-electrode voltage clamping. Anesthetic concentrations of GAs (diethylether, halothane, isoflurane, enflurane, 3α-hydroxy-5α-dihydroprogesterone and propofol) enhanced the GABA-activated current by 100-300%. This supports the hypothesis that the potentiation of $GABA_A$ response is a common action of GAs. Potentiation produced by these drugs was not suppressed by an intracellular injection of the Ca^{2+} chelator, EGTA (intracellular concentration: about 500 μM). This EGTA treatment completely blocked the 5HT-induced Ca^{2+}-dependent Cl^- current in the same oocytes. In addition, GAs alone did not induce significant current (< 5 nA), suggesting that anesthetic concentrations of GAs did not activate endogenous Ca^{2+}-dependent Cl^- channels in oocytes. These results indicated that GAs' enhancement effects of GABA-activated Cl^- current in oocytes did not require changes of intracellular Ca^{2+} concentration. However, it is possible that GAs produce actions on neuronal $[Ca^{2+}]$ that are not observed in oocytes.

318.14

POSTNATAL RAT HIPPOCAMPAL $GABA_A$ RECEPTORS WITH DIFFERENTIAL SENSITIVITY TO BENZODIAZEPINE RECEPTOR LIGANDS. J. Bormann and B. Schönrock*. Max-Planck-Institut für Hirnforschung, W-6000 Frankfurt / M. 71, Germany.

We examined the benzodiazepine pharmacology of neuronal $GABA_A$ receptors in primary cultures of newborn rat hippocampus by using the whole-cell configuration of the patch-clamp technique. After 1-7 days in culture, recordings were made from neurons with equal intra and extracellular Cl^- concentrations of 145 mM. The rapid application of GABA (10 μM) elicited inward currents at a holding potential of -70 mV. Sequentiell application of the benzodiazepine agonist flunitrazepam (FNZ) and the inverse agonist DMCM (both 1 μM) revealed different response patterns to GABA. In one set of cells, FNZ augmented the GABA response by 114%, whereas DMCM reduced the currents by 53%. This well-established effect of benzodiazepine receptor ligands on neuronal $GABA_A$ receptor channels contrasts with the observation that in some cells DMCM inhibited the GABA-induced currents, whereas FNZ was almost ineffective.

These results suggest that several isoforms of the $GABA_A$ receptor complex exist in the postnatal rat hippocampus, and that their subunit composition may change during development.

318.15

CORRELATION OF IMMUNOCYTOCHEMICAL PROPERTIES WITH GABA RESPONSES IN CULTURED SUBSTANTIA NIGRA NEURONS. K-M. Kim*, Y. Nakajima and S. Nakajima. Dept. of Pharmacology and Dept. of Anatomy and Cell Biology, Univ. of Illinois College of Medicine, Chicago, IL 60612.

We separately cultured the pars compacta and the pars reticulata of the substantia nigra of 2 to 4-day old postnatal rats. The cell cultures were double-labelled with antibodies to tyrosine hydroxylase (to show dopaminergic (DA) cells) and to GABA. Previously, we have reported that D_2 receptors are located on DA neurons and activate K-channels, whereas D_1 receptors are located on non-DA/non-GABAergic neurons and decrease K-conductance (Kim et al., Biophysical J. 59: 20A, 1991).

Using the whole-cell clamp technique, we have conducted electrophysiological studies. By applying GABA and baclofen, we have tested for the presence of GABA receptor subtypes in cultured substantia nigra neurons. For example, some cells responded to GABA with an increase in Cl^- conductance but did not respond to baclofen, suggesting the presence of $GABA_A$ receptors and the absence of $GABA_B$ receptors. After electrophysiological experiments, the same neuron was immunocytochemically treated. $GABA_A$ receptors seemed to be present in all types of cells including GABAergic cells. On the other hand, $GABA_B$ receptors were mostly located on DA neurons. Supported by PHS grants, DA05701 and NS24711.

318.16

FUNCTIONAL RECONSTITUTION OF THE $GABA_A$ RECEPTOR PURIFIED FROM BOVINE BRAIN. R.P. Thuynsma*, L.L. Duncalfe*, C. Bladen* & S.M.J. Dunn. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta T6G 2H7.

The $GABA_A$/benzodiazepine receptor complex has been purified from bovine cerebral cortex by benzodiazepine (1012S) affinity chromatography. Polyacrylamide gel electrophoresis in the presence of SDS revealed major protein species with apparent molecular weights of 55 and 59K. The purified protein was reconstituted into phospholipid vesicles with retention of many of the functional properties of a native $GABA_A$ receptor. The ability of the reconstituted preparation to mediate rapid chloride flux has been investigated by monitoring the fluorescence of a chloride-sensitive probe (6-methoxy-N-(3-sulfopropyl) quinolinium) trapped within the vesicles. Chloride influx was stimulated in the presence of micromolar concentrations of muscimol and was blocked by pretreatment of the vesicles with muscimol (desensitization), a competitive antagonist (bicuculline) or by the channel blocker (picrotoxin). The muscimol-stimulated response was potentiated in the presence of 10 μM diazepam. This reconstituted system will prove useful in the study of structure-function relationships of the $GABA_A$ receptor. (Supported by the AHFMR and the MRC of Canada).

319.1

SEQUENCE AND EXPRESSION OF A NEUROPEPTIDE Y RECEPTOR cDNA

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Neuropeptide Y (NPY) is widely distributed in the peripheral nervous system and in brain where it has been shown to influence a variety of cardiovascular, gastrointestinal, neuroendocrine, and behavioral processes. NPY ligand binding and consequent regulation of adenylate cyclase and ion channels is sensitive to guanine nucleotides and pertussis toxin treatment, suggesting that NPY receptors belong to the G protein-coupled receptor superfamily.

As a strategy to clone the NPY receptor, we have used the polymerase chain reaction to isolate cDNAs for novel members of this receptor family from locus coeruleus, a brain region enriched in the NPY system. We now report the isolation and characterization of an NPY receptor cDNA clone, referred to as LCR1, from bovine locus coeruleus. LCR1 cDNA encodes a predicted protein of 353 amino acids with a membrane topology similar to that of other G protein-coupled receptors. Expression of LCR1 in mammalian cells results in specific and high affinity (< nM) NPY ligand binding. Northern blot analysis reveals that NPY receptor mRNA is regionally distributed in both brain and peripheral tissues. This cDNA clone will be useful for studies of the regulation and function of NPY receptors and for the isolation of related NPY receptor subtypes.

319.2

ISOLATION OF NOVEL NEUROPEPTIDE RECEPTOR cDNAs FROM LOCUS COERULEUS (LC)

W.W. Xin, J.M. Rimland, E.J. Nestler, and R.S. Duman. Lab. of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508

Receptors for NPY, opiates, CRF, VIP, and substance P, as well as for other neurotransmitters have been located in LC, the major noradrenergic nucleus in brain. These receptors are thought to belong to the G protein-coupled receptor superfamily. We have used PCR and primers derived from the third and sixth transmembrane domains of previously isolated members of this receptor family in an attempt to isolate cDNA clones for these receptors. PCR amplification of bovine LC cDNA resulted in nine major amplified DNA bands which were subsequently cloned into pBluescript. These PCR clones were then used to screen an LC cDNA library to isolate full-length cDNA clones. One of these clones has been sequenced and expressed and encodes an NPY receptor (see Rimland et al., 1991, this volume). This clone, referred as LCR1, is being used as a probe to screen a randomly primed rat brain cDNA library to isolate the rat homologue of this NPY receptor and other NPY receptor subtypes. In addition, we have isolated and sequenced another cDNA clone from the bovine LC cDNA library, LCR9, which appears to be a novel G protein-coupled receptor. Northern blot analysis demonstrates a regional distribution of LCR9 mRNA in brain. Full-length cDNAs of the above clones will be subcloned and expressed in mammalian cell lines to study their functional properties.

Application of PCR to discrete brain regions like the LC offers a particular advantage in the isolation and cloning of neurotransmitter receptors.

319.3

THE NEUROPEPTIDE Y BINDING PROTEIN IN BOVINE ADRENAL MEDULLA IS LINKED TO A G-PROTEIN.

T.D. Hexum and W. Li*, Dept. of Pharmacol., Univ. Neb. Med. Ctr., Omaha, NE 68198-6260.

Neuropeptide Y (NPY), a 36 amino acid messenger, has been shown to decrease catecholamine secretion from bovine chromaffin cells (Higuchi et al., JPET 244 468, 1988). To more fully understand this phenomenon we have investigated NPY receptor structure. NPY receptors present in bovine adrenal medulla membranes were covalently cross-linked to [^{125}I]NPY via disuccinimidyl suberate (DSS, 1 mM). SDS-polyacrylamide gel electrophoresis followed by autoradiography revealed the presence of a 90 kDa NPY binding protein in crude membranes with an IC_{50} of 1.1 x 10^{-7} M for unlabeled NPY. The membranes were further purified by continuous sucrose gradient centrifugation. Binding studies indicated that the majority of the binding sites co-purified with the plasma membranes and few binding sites existed on microsomal membranes. Competitive inhibition studies indicated that purified plasma membranes have two NPY binding sites with IC_{50}'s of 1.4 x 10^{-7} M and 1.7 x 10^{-11} M. However, in the presence of 1 mM GTP, plasma membranes have only a low affinity binding site. Affinity labeling revealed the same 90 kDa binding protein in purified plasma membranes. These data suggest that the 90 kDa binding protein is a plasma membrane protein associated with a G-protein and may be the NPY receptor in this tissue. (Supported by Amer. Heart Assoc., Neb. Affil.)

319.4

VASCULAR RECEPTORS FOR NEUROPEPTIDE Y (NPY)

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We compared NPY and NPY-related analogs with respect to their ability to activate or bind to vascular NPY receptors in four experimental set-ups. Previous results have suggested the existence of: Y1 receptors requiring full-length NPY (1-36) or [Pro^{34}]NPY, and Y2 receptors recognizing also N-terminally truncated forms of NPY but not [Pro^{34}]NPY. NPY and [Pro^{34}]NPY increased arterial pressure in the anesthetized rat with a similar magnitude and potency. NPY 2-36 was much less potent than NPY. NPY 4-36 was inactive. NPY, [Pro^{34}]NPY, NPY 2-36 and NPY 5-36 increased the coronary resistance in the perfused rat heart. NPY and [Pro^{34}]NPY were equipotent, while NPY 2-36 and NPY 5-36 were 7 and 20 times less potent. At 0.3 μM, NPY 11-36 and NPY 22-36 induced a slight contraction while NPY 23-36 was inactive. NPY, [Pro^{34}]NPY, NPY 2-36, NPY 4-36, NPY 5-36 and NPY 11-36 evoked contractions in the inferior caval vein of the rat and guinea pig. [Pro^{34}]NPY was more potent than NPY. NPY 2-36 was equipotent with NPY, while NPY 4-36, NPY 5-36 and NPY 11-36 were 30 times less potent. [Pro^{34}]NPY was equipotent with NPY 1-36 in displacing ^{125}I-PYY from rat aortic smooth muscle cells, while NPY 2-36 and shorter forms were much less potent. In caval vein smooth muscle cells, the displacement pattern was more complex, in that both [Pro^{34}]NPY and NPY 13-36 displaced the radioligand, albeit none of them completely. The bioactivity and the binding affinity of the NPY-related peptides suggest the presence of Y1 receptors in all vascular systems studied. In addition, contraction-coupled Y2 receptors seems to be present in the caval vein.

319.5

SK-N-MC: MODEL SYSTEM FOR MOLECULAR STUDIES OF THE NEUROPEPTIDE (NPY) Y1-RECEPTOR.

F. Yee[1], A.G. Blomqvist[2], D. Larhammar[2], D.J. Reis[1] and C. Wahlestedt[1]. [1]Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021; [2]Dept. of Med. Genetics, Uppsala Univ., S-751 23 Uppsala, Sweden.

The Y1-receptor is the principal NPY receptor subtype in blood vessels and frontoparietal cortex. It is the only NPY receptor expressed in the human neuroblastoma cell line, SK-N-MC, making it a suitable model system to study Y1-receptor biology (Wahlestedt, et al., *Ann. NY Acad. Sci.* 611:7, 1990). In SK-N-MC cells, the Y1-receptor appears to be G-protein coupled since the number of specific ^{125}I-NPY binding sites (95,000 sites/cell) was reduced to 50% by GTP in a concentration-dependent manner. In addition, NPY and the Y1-selective agonist, [Pro^{34}]NPY, attenuated forskolin-stimulated cAMP levels, along with eliciting Ca^{2+} release from a thapsigargin-sensitive pool with potencies in the nM range. The Ca^{2+} mobilization evoked by NPY and carbachol (an Ins[1,4,5]P_3-generating agonist) do not cross-desensitize, suggesting that the two agonists liberate Ca^{2+} from distinct intracellular pools.

The G-protein-coupled receptors have conserved transmembrane (TM) domains, and on the basis of these homologies, combinations of degenerate primers corresponding to the TM2, TM3, TM6 and TM7 regions, were used to clone the Y1-receptor from SK-N-MC cells by PCR. We have selected 33 PCR-generated fragments (350-600 bp) as potential receptor candidates and these are currently in the process of being characterized.

319.6

2-NAPHTHYLBENEXTRAMINE (2-NapBXT): A PRELUDE TO THE DEVELOPMENT OF SELECTIVE AND POTENT NON-PEPTIDE NEUROPEPTIDE-Y (NPY) ANTAGONISTS.

C. S. Chaurasia, K. Li* and M. B. Doughty*, Dept. of Medicinal Chemistry, Univ. of Kansas, Lawrence, KS 66045.

NPY is a 36-amino acid peptide messenger that has been implicated in a variety of physiological and pharmacological actions including vasoconstriction and norepinephrine modulation. However, lack of a selective NPY antagonist has hampered evaluation of the functional role of NPY's central and peripheral-mediated actions. Benextramine (BXT), an irreversible α-adrenoceptor antagonist, has been shown to exhibit binding affinity to NPY receptors, and to inhibit in vivo NPY's pressor activity (Doughty et al. Eur J. Pharmacol. **185**, 1990, 113). However, its affinity is rather low, and it is not selective for the NPY receptor. Based on the structural features shared between BXT and NPY, we have hypothesized common sites of interaction for these molecules at the NPY receptor. Furthermore, based on the existing structure-activity relationship studies of BXT analogs at the α-adrenoceptor, we synthesized a number of BXT derivatives in our quest to develop more selective and potent NPY receptor antagonists. The pharmacological evaluation of these analogs were performed using [^{3}H]NPY bound to rat brain membranes. Result of our studies indicates a 2-fold higher affinity for 2-NapBXT at NPY sites as compared to that of BXT. A significantly lower α-adrenoceptor antagonist potency of 2-NapBXT makes this compound a potential candidate for the development of selective and potent non-peptide NPY antagonist (supported in part by the American Heart Association, National Center).

319.7

LOCALIZATION OF NEUROPEPTIDE Y BINDING SITES TO SMALL ARTERIOLES OF THE HEART, DORSAL ROOT GANGLIA NEURONS, AND POST-GANGLIONIC SYMPATHETIC NEURONS SUGGESTS POSSIBLE NPY ACTIONS ON ALL THREE CELL TYPES DURING MYOCARDIAL ISCHEMIA. C.Allen, M. Labenski, J. Ghilardi, M. Catton, P.J.Mannon, I.L.Taylor, S.R.Vigna, J.E. Maggio, P.W. Mantyh Mol. Neurobiology Lab (151) VA Medical Center, Mpls., MN 55417; Gastroenterology Div., VA Medical Center, Durham, N.C. 27705; Dept. of Biol. Chem. and Mol. Pharm., Harvard Med. School, Boston, MA 02115

Neuropeptide Y (NPY) is a 36 amino acid peptide that is synthesized and released by post-ganglionic sympathetic neurons and several reports have suggested that the peptides may exert potent direct and indirect effects on the heart. To identify possible sites of NPY actions on the heart and peripheral neurons which innervate the heart we have used receptor autoradiography to examine the localization of NPY receptor binding sites in the dorsal root ganglion, sympathetic ganglion and heart. Standard receptor autoradiographic methods were used along with an ^{125}I-PYY ligand which has been shown to bind to both NPY and PYY receptor binding sites. NPY receptor binding sites were found to be expressed by a substantial population of dorsal root ganglion neurons, by post-ganglionic sympathetic neurons and by small arterioles throughout the rat heart.

These results suggest that NPY released by post-ganglionic sympathetic neurons could act through these specific binding sites to mediate; dorsal root ganglion activity which is known to convey nociceptive information from the heart, vasoconstriction of myocardial arterioles, and sympathetic tone in the heart.

Supported by NIH grant NS-23970, NS-22961, DK-38216 and a VA Merit Review.

319.8

POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION OF mRNA ISOLATED FROM AtT-20/D16 CELLS USING GUANINE NUCLEOTIDE BINDING PROTEIN-LINKED RECEPTOR OLIGONUCLEOTIDE PRIMERS: PRELIMINARY ISOLATION AND CHARACTERIZATION. R.C. Thompson, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

Our laboratory has a long standing interest in the anatomy, physiology and function of neuropeptides in the central nervous system particularly those involved in the regulation of hypothalamic-pituitary-adrenal functions. Many of these neuropeptides are thought to exert their function on postsynaptic elements and/or target cells by interacting with membrane bound receptors. These receptors in turn interact with guanine nucleotide binding proteins (G-proteins) which link ligand/receptor binding to activation of intercellular second messenger systems. AtT-20 cells, mouse corticotrophic tumor cells, have been extensively studied as a model system of Proopiomelanocortin (POMC) peptide (ACTH, β-LPH, and some β-endorphin) secretion and gene regulation. These cells appear to respond to many of the same secretagogues as endogenous anterior pituitary corticotroph cells and thus express many of the receptors for these ligands. Libert, et al. in 1989 described a PCR process and the use of degenerate oligonucleotide primers to isolate cDNA clones encoding G-protein linked receptors. Using these primers, we have begun to investigate the G-protein linked receptors expressed in AtT-20 cells. In this report, we present our data on two PCR clones which have homology to other members of the G-protein receptor superfamily. The cellular and regional distribution of the mRNAs encoding these two clones is also presented as analyzed by Northern blot and in situ hybridization. R.C.T. is supported by MH09904-02. This work supported in part by MH422251-04 (to S.J.W.).

319.9

NEUROMEDIN B STIMULATES THE GROWTH OF C6 GLIOBLASTOMA CELLS. P. Duncan*, J. Rosenstein, R.T. Jensen*, J. Battey and T.W. Moody. Depts. Anatomy, Neuroscience, Biochemistry and Molecular Biology, George Washington Univ. Med. Ctr., Washington, D.C. 20037, Digestive Disease Branch, NIDKD and Lab. Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

Two bombesin (BN) receptor subtypes have been identified which prefer gastrin releasing peptide (GRP) or neuromedin B (NMB). These G-protein coupled receptors have been cloned and have 54% sequence homology (Wada et al., Neuron, 6:421, 1991). Previously, we found that human U118 glioblastoma cells have GRP receptors (Moody et al., J. Mol. Neurosci. 1:235, 1989) and here we report that C6 cells have NMB receptors. Radioreceptor assays indicated that (^{125}I-Tyr^4)BN bound with high affinity (Kd = 3 nM) to a single class of sites (Bmax = 11,000/cell) using C6 cells. Specific (^{125}I-Tyr^4)BN binding was inhibited with high affinity by NMB, GRP and BN (IC_{50} = 0.3, 100 and 50 nM respectively) but not (D-Phe^6)BN$^{6-13}$methyl ester, (Psi^{13,14}, Leu^{14})BN or GRP$^{1-16}$ (IC_{50} = 3, 3 and >10 μM). Also, C6 cells formed grafts when transplanted into the adult rat cortex. NMB elevated the cytosolic Ca^{2+} and stimulated the growth of C6 cells in a dose dependent manner. These data indicate that NMB receptors may have signal tranduction mechanisms similar to GRP receptors resulting in cellular proliferation. Supported in part by NSF grant BNS88-15133 and NIH grant NS-17468.

319.10

EFFECTS OF REDUCED PEPTIDE BOND AND DesMet ANALOGUES OF BOMBESIN (Bn) IN ANTAGONIZING THE CNS EFFECTS OF Bn. Z. Merali[1,2] and H. Plamondon*[1]. [1]School of Psychology and [2]Dept. of Pharmacology, University of Ottawa, Ottawa, Ont. Canada. K1N 9A9.

This study assessed the potency and efficacy of [DPhe^6,Cpa^{14},Ψ13−14]Bn6−14 (ΨCpaBn) and [DPhe^6]Bn6-13methyl ester (DesMetBn), two of the most potent Bn antagonists at the pancreatic acini, in blocking some of the centrally mediated effects of Bn. The in vitro receptor binding studies on rat brain membranes revealed that both the antagonists displaced (^{125}I-Tyr^4)Bn with IC_{50} values in the nano molar range, with DesMet Bn being more potent than ΨCpaBn. For the in vivo studies, male Sprague-Dawley rats (300-400g) implanted with 3rd ventricular cannulae were pretreated with ΨCpaBn (0, 0.5, 2.5 or 5.0 μg; i.c.v.) or DesMetBn (0, 0.1, 0.5 or 1.0 μg; i.c.v.), and then injected with Bn (0.5 μg; i.c.v.)15 min later. The most dominant behavior elicited by Bn was scratching directed at the head/nape region. This was antagonized more potently by DesMetBn however, in terms of efficacy, ΨCpaBn was superior as it completely blocked Bn-elicited scratching. Bn reduced the core body temperature and this effect was antagonized by the lower (0.1 and 0.5 μg) but not the higher (1.0 μg) doses of DesMetBn. At the doses used, ΨCpaBn failed to alter Bn-induced hypothermia. DesMetBn only partially antagonized Bn-induced satiety and only at the 1.0 μg dose. The effects of ΨCpaBn on ingestiion are currently being assessed. Whereas DesMetBn failed to affect Bn-induced exploratory activity, ΨCpaBn potentiated this effect of Bn. These data demonstrate that various physiological and behavioral end points were differentially affected by the two antagonists. The dose-response curves obtained with DesMetBn were bell-shaped with Bn-antagonism usually being apparent at the lower doses. Since these antagonists do not have a high affinity for the neuromedin B-type receptors, these data may suggest subtype(s) within the Bn/GRP-type receptors. (Supported by MRC).

319.11

SPECIFIC ANTAGONISTS AND AGONISTS DIFFERENTIATE BOMBESIN RECEPTOR SUBTYPES IN THE RAT BRAIN. E.E. Ladenheim, R.T. Jensen*, D.H. Coy*, S.A. Mantey* and T.H. Moran, Dept. Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205, NIDDKD, NIH, Bethesda, MD 20892 & Peptide Res. Lab., Tulane Univ. Med. Ctr., New Orleans, LA 70112.

The rat CNS contains two distinct bombesin (BN) receptor subtypes. One, similar to the pancreatic BN subtype, has a high affinity for gastrin-releasing peptide (GRP) and the other has a high affinity for neuromedin B (NMB). In this study, we used *in vitro* receptor autoradiography to examine the ability of NMB, GRP, selective antagonists [D-Phe^6]BN(6-13)ethyl ester, [D-Phe^6, Cpa^{14},Ψ13-14]BN(6-14) and a selective agonist [D-Phe^6]BN(6-13)butyl amide to inhibit binding of ^{125}I-(Tyr^4)BN to the anterior olfactory nucleus (AON), a brain region we have previously characterized as containing the BN receptor subtype with a high affinity for NMB. Our results indicate that NMB was the most potent in inhibiting binding of ^{125}I-(Tyr^4)BN to this receptor subtype, causing half-maximal inhibition at 8.1 nM and was 50-fold more potent than GRP, with half-maximal inhibition at 420 nM. The agonist and antagonists selective for the pancreatic BN receptor subtype were extremely ineffective in inhibiting binding of the BN radioligand to the AON with half-maximal inhibition occurring at >5500 nM. This binding profile strongly contrasts that previously reported for the pancreatic BN receptor subtype (von Schrenck et al., 1990). As with peripheral receptors, the use of these potent and specific BN antagonists and agonists will be useful in defining the binding characteristics of BN receptor subtypes in the rat CNS.

319.12

COMPARATIVE DISTRIBUTION OF GRP-PREFERRING AND NMB-PREFERRING BOMBESIN RECEPTOR mRNAs IN ADULT AND EMBRYONIC RAT. E. Wada, J. Way, S. Key, S. Wray, and J. Battey, Laboratory of Neurochemistry, NINDS, NIH Bethesda, MD 20892

Two bombesin-like peptides, gastrin releasing peptide (GRP) and neuromedin B (NMB), have been identified in mammalian neural and neuroendocrine cells. In previous studies, cDNA clones were isolated for two distinct mammalian bombesin receptors, one from mouse fibroblasts which has higher affinity for GRP than NMB (GRP-R) (Battey et al., Proc. Natl. Acad. Sci. USA 88:395, 1991) and a distinct receptor from rat esophagus with higher affinity for NMB than GRP (NMB-R) (Wada et al., Neuron 6:421, 1991). In this study, we analyzed and compared the distribution of GRP-R and NMB-R mRNA at various developmental stages in both brain and peripheral tissues by in situ hybridization. In the adult rat brain, GRP-R was expressed at highest levels in hypothalamic regions including the paraventricular nucleus, suprachiasmatic nucleus, magnocellular preoptic nucleus. In contrast, NMB-R mRNA was prominent in olfactory regions including the anterior olfactory nucleus, piriform cortex, tenia tecta, as well as in the thalamic regions (paraventricular, mediodorsal, centrolateral, and centromedial thalamic nuclei). Both GRP-R and NMB-R expression was observed in the gastrointestinal tract, including stomach, small intestine, and colon. At late embryonic stages (E18), both GRP-R and NMB-R mRNA was expressed in the brain, while only GRP-R is observed in the gastrointestinal tract. The distinct distribution of GRP-R and NMB-R mRNAs suggests that these two bombesin receptor subtypes play independent roles in mediating the biological effects associated with bombesin peptide agonists in different organs.

319.13

THE DISTRIBUTION OF VASOPRESSIN V_1 RECEPTOR mRNA IN RAT BRAIN. N.L. Ostrowski, W.S. Young, III, A.-M. O'Carroll*, and S.J. Lolait*. Lab Cell Biology, NIMH, Bethesda, MD 20892

A pressor-type vasopressin receptor (V_1) cDNA has been cloned from a liver library (Lolait et al., in preparation). In order to study the distribution of the V_1 receptor's expression in the rat brain, a 650bp fragment from the 5' end of the V_1 cDNA was subcloned into pGEM3Z from which [^{35}S]-riboprobes (sense and antisense) were generated for use in hybridization histochemistry. Preliminary studies with male rat brain sections revealed V_1 receptor transcripts in brain regions including olfactory bulb, hypothalamus, lateral septal area, habenula, pineal, and neocortex. Liver sections showed very high levels of V_1 transcripts, whereas kidney sections showed lower levels, especially in the medulla.

These studies confirm the presence of V_1 receptors in some areas of the rat brain, such as the septum, and raise questions about their role in other areas. Further studies are in progress to investigate the expression of this receptor gene in female and Brattleboro rats, and in rats undergoing hormonal manipulations.

319.14

GALANIN RECEPTOR AGONISTS AND ANTAGONIST REGULATE THE RELEASE OF ACETYLCHOLINE IN THE RAT HIPPOCAMPUS. G. Fisone, T. Land*, Ü. Langel*, T. Bartfai, S. Consolo*, R. Bertorelli*, P. Girotti* and T. Hökfelt. Dept. of Histology, University of Stockholm, S-106 91 Stockholm, Sweden; "Mario Negri" Institute for Pharmacological Research, Milan, Italy and Dept. of Histology and Neurobiology, Karolinska Institute, Stockholm, Sweden.

The 29 amino acid long neuropeptide galanin (GAL) coexists with the classical neurotransmitter acetylcholine (ACh) in septo-hippocampal neurons of rat and monkey brain. In the rat and monkey ventral hippocampus, GAL, acting on specific pre- and postsynaptic receptors, inhibits the evoked release of ACh (*Proc. Natl. Acad. Sci. USA* 84: 7339, 1987; *Eur. J. Pharmacol.* 164: 355, 1989) and the carbachol-stimulated phosphoinositide turnover (*Eur. J. Pharmacol.* 148: 479,1988).

We have identified the N-terminal fragments of GAL (1-9, 1-12 and 1-16) as the part of the molecule responsible for the expression of biological activity (cf. *Proc. Natl. Acad. Sci USA* 86: 9588, 1989). When applied intracerebroventricularly (i.c.v.), the short N-terminal fragments GAL(1-9) and GAL(1-12) potently inhibit the evoked release of ACh, acting as GAL agonists. We have also synthesized a peptide which possesses the properties of a GAL receptor antagonist, i.e. it binds with high affinity to the hippocampal GAL receptors (IC_{50} < 0.5 nM), it does not inhibit the evoked ACh release, but it antagonizes the effects of GAL applied i.c.v.

These findings represent a first step in the design of GAL receptor antagonists of possible therapeutical value in the treatment of neurodegenerative disorders, like Alzheimer's disease (cf. *Experientia* 43: 768, 1987). (Supported by grants from the Swedish Medical Research Council).

319.15

ACTH/MSH-LIKE PEPTIDES STIMULATE [^{3}H]DOPAMINE RELEASE AND cAMP PRODUCTION IN BRAIN SLICES; EVIDENCE FOR A MSH RECEPTOR-COUPLED MECHANISM. W.J.Florijn*, A.H. Mulder*, A.B.A. Kroese and D.H.G. Versteeg*. Dept. of Pharmacol., Med. Fac., Free University Amsterdam, Agricultural Univ. Wageningen and Rudolf Magnus Inst., Univ. of Utrecht, Vondellaan 6, 3521 GD, Utrecht, NL.

ACTH-(1-24) and related peptides potently stimulate grooming behavior after intra ventricular administration, an effect thought to involve the dopamine system. The present study was designed to study the effect of ACTH-(1-24) on transmitter release and second messenger formation in vitro using a superfusion technique. ACTH-(1-24) concentration-dependently (IC_{50} value= 10 nM) enhanced the electrically stimulated release of [^{3}H]dopamine, but not of [^{3}H]noradrenaline, from rat septal slices with approximately 20%.

ACTH-(1-24) enhanced the basal and forskolin-stimulated cAMP production in septal and striatal slices, also with an IC_{50} value of 10 nM and a maximal effect of 20%, but not the dopamine-stimulated cAMP production. Preliminary data suggest that α-MSH and ACTH-(1-24) are equipotent in enhancing cAMP formation. The structure-activity relationship resembles the one found for excessive grooming.

319.16

Stimulation of [^{32}P]GDP release from G proteins in the rat anterior pituitary lobe by gonadotropin-releasing hormone. R. Ravindra and R. S. Aronstam, Department of Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

The release of GDP is the rate limiting step of the G protein cycle, and it is this step that is catalyzed by an interaction with hormonal receptors. Gonadotropin-releasing hormone (GnRH) stimulation of [^{32}P]GDP release from G proteins was studied in plasma membranes purified from the anterior pituitary lobes of adult male rats. Membranes (1-2 mg protein) were incubated with 0.6 μM [α-^{32}P]GTP for 60 min at 37°C. Following this incubation, the predominant form of labelled nucleotide bound to the membranes was [^{32}P]GDP. The release of [^{32}P]GDP from the membranes was monitored at 37°C using thin layer chromatography. The amount of [^{32}P]GDP released was proportional to the protein concentration and increased as a function of time. GnRH agonist (0.1 μM) maximally stimulated the [^{32}P]GDP release by 31-38%. The GnRH agonist (0.1 μM) stimulated GDP release by 21%, 24%, 17%, and 14% at 30 sec, 1 , 2, and 5 min, respectively. A GnRH antagonist also stimulated [^{32}P]GDP release, albeit to a somewhat lesser extent than the GnRH agonist. The antagonist did not inhibit agonist stimulation of GDP release. These results indicate that ligand binding to the GnRH receptors promotes an interaction of the receptor with a G protein and activation of a GTP/GDP exchange. This GDP release represents an early biochemical marker of GnRH receptor stimulation. (Supported by USPHS grants GM-37948 and AA-07698).

319.17

BIOCHEMICAL CHARACTERISTICS OF [^{125}I]hCGRPα BINDING SITES IN RAT BRAIN AND PERIPHERAL TISSUES: FOCUS ON DISULFIDE BRIDGES AND MODULATION BY GTP ANALOGUE. D. van Rossum[1], Y. Dumont[1], A. Fournier[2], S. St-Pierre[2] and R. Quirion[1]. (1) Douglas Hosp. Res. Ctr. and Dept. Pharmacol. & Therap., McGill University, Montreal, Que., Canada (2) INRS-Santé, Pointe-Claire, Que., Canada.

The purpose of the following study was to better determine the biochemical characteristics of the putative calcitonin gene-related peptide (CGRP) receptor subtypes present in rat brain and lung and guinea pig atria and vas deferens. The effects of the reducing agent dithiothreitol (DTT) (0.1 to 10000 μM) and of the non-hydrolysable GTP analogue, Gpp(NH)p (100 μM), on the binding of [^{125}I]hCGRPα (40 pM) were evaluated in these tissues. Treatment with DTT markedly decreased, in a concentration dependent manner, specific [^{125}I]hCGRPα binding in all preparations. Decrements were associated with losses in maximal binding capacities without apparent alterations of binding affinities. However, DTT did not completely inhibit [^{125}I]hCGRPα binding even at very high concentrations (10 mM). Treatment of membrane preparations with Gpp(NH)p decreased [^{125}I]hCGRPα binding in rat lung. In contrast, Gpp(NH)p was totally ineffective on [^{125}I]hCGRPα binding in rat brain, and guinea pig atria and vas deferens. Globally, our results suggest the presence of disulfide bridges on CGRP receptor in all these preparations. However, except for the rat lung, it appears that CGRP receptors are not directly coupled to a GTP-binding protein, in contrast to most other peptide receptor subtypes studied thus far. Supported by the MRC of Canada.

319.18

EFFECT OF 5'-GUANYLYLIMIDODIPHOSPHATE AND SULFHYDRYL-MODIFYING REAGENTS ON [^{125}I-TYR^{10}]H GROWTH HORMONE-RELEASING FACTOR (1-44)NH_2 BINDING IN RAT PITUITARY. L. Lefrançois*, T. Abribat, L. Boulanger and P. Gaudreau. Neuroendocrinology Laboratory, Notre-Dame Hospital Research Center, Montreal, Canada, H2L 4M1.

High and low affinity binding sites for GRF have been identified in young rat anterior pituitaries. Binding of GRF to its cell surface receptors results in the activation of adenylate cyclase, presumably, through a guanine nucleotide-binding protein. In old rat pituitaries, a diminution of GRF-induced adenylate cyclase activity and an alteration of GRF binding parameters have been documented. To reproduce such a condition in young pituitaries and to investigate possible biochemical receptor modifications that could prevent G-protein-receptor coupling, modulation of GRF receptor affinity was evaluated in presence of Gpp(NH)p, DTT and glutathione. Gpp(NH)p (0.01-1mM), DTT and glutathione (1-50 mM), inhibited GRF specific binding in a concentration-dependent manner. Saturation experiments with 1 mM Gpp (NH)p, 1mM DTT or 10 mM glutathione revealed only one class of GRF binding sites, exhibiting a similar affinity to that found in old pituitaries. Interestingly, 1mM Gpp(NH)p did not modify GRF affinity in old pituitaries. It is suggested that G-protein-GRF receptor uncoupling is responsible for the decreased GRF receptor affinity observed in aged rats, however, the biochemical nature of receptor alterations involved in that phenomenon remains to be elucidated.

319.19

MODULATION OF ATRIAL NATRIURETIC PEPTIDE B RECEPTOR FUNCTION BY PROTEIN KINASE C. A. Rathinavelu, G. Pavlakovic, P-W. Sun, R.P. Maickel and G.E. Isom. Dept. of Pharmacol. & Toxicol., Sch. of Pharm. & Pharmacal Sci., Purdue Univ., West Lafayette, IN 47907.

ANP acts by binding to specific receptors in the periphery and central nervous system. cGMP is the second messenger for ANP-B receptors which are guanylate cyclase coupled, bifunctional proteins. Involvement of protein kinase C (PKC) in modulating ANP-B receptor function was explored in rat diencephalon slices and in PC12 cells. When slices were treated with a saturating concentration of (5 μM) ANP (99-126) for 60 min, PKC activity in the cytosolic fraction increased significantly (28.2%). This increase was not observed when slices were treated with the same concentration of ANP for a shorter time (15 min). A similar increase in the cytosolic PKC activity was observed in PC12 cells treated with 5 μM ANP. Increase in PKC activity did not occur when the slices and cells were treated with lower concentrations (0.5 and 1.0 μM) of ANP. Quantitation of cGMP, by HPLC method, following the above treatments indicated that 0.5 μM ANP elevated cGMP level by 321% and the increase was 85.6% with 1.0 μM ANP. 5 μM ANP treatment showed only 38.0% increase in the cGMP content. These results indicate PKC is induced when the ANP receptors are over stimulated and this may negatively modulate function of the ANP-B receptor.

319.20

MAPPING THE "CLEARANCE" ANP RECEPTORS IN WISTAR KYOTO RAT BRAIN. S. Zorad, K. Tsutsumi and J.M. Saavedra. Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

New conditions for quantitative autoradiographic determination of atrial natriuretic peptide (ANP) binding in rat brain were developed in order to block hormone degradation during incubation without the use of proteinase inhibitors. These conditions comprise 1 hr preincubation of brain slices in hypotonic solution in the presence of 2% protease-free bovine serum albumin (pfBSA) and incubation in isotonic buffer at 4°C in the presence of 4% pfBSA. HPLC revealed only 2% degraded ANP after 90 min incubation when the binding to choroid plexus and subfornical organ of Wistar Kyoto rats (WKY) had reached equilibrium. Using above conditions and the C-ANP(4-23) a truncated ANP analog selective for C (clearance) ANP receptors, we were able to displace about 30% ^{125}-I-ANP binding to choroid plexus at 10^{-6}M concentration of C-ANP. The competition curve reached plateau at the $6x10^{-8}$M concentration of C-ANP. Consequently the concentration 10^{-6}M of C-ANP was used for mapping the presence of C receptors in rat brain. C-ANP competed for ANP binding only in external plexiform layer of the olfactory bulb, arachnoid mater and the choroid plexus. Apparent amounts of C receptors in these brain structures were 40, 76 and 30% respectively of the total ANP receptors. Other studied brain structures in adult (14 weeks old) WKY rats contained only B ANP receptors.

319.21

SIGNALLING PATHWAYS OF THE EPIDERMAL GROWTH FACTOR RECEPTOR IN RAT HIPPOCAMPAL CELL LINES. M.S. Tucker and M.R. Rosner*. The University of Chicago, Chicago, IL 60637.

Epidermal growth factor (EGF) receptor has been identified in hippocampal pyramidal neurons. To investigate EGF receptor function during hippocampal neuron development, we utilized two hippocampal cell lines, H19-7 and WH19-4. Differentiation of H19-7 and WH19-4 cells coincides with an increase and decrease in EGF receptor number, respectively. MAP-2 kinase is also expressed in both cell lines. EGF strongly stimulates phosphorylation of myelin basic protein (MBP), a substrate for MAP-2 kinase, in undifferentiated cells of both lines. In the differentiated cell line that expresses lower EGF receptor levels, EGF induces a low level of MBP phosphorylation. In contrast, no induction of MBP phosphorylation by EGF is observed in either the differentiated line expressing higher EGF receptor levels or in primary hippocampal neurons, which both contain a constitutively activated MBP kinase. Thus, the different levels of EGF receptor expression and the different abilities of EGF to stimulate MBP phosphorylation in the cell lines suggest that the EGF receptor may have alternate signalling pathways in developing and mature neurons as well as in different CNS cell types. (Supported by 5 T32 GM 07151-14 and American Cancer Society.)

319.22

MECHANICAL AND CHEMICAL LESIONS INDUCE INSULIN-LIKE GROWTH FACTOR-2 (IGF-2) BUT NOT IGF-1 RECEPTOR BINDING IN THE ADULT RAT BRAIN. C.R. Breese, A. D'Costa, R.L. Boyd*, J.E. Lenham*, R.M. Booze, and W.E. Sonntag, Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

The IGF-2 receptor is a single chain glycoprotein (~250kDa) which has been shown to be identical to the cation independent mannose-6-phosphate receptor. Previous reports have shown that regenerating hepatocytes (Scott) and glioblastoma cells (Sara) have increased numbers of IGF-2/Man-6-P receptors. It was therefore hypothesized that IGF-2 receptor binding would be increased in the brain following insult. Unilateral knife lesions to cortical regions or colchicine lesions to the dentate gyrus of the hippocampus were performed and the animals sacrificed after 4 days. Brains were removed and cryostat cut for receptor autoradiography. Mounted sections were incubated in ^{125}I-IGF-1 and ^{125}I-IGF-2, washed, and apposed to film. Nissl stained sections revealed an ablation of the granular cell layer of the dentate gyrus, and extensive glial ingrowth into both lesioned regions. IGF-1 receptor autoradiography revealed an absence of normal binding in the dentate molecular layers, further supporting a loss of dentate granular cells. IGF-1 binding in the lesioned regions of the cortex was not increased as compared to the contralateral side. Conversely, IGF-2 binding was dramatically increased in lesioned areas. In the dentate gyrus, IGF-2 binding was increased 42% over normal binding, in spite of a complete loss of granular cells. In the lesioned cortical areas, IGF-2 binding was increased 350% over that normally seen in this region. While it unclear at this time the nature of the increased binding, it is clear that expression of IGF-2 receptors by astroglia following neuronal insult is important to tissue remodelling seen following brain injury. (supported by NIH grant AG07752 to WES).

319.23

6-HYDROXYDOPAMINE (6-OHDA) LESIONS OF THE NIGROSTRIATAL DOPAMINE PATHWAY DIFFERENTIALLY REGULATE SOMATOSTATIN RECEPTOR SUBTYPES IN RAT STRIATUM. J. Zhong, K. Raynor, J-J. Soghomonian, M-F.Chesselet and T. Reisine, Dept. Pharmacol., Univ. Pennsylvania, Philadelphia, PA 19104.

Somatostatin (SRIF) is a neurotransmitter. It induces its biological effects by interacting with multiple receptor subtypes. Two SRIF receptor subtypes can be selectively labeled with the SRIF agonists [125I] MK 678 and [125I] CGP 23996. To explore the interaction of the dopamine-SRIF transmitter systems in the basal ganglia, we have investigated the effect of depletion of dopamine in the striatum on the expression of SRIF receptors in this brain region. Unilateral destruction of the nigrostriatal dopamine pathway with 6-OHDA did not alter [125I] CGP 23996 binding sites in the striatum. However, such lesions increased the level of [125I] MK 678 binding sites in the ipsilateral striatum. The elevated binding is due to an increase in density of [125I] MK 678 binding sites. Analysis of the distribution of [125I] MK 678 binding sites in the striatum of control animals indicated that a gradient of binding sites exists with ventromedial aspects of the striatum exhibiting the highest levels of MK 678-sensitive SRIF receptors and dorsolateral regions having the lowest. Following 6-OHDA lesions, the distribution of [125I] MK 678 binding sites was more homogeneous in the ipsilateral striatum suggesting that the greatest increases in SRIF receptor expression occurred in dorsolateral aspects of the striatum. The enhanced expression of the [125I] MK 678 binding sites may be an adaptive response to diminished synthesis of SRIF in the striatum since similar lesions decrease SRIF mRNA levels in the ipsilateral striatum. The results of these studies indicate that the expression of SRIF receptor subtypes can be differentially regulated and further reveal the close interaction of SRIF and dopamine transmitter systems in the basal ganglia. Supported by NIMH grants 45533 (TR) and 44894 (MFC).

319.24

COMPARTMENTAL LOCALIZATION OF SOMATOSTATIN (SRIF) RECEPTORS IN THE STRIATUM. H. Kong, J. Zhong, K. Raynor, C. Gonzales, Y.Qin, M.F. Chesselet and T. Reisine, Dept. Pharmacol., Univ. Pennsylvania, Philadelphia, PA 19104

The distribution of SRIF receptors in the striatum was measured by autoradiography using the highly selective SRIF agonist [125I] MK 678. [125I] MK 678 binding sites were expressed in an uneven pattern within the rat striatum similar to the distribution of SRIF-like immunoreactivity. To determine whether the distribution of SRIF receptors corresponded to a localization to striosomes or extrastriosomal matrix compartments of the striatum, adjacent sections of rat striatum were analyzed for [125I] MK 678 binding or [^{3}H] naloxone binding, which is a marker for the striosomal compartment of the rat striatum. From these studies it was determined that SRIF receptors are more highly expressed in the extrastriosomal matrix compartment than striosomes in rat striatum. A similar localization of SRIF receptors was observed in the cat caudate nucleus, that is [125I] MK 678 binding sites were predominantly localized to acetylcholinesterase-rich areas, consistent with an extrastriosomal matrix localization. In contrast, [125I] MK 678 binding sites exhibited a patchy distribution in Rhesus monkey caudate nucleus and may be predominantly expressed in striosomes in this species. These species variations in the distribution of SRIF receptors in the striatum may be related to differences in the expression of SRIF. The findings of these studies suggest that SRIF receptors have a compartmental localization in the striatum which may contribute to specifying the functions mediated by SRIF in the striatum. This work was supported by NIMH grants 45533 (TR) and 44894 (MFC).

319.25

ANALOGS OF SOMATOSTATIN SELECTIVELY INTERACT WITH SUBTYPES OF SRIF RECEPTORS IN THE CNS. Karen Raynor and Terry Reisine. Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Somatostatin (SRIF) is a neurotransmitter which produces its multiple biological effects in the central nervous system (CNS) through interactions with membrane-bound receptors. Subtypes of SRIF receptors are found in the CNS which are distinguished by their sensitivities to the cyclic hexapeptide MK 678, as the displacement of [125I]Tyr11SRIF binding by MK 678 was incomplete. The inhibition of [125I]MK 678 binding by SRIF, CGP 23996 and MK 678 was monophasic, consistent with the highly selective labeling of a subtype of SRIF receptor. The inhibition of the binding of [125I]Tyr11SRIF and [125I]CGP 23996 by CGP 23996 was biphasic, indicating that CGP 23996 has differing affinities for subtypes of SRIF receptors. The MK 678-sensitive receptor subtype was labeled with [125I]MK 678 and the binding was potently and monophasically displaced by disulphide bridge-containing octapeptides, such as SMS 201-995, and by other cyclic hexapeptide analogs. The binding was unaffected by dicarba-containing analogs, indicating they do not interact with the MK 678-sensitive receptor. The displacement of [125I]CGP 23996 binding by the octapeptides was biphasic, consistent with the labeling of multiple SRIF receptor subpopulations. The displacement of [125I]CGP 23996 binding by either the hexapeptides or the dicarba-containing analogs was incomplete. The results of inhibition studies showed that the hexapeptides and dicarba-containing compounds were additive in displacing [125I] CGP 23996 binding indicating selective interaction of these compounds with different receptor subpopulations. [125I]MK 678 binding was abolished by 100 uM GTPγS, but [125I]CGP 23996 binding was only partially affected and this corresponded to that component of labeling which was MK 678-sensitive. Thus, two SRIF receptor subpopulations exist in the CNS which are differentially sensitive to the cyclic hexapeptides and dicarba-containing analogs. They are further differentiated by their sensitivities to GTPγS. These peptide analogs will be useful in the future characterization of the functions mediated by SRIF receptor subtypes in the CNS. Supported by MH45533.

319.26

ANTISERUM AGAINST Gia BLOCKS SOMATOSTATIN INHIBITION OF ADENYLYL CYCLASE ACTIVITY Melanie Tallent and Terry Reisine. Inst. of Neurol. Sci. and Dept. Pharmacol., Univ. Pennsylvania, Philadelphia, PA 19104

The neuropeptide somatostatin (SRIF) is a major inhibitor of hormone secretion from the anterior pituitary. It induces its effects, in part, by reducing the synthesis of the second messenger, cAMP. SRIF receptors are believed to be coupled in an inhibitory fashion to the catalytic subunit of adenylyl cyclase via the GTP binding regulatory (G) protein, Gi. In previous studies, we have shown that SRIF receptor/Gi complexes from the pituitary cell line AtT-20 can be immunoprecipitated with an antiserum (8730) directed against the C-terminal region of the alpha subunit of Gi (Gia). In the present study, we show that antiserum 8730 can also dis rupt functional coupling of the SRIF receptor/Gia complex with the catalytic subunit of adenylyl cyclase. In membranes pretreated with antiserum 8730, the inhibitory effect of SRIF on adenylyl cyclase activity was attenuated by 42%. The blockade by antiserum 8730 was specific since it could be prevented by the peptide to which the antiserum was generated. Since antiserum 8730 is directed against the C-terminal region of Gia, these results suggest that this region of the alpha subunit is critical for G protein/adenylyl cyclase coupling. Studies are in progress using antiserum directed against different subtypes of Gia to investigate whether different Gia's direct the receptor to divergent cellular effector systems. Supported by grant MH 45533.

319.27

Interactions of Alpha,Beta,and Gamma Subunits of GTP Binding Regulatory (G) Proteins. **H.M. Juul, S.F. Law, and T. Reisine. Dept. of Pharmacology and Graduate Group in Cell Biol. Univ. of Pennsylvania, Phila. Pa. 19104**

The heterotrimeric G proteins serve to transduce signals from membrane bound receptors to intracellular second messenger systems. Multiple forms of each of the three subunits are known to exist in rat brain. Our lab is investigating the specificity of associations between the alpha, beta, and gamma subtypes of G proteins using methods developed for the elucidation of G protein coupling to the somatostatin receptor. Rat brain homogenates were solubilized with the nonionic detergent CHAPS and then size fractionated on a gel exclusion column. Fractions enriched for somatostatin receptor binding were concentrated and subjected to immunoprecipitation with G protein subtype specific antisera. The resulting immune complexes were then screened by western analysis with antisera directed against a variety of subunits of G proteins to determine the ability of the different subunits to associate. By immunoprecipitating with antisera directed against Gia, and screening the immune complexes using western analysis with antisera against beta 35 and beta 36, it was determined that both forms of beta subunits are capable of associating with Gia. Furthermore, by using antisera specific for each of the Gia subtypes, as well as Gao, we demonstrate that Gia subtypes 1,2, and 3, and Goa, can associate with beta 35 and beta 36. Beta-gamma associations were investigated by immunoprecipitating with beta specific antisera and then screening immune complexes by western analysis with antisera against gamma 2 and gamma 3. It was found that both gamma 2 and gamma 3 coimmunoprecipitate with beta 35 and beta 36, although differences were observed between the amounts of gamma 2 and gamma3 which complex with the beta subtypes. Supported by grants MH45533 and GM08076

319.28

Beta and Gamma Subunits of G Proteins Couple to the Somatostatin Receptor. **S.F. Law and T. Reisine. Dept. of Pharmacology and Graduate Group in Cell Biol. University of Pennsylvania, Phila. Pa. 19104.**

SRIF is a neuropeptide that inhibits growth hormone secretion from the anterior pituitary and is involved in the control neuronal activity. SRIF acts through its receptor to inhibit adenylyl cyclase, Ca2+ conductance and increases K+ conductance. SRIF receptors couple to their cellular effector systems via G proteins. We have previously shown in immunoprecipitation studies utilizing peptide directed antisera against the alpha subunit of G proteins, that Gia1 and Gia3 are associated with the SRIF receptor from rat brain , but Gia2 was not. This same immunoprecipitation protocol was used to study the beta and gamma subunits associated with the SRIF receptor. The SRIF receptor was solubilized from rat brain with the nonionic detergent CHAPS and fractionated by gel exclusion chromatography. The solubilized fractionated SRIF receptor was exposed to peptide directed antisera against the beta subunits of G proteins and precipitated with protein A sepharose. SRIF receptors in the immune complex were detected by the high affinity SRIF agonist 125I MK678. Antisera directed against beta36 specifically immunoprecipitated 40% of 125I MK678 sensitive SRIF receptors whereas antisera directed against beta35 did not. The ability of the beta36 antisera to immunoprecipitate SRIF receptor binding was selectively blocked by the peptide to which they were directed. Along with beta36, gamma2 and gamma3 but not gamma1 immunoreactivity was coimmunoprecipitated with the SRIF receptor. These results indicate that the SRIF receptor from rat brain is associated with beta36 and gamma2 and/or gamma3. Work supported by grant MH45533.

319.29

DEVELOPMENT OF ANTIBODIES AGAINST THE SOMATOSTATIN RECEPTOR

Magali Thévenіau, Stephanie Rens-Domiano, Susan F. Law, Geneviève Rougon and Terry Reisine. Dept. Pharmacol. Univ. of Pennsylvania, PA and URA CNRS 179, Marseille France.

Somatostatin (SRIF) is a neuroregulator of pituitary secretion and in the brain a mediator of different behaviors such as cognition and motor activity. Its effect are triggered through specific G-protein coupled receptors. Polyclonal antibodies directed against the active form of the solubilized brain SRIF receptor have been developed to investigate the molecular mechanisms responsible for the physiological actions of SRIF. These antibodies were purified from immunoblots and characterized. The antibodies are able to immunoprecipitate SRIF receptors from rat brain as well as the pituitary cell line AtT20. In tissues and cell lines that express the SRIF receptor, such as rat brain, AtT20 and GH3, a 55 kDa protein band is specifically detected with the anti-SRIF receptor antibodies by immunoblotting. A similar size protein is also immuno-precipitated from AtT20 cells prelabeled with ^{35}S-methionine. This protein is absent from membrane preparations of cells wich do not express the SRIF receptor.

Since the antibodies specifically react with the SRIF receptor and are able to immunoprecipitate it, they are useful in studying the physical properties of the receptor and its coupling to G-proteins. Furthermore, these antibodies may allow for the cloning of the gene(s) encoding the SRIF receptors.

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319.30

FUNCTIONAL ROLE OF SIALIC ACID RESIDUES ON SOMATOSTATIN (SRIF) RECEPTORS. S. Rens-Domiano and T. Reisine. Dept of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

SRIF is a neurotransmitter in the brain whose physiological actions are mediated by cell surface receptors. We have previously shown SRIF receptors to be glycoproteins and that treatment of solubilized SRIF receptors with neuraminidase removes sialic acid residues from the receptor and diminishes high affinity agonist binding to the receptor. Further studies have been conducted to understand the functional role of sialic acid residues on SRIF receptors. Overnight treatment of intact AtT20 cells, a pituitary tumor cell line, with neuraminidase resulted in the reduction of high affinity binding of the stable SRIF agonist [125I]MK 678 to SRIF receptors. This treatment did not effect the maximal ability of SRIF to inhibit forskolin-stimulated cAMP production in AtT20 cells. Treatment of AtT20 cell membranes with neuraminidase for 5 hours resulted in a 75% decrease in specific, high affinity [125I]MK 678 binding sites. However, when the membranes were pretreated with SRIF agonist, neuraminidase was only able to decrease high affinity binding by 24%. These data would suggest that the desialylated SRIF receptors are functionally active and remain coupled with G-proteins, but exhibit a reduced affinity for agonist. The protection from the effects of neuraminidase on SRIF receptors afforded by SRIF agonist occupancy suggests that the sialic acids may be associated with the ligand bind site of SRIF receptors. Specifically, the negatively charged sialic acid residues may promote high affinity agonist binding to SRIF receptors through electrostatic interactions since SRIF contains a charged lysine residue critical for high affinity binding. Supported by NIMH grant 45533 and NRSA Post-doctoral Fellowship NS 09002

319.31

REGULATION OF SOMATOSTATIN RECEPTORS BY BETA-ADRENERGIC RECEPTOR KINASE (BARK). J. Delfs, S. Rens-Domiano, J. Benovic* and T. Reisine, Dept. Pharmacol. and Inst. Neurol. Sci., Univ. Pennsylvania, Philadelphia PA, 19104 and the Fels Research Inst., Philadelphia, PA, 19140.

The neuropeptide somatostatin (SRIF) is an important physiological regulator of hormone secretion from the pituitary. It induces its actions by stimulating membrane associated receptors. Continuous application of SRIF to pituitary cells desensitizes SRIF receptors. It has been postulated that SRIF desensitization may involve the phosphorylation of the receptor and that this phosphorylation may be catalyzed by the enzyme BARK. To test this hypothesis, SRIF receptors from the pituitary cell line AtT-20 were reacted with purified BARK, SRIF agonist and ATP and the loss of specific agonist binding to the receptor due to phosphorylation was measured. BARK caused a decrease (20%) in the binding of the high affinity, SRIF agonist [125I] MK 678 to SRIF receptors. This effect was blocked by an inhibitor of BARK, dextran sulfate, and required that the receptor be occupied by agonist. These findings are the first direct evidence that BARK may have a role in SRIF desensitization and support the hypothesis that SRIF desensitization may involve the phosphorylation of the SRIF receptor. This work was supported by grants MH 45533, GM 34781, GM 44944 and training grant MH 17168.

PEPTIDES: RECEPTORS III

320.1

CP-96,345 A SELECTIVE NONPEPTIDE TACHYKININ NK1 RECEPTOR ANTAGONIST. A.H. Ganong, L.S. Reynolds, C.J. Siok, D.K. Bryce* and S. McLean, Dept of Neuroscience, Central Research Division, Pfizer Inc., Groton, CT 06344.

CP-96,345 is a recently described nonpeptide NK_1 tachykinin receptor antagonist (*Science* 251:434-439,1991). Using radioligand binding assays and extracellular recordings from locus coeruleus (LC) neurons in a slice preparation, the selectivity and functional activity of CP-96,345 at the NK_1 receptor is described. For the binding assays, membranes (P_2) from guinea pig striatum (NK_1), hamster bladder (NK_2), and guinea pig cortex (NK_3) were incubated with ^{3}H-SP (2.3 nM), ^{125}I-iodohistidyl-NKA (0.1 nM) and ^{125}I-BH-eledoisin (0.1 nM), respectively.

The rank order of potency in displacing ^{3}H-SP binding to the NK_1 receptor was CP-96,345>SP>physalaemin>eledoisin>NKA>NKB. In contrast, the rank order for displacing ^{125}I-NKA binding was NKA>SP>eledoisin>>CP-96,345. Similarly, CP-96,345 was relatively weak in displacing ^{125}I-BH-eledoisin binding with the rank order of potency senktide>eledoisin>NKB>NKA>SP>CP-96,345. CP-96,345 is atleast 100-fold selective for the NK_1 receptor with IC_{50} values of 5 nM for the NK_1 receptor and, >2000 nM at the NK_2, and NK_3 receptors.

The NK_1 agonist substance P produced a dose-dependent increase in firing rates of locus coeruleus neurons recorded extracellularly in guinea pig brainstem slices; maximal firing rate increases of ~500% were acheived with 1-10 μM SP. Excitations induced by 100 nM SP were inhibited dose-dependently by CP-96,345 with an IC_{50} value of 90 nM. Excitations produced by the NK_3 agonist senktide were not significantly inhibited bby 1 μM CP-96,345.

CP-96,345 will be a useful pharmacological tool for elucidating the role of tachykinin receptor subtypes in the physiological actions of endogenous tachykinin transmitter and the utility of NK_1 antagonists as therapeutic drugs.

320.2

DIFFERENTIAL BINDING OF CP-96345 ACROSS SPECIES: POSSIBLE HETEROGENEITY OF THE NK1 TACHYKININ RECEPTOR. D. K. Bryce* and S. McLean Dept of Neuroscience, Central Research Division, Pfizer Inc., Groton, CT 06340.

The recent discovery of CP-96345, a potent and selective non-peptide NK_1 receptor antagonist provides a valuable tool for the study of the pharmacology of NK_1 receptors. As suggested by Snider et al. (*Science*.251: 435-437, 1991), species differences exist at the tachykinin NK_1 receptor. Investigation of this heterogeneity was carried out using [^{3}H]-substance P (SP), [^{3}H]-CP-96345, and [^{125}I]-Bolton-Hunter (BH) SP, in radioligand binding experiments using P_2 preparations of mouse, rat, guinea pig, and marmoset whole forebrain. Saturation studies indicated that the affinity and receptor density for [^{3}H]-SP was similar across species, consistent with values reported previously (see Dam and Quirion, *Peptides*. 7: 855-864, 1986). In contrast, the binding affinity of [^{3}H]-CP-96345 differed markedly across species. In the guinea pig and marmoset [^{3}H]-CP-96345 bound with high affinity (Kd = ~1.0nM), while in the rat and mouse binding affinity was decreased 10-30 fold. Differences between species were also obtained in competition studies. CP-96345 was 10-20 fold more potent in displacing the binding of [^{125}I]-BH-SP to guinea pig and marmoset brain than to rat or mouse brain while SP did not discriminate among the species (IC_{50}s=1-2 nM). Although agonist binding does not differentiate among the species, the selective NK_1 receptor antagonist CP-96,345 exhibits marked differences in potency.

320.3

HYPERLOCOMOTION INDUCED BY ICV [SAR^9, MET(O$_2$)^{11}]-SP IS REVERSED BY THE SELECTIVE NK-1 ANTAGONIST, CP-96,345. P. A. Seymour, G. L. Robinson* and D. K. Bryce*. Dept. of Neuroscience, Central Research Division, Pfizer Inc., Groton, CT 06340.

Studies have shown that ICV administration of Substance P (SP) induces hyperlocomotion in rats (e.g. Elliot and Iversen, Brain Res. 381,1986, 68) and guinea pigs (Brent et al., Neuropharm. 27:7,1988, 743). Since the SP analog, [SAR^9, MET (O$_2$)^{11}]-SP has been shown to be a potent and selective agonist at NK-1 receptors (Drapeau et al., Neuropeptides 10,1987, 43) these studies were aimed at elucidating the role of NK-1 receptors in the hyperactivity response to SP. Guide cannulae were implanted in the lateral ventricles of anesthetized guinea pigs and after recovery from surgery, animals were placed into automated behavioral chambers for overnight habituation. [SAR^9, MET (O$_2$)^{11}]-SP was infused into the ventricles and activity was recorded for several hours. Data analysis showed that this compound significantly increased horizontal locomotor activity in a dose related manner (3.2-32 μg/side) for approximately 2 hours. In antagonism experiments compounds were given s.c. 30 minutes after ICV [SAR^9, MET (O$_2$)^{11}]-SP (25 μg) and data were recorded for 1.5 hours. These studies showed that the nonpeptidic NK-1 antagonist, CP-96,345, significantly and completely antagonized the hyperactivity response with an ED50 of 1.9 mg/kg. In contrast, the enantiomer of CP-96,345, which lacks appreciable NK-1 receptor affinity, was without effect. These results support the hypothesis that SP increases locomotor activity by agonist activity at NK-1 receptors, and demonstrate that CP-96,345 acts as an antagonist at brain NK-1 receptors in vivo.

320.4

QUANTITATIVE AUTORADIOGRAPHY OF THE SUBSTANCE P (NK_1) RECEPTOR WITH A NEW NON-PEPTIDE ANTAGONIST LIGAND: 3H-CP-96,345. T. F. Seeger and K. G. Pratt*, Neuroscience Dept., Pfizer Central Research, Groton, CT 06340.

CP-96,345 is the first potent non-peptide antagonist of the Substance P (SP) NK_1 receptor (Science 251:435-439). CP-96,345 was tritiated and binding conditions optimized for autoradiography in hamster brain sections. 3H-CP-96,345 demonstrated saturable binding (Kd = 1.9 nM) to sections that was displaceable by SP, but not by Substance K or eledoisin at submicromolar concentrations, suggesting selective interaction with the NK_1 receptor. The distribution of 3H-CP-96,345 binding closely matched the pattern of 3H-SP binding visualized in adjacent sections. Dense binding was noted in striatum, nucleus accumbens, bed nucleus, cortical amygdala, habenula, and the septohippocampal, amygdalohippocampal and periventricular nuclei. Moderate levels were seen in hypothalamus, superior colliculus, medial geniculate, subiculum, and central gray. Little specific binding was noted in cortex, hippocampus, cerebellum, and most thalamic nuclei. Similar patterns of 3H-CP-96,345 binding were seen in guinea pig and marmoset brain. 3H-CP-96,345 provides a valuable tool for the study of the mammalian NK_1 tachykinin receptor.

320.5

NOVEL NON-PEPTIDE SUBSTANCE P ANTAGONISTS IN THE RAT. L.D. Aimone, K.C. Appell*, S.C. Chippari*, A.L. Harris* and S.J. Ward, Sterling Research Group, 81 Columbia Turnpike, Rensselaer, NY 12144, 9 Great Valley Parkway, Malvern, PA 19087

We describe the discovery of WIN 51708 and WIN 62577, novel non-peptide substance P (SP) antagonists with selectivity for the NK-1 receptor. NK-1 activity was defined against [125I] SP in a rat forebrain binding assay. WIN 51708 and WIN 62577 both bind competitively at the NK-1 site with a Ki of 22 ± 5 nM (n=3) and 21 ± 4 nM (n=3), respectively. These compounds have Kis of 1.3 and 4.5 uM against [125I] neurokinin A binding at the NK-2 site in the rat duodenum.

Plasma extravasation (PE) of Evans Blue dye was induced by the intraplantar injection of SP (2.5 nM). Antagonists (0.1 to 3.0 mg/kg, IV) were administered 2 min prior to SP. WIN 51708 and WIN 62577 produced a dose-dependent inhibition of SP-induced PE with IC50s of 0.9 ± 1.0 and 2.2 ± 0.8 mg/kg. Neither compound was effective in inhibiting PE induced by bradykinin (2.5 nM). An in-vivo time course revealed WIN 51708 to be active in the PE assay for greater than 45 min.

WIN 51708 and WIN 62577, novel non-peptide NK-1 selective antagonists, will be useful in defining the physiological role of NK-1 receptors.

320.6

CHARACTERIZATION OF THE SUBSTANCE P RECEPTOR IN TUNICAMYCIN-TREATED TRANSFECTED CELLS USING A PHOTOAFFINITY ANALOG. R. Kage*[1], A.D. Hershey[2], J.E. Krause[2], N.D. Boyd*[1], S.E. Leeman[1]. [1]Univ. of Mass. Medical Center, Worcester, MA 01655; [2]Washington Univ. School of Medicine, St.Louis, MO 6311A

A photoaffinity analog of substance P (SP), p-benzoyl-L-phenylalanine-substance P [Phe^8(pBz)]SP, has recently been described (Boyd et.al., Biochemistry 30, 336, 1991). CHO cells transfected with the complete coding region of the rat substance P receptor (SPR) cDNA were treated with 5 µg/ml tunicamycin for two days. Increasing concentrations of SP inhibited the binding of ^{125}I-[Phe^8(pBz)SP] to non-treated and tunicamycin-treated cells with the same IC_{50} (0.3 nM). Photolabelling of tunicamycin-treated CHO cells with ^{125}I-[Phe^8(pBz)SP] followed by SDS-PAGE and autoradiography showed two protein bands; one broad band centered at a mol.wt. of about 80,000 corresponding to the SPR in non-treated cells and a second narrower band with a mol.wt. of 48,000 corresponding to the mol.wt. of the photolabelled SPR which has been incubated with endoglycosidase F to remove N-linked oligosaccharides. Tunicamycin-treated CHO cells were photolabelled in the presence of increasing concentrations of SP and the radioactive SPR bands quantitatively analyzed after SDS-PAGE and autoradiography. These experiments give the first direct evidence that in CHO cells expressing the SPR the non-glycosylated receptor is able to bind SP with an affinity that is not significantly different from the glycosylated receptor. (Supported by grants DK29876, NS21937, and NS25151.)

320.7

CHARACTERIZATION OF ELECTROPHYSIOLOGICAL RESPONSE TO SUBSTANCE P IN HUMAN ASTROCYTOME CELL LINE U373. L. Pradier, S. Le Guern*, M. Laville*, A. Doble, Rhone-Poulenc Rorer, CRVA, 13 Quai J. Guesde, 94400 Vitry, France.

The neuropeptide substance P (SP) is a member of the tachykinine family. Its function in certain smooth muscle stimulation has long been known and its presence in sensory C afferent fibers and dorsal horn of the spinal chord has made SP a prime candidate as a pain neurotransmitter. However functions in the higher structures of the central nervous system have remained elusive. We report studies of SP responses in the human astrocytome cell line U373 using the whole-cell patch-clamp technique. Reproducible responses to brief application of SP (10 nM) could be obtained with little desensitization and were shown pharmacologically to be mediated by an NK1 type receptor. At -30 mV, the biphasique SP response was composed of an initial large outward current followed by a slow inward component. Both currents were carried by K ions as demonstrated by shifts in the reversal potential upon external [K^+] modification. The two K channels could be differentiated pharmacologically, the first component being likely activated by the rise in internal [Ca^{2+}]. Responses could be elicited in the absence of external Ca although they displayed a marked desensitization under those conditions. The human U373 cell line may thus represent a convenient system to study the pharmacology of the NK1 receptor in the human brain.

320.8

NEUROKININ RECEPTOR-MEDIATED DEPOLARISATION OF NEURONES IN RAT SUPERIOR CERVICAL GANGLIA. Seabrook G.R., Main M.J.*, Hill R.G., Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR, U.K.

At least 3 types of neurokinin receptors are present on neurones. It has been reported that the rank order of potency of neurokinin agonists at producing depolarisations in rat superior cervical ganglia (SCG) are inconsistent with described receptor subtypes (Brown et al 1983). Here we provide evidence for a heterogeneous population of neurokinin receptors in rat SCG which can account for this atypical agonist profile.

DC recordings were made from isolated rat SCG using grease-gap methodology in the presence of tetrodotoxin (0.1 µM). The rank order of agonist potency was as follows (EC50 in nM): senktide (4) > substance P (12) > substance P methyl ester (30) = eledoisin (33) > neurokinin B (118) > neurokinin A (267). It was apparent that the maximal depolarisation induced by the NK3 selective agonist, senktide, was less than that of the other agonists (27% that of 1 µM eledoisin). Similarly, the NK1 selective ligands substance P and its methyl ester had submaximal efficacies of 72% and 77% respectively. The depolarisations elicited by senktide and eledoisin were not blocked by atropine (1 µM).

It is evident from the above data that rat SCG possess both NK1 and NK3 receptors. The need for determination of both agonist potency and efficacy in characterisation of neurokinin receptor subtypes is emphasised.

Brown et al. 1983 J. Physiol. (Lond.) 334; 91P

320.9

A NOVEL NK_2 RECEPTOR SUBTYPE IN FIBROBLASTS TRANSFECTED WITH BOVINE cDNA EXPRESSING NK_2 RECEPTORS A.K. Henderson, P.L.M. van Giersbergen[1,2], S.H. Buck[1], W.R. Roeske and H.I. Yamamura Departments of Pharmacology & Internal Medicine, University of Arizona, Tucson, AZ 85724; [1]Marion Merrell Dow Research Institute, Cincinnati, OH 45215; [2]Department of Pharmacology & Cell Biophysics, University of Cincinnati, Cincinnati, OH 45267.

Tachykinin receptors have been classified into three distinct subtypes by their rank order of affinity to neurokinin agonists. Recently two antagonists for the neurokinin A receptor (NK_2), [Tyr^5, D-Trp^{6,8,9}, Arg^{10}]-NKA(4-10) (MEN 10207) and [Tyr^5, D-Trp^{6,8,9}, Arg^{10}]-NKA(3-10) (MEN 10208), demonstrated heterogeneity among NK_2 receptors in rabbit pulmonary artery (RPA) and hamster trachea (HT). Both antagonists had high affinity for NK_2 receptors in RPA and low affinity for these receptors in HT (Maggi, 1990). In contrast, these ligands had different binding affinities for NK_2 receptors expressed by SKLKB82#3 cells, fibroblasts transfected with bovine stomach cDNA encoding for this receptor. In competitive ligand/[^{125}I]NKA assays with SKLKB82#3 cells, the IC_{50} value was 21 nM for MEN 10207 and 691 nM for MEN 10208. This difference was also observed in bovine stomach membranes which had an IC_{50} value of 54 nM and 1560 nM, respectively. In addition, in the SKLKB82#3 cells the pA_2 values were 7.96 for MEN 10207 and 6.59 for MEN 10208 in inhibiting NKA induced [^{3}H]IP_1 accumulation. The different affinities of MEN 10207 and MEN 10208 for the NK_2 receptor in RPA, HT or bovine stomach may be a result of a species difference or indicate distinct NK_2 receptor subtypes.

(Maggi et al., Br. J. Pharmacol. 100, 588, 1990)

320.10

NEUROPEPTIDE γ-PREFERING SUBTYPES OF NK2 RECEPTORS IN RAT FUNDUS AND DOG URINARY BLADDER. E. Burcher and C.J. Mussap*, School of Physiology and Pharmacology, University of New South Wales, Sydney, NSW 2033, Australia.

Recent work with novel antagonists has suggested that subtypes of the tachykinin NK2 receptor exist. L-659,877 and MDL 29,913 show preference for one postulated subtype; MEN 10207 shows preference for another. In this study, we have used radioligand binding and isolated organ techniques to characterize the NK2 receptors found in rat fundus and dog bladder. In homogenates of rat fundus, binding of 100 pM [^{125}I]-iodohistidyl NKA (INKA) was inhibited by neuropeptide γ (NPγ) = neuropeptide K (NPK) > NKA > kassinin > MDL 29,913 ≥ [Sar^9,Met(O_2)^{11}]-SP ≥ neurokinin B (NKB) ≥ substance P (SP) >> MEN 10207 >> senktide. The potency order for contraction of the isolated fundus strip was NPγ > kassinin ≥ NKA ≥ NPK ≥ [Lys^5,MeLeu^9,Nle^{10}]-NKA(4-10) > NKB, with senktide and [Sar^9,Met(O_2)^{11}]-SP ineffective. MDL 29,913 competitively antagonized responses to NPγ (pA_2 7.2) and other tachykinins. In homogenates of dog bladder (minus mucosa), INKA binding was inhibited by NPγ >> NKA = kassinin = NPK >> NKB ≥ SP > L-659,877 > MDL 29,913 > MEN 10207 >> senktide. In isolated strips, the rank potency order was NPγ ≥ NKA ≥ kassinin ≥ [Lys^5,MeLeu^9,Nle^{10}]-NKA(4-10) = NPK = NKB. Contractile responses were diminished by atropine or mepyramine and were enhanced by phosphoramidon. Responses to NPγ, NPK and [Lys^5,MeLeu^9,Nle^{10}]-NKA(4-10) were antagonized by MDL 29,913 in a competitive manner (pA_2 6.4 - 6.6). Autoradiographic studies showed binding sites for INKA over the circular muscle and muscularis mucosae of rat fundus and over the muscularis and blood vessels of dog bladder. These data provide evidence for "classical" MDL 29,913-preferring NK2 subtypes in rat fundus. The NK2 receptors in dog bladder are less easy to categorize. NK2 receptors in both tissues have high affinity for NPγ.

320.11

NEUROPEPTIDE RECEPTORS IN DEVELOPING AND ADULT RAT SPINAL CORD: AN *IN VITRO* QUANTITATIVE AUTORADIOGRAPHY STUDY. S. Kar, J. -G. Chabot and R. Quirion. Douglas Hospital Research Center, Department of Psychiatry, McGill University, Montreal, Canada H4H 1R3.

The spinal cord which forms a vital link in relaying information to and from the brain, is endowed with a number of neuropeptides. These peptides are considered to function as neurotransmitters/neuromodulators in spinal synaptic transmission and are localized primarily in the superficial layers of the dorsal horn, intermediolateral cell column and in the ventral horn of the spinal cord. To provide a substrate for better understanding of neuropeptide functions, we have studied the distribution as well as the postnatal development of calcitonin gene-related peptide (CGRP), neurokinin/substance P (NK-1 and NK-3), galanin (GAL), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), peptide YY (PYY) and μ opioid receptor binding sites in the spinal cord of rat using *in vitro* quantitative receptor autoradiography. The distribution of the receptor binding sites for each peptide demonstrated a characteristic pattern in the developing and adult spinal cord. At postnatal day 1, notwithstanding the variation in the density of labelling, the receptor binding sites of all the peptides studied were observed throughout the grey matter as well as the white matter of the spinal cord. Changes in the pattern of distribution of binding sites were not noticed until day 7 of postnatal development. However, the receptor binding sites of each neuropeptide subsequently exhibited dramatic alterations at all regions of the spinal cord and attained the adult pattern of distribution during third week of postnatal development. In the adult rat spinal cord, high density of receptor binding sites of all the peptides, except CGRP, were found primarily in the superficial layers (laminae I-II) of the dorsal horn. Labelling in areas around the central canal, intermediolateral cell column and in the ventral horn was relatively low and varies according to the neuropeptides as follows: NK-1>CGRP>GAL>PYY>μ opioid>NPY>NK-3>VIP. This study thus reveals that the receptor binding sites of the peptides differ in terms of their distribution and pattern of development. Early appearance of binding sites and their dramatic variation during the course of development suggest an important role for these peptides in early maturation and organization of the spinal cord. Furthermore, the presence of receptor binding sites in discrete areas of the adult spinal cord strongly support the implication of neuropeptides in various sensory, autonomic and motor functions of the spinal cord.

320.12

DISTRIBUTION OF SUBSTANCE P BINDING IN THE GASTROINTESTINAL TRACT OF BB RATS - AN ANIMAL MODEL OF DIABETES MELLITUS. Q. Yu*, D.H. Silberberg, and A. Ouyang. Gastrointestinal Section, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6144, U.S.A.

Introduction: In patients with diabetes mellitus, neuropathy in the gastrointestinal tract is a common occurrence, and the disease is associated with gastroparesis and altered bowel habits. Previous reports have shown that the substance P (SP) content of the peripheral nerves was reduced in diabetic patients and in animal models of diabetes, e.g., the BioBrecding (BB) rats. The present study investigated the SP binding in the stomach, (pylorus and antrum) and the colon (proximal and distal regions)

Methods: BB rats (4 - 6 months old) were anesthetized with pentobarbital and the selected regions of the gut were dissected, cleaned and quick frozen in liquid nitrogen. Ten micron sections were cut and the binding assay was performed at room temperature with 100 pM ^{125}I-SP for 2 hr and passed through 4 washes. Hyperfilm^{TM}-^{3}H (Amersham) were then exposed to these dried ^{125}I-SP bound gut slices for 7 days. The circular muscle layers of gut autoradiograms were analyzed.

Results: In the diabetic (D) BB rats, SP binding (nCi/mg protein ± SEM) was found to be higher in the pyloric stomach and the distal colon than in the non-diabetic (ND) BB rats (Pyloric stomach: D: 110.47 ± 2.65, N = 4; ND: 90.58 ± 2.94 , N = 5; P = 0.002, 2-tailed Student's t-test. Distal colon: D; 131.05 ± 3.25, N = 4; ND: 97.638 ± 7.14, N = 5; P = 0.006). There were no observable changes between the ND and the D rats in the antral stomach (D;: 82.18 ± 4.25, N=4; ND: 79.38 ± 3.88, N = 3; P > 0.05) and the proximal colon (D; 112.01 ± 7.25, N=4; ND: 123.64 ± 2.96, N = 5; P >0.05).

Conclusion: The present data suggest that, in the circular muscle layer of the gastrointestinal tract of the BB rats, there are regional differences in SP binding between ND and D rats, a condition which may be contributing to physiologic abnormalities of intestinal function in diabetes mellitus.

320.13

DISTRIBUTION OF NEUROKININ RECEPTOR GENE EXPRESSION IN THE RAT BRAIN. M. S. Poosch, D.J. Goebel and M.J. Bannon. Dept. of Psychiatry, Wayne State University, Detroit, MI 48201.

The neurokinin receptors NK1, NK2 and NK3 have been classified based on their differing affinities for various tachykinin peptide ligands. Recently, the expression of these receptor genes in the brain and peripheral tissues has been confirmed by solution hybridization techniques. NK1 and NK3 clones (obtained by PCR amplification of rat cortex cDNA) as well as an NK2 clone (courtesy of J. Krause) were used to detect gene expression of each receptor type by simultaneous solution hybridization using ^{32}P labeled probes. The rank order of NK1 receptor mRNA expression in selected areas of the rat brain was as follows: hypothalamus> striatum> olfactory bulb> substantia nigra= hippocampus> cortex>> cerebellum. Expression of the NK2 receptor was limited to the hippocampus, hypothalamus and substantia nigra, accounting for only 1% of the total amount of neurokinin receptor mRNA in these regions. The NK3 receptor was the most predominantly expressed of the three receptor types, with substantia nigra and cerebellum exhibiting the highest concentration followed by the cortex, hypothalamus, striatum, hippocampus and olfactory bulb. The cellular localization of neurokinin receptor gene expression is under investigation using *in situ* hybridization and lesion experiments.
Supported by: grant MH 43026.

320.14

HYBRID CELLS DERIVED FROM SEPTAL CHOLINERGIC NEURONS EXPRESS NEUROTENSIN RECEPTORS. M-P. Faure*, I. Shaw*, D.N. Hammond, N.R. Cashman and A. Beaudet. Lab. of Neuroanatomy, Montreal Neurological Institute, McGill Univeresity, Montreal, Quebec, Canada, H3A 2B4.

Autoradiographic studies from our laboratory have demonstrated a selective association of high affinity neurotensin (NT) binding sites with basal forebrain cholinergic neurons. In an attempt to establish an in vitro model for further characterization of these sites, the binding of 125-I-Tyr3 NT and of fluorescein isothiocyanate (FITC)-conjugated NT was examined in hybridomas SN6 and SN17, produced by fusion of embryonic murine septal cells with a murine neuroblastoma (Science, 1986). Both cell lines showed saturable, high affinity NT binding. Flow cytometric histograms indicated that 97.4% of cells in SN6 and 99.2% of cells in SN17 stained positively for NT-FITC. Scatchard analysis of 125-I-NT binding at 4°C yielded a linear plot for SN6 (Kd=1.7 nM; Bmax=180 fmol/mg prot) and a biphasic plot for SN17 (Kd=1.5 and 5.0 nM; Bmax=120 and 281 fmol/mg prot) suggesting that the SN17 cell line may be expressing two different types of binding sites. Whereas most of the radioactivity bound at 4°C was detached from either type of cells by an acidic wash, more than 50% of the ligand bound at 37°C resisted such treatment, suggesting that a large fraction of the radioactivity had been internalized. Confocal microscopic examination of cells incubated with NT-FITC at 37°C confirmed the presence of "hot spots" of internalized ligand molecules. These results indicate that both SN6 and SN17 cell lines express high affinity NT binding sites and that these sites may be internalized upon ligand interaction, suggesting that they correspond to functional receptors.

320.15

AFFINITY CHANGES OF NEUROTENSIN RECEPTORS AFTER CHRONIC ETHANOL: CORRELATIONS WITH TOLERANCE. A.D. Campbell and V.G. Erwin. School of Pharmacy and Alcohol Research Center, University of Colorado, Boulder, CO 80309.

Neurotensin (NT), a tridecapeptide widely distributed in brain, acts as a neurotransmitter or neuromodulator, and may mediate some of the effects of ethanol. This laboratory has shown that NT produces a dose-dependent increase in ethanol sensitivity in short sleep (SS) but not long sleep (LS) mice, which were selectively bred for differences in ethanol sensitivity. The NT receptor system in these mice is best described by a two site model, with high (NT_H) and low (NT_L) affinity receptors being present, the latter being sensitive to the antihstamine, levocabastine. The LS and SS mice have been shown to differ in NT receptor density in several brain regions. The present study was conducted to determine the effects of chronic ethanol adminlstration on NT receptor systems in these lines of mice. After two weeks of ethanol consumption, receptor binding was best described by a one site model, and effects at three or four weeks were not different from two weeks. Unlike control K_D values (0.3 and 3.5 nM), Scatchard analysis of [^{3}H]NT binding in ventral midbrain and entorhinal cortex membranes from chronically treated mice revealed a one site model, with a K_D value of 1 nM. The B_{max} values, however, were not changed by this treatment. Additionally, the numbers of levocabastine- sensitive and insensitive receptors were not changed, suggesting a shift in the affinity of NT_H and/or NT_L receptors. The effects of ethanol administration on the affinity of NT receptors closely paralleled the development of behavioral tolerance and the disappearance of tolerance after withdrawal. Indeed, after four weeks of withdrawal from ethanol, the receptor characteristics appeared to return to control values. The finding that chronic ethanol markedly affects the NT receptor systems further supports the hypothesis that ethanol's actions may be mediated in part by neurotensinergic systems. (Supported by NIAAA Grants 00079, 30527, and 07330).

320.16

CHANGES IN NEUROTENSIN RECEPTOR DENSITY IN RATS CHRONICALLY TREATED WITH HALOPERIDOL AND SIGMA ANTAGONIST BMY 14802. J.T. Clapacs*, S.T. Cain, D.L. Knight, W.E. Smith and C.B. Nemeroff. Departments of Psychiatry and Pharmacology, Duke University Medical Center.

Neurotensin (NT) is an endogenous tridecapeptide found in the mamalian CNS which fulfills many of the neurotransmitter criteria. NT concentrations have been shown to be increased in the striatum and nucleus accumbens of rats treated chronically with clinically effective antipsychotic drugs. Similar changes in NT concentrations in the nucleus accumbens and caudate nucleus have been observed after treatment with the sigma receptor antagonist BMY 14802. Male Sprague-Dawley rats were treated daily with either haloperidol (1 mg/kg/day) or BMY 14802 (35 mg/kg/day) for 21 days. On day 22 all animals were killed by decapitation and their brains rapidly dissected and frozen. Frontal cortex, striatum, nucleus accumbens, hypothalamus and VTA-substantia nigra were later dissected from the frozen brains. Single-point binding was performed (at a concentration of 0.4nM NT) on each sample using mono-iodinated I^{125}-(Tyr^3) NT prepared by the lactoperoxidase reaction. Incubation with 1 μM unlabeled NT was used to define non-specific binding. Data was expressed as fmoles/mg protein. NT receptor binding was significantly decreased in frontal cortex of haloperidol treated animals compared to controls. A similar trend toward a decrease in frontal cortex NT binding was seen in BMY 14802 treated animals. No significant changes in NT binding were found in any other regions in either treatment group. These results indicate that the NT concentration increases observed after chronic antipsychotic drug treatment may not be associated with increased release of NT, because this would presumably result in NT receptor down regulation. Delineating the complex interactions between NT and dopamine systems remains a critical focus of investigation. Supported by NIMH MH-39415.

320.17

INJECTION OF MRNA FROM PANCREAS AND BRAIN INDUCE DIFFERENT TYPES OF CCK RECEPTORS IN XENOPUS OOCYTES. K.Kawasaki and Y.Shigeri*, Div. of Pharmacol., Shionogi Res. Labs., Shionogi & Co., Ltd., Osaka 553, Japan

Microinjection of mRNA from rabbit pancreas and rat hippocampus into Xenopus oocytes induced different types of CCK receptors, the peripheral- (CCK_A-R) and central-type (CCK_B-R) of CCK receptors. CCK-8 induced oscillating inward current mediated by CCK_A-R (CCK_A-responses) and CCK_B-R (CCK_B-responses). CCK_A- and CCK_B-responses were selectively antagonized by L-364718 and by L-365260, respectively. Non-sulphated CCK-8, CCK-4 and gastrin I had weak agonistic actions only in oocytes injected with hippocampal mRNA. Ramp clamp analysis showed that the equilibrium potential of CCK_A-responses was a little more positive (-15 ~ -20 mV) than that of CCK_B-responses. Although intracellular injection of EGTA completely abolished CCK_B-responses, slow inward current in CCK_A-responses remained after EGTA. The equilibrium potential of this slow current was -5 ~ 0 mV. Lowering $[Ca^{++}]_o$ reduced CCK_A-responses, while CCK_B-responses were relatively resistant to $[Ca^{++}]_o$ change. CCK_B-responses were more easily desensitized than CCK_A-responses. These results indicate that injection of mRNA from rabbit pancreas or from rat hippocampus induces pharmacologically different CCK receptors, CCK_A-R or CCK_B-R subtype, respectively. Activation of CCK_B-R opens Ca^{++}-dependent Cl^- channels, while that of CCK_A induces opening of non-specific cation channels as well as Ca^{++}-dependent Cl^- channels in mRNA injected Xenopus oocytes.

320.18

RABBIT VAGUS NERVE CONTAINS BOTH CCK-A AND CCK-B RECEPTORS. T. R. Miller*, D.G. Witte*, M. Holladay*, A. M. Nadzan, and C.W. Lin. Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064.

Cholecystokinin (CCK) receptors on vagal afferents have been implicated in mediating many of the actions of the brain-gut peptide, CCK, including satiety, release of pituitary hormones and enhancement of memory. CCK receptors have been classified as CCK-A and CCK-B based on affinities for CCK, gastrin and related peptides. Autoradiographic studies in rat have indicated the predominance of CCK-A receptors in vagus. Here, using [125I]-Bolton-Hunter(BH)-CCK-8 as the radioligand, we have characterized CCK receptors in membranes prepared from rabbit vagus nerve. [125I]-BH-CCK-8 binding was inhibited by the guanine nucleotide, Gpp(NH)p, with an IC50 of 140 nM. Competition experiments showed that CCK-8 (IC50= 0.25 nM) was about 280-fold more potent than desulfated CCK-8, suggestive of the CCK-A receptor. Competitive binding studies using A-71378, a selective CCK-A agonist, exhibited biphasic inhibition curves, with the high affinity portion (<10 nM) at ~60% and low affinity portion at ~40%. Conversely, competitive binding studies using A-63387, a selective CCK-B receptor agonist, also showed biphasic displacement curves with the high affinity portion at ~35% and low affinity portion at ~60%. These results suggest that rabbit vagal CCK receptors contain both A and B subtypes, at ~60 and ~40 %, respectively, which are linked to G protein. The presence of CCK-B/gastrin receptors on vagus may have implications for many of the behavioral effects of selective CCK-B peptides, such as anxiety.

320.19

LOCALIZATION OF CHOLECYSTOKININ RECEPTORS IN THE BRAZILIAN OPOSSUM BRAIN. M. C. Kuehl, L. R. Ross*, J. K. Elmquist, C. A. Fox and C. D. Jacobson. Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

We have studied the anatomical distribution of cholecystokinin (CCK) receptors in the brain of the adult Brazilian short-tailed opossum, *Monodelphis domestica*. *Monodelphis* is a small marsupial whose young are born in an extremely immature state making them an excellent model for studying development. CCK receptors were localized in tissue sections with Bolton-Hunter I^{125} labeled CCK. Adult Brazilian opossums were decapitated and the brains were rapidly isolated, frozen on dry ice, sectioned on a cryostat, and thaw-mounted onto slides. Sections were incubated with buffer containing I^{125} CCK, washed, and subsequently exposed to LKB ultrofilm. Following film exposure, slides were processed for autoradiography. Brain regions were characterized as having CCK receptors if they contained greater densities of silver grains (on film & on the slides) compared to adjacent tissue sections that were exposed to I^{125} CCK and unlabeled CCK. In the forebrain, CCK receptors were observed in the CA1 region of the hippocampus, neocortex, corpus striatum, medial preoptic area, posterior amygdaloid area, and ventromedial hypothalamic nucleus. In the brainstem, CCK receptors were observed in the nucleus of the solitary tract and in the spinal nucleus of the trigeminal nerve. The anatomical distribution of CCK receptors in *Monodelphis* is similar to the distribution patterns described for CCK receptors in other mammals and supports the use of *Monodelphis* in studies on the developmental expression of CCK receptors in the brain.

320.20

PARTIAL PURIFICATION AND CHARACTERISATION OF THE CHOLECYSTOKININ TYPE B RECEPTOR FROM PORCINE CEREBRAL CORTEX. J C Hunter[1], K Forde*[1], J Hughes[2], E A Barnard[1] and S H Gut[1]. [1] MRC Molecular Neurobiology Unit, MRC Center, Hills Road, Cambridge CB2 2QH, U.K.; [2] Parke-Davis Research Unit, Hills Road, Cambridge CB2 2QB, U.K.

Cholecystokinin (CCK) receptors can be divided into either CCK_A or CCK_B /gastrin on the basis of both anatomical localization and pattern of selectivity to agonists and antagonists. CCK_A receptors are predominant in peripheral tissues and show enhanced sensitivity to CCK-8S and L-364,718 while those of the CCK_B type predominate in the CNS and show particular affinity for pentagastrin, L-365,260 and PD 134308 (CI-988) (Hughes et al., PNAS 87, 6728-6732, 1990) . CCK_B sites were digitonin (2%) solubilized from pig cerebral cortex membranes (Gut et al., Eur J Pharmacol 172, 339-346, 1989) and purified by a four-step chromatographic procedure involving 1) anion exchange (AEC, sepharose Q), 2) Wheat Germ Agglutinin sepharose, 3) Affigel-15-pentagastrin, 4) Phenyl-sepharose. Following this procedure a final purification was estimated at approximately 7000. The affinity purified protein was also re-chromatographed by AEC, separated into several individual fractions and, following SDS-PAGE and silver staining, identified as a major band of either 70kDa or the deglycosylated core protein of around 45kDa. The density of the band corresponded directly to the level of specific [^{125}I]-CCK-8S binding in each fraction. The rank order of potency for the inhibition of [^{125}I]-CCK-8S binding to this purified form of the receptor (CCK_{8S} ≃ gastrin $_{1-17}$ > CI-988 > L-365,260 >> L-364,718) confirmed its identity as the CCK_B receptor.

320.21

OPIOID RECEPTOR INTERACTION AS A MECHANISM OF CCK PEPTIDE ANALGESIC ACTIVITY. J. Slaninova, R.J. Knapp, S.-N. Fang, T. Kramer, T.F. Burks, V.J. Hruby, and H.I. Yamamura. Univ. of AZ Coll. of Med, Pharmacol. and Chem. Depts, Tucson, AZ 85724.

The opioid receptor binding affinities of CCK-8 and four analogues having analgesic potency after i.c.v. administration and high affinity for CCK-B receptors were measured using the selective radioligands [^{3}H]CTOP (μ), [^{3}H][D-Pen^2, 4'-Cl-Phe^4, D-Pen^5]enkephalin (δ), and [^{3}H]U-69,593 (κ). These analogues included nonsulfated [N-MeNle^3, N-MeNle^7]CCK-8, [threo-L-β-MePhe^3, N-MeNle^7]CCK-8, [D-Phe^3, N-MeNle^7]CCK-8 and bis-sulfated [threo-L-β-MePhe^3, N-MeNle^7]CCK-8. None of the peptides tested have high affinity for either μ- or κ-receptors (IC_{50} values > 0.7 μM), but their IC_{50} values for δ-receptors range from 29 to 1,023 nM. The analogue ([D-Phe^3, N-MeNle^7]CCK-8) with the greatest analgesic activity (A-50 = 0.5 nmole i.c.v.) has high affinity for δ opioid receptors (IC-50 = 29 nM and CCK-B receptors (IC-50 = 0.79 nM). Nonsulfated [threo-L-β-MePhe^3, N-MeNle^7]CCK-8 showed the lowest analgesic potency (A-50 = 232 nmole) but also had high CCK-B receptor affinity (IC-50 = 2.1 nM). Thus, distinct receptor mechanisms (CCK-B and opioid) may be responsible for the analgesia observed.

320.22

A-73559: A FIRST GENERATION IRREVERSIBLE CCK-A ANTAGONIST. J.F. Kerwin, Jr., C.W. Lin, F. Wagenaar*, D. Witte*, T. Miller*, A.M. Nadzan. Neuroscience Research Division, D-47H, Abbott Laboratories, Abbott Park, IL 60064.

We have developed selective and novel CCK-A antagonists (eg. A-65186, A-67396) and a model of the pharmacophores necessary for affinity and selectivity. We became interested in irreversible CCK antagonists as a tool for studying the pharmacology associated with CCK-A receptors in the CNS. Using the prototypical CCK-A antagonist A-69457 as a base structure, we prepared alkylative analogs. All of these possess affinities for the CCK-A receptor (guinea pig pancreatic acini) in the 100-200 nM range. Compound A-73539 (a disulfide) was studied further. At 20 μM (20 min) it reduced radioligand binding of [^{3}H]-L-364,718 and [^{125}I]-BH-CCK-8 by 65%. This effect appears to be specific for CCK-A ligands since [^{3}H] scopalamine binding was unaffected. In amylase release studies in guinea pig pancreatic acini response was reduced for 1 nM CCK-8 (>90%) but not for VIP, PMA, carbachol and bombesin. These studies suggest that A-73539 (20 μM, 20 min) is a selective CCK-A irreversible antagonist. However, A-73539 reduced PI breakdown in guinea pig pancreatic acini induced by CCK-8 (48%), carbachol (40%) and NaF. Alkylation of the CCK-A receptor by A-73539 (20 μM) could not be prevented with CCK-8 (5 μM). The specificity of A-73539 is condition dependent and further improvements are needed for a practical CCK-A irreversible antagonist.

320.23

L-365,260 BLOCKS CHOLECYSTOKININ-INDUCED DEPOLARIZATIONS IN SUPRAOPTIC MAGNOCELLULAR NEURONS. C.R. Jarvis, C.W. Bourque and L.P. Renaud, Neuroscience Unit, Loeb Research Institute, Ottawa Civic Hospital, 1053 Carling Ave., Ottawa, Ontario, Canada, K1Y 4E9.

Exogenous application of sulphated cholecystokinin octapeptide 26-33 (CCK-8S) or cholecystokinin tetrapeptide 30-33 (CCK-4) (Bachem) induces a prominent depolarization and an increase in a membrane cationic conductance in rat supraoptic magnocellular neurons. The present study sought to determine whether this depolarizing action of CCK is mediated by central (CCK-B) or peripheral (CCK-A) type CCK receptors. Intracellular recordings were obtained from supraoptic neurons in explants of basal hypothalamus superfused with oxygenated artificial cerebral spinal fluid and maintained at 34°C. Applications of CCK-4 (0.1-25 μM) induced reversible depolarizations, similar to those evoked by CCK-8S. Bath application of 200 nM L-365,260 (Merck Sharp and Dohme), a potent and selective antagonist for central (CCK-B) and gastrin receptors (Lotti and Chang, 1989 Eur. J. Pharmacol. 162:273-280), largely blocked the depolarizing response to CCK-8S (5-20 μM). The effectiveness of low concentrations of CCK-4 and L-365,260 in mimicking and antagonizing the effects of CCK-8S respectively suggests that the depolarizing action of CCK on magnocellular neurons is mediated by central (CCK-B/gastrin) receptors. *Supported by MRC and FCAR.*

320.24

SPECIES DIFFERENCES IN BRAIN NEUROPEPTIDE RECEPTOR BINDING. N. R. Mason, R. F. Bruns, J. J. Howbert, and M. J. Yu*. Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285.

In the course of comparing affinities of non-peptide CCK antagonists, for CCK-B and gastrin receptors, species differences in affinity were observed. Several quinazolinone CCK-B antagonists appeared to be highly selective for mouse brain 125I-CCK-8S binding (CCK-B receptor) compared to 125I-gastrin binding in the guinea pig stomach; however, in guinea pig brain CCK-B binding these compounds had about 1/10 the activity seen in mouse brain and little selectivity for the CCK-B receptor compared to the gastrin receptor. Neither CCK-8S or L-365,260 showed any significant difference in affinity between mouse and guinea pig brain CCK-B receptors. Several pyrazolidinone CCK antagonists had only 2-4 times more affinity for mouse brain CCK-B binding as compared to guinea pig brain. However, the affinity of these compounds was markedly affected by the pH of the medium. CCK-B and gastrin receptor affinities were similar at pH 7.4. Thus, the CCK-B and gastrin receptors appear to be very similar or identical. Species differences were also noted in the binding of non-peptide antagonists to brain NK-1 (Substance P) receptors. Although Substance P itself showed no difference between species, the non-peptide antagonist (±)CP-96,345 showed a marked species difference with high affinity versus 125I-Substance P binding in guinea pig, rabbit, bovine, and hamster brain membranes, but had 100-500 fold less affinity in rat, mouse or chicken brain. These results show that the affinities of non-peptide compounds for peptide receptors may be highly species-dependent. This should be taken into consideration when comparing results from different tissues and when choosing an appropriate species for *in vivo* experiments.

PEPTIDES: RECEPTORS IV

321.1

ANGIOTENSIN II DEPRESSES GLUTAMATE-INDUCED DEPOLARIZATION AND SYNAPTIC TRANSMISSION OF LOCUS COERULEUS NEURONS

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We have earlier shown that iontophoretically applied angiotensin II (AII) specifically depresses the depolarizing action of L-glutamate (Glu) on locus coeruleus (LC) neurons in a transverse brain stem slice preparation (Neurosci. Lett. 118:261, 1990). We have further studied the effect of AII on Glu in LC neurons recorded intracellularly from coronal pontine-medullary brain slices in which EPSPs can be readily evoked by electrical stimulation. Consistent with our previous observation, AII was found to depress Glu excitation by more than 30% in 77 out of 87 LC neurons, in the absence of other effects on membrane properties. Application of hydrogen ions (H^+)from a pH control barrel depressed 40 out of 87 LC neurons. The correlation between H^+ and AII effects on the same cells was poor, indicating that the AII effects are not attributable to actions of co-expressed H^+.The effect of AII on Glu appears to be specific to the LC neurons since it had little or no effect when tested on non-LC neurons (e.g. neurons of Barrington's or lateral vestibular nuclei). Sar^1-AII, which is resistant to peptidase degradation, had similar effects to AII when iontophoretically applied, and with bath application it was also found to depress Glu excitation in the LC neurons in a dosage dependent manner (IC_{50}=10μM,n=14). In addition, when AII was tested by pressure ejection, the result was consistent with that observed using iontophoresis.

EPSPs evoked by electrical stimulation and blocked by an excitatory aminoacid antagonist(kynurenic acid) were also partially depressed by bath application or pressure ejection of AII (n = 8).

It has been reported that LC neurons contain three subtypes of Glu receptors, but we observed that AII can in some cases entirely eliminate the Glu response. Our tests to find which subtype of Glu receptors was affected by AII suggest that the mechanism is complex. (Supported by the Medical Research Council of Canada)

321.2

ANGIOTENSIN II (AII) MODULATED NET OUTWARD CURRENT(i_{no}) IN CULTURED NEURONS FROM RAT BRAIN: EFFECTS OF NON-PEPTIDE AII RECEPTOR BLOCKERS. P. Posner*, J. Kang* and C. Sumners. Dept. of Physiology, JHMHC, Gainesville, Fl 32610

The hypothalamus and brainstem contain the major concentrations of AII neurons and receptors in the brain. By using whole cell voltage clamp, we have found that AII modulates i_{no} in neurons cultured from these brain regions of 1 day old rats. Of 53 cells studied, AII (0.01nM-1μM) either caused an increase or a decrease in i_{no} in 83% of the cells. To characterize which AII receptor subtype(s) are involved, we tested the effects of non-peptide AII receptor blockers on these responses. The AII-1 (AT_1) receptor blocker DuP 753 (DuP,1μM), but not the AII-2 (AT_2) receptor blocker PD 123177 (PD,1μM) reversibly blocked the AII induced inhibition of i_{no}. Both PD and DuP were able to reversibly block the stimulatory effect of AII on i_{no}. However, PD was effective when given prior to, simultaneously with or after AII, while DuP was only effective when given before AII. DuP and PD alone had no effect on i_{no}. These data suggest that AT_1 receptors mediate the inhibitory effect of AII on i_{no}. The stimulatory effect is probably mediated by AT_2 receptors. The blocking action of DuP may be non-selective at these doses. However, the possibility that both AT_2 and AT_1 receptors mediate AII induced increases in i_{no} cannot be ruled out at this time.

321.3

EFFECTS OF ANGIOTENSIN II (AII) ON NET OUTWARD CURRENT (i_{no}) IN CULTURED NEURONS FROM RAT BRAIN. J. Kang*, C. Sumners, and P. Posner* . (Spon: R.L. Casto) Dept. of Physiology, JHMHC, Gainesville, Fl 32610

The hypothalamus and brainstem contain the major concentrations of AII neurons and receptors in the brain.In this study we have utilized standard whole cell voltage clamp techniques to study the effects of AII on the net outward current of neurons cultured from these brain regions of one day old rats. The i_{no} was elicited at room temperature by using 50 msec steps from a holding potential (HP) of -80 mV to +10 mV, every 10 seconds. The i_{Na} was blocked with TTX (10μM). The i-v relationship was determined using 10 mV steps over a range of -100 mV to +90 mV, from a HP of -80 mV. We have identified two morphologically distinct populations of neurons (bipolar cells and multipolar cells) which respond to AII. Of 22 bipolar cells studied, 18 responded to AII with a reversible inhibition of i_{no}. Of 31 multipolar cells studied, 27 responded to AII with a reversible increase in i_{no}. Both types of neurons responded to AII in a dose-dependent manner. The threshold dose was 0.01 nM AII, while maximal responses occurred at 1 μM. The current changes were independent of shifts in the i-v curve. Further characterization of i_{no} is in progress.

321.4

FUNCTIONAL PROPERTIES OF TYPE 1 AND TYPE 2 ANGIOTENSIN II RECEPTORS IN NEUROBLASTOMA AND LIVER EPITHELIAL CELLS. R. Makl, A. Ades*, R. Mir*, S. Mah* and S.J. Fluharty. Dept. of Animal Biology and Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

The intracellular actions of angiotensin II (AngII) are mediated by two distinct receptors (Type 1 and Type 2). We examined the coupling of these receptor subtypes to phosphoinositide (PI) hydrolysis and adenylate cyclase (AC) in neuron-like (NG108-15 and N1E-115 cells) and non-neuronal (WB cells) cell lines. In all cells examined, AngII caused a dose-related rise in inositol trisphosphate ($InsP_3$) production, and promoted the translocation of protein kinase C (PKC) from cytosol to membrane. Moreover, the $InsP_3$ response was reduced by the Type 1 selective antagonist DUP 753 but not by the Type 2 antagonist CGP 42112A. In addition to stimulatory effects on PI hydrolysis, AngII (1 μM) inhibited AC activity by 12% and lowered cAMP levels by 57.3 ± 31.2 pmols/mg prot in WB cells. In the presence of DUP 753, however, the inhibition of AC was greater (33%) and cAMP levels were reduced by 201.1 ± 52.3 pmols/mg prot, suggesting that some action of AngII at Type 1 receptors might interfere with its inhibition of AC. In this regard, the catalytic activity of AC was rapidly increased when PKC was activated by the phorbol ester PMA. In contrast, AngII increased AC activity and cAMP levels when Type 2 receptors were blocked with CGP 42112A perhaps because potentiation of cyclase by Type 1-mediated activation of PKC was unopposed by Type 2-mediated inhibition of AC. Finally, while AngII potently stimulates PI hydrolysis in neuroblastoma cells, it did not inhibit AC or reduce cAMP levels. These results suggest that the intracellular actions of AngII may involve antagonistic interactions between Type 1 and Type 2 receptors, and that the function of the Type 2 subtype is not identical in neuronal and non-neuronal cells. Supported by NS23986 and MH43787.

321.5

THE APPARENT ASSOCIATION OF SOLUBILIZED ANGIOTENSIN II RECEPTORS WITH G-PROTEINS AND PHOSPHOLIPASE C α IN N1E-115 CELLS. I.R. Siemens, K. Addya*, S. Mah*, D.R. Manning* and S.J. Fluharty*. Departments of Animal Biology, Pharmacology, and Institute of Neurological Sciences, University of Pennsylvanian, Philadelphia, PA 19104.

The murine neuroblastoma N1E-115 cell line possess membrane associated Type 1 and Type 2 angiotensin II receptors (AngII-Rs). While both of these receptor subtypes can be solubilized in CHAPS, the Type 2 receptor appears more stable in this detergent. Size exclusion chromatography of the solubilized membranes revealed that substantial amounts of AngII binding activity was present in large molecular weight complexes ranging from 250-350 kDa. In order to further examine the composition of these complexes, solubilized N1E-115 membranes were subjected to Heparin-Sepharose (HS) chromatography. Three peaks of specific ^{125}I-AngII binding activity eluted from the Heparin Sepharose column, the third of which was strongly retained by the column and required 1.5M NaCl for complete elution. Each binding peak was assayed for the presence of Type 1 and Type 2 receptors by using DUP753 and CGP42112A, respectively. The first and second peak contains equal amounts of binding activity, while the third peak exhibited 3-5x greater activity. In all three cases, the binding was predominantly at Type 2 sites, however, DUP753 appeared to stimulate binding at Type 2 sites in peak 3. Finally, we used antisera selective for the α-subunits of G-proteins and for a particular isozyme of phosphoinositide specific phospholipase C (PLC-α) to determine if these proteins co-eluted with AngII-Rs on the HS column. The majority of immunoreactive G-protein and PLC-α was present in peaks 2 and 3. Collectively, these data suggest that Type 2 AngII-Rs can associate with a heterotrimeric G-protein and PLC-α in a neuron-like cell line. Supported by NS23983 and MH43787.

321.6

CHARACTERIZATION AND DEVELOPMENTAL EXPRESSION OF AT_2 ANGIOTENSIN II RECEPTORS IN RAT CEREBRAL ARTERIES. K. Tsutsumi and J.M. Saavedra. Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

We characterized Angiotensin II (AT) receptor subtypes in the cerebral arteries of young (2-weeks-old) and adult (8-weeks-old) rats by displacement with the specific AT_1 receptor antagonist DuP 753 and the AT_2 selective displacer CGP 42112A, using quantitative in vitro autoradiography. ^{125}I Sar^1-AT binding was located throughout the arterial wall. AT receptors in the chiasmal segment of the anterior cerebral artery from young rats were completely displaced by CGP 42112A with an IC_{50} of $6 \pm 1 \times 10^{-10}$M, whereas DuP 753 did not compete in concentrations up to 10^{-4}M. Incubation in the presence of a single concentration of CGP 42112A (10^{-7}M), which totally eliminated AT binding from AT_2 receptors, resulted in complete displacement of AT binding in the orbital segment of the anterior cerebral artery, the sphenoid wing segment of the middle cerebral artery and the posterior communicating artery from both young and adult rats. A single concentration of DuP 753 (10^{-5}M) failed to displace AT binding from these arteries. Thus, larger cerebral arteries of the rats have AT_2 receptors. The apparent concentrations of AT binding in the cerebral arteries of young rats were several times higher than those of adult animals. These findings suggest that AT may exert its effects on cerebral circulation by stimulation of AT_2 receptors, and that these receptors may play a role during cerebrovascular development.

321.7

LOCALIZATION OF THE AT_2 ANGIOTENSIN RECEPTOR SUBTYPE IN THE BRAIN OF THE DEVELOPING RAT FETUS. V.I. Cook, K.L. Grove, K.M. McMenamin, J.W. Harding and R.C. Speth. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Angiotensin II (AII) receptor subtypes were examined by in vitro autoradiography in the brains of 15, 18, and 21-day gestation fetal and 2-day postnatal rats. Two nonpeptide AII antagonists were used: Dup 753 is type 1-specific (AT_1) and PD 123177 which is type 2-specific (AT_2). The AT_1 receptor subtype is known to mediate AII effects on cardiovascular regulation, while no physiological function has yet been established for the AT_2 receptor subtype. Our lab has recently shown that specific AII binding sites exist in the brain of rat fetus as early as the 13th day of gestation. We were therefore interested in the specific subtype of these early receptors. In the fetal rat brain, the AT_2 receptor subtype predominates throughout, even in areas that are mostly of the AT_1 receptor subtype in the adult rat, such as in the area nucleus tractus solitarius (NTS). It is possible that the AT_2 receptor subtype has a role in the development of fetal brain.

321.8

DIFFERENTIAL REGULATION OF TYPE 1 AND TYPE 2 ANGIOTENSIN II RECEPTORS IN MURINE NEUROBLASTOMA N1E-115 CELLS. A.M. Ades*, F. Slogoff* and S.J. Fluharty. Department of Animal Biology and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

The murine neuroblastoma N1E-115 cell line contains a high density of membrane associated angiotensin II receptors (AngII-Rs). The heterogenity of the binding of some AngII-related peptides suggested the possibility that these cells possess distinct subtypes of AngII-Rs, and this hypothesis has been confirmed using two newly developed antagonists, DUP 753 and CGP 42112A. When N1E-115 membranes were labelled with ^{125}I-AngII, DUP 753 exhibited high affinity binding at Type 1 receptors (K_D = 63 nM), while CGP 42112A primarily interacted with Type 2 receptors (K_D = 2.5 nM). Moreover, while most AngII-related peptides exhibited similar affinity at both AngII-R subtypes, AngIII and the N-terminally extended peptide Crinia-AngII had significantly higher affinity at Type 2 receptors, and sarcosine-substituted peptides displayed reduced affinity at these same sites. In addition to pharmacological distinctions between Type 1 and Type 2 subtypes, it also appears that these proteins are differentially regulated in N1E-115 cells. For instance, in vitro differentiation selectively increased the density of Type 2 receptors from 24.8 $\pm$ 2.1 to 143.2 $\pm$ 15.2 fmols/mg prot, without altering Type 1 receptors (65.6 $\pm$ 2.9 vs 61.9 $\pm$ 1.7 fmols/mg prot). On the other hand, the Type 1 subtype appeared to be more susceptible to downregulation induced by brief exposure of intact cells to AngII (10 nM; 5 min). Collectively, these data demonstrate that the density of Type 1 and Type 2 AngII-R subtypes can be selectively altered, and that N1E-115 cells are an ideal system in which to examine the expression of AngII-R subtypes. Supported by NS23986 and MH43787.

321.9

BINDING CHARACTERISTICS OF ANGIOTENSIN RECEPTOR SUBTYPES IN INDIVIDUAL RAT BRAIN NUCLEI. B.P. Rowe, R.C. Speth, and D.L. Saylor* East Tennessee State Univ., Johnson City, TN 37614 0002, and Washington State Univ., Pullman, WA 99164 6520.

In vitro autoradiography studies of angiotensin II (AII) receptor (AIIR) subtype distributions in the brain routinely use a single subsaturating concentration of radioligand, assuming that it binds with equal affinity to all receptor subtypes. This prompted us to determine the affinity of brain AIIR subtypes for ^{125}I sar^{1}ile^8 AII (^{125}I SIAII). Rat brain sections were incubated with ^{125}I SIAII (0.16-2.51 nM) to determine the K_D in 21 brain regions. The K_D at 10 nuclei previously characterized as having predominantly the AT_1 subtype was 0.66nM (range 0.5-0.9nM) while the anterior pituitary (also AT_1) K_D was 0.24nM. In contrast, the K_D at 7 nuclei having predominantly the AT_2 subtype was estimated to be 2.6nM (range 1.2-4.3nM). Estimates of K_D for AT_2 sites are subject to considerable error, but ^{125}I SIAII clearly shows selectivity (approx 4 fold) for the AT_1 subtype and previous competition studies therefore underestimate the proportion of AT_2 sites. Finally, these differences in affinity have not been observed in peripheral tissues, which might indicate differences between brain and peripheral AIIR subtypes.

321.10

IDENTIFICATION OF A NEW ANGIOTENSIN RECEPTOR SUBTYPE IN GUINEA PIG BRAIN. J.W. Harding, J.M. Hanesworth*, J.N. Swanson*, V.I. Cook, A.V. Wing*, J.W. Stobb*, L.L. Jensen, M. Sardinia*, and J.W. Wright. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Recent reports have demonstrated the existence of two distinct angiotensin receptor subtypes designated AT1 and AT2. Both receptor subtypes bind AII, AIII, and Sar^1-substituted angiotensins. We report here the discovery of a previously unrecognized angiotensin receptor subtype specific for the hexapeptide fragment, AI(3-8), which is different from AT1 or AT2. This receptor is highly localized in cardiovascular tissues including aorta, heart, kidney, adrenals, and brain. In addition, it can be found in almost all mammalian species examined including guinea pig, rabbit, cat, dog, pig, horse, and cow. The receptor has been solubilized and partially purified. Preliminary biochemical studies indicate that the receptor is very acidic, heat stable in the presence of divalent cations, and possesses a molecular weight in the range of 150,000 daltons. The receptor is extremely specific, saturable, and reversible. The N-terminal structure of the binding ligand is paramount in determining binding affinity. While the binding of AII(3-8) is very tight (K_d = .4 nM), the binding of Sar^1,Ile^8-AII, Sar^1-AII, AII and AIII is all but nonexistent (K_d>10^{-6}M). Small changes in the N-terminal valine dramatically reduce affinity, usually, 2,000 to 10,000 fold. These changes include substitution of L-val with D-val or Sar, removal of L-val to produce the pentapeptide and extension of the N-terminal with additional amino acids. The function of this receptor and its accompanying peptide system is unknown and presently under investigation.

321.11

INTERACTIONS BETWEEN ANGIOTENSIN II AND CORTICOSTEROID RECEPTORS IN NEUROBLASTOMA CELLS. S.Y. Chow, R.R. Sakai, L.P. Reagan, B.S. McEwen, and S.J. Fluharty. Depts. of Psychology, Animal Biology, and Inst. of Neurological Sciences, Univ. of Penna., Phila., PA 19104, and Lab. of Neuroendocrinology, Rockefeller Univ., New York, NY 10021.

The central actions of angiotensin II (AngII), particularly its ability to elicit sodium appetite, are potentiated by both mineralocorticoids and glucocorticoids in the rat. Moreover, it appears that this potentiation may result from an upregulation of AngII receptors (AngII-Rs). We have examined the interactions between AngII-R subtypes and the cytosolic mineralocorticoid (MR) and glucocorticoid (GR) receptors in cultured neuron-like cells. Murine neuroblastoma N1E-115 cells possess both Type 1 and Type 2 AngII-Rs. However, only the Type 2 receptor increases during in vitro differentiation. A similar pattern of receptor changes was noted in NG108-15 neuroblastoma x glioma hybrid cells. N1E-115 and NG108-15 cells also contain both MR and GR receptors. In undifferentiated neuroblastoma cells the density of GR receptors (B_{max} = 100-250 fmols/mg prot) was 10-20x greater than the level of MR binding, and unaffected by differentiation. However, in NG108-15, but not N1E-115 cells in vitro differentiation substantially increased MR receptors. Moreover, aldosterone, corticosterone and RU28362 upregulated AngII-Rs in NG108-15 cells, but only corticosterone and RU28362 produced this change in N1E-115 cells. The upregulation of AngII-Rs resulted from a small rise in Type 1 receptors and a 3-5x increase in Type 2 receptors. Finally, it is likely that the steroid induced upregulation of AngII-Rs results from genomic changes because they were blocked by actinomycin. Collectively, these results suggest that the gene(s) encoding for the multiple subtypes of AngII-Rs possess a steroid responsive element. Supported by NS23986 and MH43787.

321.12

ANGIOTENSIN RECEPTOR SUBTYPES IN THE ALBINO RABBIT EYE. P. Mallorga, M. Whitfield* and M. F. Sugrue*. Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Receptors for Angiotensin II (AII) have been divided into the subtype I, which possesses a nM affinity for the nonpeptide antagonist DuP-753 and a μM affinity for another antagonist WL-19, and the subtype II which displays the inverse profile, i.e., having a high affinity for WL-19 and a lower affinity for DuP-753. The two subtypes have been shown to be present in rat brain membranes. Only the subtype I was found in the rabbit cerebral cortex. In the rabbit eye AII binding sites are present in the ciliary process and the retina. In vitro experiments were undertaken to characterize the subtype(s) present in these tissues, as assessed by the abilities of DuP-753 and WL-19 to displace the antagonist ^{125}I-[Sar,Ile]-AII (SARILE) from its binding sites in these tissues. Scatchard plots of SARILE binding were linear in both tissues and Kd values of 152 pM and 49 pM were found for the ciliary process and the retina, respectively. The corresponding Bmax values were 35 and 22 fmol/mg protein. In both tissues, Hill coefficients for the displacement curves by DuP-753 and WL-19 were greater than 0.9 indicating the presence of one population of binding sites. The Ki values of DuP-753 for the ciliary process and retina were 50 nM and 16 nM, respectively. In contrast, the corresponding Ki values for WL-19 in these tissues were 76 μM and 74 μM. Based on the relative selectivities of DuP-753 and WL-19 for AII receptor subtypes 1 and 2, these studies indicate that the great majority of AII receptors present in both the ciliary process and the retina of the rabbit are of the subtype 1 and in this respect resemble the cerebral cortex.

321.13

PRELIMINARY CHARACTERIZATION OF ^{3}H-DuP 753 BINDING IN THE RAT BRAIN AND LIVER: EVIDENCE FOR ANGIOTENSIN II AND NON-ANGIOTENSIN II DISPLACEABLE BINDING SITES. K.L. Grove and R.C. Speth. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Multiple subtypes of angiotensin II (AII) receptors (AIIR) have been identified in the rat brain and other tissues using highly selective non-peptidic AIIR antagonists and other agents. This study examines the possibility of using the selective AT_1 antagonist, DuP 753, which displays antihypertensive action, as a radioligand (^{3}H-DuP 753) to selectively radiolabel the AT_1 subtype. Initial studies carried out in rat liver membranes revealed a high affinity binding site for ^{3}H-DuP 753, with a dissociation constant (K_D) of approximately 20 nM. Competition by AII for ^{3}H-DuP 753 binding accounted for only 15-20% of specific (10 μM DuP 753 displaceable) binding in the liver. Specific binding of ^{3}H-DuP 753 in the rat brain was of slightly lower affinity than in the liver, with a similar proportion of this binding inhibited by AII. To characterize the non-AII binding site of ^{3}H-DuP 753 a number of transmitter receptor specific ligands, e.g., adrenergic, serotonergic, cholinergic, were tested and displayed little competition for ^{3}H-DuP 753 binding sites. Several ion channel blockers, e.g., verapamil, quinidine, d-propranolol, lidocaine, caused moderate (25-50%) reductions in ^{3}H-DuP 753 binding in the liver at high (100 μM) concentrations that were additive with the competition by AII. However, the ion channel blockers were ineffective against ^{3}H-DuP 753 binding in the brain. These observations suggest that DuP 753 may bind to sites other than the AT_1 receptor subtype in the brain and other tissues.

321.14

A TYPE I BUT NOT TYPE 2 ANGIOTENSIN II ANTAGONIST INHIBITS WATER AND 3% NaCl INTAKE INDUCED BY INTRACEREBROVENTRICULAR (pICV) RENIN OR SODIUM DEPLETION. C. Polidori, S.Y. Chow, S.J. Fluharty and A.N. Epstein, Departments of Biology, Psychology, Animal Biology, and the Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Central pulse injection of renin (pICV) or sodium depletion elicits water and salt intake that is mediated by angiotensin II (AngII) acting in the brain. Recently, the existence of two AngII receptors subtypes (Type 1 and Type 2 receptors) has been demonstrated in rat brain. We tested the effects of DUP 753 (a selective antagonist for the Type 1 receptors), and the CGP 42112A (selective for Type 2 receptors) on the drinking behaviors induced by pICV renin (50 ng/μl), and furosemide-induced sodium depletion (10 mg/rat/sc, furosemide). After pICV renin, DUP 753, at the doses of 25, 250 and 2,500 ng/5 μl, reduced the water intake by 24, 64 and 100%, and the salt intake by 2, 20 and 90%, respectively. After sodium depletion, DUP 753 at 2,500 ng/5 μl reduced the water and 3% NaCl intake by 50%. On the other hand, the same doses of CGP 42112A had no effect on renin-induced water and salt intake and at the higher dose showed signs of toxicity. To verify that DUP 753 and CGP 42112A were selective for AngII receptors we tested them on the dipsogenic effect of pICV carbachol (300 ng/μl). Both DUP 753 and CGP 42112A had no effect on carbachol-induced water intake. These results suggest that the Type 1 AngII receptor is involved in water and salt intake, and that the Type 2 receptor does not appear to be involved in these behaviors. Supported by NS23986 and MH43787.

321.15

STRUCTURE-ACTIVITY STUDIES OF ENDOTHELIN ON A_{10} SMOOTH MUSCLE CELLS. M. Knight, K. Takahashi* and P.M. Sweetnam+. Peptide Technologies Corporation, 125 Michigan Ave. NE, Washington, DC 20017 and +Nova Pharmaceutical Corporation, 6200 Freeport Centre, Baltimore, MD 21224

Structure-activity studies of the recently discovered cardiovascular peptide, endothelin, will establish the basis of its biological actions. Endothelin-1, (Ent-1) a 21 residue peptide, is released from endothelial cells and stimulates potent contraction of smooth muscle cells lining the vascular wall. An initial vasodilatory component has been described in intact vascular beds. Receptors for Ent-1 have been characterized in the cardiovascular system and, interestingly, in the nervous system. We have measured receptors in neuronal and non-neuronal cells and in thoracic A_{10} cultured smooth muscle cells.

To develop a specific Ent-1 receptor inhibitor, single amino acid substitutions at positions, 12 through 14 of Ent-1 were applied systematically in the design of Ent-1 analogs. These along with fragments of Ent-1 and a peptide with the carboxyl hexapeptide region modified were measured for inhibition of ^{125}I-Ent-1 binding to the cultured smooth muscle cells. The results indicate that the 21-mer analogs displayed high affinities (values of $IC_{50} < 10^{-8}$M), whereas the Ent-1 [1-15] bicylic portion and the Ent-1 [16-21] hexapeptide fragment both interacted with the receptors with low potencies, IC_{50} values > 10^{-7}M. The effect of changes in amino acid positions 12 to 14 of the Ent-1 molecule on its binding to receptors will be discussed.

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321.16

ENDOTHELIN-MEDIATED STIMULATION OF PHOSPHOLIPASE C IS MEDIATED BY GUANINE NUCLEOTIDE BINDING PROTEIN(S) F. Gusovsky LBC, NIDDK, NIH, Bethesda, MD 20892

In permeabilized NIH 3T3 fibroblasts and C6 glioma cells, the peptide endothelin 1 (ET-1) in combination with GTPγS stimulates the formation of inositol phosphates. In the presence of 10 μM GTPγS, ET-1 induces the formation of IP2 + IP3 with an EC50 value of 6 nM for NIH 3T3 cells and 2 nM for C6 glioma cells. The analogous peptide ET-3 is less potent than ET-1 in such action. ET-1-induced effects were unaffected by pretreatment of the cells with pertussis toxin (1 μg/ml) in NIH 3T3 cells, but were partially reduced in C6 cells. In binding studies in whole NIH 3T3 cells and C6 cells, specific binding for [^{125}I]ET-1 was detected. Cross linking of [^{125}I]ET-1 in whole C6 cells revealed the presence of two binding proteins for ET-1 of 74 kD and 55 kD. ET-1 at 100 nM inhibited the labelling of both proteins by [^{125}I]ET-1. However, ET-3 inhibited the labelling of the 55 kD protein only. The results indicate that in NIH 3T3 cells and C6 glioma cells, receptors for endothelin are present, which are coupled to phospholipase C through guanine nucleotide binding (G) proteins. In C6 cells, endothelin-mediated phospholipase C activation is partially inhibited by pertussis toxin pretreatment, but in NIH 3T3 cells stimulation is not affected by pertussis toxin pretreatment. The endothelin receptor involved in phospholipase C stimulation in C6 cells seems to correspond to a 74 kD protein which binds ET-1 but not ET-3.

321.17

NALOXONE-REVERSIBLE INHIBITION OF CYCLIC AMP PRODUCTION BY TYR-MIF-1 IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS. J.E. Zadina, S.L. Chang[1], L.J.Ge* and A.J. Kastin. VAMC, Dept. of Medicine and Neuroscience Program, Tulane U. Sch of. Med., New Orleans, LA 70146 and [1]Dept. of Physiology, LSU School of Dentistry.

Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) is a peptide isolated from brain that binds to mu opiate receptors (PB&B 25: 1305, 1986) and its own non-opiate sites (Life Sci 47: PL25, 1990). Tyr-MIF-1 has been shown to antagonize the effects of opiates in several tests (Life Sci 39:2153,1986). In this study, we observed an opiate agonist-like effect of Tyr-MIF-1, inhibition of forskolin-stimulated cyclic AMP production, in a neuroblastoma cell line (SH-SY5Y) that expresses both mu opiate receptors and Tyr-MIF-1 binding sites (Excerpta Medica 914:151,1990). In cells differentiated by retinoic acid for 5-6 days, Tyr-MIF-1 inhibited cAMP with an IC50 of about 8 μM. Similar results were observed in undifferentiated cells. At high (100μM) doses, Tyr-MIF-1 was able to induce inhibition (60-70%) comparable to that seen with high doses of morphine. The inhibition was reversible by naloxone. Thus, at concentrations consistent with occupation of the mu opiate receptor, Tyr-MIF-1 is capable of a full agonist response. Actions of Tyr-MIF-1 that are antagonistic to opiates may occur at sites other than the mu receptor, including its own non-opiate site.

321.18

^{125}I-LHRH ANALOGUE DISPLACEMENT FROM HIPPOCAMPAL MEMBRANES, T.L. Thompson & R.L. Moss, Dept. of Physiol. Univ. Texas Southwestern Med. Cntr. Dallas, TX 75235

Autoradiographic evidence has shown that high affinity luteinizing hormone releasing hormone (LHRH) receptors are present in rat hippocampus. Pharmacologically these receptors appear very similar to those found in the pituitary. Evidence from our lab suggests that more than one binding site for LHRH may be present in rat brain. This is based on the inability of a potent LHRH antagonist to inhibit both Ac-LHRH$^{5-10}$ fragment induced mating behavior and inositol phosphate accumulation while effectively inhibiting similar LHRH induced responses. To determine whether more than one binding site exists for neuronal LHRH, we have initiated a series of experiments examining the characteristics of ^{125}I-[D-Ala^6,N Me-Leu^7,Pro^9,NET]-LHRH (a stable analogue of LHRH) binding to hippocampal membranes prepared from female rats. Competition curves were performed by adding increasing concentrations of native LHRH or Ac-LHRH$^{5-10}$ fragment ($2x10^{-7}$M-$1x10^{-11}$M). The LHRH competition curve is of "normal steepness" and bound ^{125}I-LHRH analogue was completely displaced by the native peptide. Preliminary findings suggest that the Ac-LHRH$^{5-10}$ fragment is unable to completely displace the radiolabeled LHRH (80%) and exhibited a very shallow competition curve. Logit-log analysis of the competition data reveal on IC_{50} of 3.5nM for LHRH while the fragment has an IC_{50} of .7nM. This suggests that the fragment has a 5 fold greater affinity for the hippocampal receptor than native LHRH. Whether or not this increased affinity alone can account for the inability of the LHRH antagonist to block the effect of Ac-LHRH$^{5-10}$ fragment has yet to be determined. However, the shallowness of the competition curve suggests there may be more than one binding site for LHRH in rat hippocampus. A more complete kinetic analysis of LHRH receptor binding and competition by the fragment is being completed. This work is supported by NIH Grant MH47418.

321.19

FRAGMENTS OF HUMAN DIAZEPAM BINDING INHIBITOR (H-DBI) REVEAL HETEROGENEITY OF BENZODIAZEPINE RECEPTORS (BZ) IN HUMAN LYMPHOCYTES.

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In rat glial cells the binding of [^{3}H]Ro5-4864 and [^{3}H]PK11195 is displaced by DBI(1-86) and by DBI(17-51)-TTN, a processing product of DBI (Mol.Pharm.37:164, 1990). Since DBI and DBI derivatives are found in human CSF and plasma, we studied whether synthetic peptides, which may be processing products of human DBI, displace [^{3}H]PK11195 and [^{3}H]Ro5-4864 from human lymphocytes. In intact lymphocytes, DBI(37-70) and DBI(37-80) displaced both ligands with a K_i of 2-5uM, whereas, using crude membranes the K_i was 10 times higher. However, protoporphyrin IX binding had a low K_i on membranes and practically failed to displace the labeled ligand bound to intact cells. Both protoporphyrin IX and DBI fragments do not readily cross the cell membrane, and the activity of both compounds does not change in several different ionic media tested. Therefore we hypothesize that the difference in behavior of the two classes of ligands may be due to a complex receptor structure involving at least two binding sites having different selectivity for protoporphyrin IX and H-DBI fragments.

321.20

QUINPIROLE INHIBITS THE REFILLING OF THE INTERNAL CALCIUM POOL MOBILIZED BY THYROTROPIN-RELEASING HORMONE IN BOVINE ANTERIOR PITUITARY CELLS S.L. Shorte* and J.G. Schofield* (SPON: Brain Research Association). Dept. of Biochemistry, University Walk, Bristol, BS8 1TD, U.K.

The cytosolic calcium concentration ($[Ca^{2+}]_i$) was measured in single bovine anterior pituitary cells using fura-2 and fluorescence microscopy (Shorte et al., 1991). TRH-sensitive internal calcium pools were depleted by exposure to TRH (20 nM) in the presence of only 0.1 μM external calcium as previously described (Shorte et al., 1990). Under these conditions, a subsequent application of agonist did not result in a rise in $[Ca^{2+}]_i$ unless cells were exposed to millimolar external calcium. However, if the D_2-receptor agonist, quinpirole (1 μM), was present only during the transient re-exposure to 1 mM external calcium, the amount of calcium released during a subsequent exposure to TRH was significantly reduced. The effect was reversed when the concentration of K^+ in the bathing medium was also transiently raised from 5 mM to 60 mM during the re-exposure to external calcium and quinpirole. Cells pre-treated with pertussis toxin (20 hrs; 100 ng/ml), which reverses the inhibitory action of dopamine on PRL secretion (Schofield et al., 1988), also displayed a reversal of the effect of quinpirole on pool refilling, and did not differ from control observations.

We interpret these data to indicate that activation of the D_2-receptor, present on the membranes of normal bovine anterior pituitary lactotropes, reduces the entry of calcium through voltage-activated calcium channels and that this precludes the refilling of TRH-depleted internal calcium stores.

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321.21

POSTSYNAPTIC LOCALIZATION OF FUNCTIONAL OXYTOCIN RECEPTORS ON PREGANGLIONIC NEURONS IN THE RAT DORSAL MOTOR NUCLEUS OF THE VAGUS NERVE. M. Dubois-Dauphin*, M. Raggenbass*, E. Tribollet*, J.J. Dreifuss. Dept of Physiology, Univ. Med. Cent., 1211 Geneva 4, Switzerland.

The dorsal motor nucleus of the vagus nerve (DMN) of the rat receives oxytocin (OT) immunoreactive axons of hypothalamic origin. Vagal motoneurons send their axon to visceral ganglia through the ipsilateral vagus nerve. OT binding sites are detected in the DMN, whereas the neighbouring nucleus of the solitary tract (NST) is endowed with vasopressin (AVP) binding sites. To assess the pre- or postsynaptic location of the binding sites in DMN, we cut unilaterally the vagus nerve distally to the nodose ganglion. Two weeks after axotomy, the presence of binding sites was examined by film autoradiography using [^{3}H]AVP and [^{125}I]OTA as ligands. In lesioned animals, as in controls, the same density of AVP binding was present bilaterally in the NST. In contrast, OT binding sites were fewer or absent altogether from the DMN ipsilateral to the cut vagus nerve. A strong axon reaction was revealed by choline acetyltransferase immunocytochemistry in the ipsilateral DMN. In parallel, the sensitivity to OT of antidromically identified vagal motoneurons was tested by intracellular recordings on brainstem slices. OT applied at 0.1-1.0 μM caused a reversible depolarization and generated, under voltage-clamp conditions, a transient inward current which was TTX-resistant and which was not synaptically mediated, since it persisted in a low-calcium, high-magnesium solution. Our observations favour the notion that OT of hypothalamic origin acts directly rather than indirectly on rat vagal motoneurons.

322.1

AFFINITY LABELING OF BRAIN MU OPIOID RECEPTORS WITH [^{3}H]14BETA-BROMOACETAMIDO-7,8-DIHYDROMORPHINE. J.M. Bidlack[1], R.A. Kaplan[1*], R. Subbramanian[1*], A. Seyed-Mozaffari[2*] and S. Archer[2*]. [1]Dept. of Pharmacology, University of Rochester, Rochester, NY 14642 and [2]Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12181.

[^{3}H]14β-Bromoacetamido-7,8-dihydromorphine (H_2BAM) bound to rat and bovine striatal membranes with high affinity and selectivity for the μ opioid binding site. In the absence of a disulfide bond reducing reagent, [^{3}H]H_2BAM did not bind covalently to the μ binding site. However, after treating membranes with dithiothreitol (DTT), followed by the addition of [^{3}H]H_2BAM, the affinity ligand bound irreversibly to the μ binding site, as determined by solubilization and subsequent precipitation of brain membranes. The specificity of labeling was dependent on the DTT and [^{3}H]H_2BAM concentrations, and the time and temperature of the incubation. Opioids, selective for the μ opioid receptor, blocked the specific alkylation of the binding site, while δ- and κ-selective ligands did not protect the site from alkylation with [^{3}H]H_2BAM. In order to determine which protein(s) were specifically alkylated, [^{3}H]H_2BAM-labeled brain membranes were separated by SDS-polyacrylamide gel electrophoresis, followed by either fluorography or gel slicing. A prominent protein with a molecular weight of 54,000 was specifically labeled in both rat and bovine brain membranes. The labeling of this protein was reduced by μ-selective opioids. These studies demonstrated that after reduction of a disulfide bond at the μ opioid binding site, [^{3}H]H_2BAM specifically alkylated the μ opioid binding site, which is localized in a 54,000 dalton protein in both rat and bovine striatal membranes. (Supported by USPHS grants DA03742 and DA01674.)

322.2

IRREVERSIBLE MU-SELECTIVE OPIOID BINDING OF 14-BETA-THIOGLYCOLAMIDO-7,8-DIHYDROMORPHINONE. O. Jiang[1], A. Seyed-Mozaffari[2*], S. Archer[2*], and J. M. Bidlack[1], [1]Dept. of Pharmacology, Univ. of Rochester, Rochester, NY 14642, and [2]Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12181.

Affinity labeling of opioid receptors has been studied in order to determine the pharmacological and molecular properties of these receptors. Previous studies have shown that after treating brain membranes with the disulfide-bond reducing reagent dithiothreitol, 14β-bromoacetamido derivatives of morphine and morphinone irreversibly inhibited μ opioid binding, suggesting the presence of a disulfide bond at or near the μ binding site. The present study was designed to characterize the affinity, selectivity, and irreversibility of the sulfhydryl-containing affinity ligand 14β-thioglycolamido-7,8-dihydromorphinone (TAMO). TAMO IC_{50} values for inhibiting the binding of 0.25 nM [^{3}H][D-Ala^2,(Me)Phe^4,Gly(ol)^5]enkephalin (DAGO), a μ-selective peptide, 0.2 nM [^{3}H][D-Pen^2,pCl-Pen^5]enkephalin (pCl-DPDPE), a δ-selective peptide, and κ binding, as measured by either 0.2 nM [^{3}H]bremazocine in the presence of μ and δ blockers, or 1 nM [^{3}H]U69,593, were determined using bovine striatal membranes. The IC_{50} values for TAMO were 1.58 ± 0.1 nM for 0.25 nM [^{3}H]DAGO, 22.4 ± 2.1 nM for 0.2 nM [^{3}H]pCl-DPDPE, 35.6 ± 2.3 nM and 52.4 ± 7.4 nM for inhibition of 0.2 nM [^{3}H]bremazocine and 1 nM [^{3}H]U69,593 binding, respectively. Preincubation of membranes with TAMO, followed by extensive washing, revealed a concentration-dependent irreversible inhibition of [^{3}H]DAGO binding. Therefore, these studies suggest that TAMO selectively binds to μ opioid binding sites and produces covalent binding by attacking the disulfide bond at the μ binding site to form a disulfide bond with the binding site. (Supported by USPHS grants DA03742 and DA01674).

322.3

Potential mu-opioid receptor subtype specificity of ^{125}I labelled DAGO, [D-ala^2, NME-Phe^4, Glyol] enkephalin. D.S. Roane[1] and J.W. Crim[2]. [1]Div. Pharm/Tox, NE La. Univ., Monroe, La. 71209 and [2]Dept. of Zoology, Univ. of Ga., Athens, Ga. 30309.

125I-DAGO, [D-ala^2, NME-Phe^4, Glyol] enkephalin has been used in numerous studies as a valuable tool in the autoradiographic measurement of mu-opioid receptors. However, the binding properties of this ligand have not been thoroughly investigated as compared to the better characterized 3H-Dago. In a recent series of comparative saturation binding studies we have found substantial differences in the Bmax and Kd of 125I- and 3H-Dago.

125I-Dago, prepared by the chloramine-T method, was purified to the mono-iodo fraction by HPLC. Binding studies for both 125I and 3H-Dago were completed over a 0.04-2.0 nM range of labelled ligand using membranes split from a single preparations. Kd and Bmax values for 125I-Dago, respectively, were 1.65 nM and 3.82e-11 M, while the values for 3H-Dago were 0.39 nM and 6.09e-11 M. With the higher Kd and lower Bmax, the data suggests the 125I-Dago binds the lower affinity mu subtype receptor. This is supported by an r2 value of 0.97 on a linear regression of the Scatchard plot of the 125I-Dago and is consistent with a one-site fit.

322.4

EFFECT OF CHRONIC MORPHINE EXPOSURE ON μ-OPIOID RECEPTOR REGULATED CYCLIC AMP PRODUCTION IN RAT STRIATAL NEURONS

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Rat striatal neurons cultured in serum-free, hormone-supplemented medium, were exposed to 10 μM morphine for several hours or days before intracellular cyclic AMP production was measured. Dopamine D_1 receptor- and β-adrenoceptor- stimulated cyclic AMP production were profoundly increased upon morphine treatment (up to 150 % of control). In contrast, cyclic AMP production induced by direct activation of the catalytic unit of adenylate cyclase with forskolin remained unaffected. Interestingly, the relative inhibitory effect of the μ-opioid receptor agonist DAGO on dopamine D_1 receptor- and β-adrenoceptor-stimulated cyclic AMP production was unchanged after exposure to morphine. On the other hand, unlike μ-opioid receptors chronically exposed to morphine, β-adrenoceptors mediating activation of adenylate cyclase were rapidly desensitized upon prolonged exposure of the neurons to isoprenaline.

It is suggested that tolerance to morphine is not caused by desensitization of central μ receptors, but by the fact that morphine is acting against upregulated signal transduction mechanisms. The adaptive changes following morphine treatment may involve enhanced expression and / or biochemical modification of dopamine D_1 receptors, β-adrenoceptors or G proteins, independent of possible changes at the level of dopaminergic or noradrenergic nerve terminals, which are not present in primary cultures of rat striatum.

322.5

COMPARATIVE QUANTITATIVE AUTORADIOGRAPHIC DISTRIBUTION OF μ OPIOID RECEPTORS IN THE BRAINS OF SUPEROXIDE DISMUTASE-TRANSGENIC (SOD-Tg) MICE AND OF THEIR NON-TRANSGENIC (Non-Tg) LITTERMATES : AGE EFFECTS. K. Kuilrai, V. Jackson-Lewis, S. Fahn, E. Carlson, C. J. Epstein and J. L. Cadet. Dept. of Neurology, Columbia Univ., New York, NY 10032.

Oxygen-based radicals are thought to be an important factor in the aging process. It is thus possible that some of the changes observed in the concentration of neurotransmitter receptors may be secondary to deleterious effects of these free radicals. In order to test that possibility, we used receptor autoradiographic techniques to compare the distribution of opioid μ receptors labeled with [^{3}H]DAGO (Tyr-D-Ala-Gly-NMe-Phe-Gly-ol) in SOD-Tg and non-Tg mice at 6 weeks and 20 months of age. Saturation analysis revealed that the shell subdivision of the nucleus accumbens (NAc) of SOD-Tg mice contained higher maximal binding capacity (Bmax) of μ receptors in comparison to that of non-Tg mice at both ages. With aging, only non-Tg mice showed significant decreases in Bmax. These decreases in Bmax were associated with an increase in receptor affinity. In contrast, there were no significant differences in Bmax or in affinity in either the patches nor the matrices of the caudate-putamen of the two strains at any age. Interestingly, whereas several brain structures showed age-related decreases in μ receptors only in the non-Tg mice, both the substantia nigra pars compacta and the ventral tegmental area showed significant age-related decreases only in the SOD-Tg mice. These results suggest that increased SOD activity may affect expression of opioid receptors in a complex fashion.

322.6

BINDING OF THE DERMORPHIN ANALOG TYR-(D-ARG)^2-PHE-SAR-OH (TAPS) TO μ_1- AND μ_2-OPIOID RECEPTOR SUBTYPES. S. Vonhof, P. Paakkari, G.Z. Feuerstein, and A.-L. Sirén; Dept. of Neurology, USUHS, Bethesda, MD 20814 and Dept. Cardiovasc. Pharmacol., SmithKline Beecham, King of Prussia, PA 19406, USA.

TAPS, a D-Arg^2-dermorphin derived tetrapeptide was recently shown to exert an enhanced analgetic potency compared to morphine (Sasaki et al., BBRC 120: 214-218, 1984). Opposed to morphine, however, TAPS increased ventilatory minute volume at equianalgetic doses (Paakkari et al., Proc. of the INRC, 385-388, 1990). This effect was attenuated by the μ_1-opioid receptor antagonist naloxonazine, suggesting a μ_1-opioid related mechanism. Additionally, TAPS antagonized the respiratory depression induced by the selective μ-agonist dermorphin in naloxonazine pretreated animals, congruent with an antagonistic mode of action at the μ_2-opioid site. In order to determine the selectivity of TAPS at μ-opioid binding sites, μ_1- and μ_2-selective binding assays (Clark et al., Mol. Pharmacol. 34: 308-317, 1988) were performed in calf thalamus homogenates, using [^{3}H]DADL (μ_1) and [^{3}H]DAMGO (μ_2). The K_i-values for TAPS at μ_1- and μ_2-opioid binding sites were 0.4 and 1.3 nM respectively. Addition of 10 μM Gpp(NH)p in the presence of 100 mM NaCl reduced the affinity of TAPS 49-fold at the μ_1-site but significantly less (4-fold) at the μ_2-subtype. The results indicate that TAPS exerts moderate selectivity to the μ_1-opioid binding site with a strong agonistic property. The comparatively small reduction of affinity in the presence of guanosine nucleotides may indicate a partial agonistic/antagonistic activity at the μ_2-opioid binding site.

322.7

PHARMACOLOGICAL CHARACTERIZATION OF THE ANALGESIC EFFECTS OF LEVORPHANOL. L. Tive, K. Ginsberg, C.G. Pick and G.W. Pasternak. The Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neurology and Pharmacology, Cornell U. Medical College, New York, NY 10021.

We have previously shown that there is unidirectional cross-tolerance between morphine and levorphanol in the rat, prompting us to study the receptor mechanisms of levorphanol analgesia in a mouse model. Levorphanol produced a dose-dependent analgesia in the mouse which was partially sensitive to the μ_1 antagonist naloxonazine. Although naloxonazine shifted the dose-respnose curve to the right, the shift was far smaller than that seen with traditional mu drugs. Furthermore, naloxonazine imposed a ceiling effect not seen in control animals. Levorphanol produced analgesia following either spinal or supraspinal injections. Isobolographic analysis of simultaneous i.c.v. and i.t. injections revealed synergistic interactions similar to those previously reported for morphine. There was no cross-tolerance between levorphanol and U50,488H, suggesting the lack of a signficant κ_1 component in levorphanol analgesia. However, mice tolerant to the κ_3 agonist NalBzoH showed some cross tolerance to levorphanol, implying a role for κ_3 receptors. In conclusion, levorphanol analgesia appears to comprise a complex interaction of μ and κ_3 receptor mechanisms.

322.8

SODIUM POTENTIATES THE INHIBITION OF OPIOID AGONIST BINDING BUT ANTAGONIZES THE INHIBITION OF OPIOID ANTAGONIST BINDING BY A BENZODIAZEPINE IN THE RAT SPINAL CORD. Gopi A. Tejwani, Anil K. Rattan* and Anne-Marie Duchemin. Depts. of Pharmacology and Anesthesiology, College of Medicine, The Ohio State University, Columbus, OH 43210.

Midazolam, a benzodiazepine receptor agonist can either enhance or decrease the antinociception produced by intrathecal administration of morphine in the rat. Furthermore, midazolam at high doses can inhibit the binding of several opioid ligands to the spinal opioid receptors in vitro (Rattan et al. Anesthesia & Analgesia, in press). We now report that sodium ions can either antagonize or potentiate the inhibition of spinal opioid receptors by midazolam depending upon the agonistic\antagonistic nature of the opioid ligand used. The specific binding of radioactive opioid ligands to spinal membrane preparations was performed as described before (Tejwani and Hanissian Neuropharmacology, 29 445, 1990). Binding of opioid antagonist [^{3}H]-naloxone (0.5-10 nM) to the rat spinal opioid receptors was increased in presence of 100 mM NaCl alone. Midazolam (100 μM) decreased the binding of [^{3}H]-naloxone significantly at nine different concentrations. However, in the presence of 100 mM NaCl this inhibition by midazolam was not observed. The binding of mu receptor agonist [^{3}H]-DAGO (Tyr-D-Ala-Gly-Methyl-Phe-Glyol-enkephalin) (1-20 nM) was inhibited by 100 mM NaCl or 100 μM midazolam, independently. However, when NaCl and midazolam were used together they inhibited the binding of [^{3}H]-DAGO much more than that observed when NaCl or midazolam alone was used. These results suggest that sodium ions play an important role in the modulation of spinal opioid receptors by benzodiazepines.

322.9

MU AND KAPPA RECEPTOR STIMULATION DIFFERENTIALLY AFFECT IN VIVO D2 RECEPTOR BINDING IN NEONATAL RATS. P. KEHOE, K.M. WARD* and C.B. BOYLAN*. Dept. of Psychology, Trinity Coll., Hartford, CT 06016.

Previous research has indicated that endogenous opioids modulate isolation vocalizations, analgesia and reward in neonatal rats. Activation of specific opioid receptor subtypes, however, produces differing effects on these behaviors. Mu receptor stimulation with morphine promotes both a decreased rate of ultrasonic calling and positive associations. Conversely, the kappa receptor agonist, U50,488H, increases the rate of vocalizing and does not support preference behavior. Both systems produce neonatal analgesia. This differential functioning may be due to differences in opioid-dopamine interactions that may depend on the specific receptor subtype. To assess this interaction, in vivo striatal D2 receptor binding was done on 10-day-old rats treated with ip morphine (1.0 mg/kg), U50,488H (0.25 mg/kg) or saline and then injected icv with the D2 receptor antagonist, ^{3}H-Raclopride (0.2uCi/10ul). Following icv injection and a brief isolation, during which the pups' vocalizations were recorded, the pups were decapitated and their brains dissected for striatal and cerebellar tissue. Morphine-treated pups had the least striatal ^{3}H-Raclopride binding, while U50,488H had the most. These results suggest that U50,488H caused significantly less striatal D2 receptor occupation than controls, while morphine produced the highest, presumably through the release of dopamine at these terminals.

322.10

2-CHLOROPROCAINE INTERACTIONS AT MU AND KAPPA OPIOID RECEPTORS. B. Coda*, S. Bausch*, M. Haas*, H. Hill*, C. Arnett, C. Chavkin. Depts. of Anesth., Pharm., and Psychiatry, Univ. of Wash. and Fred Hutchinson Cancer Research Ctr. Seattle, WA 98104.

Clinically, epidural 2-chloroprocaine (2CP), but not lidocaine, can decrease the analgesic effect of subsequently administered epidural fentanyl. In contrast, 2CP does not antagonize epidural butorphanol in humans. In the present study, we investigated possible opioid receptor interactions with these local anesthetics which may explain these observations.

The potencies of 2CP and lidocaine for displacing selective ligands were determined in two *in vitro* membrane binding assays using either [^{3}H]-DAGO or [^{3}H]-U69,593 to label mu or kappa-opioid receptors respectively. 2CP caused displacement of [^{3}H]-DAGO binding up to 100% at highest concentrations, with an EC_{50} of 810±290 μM, while lidocaine reduced [^{3}H]-DAGO binding only partially even at 10 mM. Both were more potent at the kappa site: 2CP displaced [^{3}H]-U69,593 completely with an EC_{50} of 177±47 μM, while the EC_{50} for lidocaine was 2.53±0.48 mM. Thus, 2CP was about 4.5 times more potent at kappa than mu sites, and 2CP was 14 times more potent than lidocaine at kappa sites. 2CP is likely to have significant occupancy at kappa as well as mu receptors at clinically relevant doses, whereas lidocaine is unlikely to reach a high enough concentration to bind either of these opioid receptors.

Using extracellular electrophysiologic recording in an *in vitro* hippocampal slice preparation, we found that chloroprocaine reduced opioid agonist induced responses in CA1 region (mu) and dentate gyrus (kappa). However, these changes were not due to specific mu or kappa receptor actions, but were typical of local anesthetic effects.

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322.11

OPIOID MECHANISMS IN RATS BRED FOR LEARNED HELPLESSNESS E. Edwards, J. Muneyyirci*, P. Van Houten*, C. Michel* and F.A.Henn. SUNY at Stony Brook, NY 11794

We have examined various parameters of opioids mechanisms in rats bred for Learned Helplessness (LH; generation 21st rats used without any exposure to the learned helpless paradigm). Plasma ß-endorphin levels of LH strain rats were decreased as compared to levels detected in NLH and naive control rats (17.5 ± 1.6, LH vs 26.25 ± 2.6,NLH & 25.5 ± 2.4, NC; data expressed in pg/ml ± S.E.M, n=17/group).

In LH strain rats, μ receptor densities measured with binding of ^{3}H-DAGO to brain homogenates, were significantly up-regulated in all limbic regions tested (CX,HPC,Ht,Sep: 40-300%)

Tail-flick latencies were measured for both LH and NLH strain rats in response to exposure to a controlled heat source and were significantly lower in the LH strain rats as compared to NLH strain rats (LH : 4.6 ± 0.4 secs; NLH : 7.8 ± 0.6 secs, n=10/group).

LH strain rats also exhibited significantly reduced precipitated withdrawal symptoms after exposure to opiate agonists as compared to NLH strain rats (LH: 6 ± 0.4;NLH: 17 ± 1.5 escape attempts, n=10/group).

The dysregulation in opioid mechanisms in the LH strain rats supports the hypothesis of an involvement of endorphin systems in the pathophysiology of depression.

322.12

THE ROLE OF OPIATE RECEPTOR SUBTYPES IN THE β-ENDORPHIN INDUCED PROLACTIN INCREASE IN LACTATING AND CYCLING FEMALE RATS. J. Janik and P. Callahan, Miami University, Oxford, OH 45056.

We have previously reported that the increase in prolactin following β-endorphin administration is potently antagonized by the specific k antagonist, nor-Binaltorphimine (nor-BNI). However, it is the μ site which has been identified in morphine induced prolactin release. The purpose of this study was to determine the role of the μ site in the β-endorphin induced prolactin secretory response in female rats.

Post-partum, lactating or diestrous female Sprague-Dawley rats were used for all experiments. Animals were implanted with chronic intraventricular (ivt) cannulae into the lateral ventricle of the brain. Following a 5 - 7 day recovery period and one day prior to an experiment, each animal was implanted with a chronic jugular cannulae. Animals were administered saline or β-funaltrexamine (β-FNA, 1 or 5 μg, ivt) 4 hours prior to saline or β-endorphin (0.025 μg, ivt) administration. Both these doses of β-FNA significantly antagonized the morphine induced prolactin increase.

The prolactin secretory response to β-endorphin was blocked by the 5 μg β-FNA dose only: the 1 μg dose was completely ineffective in blocking the action of β-endorphin. It seems that β-endorphin not only acts through a κ site to elicit a prolactin secretory response but also acts via the μ opiate receptor subtype in both lactating and diestrous female rats.

322.13

IMMUNOBLOTS WITH RHODOPSIN ANTISERA PROVIDE EVIDENCE THAT A MU OPIOID BINDING PROTEIN BELONGS TO THE G-PROTEIN-COUPLED RECEPTOR FAMILY. T.L. Gioannini*[1,2], E.R. Weiss*[3], G.L. Johnson*[4], J.M. Hiller*[2] and E.J. Simon[2]. [1]Baruch College, NY,NY 10017; [2]NYU Med. Ctr., NY, NY 10016; [3]Univ. of North Carolina, Chapel Hill, NC 27599; [4]Nat. Jewish Center for Immun. and Resp. Med., Denver, CO 80206.

A mu opioid binding protein (OBP) purified to homogeneity from bovine striatal membranes (Gioannini et al J. Biol. Chem. 260:15117, 1985) was investigated in immunoblots using antisera against bovine rhodopsin. One antibody was produced against membrane-bound rhodopsin while 5 others were made against defined regions in the rhodopsin molecule, the sequence of which is known. Three of the antibodies produced positive signals (2 strong, 1 weak), while three others did not recognize OBP. The pattern and degree of crossreactivity mimics strikingly the crossreactivity with the same antisera previously observed with the beta adrenergic receptor. These results indicate that mu-OBP contains regions that are antigenically similar to those in two G-protein coupled receptors. The findings strongly support the hypothesis that OBP is a member of the family of G-protein coupled receptors and, when sequenced, will be found to have the structure, including the 7 transmembrane domains, characteristic of such molecules.

322.14

EFFECTS OF NALOXONE ON [11-C] BUPRENORPHINE BINDING IN BABOON BRAIN. I. Galynker*[1], D. Schlyer*[2], S.L. Dewey[2], J.S. Fowler*[2], M.J. Holland*[3], J.D. Brodie*[3], E. Simon*[3], R.R. McGregor*[2], R. Ferrieri*[2] and A.P. Wolf*[2] [1]Mount Sinai Medical Center, New York, NY, [2]Brookhaven National Laboratory, Upton, NY, and [3]NYU Medical School, New York, NY

Buprenorphine is a partial opiate agonist used clinically as an analgesic, and is of interest in the treatment of some forms of psychosis and opiate and cocaine abuse. In the present study the effects of naloxone administration on O-[11-C]-buprenorphine (11-C BPN) in female baboons (Papio anubis, N=3) were examined using positron emission tomography (PET). 11-C BPN (specific activity of 1.0-2.0 Ci/μmol at EOB) was synthesized using a modified method of Lever et al (1990) and 2.5-5.0 mCi in doses of 0.001-0.002 mg/kg was administered to the baboons followed in 30-40 min by a bolus of 1.0 mg/kg of naloxone. In all cases naloxone reduced buprenorphine binding in thalamus, striatum, cingulate gyrus, and frontal cortex by 40-70% with minimal effects on occipital cortex and cerebellum. Patlak plots using a metabolite-corrected plasma integral or a nonspecific binding region gave similar results: analysis of buprenorphine binding in untreated baboons produced linear plots (slope 0.066 ± 0.0016 min^{-1} for combined specific binding) up to the point of naloxone injection, at which time the slope decreased significantly and was similar to the one obtained with naloxone pretreatment (slope 0.031 ± 0.0008 min^{-1}, $p<0.01$). This change indicated a decrease in the number of available opiate receptors and possible 11-C BPN displacement by naloxone. These results indicate feasibility of displacement as well as pretreatment methodology for receptor studies with PET. They are of particular interest in view of reported partial reversibility of buprenorphine-induced respiratory depression with naloxone and lack of abstinence induction by naloxone in buprenorphine-using subjects (Jasinsky et al, 1978). USDOE, NS-15638.

322.15

N-SUBSTITUTED DERIVATIVES OF NORMETAZOCINE: DIFFERENTIATION OF SIGMA-1 AND SIGMA-2 RECEPTORS. L. Di Paolo[1]*, F.I. Carroll[2]*, P. Abraham[2]*, X. Bai[2]*, K. Parham[2]*, S.W. Mascarella[2]*, X. Zhang[2]*, P. Wallace[1]*, J.M. Walker[1], and W.D. Bowen[1]. [1]Brown University, Providence, RI 02912 and [2]Chemistry & Life Sci., Research Triangle Institute, Research Triangle Park, NC 27709

Sigma receptors can be divided into sigma-1 and sigma-2 subtypes. While these sites bind [^{3}H]DTG with equal affinity, they can be differentiated by stereoselectivity for opiate benzomorphans: sigma-1, (+)-enantiomer > (-)-enantiomer; sigma-2, (-) > (+). Sigma-1 sites are enriched in guinea pig brain and are selectively labeled by 3 nM [^{3}H](+)-pentazocine. Sigma-2 sites are enriched in rat brain, rat liver, PC12 cells, and some neuronal cell lines (*Brain Res.* 527:244-253, 1990; *Soc. Neurosci. Abstr.* 16:370, 1990; and abstr. this meeting) and are labeled using 5 nM [^{3}H]DTG in the presence of 1 uM dextrallorphan to mask sigma-1 sites. Here we further test this model by characterizing a novel series of enantiomeric benzomorphans. (+)- or (-)-*cis*-Normetazocine was N-alkylated with various alkyl halides. Typical results for sigma-1 were, K_i (nM): N-benzyl, (+)=0.67/(-)=36.5; N-4'-phenylbutyl, (+)=3.2/(-)=85.2; N-5'-phenylpentyl, (+)=2.6/(-)=38.5. However, at sigma-2 sites (rat liver), reversed stereoselectivity was observed: N-benzyl, (+)=1379/(-)=217; N-4'-phenylbutyl, (+)=1204/(-)=202; N-5'-phenylpentyl, (+)=820/(-)=69.2. Similar results were obtained for other members of this series. These results are consistent with those obtained previously with the normetazocine derivatives pentazocine and SKF-10,047 and support the proposed classification scheme. The high affinity of these novel benzomorphans at sigma-1 sites suggests their utility in further studies of sigma receptors. (Supported by PHS Grant DA-05721)

322.16

CHARACTERIZATION OF N-SUBSTITUTED *cis*-N-[2-(3,4-DICHLOROPHENYL)ETHYL]-2-(1-PYRROLIDINYL)CYCLOHEXYLAMINES AND N-[2-(3,4-DICHLOROPHENYL)ETHYL]-2-(1-PYRROLIDINYL)ETHYLAMINES: NOVEL COMPOUNDS WITH HIGH SIGMA RECEPTOR AFFINITY AND SELECTIVITY. K. Hsu[1], B.R. de Costa[2]*, L. Radesca[2]*, L. Di Paolo[1]*, and W.D. Bowen[1]. [1]Div. Biol. & Med., Brown Univ., Providence, RI 02912 and [2]Lab. Med. Chem., NIDDK, Bethesda, MD 20892.

We have previously shown that 1*S*,2*R*-(-)-*cis*-U50,488 exhibited a sigma K_i of 81 nM, and lacked affinity for kappa opioid receptors labeled by [^{3}H]bremazocine (*J. Med. Chem.* 32:1996, 1989). Reduction of the carbonyl moiety produced BD737, with sigma K_i of 1.3 nM (*J. Med. Chem.* 33:3100, 1990). Here we investigate a related series of (-)-*cis*-N-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamines by changing the N-alkyl substituent, and measuring ability to displace [^{3}H](+)-3-PPP from guinea pig brain membranes. For the indicated N-substituent, the following affinites were observed (K_i, nM): H, 0.49; Me, 1.3; Et, 3.5; Pr, 55.7, CyPrMe, 38.1. Removal of the cyclohexane ring produced a series of N-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)ethylamines. N-Substituted derivatives in this series are, to date, some of the most potent sigma ligands known: H, 1.11; Me, 0.34; Et, 0.51; n-Pr, 1.35; allyl, 1.39; n-Bu, 11.7. Other modifications such as opening of the pyrrolidine ring and varying substitution at the remaining N-atom have also produced ligands with sub-nanomolar affinity at sigma receptors. Most of these ligands lack affinity for PCP, dopamine, and kappa opiate receptors. Selectivity studies and further SAR data will be discussed. Compounds in this series will prove useful in structural and functional studies of sigma receptors. (Supported by PHS Grants NS-26746 and DA-04988)

322.17

IDENTIFICATION AND EXPLOITATION OF SIGMA LIGAND PHARMACOPHORES. J.B. Fischer*, K. Burke Howie*+, A. C. Server*, M. El-Ashmawy*, A. Ismaiel*, M. Yousif*, S. Ablordeppey* and R.A. Glennon*; *Cambridge NeuroScience, Inc. Cambridge, MA 02139 and Department of Medicinal Chemistry, MCV/VC; Richmond, VA 23298-0540.

Sigma receptors offer a new approach to the development of agents for the treatment of mental disorders. Haloperidol, 3-PPP, and sigma-opiates bind at, but lack selectivity for, sigma receptors. We have identified the primary pharmacophore of the sigma-opiates (such as NANM; Ki = 430 nM) as being an N-substituted 2-phenylaminoethane (J. Med. Chem. 1991, 34:1094), a pharmacophore that is common to 3-PPP-like agents. For example, N-(3-phenylpropyl)-1-phenyl-2-aminopropane (PPAP) binds at sigma receptors with increased affinity (Ki *ca* 20 nM). Further manipulation of structure results in analogs with even higher affinity. Unlike other sigma agents, these do not bind at PCP sites or dopamine receptors. Phenylpiperazine (Ki > 10,000 nM) can serve as a phenylaminoethane-mimic; incorporation of PPAP substituents increases sigma affinity by more than 10,000-fold and has allowed identification of a sigma-selective pharmacophore of haloperidol (Ki - 0.8 nM relative to 10 nM for haloperidol) with reduced affinity for dopamine receptors. Thus, it is possible to account for the binding of these major classes of sigma ligands via structurally similar pharmacophores.

322.18

FURTHER STUDIES ON THE IDENTITY OF SIGMA RECEPTORS IN RODENT LIVER AND BRAIN. M.S. Sonders and E. Weber. Department of Pharmacology, UC Irvine, Irvine, CA 92717.

The most recognizable feature of the sigma receptor [haloperidol-sensitive, sigma-1, or DM/sigma] is its ability to bind with high affinity a limited number of drugs from several disparate structural and pharmacological categories. This broad ligand specificity and the high density of binding sites in rodent liver with a "sigma-like" pharmacological profile have led several groups to propose that the sigma receptor is a member of the cytochrome P-450 enzyme superfamily. Consistent with this hypothesis is the finding that cytochrome P-450 inhibitor SKF-525A can inhibit the binding of several different radioligands in sigma binding assays employing liver and brain tissue.

We have sought to characterize and purify the binding sites for the sigma ligand DTG (di-ortho-tolyl-guanidine) from guinea pig and rat livers. Subcellular fractionation studies have yielded data consistent with previously-reported results indicating that activity is predominantly associated with the microsomal and mitochondrial fractions. [^{3}H]-DTG radioreceptor assays on rat microsomal fractions displayed a pharmacological profile quite similar to that seen in brain membrane assays though the affinity for haloperidol was somewhat lower. (+)Pentazocine and SKF-525A inhibition curves were markedly biphasic, suggesting that [^{3}H]-DTG may bind to more than site, though scatchard analyses detected only one.

Preliminary studies on the effects of phenobarbital administered to male Sprague-Dawley rats (15 mg i.p./rat/day X 5 days) showed no significant change in the level of [^{3}H]-DTG binding sites in either liver or brain membranes when compared to vehicle-injected rats. Should a cytochrome P-450 comprise some of the [^{3}H]-DTG binding sites, it is non-inducible by phenobarbital.

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322.19

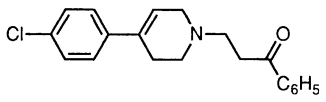
[^{3}H]-DTG SELECTIVELY LABELS "SIGMA-1" SITES IN SOLUBILIZED RAT LIVER HOMOGENATES. G. K. Ehrlich, G. Lem*, G. Singh*, R. B. Murphy, D.I. Schuster. New York University, Dept. of Chemistry and Center for Neural Science, New York, NY 10003.

[^{3}H]-DTG is known to bind to two distinct pharmacological sites in rat liver homogenates, which have been suggested to represent components of the sigma receptor/binding site (SRBS). These two components are differentiated by their enantiomeric selectivities for benzomorphans. While both subtypes exhibit high affinities toward [^{3}H]-DTG, [^{3}H]-haloperidol and [^{3}H]-(+)-PPP, "SIGMA-1" sites bind (+)-benzomorphans with higher affinities than (-)-benzomorphans. In both CHAPS and sodium cholate solubilized rat liver preparations, we have demonstrated the selective labeling of a sigma subtype with [^{3}H]-DTG which retains the pharmacological characteristics of the putative "SIGMA-1" site. This selective labeling with [^{3}H]-DTG is also observed in solubilized bovine cerebellar homogenates. Moreover [^{3}H]-haloperidol does not discriminate between subtypes of similar solubilized preparations. Hence, these results suggest that [^{3}H]-DTG can be used as a selective label in the characterization of "SIGMA-1" sites in tissues which contain a heterogeneous population of sigma sites. This work was supported by NIDA grant 1 R01 DA 05728.

322.20

PREPARATION AND CHARACTERIZATION OF SUBSTITUTED 3'-[N- (4- PHENYL- 1,2,3,6-TETRAHYDROPYRIDINE)] PROPIOPHENONES AS POTENT AND SELECTIVE SIGMA LIGANDS. G. Singh*, W. E. Frietze*, A. Khodabakhsh*, H. Zhao*, R. B. Murphy, D.I. Schuster. New York University, Dept. of Chemistry, New York, NY 10003.

We have prepared a series of substituted 3'-[N-(4-phenyl-1,2,3,6-tetrahydropyridine)] propiophenones, with very high affinities toward sigma receptor/binding sites from bovine cerebellar homogenates labelled with [^{3}H]-haloperidol and [^{3}H]-DTG, and with very low affinity toward D_2 receptors in rat striatal preparations. These compounds were synthesized through Mannich reactions of substituted 4-phenyl-1,2,3,6-tetrahydropyridines or 4-phenylpiperidines with acetophenones and other enolizable methyl ketones. The relationship of the structures of this series of compounds with their affinities toward sigma receptor/binding sites will be presented, along with their pharmacological profiles. The compound displaying the highest sigma activity in the series (IC_{50} = 1 nM) is 3'-[N-(4-(*p*-chlorophenyl-1,2,3,6-tetrahydropyridine)] propiophenone hydrochloride, shown below. These compounds are of potential interest as novel, selective ligands for the sigma site. This work was supported by NIDA grant 1 R01 DA 05728.



CATECHOLAMINE RECEPTORS: DOPAMINE III

323.1

SITE-DIRECTED MUTAGENESIS OF DOPAMINE D2 RECEPTORS. K.A. Neve, B.A. Tester, R.A. Henningsen, G.C. Hughes, A. Spanoyannis, and R.L. Neve. VAMC, Portland, OR; and Univ. of Calif., Irvine, CA.

We are characterizing three sets of mutations introduced into rat D2 receptors, to determine amino acids that contribute to the binding of ligands to D2 receptors or to the interaction of the receptors with G proteins to modulate second messengers. All mutants were stably expressed in C_6 glioma cells. One set of mutations involves potential alpha helical regions from the third cytoplasmic loop of D2 receptors. Deletion of one such region, amino acids (AA) 231-255 of $D2_{415}$, prevents inhibition of adenylyl cyclase by dopamine without substantially altering the affinity of antagonist ligands. Deletion of AA 271-283 of $D2_{444}$ had very different consequences, since this mutant was capable of inhibiting adenylyl cyclase but had markedly lower affinity for several antagonist ligands. This difference is striking because 13 of the residues missing in del231-255 (242-254) are those deleted in del271-283 of $D2_{444}$. The second set of mutations consists of three conserved aspartate residues: D80, D114, and D131. Perhaps the most interesting effects resulted from changing Asp-80 to Ala (D80A). D80A had slightly-to-moderately decreased affinity for many ligands, but a complete loss of sodium-sensitive binding of substituted benzamides and agonists. Also, D80A was unable to inhibit of adenylyl cyclase. The third set of mutations consists of 4 conserved serine residues: S193, S194, S197, and S391. S193 contributed most and S194 least to the binding affinity of dopamine. (MH 45372, HD 18658, VA Merit Review Program)

323.2

FLAG-$D2_{415}$ AND FLAG-$D2_{444}$, UNIQUE MUTANTS OF DOPAMINE D2 RECEPTORS $D2_{415}$ AND $D2_{444}$, OFFER A NOVEL APPROACH FOR RAPID PURIFICATION OF D2 RECEPTORS. B.A. Tester, R.A. Henningsen, A. Spanoyannis, R.L. Neve, and K.A. Neve. VA Medical Center, Portland, OR, 97201; UC Irvine, CA, 92707.

We have constructed cDNAs in which a short sequence (Flag) is attached to the amino terminus of each molecular form of dopamine (DA) D2 receptors. A commercially available anti-Flag antibody can be used to purify proteins bearing the Flag sequence (MDYKDDDDKS) from solubilized membranes. $D2_{415}$, $D2_{444}$, Flag-$D2_{415}$ and Flag-$D2_{444}$, have been stably expressed in C_6 glioma cells. Binding studies on membrane preparations of the transfected cell lines demonstrate that the attached Flag sequence does not change agonist or antagonist binding. Flag-$D2_{415}$ and Flag-$D2_{444}$ receptors bind [^{125}I]epidepride and other antagonists with equal affinity, and these values are equivalent to those of wild-type D2 receptors. Competition curves for inhibition of the binding of [^{125}I]epidepride by DA shift from two sites in the absence of GTP to one low affinity site in the presence of GTP, suggesting coupling of G proteins to the Flag-$D2_{415}$ and Flag-$D2_{444}$ receptors. These mutants will be useful tools for selective purification of $D2_{415}$ and $D2_{444}$ receptors. Potential applications include analysis of post-translational modifications of the receptors, as well as analysis by co-purification of selective coupling of Flag-$D2_{415}$ and Flag-$D2_{444}$ to G proteins. (MH45372, HD18658, VA Merit Review Program)

323.3

STRUCTURE:FUNCTION PREDICTIONS FOR G_i-PROTEIN COUPLING BY THE D_2 DOPAMINE RECEPTOR. S.D. HANDRAN and J.R. STARKEY*. Department of Microbiology, Montana State University, Bozeman, MT 59717.

Predictions for intracellular D_2 dopamine receptor sequences likely to interact with G_i-protein domains during receptor signal transduction were sought by comparing the published D_2 amino acid sequence with the sequences for numerous other G_i coupled membrane receptors. Using "Alimat", a program which looks for structure:function homologies, the D_2 receptor was found to exhibit most overall homology with the α2-adrenergic receptor and a lesser homology with the m2-muscarinic receptor. The homology with the α2-adrenergic receptor included a highly conserved region in the putative third cytoplasmic loop (D_2 receptor residues 291-356; α2-adrenergic receptor residues 277-342), and this region of homology is suspected to mediate a major portion of the G-protein coupling interaction. Secondary structure predictions from the "Stroud" and "Mseq" programs were used with InsightII and Discover programs to model the third cytoplasmic loop of the D_2 receptor and the putative interacting domain of the $G_{i\alpha}$ subunit. These models are being used to guide docking and peptide mapping experiments to confirm the predictions for the protein:protein interacting sequences. Supported by the Montana Technology Development Trust and NSF grant # RII-8921978.

323.4

VARIABLE EXPRESSION OF THE D_2 DOPAMINE RECEPTOR (D_2R) IN GH_4C_1 PITUITARY TUMOR CELLS: EFFECTS ON FUNCTIONAL RESPONSE. E. Meller, T. Puza*, J.C. Miller, W. Chan*, D. Filer*, A.J. Friedhoff, Y. Wang* and M. Armour*. Dept. of Psychiatry, NYU Medical Center, New York, NY 10016.

We have recently shown that dopaminergic inhibition of prolactin (PRL) secretion in the rat anterior pituitary displays very efficient receptor/effector coupling, as evidenced by a large receptor reserve for full dopamine agonists both *in vivo* and *in vitro* (Meller et al., JPET 257:668-675, 1991). While the D_2R exists in both short and long forms due to alternative splicing, only the long form (D_2R_L) is expressed in the rat anterior pituitary (Dal Toso et al., EMBO J. 8:4025-4034, 1989; Giros et al., Nature 342:923-926, 1989). GH_4C_1 pituitary tumor cells secrete PRL but are devoid of D_2 receptors. We have therefore transfected GH_4C_1 cells with a cDNA encoding the D_2R_L isoform in order to examine the relationship between functional response and D_2R density.

The complete cDNA for the coding region of D_2R_L was amplified by the polymerase chain reaction and the insert was subcloned into the pRSV expression vector. DNA sequence analysis of the D_2R_L insert was found to be complete and correct. GH_4C_1 cells were co-transfected with pRSV(D_2R_L) and another expression vector (p*SVneo*) containing the neomycin resistance gene. Single colonies expressing neomycin resistance were selected and subcultured. Cell membranes from individual clones were subjected to saturation analysis of radioligand binding with ^{3}H-spiperone. In transfectants, D_2R density varied over an approximately 8-fold range (70-570 fmol/mg protein), whereas untransfected cells displayed no detectable binding. Dopaminergic inhibition of basal as well as stimulated PRL secretion in these transfectants is currently being examined. The relationship between expressed D_2R_L density and functional responsiveness will be reported. Supported in part by BRSG SO7-RR5399-29.

323.5

DETECTION OF RNA TRANSCRIPTS FOR BOTH ISOFORMS OF THE DOPAMINE D2 RECEPTOR IN SEVERAL NEURAL CELL LINES

Christina A. Harrington and Noel J. Buckley*, Department of Physical Biochemistry, National Institute for Medical Research, London NW7 1AA

A panel of neural cell lines has been screened for the presence of dopamine D2 receptor RNA. Oligomer primers flanking the alternative exon between transmembrane regions V and VI were used in PCR amplification reactions with cell line RNA. Among the lines tested, IMR 32 cells, 1321N1 cells, and SH-SY5Y cells contained RNA transcripts for the D2 receptor. The detection of D2 transcripts in the IMR 32 line is consistent with the report of Monsma et al. (Brain Research, 1989, 492:314-324) that significant levels of specific receptor binding by the D2 antagonist [^{3}H]methylspiperone are present in these cells. Transcripts for both isoforms of the D2 receptor are detected in the lines expressing D2 RNA. In general, the longer form of the D2 receptor transcript, $D2_{(444)}$, is present in higher abundance than the $D2_{(415)}$ isoform transcript.

Neural cell lines expressing the dopamine D2 receptor will provide useful assay systems for initial characterization of the promoter for the D2 receptor gene. These lines may also be useful for studying other aspects of the regulation of dopamine receptor RNA. Experiments are currently in progress to determine whether D2 transcript levels or isoform type can be varied with cell treatment.

323.6

STRIATAL DOPAMINE D_2 RECEPTOR-REGULATED GENE EXPRESSION IN MICE FOLLOWING TREATMENT WITH 6-HYDROXYDOPAMINE AND RELATED NEUROTOXINS.

S. HOWARD, J. JOYNER*, D. BLANK*, and C.L.BLANK†, Department of Pharmacology, UCLA, Los Angeles, CA, 90024 and †Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK, USA, 73019.

6-Hydroxydopamine and related species exhibit neurotoxicity towards both noradrenergic and, usually, in higher doses, toward dopaminergic neurons. In the current study, the loss of endogenous dopamine and related metabolite levels in the striatum of the mouse was investitgated at vatious times up to one week following intraventricular treatment with the neurotoxin. These level measurements were then correlated with the specific expression of messenger RNAs encoding the D_2 dopamine receptor. Results were found to be enhanced through the utilzation of euthanasia by microwave irradiation, which decreased the post mortem enzymatic activities.

Levels of catecholamines, indoleamines, acetylcholine, and related metabolites were determined using multiple column, multiple detector liquid chromatography with electrochemical detection similar to that described for a single column approach in Lin *et al.*, J. Liq. Chromatogr., **7**, 509-538 (1984).

Measurement of the specific expression of messenger RNAs encoding for the D_2 receptor employed a Northern Blot procedure with quantitation by densitometry using random primed, radiolabelled oligonucleotide probes which were complementary to the mRNA similar to that described by Gerfen *et al.*, Science, **250**, 1429-1433 (1990). *In situ* hybridization approaches were also attempted to assess localization.

323.7

6-OHDA LESION INCREASES RAT STRIATAL D_2 RECEPTOR mRNA LEVELS WHILE CHRONIC RECEPTOR BLOCKADE DOES NOT.

S. Xu and Ian Creese Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102.

Chronic receptor blockade by antagonists, denervation by 6-hydroxydopamine (6-OHDA) and depletion of dopamine by reserpine have been considered to be functionally equivalent, leading to an increase in striatal D_2 receptor B_{max}, as well as behavioral supersensitivity to dopaminergic agonists. cDNAs encoding two forms of the rat D_2 receptor ($D_{2L\&S}$) derived from alternative splicing have been isolated and allow us to investigate directly the regulation of dopamine receptor gene transcription following chronic treatment with antagonists or denervation. Treatment of rats with haloperidol HCl (0.5 mg/kg/day, s.c., for 2 h, 7, 14, 21 days and 21 days+3 days withdrawal) had no effect on D_{2L} or D_{2total} mRNA levels, in spite of significant increases in striatal receptor density at the later time points. In contrast, 21 days following unilateral nigro-striatal pathway lesion with 6-OHDA HCl (8 µg), striatal D_{2L} and D_{2total} receptor mRNA levels were increased by 54.1 and 52.4%, respectively ($p < 0.05$, Dunnett *t* test), along with a significant increase of 38.2% in striatal D_2 receptors. These findings suggest that different molecular mechanisms can be responsible for D_2 receptor up-regulation and that denervation, but not chronic receptor blockade by antagonists, results in an increase in D_2 receptor gene transcription.

Supported by MH 44211, MH 00316 and DA 04612

323.8

CHANGES OF THE mRNAs FOR TWO DOPAMINE D2 RECEPTOR ISOFORMS IN RAT BRAIN BY HALOPERIDOL OR METHAMPHETAMINE TREATMENT.

I.Sora, Y.Fujiwara, H.Tomita*, H.Ishizu*, K.Akiyama, S.Otsuki*, H. I. Yamamura. Dept. of Pharmacology, Univ. of Arizona, Tucson, AZ 85724

Dopamine D_2 receptors are the target for drugs used in the treatment of several neurological disorders. The cDNA of a rat D_2 receptor, $D_{2(415)}$ has been cloned and that was followed by the finding that two D_2 receptor isoforms; $D_{2(444)}$ and $D_{2(415)}$. These isoforms are generated by alternative RNA splicing. To investigate changes of the two mRNAs encoding the D_2 receptor isoforms by haloperidol or methamphetamine treatment, we performed in situ hybridization histochemistry in the rat brain with the two oligonucleotide probes, an "insert probe" hybridizing to the longer $D_{2(444)}$ mRNA, and a "spanning probe" hybridizing to the shorter $D_{2(415)}$ mRNA. Both $D_{2(415)}$ and $D_{2(444)}$ mRNAs were found in the striatum, accumbens nucleus and substantia nigra in all rats treated with the drugs and these treatments did not change the distribution of mRNA for two D_2 isoforms. The changes of the two mRNAs were in broad agreement with descriptions of the receptor binding experiments and there were not any evidence that one of two D_2 receptor isoforms alone has relation with effects of these drugs.

323.9

AGE-RELATED DECREASES IN DOPAMINE D2 RECEPTOR mRNA EXPRESSION IN RAT NEOSTRIATUM.

D.M. Dorsa, K.M. Merchant, D.J. Dobie and M.W. Hamblin. GRECC, Seattle VA Medical Center; Depts. of Medicine, Pharmacology and Psychiatry, Univ. of Washington, Seattle, WA 98195.

Expression of dopamine D2 receptor mRNA in young (3 mo), middle aged (12 mo) and old (24 mo) male Fisher 344 rats was examined by *in situ* hybridization histochemistry. A ^{35}S-labeled antisense RNA probe derived from the 3'-untranslated region of the D2 receptor cDNA was employed to detect the expression of both long and short transcripts of the D2 gene. Film autoradiograms were generated by apposing the hybridized sections to Hyperfilm ßmax. Densitometric analysis of the four quadrants of the neostriatum of the young and middle aged rats showed that the expression of D2 receptor transcripts was greater in the lateral quadrants compared to that in the medial regions. In the senescent rat, there was a significant reduction in D2 receptor mRNA expression. Although the decreases were evident in all four quadrants of the neostriatum, they were maximal in the ventrolateral region thereby greatly attenuating the lateral-medial gradient observed in the young rats. These data are consistent with previously reported age-related decline in D2 receptor binding. Potential differential decline in the expression D2-short and D2-long transcripts during aging is being investigated. (Supported by Washington Institute for Mental Illness; Research Service Department of Veterans Affairs; NS 20311).

323.10

IDENTIFICATION AND REGULATION OF DOPAMINE D_2 RECEPTORS (D_2R), G PROTEINS AND TIS mRNA'S IN MMQ CELLS; A PROLACTIN-SECRETING CELL LINE.

H. Zawadzka*, X. He*, L. Li*, D. Tang*, D. Filer*, J.Y. Lew and M. Goldstein. Neurochem. Res.Lab, N.Y. Univ. Med. Ctr., N.Y., N.Y. 10016

To study the effects of DA agonists at the gene level we have characterized the D_2 R protein and examined the gene expression of D_2 R and of genes which might modulate these responses (G proteins and TIS [early response] genes in MMQ cells. The D_2 R in the MMQ cells was identified by photoaffinity labeling with ^{125}I-N-(p-azido m)[^{125}I]iodophenethyl)spiperone of membrane preparations. The major labeled D_2 R binding protein has a M.W. of approx. 32-34 KDa. The analysis of D_2 mRNA shows that its size is similar in the MMQ cells as in the 7315a tumor from which these cells were derived, as well as in the striatum. In addition, $Gi2_\alpha$ and Gs_α and TIS (c-Fos and TIS1 and TIS8) genes are expressed in the MMQ cells. Exposure of the cells to the D_2 agonist quinpirole (10^{-6}M) for 24 hrs results in a decreased D_2 R mRNA, suggesting an agonist-induced down-regulation. The effects of quinpirole on G proteins and TIS mRNA's levels are under investigation. Thus, these cells provide a useful model for investigating D_2 R regulation.

Supported by NIMH 02727 and NINCDS 06801.

323.11

REGULATION OF D2 RECEPTOR mRNA BY ESTRADIOL IN THE RAT PITUITARY. Y. Shiffman*, M. Jakubowski*, M. Shiffman, J.L. Roberts, S.C. Sealfon. Fishberg Research Center for Neurobiology and Dept. of Neurology. Mount Sinai Medical School. New York, NY 10029

17β-estradiol (E2) increases the number of dopamine receptors (D2R) in the anterior pituitary (AP) of ovarietomized rats (Endo 119, 2484:1986). We investigated whether altered D2R mRNA levels contribute to this increase in receptor number. Two weeks following ovariectomy, rats received either E2 or empty silastic implants for 72 h. Cytoplasmic AP and neurointermediate lobe (NIL) RNAs from each group were assayed using a multiplex nuclease protection assay, enabling simultaneous measurement of D2R, prolactin, POMC and cyclophilin mRNAs. Data were expressed as fg mRNA/ug DNA. In the NIL, D2R, POMC and cyclophilin mRNAs increased slightly, but not significantly, with E2. In the AP, however, E2 increased D2R mRNA ~2-fold and prolactin mRNA ~6-fold; cyclophilin and POMC mRNA levels did not change significantly. The E2-induced rise in D2R mRNA levels in the AP suggests that the reported E2-stimulated increase in D2R number is due, at least in part, to increased biosynthesis. Supported by NIH grants K11 DK01854 and MH 45212.

323.12

SEX DIFFERENCES IN DOPAMINE D_2 RECEPTORS IN THE RAT PREOPTIC AREA. C.E. Roselli, T.A. Fasasi* and A. Janowsky. Dept. Physiol., Ore. Hlth. Sci. U., Portland, OR 97201.

The incertohypothalamic dopamine (DA) system (A13) is involved in the hormone-dependent regulation of both copulatory behavior and gonadotropin secretion. The preoptic area (POA) receives projections from A13 neurons involved in these functions. We used [^{125}I] epidepride to characterize DA D_2 receptors in the POA and investigate whether they are influenced by sex or androgen status. Scatchard analyses of epidepride binding to POA membranes derived from gonadally-intact male rats demonstrated a Kd of 70.9 ± 23.2 pM and a Bmax of 18.5 ± 3.6 fmol/mg protein (n=6). Neither binding nor receptor affinity was altered 1 wk after males were gonadectomized (GX) (Bmax = 19.2 fmol/mg protein; Kd = 109.1 ± 14.3 pM; n=5). By contrast, D_2 receptor levels (Bmax = 45.2 fmol/mg protein; Kd = 120 ± 27.2 pM; n=5) in age-matched GX females were significantly greater than in males ($p < 0.01$). The pharmacological profile of binding in POA was similar to that in striatum (epidepride > spiperone > +butaclamol > sulpiride). Binding in POA was higher than in hypothalamus, amygdala, or frontal cortex. POA was the only area where a sex difference in binding was evident. These sex-related differences in POA D_2 receptor density could lead to the differential modulation of POA activity in males and females. Supported by HD23293.

323.13

EPIDERMAL GROWTH FACTOR INDUCES THE EXPRESSION OF D-2 DOPAMINE RECEPTORS IN THE GH-3 CELL LINE. C.Missale, L.Castelletti*, F.Boroni*, S.Sigala*, R.Dal Toso, P.F.Spano. Inst. of Pharmacol. Exp. Ther., Univ. of Brescia and Fidia Res. Lab., Abano Terme, Italy.

GH-3 is a prolactin (PRL) secreting cell clone which lacks receptors for dopamine. Exposure of GH-3 cells to epidermal growth factor (EGF) induced the expression of dopamine (DA) D-2 receptors. The polymerase chain reaction (PCR) was utilized to amplify D-2 related fragments from GH-3 cell cDNA; evaluation of the products suggested that both the D-2(415) and the D-2(444) isoforms were 7-fold increased by EGF. These changes were paralleled by the expression of the receptor protein as shown by the appearance of a saturable, high affinity binding for 3H-spiroperidol (Kd = 0.8 ± 0.2 nM; Bmax = 116 ± 10 fmoles/5 x 10^5 cells) with a D-2-peculiar pharmacological profile. This receptor was functionally active in controlling PRL secretion; the selective D-2 agonist quinpirole, which was inactive in naive GH-3 cells, recovered indeed the property to inhibit PRL release in the cell cultures exposed to the neurotrophic factor.

323.14

CHARACTERIZATION OF THE LONG FORM OF THE DOPAMINE (DA) D_2 (D_{2L}) RECEPTOR IN MOUSE FIBROBLAST LTK$^-$ CELLS. J.Y. Lew, G.T. Saez*, D. Filer*, A. Pellicer*, J. Grebb* and M. Goldstein. Departments of Psychiatry and Pathology, N.Y.U. Med. Ctr., N.Y., N.Y. 10016.

The D_{2L} receptor was stably expressed in mouse fibroblast LTK$^-$ cells, and subsequently characterized using ^{125}I- N-(p-azido-m)[^{125}I]iodophenethyl spiperone (^{125}I- NAPS) for photoaffinity labeling and antipeptide antibodies for immunoblotting. In the membrane fraction from LTK$^-$ cells that were transfected with the D_{2L} receptor, ^{125}I- NAPS labelled a single, major protein with an approx. M.W. of 48 KDa. ^{125}I- NAPS also labeled several minor, higher M.W. proteins, which may be partially glycosylated forms of the D_{2L} receptor. ^{125}I- NAPS did not lable any of these proteins in non-transfected LTK$^-$ cells. Proteins with the same MW's were detected, however, by immunoblotting of the membrane fraction of transfected LTK$^-$ cells using an affinity-purified antibody that had been raised against a peptide containing the 18 amino terminal amino acids of DA D_{2L}.

The specificity of this antibody was also demonstrated by immunoblot analysis of solubilized rat brain membranes. The antibody recognized a protein with an approx. MW of 92-94 KDa in striatal but not cerebellar membranes. Thus, these data show that transfected LTK$^-$ cells produce a DA D_{2L} receptor which can be identified by both photoaffinity labeling with ^{125}I- NAPS and immunoblotting with an antipeptide antibody. Supported by NIMH 02717 and NIMH 43230.

323.15

SELECTIVE IMMUNOPRECIPITATION OF THE D-2 DOPAMINE RECEPTOR ISOFORMS: DETECTION OF PHOSPHORYLATED RECEPTOR PROTEIN. P.L. Olinger*, C.L. Chio*, I. Abraham, R.M. Huff*, Cell Biology, The Upjohn Co., Kalamazoo, MI 49001.

The recent cloning of dopamine receptor subtypes has enabled characterization of these proteins in a manner not previously possible. Two molecular forms of the D-2 receptor resulting from differential splicing of a single gene have been identified. These two proteins differ by 29 amino acids present (RD-2A) or absent (RD-2B) in an intracellular loop of the receptor known as IC3. We have developed antibodies that are specific for either D-2A or D-2B receptors. These antibodies have been used to selectively immunoprecipitate either receptor protein from CHO cells transfected with the D-2A or D-2B dopamine receptor cDNA. We have found that both receptor isoforms are phosphorylated following pretreatment of the cells with dopaminergic agonists or by activation of PKC but not by activation of PKA. Activation of the D-2 receptors in these cells does not lead to increased production of inositol phosphates so we do not think stimulation of PKC is part of the dopaminergic signalling pathway. The agonist- and/or the PKC-induced phosphorylation may be related to changes in receptor function such as desensitization.

323.16

CHARACTERIZATION OF [^{3}H]QUINPIROLE BINDING IN RAT STRIATUM. B. Levant, D.E. Grigoriadis, E.B. De Souza. Central Nervous System Diseases Research, The DuPont Merck Pharmaceutical Company, Wilmington, DE 19880-0400.

The putative D_2 dopamine receptor agonist quinpirole (LY 171,555) has been extensively employed in a variety of *in vivo* and *in vitro* studies of D_2 receptor mediated effects. In addition to possessing agonist activity at the D_2 receptor, quinpirole may have even higher affinity for the recently described D3 dopamine receptor (Sokoloff et al., Nature, 347:146-151). The present study characterizes the *in vitro* binding properties of newly developed [^{3}H]quinpirole in rat striatum. [^{3}H]Quinpirole binding was assessed in homogenized striatal membrane preparations using a filtration assay. Nonspecific binding was defined in the presence of 1 μM (+)butaclamol. Data were analyzed using Ligand (Munson and Rodbard, 1980). Specific [^{3}H]quinpirole binding was saturable, membrane concentration dependent, temperature dependent, stereoselective ((+)butaclamol >> (-)butaclamol and (-)sulpiride >> (+)sulpiride), and of high affinity (K_D=2-10 nM). The affinity was confirmed by association-dissociation kinetics. The pharmacological profile of [^{3}H]quinpirole binding in striatum was: (-)NPA ≥ 6,7-ADTN ≥ quinpirole > bromocriptine > dopamine > SKF 38393 >> 5-HT for agonists; spiperone > (+)butaclamol > (-)sulpiride > clozapine ≥ SCH 23390 > cinanserin for antagonists. These data demonstrate that [^{3}H]quinpirole exhibits dopaminergic pharmacology. Further investigation is required to determine the specific characteristics of the receptor binding site for [^{3}H]quinpirole.

323.17

DIHYDREXIDINE, A SELECTIVE DOPAMINE RECEPTOR AGONIST THAT MAY DISCRIMINATE POSTSYNAPTIC D2 RECEPTORS. D.M. Mottola[1], L.L. Cook[1], S.R. Jones[1], R.G. Booth[1], D.E. Nichols[2] and R.B. Mailman[1]. University of North Carolina[1], Chapel Hill, N.C. 27599. Purdue University[2], West Lafayette, IN. 47907

Dihydrexidine (DHX) is a member of a novel structural class of dopamine receptor ligands (trans-hexahydrobenzo[a]phenanthridines). As we previously reported (J. Med. Chem. 33:1756, 1990), DHX is a high potency (IC50 = 10 nM), full efficacy D_1 receptor agonist with moderate potency (IC50 = 120 nM) for D_2 receptors. The present study was designed to address: i) the overall selectivity of DHX for dopamine receptors and ii) the functional activity of DHX at D_2 receptors.

DHX was tested in 31 different radioreceptor binding assays, as well as eight other binding assays related to loci associated with cell signaling. This series of tests indicated that DHX, while active at dopamine receptors, was essentially inactive (IC50 $\geq$ 10 μM) at all other tested binding sites, with the sole exception of the α_2 adrenergic receptor (IC50 $\approx$ 1 μM). These data demonstrate that DHX possesses a high degree of selectivity for dopamine receptors. We also sought to resolve whether DHX was acting as an agonist or antagonist at D_2 receptors. Data from radioligand (^{3}H-spiperone) competition analysis resulted in a Hill coefficient (n_H) < 1, consistent with the behavior of other D_2 agonists in this preparation. In addition, *in vivo* behavioral testing of DHX (Darney et al. Neuropsychopharmacology 1991, in press) found that this agent, like the D_2 agonist quinpirole, induced locomotion; this effect was blocked by the D_2 antagonist remoxipride. This effect of DHX apparently is not due to its action as an indirect agonist since it is a weak competitor for the dopamine uptake site (IC50 $\approx$ 50 μM). Furthermore, in preliminary experiments DHX did not alter dopamine release in striatal slices. In tyrosine hydroxylase (TH) assays, DHX inhibited TH, but these effects were not blocked by D_2 antagonists. These inhibitory effects of DHX on TH are probably due to direct actions on the enzyme, rather than being mediated via autoreceptors. Together, these data suggest that DHX does not act on D_2 autoreceptors which regulate release or synthesis but does affect postsynaptic receptors having D_2 pharmacological charateristics. Coupled with its potent D_1 activity, DHX should be an important tool to define the activity of dopamine receptors.

323.18

EFFECTS OF DOPAMINE D2 RECEPTOR ACTIVATION MEASURED BY A NOVEL TECHNIQUE: MICROPHYSIOMETRY. M.P. Rosser, M.R. Kozlowski, and K.A. Neve. Dept. of Screening and Biochemical Research, Bristol-Myers Squibb, Wallingford, CT 06492 and Research Service, VA Medical Center, Portland, OR 97207.

The dopamine (DA) D2 receptor is involved in the pathology of a number of neurological and psychiatric disorders including Parkinsonism, schizophrenia and drug addiction. The effects of D2 receptor stimulation may be mediated at least in part by a Na^+/H^+ exchanger. This possibility was examined by monitoring an indicant of proton export, the rate of acidification of the medium, in response to cellular D2 receptor stimulation. These measurements were made with the aid of a device called a microphysiometer (Molecular Devices Corp., Menlo Park, CA). This device employs a light addressable potentiometric sensor (LAPS) to measure the acidity of the medium bathing the cells.

A glioma cell line (C6) transfected with the D2 receptor (D2-415) was cultured in a flow chamber in contact with the LAPS. Drugs were introduced into the medium perfusing the chamber and the response was measured. Introduction of the D2-selective agonist quinpirole resulted in a dose-dependent increase in the rate of acidification relative to non-drug-treated controls. The maximum effect was obtained with 100nM quinpirole. This effect was blocked by the dopamine antagonist spiperone (10μM). No effect of quinpirole was seen on the non-transfected parent cell line.

323.19

STIMULATION OF MITOGENESIS IN TRANSFECTED CHO CELLS BY ACTIVATION OF THE D2 RECEPTOR. M.E. Lajiness*[1], C.L. Chio*[1], D.K. Hyslop[2], and R.M. Huff*[1] [1]Cell Biology and [2]CNS Research, The Upjohn Co., Kalamazoo, MI 49001.

The dopamine D2 receptor is a member of the G protein coupled receptor family. Other receptors in this family have been shown to mediate growth by agonist activation of the receptor/G protein complex. We used CHO cells which express rat D2 receptors to assess growth stimulation by activation of D2 receptors. Treatment of these cells with D2 agonists causes an increase in mitogenesis as measured by ^{3}H-thymidine incorporation during a two hour labeling period. EC_{50} values for D2 selective agonists correlate with K_{hi} values determined by binding analysis. The EC_{50} values also correlate with the IC_{50} values for inhibition of forskolin-stimulated cAMP accumulation. Agonist induced mitogenesis and inhibition of cAMP accumulation are both sensitive to pertussis toxin. Treatment of the cells with 8-(4-Chlorophenylthio) cAMP inhibits growth and prevents mitogenic effects of dopaminergic agonists. Similar dose response curves and pertussis toxin sensitivity indicate that the mitogenic response could be related to cAMP levels.

323.20

COMPARISON OF THE EFFECTS OF REMOXIPRIDE, RACLOPRIDE AND HALOPERIDOL ON THE ACTIVITY OF CENTRAL DOPAMINERGIC NEURONS AND HORMONESECRETION FROM THE PITUITARY. M.J. Eaton, Y. Tian, K.J. Lookingland and K.E. Moore Dept. of Pharmacol. and Toxicol. Michigan State University East Lansing, MI 48824

Remoxipride (REM) and raclopride (RAC) are selective D2 dopamine (DA) receptor antagonists currently being investigated for clinical use as antipsychotic drugs. The present study was designed to compare the time course and dose response of REM, RAC and haloperidol (HAL) on central DA neuronal activity, as well as their ability to alter plasma levels of prolactin (PRL) and α-melanocyte stimulating hormone (α-MSH) in male rats. DA neuronal activity was estimated by measuring the DOPAC/DA ratio in brain regions containing terminals of different DA neurons. RAC (1.0 mg/kg) and HAL (0.1 mg/kg) increased PRL, α-MSH and the activity of mesolimbic, nigrostriatal and incertohypothalamic DA neurons in a comparable manner. In contrast, REM (3.0 mg/kg) increased PRL and the activity of mesolimbic and nigrostriatal DA neurons, but did not alter α-MSH or the activity of incertohypothalamic DA neurons. These data suggest that there may be more than one D2 receptor subtype functioning in the rat with REM selectively antagonizing the receptor type found on lactrotrophs and mesotelencephalic DA neurons, but not those on melanotrophs and some hypothalamic DA neurons. RAC and HAL recognize D2 receptors on all of these cell types. (Supported by NIH grants NS 15911 and NS 07279)

323.21

COMBINED EFFECTS OF CONTINUAL D_2 AGONISM AND PULSATILE D_1 ANTAGONISM IN A PARKINSONIAN ANIMAL MODEL. J. Hubble, T. Basham, A.R. Dick, and W. Koller. University of Kansas Medical Center, Kansas City, KS 66103.

The antiparkinson effect of dopamine agonists appears chiefly dependent on D_2 receptor activation with D_1 stimulation playing a lesser role. The precise means by which "ideal" receptor co-activation can be achieved in parkinsonism remains undetermined. In the 6-hydroxydopamine lesioned animal model, we have previously shown that continuous s.c. D_2 agonist therapy [(+)-4-propyl-9-hydroxynaphoxazine (PHNO)] produces antiparkinson effects (contralateral turning) in rats thruout a 7-day pump period; turning is maximal by day 3, then plateaus or decreases coincidental with the appearance of stereotypic behaviors. To clarify the role of D_1 receptor activity in this behavioral paradigm, we administered pulsatile D_1 antagonist (SCH 23390) therapy in rats receiving continual s.c. PHNO for 14 days. Initially (days 2,6) no difference was observed, by days 10 & 13 SCH 23390 (10 ug/kg i.p.) produced significantly greater turns in the 72-min time interval following injection compared to PHNO-only controls (12.3±2.2 vs 5.9±2.0 turns/min, p<.01 ANOVA/Newman-Keuls posthoc test). Smaller SCH 23390 doses (0.03,0.3,3.0 ug/kg i.p.) did not enhance turning; rather, 0.3 ug/kg appeared to suppress turning but did not reach statistical significance. In conclusion, our results suggest that D_1 antagonists could potentially serve an adjunctive role in the pharmacological management of parkinsonism.

323.22

DIFFERENTIAL EFFECTS OF NEUROLEPTICS ON SLEEP-WAKING STATES IN THE RAT. M. Trampus, N. Ferri* and E. Ongini. Research Laboratories, Schering-Plough S.p.A., I-20060 Comazzo (Milan), Italy.

Sedation is a common side effect encountered during therapy with classic neuroleptic agents. We studied a series of neuroleptics, having different selectivity for dopamine D1 and D2 receptors, on the sleep-waking patterns in the rat. Haloperidol, a D2 antagonist which also interacts with other receptors, markedly increased the time spent in non-rapid eye movement (non-REM) sleep (mean effect = +81% of control). Chlorpromazine, a mixed D1/D2 antagonist, and the atypical neuroleptic clozapine, tended to increase non-REM slightly. The selective D2 blockers, raclopride and remoxipride, did not modify the duration of non-REM sleep. Regarding D1 antagonists, SCH 23390 enhanced non-REM markedly (+81%), whereas SCH 39166 had no effect on this stage. Neuroleptics are reported either to not affect or reduce duration of REM. Accordingly, in our studies, haloperidol and raclopride did not modify this stage, whereas clozapine, remoxipride and chlorpromazine tended to reduce it. Conversely, both SCH 23390 and SCH 39166 enhanced REM (+123 and +58%, respectively).

From the data, D2 selective neuroleptics appear to have little sedative potential, whereas D1 selectivity seems to play a role in the regulation of important sleep stages, such as REM and non-REM.

323.23

CHRONIC HALOPERIDOL TREATMENT CAUSES CHANGES IN CHOLECYSTOKININ AND TYROSINE HYDROXYLASE mRNA LEVELS IN RAT BRAIN. V. McKibbon*, D.J.S. Sirinathsinghji and J. Hughes.
Parke Davis Research Unit, Cambridge, England. CB2 2QB.

There is substantial evidence for the co-localization of cholecystokinin (CCK) within dopamine (DA) neurons in the ventral midbrain, suggesting a role for CCK in the modulation of forebrain (mesencephalic) DA neurotransmission. This report futher investigates this interaction at the molecular level through the study of gene expression. Chronic neuroleptic treatment has been shown to alter the levels of both synthetic enzymes such as tyrosine hydroxylase (TH), and the DA D2 receptor complex. Here, the effects of haloperidol (administered via osmotic minipumps at 2.4 mg/Kg for 3 and 7 days) on mRNA levels for TH, DA D2 isoforms and DA D1 receptors and CCK was examined by in situ hybridization using oligonucleotide probes. Areas of the CNS containing mRNA were visualised by autoradiography and measured using a Quantimet densitometer. The experiments showed increases in mRNA for TH (70% $p < 0.05$), at the level of the substantia nigra and increases in mRNA for the DA D2 receptor isoforms. In addition to these expected changes in the DA system, there was a significant decrease in CCK mRNA in the prefrontal cortex (30% $p < 0.05$). Although difficult to interpret, these changes indicate a potential interaction between components of the DA system and CCK at the level of mRNA.

CATECHOLAMINE RECEPTORS: DOPAMINE IV

324.1

QUANTITATIVE AUTORADIOGRAPHY OF IODINATED DOPAMINE LIGANDS: POTENTIAL PITFALLS IN THE USE OF IODINATED BENZAMIDES. I.A. Wortman*, D.C. Rice*, R.D. Burwell, C.P. Lawler, V.J. Watts*, C.A. Mathis*, P.Morell, M.H. Lewis, R.B. Mailman. University of North Carolina, Chapel Hill, NC 27599; Health and Welfare Canada, Ottawa K1A OL2; and Lawrence Berkeley Laboratory, University of California, Berkeley CA 94720.

[^{125}I]epidepride (EPI), a benzamide with high affinity and selectivity for the D_2-type of dopamine receptor, has been suggested as being especially suited for quantitative autoradiography. Consistent with recent reports, saturation analysis of [^{125}I]EPI indicated that 50 mM NaCl decreased the K_D (to 38 pM), and increased the B_{max} (to 220 fmol/mg protein). This large effect of sodium, coupled with some difference between the K_D of [^{125}I]EPI vs. the K_i of unlabeled epidepride in competing for [^{3}H]spiperone binding sites, suggested the need to characterize carefully the binding of [^{125}I]EPI in quantitative autoradiographic assays. Thus, the association of three ^{125}I labelled dopaminergic ligands was compared as a function of time and temperature in 15 μm sections of rat striatal tissue using three radioligands: the D_2 ligands [^{125}I]EPI and [^{125}I]iodosulpiride (IS); and the D_1 ligand [^{125}I]SCH23982 (SCH). Autoradiographic studies were done 4°, 25°, and 37°C, and time-sampled at 0.25, 0.5, 1, 2, 9, and 18 h. At 22°, the binding of [^{125}I]SCH reached equilibrium at 2 h, remained stable until 9 h, and then rapidly declined by 18 h. At 4°, the binding of SCH did not reach equilibrium until 18 h. At 37°, SCH binding was maximal at 0.5 h, but reached a level only ca. 50% of that measured at the lower temperatures; binding then declined slowly through 18 h. The behavior of the D_2 ligands was particularly interesting. At 4° or 22°, the binding of [^{125}I]EPI increased throughout the time course of this experiment (18 h). There was ca. a 35% increase between 9 and 18 h. At 37°, binding was maximal at 2 h (but lower than at 4° or 22°), and decreased significantly with time. A similar pattern was seen with [^{125}I]IS, although maximum binding at 22° was ca. 50% on the 4° maximum. These data indicate that the incubation times commonly used with these ligands may not reflect equilibrium, and that time-temperature conditions may differ markedly from other radioligands. Thus, while [^{125}I]EPI may be a radioligand of potential utility, its binding must be carefully characterized prior to use in quantitative autoradiography.

324.2

REGIONAL LIGHT AND ELECTRON MICROSCOPIC LOCALIZATION OF IMMUNOREACTIVITY FOR DOPAMINE D2 RECEPTOR PEPTIDE IN THE RAT BRAIN. S.R. Sesack, C.J. Aoki, and V.M. Pickel. Dept. Neurology & Neuroscience, Cornell Univ. Med. Coll. New York, NY 10021.

We examined the light (LM) and electron microscopic (EM) localization of immunoreactivity for a rat polyclonal antiserum raised against amino acids 273-287 of the dopamine D2 receptor protein. By immunoblot analysis, this antiserum was specific for the parent peptide and did not recognize fragments from adjacent positions on the D2 or β-adrenergic receptors. In acrolein fixed rat brain sections, specific peroxidase labeling for D2 peptide-like immunoreactivity (D2-LI) was detected upon incubation with immune serum, but not with preimmune serum or with immune serum preadsorbed with 100 µg/mL of D2 peptide. By LM, D2-LI was localized (1) to cell bodies and thick processes in regions of dopaminergic perikarya (the substantia nigra and ventral tegmental area), and (2) to diffuse punctate processes in dopaminergic target regions (the striatum and prefrontal cortex). These immunoreactive elements were identified by EM as, respectively (1) perikarya and dendrites, and (2) spines, dendrites and terminals. Within dendrites, D2-LI was most densely associated with the smooth endoplasmic reticulum, mitochondrial and plasma membranes and postsynaptic densities. Within terminals, labeling was additionally associated with some, but not all vesicles. Terminals containing D2-LI formed primarily symmetric junctions or failed to exhibit synapses in single sections. The distribution of D2-LI by LM and EM (1) suggests that the peptide antiserum is specific for dopamine D2 receptors and (2) indicates a number of cellular sites for dopamine action within midbrain and forebrain circuitry. (Support: NS08193, EY08055, MH40342 and NARSAD).

324.3

NOVEL F-18 LABELED ANALOGUES OF BENPERIDOL FOR PET STUDY OF DOPAMINERGIC D-2 RECEPTOR BINDING IN VIVO. S.M. Moerlein, J.S. Perlmutter and D. Parkinson. Mallinckrodt Institute of Radiology, Department of Neurology and Neurological Surgery, and Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110

Benperidol is a butyrophenone ligand that binds to dopaminergic D-2 receptors with high affinity and high selectivity. With the goal of developing an F-18 ($t_{1/2}$ = 110 min) labeled tracer with improved selectivity for binding to D-2 receptors in-vivo, we have synthesized novel radiofluorinated analogues of benperidol for evaluation as PET tracers. In particular, synthetic pathways to F-18 labeled benperidol ([F-18]B), N-methyl benperidol ([F-18]MB), N-fluoroethyl benperidol ([F-18]FEB) were developed. [F-18]B and [F-18]MB have the radiolabel attached to an aromatic site, whereas [F-18]FEB has the radiolabel attached at an alkyl location. Differences in the metabolic behavior of these tracers is thus anticipated. In-vitro binding assays using primate cerebral tissues indicate that these ligands have high affinity for binding to D-2 receptors (K_i = 0.3, 3.6, and 5.2 nM for B, MB, and FEB, respectively) and relatively poor affinity for S-2 receptors (K_i = 33, 89, and 31 nM for B, MB and FEB, respectively). No-carrier-added [F-18]B and [F-18]MB were prepared using a three-step procedure in 5-7% radiochemical yield and specific activity >1000 Ci/mmol within an overall preparation time (including HPLC purification) of 100 min. [F-18]FEB was produced at the no-carrier-added level via a two-step, one-pot synthetic sequence. Radiochemical yields of 25-30% and specific activities >1000 Ci/mmol were accomplished within an overall preparation time of 110 min. The results of in-vivo experiments with these promising D-2 receptor binding PET tracers will be presented.

324.4

F-18 LABELED BENZAMIDE ANALOGS AS POTENTIAL LIGANDS FOR STUDYING THE DOPAMINE D2 RECEPTOR IN VIVO WITH POSITRON EMISSION TOMOGRAPHY (PET). RH Mach,* JG Scripko,* RL Ehrenkaufer,* PA Nowak,* RR Luedtke, PB Molinoff, and M Reivich. Univ. of Pennsylvania, Philadelphia, PA.

Two fluorine-containing benzamide analogs were prepared as potential candidates for studying dopamine D2 receptors in vivo with PET. In vitro binding experiments indicate that both compounds, 2,3-dimethoxy-N-(p-fluorobenzyl)piperidin-4-yl benzamide (MBP) and 2,3-dimethoxy-N-(9-(p-fluorobenzyl)-9-azabicyclo[3.3.1]nonan-3ß-yl benzamide (MABN), possess a sub-nanomolar affinity for D2 receptors (Ki for inhibition of [I-125]NCQ 298 binding to rat striatal tissue = 0.4 nM for MBP and 0.03 nM for MABN). In vivo studies in rats indicate that both [F-18]MBP and [F-18]MABN have a high brain uptake and a regional distribution consistent with that of D2 receptors. The striatum:cerebellum ratio for [F-18]MBP and [F-18]MABN, 180 min post-iv-injection, was 20 and 45, respectively. The striatal uptake of both analogs was blocked by co-injection of spiperone, a potent D2 antagonist. These data indicate that [F-18]MBP and [F-18]MABN are potential candidates for studies of dopamine D2 receptors in vivo with PET.

This research was supported by USPHS Grants NS14867, MH43880, NS18591 and GM34781.

324.5

D_3 DOPAMINE RECEPTOR LOCALIZATION USING ANTI-PEPTIDE ANTISERA: COMPARISON TO OTHER CNS DOPAMINE RECEPTORS. M.A. Ariano, E. M.Smyk-Randall[*1], and D.R. Sibley[1]. Anatomy and Neurobiology, UVM College of Medicine, Burlington, VT 05405; and [1] Molecular Pharmacology Unit, ETB, NINDS, Bethesda, MD 20892.

We have developed antisera against synthetic peptides corresponding to sequences for the rat D_1, D_2, and D_3 dopamine receptor subtypes. These antisera are directed against both extracellular and intracellular portions of the receptor proteins, and were conjugated to thyroglobulin prior to immunization in rabbits. Their titers and specificities were determined by ELISA analysis of the sera and immunofluorescent detection of the recombinant receptor proteins expressed in stably transfected Chinese Hamster Ovary (CHO) cells.

Medium sized **striatal** cells were immunofluorescent for all three receptor subtypes, with $D_1 > D_2 > D_3$ staining. D_1 receptor subtype expression was also robust within the neuropil. Staining in the **olfactory tubercle** was most obvious for the D_3 receptor antisera, and showed an intense reaction at the cell membrane and initial processes in these neurons. The **hippocampus** exhibited D_3 and D_2 membrane staining in pyramidal neurons, while D_1 expression was less abundant. The **substantia nigra** stained for all three receptor proteins; D_3 and D_2 were especially robust in compacta neurons. The regional distribution of the receptor subtypes using these antibody reagents suggests that overlap of the different dopamine receptor subtypes occurs in the CNS areas examined. Further analyses will determine the potential cellular coincidence of the receptor subtypes.

This work was supported in part by USPHS NS 23079 to MAA.

324.6

D2 DOPAMINE RECEPTOR LOCALIZATION USING ANTIPEPTIDE ANTISERUM: MORPHOLOGICAL DIVERSITY OF RECEPTOR-BEARING NEURONS IN RODENT NEOSTRIATUM DEMONSTRATED BY COMBINED IMMUNOHISTOCHEMISTRY/GOLGI-GOLD TONING. R.S. Fisher, D. Birt, ●M.A. Ariano, ●●D.R. Sibley and M.S. Levine. MRRC & Brain Res. Inst., UCLA, Los Angeles, CA 90024, ●Dept. Anat. & Neurobiol., UVM Coll. Med., Burlington, VT 05405, ●●Mol. Pharmacol. Unit, ETB, NINDS, Bethesda, MD 20892.

Receptor-binding and immunohistochemical studies suggest that the D2 dopamine receptor is located in the targets of dopamine innervation including medium and large neurons in rodent neostriatum as well as the dopamine neurons of the substantia nigra-compact zone ("autoreceptors"). Others have implied that medium spiny neurons are the principal targets of dopamine inputs to neostriatum. We tested the hypothesis of preferential dopamine receptor localization in the medium spiny neostriatal targets of nigrostriatal dopamine inputs. Morphological methods were combined to label D2 dopamine receptor in the perikaryal cytoplasm of neurons (polyclonal antiserum directed against a 54-peptide sequence of D2 receptor, ABC immunohistochemistry) and somatodendritic parts of neurons (single-slice Golgi impregnation/gold-toning for translucent labeling) in the same aldehyde-fixed Vibratome sections of rodent neostriatum.

We observed specific and consistent double-labeling of three types of D2 receptor-bearing neurons distributed throughout the neostriatum. 1)Medium spiny neurons were frequent (>95% of doubly labeled cells, somatic diameters = 12-25μm). Some of these cells also had somatic and proximal dendrite spines. 2)Medium sparsely spiny neurons were infrequent (2-3% of doubly labeled cells, somatic diameters = 12-22μm). 3)Large sparsely spiny or aspiny neurons were infrequent (1-3% of doubly labeled cells, somatic diameters = 30-50μm. We conclude that diverse morphologic types of neostriatal neurons contain the D2 dopamine receptor. Thus, all major types of neostriatal neurons have the potential to be modulated via the D2 dopamine receptor. Supported by USPHS HD05958.

324.7

LOCALIZATION OF ^{3}H-QUINPIROLE BINDING TO D-2 AND D-3 RECEPTORS IN RAT BRAIN. D.R. Gehlert, S.L. Gackenheimer, P. Seeman and J.M. Schaus. CNS Pharmacology, Lilly Research Laboratories, Indianapolis, IN 46285 and Departments of Pharmacology and Psychiatry, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Until recently, receptors for dopamine were divided into biochemical subtypes based on their ability to modulate the activity of adenylate cyclase. Using molecular biological techniques, Sokoloff and coworkers (Nature 347: 146, 1990) have reported that a third, genetically distinct subtype of dopamine receptor gene exists with a unique distribution of mRNA in the brain. While mRNA encoding for D-1 and D-2 can be found in high density in the basal ganglia, D-3 mRNA was found principally in the islets of Calleja, nucleus accumbens and olfactory tubercle. When the gene was expressed in COS cells, the dopaminergic agonist, quinpirole, was found to be highly selective for D-3 . In order to detect the D-3 receptor in the brain, we have synthesized a radiolabelled form of quinpirole for use in autoradiographic studies.

^{3}H-quinpirole was synthesized by tritium gas hydrogenation of trans-(-)-(4aR, 8aR)-4, 4a, 5, 6, 7, 8, 8a, 9-octahydro-5-allyl-1H-pyrazolo[3,4-g]quinoline hydrochloride [LY 275947] (Amersham and DuPont-NEN). Sections of rat forebrain were labelled with ^{3}H-quinpirole using standard techniques. After a 60 day exposure to tritium sensitive film, the binding to sections was evaluated using quantitative image analysis. The binding of ^{3}H-quinpirole to sections of rat forebrain had a K_d of 9.5 nM and a B_{max} of 230 fmoles/mg tissue dry weight. At a concentration of 10 nM, the autoradiographic distribution of ^{3}H-quinpirole binding sites was similar to that reported for other D-2 selective ligands being highly localized to the caudate-putamen, nucleus accumbens and olfactory tubercle. The inclusion of 10 μM Gpp(NH)p in the incubation media markedly reduced binding to most regions of the brain; however, binding could be detected in the islets of Calleja and, to a much lesser extent, the nucleus accumbens.

These data indicate that ^{3}H-quinpirole binds to the high affinity agonist conformation of the D-2 receptor as well as the D-3 receptor in rat brain.

324.8

DEVELOPMENT OF ANTI-RAT D2 DOPAMINE RECEPTOR ANTIBODIES USING SEQUENCE-SPECIFIC PEPTIDES. V. A. Boundy, R. R. Luedtke and P. B. Molinoff. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Peptides corresponding to portions of the third intracellular loop of the rat D2 dopamine receptor were synthesized and used to produce polyclonal anti-peptide antisera able to recognize the two D2 receptor isoforms. Peptides D2-244 (aa 244-270, unique to the $D2_L$ isoform) and D2-284 (aa 284-311, common to both the $D2_L$ and the $D2_S$ isoforms) were covalently coupled to keyhole limpet hemocyanin (KLH). Rabbits were immunized monthly with peptide-KLH conjugates. A solid phase RIA was used to verify the immunogenicity of the peptides. Both immunogens produced antisera that were effective at dilutions ranging from $1:10^4$ to $1:10^5$ in a solid phase RIA within 10 weeks of the initial immunization. Specificity of the antisera was demonstrated by lack of reactivity of preimmune antisera and by the absence of cross-reactivity to the other (non-immunization) peptide. An immunoprecipitation assay was used to determine if the antipeptide antisera could recognize solubilized D2 receptors. Rat and canine caudate tissue was solubilized using 1% digitonin, and solubilized preparations were enriched chromatographically using a heparin-agarose column. Solubilized receptors were incubated with anti-peptide antisera and ^{125}I-NCQ298, a high-affinity antagonist selective for D2 receptors. The antibody/receptor/^{125}I-NCQ298 complex was precipitated with Pansorbin. The anti-(D2-284) antisera were found to quantitatively precipitate ^{125}I-NCQ298 binding sites from both rat and canine solubilized receptor preparations while the antisera against peptide D2-244 were found to immunoprecipitate the D2 receptor solubilized from rat but not from canine caudate. (USPHS GM 34781 and NS 18591)

324.9

PRODUCTION OF D2 RECEPTOR-UBIQUITIN FUSION PROTEINS FOR THE DEVELOPMENT OF POLYCLONAL ANTI-RECEPTOR ANTIBODIES. R. R. Luedtke, V. A. Boundy and P. B. Molinoff. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The sequence encoding the third intracellular loop (i3) of each of the isoforms of the D2 dopamine receptor ($D2_L$ and $D2_S$) was subcloned into the Eco RI site of the pNMHUBpoly (pNM) vector. The plasmid pNM is an expression vector containing the heat-inducible λP_L promoter and the ubiquitin gene. Following induction, this vector will overexpress fusion proteins consisting of ubiquitin and the peptide corresponding to the introduced cDNA. The pNM-i3$_L$ and pNM-i3$_S$ ligation products were used to transform C1+ *E. coli*, and colonies with DNA inserts in the appropriate orientation were identified by restriction mapping. AR58 *E. coli* were transformed with the pNM-i3 clones. In AR58 *E. coli* transformed with the nonrecombinant plasmid, a 31.5 kDa protein was expressed. Proteins with molecular weights of 25.6 kDa and 22.3 kDa were expressed after transformation with pNM-i3$_L$ and pNM-i3$_S$, respectively. Cell lysates were electrophoresed on preparative SDS-PAGE, and proteins were visualized with $CuCl_2$. Bands were excised and used for the monthly immunization of rabbits. A solid phase RIA using synthetic peptides corresponding to portions of the i3 loop was used to monitor the production of antibodies. Both fusion protein immunogens produced antisera which were reactive in the solid phase RIA at a dilution of $1:10^4$. An immunoprecipitation assay was used to determine if the antisera could recognize solubilized D2 receptors. Digitonin-solubilized extracts of rat caudate were incubated with anti-ubiquitin-i3 antisera and ^{125}I-NCQ298, a high-affinity antagonist selective for D2 receptors. The antibody/receptor/^{125}I-NCQ298 complex was precipitated with Pansorbin. Both anti-ubiquitin-i3 antisera were shown to precipitate ^{125}I-NCQ298 binding sites from preparations of receptor solubilized from rat caudate. (USPHS GM 34781 and NS 18591)

324.10

PHARMACOLOGICAL CHARACTERIZATION OF "D_3" DOPAMINE RECEPTORS IN LIMBIC AND NON-LIMBIC AREAS OF THE RAT BRAIN BY QUANTITATIVE AUTORADIOGRAPHY. M.L. Coco and C.D. Kilts. Depts. of Psychiatry and Pharmacology, Duke Univ. Med. Ctr. Durham, NC 27710.

The recently cloned D_3 receptor has been shown to bind D_2 receptor ligands when expressed in CHO or COS-7 cells (Sokoloff et al., 1990), with particular compounds demonstrating preferential affinity for either the D_3 or D_2 site. In situ hybridization of D_3 receptor mRNA suggests a primarily limbic system localization in the rat, whereas D_1 and D_2 receptors are more widely expressed. We sought to examine the D_3-like properties of receptors located in limbic and non-limbic areas of the rat brain by using quinpirole (a D_2 agonist with a reported 113-fold greater affinity for D_3) and domperidone (a D_2 antagonist with a reported 32-fold greater affinity for D_2) to displace [^{125}I]iodosulpiride binding in 10 μm thick coronal brain sections. Initial results fail to demonstrate D_3-like properties of limbic-localized receptors compared to non-limbic [^{125}I]iodosulpiride-binding receptors when characterized by these pharmacological means. The K_i's obtained from autoradiographs of either quinpirole- or domperidone-generated competition curves did not significantly differ between various divisions of the caudate-putamen, nucleus accumbens, olfactory tubercle or central or intercalated nuclei of the amygdala. Quinpirole showed an approximate 7-fold greater affinity for [^{125}I]iodosulpiride binding sites in the lateral septal nucleus or bed nucleus of the stria terminalis compared to dorsal caudate.

324.11

DOPAMINE RE-UPTAKE SITES IN HUMAN BRAIN: AUTORADIOGRAPHY WITH ^{3}H-CFT. M.B. Knable, M.F. Casanova, J.E. Kleinman, and D.R. Weinberger. Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.

The distribution of dopamine re-uptake sites in the human brain was examined using full hemisphere autoradiography with the ^{3}H-CFT ligand. Brains from four patients without brain pathology or neuropsychiatric history were studied. Tissue sections were incubated with 3uM ^{3}H-CFT. Nonspecific binding was determined using 30uM cocaine as a blocker. Computerized image densitometry was used to measure ^{3}H-CFT binding sites. Specific binding was observed in the caudate, putamen, anterior cingulate gyrus and entorhinal cortex. A regional gradient of dopamine re-uptake sites was found in the putamen with the highest concentration in its ventral portion. Detectable, but lower levels of binding were observed in the amygdala and dorsomedial thalamus. Binding was specific since it exhibited saturability or a cortical laminar preference. The mapping of ^{3}H-CFT sites in limbic areas provides a basis for the study of illnesses with postulated abnormalities of dopamine re-uptake.

324.12

Regulation of Binding to the Dopamine Uptake Complex by Metal Cations. E.K. Richfield. Departments of Neurology and Pharmacology, University of Rochester, Rochester, NY 14620

The dopamine uptake complex (DAUC) is responsible for the reuptake of dopamine (DA) after release by DAergic terminals. Cocaine binding to the DAUC is responsible for many behavioral effects of this drug. A quantitative autoradiographic assay using [^{3}H]GBR 12935 was used to study the effects of metal cations on the DAUC in rat and human brain.

Metal cations were found to have variable effects on binding to the DAUC. Cations known to be highly reactive to sulfhydryl groups (Hg^{2+}, Cu^{2+}, and Cd^{2+}) resulted in dramatic loss of binding of [^{3}H]GBR 12935 to the DAUC. These effects could be partially reversed by the use of the reducing agent dithiothreitol. Three metal cations (Mn^{2+}, Mg^{2+}, and Ca^{2+}) had no effect on the binding of [^{3}H]GBR 12935. Three transitional metal cations (Ni^{2+}, Zn^{2+}, and Co^{2+}) resulted in marked increases in binding to the DAUC. Of these 3 ions, zinc had the highest affinity for the DAUC and produced the greatest increase in binding.

The increased binding of [^{3}H]GBR 12935 to the DAUC was due to an increase in the affinity of the compound (K_d) and not to an increase in the density (B_{max}). Cocaine and WIN 35,428 both compete for binding at the DAUC with two affinities. Zinc increased the high affinity (K_H) for both compounds and increased the proportion of sites present in the high affinity state (R_H).

These findings suggest a sulfhydryl group may be important for binding of certain drugs at the DAUC. Zinc plays an important role in regulating binding to the DAUC. These findings will be important in designing drugs active at the DAUC.

CATECHOLAMINES: DOPAMINE I

325.1

EFFECTS OF APOMORPHINE ON REGIONAL GLUCOSE METABOLISM IN NORMAL AND 6-OH DOPAMINE LESIONED ANIMALS. C.A.Ray*, S.Koch*, N.F.Nichols, and M.F.Piercey, The Upjohn Co., Kalamazoo, MI 49001.

Dopaminergic (DA) neurons in the Substantia Nigra Pars Compacta (SNPC) and adjacent lateral Ventral Tegmental Area (VTA) were lesioned unilaterally with 6-OH DA injections. Animals which turned following 1 mg/kg apomorphine (APO) 1 wk after 6-OHDA injections were considered successfully lesioned. Lesioned animals had reduced glucose metabolism in the anterior caudate (Cd) and several regions of the anterior cerebral cortex. With the exception of some cortical regions, ipsilateral and contralateral sides of lesioned animals were not significantly different, indicating predominantly bilateral effects. In unlesioned animals, APO (1 mg/kg i.v.) stimulated metabolism most significantly in the anterior VTA and Substantia Nigra Pars Reticulata (SNPR) but also stimulated the Globus Pallidus (GP) Tuberculum Olfactorium (Tub Olf), and the lateral Cd. In lesioned animals, APO reversed most lesion effects and stimulated lateral Cd as well as both posterior and anterior VTA and SNPR, with the ipsilateral posterior SNPR being significantly more stimulated than observed either on the contralateral side or in control animals. It is concluded that unilateral DA lesions have bilateral effects and that turning may be due to supersensitive DA receptors in striatonigral pathways.

325.2

AUGMENTED DOPAMINE RECEPTOR SENSITIVITY IN LATERAL, BUT NOT MEDIAL, CAUDATE FOLLOWING NEONATAL 6-HYDROXYDOPAMINE LESIONS. H.E. Criswell, P.E. Simson, and G.R. Breese. Department of Psychiatry, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC 27599.

Extracellular single unit recording and microiontophoretic techniques were employed to determine the sensitivity of D1- and D2-dopamine (DA) receptors throughout the caudate putamen (CPu) of adult rats that had received 6-hydroxydopamine lesions as neonates or as adults. Only lesioned rats displaying behavioral supersensitivity to D1- and D2-dopamine agonists, as defined by increases in locomotion and other behaviors in response to peripheral administration of D1- and D2-dopamine agonists, were included in the present study. Electrophysiological testing revealed regionally specific increases within the CPu in sensitivity to microiontophoretically applied D1- and D2-agonists in rats lesioned as neonates, but not adults. In particular, current-response curves demonstrated an augmented inhibitory response, in neonatally-lesioned rats as compared to adult-lesioned and control rats, to both the selective D1 agonist SKF-38393 and the selective D2 agonist quinpirole in lateral, but not medial, portions of the CPu. At least 60% of neurons in both lateral and medial portions of CPu responded to both D1- and D2-agonists across all three groups. This is consistent with co-localization of D_1- and D_2-receptors. However, despite this extensive D1/D2-receptor co-responsiveness, there was no evidence for a purported supra-additive D1/D2 interaction. Supported by HD23042; NS21345.

325.3

COMPARISON OF DOPAMINE RECEPTOR LIGAND BINDING AND mRNA EXPRESSION IN TWO DISTINCT MODELS OF 6-OHDA-INDUCED CENTRAL DENERVATION . C.P. Lawler, J. H. Gilmore, A.M. Eaton, S.B. Southerland, M.H. Lewis and R.B. Mailman. University of North Carolina, Chapel Hill, N.C. 27599.

The prototypical rodent model used to study denervation responses of central dopamine systems is the 6-OHDA-induced unilateral lesion of the substantia nigra. This lesion causes a behavioral supersensitivity to direct agonists (turning) accompanied by an increase in D_2 receptor density on the lesioned side. We have recently shown [Mileson et al., Brain Research (in press)] that either bilateral or intracisternal 6-OHDA lesions cause supersensitive behavioral responses without causing changes in either D_1 or D_2 receptor density or affinity. The present studies used molecular biological techniques to characterize further these important differences in dopamine receptor adaptation produced by two distinct models of dopamine denervation. Rats were lesioned by unilateral injection of 6-OHDA (8 μg) into the right substantia nigra or by two successive injections of 6-OHDA (200 μl) into the cisterna magna. Rats were killed ca. 2-3 weeks post lesion. HPLC-EC analysis of tissue samples from both groups of lesioned rats confirmed that dopamine was depleted by greater than 90% in the lesioned striatum, compared to the appropriate sham-lesioned controls. *In situ* hybridization was performed on 15 μm coronal brain slices at a mid-caudate level, using a mixture of ^{35}S-labeled oligoprobes directed at unique regions of either D_1 or D_2 mRNA. Adjacent sections were used for receptor autoradiographic assays of D_1 and D_2 receptors, labeled with [^{125}I] SCH23982 and [^{125}I] iodosulpiride, respectively. Consistent with earlier findings from this laboratory, D_2 receptor density was increased significantly on the lesioned side in the unilateral subjects, while D_1 receptor density was unchanged. Neither D_1 nor D_2 receptor binding was altered in the intracisternally lesioned rats. Initial results from densitometric analysis of D_1 *in situ* labeling in dorsal lateral caudate nucleus indicated that the overall D_1 mRNA levels did not change in either unilaterally or intracisternally lesioned rats. A frequency distribution analysis of neurons expressing D_1 message indicated, however, a trend toward lesion-induced differences in D_1 message expression in subpopulations of labeled neurons for both groups of subjects, suggesting a possible heterogeneity of neural response to dopamine denervation.

325.4

SPECIFIC DOPAMINERGIC NEUROTOXICITY OF MPP$^+$ AND ANALOGS IN CULTURED MESENCEPHALON: ROLE OF THE DOPAMINE UPTAKE SYSTEM AND INHIBITION OF MITOCHONDRIAL RESPIRATION. M.S. Saporito, H.M. Geller, S.K. Youngster, W.J. Nicklas, A.N. Basma and R.E. Heikkila. UMDNJ-Robert Wood Johnson Medical School, Piscataway, N.J. 08854.

Analogs of MPP$^+$ were synthesized and evaluated for their neurotoxic potential in cultured neurons from mouse mesencephalon. These same compounds were evaluated for their affinity for the dopamine (DA) uptake system and ability to inhibit complex I of the electron transport chain from isolated mitochondria. Simultaneous [^{3}H]-DA and [^{14}C]-GABA uptake, as well as immunocytochemical staining for tyrosine hydroxylase and GABA, were used to quantify DA and GABA neurons. MPP$^+$, which possessed a high affinity for DA uptake system and is an inhibitor of complex I respiration, caused a time and concentration dependent loss of DA neurons with an LD_{50} of less then 1 μM. MPP$^+$ did not affect GABAergic neurons up to a concentration of 30 μM. Analogs of MPP$^+$ which had high affinity for the DA transporter and were similar to MPP$^+$ in inhibiting mitochondrial respiration also caused a selective DA neurotoxicity. The pyrimidinium analog of MPP$^+$ which had a high affinity for the DA uptake system, but did not inhibit mitochondrial respiration, was not neurotoxic up to a concentration of 100 μM. The 4'alkylated analogs of MPP$^+$, which were poor substrates for DA uptake system but potent inhibitors of mitochondrial respiration, caused neurotoxicity to both DA and GABA neurons. This study describes the neurotoxicity of a number of analogs of MPP$^+$ and highlights the importance of the DA uptake system and the ability of MPP$^+$ to inhibit mitochondrial respiration as critical processes in conferring selectivity and neurotoxicity, respectively, to MPP$^+$ and analogs for DA neurons in culture.

325.5

THE EFFECTS OF NEUROSTEROIDS ON THE STRESS-INDUCED ACTIVATION OF DOPAMINE SYSTEMS IN THE PREFRONTAL CORTEX. A. Chistina Grobin, Robert H. Roth, and Ariel Y. Deutch. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Neurosteroids can be formed endogenously in the brain, independent of adrenal release. These include allopregnanalone and the A-ring metabolite of deoxycorticosterone, 3α,5α-tetrahydrodeoxycorticosterone (THDOC). THDOC is a potent positive modulator of the $GABA_A$ receptor. This is consistent with behavioral investigations which suggest that neurosteroids have anxiolytic properties. Moreover, brain and plasma levels of THDOC increase after swim stress. We have therefore examined the effects of intraventricular administration of THDOC on the stress-induced activation of the dopamine (DA) innervation of the prefrontal cortex (PFC). Animals with chronic indwelling cannulae in the lateral ventricle received THDOC (0.5-25.0 μg) or vehicle, and were sacrificed 30 min later. THDOC selectively reduced DA metabolism in the PFC in a dose-dependent fashion; other mesotelencephalic DA regions were not affected. Stress increased DA metabolism in the PFC; this effect was partially reversed by 25 μg THDOC. These data indicate that a neurosteroid selectively alters dopaminergic function in the PFC, and are consistent with the suggestion that THDOC is an anxiolytic agent. Supported by MH-45124, MH-14092, GM-07324, and the National Parkinson Foundation Center at Yale University.

325.6

ONSET OF DOPAMINERGIC INNERVATION OF THE RAT PITUITARY INTERMEDIATE LOBE DECREASES MITOTIC RATE IN DEVELOPING MELANOTROPES. K.A. Gary and B.M. Chronwall, School of Basic Life Sciences, University of Missouri-Kansas City, Kansas City, MO 64108.

The intermediate lobe (IL) of the rat pituitary is innervated by axons of dopaminergic neurons located in the mediobasal hypothalamus. Immunohistochemical studies indicate that both tyrosine hydroxylase (TH) and dopamine (DA) are first detected on post-natal (PN) day 3 during development. *In vitro* and *in vivo* studies utilizing D_2 receptor agonists and antagonists have established that dopamine receptor activation decreases the mitotic rate of melanotropes in adult rat IL. In this study, we have examined the effects of DA innervation on melanotrope mitotic rate during ontogeny. Rat pups were injected at PN 2-14, with [^{3}H]thymidine and sacrificed two hours later. Pituitaries were harvested, osmicated, dehydrated, embedded in plastic, sectioned, and processed for autoradiography. Analysis of labeled melanotropes indicates mitotic rate decreases 48% at PN 3, concomitant with the appearance of DA-immunoreactive (IR) axon terminals. To correlate DA expression and post-synaptic action with decreased mitotic rate, rat pups of ages indicated above were injected intracisternally with 200 mg 6-OHDA in 25 ul normal saline with 0.01% ascorbic acid. TH-IR was decreased within the IL and neural lobe of all injected pups at each time point. DA levels were assayed by reverse phase HPLC with electrochemical detection (detection limit 100 fg). DA levels were decreased to 48% of controls at PN 3, and to 20% at later stages. 6-OHDA blocked the reduction of mitotic rate normally occurring on PN 3; the mitotic rate remained similar to that observed in normal pups prior to onset of innervation. Thus, we have shown that the onset of DA expression occurring with innervation directly decreases mitotic rate of the melanotropes.

325.7

EVIDENCE THAT $5HT_2$ RECEPTORS MEDIATE THE INHIBITORY EFFECTS OF STRESS ON THE ACTIVITY OF TUBEROINFUNDIBULAR AND TUBEROHYPOPHYSIAL DOPAMINERGIC NEURONS IN FEMALE RATS. J.L.Goudreau, J.Manzanares, K.J.Lookingland and K.E.Moore, Dept of Pharmacology and Toxicology, Michigan State University, East Lansing, MI, 48824

The purpose of the present study was to examine the role of 5-hydroxytryptamine (5HT) neurons and their receptor subtypes in mediating the inhibitory effects of stress on the activity of tuberoinfundibular (TIDA) and tuberohypophysial dopaminergic (THDA) neurons in female rats. TIDA and THDA neuronal activity was estimated by measuring the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the median eminence and intermediate lobe of the pituitary. Brief exposure to diethylether (2 min) followed by 30 min of supine restraint decreased the activity of TIDA and THDA neurons, and this effect was blocked by either neurotoxin-induced lesions of 5HT neurons (5,7-dihydroxytryptamine; 200 μg/rat; i.c.v.; 7 days) or inhibition of 5HT neuronal activity following administration of the $5HT_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT; 0.3 mg/kg; s.c.; 30 min). Taken together, these results indicate that 5HT neurons mediate the inhibitory effects of stress on TIDA and THDA neurons. Pretreatment of rats with the $5HT_2$ receptor antagonist MDL-11,939 (1.0 mg/kg; i.p.; 30 min) blocked the inhibitory effects of stress on the activity of TIDA and THDA neurons, whereas the $5HT_3$ receptor antagonist ondansetron (0.1 mg/kg; s.c.; 30 min) was without effect. These results indicate that the stress-induced decrease in the activity of TIDA and THDA neurons in female rats is mediated by postsynaptic $5HT_2$ receptors. (Supported by NIH grant NS 15911)

325.8

EFFECT OF ACUTE MORPHINE TREATMENT ON INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS: THE ROLE OF 5-HYDROXYTRYPTAMINERGIC NEURONS Y. Tian, M.J. Eaton, K.J. Lookingland and K.E. Moore. Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824

The purpose of the present study was to characterize the acute effect of morphine on incertohypothalamic dopaminergic (IHDA) neurons. DA neuronal activity was estimated by measuring concentrations of the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in medial zona incerta (MZI) and dorsomedial hypothalamic nucleus (DMN) which contain cell bodies and terminals of IHDA neurons. Morphine increased DOPAC concentration in MZI and DMN in a dose- and time-related fashion. No difference was observed in either basal activity or in response to acute morphine treatment of IHDA neurons between male and female rats. The stimulatory effect of morphine on IHDA neurons was blocked by pretreatment of animals with naltrexone. Morphine also increased 5-hydroxyindoleacetic acid (5HIAA) concentrations in MZI and DMN, which was blocked by pretreatment with naltrexone, indicating a stimulatory effect of morphine on 5-hydroxytryptamine (5HT) neurons. These data suggest that 5HT neurons may mediate the stimulatory effect of morphine on IHDA neurons. One week after an i.c.v. injection of the 5HT neurotoxin, 5,7-dihydroxytryptamine, 5HT concentrations in the MZI and DMN were undetectable. In these 5HT-depleted animals morphine still increased DOPAC concentration in the MZI and DMN. These results indicate that morphine stimulates IHDA neurons, but this stimulatory effect is not mediated through 5HT neurons. (Supported by NIH grant NS 15911.)

325.9

CHRONIC MORPHINE INCREASES SYNAPTIC DOPAMINE IN THE NUCLEUS ACCUMBENS AND NALOXONE DECREASES IT UNLESS WITHDRAWAL IS BLOCKED WITH CLONIDINE. E. Pothos, P. Rada*, G.P. Mark & B.G. Hoebel.Dept. Psych.,Princeton U.,Princeton,NJ 08544

Microdialysis was used to measure extracellular DA in the NAC of freely moving rats before and after morphine dependence, as well as after naloxone-precipitated withdrawal with and without clonidine therapy. All subjects were given daily injections of morphine for 7 days and naloxone on day 8 (20 mg/kg i.p.). After naloxone administration, all subjects showed symptoms of opiate withdrawal (wet dog shakes, diarrhea, teeth-chattering). On day 1, morphine resulted in an average 50% increase in DA for 3 hours after injection (n=8, p<.05). On day 7, basal DA was still normal and morphine increased DA again by an average 50% in the contralateral NAC (p<.05). On day 8, naloxone decreased DA by 40% 1.5 hr. after injection (p<.05). By comparison, naloxone did not alter DA levels in non-dependent animals. Pretreatment of dependent subjects with clonidine (200 ug/kg i.p.) blocked the naloxone-induced DA depression and attenuated the above withdrawal symptoms. These results demonstrate (a) similar increases in NAC DA before and after morphine dependence, (b) precipitated reduction in DA during withdrawal, and (c) blockade of the DA withdrawal effect by a noradrenergic agonist. Consequently, NAC DA may play a role in morphine withdrawal and therefore addiction.
Supported by USPHS grant DA-03597

325.10

CHRONIC MORPHINE AND CHRONIC COCAINE DECREASE NEUROFILAMENT LEVELS IN THE RAT VENTRAL TEGMENTAL AREA. D. Beitner-Johnson, X. Guitart, and E.J. Nestler, Laboratory of Molecular Psychiatry, Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Dopaminergic neurons in the ventral tegmental area (VTA) are thought to mediate some of the reinforcing properties of opiates and cocaine. We have demonstrated previously that chronic morphine and cocaine exert common actions on tyrosine hydroxylase in the VTA and in one of its projection areas, the nucleus accumbens (*J Neurochem*, 57:344, 1991). We now report that neurofilament (NF) proteins of 200, 160, and 68 kD are also regulated in the same way by chronic morphine and chronic cocaine in the VTA. NF levels were studied by immunolabeling, protein staining, and back phosphorylation. Each method indicated that chronic morphine and cocaine decreased the total amounts of the various NFs by between 15-50% in the VTA, but not in some other regions of the central nervous system studied. NF levels were not altered by chronic haloperidol or imipramine, drugs without reinforcing properties, or by acute morphine or cocaine treatment.

In the course of these studies, we found by use of microdissection techniques that NF proteins are highly enriched in the VTA, and to a lesser extent in the substantia nigra and locus coeruleus (also catecholaminergic nuclei), compared to a large number of other brain regions examined. The possibility that NFs are highly expressed in VTA neurons suggests that these dopaminergic cells display some specialized function subserved by NF proteins. Decreased levels of NFs in the VTA elicited by chronic morphine and cocaine may contribute to drug-induced functional alterations in these neurons that are part of the molecular basis of morphine and cocaine addiction and craving.

325.11

DOPAMINERGIC BRAIN REWARD REGIONS OF LEWIS AND FISCHER 344 RATS CONTAIN DIFFERENT LEVELS OF TYROSINE HYDROXYLASE AND NEUROFILAMENTS. X. Guitart, D. Beitner-Johnson, and E.J. Nestler, Laboratory of Molecular Psychiatry, Depts. of Pharmacology and Psychiatry, Yale Univ. School of Medicine, New Haven, CT 06508.

The ventral tegmental area (VTA) and one of its projections, the nucleus accumbens (NAc), are considered brain reward regions that mediate some of the reinforcing actions of drugs of abuse. Lewis (LEW) and Fischer (F344) rats are genetically inbred strains that show different levels of self-administration of several types of abused substances. Since chronic morphine and cocaine treatments regulate tyrosine hydroxylase (TH) and neurofilaments (NFs) specifically in the VTA-NAc pathway (see Beitner-Johnson et al., this volume), we studied these phosphoproteins in LEW and F344 rats to better understand possible genetic factors involved in drug addiction.

Major differences were found in levels of these proteins in the VTA and NAc of LEW vs F344 rats. LEW rats (the drug-preferring strain), compared to F344, had 40% *higher* levels of TH immunoreactivity in the VTA, and 50% *lower* levels of TH in the NAc. LEW (vs. F344) rats also had about 40% *lower* levels of the NF subunit proteins of 200, 160, and 68 kD in the VTA, as measured by immunolabeling, protein staining, and back phosphorylation. Each of these strain differences are strikingly similar to the effects of chronic morphine and chronic cocaine on the levels of TH and NFs in these neurons. The strain differences in levels of TH and NFs exhibited regional specificity within the central nervous system.

These findings suggest that levels of TH and NF expression in the VTA-NAc pathway may reflect part of the biochemical basis of individual genetic vulnerability to drug addiction.

325.12

LEWIS AND FISCHER 344 RATS AND DRUG ADDICTION: BEHAVIORAL AND BIOCHEMICAL CORRELATES. R.Z. Terwilliger, C. Bradberry, X. Guitart, D. Beitner-Johnson, D. Marby, T.A. Kosten, R.H. Roth, and E.J. Nestler, Lab. of Molecular Psychiatry, Depts. of Pharmacology and Psychiatry, Yale Univ. School of Medicine, New Haven, CT 06508.

Lewis (LEW) and Fischer (F344) rats are inbred genetic strains that show different levels of self-administration of several types of drugs of abuse, and can therefore be used to study genetic factors that contribute to drug addiction. First, we confirmed this strain difference in drug preference by showing that LEW rats develop >2-fold more conditioned place preference to morphine and to cocaine compared to F344 rats. We then studied possible biochemical factors involved.

We have reported that chronic morphine and cocaine decrease levels of Giα, and increase levels of adenylate cyclase (AC) and cyclic AMP-dependent protein kinase (cA-K), in the nucleus accumbens (NAc) (*Brain Res*, 548:100, 1991), a brain region implicated in drug reward. In the current study, we found, analogous to the drug effects, that LEW compared to F344 rats exhibit 50% lower levels of Giα, and 30-40% higher levels of AC and cA-K, specifically in the NAc. We also studied extracellular levels of dopamine in the NAc of LEW and F344 rats by in vivo microdialysis. Acute administration of cocaine elicits dramatically smaller increases in extracellular dopamine in the NAc in LEW vs F344 rats. This could be related to strain differences in levels of tyrosine hydroxylase in the NAc (see Guitart et al., this volume).

Strain differences observed in G-proteins, the cyclic AMP pathway, TH and dopamine levels, and some other phosphoproteins (see Guitart et al.) could in part underlie the strain difference in drug preference. These studies will lead to the identification of molecular mechanisms involved in individual genetic vulnerability to drug addiction.

325.13

EFFECTS OF PRE-& POST-NATAL COCAINE EXPOSURE ON THE STRIATAL DOPAMINERGIC SYSTEM IN NEWBORN MICE. E. Yablonsky-Alter, Y.Belenky*, M.A. Nathan, I. Glezer, T.I. Lidsky and S.P. Banerjee. Inst. of Brain Research, Staten Island, N.Y. and CUNY Medical School, N.Y., N.Y. 10031

Cocaine (Coc) was injected (10mg/kg, i.p.) at an interval of 24 hrs to a group of pregnant mice at the beginning of gestation. A control group of mice received equal volumes of saline. Administration of saline and Coc was continued for another 21 days after the delivery, and the female mice were allowed to feed milk to their newborn pups. The pups were sacrificed at the ages of 3, 7, 11, and 21 days and striatal dopamine levels were measured with high pressure liquid chromatography. Administration of Coc to pregnant mice decreased the striated content of dopamine as compared to control by 28%, 34%, 29% and 33% in 3, 7, 11, and 21 day old pups respectively. These results indicate that a third of striatal dopamine is lost due to prenatal Coc exposure. No further damage of the striatal dopaminergic system could be seen during the post-natal exposure to Coc. Thus, the Coc-induced insult to the striatal dopaminergic system appears to be much greater during prenatal period as compared to the postnatal stage.

325.14

SYSTEMIC COCAINE CHALLENGE AFTER CHRONIC COCAINE TREATMENT YIELDS COCAINE SENSITIZATION OF EXTRACELLULAR DOPAMINE CONTENT IN NUCLEUS ACCUMBENS BUT DIRECT COCAINE PERFUSION INTO NUCLEUS ACCUMBENS DOES NOT - *IN VIVO* MICRODIALYSIS STUDIES. J. Chen, R. Marmur*, W. Paredes*, A. Pulles* and E.L. Gardner, Depts of Psychiatry and Neuroscience, Albert Einstein Col. of Med., New York, NY 10461

Chronic cocaine treatment produces enhanced response to subsequent cocaine challenge (e.g., Kilbey & Ellinwood, *Life Sci.* 20: 1063, 1977). Many hypotheses have been proposed to explain this cocaine "sensitization", including enhanced DA release, DA autoreceptor desensitization, and postsynaptic DA receptor alterations (e.g., Kalivas et al., *J. Pharm. Exp. Ther.* 245:485, 1988; Zahniser et al., *NIDA Res. Monogr. Ser.* 88:55, 1988). In the present study, rats were treated with cocaine (20 mg/kg/day, i.p.) for 16 days followed by 7 days wash-out. On the 24th day, rats were challenged with the same dose of cocaine and extracellular DA in nucleus accumbens (Acc) was measured by *in vivo* microdialysis. Rats challenged with systemic cocaine showed enhanced Acc extracellular DA content (compared to rats not receiving chronic cocaine), but those challenged with cocaine by perfusion of 10^{-5} M cocaine directly into the Acc did not. These data suggest that cocaine sensitization, as manifested by enhanced Acc DA content to acute cocaine challenge, does not involve neural mechanisms occurring locally within the Acc. Thus, cocaine sensitization may be due to changes at other neural loci or may be due to pharmacokinetic factors (see, e.g., Pettit et al., *J. Neurochem.* 55:798, 1990).

(Supported by a grant from the Aaron Diamond Foundation)

325.15

THE PROGRESSIVE CHANGES OF NEURONAL ACTIVITIES OF THE NIGRAL DOPAMINERGIC NEURONS UPON WITHDRAWAL FROM CONTINUOUS INFUSION OF COCAINE. H.Zhang*, T.H.Lee* and E.H.Ellinwood. Dept. of Psychiatry, Box 3870, Duke Univ. Med. Ctr., Durham, NC 27710.

We hypothesized that the residual withdrawal effects of continuously chronic cocaine infusion would increase the autoreceptor sensitivity of the dopaminergic neurons in the substantia nigra pars compacta (SNpc) to apomorphine upon cocaine withdrawal. Previously we found that after chronic amphetamine, apomorphine inhibition of SNpc neuron firing goes from subsensitivity on day 1 after withdrawal to supersensitivity on day 7 (Lee and Ellinwood, 1989). In the present study, rats (S-D, male) were pretreated with cocaine (40 mg/kg/day) or saline via subcutaneous osmotic minipumps for two weeks. One, 7 or 14 days after the removal of the minipumps, the spontaneous discharges of the dopaminergic neurons in the SNpc was recorded, and the effect of intravenous injection of apomorphine (expressed as percentage inhibition of the baseline firing rate) was examined. A hyposensitivity to apomorphine was observed on the first day of cocaine withdrawal (ID_{50} 16 ±3 (5) *vs.* 11 ±2 (10) μg/kg, i.v. in control). A hypersensitivity was displayed on day 7 of cocaine withdrawal (ID_{50} 6±3 (7) μg/kg, i.v.). In addition, a significant increase of the baseline firing rate (6.0 ±1.2 *vs.* 3.1±1.2 spikes/sec in control animals) was also present among the neurons recorded 7 days after the cocaine withdrawal, while a slight increase (3.9±1.5 spikes/sec) was seen among the rats tested on the first day of withdrawal. Both the baseline firing rate and the neuronal sensitivity returned to the control level on day 14 of cocaine withdrawal. We propose that an altered sensitivity of the dopamine autoreceptor (D_2) and the neural pathways to which the D_2 receptors are related are responsible for the changes of the neuronal activities induced by continuous cocaine treatment.

325.16

NIGRAL DOPAMINERGIC NEURONS: CRUS CEREBRI LESIONS ALTER SPONTANEOUS UNIT ACTIVITY AND RESPONSE TO AMPHETAMINE AFTER LONG-TERM TREATMENT. B.A. Heidenreich and G.V. Rebec. Prog. Neural Science, Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Two mechanisms mediate the inhibitory effect of amphetamine (AMPH) on the activity of dopamine (DA) neurons in the substantia nigra compacta (SNC): self-inhibition via autoreceptors and post-synaptic feedback from striatal targets. We assessed their relative contribution to the subsensitivity to AMPH previously observed after repeated treatment (Kamata and Rebec, Neuropharmacol., 22:1377, 1983). Adult, male rats received electrolytic lesions in the crus cerebri or sham lesion surgery. After a week, rats received either 5.0 mg/kg d-AMPH or saline twice daily for 6 days. On the following day, single-unit activity of DA neurons was recorded in the SNC under urethane anesthesia. Mean spontaneous firing rate did not differ among the 4 treatment groups. Among both lesioned and sham-operated rats, higher doses of i.v. d-AMPH were necessary to inhibit unit activity after treatment with this drug, relative to saline treatment. In lesioned rats, mean spontaneous firing rate was highly correlated with the dose of d-AMPH necessary to inhibit unit activity (r=.92). This correlation was positive among lesioned rats after saline (r=.42) or d-AMPH (r=.95) treatment. No such relationship was present after sham lesions (r=.07). In fact, this correlation was in opposite directions among shams who received saline (r=.39) or d-AMPH (r=-.49) treatment. These results suggest that subsensitivity of SNC DA neurons to AMPH after long-term treatment represents both pre- and post-synaptic mechanisms of adaptation.

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325.17

THE EFFECTS OF PROLYL-LEUCYL-GLYCINAMIDE (PLG) ON IDENTIFIED MIDBRAIN DOPAMINE NEURONS. C. Rouillard[1], R.K. Mishra[2] and R.L. Johnson[3]. [1]Lab. of Neurobiology and Dep. Pharmacology, Laval University, Québec, Canada, G1J 1Z4, [2]Department of Psychiatry, McMaster University, Hamilton, Canada and [3]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, USA.

The tripeptide PLG has a pharmacological profile which strongly suggests that it exerts a modulatory effect on dopaminergic systems and could have antiparkinsonian properties. PLG has been demonstrated to potentiate the behavioral effects of dopamine agonists, attenuate catalepsy induced by neuroleptics and cause an increase in the binding of dopamine agonists to striatal dopamine receptors. In this study, extracellular single-unit recording techniques were used to examine the effects of PLG on the electrophysiological activity of identified nigrostriatal (NSDA) and mesoaccumbens (MADA) dopamine neurons in anesthetized rats. Intravenous administration of PLG (0.1 µg/kg-10 mg/kg) produced a dose dependent increase in the firing rate of NSDA and MADA neurons. However, pretreatment (3 min) with PLG (0.1 mg/kg or 10 mg/kg) did not alter the sensitivity of NSDA neurons to apomorphine (ED_{50}=7.5±2.42 µg/kg and 14.14 µg/kg respectively vs 9.18 µg/kg in control rats). These data indicate that PLG can modulate the activity of mesencephalic dopaminergic pathways. Additional studies are currently underway to determine the mechanism by which PLG can influence dopamine neuronal activity. (Supported by FRSQ [CR] and NIH [RKM and RLJ]).

325.18

EVIDENCE FOR NMDA AND AMPA SUBTYPES OF THE GLUTAMATE RECEPTOR ON SUBSTANTIA NIGRA DOPAMINE (DA) NEURONS. C.L. Christoffersen and L.T. Meltzer. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

The present studies evaluated whether NMDA or AMPA subtypes of the glutamate receptor mediate the excitatory effects of glutamate on DA neurons. Extracellular single-unit activity was recorded from chloral hydrate anesthetized rats. Iontophoretic administration of both AMPA and NMDA increased neuronal firing and could induce depolarization inactivation. In contrast, glutamate increased neuronal firing but did not induce depolarization block. In preliminary studies the selective NMDA antagonist CPP reduced the excitatory effects of NMDA and glutamate but not AMPA, while the selective AMPA antagonist NBQX reduced the excitatory effects of AMPA and glutamate but not NMDA. NBQX produced a greater reduction of the glutamate effect than did CPP. These data provide evidence for both NMDA and AMPA receptors on DA neurons.

325.19

ALPHA-ADRENERGIC MODULATION OF MIDBRAIN DOPAMINE CELL ACTIVITY. J. Grenhoff and T.H. Svensson. Dept. of Pharmacology, Karolinska Institute, S-104 01 Stockholm, Sweden.

Interactions between brain adrenergic and dopaminergic (DA) systems have been suggested in a number of studies. We have previously shown that stimulation of presynaptic alpha-2 adrenoceptors regularizes the firing of midbrain DA neurons.

Here we have utilized extracellular recording from single identified DA neurons in chloral hydrate-anaesthetized male rats to examine the effects of alpha-adrenergic substances on the firing rate and pattern of DA cells in the ventral tegmental area, the origin of the mesocorticolimbic DA pathway. The alpha-1 adrenoceptor antagonist prazosin (0.15-0.6 mg/kg IV) regularized the firing pattern and markedly decreased burst firing without affecting the firing rate of the neurons. In addition, the deregularizing action of the alpha-2 antagonist idazoxan was blocked by prazosin.

The present results indicate that adrenergic neurons exert a tonic modulatory influence on midbrain DA cell firing via alpha-1 receptors.

CATECHOLAMINES: BIOSYNTHESIS I

326.1

TYROSINE AND PHENYLALANINE AS SUBSTRATES FOR DOPAMINE SYNTHESIS IN PC12 CELLS. F.R. DePietro, R.P.S. Kwok and J.D. Fernstrom, Dept. Psychiatry, Univ. Pittsburgh, Pittsburgh PA 15213.

Phenylalanine (PHE) is reputed to be both a substrate for and an inhibitor of tyrosine hydroxylase (TH) and catecholamine (CAM) synthesis. It is difficult to study CAM synthesis from PHE *in vivo*, as PHE is rapidly converted to tyrosine [TYR] in the liver. To define the role of PHE in CAM synthesis more clearly, we are studying dopamine [DA] synthesis from PHE using PC-12 cell cultures. DA synthesis is estimated via ^{3}H-DA formation from ^{3}H-PHE (or ^{3}H-TYR); TYR hydroxylation is also quantitated via DOPA accumulation following inhibition of aromatic-L-amino acid decarboxylase. Samples are analyzed for endogenous DA or DOPA by HPLC/electrochemical detection; ^{3}H-DA is quantitated by counting HPLC fractions. Endogenous amino acids are measured fluorometrically using a Beckman 6300 amino acid analyzer. Labeling and DOPA results agreed well, yielding a K_m for TYR of 1-2 µM (extracellular [EC] concentration; the K_m= 60 µM using intracellular [IC] TYR concentrations) and a V_{max}= 19-25 pmol/min/mg protein. The K_m for PHE (EC concentration; IC measurements in progress) was 7.7 µM, and V_{max}= 9.9 pmol/min/mg protein using PHE as substrate. DOPA and DA synthesis rates declined when EC TYR was >10-30 µM; inhibition did not occur for PHE at EC levels ≤300 µM. TYR and PHE each inhibited DA synthesis from the other amino acid, though TYR was 10-times more potent than PHE. These results suggest that PHE shares many properties in common with TYR as a substrate for TH and CAM synthesis in the PC12 cell.

326.2

EXTRINSIC FLUORESCENCE STUDIES OF TYROSINE HYDROXYLASE L. G. Gahn and R. Roskoski, Jr. Department of Biochemistry and Molecular Biology LSU Medical Center New Orleans, Louisiana 70119

Tyrosine hydroxylase (TH) is the rate limiting enzyme in the biosynthesis of the catecholamines dopamine, norepinephrine and epinephrine. We have studied the effects of substrate and inhibitor binding on the conformation of purified rat TH (from PC12 cells), using the extrinsic fluorescence probe 8-anilino-1-naphthalene sulfonate (ANS). ANS non-covalently binds to proteins at hydrophobic sites and exhibits a characteristic fluorescence spectrum. A change in protein conformation may alter this spectrum. At pH 6.0, ANS interacting with TH resulted in a fluorescence spectrum with a maximum at 455 nm upon excitation at 380 nm. Tyrosine, a substrate, decreased the fluorescence slightly (10%) whereas 3-iodo-tyrosine, a competitive inhibitor with respect to tyrosine, increased fluorescence by 10%. In contrast to 3-iodo-tyrosine, the catecholamines are competitive inhibitors of TH with respect to the reducing cofactor tetrahydrobiopterin. All of the inhibitory catecholamines at a 100 µM concentration (dopamine, norepinephrine and isoproterenol) produced a 30% decrease in TH/ANS fluorescence. Metaproterenol, an isomer of isoproterenol which has no effect on TH activity, did not alter fluorescence. These results suggest that tyrosine and 3-iodo-tyrosine, which probably bind to TH at the same site, have only minor effects on TH conformation, while the catecholamine inhibitors all appear to significantly influence TH conformation. Supported by USPHS Grant NS-15994.

326.3

NGF- AND PHORBOL-STIMULATED PHOSPHORYLATION OF TYROSINE HYDROXYLASE AT SER[31] IS MEDIATED BY ERK1/ERK2. J.W. Haycock[1], N.G. Ahn[2], M.H. Cobb[3]*, & E.G. Krebs[2]*. [1]Dept. Biochem., Louis. State U. Med. Ctr., New Orleans, LA 70119; [2]Dept. Pharmacol., U. Wash., HHMI, Seattle, WA 98195; [3]Dept. Pharmacol., U. Texas, Southwestern Med. Ctr., Dallas, TX 75235

Tyrosine hydroxylase (TH) is phosphorylated at four sites (Ser^{8}, Ser^{19}, Ser^{31} and Ser^{40}) *in situ* and *in vivo*. The protein kinases (PK) which mediate the phosphorylation of Ser^{8}, Ser^{19} and Ser^{40} appear to be proline-directed PK, Ca^{++}/calmodulin-dependent PK II and cAMP-dependent PK, respectively. However, the PK(s) responsible for phosphorylating Ser^{31}, the NGF- and phorbol ester-sensitive phosphorylation site, has not previously been identified.

The amino acid sequence surrounding Ser^{31} (rat, -EAVTS^{31}PRF-; human, -EAIMS^{31}PRF-) did not suggest any candidate PKs, and a number of purified PKs failed to phosphorylate TH at Ser^{31} *in vitro*. However, two Ser^{31} kinase activities were identified in extracts of NGF-treated PC12 cells. Fractionation of the extracts on MonoQ columns produced two peaks of Ser^{31} kinase activity, which corresponded exactly with two peaks of myelin basic protein kinase activity. These kinase activities had been previously identified as ERK1 and ERK2--two related PKs of M_r ~45 kDa and ~42 kDa, respectively. To demonstrate that ERK activity was responsible for the Ser^{31} kinase activity, activated ERK1 was purified to homogeneity and shown to phosphorylate purified PC12 TH selectively at Ser^{31}.

In intact PC12 cells, the phosphorylation of TH at Ser^{31} is increased by a number of agents in addition to NGF. Such treatments (e.g., phorbol dibutyrate, bradykinin, barium) also increased ERK1/ERK2 activity.

Taken together, these data identify ERK1 and/or ERK2 as the effectors of Ser^{31} phosphorylation *in situ* and, as such, identify the first physiological substrate for these protein kinases.

326.4

EFFECTS OF ELECTROCONVULSIVE SHOCK ON IN VIVO HYDROXYLATION OF TYROSINE AND ON TETRAHYDROBIOPTERIN LEVELS IN THE BRAIN OF 6-HYDROXYDOPAMINE LESION RATS. Mir Ahamed Hossain*, Joseph M. Masserano and Norman Weiner. Dept. of Pharmacology, C-236, Univ. of Colorado Health Sciences Center Denver, Co. 80262.

We have evaluated the effects of electroconvulsive shock (ECS) on the rate of in vivo hydroxylation of tyrosine and the status of tetrahydrobiopterin (BH_4) levels in the nigrostriatal system in 6-hydroxydopamine (6-OHDA) lesioned (right substantia nigra) rats. Electroconvulsive shock (300 mA,0.2 sec) was administered to 6-OHDA lesioned rats 5 min after NSD-1015 (100mg/kg,i.p.) treatment and sacrificed 30 min later. Rats were sacrificed 5 min after ECS treatment for BH_4 assay. The rate of formation of DOPA was significantly decreased in the striatum (3%) and in substantia nigra (34%) of the non-shocked 6-OHDA lesioned rats as compared to the contralateral non-lesioned striatum and substantia nigra (100%). The application of ECS significantly increased the rate of formation of DOPA by 43% in the right striatum and by 40% in the right substantia nigra compared to that in the non-shocked rats. BH_4 levels in the right nigrostriatal system were significantly increased following ECS treatment. Our results indicate that in 6-OHDA lesioned rats the rate of in vivo hydroxylation of tyrosine and BH_4 levels are increased significantly after ECS treatment reflecting an enhancement of dopaminergic function in the nigrostriatal system of these rats. This result suggests that the nigrostriatal system following 6-OHDA treatment still maintains the potential for further up-regulation of dopaminergic function in response to ECS treatment. Supported by USPHS Grant NS09199.

326.5

EFFECTS OF MPTP ON THE EXPRESSION OF TYROSINE HYDROXYLASE AND TETRAHYDROBIOPTERIN (BH_4) BIOSYNTHETIC ENZYMES IN MOUSE BRAIN. P.Z. Anastasiadis, J.F. Solus2*, S.Tait*, S.K. Demetriou* & R.A. Levine. Lab Mol. Neurobiol., Cellular & Clinical Neurobiol. Pgm, Wayne State Univ. (WSU) & Lafayette Clinic, Detroit, MI, and [2]Center for Mol. Biol., WSU.

BH_4 is the essential cofactor for tyrosine hydroxylase (TH), the initial and rate-limiting enzyme in dopamine (DA) synthesis. The three major enzymes catalyzing successive reactions in BH_4 biosynthesis within DA neurons are GTP cyclohydrolase (GTP-CH), 6-pyruvoyl-tetrahydropterin (6-PPH_4), and sepiapterin reductase (SR). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a selective degeneration of nigrostriatal DA neurons. In male C57/B1 mice, MPTP (10 mg/kg i.p., once each day for 5 days; sacrifice on day 17) had the following effects in striata: 1) decreased *in vitro* TH activity by 50% and DA and metabolites by greater than 70%; 2) decreased BH_4 and GTP-CH activity by 50%, and 3) did not alter 6-PPH_4 synthase or SR *in vitro* activities. Radiolabelled cDNA probing of northern blots containing substantia nigral total RNA demonstrated a 33% reduction of TH mRNA following MPTP; preliminary experiments showed that nigral SR mRNA rose to 160% of control, indicating the potential for enhanced expression of SR in response to partial DA neuronal destruction. It is possible that striatal SR activity remains equal to control due to a compensatory enhancement of SR expression in surviving DA neurons. However, the lack of change in striatal SR and 6PPH_4 synthase activities following MPTP could also indicate that these enzymes are not predominantly localized to DA neurons and catalyze other reactions not involved in BH_4 biosynthesis, although other functions for these enzymes are unknown. Current experiments are examining the ability of MPTP to enhance the expression of the other BH_4 biosynthetic enzymes in nigrostriatal DA neurons.

326.6

CLONING, SEQUENCING, AND CHARACTERIZATION OF HUMAN BRAIN SEPIAPTERIN REDUCTASE (SR) cDNA. R.A. Levine[1,2], J. Solus[3*], J.C. States[3*], S.K. Demetriou[1*], S. Tait[1*], B. Citron[4], and S. Kaufman[4]. [1]Lab of Molecular Neurobiology, Lafayette Clinic & [2]Dept of Psychiatry, Wayne State University (WSU), Detroit, MI; [3]Center for Molecular Biology, WSU; and [4]Laboratory of Neurochemistry, National Institutes of Mental Health, Bethesda, MD.

Sepiapterin reductase (SR) catalyzes the final reaction in the synthesis of tetrahydrobiopterin (BH_4), the essential cofactor for tyrosine and tryptophan hydroxylases in dopamine and serotonin synthesis, respectively. Cloning of human SR and other BH_4-related genes will allow the study of altered genetic expression of BH_4 biosynthetic enzymes in neuropsychiatric illnesses caused by altered biogenic amine metabolism. A previously cloned rat SR cDNA was used to screen a lambda ZAP human frontal cortex cDNA library. Following screening of 300,000 clones (40% formamide, 42°C), one positive clone was identified on duplicate filters and remained positive upon re-screening. Southern blot analysis of the SR cDNA revealed a full-length size of 1.67 kb, which was confirmed by sequencing. Restriction fragments of human SR cDNA were subcloned in pBS in preparation for sequencing. Subcloned fragments were amplified using symmetric polymerase chain reaction (PCR), followed by asymmetric PCR to prepare single stranded DNA templates for dideoxy sequencing. Comparison of rat and human SR cDNA sequences revealed greater than 60% homology at the nucleotide and translated amino acid levels with no forced gaps introduced in either sequence. Northern blot analysis of rat and human liver RNA demonstrated that there was weak cross-hybridization of rat and human SR cDNA with mRNA from the opposite species. Human SR mRNA was slightly larger than rat SR mRNA. Current studies are focused on measuring SR mRNA in human tissues and isolating the human SR gene from genomic DNA libraries.

326.7

INDUCTION OF ADRENAL GAD BY RESERPINE: RELATIONSHIP TO AGE-RELATED ALTERATIONS IN TH INDUCTION C. Hale* J.W. Haycock , V. Reddy,* B. Raghu,* M. Wessels-Reiker and R. Strong. The Dept. of Pharmacological and Physiological Science and the Dept. of Internal Medicine, St. Louis Univ. School of Med. and the Geriatric Research, Education and Clinical Center, St. Louis VAMC, St. Louis, MO 63125; and Biochemistry and Molecular Biology, LSUMC, New Orleans, LA 70119.

We previously reported age-related increases in adrenal tyrosine hydroxylase (TH) gene expression, but age-associated decreases in TH induction by reserpine. Since we also reported that activation of GABA receptors inhibits TH induction, we examined the effects of aging and reserpine on adrenal glutamate decarboxylase (GAD), the enzyme that synthesizes GABA. The effects of reserpine on GAD and TH were compared simultaneously in adrenals from rats aged 2, 12 and 27 months. Reserpine treatment was associated with an increase in GAD. This is the first report that reserpine induces adrenal GAD. Moreover, although there was no effect of age on GAD in vehicle treated groups, aging was associated with super-induction of GAD. Thus in reserpine-treated groups, GAD was increased to 300% of control at 2 months, 400% of control at 12 months and 700% of control at 27 months. By contrast, TH increased with age in vehicle treated groups, but reserpine was less effective in inducing TH in older animals. Thus, reserpine increased TH by 250% in the 2 month group, increased TH 200% at 12 months, but by 27 months of age reserpine had no significant effect on TH. Moreover, regression analysis revealed a highly significant negative correlation ($r=-0.7241$; $p<0.002$) between the magnitude of TH induction and the extent of GAD induction. These data provide evidence that TH and GAD are coordinately regulated. Our previous studies showing that GABA receptors modulate reserpine induced increases in TH gene expression, together with the present findings, provide evidence that age-associated alterations in GAD expression may influence TH induction during aging.

326.8

REPEATED IMMOBILIZATION STRESS ELEVATES TYROSINE HYDROXYLASE AND DOPAMINE ß-HYDROXYLASE mRNA LEVELS IN RAT ADRENALS. A. McMahon *1 Kvetnansky *2, K. Fukuhara *2, I.J. Kopin[2], A.B. Drakontides[1], and E.L. Sabban[1]. [1]New York Med. Coll., Valhalla, NY 10595 and [2]NIHDS, NIH, Bethesda, Md. 20892.

Repeated immobilization has been shown to increase the activity of adrenal catecholamine biosynthetic enzymes. The molecular mechanism of this activation is not known, although it is known to require trans-synaptic stimuli. We examined whether alterations in adrenal mRNA levels of tyrosine hydroxylase (TH) and dopamine ß-hydroxylase (DBH) were involved in the response to immobilization stress. Immobilization for 30 min had no effect on TH or DBH mRNA levels, however, immediately following a single two hr immobilization, TH mRNA levels were elevated over 5-fold. Under the same conditions, DBH mRNA levels were unchanged. After 24 hr, TH mRNA levels had declined toward control values, and could be re-elevated by another 2 hr immobilization. The rise in TH mRNA was inhibited by actinomycin D, suggesting an increase in transcription. Repeated immobilization for 2 hr periods daily for seven consecutive days, elevated both TH and DBH mRNA levels about 4-fold.

Thus both adrenal TH and DBH mRNA levels are affected by immobilization stress, however the results indicate that the mechanism and/ or timing of the regulation of TH and DBH are not identical.

326.9

PROCESSING AND GLYCOSYLATION OF RAT DOPAMINE ß-HYDROXYLASE IN A CELL FREE SYSTEM. Z. Feng*[1], R.H. Angeletti[2], and E.L. Sabban[1].[1]Dept. Biochem. & Mol. Biol. N.Y. Med. Coll. Valhalla, NY 10595 and [2]Dept. Dev. Biol. & Cancer, Albert Einstein Sch. Med. Bronx, NY 10461.

Dopamine ß-hydroxylase (DBH) is the enzyme which catalyzes the synthesis of norepinephrine. DBH is present in both membrane-bound and soluble forms in neurosecretory vesicles. Tetramers of the membrane-bound form contain both 77K and 73K monomers, while only the latter are in soluble DBH. Cloning of rat DBH (McMahon et al., 1990, J. Neurosci. Res. 25, 395-404), revealed a single open reading frame and an unusually long putative signal sequence. If conversion of the 77K to the 73K monomer entails proteolytic processing cleavage, it must occur at the C- or N- terminus. Antisera to a synthetic peptide to the deduced amino acid sequence at the C-terminus immunoprecipitated both the 73K and 77K forms of DBH from PC12 cells. To examine processing in the ER, full length rat DBH cDNA was generated. DBH mRNA was synthesized by T3 RNA polymerase in vitro, capped and expressed in a cell free system. The mRNA coded for a translation product of about 68K apparent Mr. Translation with pancreatic membranes added co-translationally, also synthesized a band of higher apparent Mr. This band was sensitive to endo H digestion, confirming the role of glycosylation in the modification of DBH. Experiments are being directed to determining if the signal sequence was removed in the in vitro product.

326.10

EFFECTS OF cAMP AND GLUCOCORTICOID ON DOPAMINE BETA-HYDROXYLASE mRNA LEVEL AND CATECHOLAMINE CONTENT IN BOVINE CHROMAFFINE CELLS. O. Hwang, J.D. Lee* and T.H. Joh. Univ. Ulsan Coll. Med., Seoul, 138-040, Korea and Lab of Mol. Neurobiol., Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605

Dopamine beta-hydroxylase (DBH) catalyzes the conversion of dopamine to norepinephrine in catecholamine neurons and adrenal medulla. Our recent studies on the structure of DBH gene indicates the presence of GRE and CRE in the 5' upstream. In order to investigate the possibility of whether these two cis-acting elements are functional, the effects of cAMP and glucocorticoid on DBH transcription in bovine primary chromaffin cells were examined at the mRNA level, and the contents of norepinephrine and epinephrine were measured.

Treatments with various doses of forskolin and dexamethasone resulted in a small increase of approximately 30 to 50% in DBH mRNA level. On the other hand, the same treatments resulted in dramatic increases (3-4X) in both tyrosine hydroxylase and phenylethanolamine N-methyltransferase mRNA levels, as we have previously reported. Norepinephrine and epinephrine contents increased by 30 to 70%. These results suggest that in the primary culture of bovine chromaffin cells, regulation of DBH gene transcription by cAMP or glucocorticoid is not as dramatic as the other catecholamine biosynthetic enzymes. The results are different from the similar experiments being carried out in PC12 cells, which may be due to cell type or species differences.

326.11

NEUROLEPTIC ACTIVATION OF STRIATAL AROMATIC L-AMINO ACID DECARBOXYLASE (AADC) ACTIVITY IS NOT DUE TO *DE NOVO* PROTEIN SYNTHESIS. M.Y. Zhu*, A.V. Juorio, I. A. Paterson and A.A. Boulton, Neuropsychiatric Research Unit, University of Saskatchewan, Saskatoon, SK, Canada. S7N 0W0.

Decarboxylation of phenylalanine by AADC is the rate limiting step in the synthesis of 2-phenylethylamine (PE). which is stimulated by neuroleptics. Recent experiments have shown that blockade of D1 and D2 receptors by SCH 23390 or pimozide increase AADC activity in the rat striatum whereas D2 receptor blockade increased AADC in the mesolimbic system. The increases were observed within 30 minutes after treatment and lasted for at least 2-4 hours. Analysis of the enzyme kinetics indicated that Vmax increased with little change in the Km. The object of the present study was to test whether the changes in striatal AADC activity induced by DA antagonists is due to *de novo* synthesis of the enzyme. AADC activity was determined in rat striatum homogenates by measuring the amount of DA formed from L-DOPA with HPLC-ED. Using a 2-way ANOVA design, rats were given intraperitoneal injections of vehicle, cycloheximide (10 mg/kg), SCH 23390 (0.1 mg/kg) or pimozide (0.3 mg/kg), 30 minutes prior to death. SCH 23390 and pimozide significantly increased AADC activity in the striatum and increases were unchanged in rats treated with cycloheximide. These findings confirm further that AADC activity in the striatum is increased by D1 and D2 receptor blockers and show that these rapid changes in activity are not due to increases in protein synthesis.

Supported by Saskatchewan Health and the Saskatchewan Health Research Board.

326.12

CYCLIC AMP INCREASES DOPA BUT DECREASES DOPAMINE AND DOPA DECARBOXYLASE OF PHEOCHROMOCYTOMA PC12h CELLS. N.Nakanishi, S.Onozawa*, R.Asaumi*, and S.Yamada* Dept. of Biochem., Meikai Univ. Sch. Dent., Sakado, Saitama 350-02, Japan

We found that although NGF rose the levels of tetrahydrobiopterin (BH_4; a cofactor of tyrosine hydroxylase), dopa (DO) and dopamine (DA) of PC-12 and PC12h cells, dibutyryl cAMP (dBcAMP), cholera toxin and forskolin increased BH_4 and DO but decreased DA in the cells. We then examine the mechanism of cAMP effect on DA. Decrease in cellular DA was observed within 30 min of dBcAMP addition with the concomitant increase in DA in the medium, though dBcAMP did not stimulate the DA secretion from the cells washed with fresh culture medium or saline. In vivo and in vitro DA producing activities of the cells treated with dBcAMP for 12-24 h were lower than those of the control cells. dBcAMP gave only slight effect on DA degrading activity. These results suggest that cAMP lowers the DA level via two different ways: by increasing the leakage of cytosolic DA which is not yet transported into secretory granules; and by decreasing the dopa decarboxylase activity. (Supported in part by a Grant from the Ministry of Education, Culture and Science of Japan, No.01571027)

326.13

ANALYSIS OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE GENE PRODUCTS IN THE ADULT RAT BRAIN AND ADRENAL GLAND. T.C. Wessel, H. Baker and T.H. Joh, Lab. of Molec. Neurobiol., Cornell Univ. Med. Coll., The Burke Medical Research Insitute, White Plains, NY 10605.

The enzyme phenylethanolamine N-methyltransferase (PNMT) converts norepinephrine to epinephrine in the brain and adrenal gland. Some uncertainty exists as to the regional expression of the PNMT gene in the developing and adult brain: PNMT has been localized to the anterior olfactory nucleus, mesolimbic system as well as the hypothalamus, in addition to the less controversial sites in the brainstem. We have utilized two polyclonal rabbit antisera to purified bovine adrenal PNMT in conjunction with a 1.0 kb full-length rat PNMT cDNA probe to investigate the distribution of PNMT protein and mRNA in the adult rat brain. Cellular immunohistochemical staining and *in situ* hybridization (ISH) signal could only be detected in the ventrolateral medulla (C1 neurons) and the nucleus tractus solitarius (C2 neurons) in the brainstem; no further rostral structures could be identified. Intraventricular injection of colchicine (150 µg in 10 µl) produced more intense immunostaining of the C1 and C2 neurons, while reserpine (10 mg/kg SQ) had a less pronounced effect. These manipulations did not produce significant changes in mRNA levels as measured by grain counts. In contrast, reserpine caused increased PNMT transcription and protein synthesis in the epinephrine cells of the adrenal medulla as demonstrated by increased mRNA levels at 24 hours and enhanced immunoreactivity at 72 hours. Interestingly, reserpine injection elicited rapid c-fos and c-jun expression, within one hour, in the adrenal medulla that had a similar distribution to that of PNMT. In addition, the C1 and C2 neurons also exhibited an induction of these proto-oncogenes after reserpine which implicates a role for the Fos-Jun dimer in adrenergic cell function and possibly in PNMT gene regulation. Supported by MH44043 and MH24285.

326.14

PRESENCE OF PNMT-LIKE IMMUNOREACTIVITY IN ASTROCYTES IN PRIMARY CULTURE. M.I.Masana and I.N.Mefford. Sec. Clin. Pharm., ETB, NIMH, Bethesda, MD 20982

The involvement of glia in transmitter-related functions is becoming increasingly well documented. Astrocytes are known to possess binding sites for several neurotransmitters, high affinity uptake systems for virtually all neurotransmitters and catecholamines as well as enzymes involved in the metabolism of neurotransmitters, such as monoamine oxidase, catechol-O-methyltransferase, phenolsulpho-transferase and other methyltransferases. Using astrocytes in primary culture derived from hypothalamus, brainstem and cortex of 2 days old rats, we demonstrated the presence of astrocytes doubled staining for phenylethanolamine N-methyltransferase (PNMT) and the glial fibrilar acidic protein (GFAP). The intensity of the fluorescence for PNMT was higher in hypothalamus-derived astrocytes than astrocytes derived from other regions. The enzymatic activity determined by a radiometric assay, using norepinephrine as substrate, was 17.1 ± 1.5 pmol/h/mg prot (n=6) in astrocytes derived from hypothalamus with no regional-specificity. Only 30 % of this activity was blocked by the PNMT inhibitor DCMB(10 µM) in cortex and brainstem-derived astrocytes and 40 % in hypothalamus derived astrocytes. The enzyme seemed to be nonspecific because the activity using tryptamine as substrate was similar to that obtained with norepinephrine in most of the regions except for hypothalamus which was higher (29.9 ± 3.3 pmol/h/mg prot, n=6). These data suggest that astrocytes could be involved, perhaps in early stages of the development, in the methylation of different substrates, including norepinephrine, contributing to the formation of epinephrine in the central nervous system.

326.15

INSULIN STIMULATES ADRENAL PHENYL-ETHANOLAMINE-N-METHYL TRANSFERASE (PNMT) IN VITRO. F.N. Norflus* and J.K. Stewart. Dept. of Biology, Virginia Commonwealth University, Richmond, Virginia 23284-2012.

Previously we showed that insulin induced hypoglycemia rapidly stimulates PNMT activity in the rat adrenal, brain and spinal cord. In this study, adrenal glands from male Sprague Dawley rats were dissected free of fat, partially bisected and perifused for 2 hours in Locke solution continuously equilibrated with 95% O_2/5% CO_2. Half of the adrenals were perifused with solution containing insulin (1U/ml). PNMT activity (mean ± SE) in the 4 insulin treated adrenals was 4106 ± 701 DPM/60 min/adrenal compared to 2639 ± 80 in 5 controls ($P \leq 0.05$). These findings show that 2 hours of perifusion with pharmacological levels of insulin increase adrenal PNMT activity and suggest that insulin or insulin-like factors directly stimulate adrenal PNMT.

Supported by NIH grant NS26992.

NEURAL CONTROL OF IMMUNE FUNCTION

327.1

CRF mRNA IN THE SPLEEN. F. Aird*, M.B. Prystowsky* and E. Redei. Depts. of Psychiatry and Pathology, University of Pennsylvania, Philadelphia, PA 19104.

CRF is a primary mediator of the neuroendocrine stress response and has also been found to influence immune function directly. CRF receptors, similar to those found in the anterior pituitary and the brain, have been identified in resident macrophages of mouse spleen.

We have detected and identified CRF in the rat spleen by using gel filtration followed by specific RIA. Primary cultures of adherent cells derived from rat spleen secreted immuno- and bioactive CRF in resting state and in response to glucocorticoids. This CRF may be synthesized locally in the spleen, or alternatively, synthesized elsewhere and internalized by spleen cells. In order to distinguish between these two possibilities, we examined whether CRF mRNA is present in the spleen. Total RNA was isolated from adult mouse spleen, reverse transcribed into cDNA and amplified by the polymerase chain reaction using a set of CRF gene-specific oligonucleotide primers. A discrete band corresponding to the amplified CRF product was detected on ethidium bromide stained gels, indicating that CRF is synthesized in the spleen. We suggest that splenic CRF may function as an immunomodulator. Supported by ADAMHA grant AA07389.

327.2

THE DISTRIBUTION AND FUNCTION OF CALCITONIN GENE RELATED PEPTIDE IN THE MOUSE SPLEEN AND THYMUS. K. Bulloch, T. Radojcic*, R. Yu*, J. Hausman*, L. Lenhard* and S. Baird*. Dept. of Psych. and Path. UCSD, San Diego, CA 92093.

CGRP nerve fibers and their corresponding receptors have been characterized within the mouse thymus and spleen. In this study, the function of these receptors on immunocytes was evaluated by two **in vitro** immunological assays. Previous anatomical studies demonstrated that two populations of cells and intrathymic nerves were positive for CGRP antibody. No CGRP nerves or cells were observed in the spleen. In contrast, both the spleen and thymus demonstrated binding sites for CGRP. **In vitro** studies show that CGRP significantly suppressed a Con A induced proliferation of both murine thymocytes (by 55%) and splenocytes (by 25%) at 10^{-9}M. Furthermore, 10^{-9}M CGRP inhibited proliferation of murine responder cells by 30% in a mixed lymphocyte reaction. These doses are within the physiological range of the Kd for the receptor in both the spleen and thymus (10^{-10}M). The results of these experiments suggest that the immunological in vivo role of CGRP may be to control lymphocyte trafficking and the microenvironment in which these cells are permitted to respond to pathogens. Furthermore, in disorders such as diabetes, aberrant expression of the CGRP gene or its product may contribute to the immune abnormalities reported for this disease. Supported by ONR grant #N0014-89-J-1256 and the Kettering Foundation.

327.3

EXPRESSION AND HORMONAL REGULATION OF THE β-2 ADRENERGIC RECEPTOR GENE IN THE RAT THYMUS. B. Marchetti, M.C. Morale, *P. Paradis, M. Bouvier. Dept. of Pharmacology, Catania Univ., Med. Sch., 95125 Catania, Italy, and Dept. of Biochemistry, Montréal Univ., Québec, Canada.

Sympathetic innervation of the thymus gland constitutes a morphological link in the communication between neuro-endocrine and immune cells. The localization of a beta$_2$-adrenergic receptor (β_2AR) population in the medulla of the rat thymus, coupled with the marked hormonal modulation of β_2AR distribution, prompted us to pharmacologically characterize the β_2AR signalling system of the rat thymus and to examine the hormonal regulation of the β_2AR gene expression. We report here that the thymic β_2AR is functionally coupled to the adenylyl cyclase signalling pathway. Northern blot analysis, using a human β_2AR cDNA as a probe, revealed the presence of a mRNA of 2.3 Kb, consistent with the size of the β_2AR mRNA found in other rat tissues. Ovariectomy induces a significant decrease in both receptor number and mRNA levels, while oestrogen replacement brings the β_2AR mRNA levels even higher than those found in control animals, suggesting that the β_2AR gene expression is under transcriptional control of oestrogens in the rat thymus.

327.4

CHANGES IN INTRACELLULAR CALCIUM CONCENTRATION AND MEMBRANE POTENTIAL IN RAT THYMOCYTES IN RESPONSE TO NEUROTRANSMITTERS AND LYMPHOKINES: EXAMINATION OF POSSIBLE MECHANISMS UNDERLYING NEURO-IMMUNE COMMUNICATION. Carolyn B. Lacey[1], Martin W. Wessendorf[1], Robert Elde[1] and Jeffery L. Barker[2]. [1]Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455 and [2]Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Several neurochemicals have been implicated in the communication between the nervous and immune systems. In the present study we have begun to examine the possible mechanisms by which putative neurotransmitters may function in cellular signal transduction in rat thymocytes. To study the effects of various neurotransmitters on calcium regulation and membrane potential, we utilized the calcium indicator dye, FLUO-3, and the voltage sensitive oxonol dye, DiBAC$_4$(3), respectively. Single cell suspensions were prepared from rat thymus (100-150g) by mechanical dissociation and resuspended in physiological buffer containing 10µg/ml aprotinin. Changes in fluorescence signals were monitored utilizing a fluorescence spectrophotometer. The addition of substance P and ACTH, in concentrations ranging from 10nM to 1µM, caused an increase in the intracellular Ca^{2+} concentration. Addition of the ß-adrenergic agonist, isoproterenol (1µM to 10µM) and the lymphokine, interleukin-2 (IL-2), also independently caused decreases in the Ca^{2+} signal. In preliminary experiments using DiBAC$_4$(3), an increase in fluorescence with the addition of IL-2 (75-750U/ml) was observed, indicating relative depolarization of the cells. These results indicate possible mechanisms through which the nervous and immune systems interact. Supported by DA 06299, DA 05695, and DA 05466.

327.5

NEUTRAL ENDOPEPTIDASE 24.11 INVOLVEMENT IN IMMUNOCYTE ACTIVATION AND INHIBITION. [1]O. Duvaux-Miret*, [2]E.M.Smith*, [3]T.K.Hughes*, [4]M.K.Leung, [4]L. Mallozzi and [4]G.B.Stefano. [1]Pasteur Institute, 1, rue de la Prof. Calmatte, Lille, France; Depts. [2]Psychiatry Behavioral Sci., and [3]Microbiology. Univ. TX. Medical Branch at Galveston, TX 77550; [4]Aging CTR, SUNY/Old Westbury, Old Westbury, NY 11568-0210.

Opioid peptides, a group of transmitter substances have a high degree of phylogenic conservation, and diverse functions. For example, they have been shown to play a role in immunomodulation in both vertebrates and invertebrates. Furthermore, since we know know that neutral endopeptidase 24.11 (enkephalinase;NEP) inhibition potentiates the stimulatory effect of enkephalins, it was of interest to determine if this would hold true for other "neuropeptides". We demonstrate that authentic alpha-MSH, in a dose response manner (peak effect 10^{-7} M), can "deactivate" molluscan hemocytes, that is return them to a round conformation following stimulation by tumor necrosis factor which results in an "active" ameboid conformation. This was demonstrated using human, Mytilus, and Biomphalaria immunocytes in an in vitro computer assisted cellular assay (American Innovision Image Analysis). Addition of the NEP inhibitor, phosphoramidon (100 mM) potentiated the effect of alpha-MSH (peak 10^{-9} M). Other enzyme inhibitors e.g., captopril, were not as effective as phosphoramidon. Thus, NEP may play a critical role in modulating either signal molecule presentation to the receptor, degradation after receptor activation or both. Furthermore, it appears to be involved with both stimulation and inhibiton of cellular conformational changes. Supported by ADAMHA-17138 & 47392

327.6

EVIDENCE FOR KAPPA OPIOID BINDING SITES ON MURINE LYMPHOMA CELLS. D.M.P. Lawrence and J.M. Bidlack. Department of Pharmacology, University of Rochester Medical Center, Rochester, NY 14642.

The aim of this study was to determine if the murine lymphoma cell line R1.1 possessed opioid binding sites. Membranes prepared from R1.1 cells were resuspended in 50 mM Tris-HCl, pH 7.5. At 25°C, specific (-)[^{3}H]bremazocine binding increased linearly with protein concentration and reached equilibrium by 30 min. Nonspecific binding, which was less than 20% of total binding, was measured by inclusion of 1 μM naloxone. R1.1 cell membranes bound [^{3}H]bremazocine with a K_d value of 10.6 ± 0.2 pM and a B_{max} value of 22.5 ± 1.4 fmol/mg protein. The δ-selective peptides, [D-Pen^2,D-Pen^6]enkephalin and ICI 174,864 at concentrations up to 10 μM did not inhibit 12 pM [^{3}H]bremazocine binding, while μM concentrations of the μ-selective opioid [D-Ala^2,(Me)Phe^4,Gly(ol)^6] enkephalin were needed to inhibit binding. In contrast, the κ-selective ligand U50,488H inhibited [^{3}H]bremazocine binding with an IC_{50} value in the low nM range. [^{3}H]Bremazocine binding was stereoselectively inhibited by (-) pentazocine, which had an IC_{50} value in the low nM range, while the (+) isomer was 20 times less effective. [^{3}H]Bremazocine binding was inhibited in a concentration-dependent manner by NaCl at concentrations greater than 10 mM. Thus, R1.1 cells possess high affinity κ opioid binding sites that share many properties with brain κ opioid receptors. These cells will be very useful in the characterization of the molecular and functional properties of κ opioid receptors on lymphocytes. (Supported by USPHS grant DA04355 and DA07232.)

327.7

DECREASE IN 3H-DIHYDROALPRENOLOL BINDING SITES ON SPLENIC LYMPHOCYTES OF GENETICALLY EPILEPSY-PRONE (GEPR-9) RATS. J.A. Carr, K.A. Ortiz*, L.L. Paxton*, L.C. Saland, D.D. Savage. Department's of Anatomy and Pharmacology, University of New Mexico, School of Medicine, Albuquerque, New Mexico 87131.

Recent evidence indicates that the GEPR-9 rat exhibits a reduction in plaque-forming cell (PFC) responses after immunization in vivo with sheep erythrocytes (Rowland et al., 1991, Life Sci. 48:1821-1826). At present, the cellular events underlying this immunosuppression are not known. Since the neurotransmitter norepinephrine has been shown to act through β-adrenoreceptors to augment antibody responses, we examined β-adrenergic receptors on splenic lymphocyte membranes (SLM) obtained from 100-120 day old GEPR-9 and age-matched, non-epileptic Sprague-Dawley control rats. The antagonist dihydroalprenolol (3H-DHA) was used to label β-adrenergic receptors. Initial experiments in which SLM were incubated with 1 nM 3H-DHA in the presence or absence of 1 μM l-propranolol revealed a 33% reduction in specific binding of 3H-DHA to SLM from GEPR-9 rats. Subsequent saturation studies of 3H-DHA binding revealed a significant decrease in the maximum number (Bmax) of 3H-DHA binding sites on SLM from GEPR-9 rats. There was no difference in the mean apparent affinity constants obtained for 3H-DHA binding to SLM from GEPR-9 and control rats. Our results suggest that a deficiency in the expression of lymphocyte β-adrenoreceptors may be a contributing factor to the reduced PFC response seen in GEPR-9 rats. Supported by NS08447, RR05583, RR08139, NS21256.

327.8

EFFECTS OF KAINIC ACID INDUCED LATERAL SEPTAL AREA AND HIPPOCAMPAL LESIONS ON CELL MEDIATED IMMUNITY IN FEMALE RATS. L. Wetmore J. G. Gartner*, J. Green-Johnson* and D. M. Nance. Depts. of Pathology /Physiology, Univ. of Manitoba, Winnipeg, MB, Canada, R3E 0W3.

Previously, we have shown that kainic acid (KA) lesions of the lateral septal area (LSA) and the hippocampus have inhibitory and facilitatory effects, respectively, on the humoral immune response of female rats. Presently, we report the effects of KA (2.0 µg/µl, 0.25µl) induced LSA and hippocampal lesions on cell mediated immune responses of female rats. Animals received either bilateral KA infusions into the LSA or the hippocampus, whereas controls were infused with saline. Two weeks later spleen cells were analyzed for natural killer (NK) cell activity, T cell responsiveness to mitogen (CON A) or specific activation (using a mAB (R73) to the TCR $\alpha\beta$ chain). The results indicate that rats with KA lesions in the LSA have significantly higher NK cell activity, as compared to sham operated controls. There was a trend for T cell responsiveness to CON A to be lower in the LSA lesioned females, relative to controls, but LSA lesions had no effect on specific T cell activation. Lesions of the hippocampal region (CA3 and CA4 fields) did not affect NK cell activity nor T cell reactivity to nonspecific or specific *in vitro* stimulation. These results further demonstrate the importance of LSA-KA sensitive cells in neuro-immunoregulation and indicate that these immune alterations are specific to the class of immune cell being analyzed. Thus, different arms of the immune system are differentially modulated in LSA lesioned female rats. Although the hippocampus is involved in regulation of the humoral immune response, the absence of cell mediated immune alterations in KA hippocampal lesioned rats suggests that the hippocampus may not regulate NK cell activity or splenic T-cell responsiveness. Thus, the LSA is involved in the complex and differential regulation of immune function. Supported by MRC.

327.9

Neuromodulation of IgG secretion from plasma cells in avian lacrimal gland

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IgG secretion in the avian lacrimal gland can be transiently increased by exposure to neurotransmitters like acetylcholine (carbachol). Gland fragments containing numerous plasma cells exposed to carbachol showed in a 5 fold increase of IgG output for 10-20 minutes. The binding of the muscarinic receptor ligand ANB to sectioned glands indicates that both the acinar cells and plasma cells contain muscarinic receptors. In an effort to determine the nature of the transduction pathway for neurotransmitter stimulated immunoglobin secretion, the effects of various agents have been tested. Pretreatment of glands with atropine blocks carbachol's stimulatory effects. The presence of Cd2+ (1mM) significantly reduces carbachol stimulated IgG release. Forskolin (20 uM) also inhibits carbachol stimulated IgG release. We have previously demonstrated that plasma cells isolated from avian lacrimal gland contain maxi-K channels (Brink et al., Pflugers Arch. 417). The inhibitory effect of forskolin on IgG secretion is likely to be related to its ability to increase maxi-K channel activity which hyperpolarizes the cells. Hyperpolarization could override a potential forskolin activation of Ca2+ channels which, if occurring, should cause an enhancement of secretion. Further we present indirect evidence which suggests that plasma cells have an inward Ca2+ current. Our data support the notion that the plasma cells contain receptors which enables them to respond directly to neurotransmitters. It appears that a Ca2+ inward current is involved in the transient transmitter stimulated IgG secretion of the plasma cells. These data clearly indicate that a major interaction is possible between the CNS and the immune system. Supported by NIH grant 31299.

327.10

ADRENOCORTICOTROPIC HORMONE (ACTH) AND ACTH FRAGMENTS MODIFY PHYTOHEMAGGLUTININ-STIMULATED LYMPHOCYTE PROLIFERATION. T.L. Keadle*, D.W. Horohov* & P.A. Melrose, LSU School Vet. Med., Baton Rouge, LA 70803.

Published results have suggested that immune cell ACTH receptors are distinct from adrenocortical ACTH receptors. The present study tested for effects of ACTH and ACTH fragments on the phytohemagglutinin(PHA) proliferative response of equine lymphocytes in order to determine if carboxy and/or amine terminal fragments would mimic effects of native ACTH. Experiment I tested peptide effects on PHA-stimulated peripheral blood mononuclear cells (PBMC) from castrate male (G), experiment 2 tested effects on PHA-stimulated PBMC from intact female (F) and experiment 3 tested effects on PHA-stimulated T lymphocytes(TL) from G. In each experiment, 2 X 10^5 PBMC or TL cells were incubated with vehicle, PHA, PHA + interleukin(IL)-2 or PHA + variable amounts of peptides. ACTH(A), $ACTH_{1-24}$ (A1), $ACTH_{4-10}$(A4) or $ACTH_{18-39}$(A18) were tested at 10^{-5}, 10^{-7}, 10^{-9} and 10^{-11} M concentrations. Cells were subsequently cultured for 3 days before quantifying incorporation of radiolabeled thymidine. Data were analyzed by ANOVA and means compared using LSD. In experiment 1, 10^{-5}M A and A18 both inhibited ($P<.01$) the PHA response whereas 10^{-9}M A stimulated ($P<.01$) proliferative response and remaining treatments were ineffective. For experiment 2, all A fragments reduced ($P<.01$) proliferative response at 10^{-5}-10^{-9}M and A reduced ($P<.05$) proliferation at 10^{-5}M. Conversely, the proliferative response of TL was stimulated by 10^{-5}-10^{-11}M A4 ($P<.01$) and 10^{-7}-10^{-11} A1 ($P<.05$) whereas 10^{-5}M A inhibited ($P<.05$) proliferation. Results from these experiments suggest that ACTH receptors on PBMC and TL may respond to both carboxy and/or N-terminal ACTH fragments that extend beyond the core tetradecapeptide sequence normally associated with proopiomelanocortin peptide effects on the adrenal cortex. Further work is needed in order to determine whether differences in activity may occur due to binding variations, cell-specific responsiveness and/or multiple receptor classes.

327.11

DIURNAL COMPARISON OF ADRENAL STEROID RECEPTOR ACTIVATION IN BRAIN, PITUITARY AND IMMUNE TISSUE. R.L. Spencer, A.H. Miller, S.S. Kang*, M. Stein and B.S. McEwen. Lab. of Neuroendocrinology, The Rockefeller Univ., New York, NY 10021.

We have previously found in the rat a heterogeneity between tissues in the proportion of mineralocorticoid (Type I) and glucocorticoid (Type II) adrenal steroid receptors that are occupied and activated (as estimated by cytosolic receptor binding) under basal and acute stress conditions (Miller et al, Am J Phys 259,E405,1990). In general, we find a greater degree of receptor activation by morning basal and stress levels of glucocorticoids in brain tissue than in pituitary or immune tissue. Since basal levels of corticosterone in the rat are much higher during the evening than the morning, we have compared the in vivo activation of adrenal steroid receptors (basal vs acute stress) during both morning (AM) and evening (PM) conditions. For these studies, male Sprague-Dawley rats (270-330 g) were maintained on either a normal or reversed 12:12 h light-dark cycle with lights on or off at 7AM. Animals were sacrificed between 8 and 9 AM. Rats treated with acute stress were sacrificed immediately after 1 hr of restraint. Diurnal differences in adrenal steroid receptor activation were especially evident in the hippocampus in which the degree of both Type I and Type II receptor activation during PM basal conditions was equivalent to the degree of receptor activation after acute stress in the AM. Acute stress in the PM did not further increase hippocampal receptor activation. In immune tissue, diurnal differences in receptor activation were present in isolated peripheral blood mononuclear cells which exhibited evidence of Type II receptor activation only after acute stress in the PM. These data indicate that the relative level of adrenal steroid receptor activation by basal and stress levels of endogenous steroid may vary depending on the time of day and suggests that the relative contribution of endogenous glucocorticoids to HPA axis negative feedback regulation and immune function may vary accordingly. (Supported by MH47674)

327.12

EFFECTS OF CONTINUOUSLY ADMINISTERED NICOTINE AND HALOPERIDOL ON CLASS II MHC, CD4, CD5, AND CD8 MARKERS IN RATS. L.L. Wing, M.A. Coggiano, D.G. Kirch, Y. Shien, R.J. Wyatt and H. Kulaga. Neuropsychiatry Branch, NIMH @ St. Elizabeths, Washington, D.C. 20032.

The incidence of smoking among schizophrenic outpatients was higher than among the general population (88%) (Hughes et al., 1986). Many of these patients are treated with the DA receptor blocker haloperidol decanoate (HAL). The present study examined the change in peripheral blood mononuclear cell populations obtained from rats treated repeatedly with either nicotine (NIC) or HAL, or both.

After 28 days of treatment, animals were sacrificed and trunk bloods collected. Cells were stained for Class II MHC (B-cells, macrophages and T-cells), CD4 (T-helper response), CD5 (T-cells and some B-cells) and CD8 (cytotoxic or suppressor cells). Cell counts were analyzed by flow cytometry (Becton Dickinson FACScan). Chronic NIC resulted in a general suppression of fluorescence of the primary population of Class II MHC and CD5 cells, of a medium population of CD8 cells, and skewed the distribution of CD4 cells. Chronic HAL resulted in an increase in number for the secondary population of CD4 cells, an increase in fluorescence of a secondary population of CD5 cells, but suppression of a medium population of CD8 cells. NIC/HAL resulted in a separation of 3 populations of CD4 cells and an decrease in CD8 cells of medium brightness. Thus, chronic stimulation with a cholinergic agonist or dopaminergic antagonist alters immune responsiveness.

327.13

AUTHENTIC α-, γ-, AND ß-ENDORPHIN ARE NOT PRESENT IN HUMAN PERIPHERAL BLOOD LEUKOCYTES.
A.D. van Woudenberg*, J.P.H. Burbach*, D. de Wied*, V.M. Wiegant. Rudolf Magnus Institute, Dept. of Pharmacology, University of Utrecht, Vondellaan 6 3521 GD Utrecht, The Netherlands.

Immunoreactivity (IR) for proopiomelanocortin (POMC) derived peptides has been found in cells of the immune system, in particular when cultured in the presence of stimulatory agents, such as corticotropin releasing factor (CRF) or Newcastle disease virus (NDV). The chemical identity of this IR material has not yet been established. In the present study, endorphin-like IR in extracts of human peripheral blood mononuclear cells (PBMC), freshly isolated or cultured in the presence or absence of Concanavalin A, was analyzed. Fractions of HPLC and gelfiltration were analyzed in four region specific RIA systems. Using a RIA system recognizing the midportion of ß-endorphin (ßE) with each of the three extracts a similar HPLC-profile was observed in which a number of IR-peaks was found. Analysis of the same HPLC fractions in the other RIA systems recognizing the C-terminus of αE, γE and ßE respectively, showed that none of these substances were related to these endorphins. To further investigate the presence of a ßE-related peptide, the extracts were digested with Cathepsin D, an enzyme that cleaves the Leu^{17}-Phe^{18} bond in the ßE sequence, thereby generating the C-terminus of γE, which is detectable in the γE RIA. In none of the HPLC fractions of Cathepsin D treated extracts, however, γE-IR peaks were detectable, indicating that no ßE-like sequence had been present in the extracts. Gelfiltration of the extracts showed that the major amount of endorphin-IR, as detected with all four RIA systems, eluted as high molecular weight material. These data indicate that the endorphin-like IR found in human PBMC does not represent authentic αE, γE or ßE or other known forms of these peptides.

327.14

MORPHINE-INDUCED IMMUNOSUPPRESSION IS NOT MEDIATED BY ACTIVATION OF THE HYPOTHALAMIC-PITUITARY ADRENAL AXIS. L.R. Flores*, K. Gale, and B.M. Bayer*. Dept. of Pharmacology, Georgetown Univ. Medical School, Washington D.C., 20007.

These studies investigated the mechanism by which acute morphine administration inhibits peripheral blood lymphocyte proliferation in rats. We have previously reported that morphine (5-25 mg/kg) inhibits Concanavalin A stimulated lymphocyte proliferation in a dose-dependent manner. Maximal inhibition (80%) by morphine occurred with a dose of 10 mg/kg two hours after drug administration. Concurrent with this immunosuppressive effect was a dose-dependent increase in plasma corticosterone concentrations. Therefore, we examined the potential contribution of the hypothalamic-pituitary-adrenal (HPA) axis to the immunosuppressive effects of morphine. In order to assess the role of glucocorticoids, rats were pretreated with the steroid antagonist RU 486 (20 mg/kg) 30 min prior to morphine (10 mg/kg) and were sacrificed two hr later. In the presence or absence of RU 486, significant inhibition of lymphocyte activity occurred with morphine. Furthermore, adrenalectomy failed to attenuate the inhibitory actions of morphine. To examine the potential role of pituitary hormones in the immunosuppressive effect, morphine was administered to hypophysectomized animals. In sham or hypophysectomized animals, morphine (10 mg/kg) was found to maximally inhibit lymphocyte proliferation. These results suggest that neither intact pituitary nor adrenal glands are required for the inhibitory actions of morphine on peripheral blood lymphocytes. Supported by NIDA DA-04358.

327.15

MORPHINE ATTENUATES THE ENHANCED METASTATIC EFFECTS OF SURGERY IN RATS G.G. PAGE, S. BEN-ELIYAHU, & J.C. LIEBESKIND Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Pain and stress can inhibit immune function and enhance tumor growth in laboratory animals. We used the MADB106 tumor cell line in which the number of lung metastases is sensitive to natural killer (NK) cell control. Rats were divided into 3 groups, subjected to a standard abdominal surgery under halothane anesthesia, to anesthesia only, or to no treatment. In exp. 1, 5 hours after completion of surgery/anesthesia, all animals were injected with $3x10^5$ MADB106 cells. Lungs were taken 21 days later and surface metastases counted. Animals undergoing surgery exhibited significantly more metastases compared to both the anesthesia only and control groups (which did not differ from each other). In exp. 2, blood and spleens were harvested for the in vitro NK cytotoxicity (^{51}Cr release) assay 24 h after surgery. There was significant suppression of NK cytotoxicity in the surgery group compared to the other two groups. We are now finding that analgesic doses of morphine (MS) attenuate the enhanced metastatic effect of surgery in a 2x2 design: surgery vs anesthesia only and MS vs vehicle. MS was administered pre- and postoperatively in saline and a sustained release suspension, respectively. The surgery/MS group has developed fewer metastases than the surgery/vehicle group. Supported by the Oncology Nursing Foundation/Purdue Frederick Research Grant, the UCLA Psychoneuroimmunology Program, and NIH grant NS 07628.

327.16

STRESS-INDUCED SYMPATHETIC ACTIVATION SUPPRESSES BLOOD NATURAL KILLER CYTOTOXICITY AND INCREASES METASTATIC SPREAD IN RATS: MEDIATION BY ADRENAL EPINEPHRINE S. Ben-Eliyahu, R. Yirmiya, G. Page, H. Weiner, A. Tan, A. N. Taylor and J. C. Liebeskind Dept. of Psychology, UCLA, Los Angeles, CA 90024

Stress can suppress natural killer (NK) cell activity and increase tumor growth. We have recently provided evidence for a causal relationship between the NK-suppressive and tumorigenic effects of stress using a tumor model in which pulmonary metastases of a syngeneic mammary adenocarcinoma, MADB106, are controlled by NK cells. We now report the involvement of the sympathetic nervous system in mediating these effects. Fischer 344 rats subjected to intermittent forced swimming were compared to nonstressed rats. In both conditions rats were pretreated as follows: Injected with the ganglionic blocker, chlorisondamine (3 mg/kg i.p.) or saline; adrenal demedullated (AD) or sham operated (SO); injected with the selective ß-2 adrenergic antagonist, butoxamine (25 mg/kg i.p.) or saline. One hour after stress rats were injected i.v. with $3x10^5$ MADB106 cells. Surface lung metastases were counted 21 days later. Stress significantly increased number of metastases only in control animals. This increase was markedly attenuated in clorisondamine treated animals and completely blocked by adrenal demedullation and butoxamine. In a fourth experiment, a whole blood, 4-hour ^{51}Cr release assay against YAC-1 targets was conducted 1 hour after stress on AD and SO rats to assess cytotoxicity per unit volume of blood. Stress markedly suppressed whole blood NK cytotoxic activity in SO but not AD rats. These findings suggest that sympathetic activation, specifically adrenal epinephrine binding to ß-2 adrenergic receptors, mediates the suppressive effects of stress on cytotoxic activity in blood and that such effects are reflected in increased metastatic spread. Grants: NIH NS 07628 (J.C.L.), VA Medical Research Service (A.N.T)

327.17

PHOTOPERIOD AFFECTS IMMUNE FUNCTION AND TUMORIGENESIS. Joan M.C. Blom* and Randy J. Nelson. Dept. of Psychology, Johns Hopkins University, Baltimore, MD 21218.

Many animals display seasonal cycles in physiology and behavior. Commonly, seasonal adjustments in energy-coping systems have been studied (e.g., reproduction, thermogenesis, etc). In addition to the energy constraints imposed by winter, the stress of food restriction, low temperatures, and lack of cover may compromise immune function in rodents. Although the direct causes remain obscure, rodents die more frequently during the winter than summer. Because of this strong seasonal selective pressure, we hypothesized that rodents should physiologically anticipate winter and bolster the immune system. We hypothesized that rodents use photoperiodic information to enhance immune function. Deer mice, *Peromyscus maniculatus*, housed for 8 weeks in short days (LD 8:16) have higher white blood counts and leucocyte numbers than mice maintained in long days (LD 16:8). We tested the functional significance of this photoperiodic effect by examining the efficacy of a chemical carcinogen, 9,10-dimethylbenzanthracene (DMBA), in causing mammary gland tumors. Adult female deer mice were injected s.c. either with DMBA dissolved in DMSO (50 mg/kg) or with the vehicle alone. None of the mice maintained in short days developed mammary tumors 8 weeks after treatment. In contrast, 85 % of the animals housed in long days, developed tumors within 3-4 weeks post injection. None of the mice in either daylength developed tumors after DMSO treatment. Injection of DMBA also irritated the skin, resulting in lesions. Short day animals exhibited fewer skin lesions (60 % vs 80 %) as well as accelerated healing of the lesions when compared to long-day animals (18 vs 42 days). The difference in healing rate suggests a photoperiodic effect on cytokine activity. Taken together, these results suggest that day length can influence immune function and that risk of developing mammary tumors in response to a carcinogen is reduced in short day lengths.

327.18

BETA-ENDORPHIN LEVELS IN PERIPHERAL MONONUCLEAR CELLS OF PRIMARY HEADACHE PATIENTS. P.Sacerdote, B.Manfredi*, [1]M.Leone*, [1]G.Bussone* and A.E.Panerai. Dept. Pharmacology, School of Medicine, Un. of Milano, and [1]Istituto Neurologico "C.Besta", Milano, Italy.

Beta-Endorphin(BE) is synthesized in human peripheral mononuclear cells(PMNC), and it is regulated by some neurotrasmitters in a way similar to hypothalamic and pituitary BE. Since in migraine(M) and cluster(CH)headache, plasma and CSF levels of the opioid peptides are deranged, we studied whether also PMNC BE could be altered in this pathology. The levels of the peptide were significantly lower in all the migraine patients studied (60 M without aurea, and 27 M with aura) and in the CH patients in remission(17 patients) in comparison to 30 healthy, age and sex matched, controls; no difference was present between controls and episodic tension-type headache (20 cases). In addition a significantly lowered BE lymphocytic levels characterized the CH patients in remission in comparison to M with and without aura patients. These results have been obtained in patients out of attack and free from any prophylaxis since two weeks, so that pain and pharmacological treatments do not account for the observed differences. Our data seem to confirm previous findings showing a central opiatergic dysfunction in migraine and cluster headache and confirm the hypothesis that the lymphocyte can be considered a peripheral window to the brain.

327.19

MORPHINE-INDUCED IMMUNE ALTERATIONS: DOSE DEPENDENCY AND COMPARTMENT SPECIFICITY. V. J. Watts*, L.A. Dykstra. and D. T. Lysle, Departments of Psychology and Pharmacology, University of North Carolina, Chapel Hill, N.C. 27599

Opioids have been shown to modulate a variety of immune responses in humans and in animals. Examples include altered immune function in heroin users and suppression of immune responses in mice following implantation of controlled release morphine pellets. Given the lack of information about the doses at which these effects occur, the purpose of the present study was to determine whether morphine suppressed immune function in a dose-dependent manner. Morphine was injected subcutaneously into Lewis rats (0.0, 5.0, 10.0, 15.0 and 25.0 mg/Kg). One hour later, the spleen, mesenteric lymph nodes and a sample of peripheral blood were collected. Immune function was assessed by a variety of *in vitro* assays. For splenic lymphocytes, morphine produced a dose-dependent suppression of lymphocyte function as measured by natural killer (NK) cell cytotoxicity, and interleukin 2 (IL-2) production. Additionally, morphine suppressed mitogen-induced proliferation of spleen and blood lymphocytes in a dose-dependent manner. In contrast, the doses of morphine examined here did not alter lymphocyte proliferation or IL-2 production in the mesenteric lymph nodes. Collectively, these results show that morphine's immunomodulatory effects are dose-dependent and compartment-specific. (Supported by UPS grants DA02749, MH46284 and BSRG 2 SO7 RR07072.)

327.20

PAVLOVIAN CONDITIONING OF MORPHINE-INDUCED IMMUNE ALTERATIONS. Mary E. Coussons, Linda A. Dykstra, and Donald T. Lysle. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599-3270

Previous work has demonstrated that immune responses are affected by administration of morphine, suggesting interactions between the opiate and immune systems. Additionally, it has been shown that stimuli associated with morphine administration can become conditioned and subsequently elicit opiate-like behavioral effects when presented alone. The purpose of the present investigation was to examine the role of Pavlovian conditioning in morphine-induced alterations in immune function. Lewis rats received pairings of morphine (15mg/Kg) with a distinctive environment. Subsequent exposure to the distinctive environment alone induced morphine-like alterations in immune function including decreased T-cell and B-cell responsiveness to mitogen stimulation, decreased natural killer cell activity, and a reduction in interleukin-2 production. Control procedures showed that these alterations were the result of a conditioning process. These findings provide evidence that conditioning can play a role in opiate-induced alterations of immune function. (Supported by PHS grants DA02749, DA07244, MH46284 and BSRG 2 S07 RR07072.)

327.21

EVIDENCE THAT IMMUNE SUPPRESSION INDUCED BY A CONDITIONED AVERSIVE STIMULUS IS MEDIATED BY BETA-ADRENERGIC RECEPTORS. Linda J. Luecken & Donald T. Lysle. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599-3270

The purpose of our study is to further characterize the immunomodulatory effects of exposure to a conditioned aversive stimulus, (CS). Our research with rats investigated the role of the beta-adrenergic system in this conditioned immunomodulatory effect by administering the non-selective beta-adrenergic antagonist propranolol and the peripherally-acting beta-1 receptor antagonist atenolol prior to exposure to the CS. Both antagonists were found to attenuate the CS-induced immune suppression as measured by the mitogenic response of splenic lymphocytes to ConA, PHA, LPS, and ionomycin/phorbol. Further tests with Atenolol also showed an attenuation of the CS-induced suppression of natural-killer cell activity. These results suggest that immune suppression induced by a CS is mediated by beta-adrenergic activity at peripheral beta-1 receptor sites. (Supported by MH46284)

327.22

MODULATION OF IMMUNE FUNCTION BY A CONDITIONED AVERSIVE STIMULUS: EVIDENCE FOR MEDIATION BY CENTRAL OPIOID RECEPTORS. Donald T. Lysle, Linda J. Luecken, & Kimberly Maslonek*. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599-3270.

Our research program is designed to characterize the immunomodulatory effect of a conditioned aversive stimulus (CS) in rats. The present study investigated the effect of administration of the opiate-receptor antagonist, naltrexone, on CS-induced immune alterations. The results showed that naltrexone blocked the CS-induced suppression of the mitogenic response of splenic lymphocytes to ConA, PHA, LPS, and ionomycin/phorbol. Naltrexone also blocked the CS-induced suppression of natural-killer cell activity and interleukin-2 production. In contrast, systemic administration of the quaternary form of naltrexone, N-methylnaltrexone, did not block the immunomodulatory effects of the CS. These results indicate that CS-induced opioid activity does not directly affect lymphocytes, but modulates the sympathetic output responsible for the alterations in immune function. (Supported by MH 46284)

828.1

RECOMBINANT INTERFERON-ALPHA INHIBITS OPIOID LIGAND BINDING TO BRAIN MEMBRANES.

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Interferon-alpha (IFNα) may be part of the communication network between the central nervous system (CNS) and the immune system. This cytokine modulates a number of CNS functions such as attenuation of naloxone-precipitated withdrawal symptoms in morphine-dependent rats, cognitive behavior in humans, analgesia, and electrical activity of temperature- and glucose-sensitive neurons. We are testing the hypothesis that IFNα interacts with the CNS via opioid receptors. The ability of recombinant human IFNα (rhIFNα) to alter the binding of various radiolabelled opioid ligands is being assessed in a rat brain membrane preparation. Nonspecific binding is determined in the presence of non-labelled ligands such as levorphanol, naloxone, morphine or [met^5]enkephalin. Inhibition of ^{3}H-naloxone binding by rhIFNα is dose-dependent from 500-5000 U/ml. K_Ds for naloxone increased from 3.37 nM for controls to 6.9 nM in the presence of 5000 U/ml of rhIFNα. Intermediate K_D values were observed for 1500 and 3000 U/ml. B_{MAX} values did not change. Thus, over this concentration range, rhIFNα behaves as a competitive inhibitor to naloxone. However, only a maximum of 60-70% inhibition was observed. When ^{3}H-[D-ala^2-D-leu^2]enkephalin binding was studied, maximum inhibition was 30% as compared to naloxone. These data support the hypothesis that certain biological properties of IFNα are mediated via opioid systems. This work is supported by ADAMHA (DA05723, DA07245), and USF Research/Creative Scholarship grant (6118-932-RO).

828.2

MORPHINE IMPAIRS MEASURES OF THE IMMUNE SYSTEM IN SPINAL CORD-INJURED RATS.

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Morphine's effects upon the immune system of spinal cord transected rats were studied. Adult male Sprague Dawley rats were anesthetized and a dorsal midline incision was made in the mid-thoracic region. A laminectomy was performed at the T5-T6 vertebral level, and the spinal cord was transected with a scalpel. A 75 mg pellet of morphine was implanted sc at the time of surgery which results in sustained plasma levels in excess of 250 ng/ml. Sham-surgery and unhandled control rats were also included. Seventy-two hours following transection and morphine administration, a significant reduction in thymic weight was observed in the transected morphine-treated rats compared with all other groups ($p<.05$). Reductions in splenic lymphocyte responsiveness to Con-A and LPS were also observed. Spinal levels of IL-1 adjacent to the transection were significantly reduced, but whether this represented an effect of morphine upon glial or monocytic sources of IL-1 is currently being determined. A high incidence of cannibalism was also observed in the transected morphine-treated rats. In other studies in this laboratory using non-transected rats, morphine either enhanced or had no effect upon the immune system depending upon the measure. These combined results suggest that spinal injury creates a microenvironment within which morphine is able to suppress the immune system. These studies were supported in part by grants from ADAMHA (DA05723, DA072450).

828.3

ALTERATIONS IN PERIPHERAL ANTERIOR PITUITARY HORMONES ASSOCIATED WITH IMMUNE FUNCTION AFTER SEVERING ANTERIOR HYPOTHALAMIC AREA (AHA) CONNECTIONS.

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Hormonal communications between hypothalamic and immune systems were studied in males after surgical interruption of AHA neural connections. Virus free SD rats (Harlan, 380-400g) maintained under 12:12LD with jugular catheters were habituated to daily handling and sampling arenas. Pre-operative AM and PM blood samples (bs) were drawn for assay of corticosterone (Cort) and prolactin (Prl) by RIA. Groups of rats then received either 1) knife cuts severing anterior (r=1.5mm) and lateral (2.0mm) AHA connections (LFC) 2) sham surgery (Sh) or 3) remained controls (C). Subsequent bs were taken at 3 and 4d intervals over 30d. Hematocrits, WBC counts and presence of bacterial pathogens were monitored. Spleens were removed for assay of 3d lymphocyte response to mitogens (PHA, con A and LPS) and natural killer (NK) activity using YAC-1 cells. Thymus, spleen and LFC locations in brain were examined histologically. LFC group AM Cort levels were significantly elevated vs. Sh at 7 and 14d, whereas PM Prl levels were lower only at 7d. Moreover, Cort and Prl directional changes during most days appeared synchronized, so that parallel changes occurred more frequently. All of the effects were also associated with a significant increase in core body temp (1.0±0.17°C) during the first 7d. Although Sh was associated with a significant decline in NK activity and lymphocyte response to PHA vs. C, the effects of LFC were diminished compared to C. In conclusion, although LFC of the AHA produces neuroendocrine and immune changes similar to AHA destructive lesions (Phelps, et al., Endo Mtg., 1991), they are less severe in the long term. Supported by MH46808.

828.4

EFFECT OF THE CEREBRAL INTERFERON TREATMENT ON THE GLIOMA PROGRESSION IN VIVO.

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This work is based on earlier findings that osmotic blood-brain barrier (BBB) alteration allows cerebral interferon (IFN) entry. The in vivo model of intracerebral mouse glioma G-26, developed in this laboratory was used. G-26 of astrocytic origin is positive for glial fibrillary acidic protein (GFAP). G-26 cells at concentration $3-5 \times 10^7$ cells/ml in 1 µl were implanted in the right cerebral cortex of C57BL/6 mice (23-25 gm). Histology was done at 4,7,14,21 and 28 days after implant. On day 3 post implant animals were treated once via the right carotid artery with mouse interferon MuIFN α/β (8×10^4 units in 0.1 ml) following injection of 1.6 M Arabinose or infused subcutaneously (sc) for 3 days (osmotic pumps) with MuIFN α/β (8×10^4 units). Their median survival time was compared to nontreated controls. The osmotically altered, MuIFNα/β treated mice (n=11) showed 2.9 days longer survival than nontreated controls (n=14) p=0.02 (Student's t-test). Supported by NIH-NINDS grant RO1 NS28989.

828.5

MONOAMINE LEVELS IN HYPOTHALAMUS OF 10 DAY OLD RATS WITH ACUTE CYTOMEGALOVIRUS (CMV) INFECTION.

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During the course of an immune response to a viral or bacterial infection, activity in various neurons and endocrine glands has been demonstrated to be altered. Firing rates in hypothalamic neurons were increased after antigenic stimulation. Cerebral noradrenergic metabolism was stimulated in mice that had been inoculated with Newcastle Disease Virus. Most of the studies have been performed in adult animals. Neonatal mice had elevated corticosteroid levels during acute reovirus type 3 infection which were restored to normal by administration of a thymic extract. In the present experiment, we measured hypothalamic catecholamine and indolamine levels in CMV-infected rat pups.

On the day of birth (Day 0), pups were cross-fostered and redistributed on the basis of birth weight. Test litters were comprised of 5 males and 5 females. On day 1, one group was inoculated with 10^5 units of CMV. Vehicle-injected and uninjected groups served as controls. On day 10, pups were sacrificed and hypothalami were removed. Norepinephrine, dopamine, epinephrine, serotonin and their catabolites were measured in hypothalamic homogenates by HPLC with electrochemical detection. Data are discussed in terms of brain-immune interaction and the "stress hyporesponsive period." Research was supported by NIDDK 41025.

828.6

CHARACTERIZATION OF MAJOR HISTOCOMPATIBILITY (MHC) PROTEINS AND CELLULAR INFILTRATE IN EXPERIMENTAL AUTOIMMUNE PINEALITIS (EAP).

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The pineal gland (PG) lacks a blood-brain barrier and is accessible to both humoral and cellular components of the immune system. Under normal physiological conditions, positive immunocytochemical (ICC) staining was observed for microglia/macrophages (OX42), MHC class II (OX6), MHC Class I (OX18), and lymphocytes (OX1, OX19). An enhanced immune response by EAP was induced in Lewis rats after immunization with Peptide M (an 18 a.a. pathogenic segment of S-Antigen). Rats were immunized with 50 ug Peptide M in CFA and 20ug of pertussis toxin. Control rats were uninjected or injected with CFA and pertussis alone. The autoimmune response in the PG was analyzed by ICC for OX42, OX6, helper T-cells/microglia (W3/25), cytotoxic/supressor T-cells (OX 8) and astrocytes (GFAP). Compared to controls, PG from immunized rats exhibited enhanced staining for OX42, OX6, W3/25 and OX8. The W3/25+ and OX8+ cells, which represent T-cell infiltrate, were co-localized in specific subcapsular sites within the PG. The OX42+/ OX6+ cells were localized in these same subcapsular sites. OX42+, OX6+ and W3/25+ cells were also distributed throughout the gland. Our findings suggest that MHC class II is expressed on a sub-population of resident microglia and that antigen presentation is an important mechanism of autoimmune processes of EAP. These findings are also consistent with the hypothesized role of the PG as a "gateway" for neuroimmunodmodulation. Supported by NSF (BNS 88-01726).

328.7

CORTICAL RAT NEURONS SUPPRESS MHC CLASS II INDUCIBILITY IN ASTROCYTES. U. Tontsch*, A. Hermann and H. Wekerle*, Max-Planck-Institut f. Psychiatrie, D-8033 Martinsried.

The normal brain does not express significant levels of MHC antigens. In contrast, *in vivo* after degeneration of local neurons, and *in vitro*, by treatment with gamma-interferon (IFN-γ), glia cells can be readily induced to express MHC class II gene products. Thus, rat recombinant IFN-γ (50 U/ml) applied for 72 hr induced MHC class II in 85% of primary astrocytes as determined by MAb OX6 labeling and FACS analysis.

To investigate the question whether the absence of MHC class II expression is due to mere *lack* of induction or rather due to active *suppressor* mechanisms, possibly exerted by neuronal CNS elements, we established a coculture system for astrocytes and cortical neurons. Cortical neurons were isolated from Lewis rat embryos (E14). After 3 days in culture, syngeneic astrocytes (95% GFAP positive), grown on coverslips, were added to the neurons in such a way as to allow direct cell-cell contact (upside down) and were treated with IFN-γ. Highly significant cell-mediated suppression of MHC class II inducibility could be demonstrated. On the contrary there was no downregulatory effect of soluble factors alone (astrocytes added upside up). In addition, the cortical neurons also suppressed MHC class II inducibility in the presence of activated T cells, a physiological source of multiple upregulatory factors.

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328.8

BRAIN-REACTIVE MONOCLONAL AUTOANTIBODIES: PRODUCTION AND CHARACTERIZATION. Ni A. Khin* and S.A. Hoffman. Dept. of Microbiology, Arizona State University, Tempe, AZ 85287.

Brain-reactive autoantibodies (BRAA) have been proposed as a mechanism underlying neuropsychiatric manifestations of systemic lupus erythematosus (NP-SLE). Previous studies in our laboratory have shown a diversity of BRAA in murine models of SLE. In order to detect pathogenic BRAA, it would be useful to have a library of monoclonal BRAA. These autoantibodies could be used for characterization by determining their isotype, the epitope and the location of brain antigens with which they bind, and their functional effects. We recently developed B cell hybridomas secreting monoclonal BRAA. This was done by fusion of splenocytes from autoimmune strains of mice (BXSB, MRL/1) with non-secreting myeloma cells (Sp2/0, Ag14). Monoclonal BRAA in hybridoma supernatant have been detected by their reactivity against integral brain membrane protein (BMP) using the ELISA technique. Moreover, the monoclonal BRAA have been characterized by their binding to BMP that were separated via SDS-polyacrylamide gels and transferred to nitrocellulose. Currently, we have two monoclonal antibodies which react against BMP with apparent molecular weight of 14.7 and 21.3 kD. We are in the process of identifying where these antibodies bind in brain by immunohistochemical staining of brain sections using selected monoclonal BRAA. These studies are designed to identify a subset of pathogenically important BRAA.

328.9

INFLUENZA VIRUS AND POLY I:C ENHANCE ANTIVIRAL ACTIVITY IN RABBIT SERA, BUT NOT IN RABBITS TOLERIZED TO THE VIRUS. M. Kimura-Takeuchi, J. A. Majde*, L. A. Toth, and J. M. Krueger. Univ. of TN, Memphis, TN 38163, and Off. Naval Res., Arlington, VA 22217*.

We recently found that influenza virus induces non-rapid-eye-movement sleep and fever in a rabbit model. If the virus challenge was repeated 24 h later, animals neither slept more nor fevered. Pretreatment of animals with synthetic double-stranded (ds) RNA, poly I:C, also induced physiological tolerance to the virus. We hypothesized that virally-induced sleep and fever occurred in response to cytokines (probably an interferon, or IFN) induced by viral dsRNA, and that physiological tolerance resulted from the inability of animals to elaborate IFN in response to the second challenge. In this study, we analyzed the serum levels of IFN in virally-challenged rabbits. Male rabbits were injected IV with: 1) influenza virus (20,000 mouse LD_{50} of A/PR/8/34) twice (at intervals of 24 h), or 2) poly I:C (2,5 ug/kg) followed by the virus 24 h later. Serum samples and colonic temperatures (T_{co}) were taken just before the injection (time 0), 4, and 24 h later. Serum IFN activity was determined in RK-13 cells challenged with vesicular stomatitis virus (Indiana strain). Baseline IFN activity was below detectable levels. Four hours after the first injections of the virus or poly I:C, IFN titers were 351 $\pm$ 179 IU/ml and 2600 $\pm$ 536 IU/ml, respectively, and there was an elevation of T_{co}. Twenty four hours after the injections, antiviral activities returned to baseline. However, a second injection of virus 24 h after either viral or poly I:C challenge failed to induce antiviral activity. In conclusion, poly I:C can simulate the tolerogenic action of whole virus, lending support to the hypothesis that constitutional symptoms such as excess sleep and fever in viral infections are mediated by cytokines with IFN activity triggered by virus-associated dsRNA.

328.10

RESPONSES OF NEURONES IN DORSAL VAGAL COMPLEX TO GASTROINTESTINAL ANAPHYLAXIS IN THE RAT. RJ HOLLAND*, WR EWART*, GI Science Research Unit, London Hospital Medical College, London. SPON: Brain Research Association

The aim of the present experiments was to develop a model of GI anaphylaxis in the rat, in order to study the effects of anaphylaxis and anaphylactic mediators on the interpretation of afferent activity by the dorsal vagal complex (DVC) *in vivo*. Male Hooded-Lister rats (150 g) were sensitized with an IP injection containing 10 µg ovalbumin (OA) and 10 mg AlOH in sterile saline. Sham-treated controls were also prepared. Fourteen days later, 0.3 ml of plasma was obtained from all experimental rats and passive cutaneous anaphylaxis (PCA) performed to verify the degree of sensitization. Rats were anesthetized with Equithesin (0.30 ml/100 g IP). The fundus was cannulated and a 2 ml gastric distension was able to be applied. Close arterial injections were able to be given by a cannula placed adjacent to the stomach. Using stainless steel micro-electrodes, extracellular recordings were made from single units in the DVC. Units recorded were either spontaneously active or were evoked by gastric distension. Electrode placement was verified by examination of electrolytic lesion marks. Gastric distension (GD) changed the firing rate in 23 out of 35 units studied; 39% were excited and 61% inhibited. Of the GD sensitive units, both histamine (20 ng IA, n=5) and 5HT (20 ng IA, n=9) produced changes in firing rate. One unit responded to all stimuli tested, with OA (100 µg intragastric) converted the regular frequency to a 'burst-type' configuration. Compound 48/80 (100 ng IG) modulated the activity in 1 of 3 units tested. Thus, some of the observed effects of anaphylaxis on the GI tract may be attributed to the perturbation of vago-vagal reflex activity in the brainstem.

328.11

INTRAVENOUS MORPHINE-INDUCED IMMUNOMODULATION & STEROID RELEASE. L.L. Lockwood, L.H. Silbert*, M. Fleshner, L.R. Watkins, M.L. Laudenslager, [1]K.C. Rice, [1]R.J. Weber, & S.F. Maier. Dept Psych, U CO, Boulder, CO 80309 [1]Lab Med Chem, NIDDK, NIH, Bethesda, MD 20892

Morphine can alter immune function. Use of morphine is widespread and so an understanding of these effects is important. Acute and chronic doses of morphine suppress *in vitro* measures of immune function. However, how accurately *in vitro* immune measures reflect *in vivo* immunocompetence is unknown. Chronic morphine alters *in vivo* function. This is, however, difficult to interpret due to drug tolerance and toxicity. Since i.v. morphine is widely used we determined if acute i.v. morphine would be immunomodulatory on an *in vivo* immune measure. Sprague Dawley rats were injected i.p. with 100ug of antigen, keyhole limpet hemocyanin (KLH), followed immediately by i.v. saline or 5, 10, or 15mg/kg doses of morphine sulfate. Blood samples to measure corticosterone were taken 20, 40, 60, and 120 minutes post injection and blood samples to measure antibody (AB) were taken 7, 9 14, and 21 days post injection. Serum levels of corticosterone were determined using radioimmunoassay (RIA) and serum levels of KLH AB were determined using ELISA. Morphine groups exhibited significant increases in corticosterone levels & significant decreases in anti-KLH AB production. The experiment was repeated in Fischer 344 rats in order to begin to assess generality. Again, morphine produced significant increases in corticosterone & significant decreases in AB production. These results indicate that acute morphine results in immunosuppression as measured by *in vivo* antibody production and that high levels of corticosterone are correlated with these immune system changes. Further studies examining receptor specificity, stereoselectivity, and peripheral vs. central sites of action are being performed. BNS-8808840.

328.12

(+)-PENTAZOCINE AUGMENTS ANTIGEN-SPECIFIC ANTIBODY PRODUCTION TO SHEEP RED BLOOD CELLS AND LIPOPOLYSACCHARIDE IN VIVO. D.J.J. Carr*, B.R. DeCosta*, J.E. Blalock*, and K.C. Rice. Dept. Physiology & Biophysics, UAB, Birmingham, AL 35294 and Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

(+)-Pentazocine selective binding sites have recently been described on lymphocytes. (+)-pentazocine modulates lymphocyte proliferation in vitro in response to concanavalin A, pokeweed mitogen, and lipopolysaccharide (LPS). We investigated (+)-pentazocine for immunoregulatory characteristics following administration in vivo. Female C57Bl/6 mice (n=5) treated with (+)-pentazocine (10mg/kg i.p.) following i.p. injection of sheep red blood cells (SRBCs; $1x10^7$ cells) showed a significant increase in the production of total (60%) and SRBC-specific (135%) IgM antibody by splenic lymphocytes as determined by ELISA. Mice (n=5) treated with phencyclidine (10mg/kg) following i.p. injection of SRBCs showed no apparent change in total or SRBC-specific antibody production by splenic lymphocytes. Mice (n=5/group) treated with (+)-pentazocine (10mg/kg) or phencyclidine (10mg/kg) following i.p. injection with LPS (10ug) showed a significant increase in the production of LPS-specific IgG antibody by splenic lymphocytes as determined by ELISA. The data suggest phencyclidine and (+)-pentazocine operate through different pathways in the regulation of the immune system.

328.13

THE ROLES OF SPLENIC SYMPATHETIC NERVES IN THE SUPPRESSION OF CELLULAR IMMUNITY. T. Katafuchi, S. Take*, Y. Kaizuka* and T. Hori. Dept. of Physiology, Kyushu Univ., Fac. of Med., Fukuoka 812 Japan.

We have recently demonstrated that ICV injection of interferon α (IFNα) in rats resulted in a reduction of splenic natural killer (NK) cytotoxicity, which depends on central opioid receptors and intact splenic innervation. In the present study, we further examined the central and peripheral mechanisms of the immunosuppression mediated by the sympathetic nerves.

In urethane-α chloralose anesthetized rats, ICV injecion of human reconbinat IFNα ($1.5x10^3$-10^4U/rat) produced a dose-dependent excitation of splenic sympathetic nerve activity, which was blocked by intravenous administration of naloxone. Electrical stimulation of the peripheral cut ends of splenic nerves (0.5mA, 0.5msec, 20Hz) resulted in the suppression of the cytotoxic activity of NK cells in the spleen, which was completely blocked by nadolol (β-antagonist), but not by prazosin (α-antagonist). The results suggest that IFNα-induced suppression of NK cell activity is brought about by the activation of splenic sympathetic nerves through a β-adrenergic receptor mediated process. Furthermore, bilateral stimulation of the preoptic/anterior hypothalamus (POA/AH) suppressed the activity of splenic nerves, while lesioning the POA/AH enhanced it.

These findings suggest that the neuronal network between the POA/AH and the splenic sympathetic nerves may play an important role in the suppression of splenic cellular immunity.

328.14

MECHANISMS OF CENTRAL OPIOIDS-INDUCED SUPPRESSION OF RAT PERIPHERAL CELLULAR IMMUNITY. T. Hori, S. Take*, T. Mori*, Y. Kaizuka*, N. Shimizu* and T. Katafuchi. Dept. of Physiology, Kyushu Univ., Fac. of Med., Fukuoka 812, Japan.

Inescapable stress which produces opioid dependent analgesia is known to enhance the growth of implanted tumor cells and to reduce the cytotoxic activity of splenic natural killer (NK) cells in the rat (J. Immunol. 135, 834S, 1985). To see whether the stress-induced suppression of NK activity is mediated by central action of endogenous opioids, we observed the effects of intracerebroventricular (ICV) injection of β-endorphin and interferon α (IFNα), which is known to act on hypothalamic opioid receptors (Brain Res. 454, 361, 1988), on the splenic NK activity of the Wistar rat by the standard chromium release assay. ICV, but not systemic injection of β-endorphin (10-20μg) and recombinant human IFNα (1000-2000 U) produced a dose dependent suppression of splenic NK activity, which was completely blocked by naloxone. The suppression of NK activity was abolished by splenic denervation, but not by adrenalectomy. ICV injection of α–helical CRF reversed the central IFNα-induced suppression of NK activity. ICV injection of CRF (2μg/rat) increased the noradrenaline release in the spleen, which was measured by in vivo microdialysis technique. Furthermore, inescapable immobilization stress caused a rapid increase in the noradrenaline release in conjunction with the suppression of splenic NK activity. The results suggest that activation of brain opioid system by inescapable stress activates the CRF neurons, thereby suppressing the cytotoxic activity of NK cells predominantly through splenic sympathetic innervation.

328.15

SPLENIC REDUCTION IN NOREPINEPHRINE IS FOUND IN MRL-*LPR/LPR* MICE WITH GENERAL ONSET OF LUPUS. S.M. Breneman, J.A. Moynihan, L.J. Grota, S.Y. Felten. Depts Neurobiology & Anatomy and Psychiatry, Univ. Rochester Sch. Med., Rochester, NY 14642.

The MRL-*lpr/lpr* mouse has been used extensively as a murine model of systemic lupus erythematosis. These mice develop an autoimmune disease characterized by increased anti-DNA titers, arthritis, glomerulonephritis, marked lymphadenopathy and splenomegaly. The increased size in the lymphoid organs is due to an expansion of a subset of T-cells which are CD3+, CD4-, and CD8- (double negative, DN).

Sympathetic involvement in neural communication with the immune system has been examined by our lab in other autoimmune models (arthritis). Norepinephrine was measured in spleen in *lpr/lpr* mice along with their matched controls (congenic MRL +/+) at 3, 8, 12, 16, 20, and 24 weeks of age. At eight weeks the first sign of increasing spleen weight from the expansion of the DN T-cells is evident. At 3 wks of age the animals were not different in concentration (pMoles/ g wet weight) or total amount of norepinephrine. At 8 wks, the +/+ mice had a greater total amount of NE which increased with age. In contrast a lower total amount of NE was found in *lpr/lpr* mice which was maintained through 24 weeks.

Reduction appears to be specific to lymphoid tissue, as levels of NE in salivary gland were not different between the two strains, although thymus showed a significant reduction in NE after 16 wks. Supported by Grants NIMH R37 MH42076, NIH RO1 NS25223.

328.16

DEVELOPMENT OF SYMPATHETIC INNERVATION OF *XENOPUS* SPLEEN. K.S. Kinney, S.Y. Felten, and N. Cohen*. Univ. of Rochester Sch. of Med.& Dent., Rochester, NY 14642.

In rodent models, the observation of autonomic innervation of lymphoid organs has, in part, been responsible for an awareness of the fact that immune responses can be modulated by neural activity. Extending these observations to non-mammalian species allows us to begin to understand the phylogeny of neural immune interactions, as well as to take advantage of unusual life histories (e.g. metamorphosis) of different species and address questions about the development of such interactions. For these purposes, an anuran amphibian model has a number of advantages, and we have been using the frog *Xenopus laevis* as such a model. The spleen of *Xenopus* undergoes a dramatic change in both lymphoid and neural organization at the time of metamorphic climax. The larval spleen shows no compartmentation into red pulp and white pulp, and lymphoid innervation is absent. At metamorphosis, the spleen becomes compartmentalized, and innervation develops in the lymphopoietic white pulp areas. Blocking metamorphosis via administration of the goitrogen sodium perchlorate, strikingly delays the development of the adult pattern of both compartmentation and innervation, but with time, the adult pattern eventually appears in blocked animals. This suggests that both developmental stage and chronological age are involved in the signaling of change in splenic compartmentation and innervation. We are currently determining the age at which the adult pattern emerges in perchlorate-treated animals. We are also investigating whether there is a causal relationship between innervation and lymphoid compartmentation of the spleen.

328.17

ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE MURINE IMMUNE RESPONSE TO INFLUENZA VIRUS INFECTION G.Hermann and J.F.Sheridan. Depts. Med. Micro. Immunology and Oral Biology, Ohio State University, Columbus, Ohio 43210

The murine immune response to influenza virus infection has been well-characterized. It progresses from an inflammatory phase to the induction and recruitment of virus-specific effector cells to the site of infection, i.e., both cell-mediated as well as humoral immunity to the influenza virus. Repetitive restraint stress applied during the course of the infection resulted in a decreased inflammatory response as well as depression of virus-specific cell mediated immunity (Sheridan et al., JNI, 1991).

These experiments sought to examine the role of sympathetic innervation of draining lymph nodes in the development of the immune response to influenza virus infection. Surgical resection of the superior cervical ganglia interrupted sympathetic innervation of the superficial cervical and mediastinal lymph nodes as evidenced by a significant drop in catecholamine content in these tissues. The cell-mediated and humoral responses to the infection in sympathectomized animals were quite comparable to the responses in sham-operated animals. Sympathectomized animals survived beyond day 7 of the influenza infection (N=27/29), while 6 of the 29 sham-operated animals died as a consequence of infection. Surgically sympathectomized animals appear to have a very efficient immune response to the influenza virus infection. The immunological response of sympathectomized animals subjected to restraint stress and infection are presently being examined.

328.18

THE DEMONSTRATION OF A POSSIBLE LINK FOR NEURAL-IMMUNE SYSTEM INTERACTION IN THE BELUGA WHALE. T.A. Romano, D.L. Felten, J.A. Olschowka and S.Y. Felten. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Bidirectional communication between the nervous and immune systems has been studied extensively in rodents and primates. Neural-immune interactions were studied in the beluga whale from an evolutionary standpoint, and to investigate the possibility of a nervous-immune system pathway whereby various neurotransmitters and/or neuropeptides released during periods of stress may effect immunocompetence and perhaps result in disease.

The spleen of the beluga was obtained approximatey 1-2 hours postmortem, cut into 1 cm^3 pieces and placed in 10 mls of 4% paraformaldehyde. Twenty-four hours later, the tissue was frozen, cut at 40μm and processed for tyrosine hydroxylase (TH) and neuropeptide-Y (NPY) immunocytochemistry.

Our preliminary results demonstrate both immune compartmentation and innervation of the beluga spleen. TH and NPY positive nerve fibers were found innervating the smooth muscle of the vasculature and trabeculae. Some nerve fibers were seen radiating out from central arteries and entering the periarteriolar lymphatic sheath, a T cell dependent zone. Furthermore, TH and NPY positive nerve fibers were present along the marginal zone where B lymphocytes and macrophages are abundant.

We have demonstrated an anatomical link between the nervous and immune systems in the beluga whale. This anatomical link provides a pathway whereby various physical and psychological stressors, may alter immune function of marine mammals in the wild or captivity. Supported by N00014-89-J-1896 from ONR.

328.19

CATECHOLAMINE MODULATION OF LYMPHOCYTE-ENDOTHELIAL CELL INTERACTIONS. S.L. Carlson and J.P. McGillis, Depts. of Anatomy & Neurobiology and Microbiology & Immunology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084

Lymphocyte migration from the blood into tissues requires interactions between the lymphocytes and the endothelial cells (EC) lining the blood vessels. These interactions are mediated by specific adhesion molecules, eg. homing receptors and LFA-1 on lymphocytes binding to vascular addressins and ICAM-1 on ECs. These adhesion molecules are expressed on ECs in the lymphoid tissues and are induced at sites of inflammation. Catecholamine fibers from the sympathetic nervous system form plexuses around blood vessels and innervate lymphoid tissues. We have hypothesized that catecholamines may modulate the process of lymphocyte binding to ECs and migration into tissues, and thus have examined lymphocyte binding to rat heart-derived EC monolayers *in vitro*. To determine if catecholamines can modulate lymphocyte binding to ECs, the monolayers were incubated with the catecholamine agonist isoproterenol (10μM) for 4 hours. Peripheral lymph node derived lymphocytes were subsequently allowed to adhere for 1 hour. The number of adherent cells were counted microscopically after fixing and staining the cultures. Preliminary studies have shown that ECs bind fewer lymphocytes after exposure to isoproterenol compared to untreated ECs. We have confirmed that isoproterenol can signal the ECs by measuring increased cAMP levels in ECs after isoproterenol stimulation. Studies are being conducted to examine other time points and to determine if catecholamines will alter the ability of interleukin-1 to enhance lymphocyte binding to ECs. In addition, the effect of catecholamine pretreatment of the lymphocytes prior to the EC binding assay will be explored. (Supported by Univ. Kentucky Med. Ctr. Research Fund)

328.20

ALTERATIONS OF SPLENIC MACROPHAGE INTERLEUKIN-1 SECRETION AS WELL AS SYMPATHETIC AND NEUROENDOCRINE ACTIVITY FOLLOWING SYSTEMIC INTERLEUKIN-1ß ADMINISTRATION IN RATS. C.A.Y. Vriend, L. Janz*, L. Zuo*, J. Green-Johnson,*, S. Zalcman, D. Dyck* and A.H. Greenberg*. University of Manitoba, Winnipeg, Canada.

Interleukin-1 (IL-1) appears to activate the hypothalamic-pituitary-adrenal (HPA) axis and to suppress splenic macrophage secretion of IL-1 after intracerebroventricular injection which is dependent upon an intact splenic sympathetic nerve. There is some evidence that IL-1 induced systemically by antigen administration is transported into the brain or enters at the circumventricular organs. The present investigation assessed whether systemic administration of recombinant human interleukin-1ß (rIL-1ß) (100 or 200 ng) would influence (i) splenic sympathetic activity as well as the HPA axis and, (ii) splenic macrophage IL-1 secretion in Sprague-Dawley rats. Two hours following intraperitoneal rIL-1ß administration, marked reductions of splenic macrophage IL-1 secretion were evident at maximal LPS stimulation in vitro. Moreover, large decreases in splenic norepinephrine (NE) levels were observed 2 hours post-injection as well as elevated plasma ACTH and corticosterone levels. Taken together, these data suggest changes in splenic sympathetic activity coincidental with suppression of splenic macrophage secretion of IL-1.

328.21

RESPONSE OF METABOTROPIC RECEPTORS IN DISCRETE AREAS OF THE BRAIN IN THE LEWIS RAT R. Bernardini, G. Mauceri*, V. D'Agata*, and F. Nicoletti. Institute of Pharmacology, University of Catania School of Medicine, 95125 Catania, Italy.

It has long been known that the hippocampus is as important target for the extrahypothalamic action of adrenal steroids. Hence, we have decided to study how glucocorticoids influence hippocampal neurotrasmission.

As an initial step, we have measured the responsiveness of transmitter receptors coupled to polyphosphoinositide (PPI) hydrolysis in hippocampal slices prepared from inbred Lewis (Lew/N) rats, which exhibit a reduced activity in the hypothalamic-pituitary-adrenal (HPA) axis, due to a defect in the biosynthesis and secretion of corticotropin-releasing hormone (CRH).

Stimulation of [^{3}H]-inositolmonophoshate (InsP) formation by norepinephrine (NE) in hippocampal slices was greater in Lewis rats than in histocompatible Fisher rats (F344/N). Stimulation by ibotenic acid (a glutamate receptor agonist) was slightly reduced, whereas stimulation by carbamylcholine (a muscarinic receptor agonist) did not change. The action of NE was also increased in hypothalamic slices from Lewis rats, albeit to a lesser extent. All transmitter receptor agonists stimulated [^{3}H]-InsP formation to the same extent in cortical slices from Lewis or Fisher rats.

These results suggest that a chronic impairment of the HPA axis leads to an increased activity of α_1-adrenergic receptors coupled to PPI hydrolysis in the hippocampus.

TEMPERATURE REGULATION AND FEVER

329.1

KETAMINE ANESTHESIA MODIFIES AGONIST/ANTAGONISTS EFFECTS ON TC IN RATS. Simpson, C.W. and G.E. Resch: Sch. of Basic Life Sciences, Univ. of Missouri-Kansas City, Kansas City, MO 64108-2792

Unanesthetized and unrestrained rats responded to doses of PGE_2 that were 15 orders of magnitude below those of ketamine anesthetized rats ($P<.001$). These are the lowest doses ever reported for PGE_2 in MAHPOA heat gain sites. The Tc in unanesthetized rats increased ($P<.02$) following 10[-30] g, but not 10[-33] g or 10[-36] g ($P>.48$) of PGE_2. In ketamine anesthetized rats Tc values increased following 500X10[-15] g ($P<.02$), but not at 10[-15] g, 10[-18] g or 10[-21] g ($P>.13$) of PGE_2. Ketamine anesthetized and unanesthetized rats' Tc responses following PGE_2 were antagonized by the receptor blocker, SC19220 ($P<.004$). The blockade in unanesthetized rats persisted at 50 min ($P<.001$) and at 90 min ($P<.004$), but not at 120 min. Ketamine anesthetized rats showed no PGE_2 Tc responses in SC19220 treated rats vs PGE_2 alone at 50 min ($P<.001$), or at 90, 120, and 240 min ($P<.0001$). The lowest effective dose of SC19220 tested in ketamine anesthetized rats was $1X10^{-24}$g. To test whether this anesthesia induced a similarly persistant blockade with other antagonists, hexamethonium, atropine, and methylergonovine were also tested. They exhibited the same blockade pattern as did SC19220 to their respective agonists. Therefore, the duration of blockade under ketamine appears to be a function of the anesthesia, rather than related to the characteristics of individual antagonists. Antagonists data in the present report suggest that the anesthetized rat may be much more sensitive to antagonist, which may become irreversible while anesthesia persists. These data suggest that the ketamine anesthetized rat might be an important model to investigate agonist/antagonist receptor binding changes at specific brain sites as well as a method to test anesthetic effects on membranes at functionally important brain sites. Supported by AFOSR #87-0297.

329.2

EXTRACELLULAR AND INTRACELLULAR RECORDINGS FROM THERMOSENSITIVE NEURONS IN THE RAT DORSAL MOTOR NUCLEUS OF THE VAGUS. H. Muratani*, T. Katafuchi, T. Hori[1] and T. Kosaka[2] Depts. of Physiology[1] & Anatomy[2], Kyushu Univ., Fac. of Med., Fukuoka 812 Japan.

Extracellular and intracellular recordings were made from neurons in the rat dorsal motor nucleus of the vagus (DMV) to investigate their thermosensitivity and membrane properties. Fifteen (21%) of 72 DMV neurons recorded extracellularly were warm-sensitive neurons which increase the firing rate to a rise in temperature with thermal coefficients>0.6 impulses/sec and $Q_{10}>2$ and three (4%) were cold-sensitive neurons which responded with thermal coefficients<-0.5 and $Q_{10}<0.5$. Thermosensitivity was retained during perfusion with low Ca^{2+}/high Mg^{2+} solution. Both the warm- and cold-sensitive neurons demonstrated linear and non-linear frequency-temperature response curves. Warm-sensitive neurons showed depolarization with $Q_{10}>2$ during warming, which was associated with a decrease in input resistance. The warming-induced depolarization was abolished in the presence of TTX ($5x10^{-6}$M). Cold-sensitive neurons ($Q_{10}<0.5$) depolarized with an increase in input resistance during a fall in temperature. Two current-voltage curves of a warm-sensitive neuron in hyper- and hypothermic ranges crossed at about +20 mV of membrane potential. On the other hand, those of a cold-sensitive neuron did at about -82mV. These results suggest that the depolarization of warm-sensitive neurons during warming is caused by opening the TTX sensitive Na^+ channels, while that of cold-sensitive neurons during cooling is induced by closing the K^+ channels.

329.3

THE HYPOTHERMIZING EFFECT OF INTRAPREOPTICALLY (iPO) MICRODIALYZED NOREPINEPHRINE (NE) IS MEDIATED VIA α_2-ADRENOCEPTORS. N. Quan, L. Xin, A.L. Ungar, and C.M. Blatteis. Department of Physiology & Biophysics, University of Tennessee, Memphis, TN 38163

Our previous findings suggested that the hypothermizing action of NE microdialyzed iPO may be mediated by α-adrenergic receptors. To identify the adrenoceptor subtype(s) involved, we microdialyzed into the PO of conscious guinea pigs one of the following adrenergic agonists or antagonists: the α_1-agonist methoxamine, the α_1-antagonist prazosin, the α_2-antagonists yohimbine (YOH) or rauwolscine (RAW), the β_1-agonist dobutamine, the β_1-antagonists practolol or atenolol, the β_2-agonist zintolol, the β_2-antagonist butoxamine (all dissolved in pyrogen-free saline [PFS] to 1 µg/µl), the α_2-agonist clonidine (CLO, 0.5, 5 or 10 µg/µl), or NE (10 µg/µl). Other animals received one of the following combinations of drugs: 1 µg/µl YOH+ 0.5 µg/µl CLO, 1 µg/µl RAW+ 0.5 µg/µl CLO, 1 µg/µl YOH+ 10 µg/µl NE, or 1 µg/µl RAW+ 10 µg/µl NE. The core (T_{co}) and ear skin (T_{sk}) temperatures of the animals were monitored throughout the experiments. The α_1-, β_1-, and β_2-agonists and antagonists did not induce significant T_{co} changes. The α_2-agonist CLO produced dose-dependent T_{co} falls. The α_2-antagonist YOH and RAW evoked no thermal effect *per se*, but they abolished the T_{co} fall induced by co-dialyzed CLO. The microdialysis of NE evoked, as before, a -0.7±0.2°C T_{co} fall which, too, was abolished by co-dialyzed YOH or RAW. No adrenoceptor agonist induced T_{sk} changes. These results indicate that the T_{co} fall induced by iPO microdialyzed NE is due to a reduction in metabolic heat production, mediated by α_2-adrenoceptors. (Supported by NIH NS22716.)

329.4

CHRONIC ADMINISTRATION OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)-TETRALIN (8-OH-DPAT) ALTERS THE HYPERTHERMIC RESPONSE TO HEAT STRESS. M.O. Thornton, D.A. Armstrong*, S.T. Ahlers, and J.R. Thomas Naval Medical Research Institute, Bethesda, MD 20889.

A reduction of the hyperthermic response to heat stress is a hallmark of heat acclimation. Since 5-hydroxy-tryptamine (5HT) appears to be involved in thermo-regulation, it is possible that plastic changes in specific serotonin sub-receptor types may mediate heat acclimation. Accordingly, the present study examined whether the development of tolerance to the hypothermic effects of the $5HT_{1A}$ agonist 8-OH-DPAT administered chronically would alter the response of rats to environmental heat stress. Ten rats were implanted with intra-abdominal telemetry thermistors and a baseline (PRE) hyperthermic response to a heat stress (38°C for 90 min) was obtained. Rats then received daily i.p. injections of 0.5 mg/kg 8-OH-DPAT or equivalent volume of 0.9% saline for 7 consecutive days. Rats were then re-exposed (POST) to the heat as before. Administration of 8-OH-DPAT produced a decrease of 2.0°C in core temperature on day 1 and a decrease of 0.48°C on day 7. In rats given interpolated saline injections, the difference in the magnitude of hyperthermic response between the PRE and POST heat exposures was + 0.1°C. In contrast, the group given chronic 8-OH-DPAT showed a slight attenuation of -0.38°C in the hyperthermic response during the second heat exposure which approached significance relative to the saline condition. These results suggest a possible modulatory role of the serotonin $5HT_{1A}$ sub-receptor in heat acclimation.

329.5

THERMOSENSITIVE CHARACTERISTICS OF HYPOTHALAMIC NEURONS DETERMINED BY WHOLE-CELL RECORDING. J.D. Griffin* & J.A. Boulant. Physiology Dept., The Ohio State University, Columbus, OH 43210.

Neuronal thermosensitivity was studied by tight-seal, whole-cell recordings in horizontal tissue slices of the rat hypothalamus. Warm sensitive and temperature insensitive neurons were categorized by their firing rate responses to temperature changes. Compared to previous sharp-tip recordings, the whole-cell recorded neurons displayed very high input resistances (i.e., >200 megohms), and several neurons had pacemaker potentials that could be reset by brief injections of depolarizing current. In warm sensitive neurons, the rate of rise of the pacemaker potential was strongly temperature dependent, and these neurons had sharper after-hyperpolarizations compared to the temperature insensitive neurons. In addition, neuronal morphology was determined by intracellular filling with Lucifer Yellow. While temperature insensitive neurons displayed a variety of dentritic patterns with little branching, warm sensitive neurons displayed branching bipolar dentrites projecting laterally and medially. (Supported by NIH grant NS-14644.)

329.6

OSMOSENSITIVITY AND THERMOSENSITIVITY OF NEURONS IN RAT MEDIAN PREOPTIC NUCLEUS. K.A. Travis and A.K. Johnson, Depts. of Psychology and Pharmacology, Univ. of Iowa, Iowa City, Iowa, 52242.

The median preoptic MnPO nucleus has been shown to be important in the regulation of blood pressure and body fluids. This study evaluated the sensitivity of ventral MnPO neurons of male Sprague Dawley rats to changes in perfusate osmolality and temperature. In-vitro neuronal activity was recorded in midsagittal slices perfused with control (300 mOsm/kg), hyposmotic (280 mOsm/kg) and hyperosmotic (320 mOsm/kg) media.

Of the neurons tested, 28.6% (n=16) were temperature sensitive and 71.4% (n=40) were temperature insensitive. Fourteen of the temperature sensitive and 18 of the temperature insensitive neurons were treated with one or both of the experimental media. The majority of neurons tested in the hyperosmotic solution did not change their firing rate (16/21). The remaining 5 neurons decreased their activity. The majority of neurons tested in the hyposmotic solution were also unresponsive to the treatment (16/23). Five of the 7 neurons which responded to the treatment increased their activity. There was no difference in the proportions of temperature sensitive and temperature insensitive neurons which responded to the osmotic challenges.

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329.7

CONTRASTING TEMPERATURE RESPONSES ELICITED FROM BROWN ADIPOSE TISSUE FOLLOWING PREOPTIC STIMULATION. L. Kelly* and C. Bielajew, Dept. of Psychology, University of Ottawa, Ottawa, Canada K1N 6N5.

Cooling of the preoptic hypothalamic area (PO) has been shown to activate neurons in the ventromedial hypothalamus (VMH), and through the VMH, to cause an increase in the thermogenic activity of brown adipose tissue (BAT) (Imai-Matsumura, 1987). We reasoned that those VMH sites responsible for BAT thermogenesis should be synaptically linked to PO thermosensitive cells. As a first step in addressing this hypothesis, we examined the effect of PO stimulation alone on BAT temperature response. Moveable electrodes were implanted in seven male hooded rats, and lowered in 0.16mm increments. BAT temperature was monitored at each stimulated site, with core temperature maintained at 37°C throughout. Stimulation trains comprised 25x300 A square wave cathodal pulses of 100 s duration; trains were cycled in a 500ms on/500ms off pattern for 60s per site. One site in each of two mid-PO descents yielded BAT temperature increases, in contrast to the decrease in brown fat temperature observed with two other descents located in the paraventricular hypothalamic nucleus, and just lateral to that nucleus. Temperature drops ranged from 0.5 to 0.9°C, with a return to baseline in 30 to 120 minutes. Three descents showed no BAT effect; two were found in the medial corticohypothalamic tract and one in the dorsal hypothalamic area.

329.8

INTRASCAPULAR BROWN ADIPOSE TISSUE (IBAT) RESPONSES OF ANESTHETIZED LONG EVANS OR SPRAGUE DAWLEY RATS FOLLOWING CNS ELECTRICAL STIMULATION OR NOREPINEPHRINE INFUSION. J. Thornhill, T. Hugie* and I. Halvorson*, Department of Physiology, University of Saskatchewan, Saskatoon, Saskatchewan Canada S7N 0W0

IBAT temperature, surface and colonic temperature responses were compared in weight-matched Long Evans and Sprague Dawley rats acclimated to 21°C following CNS electrical stimulation (0.5 ms pulses of 100 or 250 µA at 50 Hz for 30 sec) or following intravenous saline or noradrenaline infusion, 50 µg/kg total dose infused at 100 µl per min for 10 min. These electrical or chemical stimuli were retested following propranolol HCl administration (2.5 mg/kg iv). Rises in IBAT temperature above core occurred in the Long Evans group following either noradrenaline infusion or electrical stimulation into the ventromedial hypothalamic area (VMH) or into a lateral preoptic area (LPA), increases subsequently reduced when retested after propranolol. The only significant increase in IBAT temperature in the Sprague Dawley group occurred following noradrenaline infusion. Taken with previous results these findings suggest further that a strain difference exists between lean Long Evans and Sprague Dawley rats acclimated to 21°C in their thermogenic capacity to evoke IBAT thermogenesis following CNS electrical stimulation.

This work is supported by MRC of Canada.

329.9

AMBIENT TEMPERATURE AFFECTS MDP-INDUCED FEVER. W.Th. Perschel* and L. Amini-Sereshki. Department of Adult Health & Illness, School of Nursing and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA. 19104.

In the present preliminary experiments we have studied the effects of ambient temperature (T_a) on the characteristics of a fever induced by Muramyldipeptide (MDP) in cat. Brain temperature (T_b), subcutaneous temperature (Ts), and electromyogram (EMG) were recorded in two cats at different T_as of 10, 20 and 30°C. After MDP (25 ug/kg, i.v.) was injected, T_b started to increase, shivering occurred, and the animal assumed a curled posture. In 13/18 cases the rise in T_b preceded the onset of shivering. After the T_b reached a maximum level (T_b = 1.24-2.05°C), it started to decrease toward the normal level. The time between the injection and the rise in T_b, the onset of shivering, and the time during which T_b increased were affected by T_a, all being shorter at 10° and longer at 30°. There was no correlation between the ambient temperature and delta T_b or delta T_s in these experiements. The present study suggests that the ambient temperature may affect some characteristics of a fever reaction in cats.

329.10

CHARACTERIZATION OF THE PGE1 FEVER RESPONSE IN THE URETHANE ANESTHETIZED RAT. T.J. Malkinson* W.L. Veale, and K.E. Cooper. Faculty of Medicine University of Calgary, Calgary, Canada. T2N 4N1

In thermoregulatory physiology pharmacological agents are often administered by direct injection into a lateral cerebral ventricle (icv). We have shown that urethane anesthetized (1.5 gm/kg) adult male Wistar rats will respond in a similar manner to the conscious animal to icv PGE1. (Am. J. Physiol. 255:R73-R81, 1988). We thought it to be of interest to examine further this febrile response to icv PGE1.

Analysis of 275 fever responses to 300ng PGE1 showed that the maximum temperature reached fell on a normal distribution. If a second injection of PGE1 is given to the animal after the first fever is completed (180 min) there is no significant difference between the two sequential fevers in the same animal. (n=30 animals). We thought it to be of interest to examine if the rate of icv injection would influence the febrile response. Fifty animals each received two injections of 300 ng PGE1 at various rates ranging from instantaneous to 180 seconds. We found no correlation between the peak of the fever (r=0.03) or time until peak was reached (r=0.07) and the time taken to administer the PGE1 into the LCV.

329.11

SYSTEMIC ENDOTOXIN INDUCES C-FOS GENE EXPRESSION IN MULTIPLE BRAIN REGIONS. S.M. Sagar and K.J. Price*. Neurology Service, VA Medical Center, and Dept. of Neurology, Univ. of California, San Francisco, CA 94143.

To examine the functional neuroanatomy of the febrile response, endotoxin (lipopolysaccharide, LPS) 150 µg/kg was administered through indwelling femoral vein catheters to adult, male Long Evans rats. Controls received saline vehicle. At varying time intervals, brains were examined for Fos immunostaining with a monoclonal antibody thought to be specific for authentic Fos. LPS induced Fos nuclear immunostaining in many brain regions, including circumventricular organs (organum vasculosum of the lamina terminalis, subfornical organ and area postreme), the anterior hypothalamic area (AV3V), magnocellular neurons of the paraventricular nu. (PVN) and supraoptic nu., arcuate nu., locus coeruleus, nu. tractus solitarius, and the A1 cell group of the ventrolateral medulla. Double label immunocytochemistry demonstrated that, within the magnocellular neurons of the hypothalamus, a greater proportion of oxytocin than vasopressin immunoreactive neurons expressed Fos in response to LPS. In addition, regions that respond non-specifically to stress, including cerebral cortex, central nu. of amygdala and parvocellular neurons of the PVN, also demonstrate Fos immunostaining in response to LPS. The Fos response was seen within 1 hr of LPS injection, peaked at about 3 hr and resolved by 24 hr. These results verify the known regional anatomy of the febrile response and permit the further investigation of that response at the cellular level.

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329.12

BLOCKADE OF FEVER BY INTRAPREOPTICALLY ADMINISTERED SUBSTANCE P ANTAGONIST, BUT NOT INDOMETHACIN. C. M. Blatteis, L. Xin, A L. Ungar and N. Quan, University of Tennessee, Memphis, TN 38163

Fever is thought to result from as yet not understood actions of pyrogens on thermosensitive neurons in the preoptic area (PO). Prostaglandin E_2 (PGE) is believed to modulate this effect. We showed previously that the integrity of the *organum vasculosum laminae terminalis* (OVLT) is necessary for fever induction by bloodborne cytokines and suggested that they may interact with receptors in this site, evoking secondary signals that transduce their messages. These signals could be brain-synthesized cytokines (*e.g.*, interleukin-1 [IL1]) or PGE, or their inducers. In this study, we microdialyzed the cyclooxygenase inhibitor, indomethacin (indo, 10 µg/µl for 5 h at 2 µl/min), into the PO (iPO) of conscious guinea pigs beginning 2 h before the i.v. injection of *S. enteritidis* lipopolysaccharide (LPS, 2 µg/kg); or we microinjected iPO the substance P antagonist, [D-Pro^2, D-Phe^7, D-Trp^9]-substance P (antiSP, 20 µg/µg), just before LPS i.v. SP stimulates both IL1 and PGE release from macrophages. Indo reduced the LPS-induced febrile rise (T_{co} ↑1.5°C) by 0.5°C, without affecting its bimodal course; given i.m. (10 mg/kg), it blocked fever completely. AntiSP attenuated the febrile response by 1.3°C and abolished its bimodal pattern. SP (20 µg/µl), microinjected iPO, caused a unimodal 1.5°C T_{co} rise; given i.v.(5 µg/kg), it killed the animals. These data suggest that PGE probably is not a fever mediator within the PO, and that SP may be a pyrogenic neuromodulator in this site either by acting directly on thermosensitive neurons or by inducing IL1 locally. (Supported by NIH NS 22716.)

329.13

INTERLEUKIN-1β RECEPTOR ANTAGONIST BLOCKS THE EFFECTS OF INTERLEUKIN-1β ON NEURONAL ACTIVITY IN GUINEA PIG PREOPTIC AREA SLICES. L. Xin and C. M. Blatteis. Department of Physiology & Biophysics, University of Tennessee, Memphis, Memphis, TN 38163

Interleukin-1β (IL-1) is a cytokine that mediates many host defense responses against infection. One of these responses is fever production. The involvement of IL1 in this effect has been repeatedly substantiated by the rise in core temperature it provokes when it is injected systemically or centrally. Consequently, it has been suggested that IL-1β may be an endogenous pyrogen. The development of fever is generally attended by a decrease in the firing rates (FR) of warm-sensitive (W) neurons and an increase in those of cold-sensitive (C) neurons, particularly in the preoptic area (POA); these neurons are presumed to be the ultimate targets of endogenous pyrogens and to modulate the febrile response. Recently, a human recombinant IL-1β-receptor antagonist (hrIL-1βra) has been cloned and reported to block IL1β-induced fever and sleep in rabbits. This study was undertaken to investigate whether hrIL-1βra may block the effects of hrIL-1β on the FR of W and C neurons in guinea pig POA slices. hrIL-1β (500 ng/ml) reduced the FR of 17 W neurons and increased those of 3 C neurons recorded; it had no effect on the FR of 8 thermally insensitive neurons. hrIL-1βra (0.5 mg/ml), which had no effect by itself on the FR of all the neurons, blocked the hrIL-1β-induced FR changes of 16 of the 17 W and of all 3 C neurons when given before the cytokine. These results support the hypothesis, therefore, that IL-1β may be an endogenous pyrogen acting on POA thermosensitive neurons to induce fever production. (Supported by NIH grant NS-22716.)

329.14

PROSTAGLANDIN E_1 INDUCED FEVER DURING ESTRUS CYCLE IN THE RAT. S.M.Martin, T.J.Malkinson*, W.L.Veale and O.J.Pittman. Biology Department, Mt. St. Vincent University, Halifax, NS B3M 2J6 and Neuroscience Research Group, The University of Calgary, Calgary, AB T2N 4N1 Canada.

Fever height and duration in male rats have been shown to be influenced by the peptide arginine vasopressin (AVP) which acts on receptors in the ventral septal area (VSA). The source of this AVP is the bed nucleus of the stria terminalis (BST), a sexually dimorphic nucleus, in which the synthesis of AVP is dependent upon sex hormones. In light of differences in the levels of such hormones in males and females and throughout the various stages of the estrus cycle, we have examined the febrile response to PGE_1 in virgin, female rats.

Anesthetized, Sprague-Dawley rats were implanted with bilateral, intracerebroventricular cannulae for central injection of PGE_1 and abdominal transmitters for constant monitoring of body temperature by telemetry. After recovery from surgery, three doses of PGE_1 (2ng, 20ng, 100ng) were delivered during each of four phases of the estrus cycle, determined from inspection of vaginal smears. The peak fevers evoked for all animals in response to 2, 20, 100 ng PGE_1 were 0.73 ± 0.08°C; 1.22 ± 0.11°C; 1.87 ± 0.12°C, respectively. The fevers were higher than those observed following similar doses of PGE_1 in male rats. Other than the resting body temperature, which was higher during estrus, there was no significant difference in the evoked fevers during the different phases of the estrus cycle. Supported by MRC of Canada and Mt. St. Vincent University.

329.15

THE EFFECTS OF IONTOPHORETICALLY APPLIED IL-1β ON SINGLE UNIT ACTIVITY IN THE BED NUCLEUS OF THE STRIA TERMINALIS (BST).

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IL-1β is one of several endogenous pyrogens elaborated by immunologically stimulated phagocytes. IL-1 is also present within the CNS being expressed by neurons as well as glial cells. Nerve terminals containing IL-1 as well as IL-1 binding sites have been detected in the area of the BST. Because the BST has been implicated in the control of fever by virtue of its strong vasopressinergic projection to the ventral septal area (VSA), we sought to investigate the effects of local application of IL-1β on BST neurons demonstrating electrical connectivity with the VSA. Single unit extracellular voltage recordings were made in the BST of urethane-anesthetized (1.5g/kg,ip) S-D rats. Iontophoresis was performed via a 7 barrel pipette glued to the recording electrode. Stimulation of the VSA, via a bipolar stimulating electrode, was used to elicit antidromic or orthodromic potentials in BST neurons. Of 39 such neurons (all of which were excited by glutamate), 18 units (46%) were excited, 3 (8%) were inhibited and 18 (46%) not affected by IL-1β. BST neurons orthodromically excited or inhibited by VSA stimulation were most often excited by IL-1β (67%). In contrast BST neurons antidromically invaded following VSA stimulation were seldom affected by IL-1β (2/14,18%). Typically IL-1β evoked a slow onset (1-5 min) long lasting (> 5min) increase in firing rate. Administration of vehicle solution (PBS, pH 7.4) via a separate barrel of the electrode was without effect in each case. The effect of iontophoretic Na^+ salicylate was also tested in 7 neurons excited by IL-1β. In all cases salicylate partially or totally reversed the effects of IL-1β without affecting basal firing rate on its own. These results indicate that IL-1β, an endogenous pyrogen and neuroimmunomodulator, has potent excitatory effects on BST neurons, particularly those cells receiving orthodromic input from the VSA.

329.16

EFFECTS OF CHRONIC INFUSION OF A VASOPRESSIN ANTAGONIST INTO THE VSA OF THE RAT DURING FEVER INDUCED BY BACTERIAL INFECTION.

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Physiological evidence suggests that arginine-vasopressin (AVP) may function as an endogenous antipyretic. Perfusion of AVP within the ventral septal area (VSA) of the brain reduces the fever induced by bacterial endotoxins or prostaglandin E. The present study examines the effects of chronic infusion of a V1 receptor antagonist into the VSA during fever induced by live bacterial infection. Telemetry was used for continuous measurement of body temperature in the unhandled rat. Saline (0.5 ul/hr) or a V1 antagonist (Manning Compound; 0.5 ug/hr) was infused continuously via osmotic minipumps for 5 days into cannulae implanted in the VSA. On day 2, rats were infected by i.p. injection of bacteria (E.coli). Febrile temperature was higher in rats infused with a V1 receptor antagonist ($n=5$) compared to those infused with saline ($n=6$) for 3 days after the bacteria injection, although these increases were only significant in the early evenings of day 3 and 4 ($p<0.05$). These results suggest that infusion of a V1 receptor antagonist into the VSA may hinder the recovery of fever during bacterial infection, and they, therefore further support the hypothesis that AVP functions as an endogenous antipyretic.

329.17

DESTRUCTION OF DIFFERENT FIBER TRACTS UNDERLIES DEVELOPMENT OF LATERAL HYPOTHALAMIC LESION-INDUCED HYPERTHERMIA AND LOSS OF BOMBESIN-INDUCED HYPOTHERMIA.

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Relative roles of fibers crossing the borders of the lateral hypothalamus (LH) were assessed for involvement in LH lesion-induced hyperthermia and bombesin-induced hypothermia. 24 h food-deprived anesthetized (methohexital, 50 mg/kg ip) male Sprague-Dawley rats (300-370 g) received control surgery or bilateral coronal knife cuts on the anterior or posterior border, or parasagittal knife cuts on the medial or lateral border, of the LH. Surgery was immediately followed by intracisternal injection of bombesin (500 ng/rat) or vehicle (saline, 10 μl). Rectal temperature was measured just before anesthetization and 2 h after intracisternal injection. Cuts on the anterior border of the LH produced marked hyperthermia (+1.8°C, $p<.03$) which was attenuated by bombesin (-1.3°C, $p<.05$). Medial cuts caused marked but nonsignificant (+1.4°C, $p=.12$) hyperthermia which was also attenuated by bombesin (-2.5°C, $p<.02$). In contrast, lateral cuts did not produce hyperthermia (-0.2°C, ns) but completely prevented bombesin hypothermia (+0.3°C, ns). Posterior cuts showed moderate nonsignificant (+1.0°C, $p=.15$) hyperthermia which was moderately and nonsignificantly attenuated by bombesin (-0.6°C, $p=.13$). LH lesion-induced hyperthermia and bombesin-induced hypothermia primarily involve fibers crossing different borders of the LH, and therefore appear to involve separate thermoregulatory pathways. [Supported by NS20660 (M.W.G.), University Research Grant (C.V.G.), AM30110 (Y.T.), and NS7687 (D.N.).]

329.18

ELECTRON MICROSCOPIC ANALYSIS OF SEPTAL AREA PROJECTIONS INVOLVED IN FEVER REGULATION.

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Growing evidence suggests the ventral septal area (VSA) of the brain is involved in the regulation of febrile body temperature. The VSA contains axon terminals immunoreactive to the neuropeptide, arginine vasopressin as well as vasopressin receptors. Release of endogenous stores of vasopressin, or exogenous application of minute quantities of vasopressin within the VSA, but not the surrounding tissue, attenuate the magnitude of pyrogen-induced fever. Previous anatomical experiments using anterograde tracers placed within the vasopressin-sensitive area demonstrated septal efferent projections to CNS thermoregulatory centers. In the present study, WGA-HRP was pressure-injected (20nl, 2% solution) into the VSA of anesthetized rats. Electron microscopic analysis of labeled axons showed that VSA efferents form synaptic contacts in the lateral habenula, dorsomedial hypothalamus (DMH), arcuate nucleus of the hypothalamus (arc) and the ventral tegmental area (VTA) of the midbrain. HRP-labeled terminals with clear, rounded vesicles formed axo-dendritic, asymmetric synapses in each of the above target areas. In the lateral habenula, efferents with pleomorphic vesicles and symmetric synapses were also observed. Target nuclei for VSA efferents contain neurons that are believed to participate in the control of energy utilization, acute phase reactions and normal thermoregulatory processes. These data suggest neuroanatomical substrates for the central antipyretic actions of vasopressin. (Supported by Dalhousie Medical Research Committee Grant).

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONS: PHYSIOLOGY

330.1

SINGLE-TRIAL DETECTION OF SOMATIC EVOKED MAGNETIC FIELD FROM AN IN VIVO SWINE PREPARATION.

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A high-resolution magnetic field detector (Biomagnetic Technologies) was used to measure the magnetic field from the brain of 3 week-old farm swines (Sus scrofa) evoked by transcutaneous stimulation of the snout (10 mA, 0.3 ms). The juvenile swine was anesthetized initially with ketamine (20 mg/kg i.m.) and xylazine (1 mg/kg i.m.), the femoral vein was canulated for infusion of anesthetics and the dorsal portion of the skull was removed bilaterally. Then, sodium pentobarbital was administered (25 mg/kg i.v.) prior to recording of the somatic evoked field (SEF). The anesthetized animal was placed in a head holder and positioned under the detector. The stimulation produced an SEF predominantly over the somatosensory area of the cortex contralateral to the side of stimulation. The responses over the measurement plane (3 mm above the vertex) were sufficiently strong (as much as 10-20 picotesla) for unaveraged analysis of the SEF (bandwidth = 0.3 to 1 kHz). The field mapping at 200 locations required less than 1.5 hrs. The polarity reversal of the SEF was observed across a distance of 1 cm indicating cortical activity underlying the SEF. The SEF showed activities over a wide area of the cortex extending over 2-3 cm along the anteroposterior and lateromedial directions. The temporal waveform varied across positions over the field plane, indicating the presense of sources activated asynchronously over time, and consisted predominantly of a triphasic waveform with a latency window of 15-50 ms. Supported by NINDS NS21149, NSF Biological Instrumentation Program grant DIR-8820556 and by Dept. Veterans Affairs.

330.2

CURRENT SOURCE DENSITY ANALYSIS OF SOMATOSENSORY EVOKED POTENTIALS IN SENSORIMOTOR CORTEX OF THE MONKEY.

N.S. Nicholson, S. Seto*, J.C. Arezzo, Albert Einstein College of Medicine, Bronx, New York, 10461.

The initial cortical components of the somatosensory evoked potential (SEP) provide an important index of the integrity of primary sensory and motor cortical regions. The precise anatomical and physiological generators of these components remain controversial. The present study examines the laminar distribution of SEPs in sensorimotor cortex, along with associated multiple unit activity (MUA) and current source density (CSD). Epidural and intracranial data were collected in awake Macaca fascicularis. Stimuli consisted of 100 μsec square pulses delivered to the median nerve at the wrist. Intracortical recording utilized a multichannel (16) microelectrode with interchannel spacing of 150 μm.

The earliest "near field" activity detected within somatosensory cortex (SI) consists of a brief current sink localized (based on physiological criteria) to lamina 4. This sink has an onset latency of approximately 6-7 msec which is coincident with the initial MUA burst in SI and subjacent thalamocortical radiations. The pattern of current flow in and around lamina 4 is consistent with early activation of additional localized neural elements. Centered approximately 150-300 μm above and below this early sink are later and larger sinks. These findings suggest the initial component of the cortical SEP partially reflects the depolarization of thalamocortical terminals and the activation of lamina 4 interneurons, while the later principal cortical positivity (P1) represents the subsequent activation of laminae 3 and 5 pyramidal neurons. (Supported by MH06723 and MH15788).

330.3

THE FIRST (SI) AND SECOND (SII) SOMATOSENSORY CORTICES OF ONE HEMISPHERE DO NOT MEDIATE THE IPSILATERAL EVOKED POTENTIAL WITHIN THE SII OF THE OPPOSITE HEMISPHERE IN CAT. G.M.Murray*, H.Zhang*, M.Chan*, E.Low*, and M.J.Rowe*, School of Physiology and Pharmacology, Univ. of NSW, and *Dept. of Prosthetic Dentistry, Univ. of Sydney, Sydney, NSW, Australia.

The SII is said to contain a prominent representation of the ipsilateral body surface. The aim of this investigation was to determine whether the ipsilateral forepaw representation within the SII area depended on the integrity of the forepaw representations within the SI and SII of the opposite hemisphere. Twelve cats were anaesthetised with chloralose and the forepaw representations within the SI and SII of one side were mapped by recording surface potentials evoked by a mechanical step stimulus delivered to the contralateral forepaw; the ipsilateral focus within the SII of the same side as the mechanical stimulus was also mapped. A circular metal cooling block (7-mm diam.) equipped with a Peltier thermal cooling device was positioned over the contralateral forepaw focus within SI and another cooling block was positioned over the contralateral forepaw focus within SII. Cooling of one or other block from 38°C to 8-13°C abolished the evoked potential recorded underneath the cooled block and was evidence for inactivation of the underlying cortical region. Inactivation of either the contralateral SI or SII or both regions simultaneously did not change the latency or amplitude of the ipsilateral potential evoked within the SII on the side of the mechanical stimulus. These data suggest that the forepaw ipsilateral representation within the SII of one hemisphere does not depend on the integrity of the forepaw representations within the SI and SII of the opposite hemisphere. E. Low was supported by the Dental Alumni Society of the University of Sydney; M. Chan was supported by the Australian Dental Research Fund.

330.4

PROPERTIES OF NOCICEPTIVE NEURONS IN THE LATERAL THALAMUS OF THE SQUIRREL MONKEY. A.V. Apkarian, T. Shi*, R.T. Stevens, Kt.-D. Kniffki[1], and C.J. Hodge. Dept. Neurosurgery, SUNY Health Science Center, Syracuse, NY 13210. [1] Physiol. Institut, Univ. Würzburg, D-8700 Würzburg, Germany.

Few studies have examined nociceptive neurons (NN) in the lateral thalamus of the monkey. The squirrel monkey is ideal for this type of study since it has a large, well defined VPI distinct from VPL and PO. Therefore, this study examines NN in the squirrel monkey lateral thalamus; their frequency and locations relative to spinothalamic terminations (STT) and to cytoarchitectural boundaries.

Five days prior to recordings, animals received unilateral injections of 2% WGA-HRP in the cervical enlargement, using sterile surgery. Recordings were done in anesthetized animals (chloralose and nembutal drip). Responses to adequate somatic stimuli were determined for all isolated units. Recording sites were marked and tissue reacted for HRP.

Nociceptive neurons were found in VPL, VPI and PO. The overall incidence of NN was 17% (46 of 275). This incidence was 30% in VPI and PO and, only 13% in VPL. Most NN in VPL were wide dynamic range type (90%; 18 of 20); while most high threshold type cells were in VPI (7 of 10). Most NN had thermal and mechanical responses, and a small number of these, located at the boundary between VPL and VPI, responded to innocuous cooling. Many NN were located within 200μm of STT terminals (68%, 19 of 28). The receptive fields of NN located in VPL were in continuity, both in size and body location, with surrounding low threshold units; NN with largest receptive fields were located in PO. The results indicate segregation of nociresponsive cell types across VPL, VPI and PO, and correspondence between these cells and sites of STT terminations.

330.5

COMPARISON OF BARREL CORTEX NEURONAL RESPONSES TO WHISKER STIMULATION IN AWAKE-UNDRUGGED VS URETHANE-ANESTHETIZED RATS. D.J.Simons, G.E.Carvell, A.E.Hershey* and D.P.Bryant*. Depts. of Physiology and Physical Therapy, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Multi-unit SmI cortical responses to controlled deflections of individual vibrissae were recorded in hand-held, awake and undrugged rats. Subsequently, the same units were studied after the animal had been anesthetized with urethane (1.5g/kg). Compared to the awake condition, urethane increased the magnitude of the response evoked both by principal (PW) and by adjacent (Adj) whisker stimulation. The time of the peak response (modal latency) was virtually identical for PW stimulation in awake and urethane conditions. By contrast, in the awake condition Adj modal latency occurred ~2 msec later than the PW response but on average 13 msec later under urethane anesthesia. The response in the urethane condition may be mediated in part by the slowly depolarizing epsp that has been described in vitro following high intensity shocks to layer IV. The physiological conditions in which such circuitry is normally active remains to be determined. Supported by NS19950.

330.6

SOMATOSENSORY RECEPTIVE FIELD PROPERTIES OF CORPUS CALLOSUM FIBRES IN THE RACCOON. J.P. Guillemot, L. Richer*, M. Ptito, F. Leporé. Dept. of Kinesiology, UQAM and Dept. of Psychology, Univ. of Montréal, Montréal, Canada.

Corpus Callosum (C.C.) is involved in the interhemispheric transfer of somatosensory information. Previous anatomical experiments in the raccoon have shown that neurons belonging the cortical somatosensory areas (SI and SII) sent somesthetic information to the other hemisphere through axons crossing at the rostral portion of the C.C. In the present study callosal somesthetic receptive fields (R.F.) were identified and characterized in terms of localization and specific sensory modalities.

Acute recordings were carried out in curarized raccoon using micropipettes electrodes and N_2O:O_2 anaesthesia. The recording site in the rostral C.C. was identified visually using an operating microscope and fibre activity amplified in the conventional manner. R.F.'s were determined using von Frey calibrated hairs, puffs of air, light touch or pressure and passive movement of the limbs.

Results indicated that various functional sub-modalities were represented originating from cutaneous, deep and paciniform receptors. Some neurons also responded to hair and whisker displacements. Moreover, both slowly and rapidly adapting axonic responses were found. R.F.'s were situated in pelvic limb (4%), trunk (45%), head (33%) or thoracic limb (6%) regions. Of the latter 82% concerned the proximal limb areas whereas 18% represented the distal limb or forepaw. These neurons all represented unilateral body parts. In addition, bilateral R.F.'s were found in the face, trunk, thoracic or pelvic limbs.

These results suggest that callosal connections may be associated to different sensory functions such as midline fusion of the two halves of the body or processing of complex sensory discrimination.

330.7

ORGANIZATION AND CONNECTIONS OF SOMATOSENSORY CORTEX IN MONOTREMES. L. A. Krubitzer, P. R. Manger and J. D. Pettigrew Vision, Touch and Hearing Research Centre, University of Queensland, Queensland, Australia 4072

The organization of somatosensory cortex was investigated in echidna (*Tachyglossus aculeatus*) using standard microelectrode mapping techniques. With as many as 532 recording sites in a single animal, the detailed somatotopy of cortical fields was readily obtained. In addition, recordings were made in visual and auditory cortex. We describe at least 3 topographically organized fields in the somatosensory cortex of the echidna. The primary somatosensory area, SI, was the largest field with the tail represented most medially, adjacent to visual cortex, and the hindlimb, forelimb and face represented progressively more laterally in the representation. Within SI, the rostral 1 cm of the bill occupied a large amount of cortical space and assumed approximately 1/3 to 1/2 of the entire representation. Rostral to SI, most neurons responded to deep stimulation such as joint manipulation or pressure to body parts. This deep region contained a complete representation of the body surface with a similar medial to lateral organization as SI. We interpret this region to be a separate representation from that of SI. Finally, caudal to SI, a smaller somatosensory field with large receptive fields has been defined. This field is in the approximate location of SII described in other mammals. Like SII, this field contains a complete representation of the contralateral body surface. Embedded within caudal somatosensory cortex was auditory cortex. Within the auditory cortex there were 2 topographically organized field. Some portions of auditory cortex responded to auditory and somatosensory stimulation, auditory and visual stimulation, or auditory stimulation alone.

Injections of anatomical tracers in SI revealed connections with the ventral posterior nucleus of the thalamus. Major cortical connections were with other portions of SI, with cortex caudal to SI, in the caudal somatosensory field, and with cortex rostral to SI.

330.8

COMPARISON OF RESPONSES ELICITED IN SI AND SII SOMATOSENSORY CORTICES BY MECHANICAL STIMULATION OF THE HAND IN MONKEYS. H.Hämäläinen, A.Lähteenmäki, K.Reinikainen†, S. Carlson and A.Pertovaara. Depts. Psychol. and Physiol., Univ. Helsinki, Finland.

Recordings of multiple unit activity and electric fields, elicited by mechanical pulses and vibration delivered to the hand, were made from SI and SII cortices of two awake monkeys. In SI the multiple unit responses were quite uniform, well reflected the stimulus features, and were elicited by stimulation of the contralateral hand only. The onset latencies of the responses to mechanical pulses varied between 7-33 ms, and in 90% of the recordings a sustained response corresponding to the duration of the vibratory stimulus was measured.

The multiple unit responses elicited by similar stimuli in SII could be categorized into two groups according to the onset latency of the responses. In group A the onsets of the responses to contralaterally applied pulses varied between 7-28 ms, whereas in group B they varied between 28-113 ms. 66% of the cell groups studied had bilateral receptive areas. The response onsets varied between 47-157 ms to ipsilaterally applied pulses.

In group A the response duration corresponded to the duration of the vibratory stimulus in only 25% of the measurements. However, in group B there was no correspondence between the stimulus and response features.

330.9

SENSORY RESPONSES IN POm, BUT NOT IN VPM, DEPEND ON INPUT FROM THE SI BARREL FIELD CORTEX. M.E. Diamond, M.A. Armstrong-James, M.J. Budway* and F.F. Ebner, Center for Neural Science, Brown University, Providence, RI 02912.

We have shown that POm cells respond to whisker movement at a threefold greater latency than do VPM cells (18.7 ms vs 6.7 ms). The long latency of POm cells raised the question: Is the trigeminal complex providing the main sensory drive to this thalamic nucleus? We speculated that POm may receive its dominant sensory drive via descending projections from barrel field cortex. To test this idea, the response of cells in POm to whisker movement was measured before, during and after reversible suppression of only barrel field cortex in urethane-anesthetized rats. Cortical suppression was achieved by Mg SO_4 or cold ACSF. The criteria for "suppression" were: the ECG trace was nearly flat and all cortical single unit activity was silenced. All POm cells studied during cortical suppression lost their response to whisker stimulation (interquartile range of response supression: 86-100%). Spontaneous activity was reduced as well. The POm cells recovered their response when cortex recovered from suppression, although this could take > 1 hour. The effect of cortical suppression was significantly ($p<.001$) less pronounced in VPM (interquartile range of response supression: 15-50%). There was often a slight increase in response latency among VPM cells. We conclude that barrel field cortex provides the dominant sensory drive to POm, but only a modulatory input to VPM. POm participates in a cortico-thalamo-cortical loop whose properties are consistent with a role in enhancing communication between barrel-columns. (Supported by NS-25907)

330.10

ANESTHETIC STAGE AS A DETERMINANT OF VPM RECEPTIVE FIELD PROPERTIES IN THE RAT.
Friedberg, M.H., Lee, S.M. and Ebner, F.F. Section of Neurobiology and Center for Neural Science, Providence, RI 02912

Changes in response properties of the thalamic ventral posteromedial (VPM) nucleus were assessed quantitatively at different anesthetic depths under halothane and urethane anesthesia. Fast Fourier transform analysis of electrocorticograms (ECoG) defined the stage of anesthesia while recording unitary responses extracellularly in VPM to controlled deflection of the contralateral vibrissae.

Predictable increases in the dominant ECoG frequency were seen from stage III-4 (1-2 Hz) through III-3 (3-4 Hz) to III-2 (5-7 Hz). Receptive field (RF) size and latency of VPM cells to whisker stimulation increased from stage III-4 to III-1. A sharp increase in RF size and latency occurred from stage III-3 (RF ~2 whiskers, latency ~7 ms) to stage III-2 (RF ~6 whiskers, latency ~11 ms).

Transecting the axons of Spinal Trigeminal Interpolaris neurons altered VPM RFs. RFs of >3 whiskers disappeared in all VPM neurons after disconnecting the interpolaris input. In addition, the changes in the response properties seen at different anesthetic depths were abolished after interpolaris pathway transection.

We conclude that the nucleus principalis projection mediates the one-to-one correspondence between a VPM cell and its center RF whisker. In contrast, the interpolaris input to VPM is responsible for transmitting a different component of sensory information; characterized by large RF, long response latency, and low response magnitude and probability which can be observed in VPM neurons under light anesthesia and probably in the awake animal. (grants NS-25907 and NS-13031)

330.11

DIFFERENTIAL EFFECTS OF $GABA_A$ AND $GABA_B$ RECEPTOR-MEDIATED INHIBITION ON VPM RESPONSES TO SOMATIC STIMULI.
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We have shown previously that a kainic acid-induced lesion of the thalamic reticular nucleus in rats leads to an immediate increase in the number of whiskers which elicit responses in a thalamic ventral posteromedial (VPM) neuron from an average of 2.4 to 7.4 whiskers. Here we have assessed the basis for these changes by selectively blocking GABA-mediated inhibition with iontophoretic application of a $GABA_A$ receptor antagonist, bicuculline methiodide (BIC) or $GABA_B$ receptor antagonist, 2-hydroxy-saclofen (2-OH-S).

The effect of BIC (40-83 nA) on vibrissa-evoked responses in VPM was a preferential enhancement in the responses elicited by the vibrissa giving rise to the highest probability response (center RF whisker). The effect of 2-OH-S was nearly the opposite; significant increases in responsiveness to the less effective whiskers in the peripheral areas of the receptive field (surround RF whiskers) were seen with iontophoretic currents ranging between 40 and 80 nA. This enhancement of the SRF following the blockade of $GABA_B$-mediated inhibition resulted in a 2.3-fold increase in the average RF size of VPM neurons; no statistically significant increase in RF size was seen with BIC.

We conclude from these results that $GABA_A$ receptor-mediated inhibition in VPM regulates the temporal response characteristics of thalamic relay neurons to sensory stimulation. In contrast, $GABA_B$ receptor-mediated inhibition appears to control the effectiveness of late arriving inputs from the trigeminal complex, thereby regulating the efficacy of the sensory stimulus. (grants NS-25907 and NS-13031).

330.12

INDUCTION OF HIGH FREQUENCY ACTIVITY IN THE SOMATOSENSORY THALAMUS OF RATS RESULTS IN LONG-TERM POTENTIATION OF RESPONSES IN SI CORTEX *IN VIVO*.
F.F. Ebner and S.M. Lee, Center for Neural Science, Brown University, Providence, RI 02912.

We have previously shown that high-frequency (HF) discharge occurs in rat thalamic (VPM) neurons when GABA-mediated inhibition is blocked using bicuculline methiodide (BIC). Here, we begin to characterize the ability of this HF thalamic activity to alter the responsiveness of barrel field cortical neurons to sensory stimulation.

Computer controlled whisker stimulus-single unit responses were measured simultaneously in VPM and in barrel field neurons. Highly cross-correlated responses of these functionally-linked units to 10 msec deflections of a vibrissa were measured using a PSTH to establish a baseline level of response. Baseline was followed by the induction of HF activity in VPM (conditioning stimulus) by two methods: *i*) direct electrical stimulation of the thalamus or *ii*) whisker stimulation during thalamic BIC disinhibition. Both methods produced a conditioning paradigm of 4-spike "bursts" of high-frequency activity (50-100 Hz) with an interburst frequency of 7 Hz.

In 8 out of 9 cases following the CS, the response of cortical neurons to single vibrissa test stimuli increased by 37 - 62% over baseline values which persisted for as long as one hour. This enhancement of the cortical response was not accompanied by increased thalamic responses, suggesting that the potentiation was specific to the thalamocortical synapse.
(NIH grants #NS13031 and NS25907)

330.13

KINESTHETIC INPUT IN PRIMARY SOMATOSENSORY CORTEX (SI) DURING REACHING MOVEMENTS. M.J.L. Prud'Homme, J.F. Kalaska, Université de Montréal, Montréal, Québec, Canada, H3C 3J7

We studied SI proprioceptive cell activity in a two-dimensional reaching task in order to study the cortical mechanisms of kinesthesia. The task required monkeys to move a manipulandum from a central starting position to 8 peripheral target lights. One hundred and fifty proprioceptive cells were recorded in four monkeys. The majority (90%) of the cells showed continuously-graded changes in activity during arm movements in different directions, centered on a preferred movement direction, which varied from one cell to another. This broad directional tuning activity ressembled that recorded in motor and parietal cortex in similar task conditions. Eighty percent (120/150) of the cells showed movement-related activity and 66% showed tonic posture-related activity while the monkey held his arm over peripheral targets. A smaller proportion of the cells (40%, 58/150) also showed directional changes of activity before movement onset, but very few preceded the onset of EMG. External loads applied to the arm in different directions produced continuously graded changes in activity for 50% (25/50) of the cells tested. These loads changed the level of muscle activity but did not alter the handpath or the joint angle changes of the arm during the movements. This indicates that information about both movement kinematics and movement dynamics are represented in an SI cell population implicated in kinesthetic sense. Supported by MRC Group Grant in Neurological Sciences (JFK) and FCAR (MP).

330.14

RESPONSES TO GROOVE WIDTH, FORCE AND VELOCITY IN SOMATOSENSORY CORTICAL AREAS I (SI) & II (SII), & THALAMIC VPL NUCLEUS OF MONKEYS DURING ACTIVE TOUCH OF TEXTURED SURFACES. R. Sinclair and H. Burton, Department of Anatomy & Neurobiology, Washington University Sch. Med., St. Louis, MO 63110. (supported by NIDCD 00096)

Recordings were made from 164 SI, 150 SII, and 35 VPL neurons in 2 *M. mulatta* trained to stroke their fingertips over pairs of horizontal gratings. Downward applied force (F) and velocity (V) of stroke were measured. Groove width (Gw) varied from 500-2900μm. Ridge width was constant at 250μm. Responses proportional to Gw were previously reported in all 3 areas. Based on ANOVA and correlation analyses, all VPL cells responding to Gw were also affected by F and V, like peripheral afferent fibers. Independent responses to Gw or F were seen in SI and SII, and thus may result from cortical processing. Preliminary analysis indicates a larger proportion of SII than SI cells independently responded to Gw. Activity in one VPL neuron was proportional to F independent of Gw or V. F independent responses were previously reported as most frequent in SI area 1 SAs (slowly adapting). There were no obvious SA vs RA (rapidly adapting) distinctions in SII or VPL. Results suggest that an independent cortical representation of Gw may underlie perceptual constancy of grating roughness. Independent sensory representation of force applied during active touch may arise from subcortical processing.

330.15

PRIMARY SOMATOSENSORY CORTICAL (SI) UNITARY RESPONSES TO ACTIVE AND PASSIVE TOUCH. C.E. Chapman and S.A. Ageranioti-Bélanger. Centre de recherche en sciences neurologiques, Université de Montréal, Québec, Canada H3C 3J7.

Previous studies have shown that the transmission of cutaneous inputs to SI cortex is decreased, or gated, during movement, at least in situations in which the test signal has no relevance to the performance of the motor task. It is not known if behaviourally significant inputs are subject to the same controls. The present experiments were designed to test the latter hypothesis, comparing SI unitary responses to differently textured surfaces (smooth or smooth/rough) explored using either active touch (monkey actively moves digits, scanning the tips over the surface presented) or passive touch (monkey immobile, surface displaced passively under digit tips). The animal indicated the surface texture encountered in a trial by pushing or pulling a lever with the opposite arm. Of 76 units with cutaneous receptive fields on the digit tips contacting the surfaces (2 hemispheres in 1 monkey), 52 discharged differently over the rough and smooth surfaces. 44% of these showed a clear texture-related response with passive touch but not with active touch, suggesting that even when inputs are relevant to an animal they are subject to gating controls. Only 10% signalled texture during active, and not passive, touch. The remaining 46% signalled texture during both active and passive touch, but the texture-related response was often obscured during active touch. It is concluded that most cutaneous signals to SI cortex, relevant and irrelevant, are subject to gating controls during movement. Supported by the Canadian MRC and the FRSQ.

330.16

THE EFFECT OF ATTENTION ON THE RESPONSES OF NEURONS IN THE SECOND SOMATOSENSORY CORTEX. S.S. Hsiao, D.M. O'Shaughnesy*, K.O. Johnson. Bard Laboratories, Dept. Neuroscience, The Johns Hopkins University Sch. Medicine, Baltimore, MD. 21205.

The effects of attention on neurons in the secondary somatosensory cortex (SII) were studied in a Macaque monkey trained to perform both a tactile letter discrimination task and a visual detection task. The tactile letter discrimination task required the animal to match one of six embossed letters (ALXPHO) scanning across his fingertips (scanning velocity = 20 mm/sec) with a letter presented visually to him on a screen. The visual detection task required the animal to attend to 3 lights on a screen and to detect when one of the lights dimmed. During the recording sessions the embossed letters were continously scanned across a neuron's receptive field while the monkey was required to switch back and forth between the visual and tactile tasks.

This study investigates the effects of attention while controlling for other behavioral effects. Since both tasks required the animal to maintain a constant state of arousal and both tasks used the same reward switch, any differences in neural responses can be attributed to differences in attentional focus required to perform the tasks.

The results showed that the task had a significant effect on the responses of neurons in area SII. Although the effect varied between neurons, the general result was that SII neurons were more responsive when the animal was required to identify the letters tactually. In addition there were significant differences in the spatiotemporal spread of the responses. These results suggest that the animals attentive state may play a role in determining the form of representation at the level of SII cortex.

330.17

NEURAL CODING OF TACTILE ROUGHNESS: SUBJECTIVE ROUGHNESS IS RELATED TO SPATIAL VARIATION IN FIRING RATE. C.E. Connor, S.S. Hsiao, and K.O. Johnson, Bard Laboratories, Dept. Neuroscience, The Johns Hopkins University Sch. of Medicine, Baltimore, MD. 21205.

Plastic surfaces containing embossed dot patterns were used as texture stimuli in combined psychophysical and neurophysiological experiments. Psychophysical subjects scanned the distal pad of the index finger across the surfaces and reported the subjective magnitude of surface roughness. In neurophysiological experiments, the surfaces were scanned across the receptive fields of cutaneous mechanoreceptor afferents while recording spike activity. The two data sets were compared in an attempt to ascertain the neural code for tactile roughness.

Previous work had shown that the sensation of roughness may be related to temporal or spatial firing rate variation in slowly-adapting (SA) Merkel's and rapidly-adapting (RA) Meissner's afferents (C.E. Connor et al., J. Neurosci. 10: 3823-3836, 1990). In this study we measured temporal variation in neural responses by convolving neural records with 1-dimensional gabor filters. Spatial variation was measured by convolution with 2-dimensional gabor filters and 2-dimensional difference of gaussian (DOG) filters. Gabor filters effectively measure variation in one direction at a time while DOG filters measure variation in all directions simultaneously. The periods and sizes of the filters were varied as parameters in the analysis. Temporal variation in SA responses was correlated with subjective roughness for some but not all of the texture patterns studied. Spatial variation in SA responses was closely correlated with subjective roughness for all patterns studied. The correlation was particularly strong for spatial variation as measured by 2-dimensional gabor filters, and somewhat weaker for spatial variation measured by DOG filters. Spatial variation in RA responses was somewhat correlated with subjective roughness for all stimulus patterns. Supported by NIH grant NS18787.

330.18

SPATIAL STRUCTURE OF PRIMARY AFFERENT RECEPTIVE FIELDS IN THE SOMATOSENSORY SYSTEM. F. Vega Bermudez, K.O. Johnson, Dept. of Neuroscience, Johns Hopkins Sch. Med., Baltimore MD 21205.

A new tactile stimulator with multiple, independently controlled probes has been developed to study the spatiotemporal responses of somatosensory neurons. Responses to multi-probe stimuli are studied in peripheral afferents.

The tactile stimulator comprises seven linear motors that drive seven probes (0.5 mm diam) arranged in a hexagonal rosette (1.0 mm spacings), which is embedded within a large hexagonal array of static probes. The entire array is mounted on a three-axis translation stage allowing movement to different receptive field sites. Four stimulus sets were used to study the receptive fields: i)single probe stimuli were used to map receptive fields; ii)dual probe stimuli were used to study interactions between two probes; iii)multiple probe stimuli were used to study responses to stimuli of increasing complexity; iv)the effect of contact indentation between the array and the skin was studied by varying the baseline indentation.

The receptive fields of RA and SAI afferents vary in shape but are generally round or oval with eccentric hot spots and field borders that are very sharp. RA field sizes are more sensitive to indentation magnitude than SAI field sizes; at the larger intensities RA fields are larger than SAI fields. Neither the response magnitudes nor the spatial structure of the receptive fields were affected by baseline indentation, which suggests that primary afferent responses depend on relative rather than absolute indentation magnitudes. In both afferent types, responses to two probes presented simultaneously evoked less impulses than the maximum response to either of the probes presented alone. Accordantly, the evoked response declines monotonically with increasing numbers of active probes. At the hot spot the numbers of action potentials evoked by seven probes are 27% (SAI's) and 47% (RA's) of the numbers evoked by a single probe.

The most probable mechanism for the peripheral "surround suppression" is based on skin mechanics as additional probes relieve the strain at the hot spot. These suppressive effects in primary afferrnt responses must be taken into account when assessing responses of cortical neurons to complex stimuli.

330.19

APPROXIMATING THE PROCESSING FUNCTION OF SINGLE UNITS IN SI CORTEX USING NEURAL NETWORK MODELS. I.A. Twombly*, K.O. Johnson, S.S. Hsiao, Bard Laboratories, Dept. Neuroscience, Johns Hopkins Univ. Sch. Med., 725 N. Wolfe St., Baltimore MD 21205

Feedforward neural networks were used to model the response properties of neurons in SI cortex of macaca mulatta. The input layer of the networks was a 2-dimensional array analogous to an array of mechanoreceptive afferents on a distal phalanx. The output layer of the network was a single unit analogous to an area 3b neuron. Models were constructed utilizing both direct convergence from the input array to the output unit and convergence from the input array through a layer of intermediate units to the output unit.

Neurophysiological data from experiments using embossed letters as stimuli were used to adjust the connection weights. Impulse rates from peripheral afferents were fed into the input layer of a network, and the output was compared to the impulse rates recorded from single units in area 3b. A least squares fit to the neural responses evoked by the letters A-M was used to produce the optimal network weights. The network responses to the letters N-Z provided an indication of the ability of the network to reproduce responses that it was not trained to match.

Linear solutions were sought using a 2-layer network with a linear output function. These linear networks were unable to reproduce many of the key features of the neuronal responses. A simple nonlinear solution using a 2-layer network with a positive linear output function also failed to reproduce many of the key features of the neuronal response. A more complex nonlinear solution was sought using a three layer network with positive linear output functions at both the intermediate and output layers. This architecture proved capable of reproducing the cortical responses quite well, suggesting that many area 3b neurons are operating in their nonlinear ranges when processing complex stimuli such as scanned letters.

331.1

MECHANISMS OF COMPENSATORY PLASTICITY OF THE BRAIN AFTER CORTEX AND MIDBRAIN DAMAGE. M.S.Sinyava. Lab. Physiology of reception, Pavlov Institute of Physiology of the Acad.Sci.of the USSR, Leningrad, 199034, USSR.

The compensatory processes in S_I area of the cortex were studied on the both operated and non-operated hemispheres after one-sided brain damage. We used electrical stimulation of visceral and somatic nerves. In cats after brain damage at the midbrain level on the operated side the decrease of responding cells could be observed 2-10 hours after the operation. This process resulted in the full blockade of neuronal activity. The neurons with bilateral sensitivity occur on the non-operated side 8-10 months after the operation. The amount of active neurons increases to 80% in the cortex of the non-operated side. Compensatory process in the cortex of the non-operated side becomes similar to that in cats with brain damage at the cortical level. Earlier we showed the bilateralization of neuronal sensitivity after the damage at the cortical level. We supposed that there was a general principle of compensatory reorganization in S_I area after one-sided damage either at cortex or midbrain levels.

331.2

EFFECTS OF SENSORY DEPRIVATION ON ZINC HISTOCHEMISTRY IN THE RAT BARREL CORTEX. N.D. Akhtar and P.W. Land. Dept. of Neurobiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Zinc is released at excitatory cortical synapses, and is thought to modulate neurotransmission at the NMDA type of glutamate receptors. Zinc storage granules in rat somatosensory barrel cortex (SI) show lamina-related differences in distribution that reflect cyto- architectonic and metabolic subdivisions of that region. Interestingly, histochemical staining for zinc is particularly light in lamina IV barrels compared with the surrounding, darkly stained septa.

Here we examined the effects of sensory deprivation produced by whisker trimming on histochemical staining for zinc in the barrel cortex. Rats had one or more rows of whiskers trimmed for 5-10 weeks beginning either at birth or at 2 months of age. Trimming whiskers of adult rats does not visibly alter the zinc staining pattern in the corresponding barrels. By contrast, in rats with whiskers trimmed from birth, zinc staining is markedly enhanced in deprived barrels when compared with adjacent non-deprived barrels in the same hemisphere or with barrels in untrimmed animals. These data suggest that neonatal sensory deprivation results in increased zinc storage by normal barrel afferents and/or ingrowth of novel, presumably excitatory inputs into deprived regions of cortex. Such changes may be related to the abnormal responsiveness of neurons in neonatally deprived barrels. (Supported by NIMH grant MH09773)

331.3

REVERSIBLE CHANGES OF THE RECEPTIVE FIELDS OF DCN, VPL THALAMIC AND SI CORTICAL NEURONS FOLLOWING PERIPHERAL APPLICATION OF LIDOCAINE

H.-C. Shin, S.A. Raymond, G. R. Strichartz Anesthesia Research. Labs, Brigham & Women's Hospital, Harvard Medical School, Boston, MA, 02115.

Because the outcome of local anesthesia (LA) in the periphery is contingent on the functional effects induced in the CNS by the block, it is important to characterize the changes in CNS responsiveness caused by peipheral LA. Single or multiple units from neurons in brainstem dorsal column nuclei (DCN, n=8), ventroposterolateral (VPL, n=15) thalamus, and primary somatosensory (SI, n=16) cortex were recorded to examine the response of the cutaneous dorsal column-lemniscal somatosensory pathway to LA application to the distal extremities of anesthetized rats (n=12, 300-400g, 50-60mg/kg Na pentobarbital, i.p.). After determining stable receptive fields (RFs) for 10-50 min, lidocaine (L: 1-5ul, 0.01-1.0M) was injected subcutaneously to either the original RF or the homotopical area on the opposite side and subsequent changes of the RF were monitored. Nine of 12 SI cortical units showed a reversible block of original RF with 0.1 & 1.0M L and expansion of RF to neighbouring area of the forepaw (FP, initial expansion: 1-15 min, maximum expansion: 8-27 min, recovery: 17-62 min). Two SI units showed only abolition of original RFs. One SI unit did not exhibit any change after injection of 0.01M L. Four other SI units showed temporary expansion of RFs after injection of L at ipsilateral (Ipsi.) homotopical FP without losing original RFs. Eight of 11 VPL units also showed loss of original RFs and expansion of RFs to neighbour after contralateral (Cont.) LA (0.1 & 1.0M L). Interestingly, 3 of 4 VPL units exhibited Cont. RF plasticity after LA to Ipsi. FP. Three of 6 DCN units exhibited temporary expansion around original Ipsi. RF, which itself was blocked by L. However, DCN units (2) did not show any change of RF after L injection at Cont. homotopical FP. Overall, this study demonstrates that changes of RF following lidocaine anesthesia of the RF occur at all three levels of DC-lemniscal pathways to the SI cortex. The results also suggest that interhemispheric transfer of plasticity involves interactions between thalamus and cortex. Supported by PHS grant NIH-GM35647.

331.4

HISTOCHEMICAL COMPARTMENTS OF SQUIRREL MONKEY SOMATOSENSORY THALAMUS FOLLOWING NERVE INJURIES. L. Harrison and C.G. Cusick. Neurosci. Training Program and Anatomy Dept., Tulane Univ., New Orleans, LA 70119.

To investigate regulation of thalamic activity by somatosensory inputs, immunoreactivities for the calcium binding proteins calbindin-D 28k (CB-ir) and parvalbumin (PV-ir) were localized and compared to cytochrome oxidase staining (CO) ten weeks after injury to the median and ulnar nerves. Ipsilaterally, the non-deprived ventroposterior lateral nucleus (VPL) contains dense CO as well as CO-poor regions, many of which relate to discontinuities in the sensory representation. Dense CB-ir occupies the ventroposterior superior and inferior divisions (VPS and VPI) and anterior pulvinar (Pa). VPL proper contains little CB-ir, except for dense patches in CO-poor regions, and a population of small neurons interspersed through the nucleus. PV-ir is dense in CO-rich zones of VPL, and sparser in VPI and Pa. In the portion of VPL related to the hand, the intensity and pattern of staining for CB, PV, and CO are similar bilaterally, and similar numbers of calbindin positive cells are found. The results suggest that oxidative metabolism and expression of calcium binding proteins are maintained at nearly normal levels after nerve injuries, at a postinjury time when the deafferented zones of VPL have been completely reactivated by the radial nerve (Garraghty et al., Soc. Neurosci. Abstr., 16:831, 1990).

331.5

CHANGES IN LOCAL CIRCUIT AXON DISTRIBUTION IN MOUSE BARREL CORTEX FOLLOWING NEONATAL DENERVATION. K.L. Bernardo, J.S. McCasland, K.L. Probst*, and T.A. Woolsey. Dept. Neurosurg., Div. Exp. Neurol. & Neurosurg. and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Using a bulk-labeling HRP technique and automated image analysis, we analyzed patterns of intrinsic connections in mouse barrel cortex quantitatively. We compared normal cortices from adults with those for which the contralateral infraorbital nerve (ION) was sectioned at postnatal day 7 (PND7), a time at which the cortical representation of the whiskers has already appeared. After peripheral denervation cortical HRP-labeled fiber distribution differed significantly from controls. Control animals showed axons projecting primarily within the barrel row which was injected, as well as into the next anterior adjacent barrel row, as described previously (Bernardo et al., 1990). In animals sacrificed as adults following PND7 ION lesions we found a near-total absence of fibers projecting outside a vertical "column" defined by the HRP injection site. These results were highly significant statistically ($p=0.0003$). This is the first demonstration of dramatic alterations in intracortical connections in response to the loss of peripheral inputs after the barrel map has been established.

(Supported by NIH grants NS01399 (KLB) and NS17763)

331.6

PLASTICITY OF SOMATOSENSORY CORTEX (SI) VENTRUM REPRESENTATION AND SIZE OF VENTRUM RECEPTIVE FIELDS DURING LACTATION IN RATS. **J.M. Stern, C. Xerri*, and M.M. Merzenich. Psychology, Rutgers Univ., New Brunswick, NJ; Psychophysiol., Univ. of Provence, Marseilles, France; Coleman Labs., Physiology, UC-SF, San Francisco, CA.**

Norway rats spend ~80% of their time nursing in the first days postpartum (PP); this measure gradually declines to ~25% by day 17 PP. We asked whether this intensive, natural stimulation of the ventrum, in particular the nipples, would alter the sensitivity of the ventrum, in terms of SI representation and receptive field sizes. To date, 4 primiparous lactating rats were studied at 6, 12, 16 and 19 days PP; 5 controls included 2 virgins and 3 primiparous nonlactating PP rats (1 newly parturient before onset of nursing and 2 at 16 and 18 days PP whose litters were removed on the day of birth). In lactating rats we found a 2.2-fold increase in SI ventrum representation between days 12-16 PP and a 1.2-fold increase on days 6 and 19 PP compared to controls. Ventrum receptive fields, many nipple-centered, are much smaller on average in lactating than in control rats (4.6 vs. 13.4 cm^2 and 7.9 vs. 27.3% of ventrum surface, a 2.9 and 3.4-fold differences, respectively).

Supported by MH-40459 (JMS) and NS-29343 (MMM).

331.7

CORTICAL SOMATOTOPY AND PERIPHERAL TERMINATIONS IN BRAIN STEM AND SPINAL CORD FOLLOWING REVERSIBLE DEAFFERENTATION OF THE GLABROUS HAND IN INFANT AND ADULT MACAQUES. M. Carlson[1], S.L. Florence[2], P.E. Garraghty[2], and J.H. Kaas[2]. [1]Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115; [2]Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

In the visual system, monocular deprivation early in life has a more devastating effect than in adulthood. To explore the behavioral, physiological and anatomical consequences of sensory deprivation in the somatosensory system, we have examined the effects of median and ulnar nerve crush performed in infant and adult macaques (M. mulatta). Following the crush of a peripheral nerve, the distal processes degenerate to be replaced eventually by the regenerating fibers. Thus, during the period of regeneration, central targets of the glabrous hand are deprived of normal driven activity following crush of the medial and ulnar nerves.

We examined the somatotopy of cortical area 3b and the pattern of central terminations of skin surfaces within the glabrous surface of the hand in adults experiencing deafferentation as infants or adults. We detected no abnormalities in either somatotopic organization in area 3b or in the pattern of afferent termination in the cuneate nucleus of the brain stem in either the early or late deprived animals. These observations are consistent with the normal behavioral capacities of macaques undergoing nerve crush as infants. On the other hand, relative to normal, afferent projections to the spinal cord from transient deafferented skin sites are expanded after both infant and adult nerve crush. The fundamental relevance of these expansions, if any, are unknown. (Supported by BNS 86-17085 and 88-46157 to M.C. and NS16446 to J.K.)

331.8

REORGANIZATION IN PRIMARY AND SECONDARY SOMATOSENSORY CORTEX (SII) AFTER COMPLETE DEAFFERENTATION OF THE HAND IN RHESUS MONKEYS. A.K. Ommaya and T.P. Pons. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

Removal of all cortical hand representations in the postcentral strip (areas 3a, 3b, 1 and 2) results initially in the deactivation of the hand map in SII but eventually to reactivation of the hand map by the foot. To compare the effects of central versus peripheral deafferentation on the pattern of representation in SII, we cut and tied the three nerves normally innervating the hand and, 6-8 weeks later, recorded from both SII and the postcentral strip. We found that tissue normally devoted to the postcentral hand representations had become responsive to somatic stimulation of both the arm and face, although stimulation thresholds were abnormally high. By contrast, sites in the expected location of the SII hand representation were largely unresponsive to somatic stimulation. The few sites in this region that did respond had receptive fields and response thresholds that were virtually identical to those in the reorganized portion of the postcentral strip. The results indicate major differences in the way SII reorganizes after peripheral and central nervous system injury.

331.9

CONVERGENT SUBTHRESHOLD INPUT TO THE ULNAR NERVE REPRESENTATION IN CAT SI CORTEX MAY PLAY A ROLE IN IMMEDIATE CORTICAL REORGANIZATION: AN *IN-VIVO* INTRACELLULAR AND EXTRACELLULAR RECORDING STUDY. R.S. Waters, C.X. Li*, A. Oladehin*, C.A. McCandlish, E.F. Johnson*. Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163.

The immediate reorganization of the primary somatosensory cortex (SI) after peripheral nerve injury suggests the existence of previously undetected inputs. As a first step in understanding reorganization, we mapped the forepaw representation using extracellular recordings methods and then examined subthreshold input onto SI cortical neurons serving the ulnar nerve using intracellular recording combined with natural and electrical peripheral stimulation.

In adult cats anesthetized with Nembutal (35mg/kg), SI was exposed, and a Lucite chamber was installed on the surrounding bone. The paw was placed in a customized holder and six stimulating electrodes were inserted into selected regions of the skin. Carbon fiber electrodes were used to map the distal forepaw representation. After mapping, an intracellular electrode (2M Potassium Acetate, 50-85 MΩ) attached to a Burleigh microdrive and mounted onto the recording chamber, was inserted into the ulnar nerve representation to record threshold and subthreshold input onto individual SI neurons. By these methods, two results are noteworthy:

1. The majority of cells in the ulnar representation in SI cortex (Layer IV) received convergent input from regions of skin served by more than one peripheral nerve.
2. In many cases, PSPs could be elicited in SI cortical cells from all six stimulating electrodes suggesting that SI cortical neurons receive a wealth of subthreshold input which may serve, in part, as the underlying substrate for cortical reorganization.

Supported by NSF Grant BNS 88-02766)

331.10

PLASTICITY OF RACCOON SOMATOSENSORY CORTEX: POTENTIATION IN A CORTICOCORTICAL PATH. P. Kirchberger*, S. Witte, and P. Zarzecki. MRC Group in Sensory-Motor Physiology, Dept. of Physiology, Queen's University, Kingston, Ontario, Canada.

Plasticity of digit representations in raccoon SI cortex may depend on a corticocortical (C-C) path between two SI subregions. Normally, neurons in the glabrous zone (GZ) for each digit are activated from only that digit, while in the heterogeneous zone (HZ) convergent inputs from off-focus digits are common. We have reported that a C-C path from the HZ to the GZ is a probable route for the few off-focus inputs to the GZ. After digit removal, when reorganization of the cortical map has occurred, more neurons of the GZ respond like those of the HZ. This change occurs without an increase in incidence of C-C connections. Is there instead a strengthening of existing synapses in the C-C path that could account for the new receptive fields?

C-C epsps were recorded intracellularly in the digit 4 representation in two groups of anaesthetized raccoons; normal controls and an experimental group surviving 4 to 9 months after removal of digit 4. Short latency C-C responses to single shock ICMS of the HZ were not different in amplitude, half width, or integrated amplitude, but epsps from the experimental group had shorter latencies and faster rising phases.

In some neurons of both groups, epsps became more effective in generating action potentials during train stimulation. Epsps that generated action potentials were faster rising than immediately preceding epsps in the same train. Changes in the rising phase of C-C epsps may facilitate transfer of new somatosensory inputs to reorganized SI cortex.

331.11

CHANGES IN NISSL-STAINED AND GABA(+) NEURONS IN THE VENTROPOSTERIOR NUCLEUS (VP) AFTER ABLATION OF SOMATOSENSORY CORTICAL AREAS IN RHESUS MONKEYS. J. Chmielowska and T.P. Pons. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

Cell soma areas of Nissl-stained or GABA(+) neurons in the hand representation of VP were measured in both unoperated and operated hemispheres. The operated hemispheres received complete (areas 3a, 3b, 1 and 2) or partial ablations (e.g. 3b) of hand representations in postcentral cortex 8 weeks prior to sacrifice. In VP of unoperated hemispheres, areas of Nissl-stained cell bodies regions ranged from 20 um^2 to 550 um^2 with the majority of cells having an area of 130-150 um^2. GABA(+) cell body areas ranged from 20 um^2 to 240 um^2, with most cells having an area of 80-100 um^2. In the affected regions of operated hemispheres there was almost a total loss of Nissl-stained neurons with areas > than 160 um^2. Most of these remaining cells had areas < 100 um^2. There was no increase in the number of cells/mm^2 for any given area, and the ratio of GABA(+) to small (< 160 um^2) Nissl-stained cells remained constant, indicating a loss of neurons with relatively large areas (>180 um^2) instead of shrinkage. These results indicate that large projection neurons in VP degenerate completely after ablation of postcentral cortex and are no longer available to serve as a source of activation to other cortical areas.

331.12

MICROSTIMULATION OF RAT "BARREL" CORTEX RESULTS IN AN EXPANDED METABOLIC AND ELECTROPHYSIOLOGIC CORTICAL VIBRISSA REPRESENTATION D. Sirois and P. Hand, Dept. Animal Biology, Sch. Vet. Med. and Neurology, Sch. Med., Univ. of Pa., Phila., Pa. 19104

The cortical representation of rodent vibrissae is highly organized, with a primarily 1:1 correlation between a vibrissa and its cortical layer IV "barrel". Intra-cortical microstimulation (ICMS) of only a few hours duration has been shown to alter the cortical representation of the body surface (Recanzone and Merzenich, *Soc. Nsci. Abstr.,1989*). In this study, we examined the metabolic and electrophysiologic effects of ICMS in the first somatosensory (SI) barrel cortex of awake, adult rats using the 14C-2-deoxyglucose (2DG) and multiple site evoked potential recording techniques.

The SI barrel cortex was exposed under ketamine and xylazine anesthesia and the barrel field explored to identify 2 neighboring barrels with non-overlapping principle-whisker receptive fields (RF). Tungsten microelectrodes were placed at a depth of 700um and fixed to a connector attached to the skull. At 1-3 week post-operative intervals all whiskers were clipped bilaterally sparing only one whisker corresponding to one of the barrels containing an implanted electrode, as well as that whisker's contralateral homologue (control). Charge balanced 5uA monophasic pulses were delivered at 300pps for 40ms, 1 train every second, in the awake rat. ICMS continued for up to 6 hours and the RFs of the two barrels were examined at 1-hour intervals. After ICMS completion, the rat received an IP injection of 14C-2DG and the whiskers were then stimulated bilaterally for 45 minutes. The rat was euthanized using pentobarbital sodium and the brain was removed and prepared for 2DG autoradiography, cytochrome oxidase, and thionin staining.

Prior to ICMS the experimental whisker evoked a response in only its corresponding barrel. As ICMS continued, the whisker evoked responses in both its corresponding barrel and the neighboring, non-corresponding barrel. 2DG autoradiography revealed an expanded area of activity which included adjacent barrels, whereas activation of the contralateral "control" whisker resulted in signal restricted to its corresponding barrel.

These results demonstrate that the cortical metabolic and electrophysiologic representation of the vibrissae is dynamically maintained and that novel inputs (e.g., non-corresponding whiskers) can be selected over a short time period. Additional studies are in progress to determine if the observed plasticity is strictly a cortical phenomenon or a cortical-subcortical event. Supported by NIH Grants DE-00227 and NS-22283.

331.13

RECOVERY OF RESPONSIVENESS IN AREA 1 AFTER CHRONIC ABLATIONS OF PORTIONS OF CORTICAL AREAS 3A AND 3B IN MONKEYS. P.E. Garraghty, S.L. Florence, and J.H. Kaas. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

We have previously reported that ablating physiologically identified parts of the somatotopic maps in cortical areas 3a and 3b in New World monkeys results in the immediate loss of responsiveness to peripheral stimulation of neurons in somatotopically matched regions of area 1. We can now add that this effect in area 1 is not due to a generalized deactivation of VPL, including those neurons projecting to area 1. We have recorded in VPL before and after ablation of areas 3a, 3b, and 1, and find no obvious immediate effect of the removal of cortex. Similar multiunit receptive fields were defined, and there was no apparent change in the strength of the evoked activity. Thus, these thalamic neurons could eventually gain or regain the capacity to drive initially silenced neurons in area 1. In monkeys which were allowed to recover for 2-6 months after ablations of physiologically defined portions of areas 3a and 3b, we found that the acutely silenced zone of cortex had indeed regained responsiveness. The map which had emerged, however, differed from the one defined in area 1 prior to the ablation in that activity was evoked by stimulation of skin surfaces with intact representations in areas 3a, 3b, and 1. These results provide strong support for the hypothesis that area 1 is activated by serial cortico-cortical inputs, with thalamic inputs perhaps playing a modulatory role. (Supported by N.I.H. NS16446.)

331.14

REORGANIZATION OF PRIMARY SOMATOSENSORY CORTEX AND FUNCTIONAL RECOVERY FROM STROKE IN THE ADULT OWL MONKEY. C. Xerri *, W. M. Jenkins, M. M. Merzenich, B. Peterson* and R. Beitel.* Coleman Laboratory, UCSF, San Francisco, CA 94143-0732, USA.

A previous study conducted in our Laboratory showed that focal lesions within the area 3b of the primary somatosensory cortex (S1) resulted in a drastic reorganization of the cortical map within the zone of cutaneous representation of hand digits. Cortical remodeling consisted in 1) the emergence of a new representation of the skin surfaces formely represented within the infarcted zone, in the cortical territories surrounding the lesion; 2) abnormal topographies and receptive field sizes. The present experiment examines the hypothesis that causal association exists between representational reorganization and functional recovery of digit dexterity after a focal cortical infarct in area 3b. Owl monkeys were trained to retrieve small banana pellets from different size wells on a modified Kluver board. A map of the cortical representation of the hand used in this retrieval task (RT) was derived on the basis of multiunit recordings at the completion of testing. A vascular lesion restricted to the representation of the 2 digits primarily engaged in the RT was induced. Post-lesion functional deficits and subsequent recovery of digit dexterity were assessed by a quantitative evaluation of changes in the RT performance. Preliminary results indicate that: 1) a hand preference can develop as a result of training, depending on the degree of digital dexterity required in the RT; 2) acquisition of digital dexterity seems to selectively induce a significant enlargement of the representations of engaged digit surfaces; 3) the relatively large cortical lesion induces profound deficits in RT performance; 4) functional recovery occurs progressively. After recovery of the RT performance, remapping experiments suggest that 1) within area 3b some specific skin surface representations re-emerge in the cortical zones bordering the injury site; 2) re-emergent skin surfaces appear to be functionally related to the RT; 3) within area 3a a cutaneous representation of behaviorally engaged skin surfaces emerges; no such cutaneous representation was observed in area 3a ipsilateral to the hand used in the RT; 4) alteration of the representational features within area 1 may also be related to the functional recovery. Research supported by NIH Grant NS-10414, and Hearing Research, Inc.

331.15

STIMULUS-LOCKED ~ 10 Hz OSCILLATIONS IN VISUAL AND SOMATOSENSORY CORTICES: EXPERIMENT AND DYNAMIC THEORY. G. Schöner, H.R. Dinse, F. Spengler, K. Kopecz, Inst. Neuroinformatik, Ruhr-Univ., 4630-Bochum, Germany.

Temporally structured neural responses to photic on/off stimuli have been observed in different areas of visual cortex of cats: Between 2 and 4 activity peaks in PSTHs at periodic intervals reflect stimulus-locked oscillations in the range of 5 to 20 Hz that lead to time-dependent receptive fields (RFs) and RF properties (Dinse et al., *Concepts of Neurosci.* 1, 199-238 (1990)). Recent experiments in fully awake animals (rabbit, visual cortex) have revealed similar stimulus-locked oscillations (Dinse & Krüger, unpublished). In a new series of experiments in somatosensory cortex of urethan anesthetized rats we ask how these long-lasting oscillations (up to several hundred milliseconds) affect response to new incoming stimuli by investigating temporal interference patterns between intrinsic neural dynamics and the timing of stimulating cutaneous RFs either periodically or with a double click at different interstimulus intervals. In ~ 50 % of the cells we observe stimulus-locked oscillations at frequencies of 9 to 15 Hz (between 4 and 9 peaks in the PSTH). Our results suggest that the neural response contains two components: a strictly stimulus-locked early response, that can be understood in terms of a classical transfer function model, and an oscillatory late reponse, that is stimulus-locked only if the system is excited from a resting initial state. We propose that these oscillations can be viewed as indicative of collective states of cortical networks and build a dynamic theory of cortical neural reponse on these basic functional units.

331.16

RESPONSE PROPERTIES OF TWO DIFFERENT SETS OF NEURONS IN SmI CORTEX OF THE RACCOON. **S.D. Stoney, Jr. and G.S. Doetsch. Dept. of Physiol. & Endocrinol. and Dept. of Surg. (Sect. of Neurosurg.), Med. Coll. Ga., Augusta, GA 30912.**

Extracellular recordings were made from single neurons in glabrous skin (G) and "heterogeneous" hairy skin/claw (H) subdivisions of forepaw digit 3 somatosensory cortex in chloralose-anesthetized raccoons. Fifty G cells and 41 H cells, each receiving excitatory input from on-focus digit 3, were studied with electrical and mechanical stimulation of each digit. G and H neurons differed significantly in their input convergence--the percentage of neurons (%N) excited by stimulating off-focus digits and the number of digits from which neurons could be driven were much greater for H cells. Surprisingly, G and H neurons responsive to stimulating any one digit did not show significant differences in probability of firing (P), spikes per response (S/R), or spike latency (L). The pattern of convergent excitatory inputs from different digits to each set of neurons was symmetrical--%N and P decreased, while L increased, with distance of each off-focus digit from digit 3, indicating corresponding variations in synaptic accessibility and conduction time. S/R did not vary significantly across digits, suggesting that strength of synaptic drive is largely independent of accessibility. These finding indicate that strengthening of existing patterns of off-focus inputs to G and H neurons may contribute to the appearance of "novel" responses of digit 3 neurons following digit 3 denervation (Kelahan & Doetsch, 1984). **Supported in part by NSF Grant BNS-8419035.**

VISUAL CORTEX: ORGANIZATION AND CONNECTIONS

332.1

COMPARISON BETWEEN THE DISTRIBUTION OF THE INOSITOL TRISPHOSPHATE RECEPTOR AND CALCIUM-BINDING PROTEINS IN THE CENTRAL VISUAL SYSTEM. L.B. Nabors[1], R.R. Mize[1], N. Maeda[2*], and K. Mikoshiba.[2] Dept. Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN 38163[1] and Inst. for Protein Research, Osaka University, Osaka, Japan[2].

Many intracellular systems depend upon the maintenance of calcium homeostasis in brain cells. One is the phosphotidylinositol system, in which inositol trisphosphate (IP3) binds to its receptor causing the release of calcium. Other systems involve the calcium-binding proteins which are believed to mediate intramembrane Ca^{++} transport and buffering. Immunocytochemical studies have shown that cerebellar Purkinje cells have high concentrations of both the IP3 receptor and the calcium-binding proteins calbindin (CaBP) and parvalbumin (PV). Selected neurons in the central visual system also are labeled by CaBP and/or PV antibodies. In this study, we asked whether these neurons are also labeled by antibodies to the IP3 receptor.

In agreement with previous studies, cerebellar Purkinje neurons were intensely labeled by antibodies to CaBP, PV, and the IP3 receptor. Within the visual cortex, the IP3 receptor was concentrated primarily in small and medium-sized neurons within layer IV. By contrast, CaBP was localized primarily in small neurons in layers II-III. PV was localized in selected neurons throughout layers II-VI. No neurons were found to be labeled by the IP3 receptor antibody in the lateral geniculate nucleus, pretectum, or superior colliculus, even when antibody exposure time was increased 2 fold and/or the concentration of the antibody was increased 10 fold. By contrast, PV and CaBP positive neurons were found in all three subcortical visual structures, as we have reported previously. We conclude that within the visual system of cat the inositol trisphosphate receptor and the calcium-binding proteins must be involved in different intracellular Ca^{++} related events. Supported by NIH grant EY 02973.

332.2

LATERAL GENICULATE PROJECTIONS TO THE SUPERFICIAL LAYERS OF VISUAL CORTEX. W. Usrey, E. Muly, and D. Fitzpatrick. Dept. of Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

In recent studies of tree shrew striate cortex, we have focused on the organization of geniculate projections to layer IV and the projections from layer IV to layer III. While these pathways no doubt play a dominant role in determining the response properties of layer III neurons, there are additional pathways from the lateral geniculate nucleus that terminate directly in layer III. Previous studies using bulk injections of WGA-HRP demonstrated that the projections to layer III originate from layers 3 and 6 of the LGN and terminate in different parts of layer III. In the present study we used extracellular injections of biocytin to examine the cortical projection patterns of single neurons in LGN layers 3 and 6.

Consistent with earlier work, we found that LGN layer 3 terminates densely in layer IIIb with sparse terminations continuing to layer I, while LGN layer 6 projects heavily to lower IIIc. In addition, however, we found that both layers 3 and 6 send collaterals to other cortical layers: neurons in LGN layer 3 project sparsely to layers V and VI as well as to the middle of layer IV; neurons in LGN layer 6 project to the bottom of layer IVb and sparsely to layers I-IIIb.

Thus, both LGN layers 3 and 6 project to multiple layers of striate cortex, including layers III and IV. The patterns of projections to layers III and IV are significant in light of our studies of the connections from cortical layer IV to layer III. Individual neurons in LGN layers 3 and 6 send axonal branches to subdivisions of layers III and IV that are themselves interconnected. Therefore, LGN neurons that project to the superficial cortical layers have axons which respect the patterns of interlaminar connections established by layer IV neurons. Supported by EY06821.

332.3

THE MORPHOLOGICAL BASIS FOR BINOCULAR AND ON/OFF CONVERGENCE IN TREE SHREW STRIATE CORTEX. E. Muly and D. Fitzpatrick, Dept. Neurobio., Duke Univ.Med.Ctr., Durham NC, 27710.

Based on the pattern of lateral geniculate terminations in layer IV, we have suggested that ON/OFF and binocular convergence in the tree shrew occurs in two stages: 1) the ON pathways from the two eyes are combined in layer IVa and the OFF pathways from the two eyes in layer IVb; 2) binocular ON and OFF pathways are brought together via the projections from layer IV to the superficial layers. We tested this idea using biocytin to examine the projections of layer IV neurons.

Consistent with our view, we found that neurons in IVa and IVb have overlapping terminal fields throughout layers II and III. These projections are organized in a highly stratified, mirror-symmetric fashion: neurons located at the edges of layer IV (upper IVa and lower IVb) project to the deepest part of layer IIIc; neurons in the middle of layer IV (lower IVa and upper IVb) project to layer IIIb and above; neurons in the middle of IVa and the middle of IVb have terminal fields that are intermediate between these extremes.

The stratified nature of the projection out of layer IV resembles the pattern of ipsilateral and contralateral eye inputs to layer IV: inputs from the ipsilateral eye are limited to upper IVa and lower IVb; those from the contralateral eye terminate throughout the depth of IVa and IVb. Thus cells near the edges of layer IV receive strong input from both eyes, while those in the middle of layer IV receive mostly contralateral input. We suggest that the projections from layer IV to layer III are organized to bring together the ON and OFF pathways while matching the ocular dominance gradients established in IVa and IVb. Supported by EY06821 and EY06661.

332.4

ORGANIZATION OF VISUAL CORTEX IN THE CALIFORNIA GROUND SQUIRREL. M.I. Sereno, H.R. Rodman, and H.J. Karten, Departments of Cognitive Science and Neurosciences, U.C. San Diego, La Jolla, CA 92093.

Ground squirrels are especially suitable for studies of the mammalian visual system because of their well-developed visual pathways, color vision, diurnal habit and availability. We made extensive microelectrode maps of visual cortex in urethane-anesthetized California ground squirrels (*Spermophilus beecheyi*), and then stained the cortex with the Gallyas myelin stain after physically flattening it.

As in tree squirrels (Hall et al., 1971), these animals have an extensive representation of the lower visual field in dorsal V1. The horizontal meridian is approximately parallel to the coronal plane and the upper field representation begins near the occipital pole, curving underneath the dorsal surface. Receptive fields in V1 are as small as 2 deg. in diameter at the center of gaze and increase to about 15 deg. in the far periphery. As in rabbits, rats, and cats, the binocular visual field nearest the vertical meridian is represented bilaterally in V1. This bilaterally represented binocular zone (assayed by recordings from both hemispheres through one eye) is 5-7 deg. wide near the horizontal meridian. There are three distinct compartments in the myelin pattern within V1 that may correspond to the monocular, binocular, and bilateral binocular zones.

Progressing laterally beyond the border of V1, receptive fields move out 10-30 deg. and then reverse to approach the vertical meridian, suggesting that much of V1 is bordered by two successive areas akin to V2 and V3 in tree squirrels (Hall et al., 1971). We have no evidence for the existence of multiple visual areas *directly* adjoining dorsal V1 similar to those found in rats and other rodents. Beyond the third representation is a zone of variable responsiveness and patchy myelination containing an expanded representation of the horizontal meridian and at least two additional maps of the visual field. Lateral to this intermediate zone, responses are much more robust and at least two distinct areas are found: 1) area L has large fields and an orderly map of the contralateral hemifield, with lower fields located rostrally and upper fields caudally, and 2) ventral to L, within a heavily myelinated zone designated Tp by Hall et al., is an area with strongly responsive neurons and receptive fields covering as much as the whole contralateral lower hemifield.

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332.5

MORPHOLOGY OF NEURONS IN CAT LATERAL SUPRASYLVIAN SULCUS THAT PROJECT TO AREA 17 AND TO THE SUPERIOR COLLICULUS. G. Einstein and Y. Liu. Department of Neurobiology, Duke University, Durham N.C. 27710

The laminar organization of efferent neurons in the cat's visual association cortex is well established, but their dendritic morphology and its relation to afferent input has not been fully explored. Since it is this relationship that underlies neuronal receptive field properties, and hence the type of information neurons relay, we studied the dendritic morphology of two populations of neuron in the posterior medial lateral suprasylvian sulcus (PMLS); those that project to area 17 and those that project to the superior colliculus. We did this by combining retrograde tract tracing methods with intracellular injections of Lucifer Yellow in lightly fixed slices.

Both projection populations contained diverse morphologies of spinous neuron. Cortical-projecting neurons in layer 2/3 had medium sized somas, basilar dendrites that extended only to the top of layer 4, and apical dendrites that ascended to layer 1; in layers 4-6 they had small to medium somas of atypical pyramidal morphologies with dendrites that could be very short or could extend horizontally for long distances (up to 1 mm) remaining within the infragranular layers. In contrast, most collicular-projecting neurons had medium to large somas, basilar dendrites that extended into layer 6, and apical dendrites that ascended to layer 1. These results indicate that cortical- and collicular-projecting neurons sample input from the same afferent pool; however, because their dendritic morphologies differ, they sample it in different ways. Cortical-projecting neurons are distributed to layers 2-6 but have restricted dendritic fields; so a single neuron receives input from, at most, a few sources. Collicular-projecting neurons are restricted to layer 5 but have dendritic fields that stretch from layer 1 to layer 6; so a single neuron probably receives input from multiple sources. Thus, in association, as in primary visual cortex, the information these two populations send their respective targets is probably different. (Supported by NEI grant R29 EYO7840)

332.6

A REASSESSMENT OF THE MAP IN LATERAL SUPRASYLVIAN VISUAL CORTEX: THE LOWER VISUAL FIELD. H. Sherk and K. Mulligan. Dept. of Biological Structure, U. of Washington, Seattle, WA 98195.

The visual field representation in the cat's lateral suprasylvian cortex has been studied by a number of groups. However, we have observed retinotopic peculiarities in this region not described in any published map. We therefore reinvestigated the lower field representation in the suprasylvian area that receives striate input, the Clare-Bishop area. (It coincides approximately with the lower field representation in Palmer, Rosenquist and Tusa's area PMLS). Both physiological and anatomical mapping (by the injection of tracers in area 17) were done. The entire suprasylvian cortex was flattened graphically by computer. In experiments with large numbers of recording sites, a complete map was generated by non-linear interpolation between sites.

The map was more orderly than reported by some groups, and its overall layout was simple. The most inferior visual field was located anteriorly, and central field was posterior, at the bend of the suprasylvian sulcus. As noted by others, there was often a progression from periphery towards the vertical meridian (VM) when the electrode moved down the medial bank of the suprasylvian sulcus. However, in detail the map was not simple. Some parts of the visual field, such as the area centralis, were duplicated. Curiously, the lower VM was split into two islands, and the gap between them represented more peripheral visual field. The posterior VM island was entirely embedded within the map instead of lying along a border.

Magnification factor could not be measured directly due to the map's complexity, but was deduced by comparisons with area 19. As in area MT in the macaque, the inferior VM (beyond about 15^{o} down) was highly compressed, while the area centralis was expanded relative to area 19.

332.7

RETINOTOPIC SCATTER WITHIN CELL COLUMNS IN THE LATERAL SUPRASYLVIAN CORTEX IS REMARKABLY SMALL. K. Mulligan and H. Sherk. Dept. of Biological Structure, U. of Washington, Seattle, WA 98195.

Many electrophysiological studies of the striate-recipient region of the cat's lateral suprasylvian cortex have described its retinotopic map as disorderly. This disorder has been generally attributed to a high degree of scatter among receptive fields within cell columns perpendicular to the cortical surface. However, only tangential electrode penetrations have been used in mapping studies, so the degree of scatter *within* cell columns remains unknown. We have recorded receptive fields in penetrations parallel to columns by angling the electrode about 45^{0} from vertical, and entering the cortex from the medial side of the suprasylvian gyrus. The electrode traversed the suprasylvian gyrus from medial to lateral, passing through white matter and then through layers 6 to 2 of the lateral suprasylvian cortex. Histological reconstructions showed that 13 penetrations were nearly parallel to cell columns (on average 8.7^{0} away).

Although the receptive fields were large within each penetration, they were surprisingly tightly clustered, with many fields almost completely superimposed. At any given eccentricity the scatter of receptive field centers was much less than the area of the largest field. We also measured the scatter in the receptive fields of afferents from area 17 and 18 after silencing suprasylvian cells with local kainic acid injections. Again, we found only modest scatter.

The scatter of the receptive field centers of cells in the lateral suprasylvian cortex relative to the area of the largest field within a cell column was comparable to that reported in area 17. It was much less than that reported in areas 18 and 19. This high degree of local order suggests that the global disorder cannot be attributed to scatter within cell columns.

332.8

FEEDBACK CONNECTIONS IN VISUAL CORTEX CONTACT INHIBITORY NEURONS. R.R.Johnson and A.Burkhalter, Dept. of Neurosurgery, Washington Univ. Sch. Med., St. Louis, MO 63110.

Little is known about the role of feedback input from extrastriate cortex (area 18a) to primary visual cortex (area 17). To understand the circuitry we have examined whether inhibitory neurons are targets of feedback excitation. For this purpose we labeled feedback projections with the anterograde tracer Pha-L and examined their relationship to neurons in area 17 that were stained for glutamic acid decarboxylase (GAD) or parvalbumin.

Feedback projections from area 18a terminated in all layers of area 17 except layer 4. Boutons from feedback fibers were closely apposed to every GAD cell body within the projection zone, except in layer 1. Feedback boutons on parvalbumin labeled dendrites also indicated the presence of axodendritic contacts. Many feedback fibers appeared to terminate on unlabeled elements which may represent components of pyramidal cells. Such cells were always surrounded by a large number of GAD and parvalbumin labeled terminals.

These results suggest that inhibitory and excitatory neurons in layers 2/3, 5 and 6 of area 17 receive feedback excitation. By contrast feedback input to layer 1 appears to provide only for direct postsynaptic excitation of pyramidal cells via their apical dendrites. It seems likely that feedback input on inhibitory neurons results in the suppression of pyramidal cell activity. The results are also consistent with the possibility that feedback excitation results in both direct facilitation of pyramical cells followed by indirect inhibition mediated through GABAergic interneurons.

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332.9

SPECIALIZED VASCULARIZATION OF THE PRIMATE VISUAL CORTEX. D. Zheng, A. S. LaMantia and D. Purves Department of Neurobiology, Duke University Medical Center, Durham, NC 27710

We have analyzed the distribution of cerebral microvessels with respect to cortical modules, laminae, and cytoarchitectonic areas in the squirrel monkey visual cortex. In tangential sections of the primary visual cortex (area 17), the mean total length of microvessel profiles per unit area within blobs was 42% greater than within adjacent (interblob) areas. Similarly, microvessel length per unit area in another class of module, the stripes in the secondary visual cortex (area 18), was 27% greater than in interstripe regions. Microvessel distribution also varied systematically from layer to layer in the primary visual cortex, being greatest in lamina IVc. Finally, the overall microvessel length per unit area in sections of area 17 was 26% greater than that in area 18. These results indicate that the distribution of microvessels in the monkey visual cortex reflects the pattern of iterated cortical modules such as blobs and stripes, and major features of cortical organization such as laminae and areal boundaries. In principle, these vascular patterns should be discernible in living animals with appropriate techniques, and may facilitate non-invasive imaging of modular, laminar and areal architecture in the intact brain.

332.10

A COMPARATIVE STUDY OF THE SUBCORTICAL CONNECTIONS OF THE DORSOLATERAL AREA (V4) IN PRIMATES. G. E. Steele, R. E. Weller and J. H. Kaas. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294 and Dept. of Psychology, Vanderbilt Univ., Nashville TN 37240.

In monkeys, the major cortical source of visual information to inferior temporal cortex is an area called the Dorsolateral Area (DL) or V4. Patterns of cortical connections suggest that DL (V4) possesses caudal and rostral subdivisions (Steele et al., '91, J. Comp. Neurol., 306). The present study determined the subcortical connections of caudal DL/V4 in squirrel monkeys (Saimiri sciureus), owl monkeys (Aotus trivirgatus) and macaque monkeys (Macaca fascicularis) following sterile injections of WGA-HRP, tritiated amino acids and fluorescent dyes in anesthetized animals. The injections in caudal DL and V4 labeled similar structures, including the locus coeruleus, dorsal raphe, superior colliculus, pretectum, lateral and inferior pulvinar, lateral geniculate, intralaminar nuclei, lateral hypothalamus, basal ganglia and claustrum. These results support the hypothesis that at least caudal DL and V4 are homologous. Supported by EY07147 to REW and EY02686 to JHK.

332.11

COMPARISON OF INPUTS FROM AREAS V1 AND V2 TO AREAS V4 AND TEO IN MACAQUES. H. Nakamura*, R. Gattass, R. Desimone, and L.G. Ungerleider. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

To investigate the differential inputs to areas V4 and TEO, we injected them with multiple retrograde tracers in 4 macaques and analyzed the distributions of labeled neurons in V1 and V2 using flattened preparations of the cortex. In V1, labeled neurons were seen after injections in V4 but not TEO. V4-projecting neurons were located in the foveal representation of V1, overlapping both the cytochrome oxidase-rich blob and interblob regions. In V2, labeled neurons arising from both V4 and TEO were seen. Although TEO-projecting neurons were far sparser, they were intermingled with V4-projecting neurons. Both V4- and TEO-projecting neurons were located in stripes running orthogonal to the V1/V2 border. V4-projecting neurons were in both cytochrome oxidase-rich thin stripes and interstripe regions, confirming prior reports, and also formed a continuous band along the V1/V2 border in the foveal representation that intersected the stripes in V2. TEO-projecting neurons were also found in the interstripe regions and probably the thin stripes as well. Because similar anatomical subregions of V2 project to V4 and TEO, they may receive similar visual information. This could explain the partial sparing of color and form vision that are seen after lesions of V4.

332.12

SUBCORTICAL CONNECTIONS OF INFERIOR TEMPORAL AREAS TE AND TEO IN MACAQUES. M.J. Webster, L.G. Ungerleider, and J. Bachevalier. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

To investigate the subcortical connections of inferior temporal cortex, we injected areas TE and TEO of 6 rhesus monkeys with retrograde and anterograde tracers. TE and TEO receive nonreciprocal inputs from several thalamic nuclei, including paracentralis, ventralis anterior magnocellularis, centralis (mainly densocellularis), and limitans, and TE also receives input from reuniens. Additional nonreciprocal inputs to both areas arise from the hypothalamus, basal nucleus of Meynert, dorsal raphe, locus coeruleus and reticular formation. TE and TEO are reciprocally connected with the pulvinar and ventral portion of the claustrum. The main subcortical nonreciprocal output from TE and TEO is to the basal ganglia and from TEO to the superior colliculus. TE also sends a very limited projection to the n. medialis dorsalis magnocellularis of the thalamus. Although the connections of TE and TEO are overlapping in most subcortical structures, they are partially segregated in the pulvinar and basal ganglia. In the pulvinar, the connections of TE are located medial to those of TEO in the medial, lateral, and inferior nuclei. In the basal ganglia, the projections of TEO extend into more caudal portions of both the ventral putamen and tail of caudate than do those of TE.

332.13

SUBCORTICAL CONNECTIONS OF INFERIOR TEMPORAL AND POSTERIOR PARIETAL CORTEX IN MACAQUES. J.S. Baizer, L.G. Ungerleider, and R. Desimone. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

To investigate the subcortical connections of the object-vision and spatial-vision cortical processing pathways, we injected the inferior temporal (area TE) and posterior parietal (areas VIP and LIP) cortex of 6 rhesus monkeys with retrograde or anterograde tracers. Although several subcortical structures were found to project to both the temporal and parietal cortex, including medial and lateral pulvinar, claustrum, and n. basalis, the cells projecting to the two cortical regions were mainly located in nonoverlapping parts of those structures, except in n. basalis where they were intermingled. Likewise, the projections from temporal and parietal cortex to subcortical structures, including the caudate and putamen, were largely segregated. Finally, projections to the pons and superior colliculus arose from parietal but not temporal cortex, whereas the lateral basal and medial basal nuclei of the amygdala were found to be reciprocally connected with temporal but not parietal cortex. Additional, nonreciprocal, projections to the lateral nucleus of the amygdala arose from temporal cortex only. The results indicate that, like the cortical connections of the two visual pathways, the subcortical connections are remarkably segregated. (Supported by MH4213 and the Whitehall Fdn.)

332.14

CORTICOCORTICAL CONNECTIONS IN MACAQUE VISUAL CORTEX EXHIBIT DIFFERENTIAL PATTERNS OF NEUROFILAMENT PROTEIN DISTRIBUTION. S.B. Kupferschmid, P.R. Hof, J.H. Morrison. Fishberg Research Center for Neurobiology, Mt Sinai Sch Med, New York, NY 10029.

SMI32 is a monoclonal antibody that recognizes a nonphosphorylated epitope on neurofilament protein that, within the primate cerebral cortex, is localized largely in pyramidal neurons with a specific regional and laminar pattern. The distribution of SMI32-immunoreactive (ir) neurons in the cortex suggests that certain cortical efferents are preferentially SMI32-ir. After an injection of Fast Blue (FB) in V2 near the V1 border many of the FB-containing cells in layer 4B of V1 are double labelled with SMI32. The contralateral callosally projecting neurons in deep layer 3 are 100% double labelled. The V2 injection also shows a population of superficially placed pyramidal cells in V3 that are double labelled. While McGuire & Siegel (Soc. Nsci. Abstr., 1990, p. 5) show that the neurons in layer 6 of V1 that project to V5 (MT) are SMI32-ir, we found that the projection from V5 back to V1 lacks an SMI32-ir component. Preliminary data suggest that double labelling with FB and SMI32 is asymmetric with respect to reciprocal connections in the hierarchical forward-feedback organization of visual areas. We are quantifying our results and examining other areas in the visual cortex to understand better the relationship of neurofilament immunoreactivity to cortical organization. Finally, after FB injection in V1 we observed that the geniculocortical projection is immunoreactive for the calcium binding protein parvalbumin. Supported by NIH grant AG06647, MSTP training grant GM07280-14 and AHAF.

332.15

COMPARATIVE IMMUNOCYTOCHEMISTRY OF CYTOSKELETAL PROTEINS IN VISUAL CORTEX OF CETACEANS AND PRIMATES.

I.I. Glezer, P.R. Hof, W. Janssen, J.H. Morrison, C. Leranth and P.J.Morgane
CUNY Med. Sch., NY, NY 10031, Fishberg Res. Ctr., Mt. Sinai Sch. Med., NY, NY 10029, Yale Univ. Sch. of Med., New Haven, CT 06510, Worcester Found. Exp. Biol., Shrewsbury, MA 01545.

Light and electron microscopic immunocytochemistry combined with computerized image analysis were used to study the laminar distribution of nonphosphorylated neurofilament (SMI32-positive) and microtubule-associated (MAP2-positive) proteins. Primary (V1) and secondary (V2) visual areas in primates (Homo sapiens and Macaca fascicularis) and toothed whales (Delphinapterus leucas and Globicephala melaena) were studied. In cetacean V1 and V2 visual areas SMI32-positive pyramidal neurons are concentrated predominantly in layer V. In primate V1 and V2 visual areas SMI32-positive neurons are more uniformly distributed across all cortical layers, except layer I. There is no significant difference in overall numerical density of SMI32-positive neurons between primate and an cetacean visual cortices, although the laminar distribution is significantly different. MAP2-positive pyramidal cells in visual cortex of primates and cetaceans are present in significantly higher numbers than SMI32-positive neurons. The overall numerical density of MAP2 neurons is significantly higher in cetacean visual cortices than in primates. The ratio SMI32/MAP2 is significantly lower in cetacean visual cortex as compared to primates. The difference of this ratio between primates and cetaceans is especially prominent for upper layers (I-IV) of the visual cortex. The observed differences between primate and cetacean visual cortices in concentration and laminar distribution of cytoskeletal proteins may reflect the considerable disparity in cortical phylogenesis of these species. Supported by AHAF, NIH AG06647, and NSF grant BNS-87-42032.

VISUAL PSYCHOPHYSICS AND BEHAVIOR: BASIC PROCESSES

333.1

THE AFFERENT COMPONENT IN BLINK SUPPRESSION. W. H. Ridder,III*, A. Tomlinson* and P. Simmons.
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Previous studies of blink suppression have suggested that the mechanism is independent of the afferent signal. If this theory is correct, manipulation of the afferent visual input should not affect the magnitude of suppression. We tested this hypothesis by presenting sine-wave gratings (16 msec duration, square-wave onset and offset) at several times after the blink (post-blink, onset times of 0, 25, 50, 100, 200 and 400 msec) to measure blink suppression. The afferent signal was manipulated by changing the spatial frequency of the grating (1.0, 4.0 and 10.5 c/deg) viewed by the two subjects. The stimulus was presented on a Tektronix 608 monitor (viewed at 1.5 meters) with an Innisfree Image Synthesizer. The task was one of detection. A minimum of 20 trials were run at each of 5 contrast levels centered around threshold for each post-blink, onset time. Psychometric functions were produced from the data for each post-blink, stimulus onset and a Weibull function was fit to the data to determine threshold. As in previous studies, the magnitude of suppression was the greatest immediately following the blink and gradually declined until no suppression was evident at 100 msec after the blink. Contrast sensitivity curves were produced for the 0 and 400 msec post-blink, stimulus onset times. The difference in contrast sensitivity between the post-blink, onset times (the sensitivity at 400 msec minus that at 0 msec) was greatest for the low spatial frequencies. This suggests that the afferent signal does influence the magnitude of blink suppression. In light of this data, models of blink suppression should be modified to include an afferent component.

333.2

THE GREAT CIRCLE MODEL OF SPATIAL LOCALIZATION AND VISUAL PERCEPTION OF ELEVATION. L. Matin and W. Li*.
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Changing the pitch* of a complexly-structured visual field produces linear changes in the elevation of a target perceived to lie at eye level (VPEL). A single 63.9° pitched-from-vertical line in darkness yields an influence 83% as large. The 1-line influence increases exponentially with line length (space constant = 15°). Most other discriminations (e.g. intensity discrimination threshold) manifest spatial summation over less than about 1°; but the influence on VPEL from two parallel 12° pitched-from-vertical lines separated horizontally by 50.3° manifests summation as great as the increase with line length.

The Great Circle Model (GCM) accounts for these results: Assume a spherical approximation to the stationary eye; two principles of GCM are: (1) The influence of a line on VPEL increases monotonically with the angular distance on the central vertical retinal meridian (CVRM) between the upper pole of the eye and the point of intersection with the great circle containing the line's image. (2) The influences on VPEL of all lines whose images lie on great circles which intersect the same point on the CVRM (e.g., all parallel pitched-from-vertical lines) summate along a common exponential function. (Supported by AFOSR-91-0146, NSF BNS-8615059, and USPHS EY05929).

* Pitch = rotation of a visual field around a horizontal axis in the frontal plane of the observer.

333.3

Stabilized Retinal Perimetry with a Hemianopic Patient: Implications for Blindsight. C.M. Wessinger*, R. Fendrich and M.S. Gazzaniga. Program in Cognitive Neuroscience, Dartmouth Medical School, Hanover, N.H. 03756

Blindsight is the ability of cortically blind patients to describe certain attributes of stimuli they report no conscious awareness of seeing. Blindsight occurs in some patients but not others. One possible explanation for this discrepancy is that some patients have a small degree of cortical function remaining within their damaged visual cortex, which mediates the residual capacities in perimetrically blind regions.

We performed perimetry on a patient, CLT, rendered hemianopic by a right occipital stroke. A Purkinje image eyetracker and mirror stabilizer system were employed to eliminate eye motion artifacts. Using a two-alternative forced-choice paradigm, 40 locations in the central 15° of CLT's blind field were tested by flashing a 1° diameter black circle 3 times for 96 msec on a white CRT screen. The task was to identify during which of two consecutive 600 msec intervals the target was flashed. Stabilization insured the accurate retinal placement of the target despite the extended presentations.

Humphrey perimetry had previously shown a dense left field hemianopia with lower left quadrant macular sparing. Our stabilized perimetry revealed the scotoma had a complex structure, with a narrow zone of sparing along the vertical meridian in the blind upper left quadrant, a zone of blindness along the vertical meridian in the spared lower left macula, and a 1° island of residual vision embedded within the scotoma. The existence of this small isolated island of vision gives credence to the idea that some demonstrations of blindsight may be mediated by residual cortical function in the form of similar islands of sparing. Supported by NIH/NINDS P01 NS17778-09 and the McDonnell-Pew Foundation.

333.4

BANDWIDTH OF HUMAN ORIENTATION SENSORS MEASURED BY FILTERED NOISE STIMULI. M. Shadlen, K. Britten and W.T. Newsome, Dept. of Neurology and Neurobiogy, Stanford University, Stanford, CA. 94305

We report a new stimulus paradigm for the study of visual mechanisms underlying the perception of orientation. Stimuli are random textures in which a desired orientation is made to appear more conspicuous by filtering out unwanted noise components at distant orientations. By varying the bandwidth properties of the filter, the relative visibility of one orientation may be affected preferentially, and the difficulty of discriminating between any two orientations may be varied smoothly. At extremely narrow bandwidths, the stimulus appears as one dimensional oriented noise and is easily discriminated from a similarly filtered pattern at a dissimilar orientation. At very broad bandwidths the pattern appears more like a random texture and the intended orientation can only be guessed. Between these extremes the pattern appears crinkled and even orthogonal orientations can be discriminated only imperfectly.

We have employed such stimuli to estimate the bandwidth of orientation channels in normal human observers using standard 2AFC psychophysical methods. Results are in agreement with prior estimates employing masking/detection methodology. We expect these stimuli to be valuable in the investigation of neural mechanisms underlying simple orientation discriminations.

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333.5

SACCADIC LATENCIES TO STIMULI DEFINED ONLY BY LUMINANCE, CHROMINANCE, MOTION, AND DEPTH. R. M. McPeek*, M. A. Sommer*, and P. H. Schiller. Department of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

Bimodal saccadic reaction time distributions have been observed in visual detection tasks with targets defined by luminance information. The shorter-latency population of saccades has been called the "express saccade" population. We examined whether express saccades can be generated by a rhesus monkey to stimuli made visible only by luminance, chrominance, motion, and depth cues.

We found bimodal distributions of reaction times for luminance, chrominance, and motion detection tasks, suggesting that express saccades can be made to stimuli defined solely by these attributes. In contrast, we did not find an express saccade population of reaction times in the stereoscopic depth detection task.

These results suggest that express saccades are not generated exclusively by a pathway involving the striate cortex, area MT, and the parietal lobe, but can also be generated through other channels. This hypothesis is supported by lesion studies, in which express saccades have been observed in rhesus monkeys with MT lesions, V4 lesions, and paired MT-V4 lesions (Schiller et al, Invest. Ophthal. Vis. Sci., **31**, 1967, 1990).

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333.6

MOTION PERCEPTION AND OPTOKINESIS AT ISOLUMINANCE

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It is generally believed that motion perception is degraded at chromatic isoluminance and that moving objects appear to slow down. In this study, the oculomotor consequences at isoluminance are evaluated and the results are compared with perceived motion under similar conditions.

The stimulus was a lattice composed of 3x3 pixel tokens (3.6 min arc/pixel), randomly assigned to be red or green, and separated from neighbouring ones in all directions by 3 pixels. The background was either dark or an isoluminant gray. In all cases, pattern movement was to the right or left and restricted to an integral multiple of the period, usually 6 pixels. The luminance of the red components was fixed and the green varied. A 2-AFC direction discrimination paradigm was used for psychophysical assessment on three human subjects. Eye movements were obtained from two humans and two monkeys (Macaca mulatta) using magnetic search-coil oculography.

The results showed that optokinetic eye movements (OKN) were produced at all red/green ratios when a dark background was used, including the isoluminant condition. However, a significantly reduced OKN response was obtained with an isoluminant gray background. Perceived motion remained salient under both conditions. These results demonstrate that motion correlations based on chromatic differences alone can produce an optomotor response only if there is sufficient luminance contrast with the surround. The implications for neural processing of motion are discussed.

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333.7

RELATIVE DEPTH FROM MOTION AND STEREO CUES.

George J. Carman, Salk Institute VCL, San Diego, CA 92186.

A number of retinal cues provide information about the relative distance (depth) of surfaces in the visual environment from an observer. Here I describe how two of these cues, motion parallax and stereoscopic disparity, may be processed in similar fashion to yield a common representation of relative depth of surfaces useful for determination of the shape and trajectory of 3D objects.

When an observer moves while fixating at infinity, images of objects in the environment will have retinal velocities Vi which are inversely proportional to their depth Hi. But when an observer moves while tracking some nearby object, this relationship is broken by the additional component of retinal velocity Ve due to pursuit eye movement. However, this component of retinal motion is common to all images and constant across the retina at any given time. Thus by computing the relative velocity $Vri = (Vi\text{-}Ve)$ we obtain measures of retinal motion which are independent of pursuit eye movements, and which are inversely proportional to the depth of objects. Furthermore, ratios of this relative velocity will be invariant over changes in eye or observer motion, providing part of a mechanism for relative depth constancy, and an explanation of motion contrast effects.

A similar inverse relationship exists between the retinal disparity Di and object depth Hi for observers fixating at infinity. When observers fixate nearby objects, however, the nonzero eye convergence breaks this simple relationship by adding a global component of retinal disparity De. This component of disparity due to convergence can be removed by computing the relative disparity $Dri = (Di\text{-}De)$, providing measures which are inversely proportional to depth of objects. Furthermore, ratios of this relative disparity will be invariant over changes in convergence angle, providing another part of a mechanism for relative depth constancy, and an explanation of stereo contrast effects.

Due to the similarity of the proposed processing, both cues can be combined into a single representation constituting a map of relative depth in the visual field. The Middle Temporal (MT) area is the first representation of the primate visual pathway containing neurons selective for both retinal motion and retinal disparity, and which exhibit an antagonistic receptive field structure suitable for computing relative velocity. I will test this hypothesis by recording single MT neurons in awake, fixating macaques. I expect these neurons will exhibit supraclassical inhibitory surrounds for binocular disparity similar to those known for monocular motion. (Supported by NIH NRSA EY06179.)

333.8

HUMAN HEADING PERCEPTION DURING COMBINED TRANSLATIONAL AND ROTATIONAL SELF-MOTION.

L. S. Stone and J. A. Perrone*. Life Science Div. & Vision Group, NASA Ames Research Ctr, Moffett Field, CA 94035.

Local differential motion (LDM) of nearby points at different depths is one method for extracting heading from optic flow. To test this idea, 3 subjects made judgments of horizontal heading in response to visually-simulated forward translation plus yaw rotation under two conditions: motion toward (**A**) two superimposed vertical planes of random dots at different depths or (**B**) two non-overlapping half-planes at different depths above and below fixation. A 5.7° strip along the horizontal meridian was blank and stationary frames of **A** & **B** were indistinguishable. **A** provides LDM, **B** does not. Therefore, poor performance and higher sensitivity to rotation is predicted for **B**. However, mean systematic errors in perceived heading were small for both **A** & **B** (0.1±0.7° and 1.3±0.6° respectively for 1.5°/s yaw). Precision was similar for **A** & **B** and only weakly related to yaw rate (1.2° and 2.3° at 1°/s vs 2.0° and 2.4° at 2°/s) with performance nearly asymptotic by 200ms. We conclude that self-motion processing, thought to occur in extrastriate cortex, does not rely on LDM.

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333.9

TRANSPARENCY CONSTRAINS MOTION, FORM PERCEPTION, AND STEREOPSIS. Daniel J. Plummer and V.S. Ramachandran, UCSD, La Jolla, CA, 92093.

If two neutral density (ND) filters overlap partially the luminance of the overlapping region is given by a multiplicative relationship. We had two grey rectangles overlapping to form a "cross" and randomly varied the luminance of the intersection. Naive subjects' ratings of transparency showed a suprising consistency with the physics. A similar relationship is seen if two moving square wave gratings are superimposed. When the intersection luminances were close to the multiplicative case, component motion was seen. (Ramachandran, 1989; Stoner, Albright, and Ramachandran, *Nature*, 1990) We offer two interpretations. (a) the visual system has access to 'tacit knowledge' of transparency, shadows, and veiling luminances. (b) if you assume a log signal compression in the retinae, multiplying the grating luminances is equivalent to adding them, and hence there would be zero Fourier energy at the blobs. But if you simply add the gratings you would inadvertantly introduce Fourier Energy of the blobs and this would cause 'motion capture'. (Ramachandran and Inada, 1985) The critical experiment is, therefore, to vary the intersection luminances independently of the gratings. We find this nulls out the energy at the blobs to produce component motion. We regard (a) and (b) as being complementary rather than mutually exclusive accounts. We also show that transparency powerfully constains 1) stereopsis. 2) 'Form Perception' in preattentive search or pop-out; alphabetical letters could be clearly discerned from background splotches if they were transparent, but were 'camouflaged' if luminances were incompatible. (Ramachandran, 1990) Supported by AFOSR 89-0414.

333.10

ON FILLING THE BLIND SPOT AND OTHER MEDICAL MARVELS V. S. Ramachandran and W. H. Aiken, UCSD, La Jolla, CA 92093, USA

Does filling in the blind spot involve creating an actual neural representation of the surround? We designed several new stimuli to answer this question. 1) A line running through the blind spot appeared continuous. This interpolation occurred even if the line segments on the two sides of the blind spot were colored differently. 1) If the center of a "star" fell in the blind spot, the pattern appeared complete. 3) If part of a circle was placed in the blind spot it appeared "chopped off", i.e. it was not completed. 4) A vertical column of dots was placed on the blind spot. At low spatial frequencies a "missing dot" was noticeable, i.e. no completion occurred. 5) A red ring (annulus) placed on the blind spot with its inner margin overlapping the outer edge of the blind spot looked like a homogenous red disk. Yet if a set of thin concentric rings were added around the annulus the blind spot appeared filled in with rings instead of homogenous red! Thus what fills in is not merely what is in the immediate surround but also the pattern from the remote surround. 6) A ring was placed on the blindspot so that it looked like a disc. If several other rings were scattered in the visual field, the ring in the blind spot region "popped out" preattentively. 7) A vertical line passed through the bind spot. The "terminators" created by the blind spot, interrupting the line and could not be used for motion correspondence.

What causes the "filling in?" We suggest that the effect arises partly in VI itself and partly from the activity of extrastriate neurons whose large receptive fields may straddle the blind spot.

333.11

PHANTOM CONTOURS: STIMULI THAT SELECTIVELY ACTIVATE THE MAGNOCELLULAR PATHWAY. D. Rogers-Ramachandran and V. S. Ramachandran. UCSD, La Jolla, CA 92093.

Equiluminous contours mainly excite the parvo (P) pathway and provide only a weak input to motion (Ramachandran and Gregory, 1978). Here we report a novel technique for selectively stimulating the magnocellular (M) pathway. The stimulus (Frame 1) was a "texture border" between black and white spots on a grey field. This was followed by frame 2 in which all the black spots were replaced with white, and white with black. At 20 Hz, subjects could not discriminate between the spots but could nevertheless see a "phantom contour" separating the two indiscriminable regions. On occluding the phantom contour the difference between the spots could be discriminated only at about 7 Hz. Since the M pathway can follow high flicker rates but is insensitive to the sign of the border the phantom border is "seen" exclusively by the M system. The P system can report the sign of the border but only at low flicker rates. Phantom contours were enhanced by blur, tolerated low contrasts (<10%) and vanished if red/green equiluminous spots were used.

The use of phase-reversing edges to stimulate the M pathway was first suggested by Livingstone & Hubel (J. Neuroscience, 1987). Our stimulus differs from theirs in using a texture border which effectively eliminates any edge artifacts (e.g. Mach Bands) that might arise from "real" contours.

Phantom contours allows us to "lesion" the P pathway in man. We find that the tilt illusion, "barber pole" illusion, size aftereffect and McCollough effect can be conveyed by phantom contours but not binocular rivalry. Phantom contours also provide a clinical test for glaucoma.

333.12

DOG AND CATS: THEY MAY LOOK DIFFERENT BUT THEY SEE THE SAME. M.S. Loop and M.M. Martin. Dept. of Physiological Optics, School of Optometry, University of Alabama at Birmingham, B'ham, AL 35294.

We have extensively examined the behavioral spatial contrast sensitivity and reaction time of a dog in a testing situation previously used with cats. To a flashed (70 cd/m^2, 500 msec, 10°, vertical, sine-wave) grating the dog was most sensitive around 1.0 cpd with a cut off sensitivity around 3.0 cpd. At equal physical contrasts the dog detected 0.25 cpd faster than he detected 2.5 cpd. At threshold equivalent contrasts 0.25 and 2.5 cpd were detected with the same latency around threshold but faster detection of the low spatial frequency emerged above threshold. These results were quite similar to those found with cats.

When grating duration was varied (100 or 1000 msec) at 0.25 cpd it had no effect upon sensitivity and no effect upon reaction times. However, at 2.0 cpd the dog was reliably more sensitive to the 1000 msec flash yet detected the 100 msec flash faster. Thus the dog behaved as if he (1) detected low spatial frequencies with a mechanism which responded relatively quickly and in a transient fashion; and (2) detected high spatial frequencies with a relatively slower mechanism which responded in a sustained fashion.

These data complement the reported similarities in ganglion cell morphologies between dogs and cats (Peichl, 1989, Neuroscience Abstracts, p. 1207).

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333.13

VISUAL REVERSAL-LEARNING DEFICITS AFTER TELENCEPHALIC VISUAL-SYSTEM LESIONS IN PIGEONS. L. Chaves* and W. Hodos, Dept. of Psychology, Univ. of Maryland, College Park, MD 20742.

Lesions of the telencephalic targets of the thalamofugal visual pathway (laminae IHA and HD of the visual wulst) in pigeons result in disrupted performance on a color reversal-learning task (Shimizu and Hodos, Behav. Neurosci., 1989). A subsequent study (Chaves and Hodos, Neurosci. Abstr., 1989) found that lesions of the OPT complex, the thalamic equivalent of the lateral geniculate, and source of afferents to the visual wulst, had no effect on this task. In contrast, lesions of nucleus rotundus, the thalamic component of the tectofugal visual pathway, resulted in greater reversal-learning impairments than did visual wulst lesions. We report here that lesions of ectostriatum, the telencephalic component of the tectofugal visual pathway and target of n. rotundus, produce deficits comparable to those observed after lesions of n. rotundus. This finding suggests that the deficits in reversal-learning after wulst lesions are not the result of interruption of the thalamofugal visual pathway, but rather that those effects are a consequence of the wulst's connections to the tectofugal pathway.

333.14

Visual and Auditory Cortex Ablations Following Cross-Modal Transfer and Recovery of Function in the Rat. R.A. Salazar*, R.B. Wallace and G.L. Baker-Salazar*, Depts. of Psychology, Univ. of New Mexico, Albuquerque, NM. 87131 & Univ. of Hartford, West, Hartford, Ct.06110.

Twenty Long-Evans Hooded rats were trained either under visual-auditory (V-A) or auditory-visual (A-V) Cross-Modal Transfer. Prior to the second training session, 5 of the A-V animals received auditory ablations and 5 V-A animals received visual ablations. The other ten control animals received sham operations, whereby they went through the same surgical procedures as the experimental animals but no ablations were performed. All animals were given two weeks to recover. Testing revealed significance using a 2X2 ANOVA during the second training session for the CTL versus EXP groups, the CS Modality groups, and the interaction. Transfer had ocurred even though the cortex that the animal used in the original training session was ablated. Information could have been received from the cortex used during the original training session by the cortex used in the second or retraining session, just prior to the operation.

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333.15

VERNIER ACUITY DEFICITS IN MENTALLY RETARDED ADULTS Stephen Oross III and Robert Fox. Department of Psychology, Vanderbilt University, Nashville, Tennessee 37240.

Our prior investigations, using random element stereograms (RDS) and kinematograms (RDK), have found that mildly mentally retarded adults appear to have fundamental deficits in depth and motion perception. Form discrimination and detection are impaired as element density, temporal correlation and number of frames are reduced. The deficits appear to be selective as visual acuity, contrast sensitivity, and color perception are not impaired. Moreover, control conditions indicate that the deficits cannot be attributed to failures of comprehension or peripheral visual anomalies.

These data have led us to investigate vernier acuity for both luminance-defined and motion-defined stimuli. Both mentally retarded and nonretarded adults were tested using conventional paradigms that utilize forced-choice methodologies. Although the mentally retarded subjects exhibited elevated thresholds for both stimulus classes, the thresholds for the motion defined stimuli were significantly higher and were further impaired by reductions in element density.

This pattern of results is similar to those obtained in our earlier studies and suggests that mildly mentally retarded adults have substantial perceptual deficits that arise early in the visual system. Consequently, these results invite reexamination of a prevailing view in the field of mental retardation that assumes most mildly mentally retarded adults have no patent neurological impairments. To that end, we discuss the results with respect to neural network models and research relating known neurological impairments to selective perceptual impairments in other populations.

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333.16

Visual Functions Associated with Rhesus Visual Pursuit Tracking. H. Zwick*[1], D.Q. Robbins[2], J. Calabrese*[1], K.R. Bloom*[1] and M. Cook*[1]. [1] Letterman Army Institute of Research, San Francisco, CA 94129 and [2] Ohio Wesleyan University, Delaware, OH 43015.

Degradation of visual performance following laser exposures has been extensively examined under a variety of different spatial and spectral viewing conditions. Much less work has been done relating the effects that suppression of foveal receptor mechanisms has on visually guided tasks such as pursuit tracking. Using various target resolution criteria we have evaluated the effects that subretinal damage threshold dosages have on visual pursuit tracking. Two rhesus monkeys were trained on a pursuit tracking task which required the animals to continuously track a horizontally moving annulus by maintaining a small spot from a HeNe laser pointer in the center of this annulus. By changing the wavelength of the annulus, intensity tracking performance functions for time on target could be obtained. Spectral sensitivity functions derived for high resolution requirements were more easily fitted with the 575 nm photopigment than were those requiring lower resolution. Laser flash effects measured for either on- or off-axis exposure revealed a significant parafoveal component related to resolution criteria. For high resolution criteria, both an on- and off-axis exposure effects were observed; for lower resolution criteria only the parafoveal function was affected. The neural motor processing of normal or degraded visual inputs may require both fine and gross neural processing mechanisms at and beyond the retinal level.

333.17

NEURAL NETWORK ANALYSIS OF VISUAL FIELD DEFECTS: WHAT IS THE NETWORK USING TO LEARN? M. Wall, M.E. Mulet*, A.D. Nelson*. Tulane University School of Medicine, Departments of Neurology and Ophthalmology, 1430 Tulane Avenue, New Orleans, LA 70112.

The visual field patterns used by neural networks to separate patterns of disease from a normal visual field is unknown. We trained a neural network (Brainmaker, California Scientific Software) on 393 visual fields to separate patients with visual loss due to pseudotumor cerebri from normal subjects. Conflicting facts were first purged from the data set. A training tolerance of 0.1 with a learning rate of 1 was used. We had 57 inputs (Humphrey program 24-2+). Test sets were made to see the influence of various nerve fiber bundle areas by equalizing test scores in various nerve fiber bundle areas. The testing tolerance was 0.20.

The network correctly categorized 85% of the subjects. With elimination of the area around the blind spot, the efficiency fell to 64%. The results for the inferior nasal area and cecocentral area were 72% and 76% respectively. We conclude pattern recognition of abnormal visual fields has a significant contribution from the peripapillary area.

333.18

THE RELEVANCE OF EYE MOVEMENTS AND BLINK MEASURES TO OBJECTIVE AND SUBJECTIVE MEASURES OF WORKLOAD. R. S. Kennedy, J. G. May, W. P. Dunlap, and M. G. Smith*. Essex Corporation, Orlando, FL 32803.

New cockpit display systems being introduced into military aircraft make high demands on human information processing. A need exists to link cognitive and motor performance to objectives measures of workload, perhaps by indexing neurophysiological events to task demands of the situation. Using a within-subjects design, bioelectric measures of various aspects of eye movements and blink (frequency, acceleration, displacement, etc.) were measured while visual and auditory (in the dark) tasks were performed. Oculomotor task demands and a workload metric were assessed independently and found to be related to the ocular measures. The general findings are that elements of eye activity (range of movement, number of blinks, and blink duration) correlated at a statistically meaningful level with either the visual task demands or with the workload. This study was the first step in the design of an automated task load analysis system using eye movements as biocybernetic applications to aid in designing new aircraft cockpit display systems.

BASAL GANGLIA AND THALAMUS: ELECTROPHYSIOLOGY

334.1

VOLTAGE-DEPENDENT 40-HZ OSCILLATIONS IN RETICULAR THALAMIC NEURONS. D. Pinault and M. Deschênes, Centre de Recherche en Neurobiologie, Laval Univ. Québec, Canada G1J 1Z4.

There is now growing evidence that focused arousal is associated with the occurrence of 40-Hz field potential oscillations in the brain. As most oscillatory neuronal activities in the central nervous system depend upon the intrinsic membrane properties of the cellular populations in which oscillations develop, and because the reticular thalamic (RT) nucleus occupies a pivotal position in thalamocortical networks, one may wonder if RT cells would not possess intrinsic membrane properties that could allow them to act as pacemakers of 40-Hz oscillations in the thalamus and cortex. Recordings performed in the rat RT nucleus under urethane anaesthesia revealed that, amidst spontaneously bursting units, many neurons discharged tonically, like clocks, at rates that were remarkably fixed within a frequency range of 25-60 Hz (i.e. 40 Hz). Control recordings performed in various thalamic nuclei and cortical regions did not disclose any field potential or unitary activity in the 40-Hz range. On the other hand, intracellular recordings showed that the clock-like behavior of RT cells was driven by a subthreshold membrane potential oscillation whose frequency was voltage-dependent; membrane depolarization increased the frequency while hyperpolarization decreased it and eventually stopped completly the oscillator. In thalamic relay cells, the 40-Hz firing of RT neurons was associated with the occurrence of inhibitory postsynaptic potentials in the 40-Hz range. Given the pivotal position of RT neurons in thalamocortical networks, the present results support the hypothesis that the RT nuclear complex might play a pacemaker function in the genesis of 40-Hz field potential oscillations in the thalamus and cortex during states of focused arousal. (Supported by the MRC of Canada)

334.2

CONTROL OF 40-HZ FIRING IN RETICULAR THALAMIC CELLS BY AMINES AND EXCITATORY AMINO ACIDS. M. Deschênes and D. Pinault, Centre de Recherche en Neurobiologie, Laval Univ., Québec, Canada G1J 1Z4.

In a companion abstract we reported a 40-Hz firing mode in reticular thalamic (RT) cells in rats under urethane anaesthesia. This tonic modeof discharges was shown to depend upon an intrinsic voltage-dependent pacemaker mechanism. Since this pacemaker activity was present at membrane potentials positive to - 65 mV, we investigated which neurotransmitters might be involved in depolarizing RT neurons within the voltage range where the pacemaker activity is expressed. Experiments were conducted in urethane anaesthetized rats using extracellular recordings and local application of antagonists against neurotransmitters known to be involved in the modulation of RT cells' activity. All drugs were dissolved in a Ringer solution (pH: 7.4) and they were applied in small quantities (25-100 nL) by pressure through one barrel of a micropipette assembly (tip size: ≈ 10μm; distance between the tips: ≈70 μm). The effect of each drug was tested on at least 10 RT cells that were firing spontaneously at 40 Hz. Forty-Hz firing was abolished by the α1 antagonist prazosin (10 μM) and by kynurenate (100 μM), a wide spectrum antagonist of glutamate and aspartate. Ketanserin, at a concentration of 20 μM, also blocked tonic firing but this effect could be due to the affinity of the drug for α noradrenergic receptors. Conversely, scopolamine (100μM) exerted a permissive action on the expression of 40-Hz activities. Many spontaneously bursting units started firing at 40 Hz under the influence of this muscarinic antagonist. Given the mode of action of amines and acetylcholine on RT neurons, and the possibility of a metabotropic action of glutamate, the above results suggest that deactivation of (a) K conductance(s) is critically involved in the expression of a 40-Hz pacemaker activity in RT cells. (Supported by the MRC of Canada).

334.3

RESPONSES TO INJECTION OF RAMP CURRENTS IN NEOSTRIATAL NEURONS. Galarraga, E. and Bargas J. Depto. de Fisiología, Biofísica y Neurociencias. CINVESTAV IPN. Ap. Postal 14-740, México, D.F. 07000.

Intracellular recordings were obtained from brain slices of the rat brain. 1) Predominant ionic conductances in a given voltage range, 2) their thresholds, and 3) their actions on the firing pattern, were analyzed using membrane voltage responses after ramp currents (rate: 0.01 to 0.1 nA/ms). Quasi-steady-state I-V plots and I-F plots were performed. I-V plots were not lineal and presented a hysteresis which was analyzed under the action of different blockers (TTX, Cd, Ni, 4-AP, TEA, etc.). Sudden alterations in the voltage rate of change signaled thresholds and decreases or increases in Rslope indicated outward or inward current predominance respectively. Examples: anomalous rectification (range <= -70 mV), inward and outward subthreshold conductances (range: -60 to -40 mV), calcium spikes after TTX (threshold: -25 to -15 mV), AHP-like Cd-sensitive hyperpolarizations present upon returning from depolarizing ramps even in the absence of "all-or-none" spikes, suggesting the presence of a lower threshold gCa albeit not able to trigger autorregenerative spikes. CONACyT grants P228CCOX891576 to E. G. and P228CCOX891559 to J. B.

334.4

THE ACTION OF APAMIN ON THE MEMBRANE PROPERTIES OF THE SUBSTANTIA NIGRA PARS COMPACTA NEURONES *IN VITRO*.

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Electrophysiological studies have characterised the properties of the substantia nigra pars compacta (SNC) neurones. Intracellular recordings *in vitro* have shown action potentials with a characteristically wide action potential (approx 2 ms) and a long lasting after-hyperpolarisation (lasting approx 100 ms), which has been suggested to be in part due to a calcium dependent potassium conductance. Apamin is a polypeptide neurotoxin that selectively blocks small conductance calcium dependent potassium channels. The purpose of this study was to investigate the effects of apamin on SNC neurones *in vitro*.

Recordings were made from guinea pig brain slices by previously described methods (Harris et al. Neuroscience 31: 355, 1989). Seventy percent of the neurones recorded were spontaneously active 2.5±0.4 spikes/s), displaying a very regular firing pattern. In response to hyperpolarising current pulses voltage sensitive inward and outward rectification was seen. Apamin reduced the large after-hyperpolarisation of spontaneous and evoked action potentials. Apamin was bath applied to a total of 14 neurones at concentrations ranging from 0.2 - 1μM. Apamin had a variable effect on the firing rate of the neurones. The firing frequency of 60% of the cells were increased, but overall there was no statistically significant change in the firing rate. However, there was a significant increase on the variance of the interspike intervals following the application of apamin. These results show that a small conductance gK(Ca) is involved in the spike after-hyperpolarisation, but that blockage of this conductance does not alter the spontaneous discharge frequency of SNC neurones. Supported by the Parkinson's Disease Society (U.K.) and Bristol Myers Squibb Co.

334.5

SKF 38393 INCREASES THE EXCITABILITY OF STRIATAL NEURONS *IN VITRO* FOLLOWING CHRONIC DOPAMINERGIC DENERVATION. L. A. Thompson, J. R. Walters, and M. J. Twery. NINDS, Bethesda, MD, 20892.

Systemic administration of the D_1 receptor agonist SKF 38393 inhibits the activity of substantia nigra pars reticulata (SNR) neurons in rats with 6-hydroxydopamine (6-OHDA) lesions, but produces variable effects on SNR activity in normal rats. Since the striatum may be the site of action of systemic SKF 38393, we have investigated the effect of chronic unilateral dopaminergic denervation on the response of striatal neurons to SKF 38393 *in vitro*. Intracellular recordings both ipsilateral and contralateral to 6-OHDA lesions (6-10 wks) were obtained from coronal slices containing striatum. Since striatal neurons were silent at rest, neuronal excitability was evaluated by varying the amplitude of depolarizing current pulses and determining the number of spikes elicited. Superfusion of SKF 38393 (1-3μM) increased the excitability of neurons *ipsilateral* to the lesion (15/19 neurons), without inducing consistent changes in membrane potential (Δ Vm: 0.5$\pm$0.8 mV) or membrane resistance (% Δ: 5.2$\pm$5.6%). Four (of 19) ipsilateral neurons were inhibited by SKF 38393. In contrast, among *contralateral* neurons, SKF 38393 predominantly decreased neuronal excitability (9/15). Only three contralateral neurons exhibited increased excitability in the presence of SKF 38393 and another three neurons were apparently unaffected. Regardless of hemisphere, decreases in excitability were typically associated with a small membrane depolarization (mean: 2.0$\pm$0.47 mV, n=13). These results indicate that chronic dopaminergic denervation changes the predominant effect of D_1 stimulation in the striatum from inhibition to excitation. This change in the responsiveness of striatal neurons may contribute to the pronounced inhibitory actions of SKF 38393 observed in the SNR following dopaminergic lesions *in vivo*.

334.6

IN VITRO ELECTROPHYSIOLOGICAL CHARACTERIZATION OF STRIATAL NEURONS FOLLOWING CHRONIC UNILATERAL 6-HYDROXYDOPAMINE LESIONS OF DOPAMINE CELLS. M. J. Twery, L. A. Thompson and J. R. Walters. NINDS, NIH, Bethesda, MD 20892.

Lesions of midbrain dopamine cells produce profound changes in the neurotransmitter content and the apparent responsiveness of striatal neurons to dopaminergic stimulation. The present study investigated whether chronic (6-10 wk) unilateral dopamine cell lesions altered the passive membrane properties or excitability of striatal neurons by comparing responses in tissue slices obtained ipsilateral and contralateral to the lesion site. Intracellular recordings from striatal neurons located ipsilateral to the lesion site revealed no spontaneous activity (V_m=-77.8$\pm$1 mV, n=32). Depolarizing current pulses (0.8-2.2 nA, 0.3-3 sec) elicited predominantly repetitive firing (20/32 neurons) characterized by a spike latency dependent on pulse amplitude. Less frequently, pulses elicited an initial spike followed by a rapidly accommodating burst of spikes (6/32) or an initial single spike alone (6/32). Striatal neurons contralateral to the lesion were also silent (V_m=-77.1$\pm$1.5 mV, n=29) and exhibited a generally similar response pattern with repetitive (23/29) and initial single spike (5/29) firing. Rapid accommodation of burst firing, however, was infrequently observed (1/29). I-V relations obtained from neurons ipsilateral and contralateral to the lesion revealed no differences in slope resistance near rest (39$\pm$ 2 MΩ, n=61) associated with evoked firing patterns. This study indicates that the chronic loss of dopaminergic input to striatum does not lead to widespread changes in neuronal excitability or passive membrane properties. Postulated changes in striatal function following chronic denervation may reflect alterations in the responsiveness of neurons to neurotransmitters.

334.7

WHOLE-CELL PATCH-CLAMP ANALYSIS OF MEDIUM SPINY NEURONS IN THIN SLICES FROM RAT NEOSTRIATUM. A. Mori*, Y. Miyashita and H. Kasai. Department of Physiology, Faculty of Medicine, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, 113 Japan.

In order to characterize the biophysical properties of the principal neurons of the basal ganglia, the whole-cell patch-clamp method was applied to thin (130 μm thickness) slices of caudate nucleus from 8 to 14 day old rats. Medium sized (10-18 μm) neurons were selected under Nomarski optics and subjected to whole-cell clamp measurements. Injection of Lucifer Yellow revealed extensive dendritic trees stubbed with spines in most of these cells. Resting membrane potentials ranged between -55 and -70 mV and input resistances between 200 and 500 MΩ. Membrane depolarization activated Na and K currents. In addition, non-inactivating Ca currents were recorded at potentials larger than -40 mV, when intracellular K was replaced by Cs. Focal stimulation of slices by current pulses through a second glass pipette evoked synaptic currents as well as antidromic Na spikes. The synaptic currents were mediated by AMPA-, NMDA- and $GABA_A$-receptors. A quickly decaying component of synaptic current had a reversal potential of about 0 mV and was abolished by CNQX (10 μM) in the presence of picrotoxin (100 μM). A slowly decaying component appeared at potentials larger than -60 mV, had a reversal potential of about 0 mV and was blocked by APV (500 μM). In the presence of CNQX and APV, synaptic currents reversed at around -60 mV and were blocked by picrotoxin. Polysynaptic inputs were also eliminated by picrotoxin, suggesting the presence of collateral inputs from the axons of other medium spiny neurons. Thus, the thin-slice patch-clamp method offers a way to study the structural and biophysical properties of the principal neurons and to characterize the local circuits in the basal ganglia.

334.8

DYE-COUPLING IN TYPE I AND TYPE II STRIATAL NEURONS: ALTERATION BY APOMORPHINE AND LOCALIZATION TO THE PATCH/MATRIX. S-P. Onn, T.W. Berger and A.A. Grace. *Depts. of Behavioral Neuroscience & Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260*

Despite numerous studies examining the effects of dopamine (DA) on single neurons in the brain, only recently have studies begun to address DA on the assemblies of functional networks of neurons such as those mediated by electrical coupling. This study examined the effects of apomorphine, a DA agonist, on the extent of dye-coupling (as an index of electrical coupling) among Type I and Type II striatal neurons in adult anesthetized rats. Type I and Type II neurons were defined on the basis of their paired impulse response to stimulation of excitatory cortical afferents. All cells were recorded intracellularly *in vivo* using Lucifer yellow-filled microelectrodes. After recording and staining, the cells were also localized with respect to the patch/matrix architecture of the striatum by immunocytochemical labeling of calbindin 28 Kd, a known marker for matrix (antiserum kindly provided by Dr. M.R. Celio). In control rats, dye-coupling was found exclusively between pairs of neurons (8/43; 18%) and in all cases neurons were localized in the matrix. In each case, coupling was observed between medium spiny neurons that shared Type II response patterns (Type I: 0/15 vs Type II: 8/28). However, intravenous apomorphine (0.1mg/kg) increased the incidence of dye coupling (7/10; 70%) and the numbers of cells stained by each injection, with up to six cells being labeled after a single injection (4/7 cases showing labelling of 5-7 cells; 1 for Type I and 3 for Type II cells). Thus, in addition to its action on single cells, DA appears to play a role in regulating the intercellular communication within the striatum. (Supported by Huntington's Disease of America Grant & NS08288 to S-P.O. and NS19608, MH42217 & MH00343 to T.W.B and A.A.G.).

334.9

$GABA_B$ RECEPTORS REDUCE BOTH GLUTAMATERGIC EXCITATION AND GABAERGIC INHIBITION OF RAT STRIATAL NEURONS THROUGH PRESYNAPTIC MECHANISMS. E.S. Nisenbaum, T.W. Berger, and A.A. Grace, *Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

Recent evidence has indicated that $GABA_B$ receptors can mediate presynaptic inhibition of neurotransmitter release in several brain systems. The present experiments investigated the potential regulation by $GABA_B$ receptors of cortically-evoked glutamatergic excitation and GABA-mediated paired impulse inhibition of striatal neurons using intracellular recording techniques in an *in vitro* corticostriatal slice preparation.

In response to single stimulation of cortical afferents, a monosynaptic EPSP was recorded in striatal cells which could be completely blocked by the glutamatergic kainate/quisqualate receptor antagonist, CNQX (10 μM; n=5). In addition, when the allosteric $GABA_A$ receptor agonist, pregnanolone (5.0-7.5 μM; n=17), was used to enhance GABAergic input, paired impulse stimulation of cortical fibers selectively decreased EPSP amplitude and probability of spike discharge in response to the second impulse of ISIs of 10-30 ms. Application of the $GABA_B$ receptor agonist, baclofen (0.5-2 μM; n=23), markedly inhibited the glutamate-mediated EPSP evoked by single stimulation of cortical afferents. Similarly, in pregnanolone-treated slices, administration of baclofen produced an increase in stimulation threshold for spike discharge, and reversed the paired impulse inhibition of striatal cell responses. Baclofen did not alter either the resting membrane potential or the input resistance of striatal neurons. The baclofen-induced inhibition of glutamatergic and GABAergic responses could be blocked by the $GABA_B$ receptor antagonist, 2-OH-saclofen (100-500 μM; n=10). These results suggest that the release of glutamate and GABA in response to stimulation of cortical afferents can be regulated by presynaptic $GABA_B$ receptors. (NS19068, MH00343, MH45156, Tourette Syndrome Association, National Alliance for Research on Schizophrenia and Depression).

334.10

STRUCTURE-FUNCTION RELATION OF THE GLOBUS PALLIDUS NEURONS: AN *IN VITRO* STUDY IN GUINEA PIG BRAIN SLICES. A. Nambu and R. Llinás. Dept. of Physiology and Biophysics, New York Univ. Med. Ctr., New York, NY 10016

Intracellular recordings obtained from globus pallidus neurons revealed the existence of at least two classes of neurons on the basis of membrane properties. Type I neurons were characterized by a low threshold calcium spike, fast transient K^+-current (A-current) and a prominent spike accommodation. Continuous depolarization of type I neurons produced Ca^{2+}-dependent slow membrane oscillation (around 2 Hz), which triggered Na^+-spikes. Type II neurons were characterized by a low threshold calcium spike and capability of high frequency firings (up to 150 Hz) by intracellular depolarizing pulse. Continuous depolarization of type II neurons produced Na^+-dependent membrane oscillation (10 - 100 Hz), which triggered Na^+-spikes. Morphology of these neurons was studied by intracellular injection of biocytin and ABC method. Soma size of type I neurons (30-50 μm long and 15-30 μm wide) was larger than that of the type II neurons (20-40 μm long and 10-25 μm wide). Both types of neurons had 2-6 primary dendrites. Most dendrites had few spines and exhibited varicosities especially in their terminal branches. Axons originated from soma or dendrite. All axons were prominently beaded. Usually axons had collaterals, and in some cases, one axonal branch was followed caudally and another one was followed rostrally into the striatum. These axonal patterns suggest the inhibitory effect of the pallidal neurons on the striatal neurons as well as on the other pallidal neurons. (This work was supported by NIH grants NS13742. A.N. was also supported by the Naito Foundation.)

334.11

EXCITATORY POSTSYNAPTIC CURRENTS OF IDENTIFIED LARGE ASPINY CELLS IN THE MATRIX OF THE NEOSTRIATUM *IN VITRO*. Y. Kawaguchi. Lab. for Neural Systems, Frontier Research Program, RIKEN, Wako 351-01, Japan

Most large neurons in the neostriatum are considered as interneurons that are cholinergic. It has been suggested that they are different from medium sized spiny projection cells in firing pattern, excitatory inputs and vulnerability to excitatory amino acids. The large neurons are candidates for connecting the patch and matrix compartments of the striatum. These are studied in rat slice preparation by whole cell recording. Large neurons were identified by soma diameter larger than 20 μm and confirmed by biocytin staining. Compartment identification was made by calbindin immunohistochemistry. All large neurons stained had aspiny and sparsely spiny dendrites and were in the matrix or on the border of this compartment. Axons close to the boundaries avoided invading patches by curving or running along the border. Some dendrites close to the boundaries avoided crossing by curving but a few entered the patch and made numerous fine branches with spine-like appendages. The cells had action potentials with large AHP and inward rectification occurred only with potentials hyperpolarized by -20 mV. EPSCs had two components. A later component had depolarized rectification which was eliminated in Mg-free solution and blocked by 50 μM DL-APV. An early component was blocked by 10 μM CNQX. A few large cells with varicose dendrites had virtually no NMDA components in their EPSCs. These results show that most large neostriatal cells have NMDA and non-NMDA component EPSCs, that the axons observe the compartment organization and only specialized dendrites cross the boundary. (Supported by HFSP)

334.12

QUANTITATIVE *IN VITRO* AUTORADIOGRAPHY OF EXCITATORY AMINO ACID BINDING IN THE PRIMATE BASAL GANGLIA. A. S. Clark and P. S. Goldman-Rakic. Dept. of Psychology, Dartmouth College, Hanover, NH 03755 and Sect. of Neurobiology, Yale Univ. Sch Med., New Haven CT 06510.

The corticostriatal pathway has been implicated in numerous neurological and psychiatric disorders including schizophrenia. To understand the functions of neurotransmitter systems within the cortico-striatal-thalamo-cortical loop in the nonhuman primate, we examined glutamate binding in the basal ganglia (BG) and related structures.

Adult rhesus monkeys were anesthetized and perfused transcardially with PBS followed by 0.1% paraformaldehyde containing increasing concentrations of sucrose. The brain was removed and a block containing the BG dissected from both hemispheres and frozen in isopentane. Quantitative autoradiography of glutamate receptor subtypes was conducted on cryostat sections through the extent of the BG. Binding to NMDA and kainate sites was present at nearly equal levels in the rostral and caudal aspects of the caudate nucleus (800 fm/mg protein). Similar levels were observed in the putamen. Binding for both ligands was significantly lower in the globus pallidus (< 200 fm/mg). MK-801 binding was also observed in the caudate/putamen with highest levels in the ventral aspect of the putamen (1400 fm/mg). Finally, NMDA binding appeared higher than kainate binding in the amygdala (1200 vs. 700 fm/mg). Identifying the distribution and kinetics of glutamate receptor subtypes in the primate BG and their relationship to other neurotransmitter systems may provide insight into the neurochemical dysfunctions underlying psychiatric disorders.

334.13

INWARD RECTIFICATION AND SYNAPTIC RESPONSES OF NEURONS IN THE INTACT TURTLE STRIATUM *IN VITRO*. D.J. Rossi, P.D. Morrison, and N.T. Slater. Department of Physiology, Northwestern University Medical School, Chicago, IL 60611 U.S.A.

Despite the wealth of information obtained using intracellular recording methods in mammalian striatal neurons *in vitro* and *in vivo*, no studies to date have examined the striatum of lower vertebrates. We have employed an intact preparation of turtle striatum *in vitro* to study the membrane properties and synaptic responses to stimulation of afferent sites within the telencephalon of striatal neurons.

Intracellular recordings of the passive electrical properties and synaptic responses in striatal neurons were obtained in the intact turtle (*Chrysemys picta*) striatum *in vitro* using both conventional 'sharp' microelectrodes and 'blunt' patch electrodes for current- and voltage-clamp recording. Penetrations were confined to the rostral pole of the striatum, where medium-sized spiny cells predominate. Turtle striatal neurons displayed prominant fast inward (anomalous) rectification which was blocked by external cesium and barium, but not by TTX. Under voltage clamp, the activation of the inward rectifier current was too fast to observe inward current relaxations to hyperpolarizing voltage commands, despite coating of patch electrodes to reduce capacitative transients. Little spike adaptation or post-burst AHPs were observed following depolarizing current injection. Electrical stimulation of the dorsal (visual) cortex and the dorsal ventricular ridge (DVR) evoked both monophasic EPSPs and compound EPSP-IPSPs. Stimulation of the lateral forebrain bundle evoked primarily compound IPSPs. Both EPSPs and IPSPs could be blocked by excitatory amino acid antagonists; fast EPSPs and IPSPs were blocked by CNQX (5 μM), and a slow component of the corticostriatal EPSP was blocked by D,L-AP5 (100 μM). *I-V* curves for the EPSCs recorded with patch electrodes displayed either linear or bell-shaped curves in individual cells over the range -40 to -120 mV.

The results demonstrate the utility of the turtle preparation for the study of striatal function, and illustrate that turtle striatal neurons have similar electrical and synaptic properties to those of mammals. Supported by NS 17489 and NS 25682.

334.14

DEVELOPMENTAL FEATURES OF SODIUM CURRENTS IN RAT NEOSTRIATAL NEURONS. A. Howe and D.J. Surmeier, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

In the course of postnatal development, a number of changes take place in the integrative properties of neostriatal neurons that may be attributable to alterations in the properties of Na currents. To characterize these changes, Na currents were studied in neurons taken from embryonic and postnatal animals through the fourth week of life.

Voltage-clamp techniques were used to study Na currents in cultured and acutely dissociated neurons. Cultures were derived from embryonic striata (E17) using techniques previously published. Acutely-dissociated cells were obtained by dissecting striata from 500 μm slices, enzymatically digesting with pronase E (1.5 mg/ml at 35 C), and then mechanically dissociating. Whole-cell and excised patch recordings used conventional techniques. Most recordings were made at room temperature; some experiments, including those studying deactivation tail currents, were done at 10 C using a temperature controlled bath.

Sodium currents changed in several respects over the late embryonic and early postnatal period. The most obvious change was an increase in current density. Another change was in the characteristics of inactivation. In embryonic and early postnatal cells, inactivation at depolarized potentials was well-fit with a single exponential; at later times, a slow component of inactivation was seen in addition to the faster component seen in younger animals. In contrast, the recovery from inactivation was well-fit only with a sum of exponentials at all ages studied. This work was supported by USPHS grant NS 28889.

334.15

DOPAMINERGIC MODULATION OF SODIUM CURRENTS IN RETROGRADELY-IDENTIFIED RAT STRIATONIGRAL NEURONS. D.J. Surmeier, C.J. Wilson, A. Stefani and S.T. Kitai, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

The role of dopamine in controlling the excitability of neostriatal neurons remains controversial. One source of experimental difficulty is the existence of multiple receptor subtypes. Recent work has suggested that particular subtypes may be restricted to striatopallidal or striatonigral neurons. We have tested this hypothesis in the striatonigral population by using the dopaminergic modulation of the sodium current as an assay.

Neurons from the neostriatum of adult rats (> 4wks postnatal) with rhodamine bead injections into the substantia nigra were dissociated using techniques previously reported by our group. Briefly, the striata were dissected from 500 μm coronal slices. Tissue fragments were incubated in oxygenated saline containing pronase E (1.5mg/ml) at 35 degrees C. After a 30 minute digestion, tissue was washed and mechanically dissociated. Electrophysiological recordings used conventional techniques at room temperature. Striatonigral neurons were visualized with epi-fluorescence.

Our results indicate that in a significant percentage of striatonigral neurons, sodium currents are modulated by both D1 and D2/3 class receptors. In most neurons, D1 and D2/3 agonists both produced a shift in the voltage dependence of steady-state inactivation toward more negative membrane potentials. In some neurons, D1 and D2/3 agonist effects were antagonistic. This work was supported by USPHS grants NS 20702, NS 28889 and NS 26473.

334.16

THE DEVELOPMENT OF POTASSIUM CURRENTS IN NEOSTRIATAL GRAFTS. Z.C. Xu, C.J. Wilson, D.J. Surmeier, A. Stefani, and S.T. Kitai Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis,TN, 38163

Previous studies have shown that a slowly inactivating transient potassium current develops in neostriatal neurons over the first 2-4 weeks of life, while a similar but much more rapidly inactivating current is present at birth and continues to be seen in adults. This offers the opportunity to examine a developmentally regulated ionic conductance in neostriatal grafts, which are usually considered to be immature in their physiological properties.

A cell suspension prepared from fetal striatal primordia was implanted into the kainic acid-lesioned neostriata of adult rats. Two to six months after implantation, we employed intracellular recording in tissue slices and whole cell voltage clamp recording on acutely dissociated cells to compare transient potassium currents present in spiny neurons of the neostriatal graft with those in normal adult and neonatal cells of the same type.

Voltage-clamp recordings from graft neurons revealed both slowly and rapidly inactivating potassium currents, as seen in adult neurons. No significant differences in the voltage-dependence or kinetics of these currents were found. Responses of spiny neurons to current injection in slices confirmed the presence of the late developing current in graft neurons. Thus, while graft neurons are developmentally arrested in some respects, they do express normally the full complement of transient potassium conductances activated by depolarization. Supported by NIH grant NS26473.

334.17

ROLE OF D_1 AND D_2 DOPAMINE RECEPTORS IN MODULATION OF SYNAPTIC RESPONSES OF NEOSTRIATAL NEURONS. M. Bertolucci, C. Cepeda, N.A. Buchwald and M.S. Levine. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Dopamine (DA), that is released from axon terminals of neurons originating in the substantia nigra, has an important role in regulating responsiveness of neostriatal neurons. We previously showed that DA application decreased the amplitude of locally-evoked synaptic responses in neostriatal neurons in a dose-dependent reversible manner. The present experiments assessed whether the modulatory effects of DA were mediated by activation of D_1 or D_2 receptor subtypes. Intracellular recordings were made from neostriatal neurons in slices obtained from rats. Local extracellular stimulation elicited short-duration excitatory postsynaptic potentials (EPSPs). When domperidone (20μM), a D_2 antagonist, was added to the bath, the decrease in EPSP amplitude produced by DA was blocked (n=8), suggesting that the D_2 receptor may be involved in the mediation of this effect. Furthermore, bath application of a specific D_2-agonist, quinpirole hydrochloride (10μM, 20μM, 50μM) also induced a significant dose-dependent reduction in the amplitude of the EPSP (n=5). When the effects of the specific D_1 antagonist, SCH23390 (50μM), were assessed, only 1 cell (n=5) displayed a total blockade of the DA-induced decrease in the EPSP. Two cells showed a partial blockade and the remaining cells showed no effect. These data suggest that the D_2-receptor mediates DA-induced modulation of synaptic responsiveness in neostriatal neurons. Supported by USPHS AG 07462 and HD05958.

334.18

EFFECTS OF GABA ON RESPONSES EVOKED BY EXCITATORY AMINO ACID RECEPTOR AGONISTS IN INTRACELLULARLY RECORDED NEOSTRIATAL NEURONS. C. Cepeda, N. A. Buchwald and M. S. Levine. Ment. Retard. Res. Ctr., UCLA, Los Angeles, CA 90024.

GABA is the major inhibitory amino acid neurotransmitter in the neostriatum (NS) and is contained in the medium-sized spiny neurons. These neurons have both extrinsic and intrinsic projections. The present experiment was designed to assess the effects of GABA on responses evoked by glutamate and NMDA. Intracellular recordings were made from NS neurons in an in vitro slice preparation. Slices (400 μm) obtained from rats or cats were maintained following standard procedures. Compounds were applied iontophoretically in the vicinity of recorded cells; GABA (0.1 M, pH 5.5), glutamate (0.2 M, pH 8), NMDA (0.1 M, pH 8) and saline (as a current control). The average resting membrane potential of NS neurons was -71 mV (n=9 in rats, n=8 in cats). When GABA was applied the membrane displayed a fast depolarization which reached a plateau at the Cl^- equilibrium potential (--60 mV). If the membrane was first depolarized by intracellular injection of positive current, the response to GABA was a hyperpolarization. Changes in membrane potential were accompanied by increases in input conductance. Excitatory postsynaptic potentials evoked by local extracellular stimulation, believed to be mediated by non-NMDA receptors, decreased markedly in amplitude and duration when GABA was applied. Excitatory responses induced by glutamate (membrane depolarization and repetitive firing) were also inhibited by GABA. Excitatory responses induced by NMDA (oscillatory depolarizations and bursts of action potentials) were less affected by GABA. In some cases GABA modified the pattern of excitation induced by NMDA, blocking bursting activity and increasing input conductance, but leaving the membrane depolarization unaffected. These findings suggest that, even though GABA exerts inhibitory effects, its efficacy in altering excitatory responses induced by excitatory amino acid receptor agonists, may depend on the receptor subtype activated. (Supported by USPHS 05958).

334.19

CONTRASTING EFFECTS OF DOPAMINE ON EXCITATORY RESPONSES EVOKED BY NMDA AND GLUTAMATE IN NEOSTRIATAL NEURONS. N.A. Buchwald, C. Cepeda, Z. Radisavljevic and M.S. Levine. Ment. Ret. Res. Ctr., UCLA, Los Angeles, CA 90024.

These experiments assessed the effects of dopamine (DA) on responses evoked by excitatory amino acid receptor agonists in the neostriatum (NS). NS neurons were recorded intracellularly in a slice preparation. Slices (400 μm) were obtained from adult rats or cats and maintained according to standard procedures. The following substances were applied iontophoretically in the vicinity of the recorded neuron; DA (0.1 M, pH 4.5), glutamate (GLU) (0.2 M, pH 8), N-methyl-d-aspartate (NMDA) (0.1 M, pH 8) and saline for current control. In some cases, bicuculline was bath applied to enhance the response evoked by the excitatory amino acids. Data were obtained from 17 cells (9 from cats and 8 from rats). Average values were; resting membrane potential -77 mV, spike amplitude 72 mV and input resistance 19 MΩ. In all NS cells, responses evoked by GLU consisted of a fast rising membrane depolarization, repetitive firing but no consistent changes in input conductance. The cell recovered rapidly after discontinuation of GLU. Excitation evoked by NMDA consisted of a slow rising membrane depolarization accompanied by a decrease in input conductance, followed by membrane oscillations and bursts of action potentials. Recovery after NMDA was slow and action potentials persisted for several seconds. Concomitant iontophoretic application of DA produced contrasting effects on the excitation induced by amino acids. Repetitive firing induced by GLU was inhibited by DA. This inhibition was accompanied by a membrane hyperpolarization without noticeable changes in input conductance. Membrane oscillations and bursts induced by NMDA were enhanced when DA was applied. Bicuculline (20 μM) in the bath potentiated excitatory responses evoked by GLU. Iontophoretically applied DA was less effective at inhibiting firing induced by GLU; higher currents were required to alter spike frequency. This contrasting effect of DA may have an important role in modulating the actions mediated by different excitatory amino acid receptor subtypes. (Supported by USPHS 05958).

334.20

LONG-TERM POTENTIATION (LTP) OF EXCITATORY SYNAPTIC INPUT TO MEDIUM SPINY NEURONS OF THE RAT STRIATUM. J.P. Walsh, Division of Neurogerontology, Andrus Gerontology Center, Los Angeles, CA 90089

Excitatory synaptic input to medium spiny neurons comes largely from pyramidal neurons of the cortex. To activate this synapse, extracellular stimulating electrodes were placed at the junction of the dorsal striatum and the corpus callosum in an in vitro slice preparation. Synaptic responses were monitored using intracellular recording techniques (electrodes were filled with 2% biocytin). Control synaptic responses (> 10 mV) were obtained, followed by the delivery of a robust tetanizing stimulus (four tetani each of 1-sec duration at 100 hz at 10-sec intervals, adapted from Zalutsky and Nicoll, 1990). In normal artificial cerebral spinal fluid (ACSF), post-tetanic potentiation (PTP) was observed to last 2-3 min following the tetanizing stimulus. Thereafter, the EPSP amplitude returned to its pre-tetanic control level. Similar results were also obtained when $GABA_A$ mediated inhibition was removed by bathing the slice in ACSF + 30 μM bicuculline methiodide. Medium spiny neurons exhibit negative resting membrane potentials (> -75 mV) and possess significant voltage-dependent K^+ conductances both of which oppose the activation of voltage dependent inward currents participating in the generation of LTP (i.e. NMDA, Ca^{2+}). Slices were therefore bathed in Mg^{2+}-free ACSF to remove the voltage dependent block of NMDA receptor coupled channels. In the Mg^{2+}-free ACSF, the tetanizing stimulus elicited bursts of action potentials. The EPSP demonstrated PTP that lasted the first few minutes and LTP (an increase in the amplitude of the EPSP of greater than 30%) that lasted 60 minutes. In a second set of experiments, slices were bathed in ACSF + 30 mM tetraethyl ammonium (TEA). Similar to the Mg^{2+}-free ACSF experiment, tetanizing stimuli in the TEA treated slices elicited PTP and LTP following the same criteria of a 30% enhancement of EPSP amplitude. The present experiments illustrate that, under appropriate experimental conditions, LTP of excitatory synaptic input can be produced in the striatum.

BASAL GANGLIA AND THALAMUS: MOLECULAR

335.1

TONIC INHIBITORY ROLE OF D2 RECEPTORS IN THE REGULATION OF GAD mRNA EXPRESSION. M.J. Besson*, J. Caboche*, M.Rogard*, B. Zalc, J. Mallet*, P. Vernier* and J.-F. Julien*. Lab. de Neurochimie-Anatomie, IDN-UPMC, 75005 Paris, and Lab. de Neurobiol. Cell. Mol. & Clinique, INSERM-U134, 75013 Paris, and Lab. de Neurobiol. Cell. & Mol., CNRS, 91198 Gif-sur-Yvette.

In order to further investigate the role of DA in the regulation of striatal neurons we measured the levels of mRNA encoding glutamic acid decarboxylase (GAD, the rate-limiting enzyme in the production of GABA) and preproenkephalin (PPE, the precursor of Enkephalin) after chronic injections of typical (haloperidol: a D1 and D2 receptor antagonist) and atypical (sulpiride: a selective D2 receptor antagonist) neuroleptics. Rats were injected (s.c., twice a day, 14 d.) with haloperidol (1 mg/kg), sulpiride (80 mg/kg) or saline. After in situ hybridization with specific ^{35}S-cDNAs, densitometric measurements were performed in the whole rostro-caudal extension of the caudate-putamen (CPu), the nucleus accumbens (NAc) and the olfactory tubercles (OT). In the dorsal CPu, both GAD and PPE mRNA levels were increased after haloperidol (+30%) and sulpiride (+20%) treatments. In the NAc, GAD and PPE mRNA are distributed according to a rostro-caudal gradient. Haloperidol treatment increased GAD mRNA levels in the anterior part (+17%) and PPE mRNA levels in the posterior part (+34%) of the NAc. No changes of GAD and PPE mRNA were found after sulpiride treatment. None of these treatments produced a modulation of GAD and PPE mRNA levels in the OT.

The co-regulation of GAD and PPE mRNA expression observed in the CPu after these treatments indicates a tonic inhibitory role of DA mediated by D2 receptors and likely reflects a regulation in striato-pallidal neurons. The different regulation of GAD and PPE mRNA expression in the anterior and posterior part of the NAc could be related to the differential CCK-DA innervation of the nucleus.

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335.2

SUBCELLULAR LOCALIZATION OF THE GTP-BINDING PROTEIN, G_i, IN THE BASAL GANGLIA REVEALS ITS POTENTIAL ROLE IN BOTH SIGNAL TRANSDUCTION AND VESICLE TRAFFICKING. N. Aronin and M. DiFiglia. Dept. of Medicine, Univ. of Mass. Med. Sch., Worcester, MA 01655, and Dept. of Neurology, Mass. General Hosp., Boston, MA 02114

The G-protein, G_i, is known to mediate signal transduction in cells by inhibiting adenylyl cyclase and by affecting ion channel functions. The immunohistochemical localization of G_i was examined in the basal ganglia using an antisera directed against the alpha subunit of G_i-1 (gift of E.J. Neer). Immunoreactive G_i was present in medium-sized spiny and aspiny neurons in the neostriatum and in numerous axon terminals in the neostriatum, globus pallidus (GP) and substantia nigra (SN). Labeled axon terminals in the GP and SN formed symmetric synapses characteristic of neostriatal spiny projection neurons. G_i immunoreactivity in neostriatal somata and dendrites was present in rough and smooth endoplasmic reticulum, microtubules and vesicles in transport and along the inner aspect of plasma membranes. In axon terminals in all regions, G_i was evident at different sites along the inner face of the plasma membrane and heavily labeled the membranes of synaptic vesicles, but was not present in the axoplasm or in mitochondria. Western blot analysis with the same anti-G_i antisera demonstrated a predominant 41 kDa protein in both neostriatal and pallidal synaptosomes, confirming the presence of G_i alpha in the axon terminals.. Conclusions: G_i is localized to neostriatal projection pathways, where it may function in membrane-bound signal transduction, both pre- and post-synaptically. G_i localization in vesicle membranes points to a potential role for this G-protein in vesicle trafficking such as that recently shown for smaller molecular mass proteins. Supported by NSF BNS-8819989 and NIH NINDS 16367.

335.3

DOPAMINERGIC AND NON-DOPAMINERGIC REGULATION OF STRIATAL NEUROTENSIN mRNA EXPRESSION. K.B. Seroogy, K.H. Lundgren* and C.M. Gall. Departments of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536 and University of California, Irvine, CA 92717.

Manipulation of the dopaminergic mesotelencephalic system can profoundly modulate levels of neuropeptides and their mRNAs within striatal neurons. To determine if aberrant striatal peptide expression is strictly due to dopaminergic influence or whether other non-dopaminergic mechanisms or circuitries may be involved, adult rats were treated according to either of two paradigms: 1) acute haloperidol administration or 2) induction of limbic seizures by unilateral electrolytic lesion of the hippocampal dentate hilar region. In situ hybridization using an ^{35}S-labeled synthetic oligonucleotide probe complementary to neurotensin mRNA was used to examine possible changes in forebrain expression of this peptide, which is infrequently present within striatal neurons in the normal rat. Acute (1 hr or 3 hr) treatment with haloperidol (2 mg/kg, i.p.) resulted in a dramatic increase in the number of neurons expressing neurotensin mRNA in dorsolateral aspects of the caudate-putamen. By 24 hr post-hilus lesion, a significant increase in hybridization for neurotensin mRNA was also observed in the dorsolateral caudate-putamen and in ventral striatal regions (including medial nucleus accumbens) as well as in the septum and bed nucleus of the stria terminalis. In contrast, hybridization to the peptide mRNA appeared to be decreased in the subiculum and hippocampus following hilus lesion. These data demonstrate that dopaminergic mechanisms are not the sole factors regulating striatal peptide expression and that physiological activity radiating from other brain areas exerts a regulatory influence over striatal peptides as well.

335.4

COMPARATIVE DISTRIBUTION OF mRNAs ENCODING TWO GAD ISOFORMS IN OUTPUT NUCLEI OF THE BASAL GANGLIA IN RATS. M. Mercugliano[1], H. Nguyen*, S. Feldblum[2], M. Erlander[2], A.J. Tobin[2] and M-F Chesselet[1]. Dept. of Pharmacol., Univ. of Pennsylvania, PA19104 [1], and Dept. of Biol., UCLA, Los Angeles, CA90024 2.

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the synthesis of GABA, the neurotransmitter of basal ganglia efferent pathways. Two genes encode isoforms of GAD (M_rs 67,000, GAD67, and M_rs 65,000, GAD65) which differ in their co-factor requirement (Erlander et al. Neuron, in press). We performed in situ hybridization histochemistry with 35S-radiolabelled probes complementary to mRNAs encoding each GAD isoform in globus pallidus (GP), entopeduncular nucleus (EP) and substantia nigra pars reticulata (SNR) of adult rats. Quantification of autoradiographic signal in emulsion-coated slides was performed at the single cell level with a Morphon Image Analysis system. Average labelling per rat was calculated for each region in sections processed in parallel. Both mRNAs were expressed in projection neurons of the three regions examined. In the pallidum, however, the mean ratio of EP/GP labelling in individual rats was 0.71+.1 for GAD67 mRNA and 1.95+.17 for GAD65 mRNA (N=4), revealing marked differences in the relative expression of mRNAs encoding the two GAD isoforms in some output neurons of the basal ganglia. Supp. by MH 44894 (MFC), NS 22256 (AJT) and MH 14654.

335.5

NGFI-A mRNA EXPRESSION IS INDUCED IN THE RAT STRIATUM BY INDIRECT DOPAMINE AGONISTS

R. Moratalla, H.A. Robertson, and A.M. Graybiel. Dept. of Brain and Cognitive Science, M.I.T., Cambridge, MA.

In neurons, immediate early genes (IEGs) encode transcription factors that may be involved in the control of genomic events following neuronal stimulation. We have previously shown that cocaine and amphetamine induce c-fos and jun-B but very little c-jun mRNA in the striatum. Moreover, at rostral levels, amphetamine induces Fos-like immunoreactivity (Fos) preferentially in the striosomal compartment of the caudoputamen, whereas cocaine induces Fos about equally in striosomes and matrix. We now show that treatments with amphetamine and cocaine result in similar drug-specific patterns of NGFI-A (zif/268, erg-1, Krox-24) induction. NGFI-A mRNA was detected by in situ hybridization with oligonucleotide probes, and striosomes were identified by ^{3}H-naloxone binding in adjacent sections.

NGFI-A mRNA induction by each drug was dose dependent, being scarcely detectable at 5mg/kg and progressively stronger at l0 and l5 mg/kg (amphetamine) or 25 and 50 mg/kg (cocaine). In the rostral caudoputamen, induction by amphetamine was striosome-selective but cocaine induced NGFI-A mRNA in both striosomes and matrix. Induction in the nucleus accumbens was greater in the core than in the shell except after 50 mg/kg cocaine. Pretreatment with the D1-selective antagonist SCH 23390 (0.5 mg/kg) inhibited NGFI-A mRNA induction by either drug. We conclude that IEGs of at least two gene families can be induced in the striatum in drug-specific and striosome-matrix selective patterns.

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335.6

THE INFLUENCE OF TH-CONTAINING AFFERENTS ON THE DEVELOPMENT OF MODULAR ORGANIZATION IN EMBRYONIC STRIATAL GRAFTS. F.-C. Liu[1], S.B. Dunnett[2] and A.M. Graybiel[1], [1]Dept. Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, USA, and [2]Dept. Experimental Psychology, Univ. of Cambridge, Cambridge, CB2 3EB, UK.

The most prominent feature of embryonic striatal grafts is that they develop a modular organization in which patches enriched in neurochemical substances (P regions) are embedded in surrounds (NP regions). Of particular interest is that patches of TH-positive fibers correspond spatially to the AChE-rich P regions in which many striatal markers are concentrated. This spatial alignment suggests that the TH-containing fibers, predominantly derived from the host's substantia nigra, may be capable of regulating the development of cells in the P regions of the grafts. In the present study we sought to determine whether the ingrowing TH-positive fibers from the host striatum are important for inducing or maintaining a subpopulation of grafted cells to form P regions. Unilateral 6-OHDA lesions were performed on host rats to destroy the nigrostriatal bundle 29-32 days before grafting. The hosts further received ibotenate lesions of the denervated striatum a week prior to grafting. Cell suspensions derived from E15 striatal primordia were then injected into the degenerated striatum. Survival times for the grafted rats were 3.6-6 months. Our results indicate that the destruction of TH-containing dopaminergic afferents does not disrupt the formation of modular organization in the grafts, as patches containing AChE activity, medium-sized calbindin-positive, enkephalin-positive, DARPP-32-positive and calcineurin-positive neurons are still present in the grafts. Given that the modular organization may reflect an admixture of striatal tissue (P regions) and non-striatal tissue (NP regions), our results suggest that the ingrowing TH-positive fibers from the host striatum are probably not obligatorily involved in sorting out of striatal from non-striatal cells to form P regions in embryonic striatal grafts. Supported by NSF BNS 8720475 and NATO grant RG.85/0180.

335.7

MOLECULAR CHARACTERISTICS OF STRIATAL NEURONS THAT EXPRESS FOS ON STIMULATION BY INDIRECT DOPAMINE AGONISTS. S. Berretta,* R. Christie,* H.A. Robertson, and A.M. Graybiel. Dept. of Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139.

Dopamine (DA) regulates neurotransmitter expression in striatal neurons, and increasing evidence suggests that induction and/or repression of immediate-early genes such as c-fos could participate in this regulation. To characterize striatal neurons in which DA can activate c-fos, we acutely treated rats (n=14) with the indirect DA agonists cocaine (10,25,50 mg/kg) and amphetamine (5,10,15 mg/kg) and tested for neurochemical specializations of the medium-sized striatal cells in which nuclear Fos-like immunoreactivity (Fos) was induced. Sections from treated rat brains were doubly stained, with immunogold and DAB as distinguishable chromogens, for Fos and for enkephalin-like (ENK) or dopamine and cAMP-regulated phosphoprotein-like (DARPP-32) immunoreactivity. ENK-immunoreactive neurons have been shown to express D2 dopamine receptors, whereas DARPP-32 is thought to be a third messenger characteristic of neurons with D1 (and perhaps D5) receptors.

Fos induced by cocaine and amphetamine was nearly always colocalized with DARPP-32 but was almost never colocalized with ENK. This selective pattern of Fos induction may be related to the different patterns of DA regulation of striatal ENK, substance P and dynorphin. As ENK is the major known neuropeptide in the striato-GPe-subthalamic loop pathway, our results further suggest that indirect DA agonists may produce a signalling imbalance in the direct and indirect output pathways of the basal ganglia.

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335.8

PRIMING OF A D1-LIKE DOPAMINE RECEPTOR AND INTRASTRIATAL ACTIVATION OF IMMEDIATE-EARLY GENES.

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Repeated administration of D1 or D2 dopamine agonists can lead to a long-lasting increase in the responsiveness of a D1-like receptor, a phenomenon known as PRIMING (J.Pharm.Exp.Ther. 234:447, 1985). We have speculated that this priming response may involve activation of immediate-early genes, as these have been implicated in other long-term changes in the brain.

In rats with unilateral 6-OHDA lesions of the medial forebrain bundle, the contralateral turning response elicited by the D1-selective dopamine agonist SKF38393 (2 mg/kg,i.p.) was significantly enhanced by a single prior exposure (48 hr prior to SKF38393) to either apomorphine (0.5 mg/kg,i.p.) or the D2-selective agonist LY171555 (0.25 mg/kg,i.p.). Immunohistochemical detection of Fos-like protein demonstrated that apomorphine (but not saline) produced activation of *c-fos* throughout the denervated striatum. MK801 (0.1 mg/kg,i.p.) given 30 min prior to apomorphine, blocked the apomorphine-induced priming response, but had little effect on *c-fos* activation. Both priming and induction of *c-fos* by apomorphine were blocked by a higher (0.5 mg/kg,i.p.) dose of MK801. LY171555 was a good primer of SKF38393 behaviour, but induced little if any *c-fos* activation in the striatum. SKF38393 produced robust activation of *c-fos* both in unprimed animals and in animals exhibiting behavioural priming. These findings demonstrate that priming of the dopamine receptor, as determined by turning responses, can be dissociated from activation of *c-fos* in the striatum. Preliminary evidence from Northern blotting is consistent with the immunohistochemical results and further suggests a dissociation for at least two other immediate-early genes, *jun B* and *NGFI-A*. (supported by the MRC of Canada, NIH Javits award NS25529 and the Human Frontiers Science Program).

335.9

NEURONAL LOCALIZATION OF RC3 PROTEIN IN RODENT NEOSTRIATUM. J.B.Watson and R.S.Fisher. Mental Retardation Research Center, Department of Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.

RC3 is a 78-amino acid neuron-specific rat protein first identified by subtractive cDNA cloning. The RC3 sequence is nearly identical to bovine proteins termed neurogranin or BICKS which were isolated by properties of protein kinase C (PKC) phosphorylation and calmodulin-binding. RC3 resembles GAP-43 protein (neuromodulin, B-50) in a highly conserved sequence containing a PKC phosphorylated serine and a calmodulin-binding domain. The localization and functional roles of RC3 are largely uncharted. Our aims were to determine if neostriatal neurons express the RC3 protein and to demonstrate its cellular sites of accumulation.

Specific and equivalent immunolabeling patterns of the neostriatum of rats were obtained with two polyclonal antisera directed against synthetic peptides of different regions of RC3. Light microscopy revealed fine labeling in the neuropil. Practically all medium and large neuronal cell bodies located in immunoreactive section faces contained RC3. Immunohistochemistry/Golgi-gold toning showed RC3 expression in medium spiny, medium sparsely spiny and large sparsely spiny neurons. Correlative light/electron microscopy demonstrated subcellular sites of RC3 in nucleoplasm, perikaryal cytoplasm, and dendritic cytoplasm. Dendritic spines were the most frequently labeled profiles while labeled axons were seen sporadically .

We conclude that most, if not all, of the neostriatal neurons express the RC3 protein. RC3-positive cells of distinct morphological types always had at least a few dendritic spines. RC3 may act as a "third messenger" principally in dendritic spines of neostriatal neurons and other forebrain neurons. Supported by NICHD HD 25831.

335.10

DOPAMINERGIC REGULATION OF GLUTAMIC ACID DECARBOXYLASE mRNA IN PALLIDAL PROJECTION NEURONS. A.E. Kincaid, R.L. Albin, S.W. Newman, J.B. Penney and A.B. Young, Depts. of Anatomy and Cell Biology and Neurology, University of Michigan, Ann Arbor, MI 48109.

Fluoro-gold (FG) tract tracing and in situ hybridization were combined to study the dopaminergic regulation of GAD mRNA levels in globus pallidus (GP) projection neurons. FG was injected into the entopeduncular nucleus or substantia nigra (SN) of adult rats. After 8 days animals received an injection of 6-hydroxydopamine in the SN ipsilateral to the FG injection. Animals were perfused with 4% paraformaldehyde and sections were cut through the forebrain (12 um) and midbrain (40 um). Tyrosine hydroxylase immunocytochemistry identified complete lesions of SN. An ^{35}S-labeled 30 base oligonucleotide probe was used to identify cells containing GAD_{67} mRNA. More GP projection neurons, identified by FG labeling, contained GAD mRNA in the lesioned animals (88% vs 53% of control), and these neurons contained more mRNA than neurons from control animals. Supported by NIH NS20629 to SWN and NIH NS19613 to ABY and JBP.

335.11

THE PHOSPHOINOSITIDE SECOND MESSENGER SYSTEM IS ENRICHED IN STRIOSOMES OF THE PRIMATE STRIATUM. M. Fotuhi, T.M. Dawson, A.H. Sharp, L.J. Martin, A.M. Graybiel, and S.H. Snyder. Dept. of Neurosci, The Johns Hopkins Sch. Med., Baltimore, MD, 21205 and Dept. of Brain and Cognitive Sci (AMG), M.I.T., Camb, MA, 02139.

A large number of neuropeptides and neurotransmitter markers are preferentially distributed in either striosome or matrix compartments of the striatum. In the present study, we examined the distribution of selected components of the phosphoinositide (PI) second messenger system using immunocytochemical staining with antibodies against inositol triphosphate receptor, phospholipase C beta , and phospholipase C gamma in the striatum and in other regions of the primate basal ganglia.

The immunoreactivity for these markers appeared in distinct regions of the basal ganglia and in specific patterns. In the substantia nigra pars compacta, only a subset of neurons showed positive immunoreactivity for these epitopes. In the substantia nigra pars reticulata, as in both segments of the globus pallidus, only fibers and terminal-like punctate staining were present. In the striatum, both striosomes and matrix contained positive perikarya and neuropil staining. Striatal zones with heightened PI-IR were identified as striosomes by reference to calbindin-IR in adjacent sections.

These findings suggest that the PI second messenger system is differentially represented in basal ganglia pathways. The striosomal pattern of the PI system indicates that the striatum is compartmentalized with respect to second messengers.

335.12

DIFFERENTIAL ACTIVATION OF THE *c-FOS* PROTO-ONCOGENE IN SUBSETS OF STRIATAL NEURONS BY DOPAMINERGIC DRUGS. H. Steiner and C. R. Gerfen, Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.

Subsets of striatal neurons can be differentiated by the targets of their projection, the expression of neuropeptides, as well as by the expression of dopamine receptor subtypes. Dopamine plays a crucial role in the function of the striatum; it appears to modulate the activity of striatal neurons, the lack of which results in imbalanced basal ganglia output and, thus, in movement disorders. Long term activation of dopamine receptor subtypes has been shown to lead to specific alterations in gene expression in striatal neurons. Dopamine agonists also affect the activity of immediate-early genes such as *c-fos*, and such genes, therefore, provide a mechanism to study short term changes in gene expression under the influence of dopaminergic activity. In a series of studies, we examined *c-fos* expression induced by dopaminergic drugs in different subsets of striatal neurons. Expression of this gene was analyzed with *in situ* hybridization after application of amphetamine, cocaine and specific D1 and D2 dopamine receptor agonists, alone and in combination, in normal rats and after 6-OHDA lesions of the dopaminergic nigrostriatal projection. Co-localization was used to determine the neuronal subtype. These dopaminergic drugs led to specific regional patterns of *c-fos* activation, as well as to differential activation in neuronal subsets of the striatum.

335.13

SUBSTANCE P RECEPTOR mRNA EXPRESSED SELECTIVELY IN CHOLINERGIC NEURONS IN THE STRIATUM AND BASAL FOREBRAIN. C. Gerfen, Lab of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892

In the striatum, substance P (neurokinin-1) receptor mRNA is selectively localized in large neurons that also express mRNA encoding choline acetyl transferase (ChAT) by in situ hybridization histochemistry. Substance P receptor mRNA is also localized in ChAT mRNA containing neurons in the medial septum and basal forebrain cell groups. Thus, in the rat forebrain the substance P receptor appears to be expressed selectively by cholinergic neurons. Striatal neurons that contain substance P also utilize g-aminobutyric acid (GABA) as a transmitter. These neurons make synaptic contact with striatal cholinergic neurons (Bolam, et al., 1986, *Brain Research.*, 397: 279-289), which are shown here to express the substance P receptor, and with other GABAergic neurons in the striatum and substantia nigra, which express GABA receptors but not substance P receptors. This suggests that individual striatal neurons may differentially affect target neurons dependent on the receptors expressed by those target neurons.

335.14

INTRODUCTION OF THE *E. COLI LACZ* GENE INTO RAT NEOSTRIATAL NEURONS USING HERPES SIMPLEX VIRUS MUTANTS. Q. Huang [1], J.P. Vonsattel [1], P.A. Schaffer [2], R.L.Martuza [2], X.O. Breakefield [1] and M. DiFiglia [1]. Depts. of Neurology [1] and Neurosurgery [2], Massachusetts General Hosp., and Dana Farber Cancer Inst. [3], and Harvard Medical School [1,2,3], Boston, MA 02114.

Introducing genes into adult neurons *in vivo* may be a useful tool for understanding and modulating neuronal function. Two herpes simplex virus type 1 (HSV 1) mutants, 7134 and RH105, which have deletions of viral genes ICP0 and TK, respectively, were used to determine if adult rat neostriatal neurons can express the substituted foreign gene, *E. coli, lacZ* introduced by viral infection. After intrastriatal injection of the mutants, rats survived for 1-70 days with no apparent adverse effects except significant shrinkage of the injected hemisphere at longer postinoculation intervals. At the injection site, both mutants produced a marked focal necrosis and gliosis. Expression of the *lacZ* gene product, β-galactosidase (βGal) was maximal at three days postinoculation in all types of striatal neurons and was present in many more cells with mutant 7134 which has some capacity to replicate than with RH105 which is replication defective. βGal expression was also present in a higher than normal proportion of large neurons and in some glia. At the EM level neurons infected with mutant 7134 ranged from severely pathologic to remarkably healthy and were contacted by degenerating axon terminals. Results show that foreign genes can be delivered to neostriatal neurons by HSV 1 infection. Further minimizing the cytotoxicity of HSV 1 mutants will be important in determining their success as vectors for gene delivery. Supported by NIH grants NS16367 to MD and NS24279 to XOB and RLM.

335.15

Increased expression of fos-like immunoreactivity, preproenkephalin A and proneurotensin mRNAs in rat striatum following pertussis toxin administration.

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The effects of a unilateral intrastriatal microinjection of pertussis toxin (PT) on the expression of proneurotensin (NT) and preproenkephalin A (PPE A) mRNAs was investigated in the rat striatum using non radioactive *in situ* hybridisation. Sham injected rats received vehicle alone. Rats were allowed to recover for 22 hours following stereotaxic surgery. A single microinjection of PT resulted in a significant increase in the expression of both NT and PPE A mRNAs in the ipsilateral striatum when compared to sham injected rats. Further, cryostat sections stained to visualise fos-like immunoreactivity (-LI) demonstrated an abundance of weakly stained fos-LI cells in both the ipsilateral and contralateral sham injected striata and lateral septal nuclei. Following PT treatment, an increase in the expression of fos-LI was detected in some cells in the ipsilateral striatum. However, not all striatal cells displayed this increased expression at this time point. Co-expression studies, combining non radioactive *in situ* hybridisation and immunocytochemistry, are in progress to determine the neurochemical identity of the cells expressing an increase in fos-LI.

BASAL GANGLIA AND THALAMUS III

336.1

THE EFFECT OF POST-IMPLANTATION INTERVAL AND HALOTHANE ANESTHESIA ON DIALYSATE DOPAMINE AND AMINO ACID LEVELS IN THE DORSOLATERAL STRIATUM OF THE RAT. **K.G. Kramlinger, W.T. O'Connor*, S. Lillrank*, and U. Ungerstedt*. Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.**

In the present in vivo microdialysis study, post-implantation interval and halothane anesthesia were investigated for their effects on extracellular striatal dopamine (DA), DOPAC, HVA, 5-HIAA, GABA, glutamate (GLU), and aspartate (ASP) levels, which were measured from the same perfusate sample. Three groups of male Spraque-Dawley rats (350-400gms) were compared: A) halothane anesthetized rats 3 hours following probe implantation (n=9); B) halothane anesthetized rats 48 hours following probe implantation(n=7); and C) awake, freely moving rats 48 hours following probe implantation (n=7).

There are large differences in the dialysate concentrations of these substances: DA & GABA (2-12nM); ASP & 5-HIAA (60-200nM); DOPAC & HVA (300-700 nM); and GLU (600-4000nM). DA and GABA levels are similar in all 3 preparations. However, acute probe implantation (A vs B) is associated with elevated DOPAC (143%) and HVA (149%), but reduced GLU (21%) and ASP (32%) levels. In contrast, 5-HIAA levels are not affected by post-implantation interval, but are elevated (121%) by halothane anesthesia (B vs C).

These data suggest that use of different microdialysis preparations may systematically affect results obtained in otherwise comparable study designs and thus limit comparisons across studies.

336.2

THE DOPAMINERGIC INNERVATION OF THE DORSAL STRIATUM IN CATS RECOVERED FROM MPTP-INDUCED PARKINSONISM: A TH-IMMUNOHISTOCHEMICAL AND RETROGRADE TRANSPORT STUDY. J. S. Schneider, D. S. Rothblat and M.G. Smith*, Depts. of Neurology and Physiology and Biophysics, Hahnemann Univ., Philadelphia, PA 19102

Administration of MPTP to cats results in a parkinsonian syndrome characterized by akinesia, rigidity, and diminished responsiveness to external stimuli. Neuropathologically, there is severe loss of substantia nigra pars compacta (SNc) dopamine (DA) neurons and less loss of DA neurons in adjacent regions such as the ventral tegmental area (VTA), retrorubral area (RR), and substantia nigra pars lateralis (SNl). MPTP also causes extensive DA depletion in the striatum, with the dorsal lateral caudate (DL CD) and dorsal putamen most severely affected. Interestingly, cats spontaneously recover motor function in 4-6 wks. without significant increase in dorsal striatal DA levels or recovery of SNc neurons. TH-immunohistochemistry in symptomatic MPTP-treated cats showed extensive loss of immunoreactivity throughout the striatum with slightly less loss ventrally, medially and caudally. In cats recovered from MPTP-induced parkinsonism, there were still only scattered TH-positive fibers visible in the dorsal striatum and a continued reduction in terminal field-like staining but increased TH-positive staining in ventral caudate regions, nucleus accumbens, and ventral putamen. Retrograde transport studies with WGA-HRP injected into dorsal medial and DL CD in recovered cats showed no labeled neurons in the SNc but some scattered labeled neurons in the RR and SNl. The patterns of retrograde neuronal labeling in recovered animals were similar to those seen in normal animals, though few neurons overall were labeled in the recovered cats. These data suggest that motor recovery in the MPTP-treated cat is not accompanied by an endogenous sprouting response in the dorsal striatum originating from residual ventral mesencephalic neurons which innervate other striatal regions. Supported by NIH grant NS23980.

336.3

ELECTROPHYSIOLOGY OF THE GLOBUS PALLIDUS (GP) IN CATS SYMPTOMATIC FOR AND RECOVERED FROM MPTP-INDUCED PARKINSONISM. D. S. Rothblat and J. S. Schneider, Depts. of Neurology and Physiology and Biophysics, Hahnemann Univ., Philadelphia, PA 19102

Recent research has shown significant changes in the electrophysiology of the caudate nucleus (CD) in cats with MPTP-induced parkinsonism and severe striatal dopamine depletion. Changes observed were decreased responses to peripheral sensory stimuli and increased responsiveness to electrical stimulation of monosynaptic afferent inputs. The present study examined changes in globus pallidus (GP) neurons in cats while normal, while exhibiting parkinsonian symptoms of akinesia, rigidity and diminished response to environmental stimuli due to MPTP exposure, and while recovered from these deficits. Spontaneous firing rates of GP neurons in symptomatic cats decreased by 77% as compared to the normal condition. The spontaneous firing rates of GP neurons in recovered cats was decreased by 48% as compared to normals. Changes were also observed in the percentage of units responding to electrical stimulation of the CD. In the normal condition 54% of GP units tested responded to CD stimulation. When cats were symptomatic for MPTP-induced parkinsonism, only 40% of GP units tested responded to CD stimulation. In recovered cats, the percentage of units responding to CD stimulation returned to normal (57%). The stimulus threshold for activation of a GP unit from CD stimulation was not significantly different between the three test conditions. This differed from results from CD recording studies in symptomatic animals in which there was increased responsivity to and decreased threshold for stimulation of monosynaptic afferents. The potential significance of these findings for understanding the consequences of dopamine loss on striatal and pallidal response properties will be discussed. Additionally, the responses of GP neurons to peripheral sensory stimulation across conditions will be compared to data from CD recording studies and possible mechanism(s) for the modulation of motor function and for the mediation of dopamine-dependent sensorimotor deficits will be discussed. Supported by NIH grant NS23980

336.4

MOTOR RECOVERY IN MPTP-TREATED CATS IS ACCOMPANIED BY PARTIAL RECOVERY OF EXTRACELLULAR DOPAMINE LEVELS IN DORSAL LATERAL CAUDATE NUCLEUS. L. DiStefano and J.S. Schneider, Dept. of Neurology, Hahnemann Univ., Phila., PA. 19102.

Cats made parkinsonian by adminstration of the dopaminergic toxin MPTP tend to spontaneously recover gross motor function by 4-6 wks. after the last MPTP injection. Symptomatic and recovered cats do not have significantly different levels of dopamine (DA) (98% vs. 94% depletion, respectively) in the dorsal lateral caudate (DL CD) as measured in post mortem tissue samples. The present study was conducted to examine whether there might be more extracellular DA available in the DL CD in recovered animals than would be predicted based on post mortem measures of tissue DA levels and whether this would correspond with motor recovery. Cats were implanted with indwelling cannulae above the DL CD through which microdialysis probes could be inserted. For dialysate sampling, the cats were placed in a canvas restraint bag in which they had been previously adapted to sit quietly. Probe recovery was estimated *in vitro* at 37o C and the probe was equilibrated *in vivo* for 60 min. prior to data collection. Baseline measurements of DA, DOPAC and HVA levels were obtained prior to administration of MPTP. After MPTP administration and while cats were symptomatic, DL CD extracellualr fluid (ECF) levels of DA, DOPAC, and HVA were reduced 97%, 99% and 96%, respectively, corresponding with the tissue loss of these substances. When cats first recovered from motor impairments, ECF levels of DA, DOPAC, and HVA were depleted 54%, 94% and 82%, respectively while post-mortem tissue levels of these substances were depleted 94%, 92% and 50%, respectively. This data suggests that motor recovery in the MPTP-lesioned cat might at least in part be due to significant recovery of ECF DA levels available for use in the DL CD, the cat's sensorimotor striatum. This DA may arise from an up-regulation of DA synthesis and release from the few spared DAergic elements innervating the DL CD. Alternatively, the increased amount of ECF DA in the DL CD may originate in other, less denervated striatal regions and may reach the DL CD via enhanced diffusion due to loss of DA reuptake sites. Supported by NIH grant NS23980.

336.5

PRE AND POST-SYNAPTIC ALTERNATIONS IN STRIATUM OF CATS SYMPTOMATIC FOR AND RECOVERED FROM MPTP-INDUCED PARKINSONISM. P.A. Frohna, D.S.Rothblat, J.S. Schneider and J.N. Joyce. Depts. Psychiat. and Pharmacol.,Univ. of Pennsylvania School of Medicine and Dept of Neurology, Hahnemann Univ., Phila., PA.

Cats treated with MPTP develop parkinsonism, >95% striatal dopamine (DA) depletions and spontaneously recover motor function with no recovery of tissue DA levels in dorsal striatum but significant recovery of DA levels in ventral caudate and nucleus accumbens (NAS). The present study examined autoradiographically the regional integrity of pre-synaptic DA systems (by measuring [^{3}H]mazindol binding) and the numbers and regional distribution of D2 and D1 receptors in the striatum in cats sacrificed while symptomatic for parkinsonism and after recovery of motor function. Symptomatic and recovered cats were sacrificed 1 and 6 wks. respectively, after the last MPTP injection. One hemisphere was used for measurement of tissue DA and metabolites, and the other for autoradiographic studies. In both symptomatic and recovered cats, [^{3}H]mazindol binding was greatly reduced in the dorsal striatum (DS) (>94%) and substantia nigra (90%) and less diminished in NAS and ventral caudate. In normal cats, [^{125}I] Epidepride binding to D2 receptors was most dense in the DS where it was approximately double the density of binding in NAS and substantia nigra pars compacta (SNc). D2 binding was significantly decreased in the SNc in symptomatic and recovered cats. Ventral striatal D2 binding was decreased in symptomatic cats and further decreased in recovered cats but unchanged in DS in both conditions. In normal cats, D1 receptors labeled with [^{125}I] SCH 23982 were denser in the NAS and ventral caudate than in DS. D1 binding was increased in symptomatic and decreased in recovered cats in DS. These results suggest a continued loss of direct DAergic input to DS in recovered cats. Reduced receptor binding in recovered animals could reflect enhanced DA turnover and release in ventral striatum and increased availability of DA to DS due to loss of DA uptake sites and enhanced DA diffusion. Funded by MH43852, MH 43880, AG 09215, and NS 23980.

336.6

SOMATOSTATIN (SOM) AND GLUTAMIC ACID DECARBOXYLASE (GAD) mRNAs IN QUINOLINIC ACID-LESIONED STRIATUM OF ADULT RATS. Y. Qin, J-J Soghomonian and M-F Chesselet Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA, 19104.

Rat striata were lesioned by local injections of quinolinic acid (QA), an agonist of NMDA receptors which produces a pattern of cell loss similar to that observed in the striatum of patients with Huntington's disease. The level of expression of mRNAs encoding SOM and two isoforms of GAD (Mrs 65,000, GAD65 and 67,000, GAD67) was examined by quantitative in situ hybridization histochemistry. After 2 weeks survival, Nissl stains showed profound loss of neurons in the injected striata. With 120 nmol of QA, the lesioned area was completely devoid of SOM mRNA-positive neurons but cells expressing NADPH-diaphorase (a marker of SOM neurons in striatum) were still detectable in the same area. After 60 nmol of QA, the number of neurons expressing SOM mRNA in the lesioned area was not different from controls and their level of labelling was increased. In this group, some GAD67 mRNA-labeled interneurons were still observed in the lesioned area whereas no GAD65 mRNA labeled cells were detected. The results show that QA induces dose-dependent alterations in the expression of striatal SOM mRNA and reveal a relative sparing of GABA-ergic neurons in the QA-lesioned rat striatum. Supp. by MH 44894 and by the PMAF (JJS).

336.7

SELECTIVE SPARING OF GABA-ERGIC INTERNEURONS IN THE GERBIL STRIATUM AFTER TRANSIENT ISCHEMIA. C. Gonzales [1], R. C-S Lin [2] and M-F Chesselet [1], Dept of Pharmacol. Univ. of Pennsylvania, Phila., PA 19104 [1], and Dept of Physiol. and Bioph., Hahneman U., Phila., PA 19102 [2]

In addition to efferent GABA-ergic neurons, the striatum contains GABA-ergic interneurons, distinct from interneurons containing somatostatin, and expressing high levels of one isoform of glutamic acid decarboxylase (Mrs 67,000: GAD67) and of the calcium-binding protein parvalbumin. We have previously shown that cholinergic and somatostatinergic interneurons are spared after transient ischemia in the gerbil striatum, despite loss of efferent neurons, suggesting that striatal interneurons are less vulnerable to ischemic damage than projection neurons. In this study, Mongolian gerbils were sacrificed 4,7 and 10 days after occlusion (5min) of the common carotid. Striatal sections were processed for immunohistochemistry with a monospecifc antibody for GAD67 (A.Tobin, UCLA) and a monoclonal antibody for parvalbumin (M. Celio, Fribourg). Staining for GAD was decreased within an area of profound cell loss in Nissl stain. However, the number of neurons intensely stained for GAD67 and of parvalbumin-positive neurons, was similar in controls and in the lesioned area, indicating a relative sparing of GABA-ergic interneurons in the ischemic striatum. Supported by MH 44894 and NS 29230 (MFC), and S07-RR07-241 (RCSL).

336.8

NIGROSTRIATAL LESIONS DIFFERENTIALLY AFFECT mRNAs ENCODING GLUTAMATE-DECARBOXYLASES (GADs) IN SUBPOPULATIONS OF STRIATAL NEURONS. J-J Soghomonian, C Gonzales and M-F Chesselet. Dept.of Pharmacology, U. Pennsylvania, Philadelphia, PA, 19104.

We examined the effect of lesions of dopamine (DA) afferents to the striatum on the level of expression of mRNAs encoding two isoforms of GAD. Rats had an injection of 6-hydroxyDA in one susbtantia nigra and were sacrificed 2-3 weeks later. Sections of the striatum were processed for in situ hybridization histochemistry with radiolabeled RNA probes. Autoradiographic signals were quantified at the single-cell level with computer-assisted image analysis. Labeling for mRNA encoding GAD (M_rs 67,000) increased in medium-sized striatal neurons normally expressing low levels of this mRNA. This effect occurred in both the striosomes and the extrastriosomal matrix, and was accompanied by increased immunostaining for the corresponding protein with a monospecific antibody (Kaufman et al., J. Neurochem. '91). In contrast, labeling was decreased in neurons normally expressing high levels of mRNA encoding GAD (M_rs 67,000) (GABAergic interneurons). Labeling for mRNA encoding GAD (M_rs 65,000) (Erlander et al., Neuron '91) was not modified in the DA-depleted striatum. Results show that DA depletion differentially affects gene expression for different isoforms of GAD in subpopulations of striatal neurons. Supp. by MH 44894 and the PMAF (J-J S.).

336.9

EXPRESSION OF GAD mRNA IN THE FELINE MOTOR THALAMUS AND EFFECT OF SUBSTANTIA NIGRA LESIONS. K. Kultas-Ilinsky[1], M. Mercugliano[2], M.-F. Chesselet[2] and I.A. Ilinsky[1], [1]Dept. of Anatomy, Univ. of Iowa Coll. Med., Iowa City, IA 52242, [2]Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Previous studies (Kultas-Ilinsky et al., Brain Res., 1990, 511:197) suggest that deafferentation of the thalamus from substantia nigra (SN) induces short- and long-term changes in local thalamic GABAergic mechanisms. Glutamic acid decarboxylase (GAD)-containing local circuit neurons (LCN) represent one of the systems that may react to deafferentation. In this study we used cDNA for GAD from A. Tobin, UCLA; (Kaufman et al., Science 1986; 232:1138) to produce [^{35}S]-labeled mRNA probes to label LCN in frontal and sagittal serial sections through the cat thalamus. Intensity of labeling and distribution pattern of cells expressing GAD mRNA were analyzed both qualitatively and quantitatively in thalamic nuclei receiving basal ganglia afferents. In control unoperated animals GAD mRNA content was higher in lateral regions of the motor thalamus than in medial, which is consistent with earlier immunocytochemical studies (Kultas-Ilinsky et al., J. Neurosci., 1985; 5:1346). One year after kainic acid lesioning of pars reticularis of SN, this gradient was not detectable due to an increase in GAD mRNA in medial regions of the basal ganglia afferent territory, i.e., in the nigrothalamic projection zone. The data suggest that nigral lesions result in long-term alterations of GAD gene expression in GABAergic interneurons in thalamic regions normally receiving nigral inputs. Supported by RO1NS19280 (KKI), MH44894 (MFC) and training grant MH14654 (MM).

336.10

STRESS-INDUCED ALTERATIONS IN FOS EXPRESSION IN NEURONS INNERVATING THE VENTRAL TEGMENTAL AREA: AN APPROACH TO THE DETERMINATION OF THE STRESS CIRCUIT. Martha Gillham[1], Michael Iadarola[2], and Ariel Y. Deutch[1]. [1]Department of Psychiatry, Yale Univ. Sch. of Medicine, New Haven, CT 06510 and [2]Neurobiology and Anesthesia Branch, NIDR, Bethesda, MD 20892.

We have previously reported that restraint stress increases Fos protein expression in dopamine (DA) neurons innervating the prefrontal cortex (PFC). The increase in Fos expression is dependent upon the time interval between stress and sacrifice. We have therefore examined stress-elicited alterations in Fos expression in neurons retrogradely labelled from the ventral tegmental area (VTA). In these studies rats were sacrificed at a time point earlier than that required to increase Fos expression in the VTA. Neurons in a variety of telencephalic sites (e.g., PFC, ventral striatum, ventral pallidum, lateral hypothalamus, and amygdala) were retrogradely labelled from the VTA. Similarly, neurons in a number of brainstem sites (e.g., dorsal raphe, pedunculopontine tegmental nucleus, mesencephalic locomotor region, ventrolateral medulla) were also retrogradely labelled from the VTA. Initial examination indicates that certain of these neurons, prominently including neurons in the pons, express Fos protein at a time point prior to the expression of Fos in DA neurons projecting the PFC. This approach may lead to identification of components of the pathways through which stress gains access to the midbrain DA neurons. Supported by MH-45124 and the National Parkinson Foundation Center at Yale University.

336.11

THE EFFECTS OF ATYPICAL ANTIPSYCHOTIC DRUG ON STRIATAL FOS EXPRESSION. Maggie Lee[1], Michael J. Iadarola[2], and Ariel Y. Deutch[1]. [1]Yale University School of Medicine, New Haven, CT 06510 and [2]Neurobiol. Anesth. Branch, NIDR, Bethsda, MD 20892.

Double-blind trials indicate that both clozapine and remoxi- pride are atypical antipsychotic drugs (as defined on the basis of clinical efficacy and very low EPS liability). The former has been most extensively studied, and interacts with dopamine (DA) D_1 and 5-HT_2 as well as D_2 receptors; remoxipride has relatively high affinities for the D_2 and sigma receptor sites. We have compared the effects of remoxipride and clozapine with those of the typical antipsychotic drug haloperidol on striatal Fos protein expression. Haloperidol, as has been previously reported, dramatically increased Fos expression throughout the dorsal striatum. In contrast, an increased number of striatal neurons which express Fos after both clozapine and remoxi- pride was observed in the medial striatum, i.e., the region of the dorsal striatum receiving projections from A10 DA neurons and the medial prefrontal cortex. Moreover, the effects of remoxipride on striatal Fos expression were predominantly observed in the areas of the medial striatum which stain poorly for calbindin, i.e., the striatal patch compartment. These data suggest that atypical antipsychotic drugs may differ from the prototypical neuroleptic haloperidol by virtue of prominent effects on the striatal sector associated with A10 DA neurons and association rather than sensorimotor cortex. Supported by MH-45124 and the National Parkinson Foundation at Yale Univ.

336.12

BASIC FIBROBLAST GROWTH FACTOR MARKEDLY ENHANCES THE GROWTH OF NEOSTRIATAL GABAergic NEURONS IN VITRO. Dan Zhou* and Marian DiFiglia, Massachusetts General Hospital, Boston, MA 02114.

Basic FGF (bFGF) promotes the survival of some CNS neurons including neostriatal neurons in vitro (Wallicke, 1988). We examined the effects of bFGF on the growth of GABAergic neurons taken from the newborn caudate-putamen and cultured for 12 to 18 days. Control cultures showed three types of immunoreactive GABAergic neurons based on somatic size (small:>10 μm, medium:10-20 μm; and large:>20 μm) and distinct dendrite-like and axon-like processes in all cell types. In the presence of bFGF (6 pM), small and medium-sized GABAergic neurons showed significant increases ($p<.0005$) in five parameters of neurite growth (number of primary dendrites, dendritic-field radius, dendritic branch order, length and arborization of axons) compared to untreated neurons. Large GABAergic neurons were unaffected by treatment with bFGF. Striatal GABA neurons exposed to nerve growth factor were not different from untreated controls. The potent effects of bFGF on the neuritic outgrowth of striatal GABAergic neurons suggests a major role for bFGF in striatal cell development and regeneration. (Supported by NIH NS16367 grant to MD).

336.13

L-DOPA INDUCES STRIATAL FOS IN RAT WITH PARTIAL DOPAMINERGIC DENERVATION. D.Cole, J.Growdon, M.DiFiglia.Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

L-DOPA induces the immediate early gene product Fos in striatum in a rat model of Parkinson disease (PD) when there is dopamine (DA) receptor supersensitivity. To establish if L-DOPA also induces Fos with partial striatal DA denervation we made graded unilateral stereotaxic nigral 6-hydroxydopamine lesions in adult rats, determining extent of denervation by counting rotations after intraperitoneal (ip) apomorphine (1mg/kg) and amphetamine (5mg/kg). Two hours after ip caridopa/L-DOPA (2.5/25 mg/kg), rats were perfused. Brains were examined immunohisto- chemically. Rats with >90% denervation (rotation to apomorphine) had Fos-like immunoreactive (FosLI) neurons throughout the striatum, rats with 50-90% denervation (rotation to amphetamine but not apomorphine) had FosLI neurons only in the lateral and rostro-ventral striatum, and rats with <50% denervation (no rotation) had no FosLI neurons. Accumbens and pallidum never had FosLI neurons. Nissl counterstaining revealed that only medium-sized neurons were FosLI. L-DOPA induces Fos in this model with partial DA denervation. This effect suggests that early use of L-DOPA in PD may induce striatal cellular adaptation.

OCULOMOTOR SYSTEM IV

337.1

TOPOGRAPHY OF CONNECTIONS BETWEEN CORTICAL VISUOMOTOR AREAS IN MACAQUE. J.D. Schall, A. Morel, D.J. King*, C. Whalley*
Department of Psychology, Vanderbilt University, Nashville, TN 37240

The frontal eye fields (FEF) systematically represent small to large saccades in a ventrolateral-dorsomedial progression. We determined that lateral and medial portions of FEF have coarse topographic connections with visuomotor areas in frontal and parietal cortex by placing injections of 2 or 3 tracers (fluorescent dyes and/or WGA-HRP) in regions of FEF representing smaller (sFEF) and larger (lFEF) saccades determined through intracortical microstimulation in *Macaca mulatta*.

sFEF and lFEF connections with SEF overlapped substantially. Even so, sFEF afferents extended further caudally, and connections with lFEF, further rostrally and laterally. This rough topography is consistent with the relative amplitudes of saccades evoked by stimulation of rostral and caudal SEF (Tehovnik & Lee, 1990, *Soc. Neurosci. Abstr.* 16:900).

sFEF and lFEF connections in the intraparietal sulcus overlapped and inter- digitated, occupying both the densely myelinated LIP/VIP* and the lighter, dorsal LIP. sFEF connections extended further rostrally and lFEF, more caudally. The patchy inputs to sFEF and lFEF from the intraparietal sulcus may be related to the mapping of the upper and lower visual fields from LIP into the FEF.

These results indicate that saccade amplitude is represented topographically in SEF and LIP although with less precision than in FEF. This pattern of organization would seem to be a necessary correlate of the fact that saccade- related activity in LIP and SEF, unlike that in FEF, varies with orbital position.

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337.2

CHARACTERISTICS OF "ANTI"- AND "NORMAL" SACCADES IN MAN. Heike Weber* and Burkhart Fischer . Abt. Neurologie und Neurophysiologie, Universität Freiburg, Hansastr. 9a, 7800 Freiburg

Saccades with extremely short latencies (express saccades) can be elicited by the onset of a stimulus which at the same time is the saccade target. We instructed 4 adult human subjects to execute saccades in the opposite direction to the side where a visual stimulus appeared (anti-task). The gap (fixation point offset precedes stimulus onset by 200ms) and the overlap (fixation point remains on) paradigm were used. As control served the normal task with target-directed saccades. All subjects had been trained to produce express saccades in the normal gap task. Stimuli were computer generated on a color monitor and eye movements were recorded using infrared light reflexion. Reaction time (SRT), velocity, amplitude and duration of the saccades were analysed and compared. Distributions of SRTs in the normal gap consisted of a small group of anticipatory saccades (SRT < 80ms), followed by a large peak between 80 and 120ms (express saccades) and by another group ranging up to 180ms (regular saccades). In the anti gap anticipatory saccades and saccades with SRTs longer than 100ms were obtained. Express saccades were missing. Distributions of SRTs in the normal and anti overlap were unimodal with mean values of 216ms (normal) versus 231ms (anti). The velocity of anti saccades was slightly (10%) but significantly slower. Control experiments show that the absence of express saccades in the anti task is not due to an interhemispheric transfer time. We conclude that to program a saccade to a position where no target appears, using the onset of the visual stimulus only as a temporal trigger cannot be executed within the latency of an express saccade. These data provide further evidence for a reflex-like pathway from the retina to the oculomotor nuclei mediating express saccades.

337.3

EXPRESS AND ANTISACCADES IN SCHIZOPHRENIC, AFFECTIVE DISORDER, AND NORMAL CONTROL SUBJECTS. A.B. Sereno and P.S. Holzman*. Dept. of Psychology, Harvard Univ., Cambridge, MA 02138.

Previous work (Guitton et al, 1985; Fukushima et al, 1990) suggests that both frontal lobe lesioned patients and schizophrenic patients have difficulties in an antisaccade paradigm (where subjects must look opposite to where the target appears). All subjects performed 4 tasks. Three of the tasks examined saccadic eye movement performance: a prosaccade task (as a measure of baseline performance), an antisaccade task, and an express saccade task (i.e., a gap paradigm, where the central fixation point is removed before presentation of the target; in this study, gap duration=150 ms). The order of the saccadic eye movement tasks was counterbalanced within each subject group. Each subject also performed a smooth pursuit tracking task.

Schizophrenic subjects showed a greater increase in both saccadic response time and error rate than did normal controls in the antisaccade task. In addition, schizophrenic subjects showed a greater decrease in saccadic response time than did normal controls in the gap task. Although affective disorder subjects also showed a greater response time and error rate increase than normal controls in the antisaccade task, they did not show a greater benefit in saccadic response time than normal controls in the gap task. The relation between performance on the antisaccade and gap paradigms will be discussed and its relevance to a prefrontal deficit hypothesis of schizophrenia. In addition, the relationship between the quality of smooth pursuit and performance on these tasks will be discussed.

337.4

GRAVITY AFFECTS THE POSITION OF THE PARALYSED CAT'S EYE. **L. R. Harris, M. J. Steinbach and H. C. Goltz*, Dept. Psychology, York University, Toronto, Canada M3J 1P3**

To see whether gravity might potentially disturb fixation in the cat, we measured the position of the paralysed cat's eye. Cats were anaesthetized with sodium thiamylal (5 mg/kg/hr i.v.) and paralysed with gallamine triethiodide (10 mg/kg/hr i.v.). EKG and CO_2 were monitored. Animals were tilted in 45 deg steps around the pitch and roll axes and eye positions were subsequently measured from photographs. A laser beam reflected from a mirror held on one eye by gum tragacanth, provided an additional, translation-insensitive measure of eye rotation.

The occurrence of eye rotation was consistent with the front of the eye being heavier (to the left when tilted left ear down, towards the top of the orbit when upside down, etc...). The response to tilts in the pitch plane revealed which orientations the eye regarded as upside down and right way up. They did not correspond to the plane of the horizontal semicircular canals. These findings suggest that cat's eyes are different from human's in having the centre of mass closer and anterior to the centre of rotation (cf. Steinbach et al. IOVS Suppl. 31:533 1990). Inter-species differences may correlate with the differential occurrence of eye-muscle proprioceptors. The arrangement in both species poses a continuous challenge to fixation that depends on the direction and magnitude of the prevailing gravito-inertial forces.

337.5

ADAPTATION TO DISCONJUGATE RETINAL SLIP FOLLOWING VERTICAL SACCADES. Z. Kapoula. Lab. de Physiologie Neurosensorielle, CNRS-UPR2, Paris, France.

This study tests the ability of normal subjects to create post-saccadic eye drift (PSD) in opposite directions in the two eyes. The question is whether this can be achieved by readjusting the pulse-step saccadic signals differently in each eye.

Two slides of the same image containing fuseable contours were projected. The images (32x32 deg) were polarized 90 deg apart; subjects used polarized lenses. At first, subjects saw a single, stationary image. They were asked to saccade between two points of the image. At the end of each vertical saccade, the L eye image drifted onwards (in the same direction as the saccade), while the R eye image drifted backwards. Motion of the images was exponential (tc 50 ms) and its amplitude was 3,6 or 10% of the vertical saccade size. Three subjects were trained for 1.5 hr. During training, eye movements were monitored by EOG. Pre and post-training binocular recordings were made with the search-coil method.

At first, subjects followed the images only 45% of the time with an early backward component, more prominent in the R eye, and a later, onward, vergence component seen only in the L eye. The mean delay (from the saccade offset) was 79 ms in the R eye; that of the L eye onward motion was 174 ms. After training, responses in the direction of drifting images rose to 70%. The delays decreased particularly, in the dominant eye. The mean R eye delay dropped to 15 ms and that of the L eye to 111 ms. These decreases could be produced by adaptive changes in the timing of vergence and/or changes in PSD. To evaluate the latter we examined spontaneous saccades in the dark. Training led to an increased but conjugate backward drift. These preliminary results show no evidence for disconjugate PSD by the saccadic system.

337.6

INSPECTION OF FACES AND OBJECTS IN A VISUAL MEMORY TASK. I. Mertens*, H. Siegmund* and O.-J. Grüsser. (SPON: European Brain and Behavior Society) Inst. Physiol. Humboldt-Univ., Dept. Physiol. Freie Univ. Berlin, Germany.

The relation of face and non-face perception to parameters of eye movements was studied in a learning paradigm. 33 right-handed subjects (21-47 years) were asked to inspect a set of slides (20 faces, features only; 20 vases), each projected onto a tangent screen (7.5°x10°) for 6 s and 20 s. In the control series all pictures were left-right inverted. Eye position was recorded by an infrared system with a precision of about 0.1° (fig. 1). Fig. 2 indicates that with faces (1,2,4,5) the center of gaze remained longer in the left visual field (right half of the face), regardless of presentation time. 4 faces evoked the opposite results during inverted presentation, indicating a stimulus factor in addition to the left/right bias. Vases did not evoke the left/right bias (3,6).

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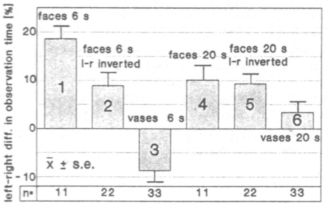
←Fig. 1 Fig. 2



337.7

ENDOGENOUSLY GENERATED AND VISUALLY GUIDED SACCADES AFTER LESIONS OF THE HUMAN FRONTAL EYE FIELDS. A. Henik*, R. Rafal and D. Rhodes, UC Davis,VAMC Martinez, CA 94553.

Latencies for endogenously generated and visually guided saccades were measured in 9 patients with unilateral lesions involving the frontal eye fields (FEF), in 7 control patients with lesions of dorsolateral prefrontal cortex not involving FEF, and in 12 normal control subjects. Visually guided saccades had shorter latencies than endogenously generated saccades in the control subjects. In the FEF lesion patients, but not in patient controls, endogenously generated saccades had longer latencies to targets in the contralesional field; whereas visually guided saccades had shorter latencies toward the contralesional field. In a control experiment in which key press responses rather than saccades were made, reaction time to contralesional targets was slower in FEF lesioned patients. Visually guided saccades did not have shorter latencies than endogenously generated saccades toward the field ipsilesional to FEF lesions. The FEF functions in generating endogenous saccades and also inhibits reflexive visually guided saccades. FEF lesions appear to disinhibit the ipsilesional superior colliculus; and this disinhibited colliculus may also inhibit the opposite colliculus to slow visually guided saccades toward the ipsilesional field.

337.8

CAFFEINE INCREASES THE MAXIMUM VELOCITY OF SACCADIC EYE MOVEMENTS. B. Schielke*, T. Mager* and T. Duka* (SPON: European Neuroscience Association). Schering Research Laboratories, Müllerstr. 170-178, 1000 Berlin 65, FRG.

There has been a longstanding discussion on the occurrence of an increase in the maximum velocity of saccadic eye movements of a given amplitude, i. e. the occurrence of a steeper rise of the main sequence in young healthy people. This was reflected by publications on faster saccades during vestibular stimulation, unchanged saccadic velocities during smooth pursuit and conflicting findings for different stimulant drugs.

Using the infrared reflection technique the amplitude, maximum velocity, duration and reaction time of saccades were measured in sixteen healthy young (18-45 years old) male volunteers after oral administration of placebo, 5 mg baclofen (Gaba-B-receptor-agonist), 5 mg diazepam (Gaba-A-receptor-agonist) and 250 mg caffeine in a four-fold cross-over double-blind study under standardized conditions in terms of diet, sleep and withdrawal of stimulants (nicotine, caffeine and other xanthine derivatives) and sedatives (urine drug screen). Saccades elicited before administration of the substances did not reveal any differences between the groups. 2 hours after application there was a significant difference between the caffeine and the placebo group as well as between the diazepam and the placebo group ($p < 0.05$). 6 hours after oral administration we could still find a significant difference between the caffeine and the diazepam group ($p < 0.05$) but not between any drug and placebo. The parameters of the baclofen group failed reaching the level of significance compared with placebo. So there is evidence that the saccadic system is not working at its optimum point even in young healthy people. However the observed differences could well be an effect of caffeine withdrawal in a population ingesting the stimulant drugs of daily living, although no difference was found comparing the subpopulations of coffee-drinkers (11) and non-coffee-drinkers (5).

337.9

THE RESPONSE OF GAZE VELOCITY PURKINJE (GVP) CELLS TO SUSTAINED VELOCITY (1-2 s) DOES NOT PREDICT THEIR RESPONSE IMMEDIATELY AFTER A RAPID HEAD ACCELERATION. L.H. Snyder and W.M. King. Department of Physiology, University of Rochester, Rochester, NY 14642.

GVP cells modulate their discharge with eye position and velocity and with head velocity when tested with sinusoidal stimuli. These data have been obtained over a limited frequency range and the transient responses of GVP cells to rapid changes of eye and head velocity have not been well characterized.

Monkeys were trained to fixate and track LED or laser targets and eye movements were monitored using search coils. GVP cells were identified by recording their discharge during the constant velocity phase (~2 s) of matched trapezoidal smooth pursuit or trapezoidal whole body rotations. During the latter paradigm, the monkeys canceled the vestibulo-ocular reflex by viewing a head fixed target. GVP head or eye velocity responses obtained with these stimuli were comparable to sinusoidal steady state responses published in the literature. To obtain transient responses of GVP cells, we applied brief pulses (~120 ms) of whole-body rotation (500 d/s/s up to 25 d/s) to the monkey immediately after extinguishing an LED target.

The majority of GVP cell responses to transient head accelerations could not be predicted by linear combinations of the head and eye velocity responses obtained with the trapezoidal stimuli. For example, in most cells, the responses measured 100 ms after the onset of head acceleration were dependent on initial eye position and in ~50% of the cells, firing rate increased regardless of the direction of rotation.

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337.10

COMPUTER SIMULATION OF PURSUIT EYE MOVEMENTS DURING DORSAL LATERAL PONTINE NUCLEUS STIMULATION. K.D. Pfann* and E.L. Keller. UCSF/UCB Grad. Group in Bioeng., Dept. of Elect. Eng., Univ. of Cal., Berkeley CA and Smith-Kettlewell Eye Res. Inst., San Francisco CA.

Pursuit eye movements may be altered by electrical microstimulation of pursuit-related cortical and brainstem areas. Two classes of models have been proposed to explain normal pursuit. Both produce realistic simulated pursuit movements, but they have radically different neural architectures. One emphasizes generation of a target velocity signal using efference copy, while the other emphasizes visual processing via parallel pathways. We modelled the effects of stimulation applied to these two systems and compared the results to actual pursuit perturbations produced in monkeys by stimulation of dorsal lateral pontine nucleus during "steady state" pursuit. We model the effects of stimulation with several variations, 1)substituting the stimulation signal for a model pathway, 2) perturbing a portion of an otherwise intact pathway or 3) altering a model state by changing the gain of an intact pathway.

Sites were found in each model that generate stimulation perturbed responses similar to the actual responses produced by pontine stimulation during open loop (OL) or closed loop (CL) tracking. However, no manipulation in either model generated both the constant acceleration (OL) and the reversal of acceleration (CL) observed with pontine stimulation. Although perturbation of part of an intact pathway in the models is an appealing way to explain the different effects during OL and CL, the resulting visual feedback error cannot completely overcome the disturbance and, therefore, cannot generate the amount of reversal seen in CL pontine stimulation. We believe the current models will require revision in order to account for the observed effects of pontine stimulation. (Supported by EY06860 and T32 GM07379)

337.11

TORSIONAL CHARACTERISTICS OF THE HUMAN OCULAR MOTOR PLANT S.H. Seidman, R.L. Tomsak*, M.P. Grant and R.J. Leigh. Depts of Biomedical Eng. and Neurol., Dept. of V.A. Med Center and Case Western Reserve Univ, Cleveland OH 44106.

To determine the properties of the ocular motor plant (OMP), we are studying the time course of the return of the eye after it has been released from a horizontal or torsional displacement. Using the magnetic search coil technique, we measured torsional, horizontal, and vertical eye movements in two humans. One eye viewed a stationary target, while the anesthetized, non-viewing eye was abducted, intorted, or extorted using ophthalmic forceps. Using techniques of non-linear parameter estimation, the return of the eye following release from forced duction was fit to a two time-constant model of the OMP as described by Robinson (1964). The shorter time-constant of this model proves difficult to estimate with accuracy, but estimation of the longer time-constant is comparatively simple. We find that the eye returns more rapidly from extorsional duction (τ=163ms,98ms) than from abduction (τ=205ms,146ms) in each subject. Returns from an intorsional starting point, however, follow a time course similar to or longer than that of abduction (τ=331ms,132ms). Data from three subjects will be presented.

Supp. By NEI EY06717 and VAMC

337.12

THE PRIMATE ROSTRAL INTERSTITIAL NUCLEUS OF THE MEDIAL LONGITUDINAL FASCICULUS. J.A. Rafols, X-J. Hu*, and W.J. Crossland. Dept. Anatomy and Cell Biology, Wayne State Univ. Sch. Med., Detroit, MI 48201.

Our morphological investigation of the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) in 50 Golgi impregnated adult (Macaca mulatta) brains revealed five (I-V) neuron types. Type I has a large polygonal cell body issuing 3-7 primary dendrites which extend up to 680μm from the soma. The large, bulbous cell body of type II neurons, the only type with many spines, issues 2-4 dendrites which radiate 120-280μm from the soma. Type III has a medium size fusiform soma and its dendrites become varicose as they extend 340-520μm from the soma. Type IV cells have small cell bodies issuing 3-4 dendrites which extend 200-250μm, possess bulbous appendages, and frequently end in axon-like arborizations. Type V has a giant fusiform cell body from which two thick dendrites arise and extend 700-800μm from the soma. Three dimensional rotation of digitized representative examples of each cell type demonstrated that the dendritic spread of the type I, III, and V cells is discoid and confined mainly to the frontal plane, well situated to intercept MLF fibers. Type II and IV cells appear to radiate their dendrites in a spherical compartment and may sample more topographically localized input (type II) or carry out inhibitory interactions (type IV). Supported by grants EY04068, GM08167, Michigan Eye Bank.

337.13

THE PRIMATE INTERSTITIAL NUCLEUS OF CAJAL AND THE NUCLEUS OF DARKSCHEWITSCH. X. J. Hu*, J. Czech*, J.A. Rafols, W.J. Crossland. Dept. Anatomy and Cell Biol., Wayne State U. Sch. Med., Detroit, MI 48201.

The neuronal organizations of the interstitial nucleus of Cajal(iC) and the nucleus of Darkschewitsch (nD) were studied in Golgi impregnated brains of adult monkeys(Macaca mulatta). The four (I-IV) neuron types distinguished in iC are similar to types I-IV in the rostral interstitial nucleus of the MLF (riMLF-see accompanying abstract). However, the spine densities of types I and II in iC are higher than those of the corresponding types in riMLF. Additionally the dendrites of types I and II in iC appear to be confined strictly to its boundaries. Except for type IV, a local interneuron, all the other types in riMLF and iC may have long projecting axons. The sole type in nD has a medium polygonal cell body which gives rise to 4-6 thick dendrites. The dendrites seldom branch, taper to 2-4um in diameter and extend linearly 450-600um from the soma. Most dendrites in nD radiate dorsolaterally and ventromedially. However, some radiate medially into the periaqueductal gray. Moderate spines densities are found along the entire dendritic length. The axon does not impregnate beyond the initial segment and may project extrinsically. This study shows that most cell types in riMLF are found in iC. It also shows that the cells of nD are morphologically homogenous and distinct from the cells in iC and riMLF. (Supported by grants EY04068, GM08167, and the Michigan Eye Bank).

337.14

FINE STRUCTURE OF THE MACAQUE ZONA INCERTA. T. P. Ma, G. A. Hoskins*, J. C. Johnson*, G. A. Mihailoff, and P. J. May. Depts. of Anatomy and Ophthalmology, University of Mississippi Medical Center, Jackson, MS 39216.

We have studied the fine structure of the zona incerta (ZI) in macaque monkeys to determine whether ZI contains the anatomical substrate necessary for its hypothesized role in orienting movements. Two cell types, similar to those observed in light microscopic preparations, could be distinguished. Putative interneurons (n=2) exhibited a highly indented nucleus, a thin rim of cytoplasm containing few organelles or Nissl bodies, and a sparse Golgi apparatus. Principal cells (n=15) had large, highly indented nuclei, and cytoplasm that contained the full range of organelles. Few synaptic contacts were found on the soma of either type. We examined 504 synaptic profiles in which clear pre- and postsynaptic membranes were present and distinguished at least three classes of synaptic profiles. Type A synapses (35%) form symmetrical contacts and exhibit considerable variability in vesicle packing density. Some Type A profiles contain large synaptic vesicles, dense-cored vesicles, and/or presynaptic grids. It is not clear whether these differences constitute distinct subtypes or simply represent normal variation. Type B synapses (6%) form asymmetrical contacts, are relatively large, and contain moderate numbers of pleiomorphic vesicles, coated vesicles, and mitochondria. Type C synapses (54%) form distinct asymmetrical contacts and may exhibit postjunctional bodies. These profiles contain vesicles of varying sizes as well as dense-cored vesicles and multivesicular bodies. The remaining 5% could not be classed. All synaptic types contact cell bodies and dendrites and may contact multiple postsynaptic structures. Only Type C boutons contact spines or other vesicle-containing profiles. In sum, two cell types and three synaptic classes were identified in the primate zona incerta. The complexity observed here is consistent with the multiple inputs known to terminate in the zona incerta and forms the substrate necessary for the control of orienting movements. *Supported in part by grants RR05386 (TPM), NS12644 (GAM), EY07166 (PJM).*

337.15

EYE MOVEMENT DEFICITS REVEALED BY STEP RAMP TRACKING ERRORS IN MONKEYS WITH MAGNO AND PARVOCELLULAR LGN LESIONS. W.K. Page*, W.M. King, W.H. Merigan, and J.H.R. Maunsell, University of Rochester Medical Center & Center for Visual Science, Rochester, NY 14642.

Cortical processing of retinal information utilizes two parallel pathways. These pathways are anatomically separated in the parvocellular and magnocellular layers of the primate lateral geniculate nucleus (LGN). Cortical areas MT and MST, which receive most of their retinal input via the magnocellular pathway, are likely involved in processing retinal image motion and provide inputs to the oculomotor system for ocular tracking. To test the contribution of the magnocellular pathway to ocular tracking of moving targets, we made selective unilateral ibotenic acid lesions of magnocellular layer 1 of the LGN in two macaque monkeys trained to pursue small target spots. Later, a selective lesion was made in parvocelluar layers 4 and 6 of the opposite LGN. By only testing the contralateral eye, lesions specific to layers 4 and 6 or layer 1 could be studied. Ocular tracking was tested several months after the lesions using step ramp target trajectories. Both animals detected, and made accurate saccades to, stationary targets stepped into either visual field, even when the target spot was extremely dim. The animals accurately pursued targets stepped onto the fovea and ramped temporally or nasally at speeds up to 20 deg/s. In contrast, smooth pursuit eye velocity had an increased latency and showed deficient tracking gain when targets were stepped into either the parvocellular or magnocellular lesioned fields, although initial pursuit acceleration appeared normal.

EY04045 (King), EY05911 (Maunsell), AFOSR890041 (Merigan)

337.16

ADRENAL AUTOTRANSPLANT EFFECTS ON EYE MOVEMENTS IN PARKINSON'S DISEASE. D. Impelman and B. Brooks-Eidleberg. Dept. of Physiol. UTHSC, S.A. TX 78284

The effects of an adrenal medullary autotransplant on saccadic and smooth pursuit eye movements were followed in a longtitudinal study of a 57 year old Parkinson(PD) patient for one year postoperatively. Presurgical motor deficits were bilateral and the right caudate was implanted. Computer generated 5°, 10°, and 20° saccadic target movements and 10° sinusoidal tracking routines were used in oculomotor measurements. The initial findings show a main effect on hypometria contralaterally. MANOVAS of bilateral frequency and interval effects show a significant decrease in the frequency of hypometric intervals less than 150 ms in PD saccades. Frequency analysis shows the number of normative saccades initiated during self-paced trials is significantly increased. Saccadic tracking is improved by a decrease in latency variability and a trend towards decreased lag times. Postsurgical smooth pursuit gain and phase lag effects are correlated with transplant effects on PD hypometria. In summary, there appears to be a decrease in saccadic inhibition shown by 1)the decrease in PD hypometria and 2)the increase of normative saccades. These implant effects are consistent with a reduction of the net GABAergic inhibition in the striatal-nigro-collicular pathway in PD eye movements.

337.17

HUMAN OCULOMOTOR SYSTEM USES BOTH EXO- AND EGOCENTRIC CUES IN THE LOCALIZATION OF SUCCESSIVE TARGETS. P. Dassonville, J. Schlag, and M. Schlag-Rey. UCLA, BRI and Dept. of Anatomy, Los Angeles, CA 90024.

Although it is well understood that, under certain conditions, egocentric cues are inadequate to allow the human *perceptual* system to accurately localize a visual target (Matin 1976), it has been assumed that these cues are sufficient to allow for accurate *oculomotor* localization (Hallett and Lightstone 1976). Recent studies have called this distinction into question: when a brief flash is presented during an initial saccade, the target is mislocalized, in the direction of the initial saccade, by the oculomotor system (Dassonville et al. 1990). The patterns of mislocalization are identical to those of the perceptual system (Honda 1990), and have led to the conclusion that the perceptual and oculomotor systems use a damped internal representation of eye position in an attempt to achieve direction constancy.

Can the oculomotor system utilize *exocentric* cues to improve its accuracy, as the perceptual system does? This question has only recently been investigated (Hayhoe et al. 1990). In the present study, five human subjects, in complete darkness, were required to make a saccade to the location of a 2 ms flash (S2) occurring near the time of an initial saccade directed to a previous flash (S1, 20° right of fixation point). A monocular magnetic search coil was used to measure eye position. In the first condition, S1 was of a short duration (5 ms) and was, therefore, extinguished well before the onset of S2. In the second condition, S1 had a longer, variable duration so that it remained on until replaced by S2. In four of five subjects, accuracy of the movement to S2 significantly improved in the latter condition ($p<0.02$), where S1 and S2 were in close temporal proximity. Accuracy was also dependent on the distance between S1 and S2: errors were smallest when the two flashes were in close spatial proximity, a result similar to those of Mateeff & Hohnsbein (1989) with perceptual localization. These results suggest that the exocentric relationship of successive flashes can be used by the oculomotor system to reduce the errors of egocentric localization - a possibility that should be considered when interpreting the results of studies utilizing double-step stimuli. (USPHS grants EY05879 & EY02305)

337.18

THREE DIMENSIONAL INTERSEGMENTAL INTEGRATOR OF EYE MOVEMENTS J.Droulez*, J. Laczko, A. Berthoz C.N.R.S. Lab. de Phisiologie Neurosensorielle, Paris 75006 France

The question of how orienting movements are generated by neural networks led us to build a model of oculomotor integration. This model is an extension of the dynamic memory model proposed by Droulez and Berthoz (P.N.A.S. , in Press and in The Oculomotor System ed.Shimazu & Shinoda.) in which the instantaneous target position is stored as the distributed activity of a retinotopic map and is continuously shifted according to the eye velocity signal. The input of the extended model are the gaze- and head velocity signals. The model solves some problems of other oculomotor integrators.

Graphical computer programs have been developed to simulate how a desired velocity can be transformed into relative position between two body segments (eye and head). The computer simulation obeys known features of eye-head coordination: 1. It considers the properties of the three dimensional rotational group. During the integration no cumulative error occurs in contrast with such models in which each dimension was integrated separately. 2. The output of the integrator is the position of the eye coded in intrinsic muscular coordinate system. This allows transformation between different frames of reference: the same model can be applied to the cephalomotor integrator to provide the adequate command for neck muscles. 3. The use of musculotopic coordinates makes it possible to formulate and maintain the rigidity of the muscular system. Rigidity is formulated by expressing each eye and head centered vectors as a linear combination of the vectorial products of the others and maintained by keeping the coefficients constant. This method offers a fast algorithm to maintain rigidity of the quasi-orthogonally organized eye muscles and it can be applied for arbitrary muscular systems with any number of muscles as well. 4. The model is compatible with Listing's law: it generates saccadic eye movements without torsional rotation component.

This computerized model is very useful to investigate properties and coding of orienting eye movements in different muscular and neural reference frames.

337.19

HEAD AND EYE MOVEMENTS IN THE MACAQUE: CONTRIBUTIONS OF THE PONS AND SUPERIOR COLLICULUS. R. J. Cowie and D. L. Robinson. Department of Anatomy, Howard University, Washington, D. C. and Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

The coordination of head and eye movements is essential for normal vision and many subcortical structures participate in the generation and integration of these motor activities. We have electrically stimulated the medial pontine reticular formation as well as the intermediate and deep layers of the superior colliculus in monkeys free to move their head and eyes. Excitation of the pons leads to brisk, saccadic-like movements of the head in a contralateral direction. Comparable head movements are evoked while the monkey performs a fixation task and during spontaneous behavior in total darkness. These evoked movements cause no significant gaze shift; the vestibulo-ocular reflex compensates for these head movements. If the head is restrained, stimulation does not evoke an eye movement. In contrast, when the superior colliculus is stimulated, large shifts in gaze occur. If the head is restrained, then contralateral saccadic eye movements result from stimulation. In the head-free condition, the majority of the gaze shift is accomplished with a saccadic eye movement; on a few trials there is a slight head movement which accompanies the saccade. At deeper collicular sites there is a significant head movement evoked which moves the head in the same direction as the eyes. Such head movements can be evoked with the head and eyes in their primary positions.

These data demonstrate that in the macaque both the superior colliculus and pontine reticular formation participate in the coordination of head and eye movements although they perform different functions. The collicular contribution is in the form of a gaze shift, most often with saccadic eye movements but head and eye movements are generated. The pons contributes in terms of movement of the head; this evoked movement does not create any gaze shift.

338.1

HUMAN HEAD SACCADIC GAIN. James H. Fuller
Dept. Oral Anatomy, Univ. of Illinois, Chicago IL 60612

Visually evoked gaze shifts were studied in nine young adults to define and examine head movement gain (head/target amplitude). Subjects (S) stepped their gaze with a free head between four randomly presented step sequences, beginning from: a, 0; b, 20; and c, 40 deg; and ending on the opposite side at: a, 40; b, 20; b' and c, 40 deg. Thus the a step is centric-eccentric, b is symmetrical; the b' and c steps start progressively eccentric. There were two tasks: the non-aligned (NA) task required: 1) gaze fixation and 2) a reaction-time, accurate, gaze step to the target; the head-aligned (HA) task required: 3) head alignment within 3 deg of fixation before the step sequence began. Gain = 0.27 NA, 0.67 HA.

It was found that S's were divided by: 1) the b step best characterized head-movers in the NA task; 2) the b step, HA task, best identified non-movers; 3) the a, b', c series in the HA task characterized the non-movers by a relatively linear increase in gain, but not head-movers. Three effects account for these results: 1) a midline-attraction effect increasing movement towards the center in head movers, and decreasing movement away from the midline in non-movers; 2) a momentum effect tending to carry the head across the midline in the b, b', and c steps; 3) an awareness/arousal effect due to the intrinsic nature of the alignment procedure.

338.2

SMOOTH PURSUIT ABNORMALITIES IN AMYOTROPHIC LATERAL SCLEROSIS (ALS). L.A. Abel, I.M. Williams*, K. Gibson* and L. Levi*. Dep't of Ophthal., Indiana Univ., Indianapolis and Neuro-Ophthalmol. Lab, Monash Univ. Dep't of Med., Melbourne, Australia.

Once held to be the only motor function spared in ALS, several studies have reported ocular motor defects, including reduced pursuit gain. To better define this latter defect, we examined ALS patients with moderate and severe clinical impairments and age-matched controls, using constant velocity and sinusoidal targets. In the former study, 17 patients and 17 controls followed 10 and 20°/s targets. Patients', but not controls', gain decreased with target speed. The severe group also showed a significant gain asymmetry at 20°/s. In another study, sinusoidal stimuli of ±5 and 10° from 0.25 to 2 Hz were presented to 9 patients and 11 controls. Targets were such that for all but one peak velocity there were two peak accelerations, allowing their separate assessment. Gain was reduced for the severes at all velocities; initially, moderates performed like controls, but at a peak velocity of 31.4°/s, went from being grouped with the normals to clustering with the severes when acceleration went from 98.6 to 197.2°/s^2. Thus, early in the disease process, an acceleration limit is present, to be joined later by a velocity limit. Pursuit impairment in ALS involves more than a simple, global gain reduction.

338.3

OCULOMOTOR DYSFUNCTIONS IN SCHIZOPHRENIA. J.A. Sweeney, G.L. Haas*, J. R. Carl*, M. Keshavan*. Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Pursuit eye movement dysfunctions are common in schizophrenic patients and their family members. We have observed significantly reduced pursuit gain in schizophrenia, bipolar disorder, psychotic and nonpsychotic unipolar depression and obsessive compulsive disorder, indicating limited specificity of this abnormality for schizophrenia. Memory-guided-saccade and anti-saccades, thought to be mediated by prefrontal cortex, were abnormal in twenty unmedicated schizophrenic patients. Latency, gain of first saccade and error of final resting position of memory guided saccades were all abnormal in schizophrenia. Saccades to target were more common and anti-saccades less accurate in schizophrenia. Neuroleptic treatment reversed right visual field advantages on some tasks. Preliminary analysis suggests that deficits on oculomotor tasks of frontal function are more specific to schizophrenia than pursuit impairments.

338.4

PURSUIT PROLONGS, WHILE FIXATION GAPS SHORTEN SACCADIC REACTION TIMES (SRT). D. Braun, D. Boman, J. Hotson. Calif. Inst. Med. Res., San Jose, CA & Stanford Univ. Sch. Med., Stanford, CA.

Changing the condition of attentive visual fixation on a stationary target alters SRT. SRT decrease when the fixation point is extinguished prior to saccade target onset and increase when the fixation point remains on after saccade target onset. The present experiments examine the affects of fixation conditions on SRT during smooth pursuit.

We compared vertical SRT during stationary fixation and during horizontal smooth pursuit using three fixation conditions: 1) Gap - fixation point offset occurred 200 msec prior to target onset, 2) Simultaneous - fixation point offset coincided with target onset, and 3) Overlap - fixation point offset occurred 200 msec after target onset. With both stationary and smooth pursuit fixation, SRT were shortest in the Gap condition and longest in the Overlap condition indicating that similar mechanisms may be involved in the disengagement of fixation from moving and stationary targets. Also, average SRT with stationary fixation were 10-40 msec shorter than during smooth pursuit under each condition. The disengagement of pursuit or the computation of its spatial displacement may slow saccade initiation.

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338.5

SMOOTH PURSUIT EYE MOVEMENTS IN PATIENTS WITH MAJOR DEPRESSION

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Eye tracking dysfunctions (ETD) are believed to be a psychophysiologic marker of schizophrenia. Although ETD is thought to be specific for schizophrenics, it has not clearly been established that patients with major depression have similar disturbances.

We analyzed smooth pursuit tracking in 17 patients with a major depressive disorder. No patient had psychopharmacological treatment at the time of the examination.

As controls, we tested 10 age- and sex-matched volunteers. Eye tracking was recorded with DC-EOG. The target was driven sinusoidally at a frequency of 0.4 Hz and an amplitude of 30° of visual arc. Beside a qualitative rating (5-point scale), a Fast Fourier Transformation (FFT) was computed and the power between 0.8 and 12.4 Hz calculated, which reflects the accuracy of tracking performance.

The mean qualitative ratings and the mean quantitative scores (FFT) of eye-tracking showed no significant difference between depressed patients and controls. This finding supports the hypothesis that ETD in psychiatric populations might be specific for schizophrenia.

338.6

COLOURED TARGETS AND LATENCIES OF EYE SACCADES.

P.E. Hallett & Christian Perron, Institute of Biomedical Engineering & Department of Physiology, University of Toronto, Ontario, Canada M5S 1A8.

The task was to track a disk target as it stepped randomly between 7 equally spaced positions on a white screen. Tracking was frequent because the target stepped shortly after each successful foveation. Disk subtense was typically 2.3° and the step 5°. Colour was randomly switched between light/dark, yellow/blue, red/green or orange/cyan during different runs.

Analyses of mean latencies and multiple linear regressions showed that latency inflating factors were low tritanopic purity difference (i.e. blue or yellow colour), low achromatic or chromatic contrast, small size, and edge blur. Screen position and retinal meridian were very weak factors. Isoluminous chromatic targets were often well tracked, especially at high chromatic contrast. Severe blur was also fairly well tolerated.

A possible model is that saccadic latency is partly, if not largely, determined by the magno pathway.

338.7

Small lesions in the frontal eye field impair predictive pursuit but preserve the memory for movements past. E.G. Keating, Dept. of Anatomy & Cell Biology, SUNY-HSC, Syracuse, N.Y. 13210

Smooth pursuit is driven by current signals (visual, oculomotor) and prior experience (predictive strategies). Previously, large ablations of the frontal eye field (FEF) have impaired visually guided and predictive pursuit. This study searched for the subset of FEF critical for the impairment, and further explored the predictive pursuit deficit.

Three monkeys tracked visual targets designed to evoke predictive pursuit. For example, if a sinusoidal target suddenly disappeared, the monkeys' tracking continued to approximate sinusoidal pursuit for several reaction times.

Small unilateral FEF ablations, approximately confined to "low-threshold" FEF, impaired visually guided and predictive epochs of smooth pursuit. The decrement in peak velocity of the predictive sine epochs was worse than for visually guided epochs.

However, the impairment was not an amnesia for prior experience. After surgery, predictive sine pursuit, albeit impaired, still varied as a function of the prior visual target's frequency. When ramp targets had the same velocity on successive trials, the monkeys still occasionally evoked anticipatory pursuit, i.e., began pursuit prior to target motion. Variance in the latency of pursuit was related to the target velocity of prior trials.

The FEF deficit seems not a loss in predictive strategies directly, but impairment of some other control signal, perhaps eye velocity, on which predictive eye movements heavily depend. NSF BNS 8603915

338.8

TRACKING EYE MOVEMENTS FOLLOWING LOOMING VISUAL STIMULATION U. Schwarz. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Tracking eye movements following brief periods of undirectional, looming motion in the central visual field were studied in monkeys. The goal was to determine to what extent motion processing structures (MT/MST) in the brain utilize pure speed information in a subsequent tracking task, which might involve modules used in object recognition (V4). Monkeys were seated 200 *mm* in front of a video display with their head fixed. Eye position was recorded with the search coil technique. The target was a filled square subtending a visual angle of 1.6°. First, it was positioned in the center of the screen. The animals were trained to fixate the center of this square and were never reinforced for responses to target movements. After successful fixation for at least 1*s* the square was radially expanded at 12, 18, or 24°/*s* as annulus (same thickness as target) subtending an angle of 360, 270, 180, or 90° during 50, 100, 150, or 200*ms*. After this expansion period the annulus was reduced back to the square which continued the movement horizontally at either the same or changed speed for a total of 750*ms*. Controls consisted in ramp movements of the fixation target only. The eye responses were robust and showed these characteristics: no movements occurred during the expansion period (eyes locked in position). Latencies were similar in all conditions, but the time from the onset of the response to the onset of the saccade was shorter for longer expansion times, and eye acceleration was slightly higher when compared to the simple ramp task. Fractioned annuli produced an angle dependent change in eye acceleration prior to the onset of the actual pursuit response. Steady-state pursuit was always close to 100% in all conditions. These findings suggest that tracking performance is not tuned by speed information that by itself does not yet need to elicit eye movements (diverging optic flow during forward movements).

338.9

THE STABILITY OF THE OCULOMOTOR SYSTEM TO TIME DELAYS D.M. WOLPERT*, G.K. KERR, R.C. MIALL* & J.F. STEIN*
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The oculomotor system has intrinsic delays (totalling about 200 msec) which represent the time taken by neuronal processing and eye dynamics. The ability of the eye to track and fixate objects accurately implies that the control system is well adapted to these intrinsic delays. We have therefore investigated the stability of the oculomotor system when additional time delays are imposed during fixation or pursuit.

We examined head restrained, human monocular tracking of a spot target using infra-red reflectometry. In order to incorporate an extra time delay of D msec into the oculomotor feedback loop, the spot was iteratively displaced from the target waveform by the angle through which the eye (sampled at 500 Hz) had moved during the previous D msec. Thus, the difference between the current and past eye positions was used to modify the target location.

Three subjects were examined whilst they performed two tasks. In the first the subject attempted to fixate a stationary target for 15 seconds during which delays of between 0 and 520 msec were added. In the second he tracked pseudorandom waveforms for 15 seconds. Delays of 0 to 280 msec were added.

The standard deviation of eye position during fixation trials increased greatly when the delay was made greater than approximately 200 msec. During pursuit the mean squared error increased greatly as delays rose above 150 msec.

These results suggest that added delays of less than 150 msec have little effect on the subjects' ability to fixate or pursue targets. This is surprising given that the system has intrinsic delays of a similar magnitude.

338.10

RAPID AND SLOW MECHANISMS FOR THE CONTROL OF OCULAR TORSION DURING VOLUNTARY GAZE SHIFTS. J.A.M. Van Gisbergen and A.W.H. Minken*. Dept. Med. Phys. & Biophys., P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

It is known that Listing's law holds reasonably well during fixation and saccades when the eyes are not converged. Tweed and Vilis have demonstrated, for fixed-axis saccades, that implementing the law dynamically requires tilting of the angular velocity axis out of Listing's plane. We have investigated whether a dynamic implementation mechanism can also be shown in strongly curved (non-fixed axis) saccades. In addition, we explored the time course of Listing's law violations in near vision.

Eye movements were recorded in human subjects with the 3D scleral coil technique. Curved saccades were elicited in five subjects across a screen at 95 cm distance by two-dimensional double-steps. In the near-vision experiments, three subjects alternated fixation between various targets at 15 and 95 cm distance.

The results show that strongly curved saccades, corrected in midflight, obey Listing's law just as well as normal fixed-axis saccades. Thus, fixed-axis rotations are not a necessary condition for the dynamic implementation of Listing's law during saccades. Our curved-saccade results can be interpreted in terms of the 3D internal feedback model proposed by Tweed and Vilis (1987). The near-vision experiments show that violations of Listing's law during refixations in depth, manifest in torsion changes, were often very slow and sometimes had a different latency than the saccadic component of the gaze shift. These results seem nicely in line with the suggestion that near-vision violations of Listing's law may be due to a system linked to the control of convergence (Allen, 1954), but binocular 3D recordings are required to investigate this possibility further.

338.11

AUDITORY FACILITATION OF VISUALLY-GUIDED SACCADES C.Lee, S.Chung*, J.Kim*, and J.Park*. Dept. of Psychology, Seoul National Univ., Seoul, Korea.

It has been shown that the latency of saccades toward a visual target is reduced when an auditory stimulus is simultaneously presented. In order to test whether this facilitation is spatially selective, we examined the effect of spatial congruence of visual and auditory stimuli on the saccade latency. Two human subjects, wearing a golden eye-ring fitted on the globe (Bour et als, IEEE BME31,1984) with heads restrained, were instructed to fixate on a front LED and look to eccentric LEDs mounted on the center of speakers. The eye position signals were obtained with the circuit described by Remmel (1984), and sampled every 4 msec. The visually-guided saccade latency was reduced by approximately 20 msec when a tone was simultaneously presented at the same location as the lit LED, confirming previous reports. A tone delivered at the opposite side to impending saccades, however, had neither positive nor negative effects on saccade latency. These results indicate that the auditory facilitation is not due to general alerting caused by an auditory signal, but to spatially-selective intermodal interaction. (Supported by Korea Science and Engineering Foundation Grant 901-0408-007-2).

338.12

PREDICTIVE SACCADES IN HEMIPARKINSONS DISEASE (HPD). J. Ventre, D. S. Zee, H. Papageorgiou, and S. Reich. The Johns Hopkins University, School of Medicine, Baltimore, MD 21205 and INSERM, Bron, France.

We recorded eye movements (EOG) in 9 patients with mild HPD and in 16 controls. Five patients were affected predominantly on the right side (RHP), and 4 on the left side (LHP). Reflexive saccades were elicited in response to the random appearance (timing and location) of a light-emitting diode (LED). Predictive saccades were elicited by alternately illuminating LEDs (R10-L10deg), at fixed frequencies of 0.25-1.0 Hz.

In the reflexive task there were no differences in saccade latency or amplitude between patients and controls. During predictive tracking, while mean saccade latency was not significantly different between patients and controls, two abnormalities were noted in timing. First, in LHP, there was a directional asymmetry in latency (L > R, eg. at 0.25 Hz, mean difference of 90ms). Secondly, in LHP, and especially in RHP, there was less consistent prediction as reflected in more variability of the mean value of saccade latency (for each group of subjects), at each point in time throughout a trial. Saccade amplitudes were also abnormal. Mean values were decreased in RHP but especially in LHP (eg. at 0.25 Hz, rightward saccades, 19 deg (1.6 sd) in controls, 14 deg (2.7 sd) in LHP, 15.7 deg (2.3 sd) in RHP).

In sum, HPD patients showed abnormalities in timing and in amplitude during predictive saccadic tracking, while more reflexive saccades were normal. The defects were most evident at low frequencies. While the defects were largely bilateral, our results suggest that the left and the right cerebral hemispheres make slightly different contributions to both the temporal (timing) and the spatial (accuracy) aspects of predictive saccadic tracking.

338.13

THE EFFECTS OF COMBINED SUPERIOR TEMPORAL POLYSENSORY AREA AND FRONTAL EYE FIELDS LESIONS ON EYE MOVEMENTS IN THE MACAQUE. J.P Skelly, T.D. Albright, H.R. Rodman, and C.G. Gross. Dept. of Psychology, Princeton University, Princeton, NJ, 08544.

We previously found (Skelly, et. al., Soc. Neurosci. Abstr. 15:475.1) that lesions of the superior temporal polysensory area (STP) cause temporary deficits in the production of saccadic eye movements. In order to define regions participating in recovery, we examined the effects of adding lesions of the frontal eye fields (FEF) in 3 macaques who had reattained preoperative performance on a saccade task after bilateral STP lesions and in 1 animal with a bilateral control lesion of inferior temporal (IT) cortex. Prior to initial surgery the animals were trained to saccade to targets appearing 8, 15, and 22 deg. to either side of and 8 deg. above and below a central fixation point (FP), and to make smooth pursuit eye movements to targets moving 5, 13, and 22 deg./sec.

After adding a unilateral FEF lesion to STP damage in one animal and a laterally asymmetric bilateral FEF lesion in another, the animals showed larger increases in saccade latency than are seen after either FEF (Lynch and Allison, Soc. Neurosci. Abstr. 11:144.9) or STP lesions. Animals often failed to make a saccade to peripheral targets within the second that they were given to respond. No effect on saccadic eye movements was found from adding a laterally symmetric FEF lesion to the third animal with bilateral STP damage or from adding a unilateral FEF lesion to the IT control. Addition of FEF lesions to STP or IT damage produced variable effects on pursuit, similar to those seen after FEF damage alone. After all of the FEF lesions there was a large initial deficit in fixating the FP, which was greatly ameliorated by turning on a dim light in the testing room. All of the deficits showed considerable recovery over time, especially within the first two weeks of testing.

The results are consistent with a contribution of the FEF to the recovery after STP damage alone.

338.14

A POPULATION CODING MODEL OF TWO DIMENSIONAL SACCADE GENERATION M. Fujita. Neural Computation Section, Communications Research Laboratory, Koganei-shi, Tokyo 184.

Saccadic eye movement shows a nonlinear characteristics in its peak velocity vs. amplitude relationship which makes saccadic duration lengthened as the amplitude increases. Saccade system must have some mechanism to stretch a weaker (horizontal or vertical) component duration of eye movement in oblique saccades so as to keep its trajectory straight.

Our model is based on the idea of population coding in the brainstem neural circuits. We assume four ensembles of an element circuit, corresponding to the four burst neuron pools supposed to be active in a saccade to one of four directions (up, down, left or right). Each element circuits consists of a pair of long-lead burst neuron and medium-lead burst neuron with local feedback system, an equivalent circuit as the one proposed by Scudder (Soc. Neurosci. Abstr., 1984). A signal from the superior colliculus (SC) traverses two of the four ensembles with a variable trajectory since its output location from the SC varies according to the SC motor map and so the number of element circuits which the signal intersects and makes synaptic contacts with changes. In our model, this number becomes proportional to the direction factor which the ensemble encodes. Since the strength of an input signal to an element circuit represents the saccadic amplitude, activity duration of the circuit or the whole ensemble depends only on the amplitude, not on the direction, thus realizing naturally the stretching phenomena. Since the total output effect of one ensemble is proportional to the product of an element circuit output and a number of active elements in the ensemble, saccade trajectory becomes straight.

Population coding idea applied in the brainstem not only leads to plausible two-dimensional saccade generation, but also bears following features: it mimics neuronal signals in the brainstem, proposes neural mechanisms to realize a trigonometric function, does not need precise timing in the activity of pause neurons, explains the horizontally-dominated curvature of normal saccades and has ability to issue microsaccades with minor modification of the model.

338.15

Relation of Purkinje cell activity in the ventral paraflocculus of alert monkey to ocular following response. M. Shidara* and K. Kawano. Neuroscience Sect., Electrotechnical Lab., Umezono, Tsukuba-shi, Ibaraki 305, JAPAN.

Previous studies, using alert monkeys, showed that neurons both in the medial superior temporal area (MST) of the cortex and dorsolateral pontine nucleus (DLPN) are activated by movements of the entire visual field, eliciting ocular following response. In order to know the efferent limb of the DLPN neurons, the simple-spike activities of Purkinje cells (P-cells) were recorded in the ventral paraflocculus (VPFL; = folia V ~ XII of flocculus as defined by Madigan & Carpenter, 1971) of two monkeys.

The animal faced a tangent screen onto which a random dot pattern was projected and moved, and its eye movements were recorded with the magnetic search coil technique. P-cells in the VPFL were activated by the movements of the entire visual scene. One group of P-cells preferred downward motion, and the other preferred motion toward the side of recording. The latency of the simple-spike response of 70% of the P-cells to the movement of the large-field visual scene was ~8 msec shorter than the latency of ocular following. At the site of each recording, electrical stimulation of single negative pulse, 5 ~ 30 μA, 0.2 ms in width was applied. 66% (19/29) of them elicited eye movements toward the preferred direction of the P-cell with a latency of 8 ~ 10 ms. These data suggest that P-cells in the VPFL participate in initiating the ocular following response.

BRAIN METABOLISM AND BLOOD FLOW IV

339.1

SYMPATHETIC EFFECTS ON RESISTANCE VESSELS OF THE BRAIN. J. A. DeMaro*, W. Finnegan*, J. Y. Wang*, and J. D. Fenstermacher. Department of Neurological Surgery, SUNY Stony Brook, Stony Brook, N.Y. 11794.

In the cerebral arteries of spontaneously hypertensive rats (SHR, luminal diameter is decreased and wall thickness and area are increased. These changes are thought to be driven be the sympathetic nervous system. To test this hypothesis, the superior cervical ganglion was unilaterally removed in one-month-old SHR and Wistar-Kyoto (WKY) rats (WKY are the normotensive control for SHR). Six months later, a set of 9 cerebral arteries were sampled and analyzed by light and electron microscopic morphometry. A similar set of arteries were also taken from age-matched intact (control) SHR and WKY. Differences, which were sizable and significant in a number of cases, were found between control SHR and WKY. In accordance with published data, luminal diameter was less and wall area and thickness were greater in SHR compared to WKY. Unilateral ganglionectomy (uGX) slightly decreased luminal diameter (D) in SHR and increased the differences in D between SHR and WKY. uGX had small and variable effects on wall area in both SHR and WKY. There were no differences in arterial wall structure between ipsilateral and contralateral sides in either uGX-SHR or uGX-WKY. In support of the hypothesis, alteration of the sympathetic nervous system at one-month of age affects cerebral arterial wall structure in adult SHR.

339.2

EFFECTS OF HYPOGLYCEMIA (H) ON AUTOREGULATORY ADJUSTMENTS OF NEWBORN PIAL ARTERIOLES (PA). T.S. Park, E.R. Gonzales*, A.R. Shah*, and J.M. Gidday. Department of Neurosurgery, Washington University, St. Louis, MO 63110

We investigated the effects of (H) on the progressive vasodilatory responses we previously observed in newborn pig PA in response to graded hypotension (GH). Two groups of newborn pigs were equipped with closed cranial windows for the observation of PA (50-100 μm dia) by videomicroscopy. In the normoglycemic controls (C), PA diameters were measured during normotension, and during GH (MABP = 50, 40 and 30 mmHg) induced by hemorrhage. A similar protocol was followed in the H group, with H induced by insulin after normotensive normoglycemia.

MABP (mmHg)	Glu (mg/dl)	C Group (μm)	MABP (mmHg)	Glu (mg/dl)	H Group (μm)
74	105	67±3	67	100	76±5
68	-	79±4	62	18	99±6 * #
50	-	85±5 *	50	16	97±7 *
40	-	88±5 *	40	19	103±8 * #
30	159	97±5 * **	30	15	107±8 *

* and ** = $p<0.05$ compared to initial and second normotensive diameters, respectively, within each group.
# = $p<0.05$ compared to corresponding C group diameter.
Thus, H caused dilation of PA during normotension, but, in contrast with C animals, no further increase in PA diameters was observed with GH to 30 mmHg, indicating an impairment in autoregulation under conditions of H.

339.3

CHANGES IN MITOCHONDRIA AND CAPILLARIES ASSOCIATED WITH SYNAPTOGENESIS AND EYE-OPENING IN RAT VISUAL CORTEX.
J.E. Black & W.T. Greenough. College Medicine, Neuroscience Prog, Depts of Psych and Cell & Structural Biol, University of Illinois, Urbana, IL 61820.

Capillaries and mitochondria in visual cortex undergo substantial growth during the postnatal period. The earliest stages of capillary and mitochondrial proliferation are probably controlled by a rigid developmental program. On the other hand, growth of both these components can be elicited of healthy, mature animals by manipulating neuropil volume and metabolic demand (e.g., Black, et al, Neurosci Lett, 1987; Black, et al, PNAS, 1990). This study examines capillaries and mitochondria in Layer IV of visual cortex during eye-opening, an interval with a sharp increase in metabolism about day 14.

Eight littermates were sacrificed in pairs on postnatal day P13, P15, P20, and P25. After perfusion and aldehyde fixation, tissue blocks from Area 17 were prepared for TEM. Montages at 32,500X were prepared from serial sections. Each set of serial montages contained a long segment of an apical dendrite from which 3-dimensional reconstructions of spines have been described (Hwang & Greenough, Soc Neurosci, 1985). Two montages from each animal were used to obtain estimates of mitochondrial volume fraction. Mean PSD length was estimated from a sampling of synapses nearby. Synapse density was estimated as Nv=Na/(D+t), where t is section thickness. Capillary density and mean capillary diameter were estimated from 1-µm toluidine-blue stained sections. The Disector method will be used to estimate Nv mitochondria.

age	Nv syn (#/100µm^3)	PSD length(µm)	mitochon (%)	cap dens(#/mm^2)
P13	47	.27	11.5	259
P15	81	.20	14.8	254
P20	81	.25	11.6	353
P25	94	.25	12.6	331

Supported by NIMH MH35321 and NIA AG101514.

339.4

METABOLIC MAP OF THE NORMAL RAT BRAIN AS REVEALED BY CYTOCHROME OXIDASE HISTOCHEMISTRY AND BIOCHEMISTRY.
S. Liu, R. Hevner and M. Wong-Riley. Dept. Cellular Biology & Anatomy , Med. Coll. Wis., Milwaukee, WI 53226.

The rat has served as a useful experimental model, and a number of studies in the past have focused on its enzymatic distributions in isolated regions of the brain. The present study was undertaken to systematically establish a metabolic map of the rat brain based on cytochrome oxidase (C.O.) histochemistry (of sections cut in three different planes) and supplemented with biochemical assays of C.O. activity in selected brain regions. Previously, Darriet et al. ('86) have found a strong correlation between the two techniques. Our analysis indicates that the distribution of C.O. activity in the rat brain is quite heterogeneous at the regional, laminar, and cellular levels. The gray matter has a much higher level of C.O. activity than the white matter (about 4-10 fold). Quantitative analyses of both histochemical and biochemical data show that there are significant differences in C.O. activity among different structures in the gray matter ($p<0.05$ to $p<0.01$) as well as among regions of the white matter ($p<0.01$). Visual cortex, caudate nucleus, and superior colliculus, for example, are more C.O. reactive than other brain regions, such as the thalamus ($p<0.01$). Optic nerves have a higher level of C.O. activity than trigeminal nerves and the corpus callosom ($p<0.01$). In conclusion, both histochemical and biochemical analyses reveal a distinct heterogeneous pattern of C.O. activity in the normal rat brain. These findings will serve as a reference for our future experimental studies. (Supported by NIH grant NS 18122 to MWR and an MCW MSTP Fellowship to RFH).

339.5

FATTY ACID UPTAKE AND ESTERIFICATION BY THE IN SITU PERFUSED RAT BRAIN.
K. Washizaki, D. Purdon*, J. DeGeorge*, P.J. Robinson, S.I. Rapoport, and Q.R. Smith, Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20892.

Radiolabeled fatty acids have been used following intravenous injection to examine cerebral lipid synthesis and turnover in awake animals. To evaluate the time course of brain precursor specific activity and the influence of plasma fatty acid concentration on brain fatty acid uptake and incorporation, pentobarbital-anesthetized rats were perfused in situ with artificial or whole rat blood containing [3H]arachidonate or [14C]palmitate, following the procedure of Takasato et al. (1984). At the end of perfusion (0-15 min), brains were microwaved and processed to quantitate tracer in brain phospholipid, triglyceride, diglyceride, phosphatidic acid, acyl-CoA and unesterified fatty acid pools using combined TLC, HPLC and GC. Fatty acid incorporation into brain phospholipid increased ~linearly with time and was shown to be measurable even with perfusion times of less than 1 min. Calculated transfer coefficients for incorporation (blood-to-brain phospholipid) equaled $1.71 \pm 0.37 \times 10^{-4}$ and $0.68 \pm 0.19 \times 10^{-4}$ ml/s/g for [3H]arachidonate and [14C]palmitate, respectively, and agreed well with reported in vivo values. Incorporation of [3H]arachidonate decreased with increasing perfusate unlabeled arachidonate concentration, as expected for competition. Preliminary estimates indicate that the half-time of free fatty acid in brain is ≤1 min. In summary, the in situ brain perfusion technique can be used to examine the dynamics of cerebral lipid metabolism in vivo. The results demonstrate that the incorporation of fatty acid label into brain phospholipid pools depends on the level of fatty acid in the perfusate and is very rapid.

339.6

BRAIN N-ACETYL-L-ASPARTATE IS A MAJOR SOURCE OF ACETYL GROUPS FOR LIPID SYNTHESIS
R. Burri, N. Herschkowitz*. University of Berne, Department of Pediatrics, CH-3010 Berne, Switzerland.

To investigate the possible function of N-acetyl-L-aspartate as a lipid precursor in the CNS, N-[14C]-acetyl-L-aspartate (NAA) was injected intracerebrally into 8, 15, 22 and 79-day-old rats. Four hours after the injections 5-20% of the injected NAA was recovered in the brain tissue. These amounts were taken as 100 % in Table 1.

Table 1: Incorporation from NAA	% recovered radioact. in the lipid fraction	% recovered radioact. in the protein fraction
8 days	42.88 ± 9.80 (4)	7.20 ± 0.91 (4)
15 days	57.95 ± 10.38 (4)	8.73 ± 0.93 (4)
22 days	65.66 ± 5.96 (4)	9.38 ± 2.41 (4)
79 days	47.29 ± 7.87 (9)	17.29 ± 3.53 (9)
ANOVA (one-way)	p<0.01	p<0.001

NAA was a more efficient lipid precursor than a protein precursor. Incorporation into both fractions was age-dependent. The recovered radioactivity in the brain lipid fraction of rats injected with NAA was the same (8, 15, 22 days) or significantly higher (79 days) than in rats injected with [14C]-acetate (AcA). However, AcA was a better precursor for protein and cholesterol synthesis than NAA.

NAA is an efficient presursor for lipid synthesis in the brain of developing and adult rats. The comparison of NAA and AcA as lipid precursors suggests that lipid synthesis from NAA may occur without an intermediate product of free acetate.

339.7

INDUCTION OF C-*FOS* WITHIN DISCRETE BRAIN AREAS FOLLOWING INTRACEREBROVENTRICULAR INJECTION OF PROPIDIUM IODIDE IN THE RAT
Sheng Chen, Marina Bentivoglio (SPON: European Brain and Behaviour Society)
Institute of Anatomy, University of Verona, Italy.

It has been reported that intracerebroventricular (icv) injection of the fluorochrome propidium iodide (PI) results immediately in behavioral abnormalities, such as nystagmus, ataxia and truncal tremor. We aimed here at the identification of the structural substrate of the movement disorders induced by PI icv administration. Previous observations indicated that after icv injection PI labels selectively the Purkinje cells of the cerebellum and the dopaminergic neurons of the substantia nigra. In the present study, a prominent induction of c-*fos* protein, as revealed by immunohistochemistry, was observed bilaterally in the deep cerebellar nuclei, inferior olivary complex and pontine nuclei after icv injection of PI (0.2%, 3-10 µl). Such induction was absent in control cases with icv injection of saline. The present results indicate that the PI selective uptake activates discrete brain circuits, thus resulting in the behavioral abnormality. These data may contribute to the understanding of the mechanisms of action of molecules circulating in the cerebrospinal fluid. Further investigations on these mechanisms are in progress.

339.8

SYNTHESIS OF IRON REGULATORY PROTEINS IN THE HUMAN CHOROID PLEXUS.
C.V. FLAHERTY*, S.L. MENZIES* AND J.R. CONNOR. Dept. of Neuroscience & Anatomy, M.S. Hershey Medical Center, Hershey, PA 17033

The choroid plexus is responsible for the synthesis, secretion, and filtration of many of the constituents of the cerebrospinal fluid (CSF). Transferrin (Tf), the iron mobilization protein, is found in high abundance in the CSF and the plasma. The source of the high levels of Tf in the CSF has been in question, although recently the mRNA for Tf was demonstrated in the rat choroid plexus. In this investigation, we use immunohistochemistry, in situ hybridization and Northern blot analysis to demonstrate the presence of Tf, ferritin, the Tf receptor, and ceruloplasmin (ferroxidase) in the adult human choroid plexus. Human choroid plexus was obtained at autopsy and prepared for either immunohistochemistry or frozen on liquid nitrogen for RNA analysis. Immunohistochemical analysis revealed the presence of Tf, ferritin, the Tf receptor, and ceruloplasmin. While immunohistochemistry does not indicate whether these proteins were synthesized locally or transported via the plasma, Northern blot analysis for all the transcripts strongly implies local synthesis. These results suggest that the choroid plexus may be sensitive to alterations in iron plasma levels and may play a role in regulating iron in the nervous system. Supported by research grant AG09063.

339.9

INHALATIONAL ANESTHETIC AGENTS DECREASE SUSCEPTIBILITY TO CORTICAL SPREADING DEPRESSION IN THE CAT. R. D. Piper, G. A. Lambert* and J. Michalicek* . Inst. of Neurological Sciences, Prince Henry Hospital and University of N.S.W, Sydney, Australia 2036.

Cortical spreading depression (CSD) has not been demonstrated in the human neocortex intraoperatively during human neurosurgery. In this study we examine the effect of halothane and isoflurane, two anesthetic agents used during human neurosurgery, on the incidence of CSD after cortical pinprick with a 26 gauge needle (depth 1-2mm) in the anesthetized cat. The presence of CSD was detected by laser Doppler, DC potential or single cell recordings, and confirmed by demonstrating abolition of the cortical dilator response to arterial hypercapnia. CSD was seen in 100% of cats anesthetized with α-chloralose (N=15). CSD was induced in 3 of 7 (42%) animals anesthetized with isoflurane alone ($p < 0.005, \chi^2$ with Yates correction) and 0% (n=6) with halothane alone ($p < 0.005, \chi^2$ with Yates correction). In all animals anesthetized with either halothane or isoflurane it was possible to initiate CSD after administering α-chloralose intravenously and ceasing the inhalational anesthetic. In animals anesthetized with α-chloralose, CSD was blocked by the addition of an inhalational anesthetic on 83% of occasions (isoflurane n=3, halothane n=3). These findings suggest that inhalational anesthetic agents decrease the threshold for the initiation of CSD. This observation may be one reason why CSD has not been reported during human neurosurgery.

339.10

BIPHASIC CEREBROVASCULAR RESPONSE TO CORTICAL SPREADING DEPRESSION IN RABBITS. M. Shibata, C.W. Leffler* and D.W. Busija*, Dept. Physiol. and Biophys. , Univ. Tennessee, Memphis, TN 38163.

Cerebrovascular response to single cortical spreading depression (CSD) was examined using a closed cranial window and intravital microscopy. CSD was induced by KCl microinjection and its propagation was monitored electrophysiologically. Prostanoid levels in cortical periarachnoid cerebrospinal fluid (CSF) were determined by radioimmunoassay. Pial arteriolar diameter increased from 76±6 to a maximum of 119±5 µm (57%) for 1.6±0.1 min when CSD reached the cortex just beneath the vessel. Sho rtly after CSD expiration from the cortex, pial arteriolar diameter decreased to a minimum of 67±5 µm (12%) for 19.5± 2.1 min. CSD was elicited again in the same animal while the cortical surface under the window was continuously superfused with artificial CSF. Pial arteriolar dilation was observed again during CSD. However, no constriction of the vessel was seen after CSD expiration. Indomethacin (INDO) enhanced the CSD-induced vasodilation from the pre-INDO levels of 59±9% to the post-INDO levels of 82±13%. In contrast, the post-CSD vasoconstriction observed before INDO (11± 2% for 16.6±2 min) was completely ablated after INDO. CSF levels of prostaglandin (PG) E2, 6-keto-PGF1α and PGF2α increased during the CSD-induced vasodilation by102%, 34% and 93%, respectivey. These prostanoid levels increased further during the post-CSD vasoconstriction: PGE2 (213%), 6-keto-PGF1α (99%), PGF2α (236%). INDO decreased the resting levels of all the prostanoids and blocked their increases during the CSD-induced vasodilation and during the post-CSD period. These results suggest that pial arteriolar dilation observed during CSD is caused by neurogenic factors, and that the post-CSD constriction of the vessel is induced by prostanoids intraparenchymally synthetized and diffused into CSF. (NIH Grant HL 30260 and HL 34059)

339.11

CORTICAL HYPOPERFUSION ASSOCIATED WITH SPREADING DEPRESSION PERSISTS IN AWAKE RATS AFTER TREATMENT WITH TIRILAZAD MESYLATE (U-74006F). R.B. Duckrow and D.C. Beard*. Department of Medicine, Division of Neurology, The Pennsylvania State University, Hershey, PA 17033.

The 21-aminosteroid antioxidant tirilazad mesylate (U-74006F) has been reported to block the delayed hypoperfusion associated with spreading cortical depression (SCD) in anesthetized rats. This suggests that oxygen radical-induced lipid peroxidation may mediate this hypoperfusion. Because the resting vascular tone is one factor which determines the cerebral blood flow (CBF) response to SCD, the effect of this drug was reassessed in awake rats. Vascular catheters were placed using halothane/nitrous oxide anesthesia. Wounds were infiltrated with procaine and swabbed with xylocaine ointment. The hip girdle was immobilized using a plaster cast and anesthesia was withdrawn. After at least one hour, SCD was induced by passing direct current through a bipolar electrode previously implanted in the left lateral frontal cortex. CBF was measured using [14C]isopropyliodoamphetamine and quantitative autoradiography. Tirilazad mesylate (1mg/kg, i.v.) was given 5 minutes before induction of SCD and CBF was measured 90 seconds later. Drug was also given 10 minutes after induction of SCD and CBF was measured 8 minutes later. In both cases, a 30% reduction in cortical CBF was measured in the wake of SCD. Oxygen radical-induced lipid peroxidation may not mediate cerebral hypoperfusion after SCD induced by focal electrical stimulation in awake rats. (Supported by PHS NS24109)

339.12

BRAIN SLICE GLUCOSE UTILIZATION (SGU) MADE SIMPLE. G. C. Newman, C. S. Patlak, F. F. Hospod and H. Qi. Depts. of Neurology and Neurological Surgery, SUNY at Stony Brook, Stony Brook, NY 11794 and Northport VAMC.

Our goal is to increase the general usefulness of the *in vitro* glucose utilization method for brain slices (SGU) by simplifying calculations and permitting flexible incubation conditions. To achieve this, we use a form of the rate equation which requires only measurement of total radioactivity in the slice and buffer after incubation of slices in tracer amounts of ^{14}C-2-deoxyglucose (2DG). We have also generated a table of coefficients which directly yield glucose utilization values when multiplied by the ratio of radioactivity in slice to bath. The table provides coefficients for incubation times of 15 to 60 minutes, rinse times of 5 seconds to 30 minutes and buffer glucose concentrations (C_p) of 4 to 10 mM.

The table of coefficients was generated using our 8 parameter kinetic model of 2DG metabolism. Rate constants for $k4^*$ to $k8^*$ were derived from our published values. To obtain values of K_1^*, k_2^* and k_3^* for all C_p between 4 and 10 mM, we measured values for V_d^* and $k3^*$ at C_p of 4, 7 and 10 mM for hippocampal or hypothalamic slices and analyzed the results assuming Michaelis-Menton kinetics.

To test this approach we compared apparent SGU with the experimental values and determined the error introduced by the use of the theoretical "universal slice" constants. For both hippocampal and hypothalamic slices, the error in SGU is about 2 % at 4 mM glucose and almost 10% at 10 mM glucose although aberations in hypothalamic slices at 4 mM suggest that glucose may be limiting.

Using this method it is now possible to measure glucose utilization of brain slices from any brain region under a wide variety of incubation conditions.

339.13

EVIDENCE FOR AN AMILORIDE-SENSITIVE COMPARTMENT IN HIPPOCAMPAL BRAIN SLICES. T.S. Whittingham[1,4,5], C.W. Lin*[2] and J.C. LaManna[3,4,5]. Depts. of Neurosurgery, Biomedical Engineering, Neurology, Neuroscience, Physiology/Biophysics, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

We have previously reported the apparent alkalinization of rat hippocampal slices in control artificial cerebrospinal fluid (ACSF) using Neutral Red (NR) ratio microspectrophotometry (resting pH_i = 7.52, n=50). We have extended these observations using an improved data processing mechanism to record the responses of the NR pH compartment to an acid load in the presence of amiloride analogs.

Hippocampal slices were pre-loaded with 50 µM NR and incubated in HEPES ACSF in an interface chamber. Each slice was trans-illuminated and the absorption spectrum calculated using stored reference spectra and a Gaussian spectral dispersion program to optimize amplitude determinations at 468 and 528 nm wavelengths. The previously determined calibration curve was verified by exposing slices to nigericin and high potassium while altering ACSF pH by known increments.

The alkaline resting pH in slices may result from activation of amiloride-sensitive Na/H antiport. Consequently, slices were acid loaded by exposing them to NH_4Cl, and the time required to recover to resting pH was monitored. The presence of 0.1 mM amiloride or 10 µM 5-Hexamethylene amiloride (Hexa) decreased the recovery slope from 0.12 to 0.8 pH unit/min, while 50 µM Hexa completely blocked pH recovery. These results suggest that amiloride-sensitive Na/H exchange is involved in pH_i regulation in the NR-detected compartment of brain slices.

339.14

INFLUENCE OF LACTATE ON K^+ TRANSPORT IN RAT HIPPOCAMPAL SLICES. E.L. Roberts, Jr. Department of Neurology, University of Miami School of Medicine, Miami, FL 33101

In this study, the contribution of glycolysis to K^+ transport in hippocampal slices following the elevation of extracellular K^+ (K^+_o) by electrical stimulation was examined. It was hypothesized that K^+ transport would be faster if cellular ATP demands were met by both oxidative phosphorylation and glycolysis rather than by oxidative phosphorylation alone. This hypothesis was tested in hippocampal slices from male Fischer 344 rats of age six months. Slices were first exposed for 45-60 min. to an ACSF containing 10 mM glucose. K^+_o was then recorded in stratum pyramidale of hippocampal subfield CA1 before, during, and after transsynaptic activation of pyramidal cells in the stratum by 40 Hz stimulus trains lasting two seconds. Intensity of stimulation was varied so that a range of values for maximum K^+_o during a stimulus train was obtained. The slices were next exposed for 45-60 min. to glucose-containing ACSF, or to an ACSF containing 20 mM sodium lactate and no glucose. 20 mM sodium lactate was used because it potentially provided as much substrate as 10 mM glucose for oxidative phosphorylation. Slices were again stimulated at 40 Hz, and K^+_o changes recorded. K^+_o was essentially unchanged between glucose- and lactate- containing ACSF. Decay of K^+_o to its original baseline following its elevation by high frequency stimulation was unaffected by time of exposure to 10 mM glucose ACSF. However, the half-life for decay of K^+_o to baseline in lactate-containing ACSF was approximately 60% longer than in glucose-containing ACSF. These results imply that energy demands for K^+ transport can be met by oxidative phosphorylation during resting (unstimulated) conditions, but are best met by a combination of glycolysis and oxidative phosphorylation following high frequency stimulation. (Supported in part by NIA grant AG08710)

339.15

TEMPORAL RELATIONSHIP BETWEEN 2-DEOXY-D-[1-^{3}H]GLUCOSE UPTAKE AND GLYCOGEN HYDROLYSIS EVOKED BY NORADRENALINE IN PRIMARY CULTURES OF MOUSE ASTROCYTES. N. Yu*, J.-L. Martin and P. J. Magistretti. Institut de Physiologie, Faculté de Médecine, Université de Lausanne, CH-1005 Lausanne, Switzerland.

Noradrenaline (NA), like other neurotransmitters such as Vasoactive Intestinal Peptide and adenosine, promotes the hydrolysis of glycogen in cultured astroglia. Recently we have observed that NA also promotes the uptake of 2-deoxy-D-[1-^{3}H]glucose (2-DG) in the same cell type. In the experimental conditions used (namely absence of serum and 5 mM glucose in the medium) basal 2-DG uptake by astrocytes corresponds to 3.5 ± 0.3 nmol/mg prot/min of glucose; NA increases in a concentration-dependent manner 2-DG uptake, with an EC_{50} of 0.5 µM and a maximal effect of 2-fold increase over basal level at 10 µM. As a comparison, NA-stimulated glycogen hydrolysis occurs at an initial rate of 5 nmol/mg prot/min.The effect of NA is mimicked by isoproterenol and inhibited by atenolol, indicating that, like for its glycogenolytic action, NA acts on β-adrenergic receptors to promote 2-DG uptake into astrocytes. However, a time lag of ≈ 5 min between NA application and stimulation of 2-DG uptake is observed; the maximal effect of NA is reached within 20 min. This is in striking contrast to the rapid glycogenolytic action of NA which reaches 50 % of its maximal value within 5 min. Furthermore, this time lag is influenced by the glycogen content of the cultures; thus, high (> 200 nmol/mg prot; normal 10-40 nmol/mg prot) glycogen further delays the effect of NA on 2-DG uptake. These results suggest that NA-induced glycogenolysis is an early event and that 2-DG uptake stimulation is revealed once glycogen stores have been depleted.

339.16

OVERINDUCTION OF GLYCOGEN RESYNTHESIS FOLLOWING GLYCOGENOLYSIS EVOKED BY VASOACTIVE INTESTINAL PEPTIDE (VIP) IN CULTURED ASTROCYTES. O. Sorg* and P.J. Magistretti. Institut de Physiologie, Faculté de Médecine, Université de Lausanne, CH-1005 Lausanne, Switzerland.

We have previously shown that VIP, noradrenaline (NA) and adenosine (Ado) promote a time- and concentration-dependent hydrolysis of glycogen in primary cultures of mouse cortical astrocytes, with EC_{50} of 3, 20 and 800 nM, respectively. The effect of VIP is mimicked by two related peptides, PHI and secretin, while that of NA is mediated by β-adrenergic receptors, since it is mimicked by isoproterenol and it is antaginzed by pindolol; the effect of Ado is inhibited by theophylline. Dibutyryl cyclic AMP, forskolin, and phorbol dibutyryl ester are also glycogenolytic.

Glycogen synthesis can also be induced by appropriate treatments. Thus, the acute exposure of the cultures to insulin 1 µM for 2 hours increases glycogen levels by 2.5-fold. Furthermore, overinduction of glycogen resynthesis is observed after exposure to glycogenolytic agents. For example, when VIP (1 µM) is applied for 2 minutes and then removed from the medium, two time-dependent phenomena are observed : during the first 30 minutes the well-documented glycogen hydrolysis occurs; this phase is followed by a time-dependent induction of glycogen resynthesis which results, after 9 hours, in glycogen levels that are 10 times higher than those observed before VIP application.

These results are consistent with at least two possible mechanisms : (1) a "rebound" effect following exposure to a glycogenolytic agent or (2) the induction by VIP of glycogen synthase in a population of astrocytes still at an early stage of differentiation.

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HUMAN COGNITION: HEMISPHERIC LATERALIZATION, GENDER DIFFERENCES

340.1

METABOLIC SUBSTRATE OF HUMAN INTEGRATIVE BEHAVIOR: EVIDENCE FROM QUANTITATIVE STEREOTACTIC PET AND MRI FOLLOWING CORPUS CALLOSOTOMY. M.F. Lévesque, L. Lebas*, E.J. Behnke*, J.X. Zhang*, R. Lufkin*. Division of Neurosurgery and Brain Research Institute, UCLA School of Medicine, Los Angeles, California, USA, 90024.

This study was aimed to investigate the relative contribution of subcortical structures to integrative behavior following callosotomy. Stereotactic 18-fluoro-deoxyglucose Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) studies were obtained before and two weeks after callosotomy in two epileptic patients. A standard battery of neuropsychological tests were also obtained followed by tachitoscopic studies. Anatomical regions of interests (ROI) were drawned base on MRI and directly transposed to the identical functional PET image using a muti-modal stereotactic image analysis system (Lévesque M, J Neurosurg, 1990). Average metabolic activity was measured for 40 ROIs per hemisphere including 6 subcortical structures(15 ROIs) for each study.. Statistical differences were found between subcortical and neocortical ROIs that could support their relative role to integrative behavior.

340.2

EVIDENCE FOR SOMESTHETIC MIDLINE FUSION IN AGENESIS OF THE CORPUS CALLOSUM. A. Schiavetto*, M. Lassonde and F.Lepore.Groupe de Recherche en Neuropsychologie Expérimentale, Univ. de Montréal, Qué., Canada.

The aim of this study was to investigate the role of the corpus callosum in midline fusion, in subjects with callosal agenesis. Three agenic subjects and nine control subjects were tested using a two-point discrimination task. Thresholds were established for both index fingers, the palms, the arms, the forehead and the back. Threshold was defined as the smallest separation at which an accuracy level of at least 80% was achieved. Results showed that the acallosal subjects did not have thresholds that were significantly different from normals in any of the distal or para-axial modalities and in the head area. However, all measures in the back (axial) were significantly larger than normals whether in midline or lateral positions, in both the vertical and horizontal axes. It is suggested that although the acallosal subjects can compensate in the distal and paraxial regions of the body and the forehead, they are unable to do so on the trunk. These results can be explained by electrophysiological data obtained in cats and monkeys which show that midline receptive fields of fibers crossing the corpus callosum are quite large. The acallosals' performance reflects the disruption that results from an abnormal callosal organization. Therefore we conclude that the corpus callosum is necessary for midline fusion in somesthesis.

340.3

DEVELOPMENTAL ASPECTS OF INTERHEMISPHERIC TRANSFER OF MOTOR LEARNING. A. J. Chicoine[1]*, L. Proteau[2]* and M. Lassonde[1],. [1]Lab. de Neuropsychologie Expérimentale, [2]Dép. d'éducation physique, Université de Montréal, Canada, H3C 3J7.

Studies addressing the issue of ontogeny of interhemispheric communication have established a parallel between the progressive morphological maturation of the corpus callosum and the concomittant increase in functional communication between the two hemispheres. There is a general consensus that the ability to effect intermanual transfer of tactuo-motor information improves gradually until the age of 11 years, at which time the myelination of the callosal commissure is presumably completed. The present work was undertaken to extend the study of the development of interhemispheric transfer to tasks involving unilateral motor learning. Three groups of right-handed subjects (6-7, 11-12 and 20-30 years of age) were required to make aiming movements from a starting position towards either the middle or the side target ipsilateral to the hand used, while maintaining central fixation. Prior to the learning phase, a pre-test was administered to determine the baseline performance of all subjects. Once the training was completed the test was repeated. In each group, half the subjects performed the pre- and post-tests with the right hand and the intermediate acquisition phase with the left and the others followed the opposite hand sequence. The results on the acquisition phase indicated that all subjects learned the aiming task as demonstrated by a reduction in spatial errors with an increasing amount of practice trials. Comparisons between performance on pre- and post-tests indicated a significant improvement of performance with learning attributable to an increase in interhemispheric transfer. This effect, however, was observed only in the 11-12 and adult groups, the 6-7 year old children showing no improvement in aiming accuracy on the post-test. The findings of the present study concur with previous works on the development of interhemispheric transfer in normal children and extend the range of information that are gradually transferred between the hemispheres to tasks involving motor learning.

340.4

ELECTROPHYSIOLOGICAL MEASURES OF INTERHEMISPHERIC TRANSFER OF VISUAL INFORMATION: STUDIES IN SPLIT-BRAIN PATIENTS. G.R. Mangun, S.J. Luck, M.S. Gazzaniga & S.A. Hillyard. Dartmouth Medical School, Hanover, NH 03756 & Univ. of California, San Diego, La Jolla, CA 92193

Event-related potential (ERP) studies in healthy human subjects have identified a series components that reflect the processing of visual information in the visual cortices. The well studied P1 and N1 components elicited by unilateral flash stimuli can be recorded from occipital electrodes located over both the contralateral and ipsilateral hemispheres. Previous research by Rugg et al. (1985) has shown that in callosal agenesis patients, the ipsilateral N1 component was absent, supporting the hypothesis that this response was mediated by callosal activation of the ipsilateral hemisphere. The present study uses a similar logic in split-brain patients to investigate the neural source(s) of the P1 and N1 components recorded from over the ipsilateral hemisphere.

Visual ERPs were recorded from 30 scalp locations in four patients that had undergone complete section of the corpus callosum for the treatment of intractable epilepsy. Stimuli consisted of small bars flashed one at a time to the four quadrants of the visual field. Brain responses to the different stimuli were plotted as CSD topographic maps in order to localize the responses to the activated hemisphere.

The lateralized flash stimuli elicited P1 and N1 peaks over the contralateral occipital cortex in the patients. In contrast to normal controls, no activity could be observed over the hemisphere ipsilateral to the stimulated visual field in the split-brain patients. These data support the idea that these early ERP components over the ipsilateral hemisphere of normal subjects reflect the callosally mediated activation of the ipsilateral hemisphere following the initial registration of the visual information in the contralateral occipital cortex. Moreover, they provide functional measures of interhemispheric transfer of visual information in the split-brain patients that can be related to MR verification of callosotomy. Supported by NINDS 2P01 NS17778-09 and NIMH 1 K21 MH00939-01.

340.5

EFFECTS OF VIOLATIONS OF A FACE SCHEMA IN THE LEFT AND RIGHT HEMISPHERES OF SPLIT-BRAIN PATIENTS AND NORMAL SUBJECTS. Dahlia W. Zaidel. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Face perception is not a unitary function. The human face is perceived or remembered by either the left or the right hemispheres of the brain, and given hemispheric specialization this suggests that the rules that make a face, a face, are different in each hemisphere. Here we studied the role that three structural parameters of the face play in face perception in each hemisphere of complete commissurotomy patients and normal subjects. These parameters are, contour frame, individual features (e.g., lips,nose), and spatial juxtaposition of these features. The hemi-field technique was used to lateralize the stimuli to the left or right visual half-field. They consisted of line-drawings of normal faces as well as of face-like arrays with systematic violations of the parameters. The task was to match an isolated facial feature (e.g., lips) with its equivelant positioned within a face or face like array. The data showed both functional symmetries and asymmetries depending on the nature of the violation or its absence, with the number of violations that could be tolerated being smaller in the right hemisphere than in the left.

340.6

LATERALITY EFFECTS IN THE PROCESSING OF HIGH AND LOW FREQUENCY AUDITORY STIMULI. P.C. Lebby, R.B. Ivry, L.C. Robertson and A.P. Shimamura*. Department of Psychology, University of California, Berkeley, CA. 94720.

The present study investigated laterality effects in the processing of auditory stimuli. In visual perception experiments, a right hemisphere advantage has been found for processing low spatial frequencies and a left hemisphere advantage for high frequencies. The present study examined whether a similar asymmetry exists in the perception of auditory stimuli. Subjects were presented monoaurally with duplex tones consisting of a high and low frequency component. On different blocks, the frequency of one of the two components was adjusted lower or higher. Subjects judged whether the target component was higher or lower in frequency. Stimuli presented to the left ear/right hemisphere were judged more accurately when the target tone was lower in frequency. In contrast, stimuli presented to the right ear/left hemisphere were judged more accurately when the target tone was higher in frequency. Additional studies using patients with right or left temporal-parietal lobe damage are being conducted.

340.7

TIMBRE PERCEPTION AFTER UNILATERAL TEMPORAL LOBECTOMY IN HUMAN. S. Samson & R.J. Zatorre. Montreal Neurological Inst., McGill Univ. Canada H3A 2B4.

Timbre is a psychological quality associated with spectral shape as well as onset and offset characteristics of sounds. Based on prior research, we hypothesized that the right temporal lobe should be predominantly involved in discriminating spectral cues but little is known regarding the processing of temporal cues. The ability to discriminate different timbres was tested in patients with anterior right (RT) and left (LT) temporal lobectomy as well as in normal control (NC) subjects. The timbre discrimination task was composed of pairs of complex tones in which spectral and temporal information was manipulated independently. The digitized stimuli differed either by the number of harmonics (which alters the spectral cue) or by the duration of the attack (affecting temporal cue). The results showed that RT patients were impaired as compared to LT and NC subjects in discriminating both types of information, suggesting the importance of RT auditory cortex in perceiving timbre. Possible differences in processing spectral vs temporal information will be discussed.

340.8

LATERALIZATION OF FRONTAL LOBE FUNCTIONS IN HUMAN MALES. K.Podell*, E.Goldberg*, R.Harner and S.Riggio*. Med. Coll. of PA, Phila, PA 19129

Prefrontal systems (PF) are critical in guiding behavior by internal representations (set maintenance-SM), and in flexible responses to environmental contingencies (set shifting-SS). We propose that SM is lateralized to the left and SS to the right PF. We developed a novel task to measure cognitive bias along the SM-SS continuum. A geometric form is presented as a target and a choice of one out of two other forms is made. Cognitive distances between targets and choices are quantified and summed across trials. Low total score means that choices are determined by target representations (SM); high score that they are determined by external multiple choice properties (SS). The task was given to right-handed males with left (N=5) and right (N=6) PF lesions (LPFL and RPFL), and 22 matched healthy controls (H). LPFL had high, RPFL low, and H intermediate scores, confirming the hypothesis. Difference between LPFL and RPFL scores was significant ($t=25$, $p<.001$). Variances were small in LPFL ($F=26.7$, $p=.006$) and RPFL ($F=49$, $p<.001$) relative to H, indicating strong lesion effects.

340.9

INTRASUBJECT CORRELATIONS OF OCCIPITAL ASYMMETRY MEASUREMENTS BASED ON CT AND MRI. C-C. Chu,* H. Damasio, & D. Tranel. Division of Cognitive Neuroscience, University of Iowa College of Medicine, Iowa City, IA 52242.

In vivo occipital asymmetry (OA) measurements have been used to infer functional asymmetries (e.g., cerebral dominance), but their intrasubject reliability has not been documented. We studied correlations between CT:CT, MR:CT, and MR:MR, using OA measurements derived by the same rater from different scans of the same subject. In MR, the effect of incidence was also studied. OA was calculated by summing *Z*-scores of occipital width, occipital petalia, and straight sinus deviation, after we found the three measures to be relatively independent. MR:CT correlations were calculated in subjects with either 0^{0} ($n=50$) or negative (minus 10 to minus 15^{0}; $n=20$) MR incidence; CT:CT correlations were calculated for 25 of these subjects; and MR:MR correlations were calculated for 20 additional subjects. The highest intrasubject correlation was for MR:MR ($r=.79$). The CT:CT value was similar ($r=.76$). CT:MR correlated nearly at the same level ($r=.71$), but only for the zero-incidence group; the correlation was small ($r=.27$) for the negative-incidence group. The findings indicate that the reliability of occipital asymmetry measurements is modest at best, setting a fairly low ceiling on the extent to which this variable can be used validly to infer other indices. OA measures based on zero-incidence MR have the best reliability.

340.10

CEREBRAL DOMINANCE AND GENDER EFFECTS ON NEUROPSYCHOLOGICAL TEST PERFORMANCE AFTER BRAIN INJURY. Elaine MacNiven and M. Alan J. Finlayson*, Chedoke-McMaster Hospitals, Hamilton, Ontario, Canada. L8N 3Z5

Differences in cognitive functioning associated with sex and hand prefer-ence are now well known (Springer & Deutsch, 1981). These differences are consistent from early in development (Witelson, 1987) and may relate to neuroanatomical asymmetries in the corpus callosum (de Lacoste-Utamsing & Holloway, 1982; Witelson, 1989). The corpus callosum is known to be macro-scopically damaged by the acceleration-deceleration forces characteristic of Closed Head Injury (CHI), with little imbalance in focal tissue damage in either hemisphere (Adams, Mitchell, Graham & Doyle, 1977; Strich, 1956). Thus, it is reasonable to inquire whether gender and hand preference have an influence on cognitive performance and recovery following brain injury.

We studied a sample of 59 CHI patients who had received an initial post-injury assessment and who had been followed after one year. Neuropsycho-logical tests of verbal, spatial, mathematical and reasoning ability and emotional functioning revealed a number of significant differences between sexes and hand preference groups. Furthermore, there were differences in the extent of recovery attained on follow-up. Of particular interest, there was no difference between the performance of right and left-handers on a reasoning test given shortly post-injury. However, on follow-up, the left-handed patients made significantly fewer errors, suggesting that their problem solving ability had recovered to a greater degree. These results suggest that CHI results in differential levels of deficit contingent on neuroanatomical and neurophysiological disparities related to gender and cerebral dominance.

340.11

SEX DIFFERENCES IN SPATIAL NEGLECT. B.Laeng* and C.M.Butter. Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI 48108.

The greater dependence of spatially-directed attention on the right as opposed to the left cerebral hemisphere may be more characteristic of the male than the female brain. This hypothesis is suggested by Kimura's findings that the left parietal lobe of women, compared to that of men, is less committed to language and praxis. To investigate this hypothesis, we tested 22 women and 21 men with infarcts involving the right parietal lobe in a line cancellation task sensitive to unilateral spatial neglect. As predicted, left neglect was less severe in the women than in the men; this effect was not due to group differences in age, extent of lesion or other factors known to affect neglect. A smaller group of patients with unilateral lesions in either parietal lobe was tested with the Weintraub & Mesulam search test. Again, among patients with right-sided lesions, the women tended to be less impaired than the men, whereas the reverse trend - also predicted by our hypothesis - appeared in the left hemisphere patients.

340.12

TESTOSTERONE ADMINISTRATION ENHANCES SPATIAL COGNITION IN OLDER MEN. J.S. Janowsky, S.K. Oviatt, J.S. Carpenter, E.S. Orwoll. Dept of Psychology, Univ. of Oregon, Eugene, OR 97403 and Dept. of Endocrinology, Oregon Health Sciences Univ., Portland, OR 97201.

Recent studies have shown that spatial cognition and motor speed change across the menstrual cycle in relation to changes in estrogen (Hampson and Kimura 1988). The role of testosterone (T) in higher order cognition is not clear. Therefore, in a double blind study we assessed cognition in 40 men (mean age 68.4 years) before T administration and again after three months during which half the subjects wore T and half placebo scrotal patches. Peak T levels increased by a mean of 150% of baseline in those men who wore T patches. Whereas, T administration did not effect verbal or visual memory, cognitive flexibility, or motor speed, a significant enhancement of spatial cognition was found on the Block Design subtest of the WAIS-R. These results support the possibility that spatial cognition is enhanced by hormonal masculinization of the brain and suggests that T may play a role in maintaining sexually dimorphic cognitive specializations.

340.13

SEX DIFFERENCES IN COGNITIVE FUNCTION VARY WITH THE SEASON. D. Kimura and C. Toussaint*. Dept. Psychology. Univ. Western Ont., London, Canada

In young adults there appears to be a non-linear relation between androgen levels and spatial ability, with an optimum androgen level somewhere above the female average and below the male average. Testosterone levels are also known to be higher in human males in autumn than in spring. Since cognitive patterns vary with monthly hormone fluctuations in women, this study investigated the possibility of seasonal fluctuations in men, with spatial skills expected to be better in spring.

Three types of cognitive tests were given in either the fall or the spring to groups of male and female undergraduates, well matched for age and study program: neutral tests, tests favoring females, & tests favoring males (spatial). Typical overall sex differences were found on non-neutral tests. In addition, there was a sex X season X test-type interaction, such that on the spatial composite, men in spring performed better than in fall; and men and women did not differ significantly in fall. Female-favoring tests tended to show the reverse pattern, but there was no effect on neutral tests. Cognitive pattern appears to be biologically dynamic.

340.14

TACTILE SPATIAL TASK UNMASKS PERFORMANCE DIFFERENCES DUE TO SEX AND LEARNING DISABILITIES. J. Thomas, R. Pariser*, S. Turco*, and N. Gaudin*. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148.

The ability of a tactile task to detect differences in spatial performance related to sex, development, or possible brain dysfunction, were studied in students classified as learning impaired (LI) or unimpaired (UI). The task used required subjects to trace a nonsense shape that was hidden from their view and then to identify a picture of the shape from an array of three pictures. The results revealed that UI males made fewer errors than UI females, while LI males made more errors than both the UI males and the UI females. The error scores of the LI males and females, however, were similar to error scores of UI females. The identification of performance differences on this task among groups of males and females with and without LI suggests that this tactile spatial task may be useful for studying how different liabilities for learning disorders develop in males and females.

LEARNING AND MEMORY—ANATOMY V

341.1

DOES A NINE SECOND INTERTRIAL INTERVAL SUPPORT LEARNING IN THE INTACT RABBIT? A.F. Nordholm, D.G. Lavond, and R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Recently Kelly et. al. (1990) reported conditioning in the decerebrate/decerebellate rabbit using a 9 sec intertrial interval (ITI) and a single training session. The purpose of the present study was to determine if conditioning using this short ITI occurs in the intact animal. Group 1 rabbits were trained according to the standard convention (i.e., no pretraining exposure to conditioning stimuli and a 30 second ITI). Group 2 received training similar to that described in the Kelly paper (i.e., 100 pretraining trials of unpaired stimuli followed by conditioning with a 9 sec ITI and a 500 Hz tone CS). Group 3 was treated identically to group 2 except that a 1000 Hz tone served as the CS. Group 4 was identical to group 3 but was given no unpaired stimulus pretraining. Our results indicated that only group 1 exhibited learning across trials ($F=4.71$, $p<.01$). Groups 2-4, in fact, showed no signs of conditioning. This indicated that conditioning using a nine second ITI in a single session does not occur in the intact animal.

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341.2

KAINIC ACID LESIONS OF N. INTERPOSITUS ABOLISH RETENTION OF CLASSICAL CONDITIONING IN RABBITS WHEN HVI STIMULATION IS THE US. R.A. Swain, P.G. Shinkman, & R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Previous experiments have shown that cerebellar HVI stimulation elicits a variety of behavioral responses which can be reliably classically conditioned to a tone CS (Brogden & Gantt, 1937, 1942; Shinkman, Swain, & Thompson, 1989). Such responses often include eyeblink, head movement or lip extension. The present experiment was designed to study the role of n. interpositus (IP) in this effect. New Zealand White rabbits (*Oryctolagus cuniculus*) were chronically implanted with bipolar stimulating electrodes in the white matter immediately underlying left lobule HVI. Cannulae were implanted approximately 1 mm above IP in each hemisphere. Following 1 week of recovery, the animals were trained using paired tone and HVI stimulation. One session past criterion performance, the rabbits received either left IP or bilateral IP injections of kainic acid (5 nmoles) depending on the nature of the response. These lesions produced abolition of both the CR and UR that persisted for the 5 days of postlesion training. These results provide additional evidence for the critical role of IP in classical conditioning of discrete motor movements. (Supported by NSF BNS8718300, ONR N0001488K0112, & McKnight to R.F. Thompson, and by Brain and Development Res. Ctr., University of North Carolina.)

341.3

LESIONS OF CEREBELLAR CORTEX IMPAIR DISCRIMINATION DURING PAVLOVIAN CONDITIONED INHIBITION OF THE RABBIT NICTITATING MEMBRANE RESPONSE. C. G. Logan & R. F. Thompson, Neurosciences Program, USC, Los Angeles, CA 90089-2520.

The role of cerebellar cortex vs. the deep cerebellar nuclei in eyeblink conditioning has been controversial. While some (Yeo et al., 1985) have reported that lesions of lobule HVI abolish a previously conditioned response, others (Lavond et al., 1987; Lavond & Steinmetz, 1989) have found that such lesions result in partial relearning of the response and severely impair, but do not prevent, acquisition in naive animals. The present study was designed to determine whether cerebellar cortex is essential for inhibitory conditioning by using a Pavlovian conditioned inhibition paradigm.

Rabbits were first trained to a light CS, then lesioned in crus I, crus II, and HVI and retrained to the light CS. While a control group relearned the CR almost immediately, the lesioned group required as long to relearn as for initial learning. These data support the idea that cerebellar cortex is involved but not essential for simple excitatory conditioning.

Following reacquisition to light, Pavlovian conditioned inhibition training consisting of differential conditioning with light as the CS^+ and a tone-light compound as the CS^- followed. The control group showed a significant effect of CS type over the 20 days of training in contrast to the lesioned group which responded at the same levels to both CSs throughout. Difference scores and relative inhibition ratios, both averaged over the last 5 days of conditioning, showed a significant difference between groups. Three other rabbits with similar lesions were given discrimination training with light as the CS^+ and tone as the CS^-. All were able to discriminate between the 2 CSs, indicating that the failure at conditioned inhibition was not merely due to an inability to discriminate between the tone and light stimuli. Rather, a role for cerebellar cortex in inhibitory conditioning is suggested. Supported by the McKnight Foundation, ONR N00014-88-K0112 and NSF BNS-8718300 to RFT.

341.4

Effects of Cerebellar Cortical Lesions on Extinction and Reacquisition of NM Conditioning in Rabbits. C. Weiss, C.G. Logan and R. F. Thompson, Program in Neuroscience, University of Southern California, Los Angeles, CA. 90089.

Lesions of the cerebellar cortex significantly retard acquisition of nictitating membrane (NM) conditioning. The present study examines the effects of such lesions on extinction and reacquisition of conditioned responses (CRs).

Rabbits with aspiration lesions aimed at HVI (n=5) and nonlesioned, but operated, controls (n=6) were run. Following one week of recovery, daily tone-airpuff conditioning sessions (108 trials/day) were given followed by tone alone extinction and then reacquisition. Criterion for acquisition and reacquisition was set as the 2nd day of $\geq$70% CRs ($\geq$0.5mm NM extension); criterion for extinction was set as the 2nd day of tone alone trials with $\leq$30% CRs.

Our results confirm that the lesion retards acquisition. They also indicate that there is no effect of the lesion on sessions to criterion for extinction or reacquisition. During initial acquisition the lesioned group exhibited an initial period of low %CRs, but then learned almost as fast as controls. On reacquisition the lesioned rabbits had fewer CRs, but reached criterion in a similar number of sessions. The latency of CRs (when they occurred) was similar between groups on acquisition and reacquisition, but during extinction, the lesioned animals had longer latencies. The amplitude of the CRs was greater in the control rabbits during most of the experiment, but especially during the initial days of extinction. On reacquisition the amplitude returned to preextinction levels for the lesioned group and increased further for the control group.

These results suggest that once the CR pattern is established it can be normally extinguished and reacquired without the cerebellar cortex. Thus, extinction and reacquisition are likely to result from modification of the existing motor program rather than creation of a new motor program. These data also suggest that the delay in initial acquisition may be due to an increase in the time for phase 1 learning. Support: BNS-8718300, ONR N0001488K0112, & McKnight to R.F.T.

341.5

ACCESSORY ABDUCENS LESIONS PRODUCE PERFORMANCE DEFICITS WITHOUT PERMANENTLY AFFECTING CONDITIONED RESPONSES. D. Ivkovich, C.G. Logan, & R.F. Thompson, Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Rabbits were classically conditioned with a 3 psi unconditioned stimulus (US) to the cornea, lesioned in the accessory abducens nucleus, and subsequently retrained. Reflexive eyeblinks to 4 different US intensity levels (1,2,3,& 4 psi) were measured over the course of training.

Lesions severely impaired all eyeblink responses initially. Conditioned responses recovered to pre-lesion levels within 3-20 days following the lesion. Extent of the lesion determined recovery time. Extensive lesions prevented full recovery of the UR up to 30 days following the lesion. These findings indicate that performance of the CR is less sensitive to direct impairment of performance than is the UR itself and, therefore, argue against the notion that cerebellar lesions selectively abolish the CR because of hypothetical effects on "performance."

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341.6

EFFECTS OF REVERSIBLE LESION OF RETICULAR OR FACIAL NEURONS DURING EYEBLINK CONDITIONING. A.A. Zhang & D.G. Lavond. Neuroscience Program, University of Southern California, Los Angels, CA 90089-2520.

The cerebellum is the most likely site for neural plasticity associated with classical eyeblink conditioning. Alternatively, the reticular formation (Ri, neurons adjacent to the caudal portion of the pars oralis of the trigeminal sensory complex or facial motor nucleus (FMN) may be essential sites for eyeblink conditioning.

Rabbits were implanted with a probe near the RF or FMN. The probe can be cooled at the tip by injecting Freon and heating the shaft. After 1 week recovery from surgery we cooled the probe while training 5 days for classical conditioning (108 trials per day; tone conditioned stimulus; airpuff unconditioned stimulus) and recorded eyelid EMG activity for behavioral measurement.

The animals with RF cooling showed conditioned responses (CRs) on the second day of acquisition training. This suggests that the RF is not an essential locus for eyeblink conditioning.

However, in the FMN group, the animals did not show any CRs during training with cooling. However, with subsequent normal training without cooling, the animals showed CRs immediately. This suggests that the FMN can not be the site of the engram, but could be essential output for eyeblink conditioning.

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341.7

Associative Eyeblink Conditioning, but not Unpaired Conditioning, Causes Morphological Changes in Purkinje Spiny Branchlets. B. Anderson, K. Relucio*, C. Mohr*, C. Logan‡, B. Knowlton†, F. Davis*, A. Hawkins*, J. Thompson†, J. Steinmetz*, R. Thompson†, & W. Greenough. Dept. of Psych., Univ. of Ill., Urbana, IL, †Neur. Inform. Behav. Sci., Univ. South. Calif., Los Angeles, CA, *Dept. of Psych., Indiana Univ., Bloomington, IN.

Purkinje cells exhibit nearly simultaneous CR-related physiological activity with the interpositus nucleus during eyeblink conditioning (McCormick & Thompson, 1984; Berthier & Moore, 1986). Rabbits with lesions of lobule HVI have impairments in CR rate and amplitude (Lavond & Steinmetz, 1989). Although lobule HVI may not be essential for eyeblink conditioning, the physiological activity is indicative of a memory trace.

Previous studies have shown morphological change in the cerebellar Purkinje cells following environmental enrichment and motor learning (Floeter & Greenough, 1979; Black, et al., 1990). The eyeblink paradigm is ideal to test whether morphological changes are related to learning or to neuronal activation arising from CS and US exposure. Conditioned rabbits were trained unilaterally using pontine stimulation (350 ms) as CS paired with a coterminating corneal air puff (100 ms) as US. Unpaired rabbits received the same number of stimulation and airpuff presentations, but at random intervals varying from 1 to 32 sec. The contralateral hemispheres served as the controls for both groups. The branching pattern of spiny branchlets, sites of parallel fiber input, of lobule HVI Purkinje cells were analyzed. Significantly fewer branches were seen at order 5 & 6 in the hemisphere receiving paired conditioning trials than in the contralateral untrained hemisphere. Overall, the paired hemisphere had 15% fewer spiny branches ($p<.05$). There were no differences in branch numbers between hemispheres in the unpaired animals. These results suggest that morphological plasticity in the cerebellum is strictly related to conditions that cause learning.

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341.8

A Model of Eyelid Conditioning Based on the Cerebellum. M.D. Mauk and N.H. Donegan. Dept. of Neurobiology and Anat., Univ. of Texas Med. Sch., Houston, TX 77225 and Dept. of Psych., Yale Univ., New Haven CT 06510.

We present a model of Pavlovianly conditioned motor responses (CRs) that is inspired by the extensive characterization of the synaptic organization and physiology of the cerebellum. At the brain system level the model consists of a cerebellar and brain-stem network thought to be responsible for the development and expression of CRs. At a cellular level the model specifies sites of plasticity and the rules for inducing plasticity that allow the larger network to generate a diverse range of important phenomena of Pavlovian conditioning.

Synaptic plasticity is assumed to occur in both the cerebellar cortex (granule to Purkinje synapses) and in the cerebellar nuclei (mossy fiber synapses). Plasticity in the cortex is assumed to take the form of heterosynaptic long-term depression (LTD) induced by coactivation of granule cell and climbing fiber inputs. In the nuclei plasticity is assumed to be Hebbian, with a heterosynaptic modulatory influence from Purkinje cell inhibition. Importantly, the induction and maintenance of the cortical plasticity is assumed to be influenced by the inhibition of climbing fiber inputs by cerebellar output. This feature is critical for explaining sustained asymptotic performance, extinction, blocking, and conditioned inhibition.

This model particularly emphasizes the cerebellar mechanisms that might account for inter-stimulus-interval (ISI) functions and for the timing of CRs. The proposed timing mechanism is an elaboration of previous suggestions that stimuli are discriminated on the basis of the different subsets of granule cells they activate and that Golgi cell inhibition of granule cells improves this discrimination (e.g. Marr, J. Physiol. 202, 1969). We extend this thinking by assuming that such mechanisms allow discrimination among temporal components of a single stimulus. Since Golgi cells are excited by mossy fibers and by granule cells, we propose Golgi feedback inhibition permits the activation of different subsets of granule cells at different times during a tonic stimulus. Thus, the subset of granule cells that is active may encode not only a particular stimulus but also a specific time during that stimulus. This, combined with the LTD-like plasticity, can provide an appropriately timed disinhibition of nuclei cells. These assumptions, plus those regarding the influence of noise on granule cell activation, allow an account for ISI functions and for the timing of conditioned responses.

341.9

NEURAL NETWORK BASED ON THE CIRCUITRY OF THE CEREBELLUM SIMULATES THE TIMING OF MOTOR RESPONSES. D.V. Buonomano and M.D. Mauk. Dept. of Neurobiology and Anat., Univ. of Texas Med. School, Houston, TX 77225.

An important aspect of motor output is its timing in relation to sensory stimuli or in relation to other components of a movement. A simple example is the learned, adaptive timing displayed by conditioned eyelid responses. An effective US can follow CS onset by 80-2000 ms, within this interval the CR will peak near US onset.

We present a neural network based on the synaptic organization of the cerebellum that generates properly timed responses after the onset of a stimulus. Contrary to previous models addressing this issue, there are no delay lines, elements with a spectrum of time constants, or periodically oscillating elements. This neural network is based on a conceptual model (see previous abstract) in which different times during a stimulus are encoded as population vectors of active granule cells.

The network consists of 10000 granule units (GrU), 900 Golgi units (GoU) and 500 mossy fiber units (MFU). Each GrU is simulated as an integrate and fire element with excitatory (from MFU) and inhibitory (from GoU) synaptic conductances, as well as a leak conductance. GoU are similarly modeled except they receive excitatory synaptic inputs from the GrUs and MFUs. A stimulus is represented by activity in a subset of the MFUs. This activity is constant and thus contains no temporal information. At the onset of a stimulus a particular subset of GrUs becomes active depending on the convergence of excitatory input from MFUs and on the activity of the population of GoUs. Since the active GrUs influence subsequent GoU activity the negative feedback between the GoU and GrU results in a dynamic population vector of active GrUs. Thus, temporal discrimination occurs because different subsets of GrUs are active at different times during a stimulus. Due to local differences in connectivity the feedback between GoUs and GrUs varies throughout the network. This provides symmetry breaking and prevents the network from oscillating. All GrUs connect to a Purkinje unit (PU), initially with identical synaptic weights. By decreasing weights of the synapses active during a US, subsequent PU activity decreases to the extent that ongoing GrU subsets are similar to the reinforced GrU subsets. This decrease in PU activity peaks at, and anticipates the target time thus mimicking the timing of a conditioned eyelid response. This model demonstrates that a network based on cerebellar circuitry can generate time specific responses by encoding time as a population vector of active GrUs.

341.10

CEREBELLAR CORTEX ABLATION DISRUPTS EXTINCTION OF CONDITIONED EYELID RESPONSES. S.P. Perrett, B.P. Ruiz*, and M.D. Mauk. Department of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

An inherent difficulty in examining the role of the cerebellar cortex in classical conditioning of motor responses arises from the degeneration of climbing fibers that results from cerebellar cortex lesions. Since climbing fibers are necessary for acquisition of conditioned responses (CRs) it is difficult to determine the contribution of the cortex to acquisition once a lesion has been made. Because extinction of CRs occurs in the absence of climbing fiber input, it is possible to investigate the role of the cerebellar cortex in extinction using cortical lesions. We report that cerebellar cortex ablation disrupts extinction of the classically conditioned eyelid response in rabbits.

Following 10 daily training sessions (109 trials each) using standard eyelid conditioning procedures, rabbits receive aspiration lesions of the ipsilateral cerebellar cortex (paramedian and ansiform lobules and the anterior lobe). Animals are then subjected to 2 additional sessions employing pre-lesion training parameters to assess the effect of the lesion on CRs. Based on previous findings (Perrett et al, **Neurosci Abstr**.115.1 1990) we define a lesion as effective when the CRs display extremely short latencies to onset and to peak. Rabbits retaining pre-lesion timing are considered controls. Rabbits are then subjected to 10 sessions of extinction training in which they receive only the conditioned stimulus. We find that effective lesions produce profound deficits in CR extinction compared to controls. While CRs are virtually extinguished by the third session in control animals, with effective lesions we observe an 80% response rate with little dimunition in CR amplitude during this session. Rabbits with effective lesions retain a 50% response rate during the tenth day of extinction, though with some reduction in amplitude.

These data provide evidence that the cerebellar cortex is required for extinction of CRs and support the hypothesis that it contributes to motor learning. These data further support the notion that acquisition and extinction of CRs involve synaptic plasticity in both the cerebellar cortex and deep nuclei, and suggest that the plasticity in the nuclei requires input from and possibly plasticity in the cerebellar cortex. We hypothesize that extinction is mediated by a decrease in strength of mossy fiber/deep nuclei synapses which is driven by Purkinje cell activity resulting from an increase in strength of granule cell/Purkinje cell synapses.

341.11

DEEP CEREBELLAR LESIONS DO NOT AFFECT THE DEVELOPMENT OF CLASSICALLY CONDITIONED JAW MOVEMENT RESPONSES IN RABBITS. C.M. Gibbs, K.L. Watson*, A.W. Gibbs* & B.M. Hester*, VA Med. Center & Univ. of South Carolina, Columbia, SC 29201.

Repeated pairings of tone (CS) with an intraoral pulse of water (US) lead to the development of learned masticatory responses (JM CRs) in rabbits. In light of current models of somatomotor learning, we sought to assess the effects of deep cerebellar lesions upon JM conditioning.

The studies included 6 New Zealand albino rabbits with extensive bilateral lesions of either the anterolateral interpositus n. or the brachium conjunctivum; another 7 animals served as sham-operated controls. Following post-operative recovery and the imposition of a 1h/day water--access regimen, animals received appetitive conditioning (1216-Hz CS; 2-s CS/US interval), followed by extinction training. Both lesion and control animals showed rapid acquisition of robust JM CRs, as well as their extinction; data from 5 additional control animals receiving unpaired CS/US presentations confirmed the associative character of the JM CRs. In contrast, lesion animals failed to acquire eyeblink CRs during subsequent aversive conditioning (304-Hz CS; eye-shock US; 0.5-s CS/US interval). Thus, the present data suggest that the lateral cerebellum is not an essential substrate for the development/expression of JM CRs associated with deglutition, a response constellation that transcends simple reflex mechanisms. (Supported by VA Institutional Research funds & NSF Grant BNS 88-20379.)

341.12

CORTICAL INHIBITION IN PAVLOVIAN CONDITIONING. I. Steele Russell, Anatomy, Texas A&M University, College Station, TX 77843.

The present experiment re-examined the role of the cerebral cortex in Pavlovian conditioning. Normal rabbits were tested for visual interocular transfer (IOT) using NM conditioning to a light stimulus (CS). They were first trained monocularly to criterion. Following this they were trained with the other eye. No signs of IOT were seen in any animal. All animals had to reacquire CRs from zero. This suggests that the normal rabbit can be used as a "functional split-brain" animal. Accordingly, a small group of unilateral visual cortex lesioned animals had the visual input, via the crossed optic fibres into the intact hemisphere; and the other half had the input into the damaged hemisphere. In rabbit, the visual control of the NM response is solely via the crossed fibre system. The ipsilateral projections are incapable of supporting this response due to the lack of connections with the NOT and other oculomotor control centres (Russell et al, *Exp. Brain Res.*, 1987). The results showed that learning was retarded using the eye that projected into the intact hemisphere. In contrast, the learning was superior when using the eye that projected into the damaged hemisphere. There acquisition was significantly faster either with respect to the other eye or to monocular learning in normal control animals. This finding of faster NM conditioning in the decorticate hemisphere indicates the possibility of cortical inhibitory control over brainstem oculomotor systems. This is strong evidence for an important inhibitory role for the cerebral cortex in Pavlovian conditioning. The view that the cerebral cortex plays no role in simple associative learning can be no longer sustained. The contribution of the cerebral cortex was missed because earlier experiments were looking for deficits. The possibility of any improvement due to removal of cortical inhibition was never considered.

341.13

ASSOCIATIVE EYEBLINK CONDITIONING IN THE INFANT RAT. J.H. Freeman[1], M.E. Stanton[1,2], and R.W. Skelton[3]. [1]Psych. Dept., UNC-Chapel Hill, NC 27514; [2]Neurotox. Div., US EPA, RTP, NC, 27711; [3]Psych. Dept., Univ. of Victoria, Victoria, BC, Canada V8W 2Y2.

The eyeblink conditioning paradigm may be well suited for studying the neurobiological bases of learning during development. Here we report associative learning of the eyeblink response in developing rat pups.

Pups were trained on delay conditioning or given unpaired training at either 17 or 24 days of age. Each animal received three sessions of 100 trials that consisted of a 280 msec tone CS and a 100 msec, 2 mA periorbital shock, delivered through subcutaneous electrodes. Responses were recorded from EMG electrodes implanted in the eyelid muscles (see Skelton, 1988, Beh Neurosci, 102, 586-590 for details). The results showed stronger conditioning at 24 days than 17 days but, at asymptote, both ages differed from their unpaired controls.

A second experiment showed that when 17- and 24-day-old rats recieved 0 mA, 1 mA, 2 mA, or 3 mA US-alone trials, UR amplitudes increased with US intensity but were the same for both ages at each US intensity.

These findings suggest that auditory conditioning of the eyeblink response develops around the time of weaning in the rat and that age differences in US-processing do not account for this effect. Further work examining sensory and parametric factors is in progress.

341.14

RED NUCLEUS PROJECTIONS TO CEREBELLAR CORTEX (HVI) IN RABBIT EXAMINED WITH WGA-HRP.

M.E. Rosenfield* and J.W. Moore. Dept of Psychol, Univ of Mass, Amherst, MA 01003.

Cerebellar cortex (Larsell's HVI) has been implicated in the generation of the classically conditioned eye blink/nictitating membrane response. The motor program for the conditioned response is presumably learned by the cerebellum and relayed to motoneurons via the red nucleus (e.g., Rosenfield, M. & Moore, J., *Behav Brain Res*, 10:393, 1983). Cat red nucleus reportedly contains neurons that project to cerebellar cortex (Dietrichs, E. & Walberg, F., *Exp Brain Res*, 50:353, 1983). Projections from the red nucleus to HVI could be important for brain stem and cerebellar processes involved in classical conditioning (e.g., Moore, J. et al, *Biol Cyber*, 62:17, 1989).

We implanted WGA-HRP (Sigma L3892) unilaterally into HVI in 4 albino rabbits (Mori, J., et al, *Brain Res Bull*, 6:19, 1981). The pipette remained *in situ* for 48 hours before sacrifice. Animals were perfused transcardially (descending aorta clamped) with approximately 2 L of .9% saline followed by .5 L of 10% formalin and then 3 L of 12% sucrose solution at 4 degrees C. Brains were blocked immediately on extraction (saving only the brain stem and cerebellum), placed in 30% sucrose in .1 M phosphate buffer (pH = 7.2), and stored at 4 degrees C for 24 h. Brain stem and cerebellum were embedded in gelatin; frozen sections were cut transversely at 60 μ, mounted on subbed slides, and reacted with tetramethylbenzidine. All HVI cases (defined by histological verification of the implantation locus and the presence of retrogradely labeled cells in the pontine nuclei, spinal trigeminal nucleus par oralis, and the dorsal accessory olivary nucleus) showed sparse retrogradely labeled cells in the more caudal (3rd nerve level) portions of the red nucleus, consistent with Dietrich and Walberg's cat study. (Supported by AFOSR grant 89-0391)

341.15

DIFFERENTIAL PROJECTIONS OF PONTINE NUCLEI TO INTERPOSITUS NUCLEUS AND LOBULE HVI. J.K. Thompson, W.J. Spangler and R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

In earlier work using both retrograde and anterograde labelling we reported that projections to the interpositus nucleus from the pontine nuclei were primarily from the ventrolateral region of the pontine nuclei. In the present study, we extended these observations and compared pontine projections to the interpositus nucleus and region HVI of cerebellar cortex. Fluoro-gold, Fast Blue or WGA-HRP were injected into the anterior interpositus nucleus or into cortical tissue of HVI overlying the interpositus. Interpositus injections resulted in heavy retrograde labelling in the ventrolateral region of both contra- and ipsilateral pontine nuclei. In contrast, retrograde labelling from HVI injections was more prominent in the dorsolateral regions of the pontine nuclei.

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LEARNING AND MEMORY—PHARMACOLOGY: OTHER I

342.1

COCAINE ENHANCES RETENTION OF A ONE-WAY AVOIDANCE RESPONSE IN MICE. S.B. Weinberger, C.A. Riedel*, P.H. Janak, and J.L. Martinez, Jr. Department of Psychology, University of California, Berkeley, CA 94720.

Post-training administration of cocaine both facilitates acquisition of an automated shelf-jump avoidance response (Janak and Martinez, this volume) and enhances retention of a trough avoidance response (Janak et al., Psychopharm., in press) in rats. In the present study we found that cocaine (10 or 30 mg/kg IP) administered to mice immediately following completion of two training trials on Day One significantly enhances performance on Day Two of a one-way trough avoidance response. Neither cocaine methiodide nor lidocaine, when administered in doses equimolar to the effective cocaine doses, altered performance on Day Two. These data indicate that cocaine's enhancement of avoidance response retention most likely is neither peripherally mediated nor attributable to its local anesthetic properties.

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342.2

POSTTRAINING COCAINE OR AMPHETAMINE ENHANCES JUMP-UP ACTIVE AVOIDANCE CONDITIONING IN RATS P.H. Janak and J.L. Martinez, Jr., Dept. of Psych., Univ. of Calif., Berkeley CA 94720

The effects of cocaine (COC) and amphetamine (AMPH) on jump-up avoidance response acquisition were examined in two experiments. For 2 days rats received 1 avoidance trial, and on the third day, received 8 avoidance trials, using a 310μA footshock. Rats escaped or avoided shock delivery by jumping onto a raised shelf. Immediately after each training trial in the 1st experiment, rats received an IP injection of either COC or saline. COC (2.75 mg/kg) facilitated acquisition of the jump-up response as compared to saline-treated rats as indicated by a difference between the slope of the acquisition functions at test on day 3 [$p<.025$]. In a 2nd experiment, AMPH (0.3 & 1.0 mg/kg) facilitated jump-up response acquisition as indicated by an effect of treatment for each dose as compared to saline-treated rats [0.3 mg/kg: $p<.02$; 1.0 mg/kg: $p<.04$]. Thus both stimulants enhance jump-up response acquisition, although the nature of the memory effects of COC and AMPH are different. (Supported by DA06192 to JLM & DA05375 to PHJ.)

342.3

D-PEN^2-[D-PEN^5]ENKEPHALIN IMPAIRS ACQUISITION AND ENHANCES RETENTION OF A ONE-WAY AVOIDANCE RESPONSE IN RATS J.L. Martinez, Jr., R.V. Hernandez*, and S.B. Weinberger. Department of Psychology, University of California, Berkeley, CA 94720.

D-Pen^2-[D-Pen^5]enkephalin (DPDPE), a delta opioid receptor selective analog of [Leu]enkephalin, impairs acquisition of an automated shelf-jump avoidance response in rats (Reg. Pep. 26:323, 1989) and acquisition of a one-way active avoidance response in mice (Behav. Neurosci. 102:678, 1988). In the present study we found that DPDPE (1.16 μg/kg IP) administered prior to presentation of two training trials on Day One also impairs acquisition of a one-way active avoidance response in rats tested 24 hrs later. On the other hand, DPDPE (0.332 μg/kg IP) administered following two training trials on Day One significantly enhances performance on Day Two. These results indicate that activation of delta opioid receptors has a modulatory effect on acqusition and retention of aversively motivated learning.

(Supported by NIDA #DA04195.)

342.4

[LEU]ENKEPHALIN (LE) AND ITS METABOLITE, TYR-GLY-GLY (TGG), IMPAIR RETENTION OF AN ACTIVE AVOIDANCE RESPONSE IN MICE. G. Schulteis & J.L. Martinez, Jr. Dept. of Psychology, University of California, Berkeley, CA, 94720.

In the present study we sought to determine whether LE and TGG affect retention of an active avoidance response. Male Swiss Webster mice were given 2 training trials in which footshock (140 μA) was delivered 10 s after placement in the dark chamber of a two-compartment avoidance apparatus; mice could terminate the footshock by escape into the lighted safe chamber. The next day, mice were given 10 additional trials in which they had 10 s to enter the safe compartment; failure to avoid within 10 s resulted in administration of footshock. In Experiment I, saline-treated mice that received 2 training trials on Day 1 made significantly more avoidances ($\bar{X}$=3.85±0.44) the following day than mice that received 0 ($\bar{X}$=2.00±0.43) or 1 ($\bar{X}$=2.00±0.48) training trial. This indicated that significant retention of the training experience had occurred in the mice receiving 2 training trials, and that pharmacological treatments could potentially modulate the degree of retention. In Experiment II, LE given just after training produced a U-shaped effect on retention; while 30 and 100 μg/kg LE impaired retention, 10 and 300 μg/kg were without effect. TGG likewise affected retention in a U-shaped manner, with 16 and 53 μg/kg (but not 160 μg/kg) impairing retention. The mechanisms of action of TGG and LE most likely differ, because TGG exhibits little opioid activity. Supported by DA04195 & DA05334.

342.5

CHARACTERIZATION OF THE EFFECTS OF DYNORPHIN A(1-13) ON PASSIVE AVOIDANCE RESPONSE IN MICE. M. Ukai, X. shan-Wu * and T. Kameyama. Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan.

The effects of dynorphin A(1-13), a kappa-selective opioid peptide, on the cycloheximide (CXM)- and scopolamine (SCOP)-induced amnesia were investigated using step-down-type passive avoidance task. Male ddY mice weighing between 30 and 35 g were employed in the study. CXM (30.0 mg/kg, s.c.) and SCOP (1.0 mg/kg, i.p.) administered 30 min before training produced amnesia in a retention test (24 hr later) as shown by short latency to step down from the platform on grid floor. Dynorphin A(1-13)(3.0 and 10.0 mg/kg, i.p.) given 15 min before either training or retention markedly prolonged step-down latency (SDL) in mice treated with CXM, demonstrating improvements of the amnesia. The above effects of dynorphin A(1-13) were reproducible, when administered the peptide (1.0 and 3.0 μg) intracerebroventricularly. U-50,488H (1.0 and 3.0 mg/kg, i.p.) also produced an anti-amnesic effect in mice treated with CXM. Dynorphin A(1-13)(1.0 and 3.0 μg, i.c.v.) produced a marked prolongation of SDL in mice treated with SCOP. These results suggest that the ligands selective for kappa opioid populations possess anti-amnesic effects.

342.6

COMBINED EFFECTS OF VAGOTOMY AND ATROPINE METHYL BROMIDE ON LEU-ENKEPHALIN-INDUCED IMPAIRMENTS IN MEMORY STORAGE PROCESSES. C. L. Williams and R. A. Jensen. Dept. of Psychology, Southern Illinois University, Carbondale, IL 62901.

These experiments investigated the role played by the vagus nerve in integrating the effects of substances which do not enter the CNS, but which alter brain functioning when administered peripherally. Four experiments were conducted to examine whether the vagus represents one pathway by which peripheral substances modulate brain mnemonic processes.

Experiment 1 determined which doses of leu-enkephalin (LE) are effective in influencing memory processes. Posttraining administration of 100.0 ug/kg of LE impaired retention performance relative to saline-treated animals or those receiving lower doses of this peptide. In Experiment 2, atropine methyl bromide (AMB), a peripherally acting anticholinergic, was administered posttraining in an inhibitory avoidance task. These results demonstrated that AMB produces bidirectional effects on memory processes. An intermediate dose of AMB improved performance relative to rats given a higher dose that produced amnesia.

Experiment 3 determined whether blockade of vagal efferents with AMB attenuates the effects of LE. The impairment in retention produced by 100 ug/kg of LE in Experiment 1 was not observed when this peptide was injected with either a low or a high dose of AMB. The final experiment examined whether vagotomy attenuates LE's effects on retention and assessed the combined effects of vagotomy and AMB administration on LE-induced memory impairments. Unoperated and sham-operated animals given 100.0 ug/kg LE after training had significantly lower retention latencies than their respective controls that received saline. The retention performance of vagotomized animals receiving 100.0 ug/kg of LE was lower but not significantly different from vagotomized rats given saline. Administration of AMB in conjunction with LE to vagotomized rats also did not result in impaired retention performance. These results suggest that vagotomy or the combination of vagotomy and AMB attenuates, but does not completely abolish the memory-impairing effects of LE.

342.7

CONDITIONED EMOTIONAL RESPONSE TO MORPHINE AS A CONDITIONED STIMULUS. N. M. Bormann* and D. A. Overton. Department of Psychology, Temple University, Philadelphia, PA 19122.

Three exps. were conducted to determine whether morphine (4 or 6 mg/kg, i.p.) can act as a conditioned stimulus (CS) when paired with a shock unconditioned stimulus (US), to produce a conditioned suppression of drinking (CR) in water deprived rats. All exps. consisted of baseline, conditioning and extinction phases. In baseline, rats were injected, with morphine or saline 15 or 25 min before a drinking test to determine baseline water consumption. Conditioning in Exps. 1 and 2 consisted of drug-shock and saline-no shock pairings with drinking tube absent. In the conditioning phase of Exp. 3, three groups of 4 rats were given a 4 min drinking test 15 min after drug injection and 0-10 minutes before shock (or no shock). One group received drug-tube-shock sessions every day, one group every third day with saline-tube-no shock on other days, and the third group every third day with drug-shock on other days. Extinction consisted of drug or saline 15 min prior to 4 min drinking tests. In Exp. 1, rats conditioned with morphine (4 mg/kg), did not suppress drinking when tested as compared with baseline. In Exp. 2, twelve rats, conditioned with morphine (6 mg/kg), drank significantly less water during testing as compared with an unconditioned control group. In Exp 3, contrary to our hypothesis, there was no difference between groups of rats, although there was a significant difference in water consumed during baseline and extinction phases. The conditioned fear CR found in Exp. 3 was to morphine + presence of drinking tube or to drinking tube alone as CS. The CR in Exp. 2 was to the morphine CS.

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342.8

REINFORCEMENT OF HIPPOCAMPAL CA1 BURSTING BY CANNABINOID RECEPTOR ACTIVATION. B. G. Xue* and L. Stein. Department of Pharmacology, UCI School of Medicine, Irvine, CA 92717.

Involvement of cannabinoid receptors in behavioral reinforcement has been demonstrated by self-administration of Δ^9-tetrahydrocannabinol (THC) and THC-induced reduction of self-stimulation reward thresholds. Cannabinoid receptors are found in high density in rat hippocampus (Michelle, et al J. Neurochem. 55, 21-26, 1990). Using a hippocampal-slice operant conditioning preparation, reinforcement of CA1 or CA3 bursting activity by extracellular applications of dopamine and opioid receptor agonists, respectively, has been described (Stein & Belluzzi, JD. Neurosci. & Biobehav. Rev. 13, 69-80, 1989). Here we report highly reliable CA1 cellular operant conditioning with the high affinity cannabinoid agonist CP-55940 used as reinforcement. A single-barrelled glass micropipette for simultaneous recording and pressure injection was filled with CP-55940 (2.5-100 μM in 165 mM Saline) and aimed at spontaneously active pyramidal cells in the CA1 layer. In reinforcement periods, the pressure injector was activated for 10 ms at 15 P.S.I. to deliver an approximately 10 μ-diameter droplet of drug. More than 55% of the tested neurons were successfully reinforced by burst-contingent applications of CP-55940 (at concentrations of 5 and 10 μM, but not at 2.5 and 100 μM). The same microinjections, administered independently of firing, did not increase bursting rate and therefore provided a control for direct pharmacological stimulation or facilitation. The results indicate that cannabinoid receptor activation can reinforce hippocampal CA1 bursting activity. Cannabinoid receptors, like dopamine and opioid receptors, may play important roles both in behavioral and cellular operant conditioning.

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342.9

VIP INDUCES THETA-LIKE CELLULAR RHYTHMS IN HIPPOCAMPAL CA1 NEURONS. Oscar Prospero-Garcia, Scott Steffensen* and Steven J. Henriksen. Dept. Neuropharmacology, Scripps Research Institute, La Jolla, CA 92037.

We have previously shown that the Vasoactive Intestinal Polypeptide (VIP) induces REM sleep and memory retention in the otherwise insomniac (PCPA pre-treated) rat. In order to more precisely evaluate VIP's cellular site of action we have iontophoretically applied VIP onto hippocampal neurons and have measured variations in spontaneous and in evoked activity of neurons located in this mnemonic structure. Studies were performed on halothane anesthetized rats. Bipolar electrodes were implanted into the perforant pathway to electrically stimulate ipsilateral dentate gyrus (DG) and a second into CA3 commissural collaterals to stimulate contralateral CA1. Multi-barrel recording/injecting electrodes were employed, with the recording pipette protruding 25 μm further than the drug barrels . Pyramidal neurons, granule cells and putative interneurons were electrophysiologically identified. Microiontophoretic application of VIP (100-200 nA for 10 min) doubled the spontaneous rate of isolated CA1 pyramidal cells and induced a regular pattern of discharge of approximately 4 Hz, determined from peri-stimulus interval spike histograms. Evoked activity of CA1 interneurons was also enhanced by VIP iontophoresis. In the DG, granule cell spontaneous activity also slightly increased following VIP administration. On the other hand, DG putative interneuron activity was reduced by this peptide. Our data suggest that VIP may favor the appearance of a regular, theta-like rhythm in CA1 by enhancing both pyramidal and interneuron activity. Conversely, theta-like activity was not induced by VIP in the DG where VIP differentially inhibited putative interneurons.

342.10

SEQUENCE-SPECIFIC EFFECTS OF THE NEUROTACHYKININ SUBSTANCE P ON MEMORY, REINFORCEMENT, AND NEURAL TRANSMISSION. J.P. Huston, R.U. Hasenöhrl*, P. Gerhardt*, F. Boix*, J. Fornaguera*, F. Adams*, & R.K.W. Schwarting. Inst.Physiol. Psychol., Univ. Düsseldorf, 4000 Düsseldorf, Germany.

There is now considerable evidence that substance P (SP) can have nootropic and neurotrophic effects. Thus, the study of its mechanisms may provide new insights with regard to learning, memory, and neurodegenerative disorders, such as Alzheimer's and Parkinson's disease. Our work shows that SP, when given peripherally, is memory-promoting (inhibitory avoidance) and reinforcing (place preference). In both paradigms, a dose of 50μg/kg has repeatedly shown to be effective. The two effects seem to be mediated by different SP sequences, as an equimolar dose of SP1-7 enhanced memory, whereas SP5-11 was reinforcing. These differential behavioral effects were paralleled by selective and site-specific changes in dopamine (DA) release, as SP or its C-terminal increased DA release in the nucleus accumbens, but not the neostriatum. The neurochemical changes lasted at least 4 hours after injection. Direct injection of SP (1ng) into the NBM was also memory-promoting and reinforcing, and again, these effects were distinctively mediated by SP1-7 and SP5-11. Furthermore, such injections differentially affected biogenic amines in the nucleus accumbens, but not the neostriatum or frontal cortex. These results are discussed with regard to the role of SP in memory and reinforcement, and its interaction with brain DA.

342.11

NONPEPTIDE ANGIOTENSIN II RECEPTOR ANTAGONIST AND ANGIOTENSIN CONVERTING ENZYME INHIBITOR: EFFECT ON A RENIN-INDUCED DEFICIT OF A PASSIVE AVOIDANCE RESPONSE IN RATS. K. F. DeNoble, V. J. DeNoble, K. R. Spencer*, A. T. Chiu*, P. C. Wong*, and P. B.M.W.M. Timmermans*. The Du Pont Merck Pharmaceutical Company, P.O. Box 80400, Wilmington, DE 19880-0400.

Nonpeptide receptor ligands with differential affinity for the angiotensin II-1 (AII-1) receptor (EXP3312, EXP3880) or the AII-2 receptor (PD123177) and an angiotensin converting enzyme (ACE) inhibitor captopril were evaluated for the ability to protect against a renin-induced performance deficit in a passive avoidance (PA) task in rats. The ability to retain a PA response was shown to decrease as the dose of intracerebroventricularly (i.c.v.) administered renin increased with maximal retention deficits occurring at 1.0 µg/5 µl i.c.v. EXP3312 (1 - 100 µg/5 µl i.c.v.) and EXP3880 (1 - 100 µg/5 µl i.c.v.) produced dose dependent increases in retention latencies when co-administered with renin. The peak effect dose (PED) for EXP3312 and EXP3880 was 3 µg and 30 µg i.c.v., respectively. In contrast, PD123177 was not effective in preventing the renin-induced decrease in retention across a broad range of doses (0.1 - 100 µg/5 µl i.c.v.). Captopril (1 - 100 µg/5 µl i.c.v.) also prevented the renin-induced performance deficit with a PED of 30 µg/5 µl i.c.v. These results suggest that renin given i.c.v. produces a deficit in performance of a PA response in rats and that this effect can be attenuated by an ACE inhibitor, AII-1 receptor ligands, but not AII-2 receptor blockers.

342.12

LEARNING FACILITATED BY ANGIOTENSIN CONVERTING ENZYME INHIBITOR HOE 065 INJECTED SYSTEMICALLY OR INTO THE BASAL FOREBRAIN. P. Gerhardt*, F.J. Hock [2], R.U. Hasenöhrl* and J.P. Huston. Inst. Physiol. Psychology, University of Düsseldorf, 4000 Düsseldorf 1, Germany. Hoechst AG, Dept. Pharmacology, 6000 Frankfurt a.M., Germany [2].

Angiotensin converting enzyme (ACE)-inhibitors can facilitate learning in rats and improve symptoms of dementia in humans. We investigated the ACE-inhibitor HOE 065, a novel compound with low antihypertensive potency, for its effects on learning after i.p.-application and injection into the NBM area in rats. Effects on habituation learning were measured in an open field by recording the number of rearings during free exploration. Like Substance P (50µg/kg) i.p. post trial administered HOE 065 (1 and 10 mg/kg) reduced the number of rearings during the next trial compared to controls, indicative of enhanced habituation learning. Effects of injection of HOE 065 or SP into the NBM were investigated on inhibitory avoidance learning, which involves punishment of a high-probability turning response on a tilted platform. Immediately after the learning trial, i.e. after a shock administered upon performing the response, the given substance was injected. Rats treated with HOE 065 (50 and 500 ng) or SP (1 ng) had longer uphill latencies during retest than controls, indicative of superior learning. Thus, HOE 065 improved learning after peripheral and central injection.

342.13

EFFECT OF A PURINE ANALOGUE ON MEMORY IN MICE
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An animal model has been proposed for human memory loss which occurs during aging. This model is based on the observation that in a T-maze once a rat or mouse enters a goal box and consumes all the food, on the next trial it will enter the other goal box. By increasing the time between trial it can be determined if the S can remember which side of the maze it entered on the previous trial. Male Swiss Webster mice, food deprived to 80% of their free feeding weight, were tested in this model. At delays of 30 or 60 seconds a correct response occurred 75% of the time. When the delay was increased to 90 seconds the correct response rate fell to chance (46%). Since there is a considerable amount of interest in comparing immune and brain function we tested a purine analogue with immunomodulatory activity, AIT0082, in this model. AIT0082 (0.5 mg/kg) improved performance at the 90 second delay to 65% correct. At higher doses (20-60 mg/kg) AIT0082 improved performance to 80% correct. The effect of the 60mg/kg dose was observed for 7 days following a single injection. The daily injection of the drug for 18 days indicated that tolerance did not develop. The injection of atropine (0.5 mg/kg) 30 min. prior to testing blocked the memory enhancing effects. Supported by VAMC Research Service & a grant from AIT.

342.14

N6-CYCLOPENTYLADENOSINE IMPAIRS INHIBITORY AVOIDANCE RETENTION BY SELECTIVE ACTION AT A1 RECEPTORS. H.J Normile[1], N. Jadallah*, G. Johnson* and R.A. Barraco. Departments of Psychiatry[1] and Physiology, Wayne State Univ. Sch. of Med., Detroit, MI 48207.

The effects of N6-cyclopentyladenosine (CPA), a highly selective agonist for adenosine A1 receptors, on retention of a one-trial inhibitory avoidance response was examined in mice. Water-deprived mice were trained to avoid drinking by pairing foot-shock with licks from a water spout. Retention was measured as the suppression of drinking (latency to drink) 48 hr following training. Administration of CPA (0.15-2.25 umols/kg) 30 min before training produced a dose-dependent impairment in retention of the original avoidance response. The CPA-elicited deficits in retention performance were blocked by pretreatment with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective A1 receptor antagonist; DPCPX (15 umols/kg) administration alone had no effect on retention. These findings suggest that selective activation of a presumably central population of A1 receptors may impair retention performance and influence information processing. [Supported by grants from NIA (AG07069), ADAMHA (MH47181 & MH17150) and NIH (GM08167-13)]

342.15

PHENCYCLIDINE DISRUPTS LEARNING OF SPATIAL INFORMATION WITHIN A CONTINUOUS RECOGNITION AND A "CHEESE" BOARD TASK. R. P. Kesner, M. Dakis, B. L. Bolland, Dept. of Psychology, Univ. of Utah, Salt Lake City, UT 84112.

Rats with saline, 1, 2 or 4 mg/kg PCP injections (i.p.) were trained either on a 12 arm maze using a continuous recognition memory procedure or on a "cheese" board (dry-land version of water maze). For the 12 arm maze task the rats for each daily session were allowed sequential access to 12 arms of the maze from the center platform. Of the 12 presentations, three or four of the arms were repeated, but did not contain reinforcement. Repeated arms were presented with lags ranging from 0 to 6 (from 0 to 6 different arm presentations between the first and the repeated presentation). All animals received 16 sessions. Results indicated that compared to rats with saline and 1 or 2 mg/kg PCP injections, the rats with 4 mg/kg PCP injections could not learn the task even at 0 lag. For the "cheese" board task rats were given 8 trials a day for 3 consecutive days to learn the correct location of food, starting at different spatial locations relative to the correct food location. Results indicated that rats with 4 mg/kg PCP injections were similar to rats with saline or 1 and 2 mg/kg PCP injections by displaying improved learning within a day, but different by displaying forgetting between days.

342.16

EFFECT OF THE ANESTHETIC AGENT DIPRIVAN (PROPOFOL) ON MEMORY PROCESSING IN MICE.
E. Rosman, R.Pang, D Quartermain & H.Turndorf
Anesthesiology & Neurology NYU Medical Center
Amnestic effects of the i.v. hypnotic agent Diprivan were studied in a single trial passive avoidance task. Mice were injected with propofol (PROP 50 & 100 mg\kg i.p.) 10 min before, or immediately after the training trial. Memory was impaired when the drug was administered before learning but not when injected after training. In a second experiment a dose response curve was demonstrated (PROP 5,25,50 & 75mg\kg) with progressive impairment of retention as the concentration increased, and significant anterograde amnesia at 50mg\kg. To determine if the anterograde amnesia resulted from state dependency, 2 groups of mice n=24 were injected with vehicle or 50mg\kg PROP before training. Before testing each group was further divided n=12 and reinjected with either PROP or vehicle. Result showed that both the PROP-Vehicle as well the PROP-PROP groups demonstrated robust memory impairment indicating that amnesia was not due to state dependent learning.

342.17

Kynurenic Acid Attenuates Cognitive and Memory Impairments Induced By An Environmental Neuroexcitotoxin. B.F. Petrie (1), C. Pinsky (2), N. Standish (1), R. Bose (2), and G. Glavin (2). (1) Department of Psychology, Red Deer College Red Deer, Alberta, Canada, T4N 5H5; (2) Department of Pharmacology, University of Manitoba, Winnipeg, Manitoba, Canada, R3E OW3.

The present study is the first to examine the effect of parenteral injection of the neuroexcitotoxin domoic acid and the broad-spectrum neuroexcitotoxin antagonist kynurenic acid, on a memory task in mice. Domoic acid impaired performance on a previously learned place task in the Morris water maze, while kynurenic acid, administered subsequent to domoic acid, prevented an initial decline in performance. The results have implications for the mammalian neuroanatomic correlates of this spatial memory behavior, and suggest that the domoic acid-treated mouse is an appropriate animal model with which to study the functional deficits associated with neurodegenerative disorders, including senile dementia of the Alzheimer's type.

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342.18

CALCIUM AND CALMODULIN ANTAGONISTS DISRUPT INTERMEDIATE -TERM MEMORY FORMATION (ITM) IN THE 2-DAY OLD CHICK. P.A. Serrano, D.R. Smith*, E.L. Bennett and M.R. Rosenzweig. Department of Psychology , Univ. of California, Berkeley, CA 94720

The one-trial peck-avoidance paradigm for the 2-day old chick has been extensively used to investigate neural mechanisms of memory formation (Gibbs & Ng, 1977; Patterson et al., 1986; Ali et al., 1988; Serrano, 1990).

Protein kinase C activity has been suggested as an important neural mechanism for long-term memory (Burchuladze et al., 1990; Lovinger et al., 1987; Serrano et al., 1990). We have investigated other protein kinase (PK) inhibitors for their role in memory formation: three calmodulin inhibitors, N-(6-aminohexyl)-5-chloro-2-napthalenesulfonamide (NACN), W-7 and W-13, an intracellular calcium inhibitor, HA-1004, and two non-specific PK inhibitors, A-3 and ML-9. HA-1004, NACN, A-3 and ML-9 produced significant amnesia at a 24 hr test ($p<.01$). HA-1004 and NACN produced significant amnesia by 30 min post-training and at subsequent test times ($p<.01$). The time courses for A-3 and ML-9 are being determined. These time courses show ITM is disrupted, further implicating calcium and calmodulin as neural mechanisms for ITM formation.
(NSF grant BNS-88-10528).

342.19

THE EFFECTS OF ALTERING BRAIN PROTEIN KINASE C ON PERFORMANCE IN THE MORRIS WATER TASK (MWT). R.Paylor, S.K. Morrison*, J.W. Rudy, L. Waltrip*, and J.M. Wehner. Institute for Behavioral Genetics, and Department of Psychology, University of Colorado, Boulder, 80309.

Protein Kinase C (PKC) has been implicated in learning and memory processes in Aplysia, Hermissenda, and rodents. More specifically, evidence has lead researchers to speculate that hippocampal PKC contributes to spatial learning and memory. This project used two approaches to test the hypothesis that brain PKC contributes to performance in a spatial learning and memory task.

If PKC is involved, then manipulations of its activity should affect performance in a spatial-learning-dependent task. Phorbol esters are known to activate PKC. Therefore, in the first study the effects of the phorbol ester, PDBu, was tested on spatial learning in adult Long-Evans rats using the hidden-platform version of the MWT. Fifteen min after an ICV injection (10ng), PDBu-treated rats were better than controls at learning the position of the platform as measured by latency to escape scores. Twenty-four hours after the last trial PDBu-treated rats showed signs of having remembered the location of the platform better than controls as measured by heading error, latency to cross the place where the platform had been located, and platform-crossing score.

In the second study, rats were reared in either a complex environment or a normal lab environment for either 6 or 12 days starting when they were 15 days old. PKC was measured in cytosolic and particulate fractions. Rearing in the complex environment for 6 days had no effect on hippocampal PKC, however, after 12 days there was an increase in hippocampal cytosolic PKC. Preliminary results suggest that the performance in the MWT was improved in rats reared in the complex environment.

These results support the notion that brain PKC is involved in performance in the Morris water task. (Supported by NSF-BNS 8820076 to J.M.W.)

342.20

CLASSICAL CONDITIONING AND PROTEIN KINASE C IN YOUNG AND OLD RABBITS TREATED WITH BMY 21502. M.H. Kreps[1], D.K. Sasse[1], J.M. Coffin[1], S.L. Moon[2], & D.S. Woodruff-Pak[1]. Deptartment of Psychology[1], Temple University, Philadelphia, PA 19122 and CNS Neuropharmacology[2], Bristol-Myers Squibb Co., Wallingford, CT 06492.

BMY 21502, a substituted pyrrolidinone, was injected in young (Y) and older (O) rabbits to see if eyeblink classical conditioning could be facilitated. A 750 msec CS-US interval in the delay paradigm was used (850 msec, 1 KHz, 85 dB tone CS followed 750 msec after its onset by a 100 msec, 3 psi, corneal airpuff US) in 10 daily acquisition sessions with 90 paired or 180 unpaired trials. Fifty-four male and female rabbits were used (30 3-month; 24 30 to 48-month retired breeders). There were 4 conditions for both age groups of rabbits: Paired tone and airpuff presentations with (1) vehicle only; (2) BMY 21502, 5 mg/kg; (3) BMY 21502, 10 mg/kg; (4) unpaired tone and airpuff presentations with BMY 21502, 5 mg/kg. BMY 21502 enhanced conditioning at both dosage levels in O but not Y rabbits. There were large age differences in conditioning in vehicle-treated animals, but O rabbits with BMY 21502 learned as well as Y rabbits. Preliminary analyses indicate that ^{3}H-PDBU binding redistributes within the layers of the hippocampus with age. In O animals the densest ^{3}H-PDBU binding in hippocampus is in the pyramidal layer.

NEURAL PLASTICITY III

343.1

EDUCATION RELATED CHANGES AND INDIVIDUAL VARIABILITY IN WERNICKE'S AREA: A QUANTITATIVE DENDRITIC ANALYSIS. B. Jacobs and A. B. Scheibel. Brain Res. Inst., UCLA, Los Angeles, CA 90024.

This investigation, which is part of a more comprehensive multivariate project, examined the relationship between the basilar dendrites of supragranular pyramidal cells in Wernicke's area of both hemispheres and environmental variables, specifically education and general personal factors. Tissue was obtained from 10 male (M_{age} = 52.2; *range* = 18-78; *SD* = 17.42) and 10 female (M_{age} = 47.8 years; *range* = 20-79; *SD* = 20.47) subjects. All subjects were determined to be neurologically nomal right-handers. Using a modified rapid Golgi technique, 10 pyramidal cells were sampled from each hemisphere (total cells = 400 cells; total segments = 16,697). The dendritic systems were evaluated according to total dendritic length (TDL), mean dendritic length (MDL), and dendritic segment count (DSC). A distinction was also made between proximal (1st, 2nd, and 3rd order) and the ontogenetically later developing distal (4th order and above) dendritic branches.

Considerable interindividual variation in dendritic systems appeared to reflect general sociocultural and/or personal experiences. Females characterized as being more active socially or occupationally tended to have greater TDL values. Males with jobs requiring manual labor tended to have TDL values below average. Education had a consistent and substantial effect such that dendritic measures tended to increase as one ascended the educational scale. Individuals with a university education typically had the highest dendritic values (TDL = 55,057 μm); those with a high school education were characterized by somewhat lower values (TDL = 51,695 μm); and individuals with less than a high school education had the lowest dendritic values (TDL = 43,397 μm). These neurohistological consequences were registered primarily in distal order segments, supporting their reported epigenetic sensitivity (Carughi et al., *J. of Nutrition, 119*: 2005-2016, 1989).

343.2

INCREASE IN THE NUMBER OF AXOSPINOUS SYNAPSES WITH SEGMENTED POSTSYNAPTIC DENSITIES AFTER THE INDUCTION OF LONG-TERM POTENTIATION (LTP). Y. Geinisman, L. deToledo-Morrell and F. Morrell. Dept. of CMS Biol., Northwestern Univ. Med. Sch. and Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL 60611.

Sustained enhancement of synaptic efficacy, which is characteristic of LTP, may be supported by an increase in synaptic numbers. Although no such change was detected in our previous study of synaptic contacts on dendritic shafts and spines, the ratio of perforated (with a discontinuous postsynaptic density, PSD) to nonperforated (with a continuous PSD) axospinous synapses was found to increase after LTP induction (Geinisman et al., Soc. Neurosci. Abstr., 1990, *16*:979). The aim of the present study was to determine if this structural alteration reflects an increment in a certain subtype of perforated synapses. Young adult rats were implanted with stimulating electrodes in the medial perforant path and recording electrodes in the hilus of the ipsilateral dentate gyrus. Potentiated animals (n = 7) were stimulated (with fifteen 20 ms bursts of 400 Hz delivered at 0.2 Hz) on each of 4 consecutive days and sacrificed 1 h after the fourth stimulation. Unpotentiated but stimulated and unstimulated but implanted rats served as controls (n = 7 in each). Synapses were examined in the middle (MML) and inner (IML) molecular layer of the dentate gyrus. Using the disector technique, estimates of the number of synapses per neuron were differentially obtained for perforated axospinous junctions distinguished by a fenestrated, horseshoe-shaped or segmented PSD. The results showed that the number of synapses with segmented PSDs was markedly and significantly increased in the MML, but not in the IML, of potentiated rats relative to controls. This highly selective modification of connectivity, which involves only one particular subtype of synapses in the potentiated synaptic field, may represent a structural substrate of the enduring augmentation of synaptic efficacy typical of LTP.

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343.3

KINDLING-INDUCED INCREASE IN THE NUMBER OF AXOSPINOUS SYNAPSES WITH SEGMENTED POSTSYNAPTIC DENSITIES. F. Morrell, Y. Geinisman and L. deToledo-Morrell. Depts. of Neurol. Sci. and Psychol., Rush Med. Coll. and Dept. of CMS Biol., Northwestern Univ. Med. Sch., Chicago, IL 60612.

Kindling, which is evoked by repeated, low-level electrical stimulation of a local brain area, is characterized by a virtually permanent augmentation of synaptic responsiveness in the stimulated circuit. Although this enduring synaptic plasticity may be due to an increase in synaptic contacts, our previous work failed to demonstrate such a change in the absolute number of axodendritic, axospinous perforated (with a discontinuous postsynaptic density, PSD) or nonperforated (with a continuous PSD) synapses; however, the relative proportion of perforated synaptic junctions was increased (Geinisman et al., *Brain Res.*, 1990, *507:* 325). Perforated synapses can be further subdivided into those having a fenestrated, horseshoe-shaped and segmented PSD. These synaptic subtypes were differentially quantified in the present study. Rats were kindled via medial perforant path stimulation (1 msec pulses at 60 Hz for 2 sec at a current level that initially induced an afterdischarge of 10 sec or less) and examined morphologically 4 weeks after reaching a criterion of 5 generalized seizures. The number of synapses per neuron was estimated both in the middle (MML) and inner molecular layer of the dentate gyrus with the disector technique. Results indicate that kindling is indeed associated with an increase in synaptic numbers relative to controls. This change is highly selective: it involves only those perforated axospinous synapses which exhibit a segmented PSD and it is restricted to the directly stimulated synaptic field (MML). Since synapses distinguished by a segmented PSD may represent specialized contacts of an unusually high efficacy, a selective increase in their numbers may provide a structural substrate of synaptic plasticity associated with kindling.

Supported by Grants AG 08794 from NIA and BNS-8819902 from NSF.

343.4

INCREASE IN DENTATE GRANULE CELL SOMATIC SYNAPSES FOLLOWING RECURRENT LIMBIC SEIZURES IN RATS. M.C.Bundman and C.M.Gall. Depts. of Pharmacology and Anatomy & Neurobiology, Univ. of Calif., Irvine, CA 92717.

We have previously shown that hilus lesion-induced, recurrent limbic seizure activity leads to a robust elaboration of somatic spines on hippocampal dentate granule cells. The spines are evident 3 hrs following the start of seizure activity and persist in significant numbers for at least one month. Many of the spines receive fully developed synapses consisting of vesicle filled terminals, asymmetric membrane specializations and PSD's. The aim of the present study was to determine whether somatic spines form at the site of pre-existing synapses or whether the somatic spine synapses represent new synapses. Seven rats received hilus lesions (HL) and were sacrificed 5 hrs postlesion along with 7 anesthetic controls (CON). Hippocampal dentate gyrus, contralateral to the lesion, was prepared for electron microscopy and high power (27,000x print magnification) montages of 9 granule cells from each animal were analyzed. There was a 6-fold increase in somatic spines with HL and approximately 30% of the spines contained synapses in the single plane of section examined. The number of synapses directly on the soma did not differ in the HL and the CON rats; however, there was a 31% increase in the total number of granule cell somatic synapses (spine synapses+synapses directly on the soma) in the HL rats. These data suggest that the newly elaborated spines do not form at sites of pre-existing synapses but, rather, that the somatic spine synapses are new synapses and thus, represent a dramatic demonstration of activity dependant synaptogenesis. The increased somatic synapses may contribute to a change in the state of granule cell excitability following heightened physiological activity. Work is now in progress to determine the source of the afferents to the newly elaborated spine synapses. This work was supported by NIMH fellowship MH09868 to MCB and NINCDS grant NS26748 to CMG.

343.5

ANATOMICAL BASIS OF RECOVERY OF SPATIAL LEARNING AFTER NEONATAL PREFRONTAL LESIONS IN RATS. B. Kolb, R. Gibb* and D. Muirhead*. Dept. of Psychology, Univ. of Lethbridge, Lethbridge, Canada, T1K 3M4.

Rats given medial prefrontal lesions on postnatal day 1 (FR1) or 10 (FR10) were trained on the Morris water task on postnatal days 19-21 or days 56-58. The animals then were sacrificed and stained with Golgi-Cox. Analysis of dendritic arborization and spine density was carried out in parietal layer II/III pyramidal cells. The results showed that the operated groups were equally impaired at the water task at 19-21 days but that the FR10 rats had recovered by 56 days. Dendritic results showed no group differences at day 22 but significant differences at day 60. The FR1 animals had less dendritic branching than the control rats whereas the FR10 rats had more than both other groups. Furthermore, there was a marked left/right asymmetry in dendritic branching, favouring the left, at Day 22 but not at Day 60. The results show (1) behavioural and anatomical outcome varies with precise age after early lesions, (2) that there is recovery rather than sparing of spatial learning after prefrontal lesions at 10 days, and (3) that the anatomical basis of the behavioural differences may be increased dendritic arbor, which develops between 22 and 60 days of age.

343.6

PLASTICITY IN AUDITORY CORTEX. P.Maldonado*, G.Gerstein and J.Altman*, Dept. of Physiology, Univ. of Pennsylvania, Philadelphia PA 19104

Changes in cortical somatosensory map structure have been observed after weak, pulsatile electrical stimulation of supragranular layers (Recanzone et al. Soc. Neurosc. Abs. 14:223, 1988). We report a similar experiment with rat auditory cortex using separable multi-neuron recording technology and analyses. Rats were anesthetized with Ketamine-Xylazine. Glued arrays of standard Tungsten electrodes (Microprobe, Parylene, 1.5Mohm) were inserted into auditory cortex. Stimuli were free field 50ms tone bursts over the range of 4-40khz, intensity 40 dB above threshold, presented through one of two loudspeakers at +/- 60 degrees, in random sequence of frequency and direction. For frequency scans tone bursts occurred every 200ms; for study of "afterdischarges" they came every 2 sec. After auditory characterization of the neurons was completed, electrical stimuli (13 0.5ms pulses of 5ua, spaced 5ms, train repeated every 0.5sec) was given for one hour through one of the microelectrodes; characterization partly repeated; another hour of electrical stimulation given; and a final series of full auditory characterizations alternating with periods of spontaneous activity. PST histograms arranged into response planes; response areas were obtained by integrating these over a selected time window. Autocorrelations quantified periodicities in afterdischarges; cross-correlations and Joint PSTH characterized neuronal interactions and their temporal modulations.

About one half the neurons showed changes in response area after electrical stimulation. In some cases the peak moved to the frequency preferred at the stimulating electrode, in direct analogy to the somatosensory remapping reports. In other cases response area of neurons at stimulated and unstimulated electrodes moved to some intermediate frequency. After electrical stimulation most neurons that were studied for afterdischarge showed changes of its strength and/or of silent period following the direct tone burst response. Changes of correlation in the afterbursts (JPST) were observed. These data examine changing neuronal organization underlying a rapidly changing cortical map. NIH MH46428.

343.7

TACTILE COMPENSATION OF THE EFFECTS OF VISUAL DEPRIVATION IN THE CAT. P. Henning and J.P. Rauschecker. NIH Animal Center, NIMH, Poolesville, MD 20837, U.S.A. and Max Planck Institute for Biological Cybernetics, Tübingen, Germany.

Visual deprivation from birth leads to a remarkable improvement of auditory capacities in cats, which can be demonstrated on the physiological, anatomical, and behavioral level. We have also observed an excessive growth of the facial vibrissae in visually deprived cats. The present study tried to address the question whether these longer whiskers actually have any behavioral advantage and whether somatosensory compensation of early blindness can indeed be demonstrated.

Cats lid sutured from birth for several years were trained to find their way through a longitudinal maze. Normal adult cats acted as controls and were tested in the light and in darkness with the aid of an infrared camera system. The maze consisted of 10 small gates in 5 different, variable positions. Electrical contacts signalled when the animals passed through each gate, so running times could be measured very precisely. After an initial training period, cats were given at least 8 test blocks (3 trials each) to determine their performance in the maze task. In the light, the normal control group naturally performed somewhat faster than the blind animals, which had to "feel" their way through the maze, but the difference was not very large. In total darkness the normal cats lost their advantage completely, and the blind animals actually outper-formed the normal ones.

It can be stated with certainty, therefore, that cats without visual experience can acquire knowledge about spatial relationships and can be trained to solve a spatial learning task. While vision obviously helps to solve such a task, the blind animals, when put in a comparable situation with sighted ones, perform at least as well if not better in such a spatial task. Since auditory cues cannot play a major role for this and odor cues were ruled out, we conclude that the somatosensory system, and in particular the vibrissae system, helps these blind animals to build a spatial representation, which is used for spatial orientation. Fundamental mechanisms of neural plasticity are assumed to form the basis for these behavioral adaptations.

343.8

COMPENSATORY CHANGES IN THE MOUSE VIBRISSA/BARREL SYSTEM AFTER EARLY BINOCULAR ENUCLEATION. J.P. Rauschecker, B. Tian, and M. Korte*. (SPON: European Brain and Behaviour Society). NIH Animal Center, NIMH, Poolesville, MD 20837, U.S.A. and Max Planck Institute for Biological Cybernetics, Tübingen, Germany.

Visual deprivation from birth in cats leads to compensatory changes in other sensory modalities. This can be demonstrated on the physiological, anatomical and behavioral level. Besides pronounced compensatory plasticity in the auditory system, we have also shown that visually deprived cats have on average longer whiskers than normal cats. With a switch in species, we were also able to demonstrate that the anatomically distinct barrels representing the mystacial vibrissae in the somatosensory cortex are enlarged in mice enucleated after birth as compared to normal littermates (Rauschecker, Kröger & Tian, 1990).

This finding, which we originally described with the cytochromoxidase technique, was followed up with Nissl staining and with GAD immunohistochemistry. We found that those barrels which correspond to the longest whiskers are the ones which expand most after visual deprivation. This fits in well with the finding that the longest whiskers are the ones which grow most in visually deprived cats.

In order to establish the missing link between the two species, we also measured whisker lengths and diameters in our binocularly enucleated mice. It turned out that both lengths and diameters are enlarged as compared to normal littermates, and this enlargement is very highly significant ($p<0.0001$). What is more, the most significant relative growth occurred again in the positions with the longest vibrissae.

343.9

THE CORTICAL METABOLIC AND BEHAVIORAL EFFECTS OF TRAINING A SPARED RAT VIBRISSA C.L. Hand, D. Noaker*, R.L. Craik, K.M. Gallo*, and W.J. Carr*. Idaho State U., Pocatello, ID 83209; Beaver College, Glenside, PA 19038; and U. of Pennsylvania, Phila., PA 19104.

The first somatosensory (SI) cortical metabolic effects and behavioral consequences of associatively-paired (AP) and disassociatively-paired (DAP) training of a spared vibrissa was examined in 28 rats. Subtotal vibrissa deafferentation involved bilateral sparing of C3 vibrissa (SC3) before postnatal day 3. AP (classically pairing vibrissa stroking with 10% sugar water administration) or DAP (unpaired vibrissa stroking and 10% sugar water administration) training of left or right SC3 was continued at 5 min/day for 60 days; controls were handled an equivalent time. Behavioral testing involving 5 days of 4 minute trials in a darkened environment and using a raised, circular maze indicated that AP rats preferred to use the AP/SC3-trained vibrissa. In contrast, no preference was noted in the DAP/SC3-trained or control rats. Subsequently, the quantitative (14C)-2DG technique revealed, in the same rats and as compared to controls, a significant increase (35 ± 14%) and decrease (37 ± 11%) in the AP/SC3 and DAP/SC3 SI cortical representations, respectively. In conclusion, these results suggest that: (1) training a peripheral receptor organ affected somatosensory related locomotor behavior in the rat and (2) "meaningful" intervention alters the extent of a peripheral lesion-induced functional cortical reorganization that has predictable behavioral consequences. Supported by NS-22283.

343.10

IMMEDIATE REORGANIZATION IN SI FOLLOWING SPINAL CORD HEMISECTION AT T12 IN SIX WEEK OLD KITTENS. M. B. Calford, L. A. Krubitzer, R. Tweedale, T. C. T. Yin* Vision, Touch and Hearing Research Centre, University of Queensland, Queensland, Australia 4072, *Univ. Wisconsin, Madison

Changes in the cortical organization of the primary somatosensory area, SI, were investigated in kittens, six weeks of age, after hemisection of the contralateral spinal cord at T12. Microelectrodes designed to record from single units and neural clusters, were used to examine responses in SI. Seventy eight to ninety four recording sites, approximately 250-500 µm apart, were made in SI in each kitten, before and after hemisection, to obtain dense maps of the hindlimb, trunk and forelimb representations. In addition, selected portions of the representations were restudied in the intact animal to confirm that the degree of error in remapping was small. Immediately after to 4 hours after the hemisection, SI was remapped in detail. During the remapping, we endeavoured to place the electrode in the same location as electrode tracks made before the transection Our results showed that neurons , previously responsive to stimulation of the hindlimb, either became unresponsive to somatic stimulation of any body part, or became responsive to stimulation of the trunk, or sometimes the forelimb. Thus, there was expansion of the trunk representation into cortex that was previously occupied by the hindlimb representation. In addition, there was a lateral shift in the boundary of the trunk representation into regions that previously were responsive only to proximal forelimb stimulation. When neurons in the representation of the forepaw were remapped, receptive fields were nearly identical to those defined before the hemisection. Within the expanded trunk representation there was a rough topography with middle portions of the trunk, adjacent to the deafferented lower trunk, being represented most medially, and the upper trunk was represented laterally, adjacent to the representation of the forelimb. There was a large expansion of the middle trunk representation.

343.11

RECOVERY PROCESS OF HINDLIMB LOCOMOTION FOLLOWING BILATERAL DOUBLE HEMISECTION OF THE SPINAL CORD IN THE RAT. J. H. Kim, W. H. Lee*, A. Chen*, The Miami Project To Cure Paralysis, Dept. of Neurol. Surg., Univ. of Miami, Miami, FL 33136

Adult cats with serial double-hemisected spinal cords recovered the ability to stand and walk without any special training if the lesions were temporally separated by more than 8 days. In this preparation, neurons in the lumbosacral spinal cord receive no direct descending input from supraspinal motor centers. Therefore, it appears that the injured spinal cord is being reorganized to compensate for the lost descending input after the initial hemisection, and that the altered cord maintains the ability to generate locomotion following the second hemisection. The goals of the present study were: (1) to investigate recovery of locomotion in adult rats following double hemisection of the spinal cord, (2) to characterize the restored locomotion, and (3) to find the specific descending input required for reorganization of the injured spinal cord. Adult Sprague-Dawley female rats (225-250 g) were used in this study. Under sterile conditions, double hemisection of spinal cord was made at C4 and T6 using a No. 11 blade. The two hemisections were separated by 2-3 weeks. Following the hemisections, animals were tested for return of normal muscle tone, response to painful stimuli, and treadmill locomotion. After the first hemisection at C4 the animals lost temporarily ipsilateral muscle tone and withdrawal reflex. These animals regained reflex activity, and finally within two weeks recovered the ability to locomote . Following the second hemisection (T6 level), muscle tone of the hindlimb was restored within a few hours and spontaneous locomotion was observed within 48 hours. Recovery was gradual but within 2-3 weeks the prelesion speed of locomotion was obtained. Coordination between fore- and hindlimbs was permanently disturbed. **(Support: NIH (NS2805), The Miami Project Research Fund)**

343.12

EVIDENCE OF ALTERED RAT NEUROMUSCULAR JUNCTION AFTER CHRONIC CORTICOSTERONE TREATMENT. Mohamed A. Fahim. Faculty of Medicine , UAE University, Al Ain, United Arab Emirates.

Chronic exposure to glucocorticoids affects both structure and function of vertebrate skeletal muscles. As less is known about the effects of such steroids on the neuromuscular junctions (NMJs) of different muscle fiber types, the influence of chronic corticosterone(CORT) administration on the ultrastructure of NMJs of soleus and extensor digitorum longus (EDL) was studied. Five intact Fischer 344 male rats were injected daily with 5-10mg CORT for 3 mo and were sacrified at 5 mo of age. Soleus and EDL muscles were bathed *in situ* for 10 min with 4% glutaraldehyde in 0.1M Phosphate buffer (PH 7.2). Fibers within 100 um of the surface were removed and placed in buffered glutaraldehyde at 4 C for 2 hrs, washed in buffer and post-fixed for 1hr in 2% buffered osmium tetroxide. Specimens were then dehydrated, embedded in Spurr, and thin sectioned (80nm). Sections were stained and examined by electron microscopy at 50, 000X. Morphometric analysis of NMJs in CORT-treated rats, revealed significant decrease in fiber diameter, nerve terminal area and synaptic vesicle density, but a significant increase in synaptic cleft.The NMJs underwent partial denervation and reinnervation processes ; as demonstrated by large areas of the presynaptic nerve terminal occupied by microtubules and electron dense granular material.Proliferation of muscle and nerve mitochondria was also observed. Subsarcolemmal electron dense vesicles of variable diameters were present near the NMJ. Treated animals exhibited prominant lysosome deposition, as well as increased nerve sprouting. These steroid-induced stress changes are similar to those observed in aging and disuse studies of NMJ. Thus, glucocorticoids hormones may play an etiological role in the homeostasis of the NMJ in response to various stimuli.

343.13

NEW FORMS OF NEUROMUSCULAR PLASTICITY IN ADULT AND OLD MICE REVEALED BY EM SERIAL SECTIONING. S. Nakashiro and N. Robbins. Dept. of Neurosciences, Case Western Reserve Univ., Cleveland, OH 44118.

In adult and especially in old mice, motor nerve terminals show ongoing outgrowth, retraction, constriction and expansion, but at any moment these events involve only a few percent of the nerve terminal area (*J.Neurocytol* 20:165, 1991; *Neurosci. Abstr.* 20:1162, 1990). Therefore, semiserial sectioning and correlated fluorescence microscopy were used in order to select and characterize the ultrastructural cellular events at these focal sites of neuromuscular plasticity. Three new findings emerged from this work: (1) Especially in old mouse neuromuscular junction(NMJ), nerve terminals showed frequent focal transitions from synaptic to axonal structure. The axonal regions were characterized by infrequent synaptic vesicles and absence of active zones, but there was no Schwann cell engulfment, and only occasional secondary folds. (2) At focal nerve terminal constrictions, the terminal was encircled by the Schwann cell and isolated from underlying secondary folds, probably indicating a prior state of synaptic differentiation before Schwann cell engulfment. Findings (1) and (2) together with previous *in vivo* data indicate that nerve terminals undergo constant and focal synaptic differentiation and dedifferentiation, affecting post-synaptic structure as well as the relation of nerve terminal to Schwann cell. (3) Satellite cells at NMJs were associated with myelin figures (in one case, clearly derived from muscle membrane) indicating that these cells may play a role in remodelling of post-synaptic membrane. Supported by NIA grants AG08886 and AG06641.

343.14

NATURALLY OCCURRING PROJECTION NEURON LOSS IN THE SONG CONTROL SYSTEM OF THE ADULT CANARY. J.R. Kirn & F. Nottebohm. The Rockefeller University Field Research Center, Millbrook, NY 12545.

The motor pathway controlling learned vocalizations in songbirds consists in part of projection neurons linking the High Vocal Center (HVC) to the robust nucleus of the archistriatum (RA). In adult canaries, the total number of RA-projecting HVC neurons remains constant, although new cells of this type are continuously produced (Kirn et al., 1991). Thus, addition of new projection neurons must be matched by loss. We now report that between spring and fall, a period encompassing the yearly transition from stable song to song plasticity in the canary, there is a massive loss and replacement of RA-projecting HVC neurons. Adult males received injections of fluorescent latex microspheres in RA in early April, thereby producing long term retrograde labelling of RA-projecting HVC neurons. RA in these birds was then re-injected with the retrograde tracer fluorogold 4 days prior to killing either in late April (n=4) or mid-October (n=5). The number of HVC neurons labelled with fluorogold remained constant, but that of double labelled cells was reduced by 50% between April and October (t-test; $p<.005$). The lateral magnocellular nucleus of the anterior neostriatum (LMAN) also projects to RA but does not recruit new neurons in adulthood. The number of double labelled RA-projecting LMAN cells did not change between April and October ($p> .05$). Thus, neuron loss was specific to RA-projecting HVC neurons. The canary appears to undergo a natural process whereby the entire HVC-to-RA circuit could be replaced within a year.

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343.15

EVALUATION OF PARAMETERS IN APHASICS: A CLINICAL CONTRIBUTION TO NEURAL PLASTICITY. Ferreira, R.R. Dept. of Physiology and Biophysics, Institute of Biology, University of Campinas, SP, BRAZIL.

The prevalent paradigm in the studies of Neuropsychology referring to Language express a reductionist approach due to historical and epistemological reasons. Usually the classical batteries of pattern-tests are employed for single evaluations or for several evaluations through short periods. The results thereby obtained are related to clinical and topographical syndromes. In the present work five aphasic adult subjects were evaluated, from weekly interviews ranging from 13 to 53 months as to their individual difficulties, and orientated in the linguistic process recovery. Aiming at this process a paradigm was worked out according to recent acquisitions which are not yet properly incorporated by Aphasiology. Concomitantly, they were submitted to a set of tests similar to those most frequently applied in aphasiological research. These observations allowed a diagnosis of a vascular disease in the left hemisphere at distinct areas as well as a set of clinical and neuropsychological symptoms regarding to the damaged area. In this methodology where diagnosis were not dissociated from therapy a significative improvement of the contextualized linguistic activities were reached by all aphasic subjects. The results are relevant not only concerning the rehabilitation but also because they offer a favorable support for a discussion beyond the present experimental conditions. In fact, these data point to the importance of the theoretical debate in the context of Vygotsky and Luria concepts in order to broad the neural plasticity knowledge.

343.16

LEFT HANDEDNESS AS RELATED TO SIDE OF FOCAL TEMPORAL-LOBE EPILEPSY (G.Leonard, A. Sapin,* B. Milner, T. Rasmussen* and W. Feindel*). Montreal Neurol. Inst., McGill Univ., Montreal, Canada H3A 2B4.

We report on a series of 276 consecutively studied patients, each of whom underwent an anterior temporal lobectomy (145 in the left hemisphere, and 131 in the right) to control pharmacologically intractable seizures. No patients showed evidence of extratemporal damage or more widespread epileptiform activity, and subjects who had motor or sensory deficits were excluded. The majority had onset of seizures before the age of five.

The total group comprised 223 strongly right-handed subjects, and 53 predominantly left-handed ones. Hand preference was assessed by means of a modified version of the questionnaire developed by CROVITZ and ZENER [Milner, 1965]. Of the left-handers, only 13 were in the right temporal-lobe group, whereas 40 had left temporal-lobe lesions (Chi-Square = 12.72, $p < .0004$). To account for the observed difference in proportion we suggest that interference in the left cerebral hemisphere, originating in the temporal lobe, can have an effect on hand preference.

343.17

HIGH RESOLUTION MAGNETIC RESONANCE IMAGING OF THE HAMSTER BRAIN. D.N. Kennedy* and D.O. Frost. Dept. of Neurology, Massachusetts General Hospital, Charlestown, MA 02129.

We are investigating the use of magnetic resonance imaging (MRI) for high resolution visualization of rodent brain structure *in situ*. Previous work in humans has demonstrated the value of this technique for determining the locus and extent of cerebral lesions, tumors, etc. in large brains.

Syrian hamsters were placed in a GE CSI Omega 4.7 Tesla imaging system equipped with Accustar self-shielded gradients and a 3 cm slotted resonator rodent head coil. An inversion recovery MRI sequence (TR=3 sec; TE=33 msec; TI=400 msec; slice thickness = 1mm; matrix = 256X128; 2 averages) was used. These parameters provided an in plane resolution of 156X312 µm. A regularly spaced series of images in the coronal plane were obtained.

In T1 weighted images using these parameters, grey matter appears bright and white matter appears dark. Major axon tracts such as the corpus callosum, subcortical white matter, internal capsule, cerebral peduncle, fornix, optic tract, and external medullary lamina are readily visible; prominent tangential fiber laminae in the neocortex can be distinguished in some regions, although contrast is not sufficient for architectonic parcellation.

We are currently using these techniques for the *in situ* evaluation, in mature hamsters, of brain lesions induced at neonatal stages. This procedure permits the identification of cases in which the outcome of the neonatal surgery is most likely to have been appropriate for the behavioral testing of novel, surgically induced neural circuits.

We anticipate that refinement of the parameters of our MRI sequence will permit slice thicknesses as low as 750 µm, improved in-plane resolution and faster imaging times.

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MOTIVATION AND EMOTION II

344.1

THE CORTICOMEDIAL AMYGDALA AND HAMSTER AGONISTIC BEHAVIOR M.Potegal, C.Ferris and L.Skaredoff* New York State Psych. Institute, NY and University of Mass. Medical Center, MA.

Field observations of many animal species have revealed short term increases in aggressive arousal ocurring during the initial stages of agonistic encounters. We have developed a laboratory model of this phenomenon: "Priming" a female hamster by allowing it a single attack on an intruder increases its aggressive arousal,ie, reduces the latency of attack over the next 30 minutes. We now report immunocytochemical evidence for an increased number of neurons expressing c-fos in the corticomedial amygdala (CMA) and preoptic area of primed animals. Other limbic and hypothalamic nuclei show no such changes. Pursuit and biting of an inanimate object, exposure to a noxious olfactory stimulus, or engaging in sexual behavior, all highly arousing as indicated by activity measures, do not induce CMA c-fos expression of the same magnitude. Radiofrequency lesions of the CMA reduced aggression demonstrating the functional significance of this region. The greatest increases in attack latency and reductions in priming were significantly correlated with larger, more anterior lesions. Other behaviors, including a locomotor practice effect in a running wheel, were unaffected by CMA lesions. Control lesions of cortex and medial septum did not affect priming. These observations suggest that CMA c-fos expression marks a process coupled to aggressive arousal and support the hypothesis that the CMA is part of the neural circuitry mediating this phenomenon.

344.2

SEX DIFFERENCES IN ANTIPREDATOR DEFENSIVE REACTIONS AND THE EFFECTS OF ANXIOLYTIC DRUGS. D.C. Blanchard, J.K.Shepherd*, R.J. Rodgers, R. Agullana*, T. Flores* and R.J. Blanchard Bekesy Laboratory of Neurobiology and Department of Psychology, Univ. of Hawaii, Honolulu, HI 96822

Antipredator defensive reactions in rats comprise a pattern of behaviors (flight/ avoidance, freezing, defensive threat/attack, risk assessment, inhibition of nondefensive behaviors) which vary with relevant situational features, intensity of threat, and immediacy and salience of the threat stimulus. Detailed characterization of these patterns in both males and females indicates fundamental sex differences in the organization of antipredator defense. When freezing is the prepotent response to predatory stimuli, it is more evident in females, and risk assessment is delayed, compared with males. Similarly, females show more defensive ultrasound, and inhibition of certain behaviors (e.g. eating) in situations associated with a predator. Sex differences are not seen in flight or defensive threat/attack, suggesting that enhanced female defensiveness is particularly associated with anticipatory defensive responses.

Sex differences are also observed in anxiolytic drug (diazepam, ethanol) effects on defensive responding. Preliminary studies with the serotonergic 5-HT_{1A} agonist, 8-OH-DPAT and the 5-HT_2 antagonist, ritanserin, indicate greater sensitivity in females, while males seem more sensitive to 5-HT_3 antagonists, MDL72222 and Ondansetron. While some of these effects can be explained in terms of baseline behavior, others seem to reflect true sex differences in drug sensitivity.

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344.3

ANTIPREDATOR ULTRASOUNDS: SEX DIFFERENCES AND DRUG EFFECTS. R.J. Blanchard, S. Weiss*, R. Agullana*, T. Flores*, & D.C. Blanchard. Bekesy Laboratory of Neurobiology, and Department of Psychology, Univ. of Hawaii, Honolulu HI 96822

Brief exposure to a predator for rats living in groups in a burrow system affording concealment elicits 18-27 kHz ultrasounds which may persist for up to an hour following such exposure. When each animal in 12, 1M,1F groups was tested individually with the cat, with ultrasonic cries recorded on a RACAL instrumentation recorder and analyzed with a Kay 5500 digital sonograph, a pattern of sex differences emerged. Males made reliably fewer ultrasonic pulses. However, the mean durations of these were reliably longer, and the base frequency lower than for females. About 70% of male pulses, but less than 25% of female pulses, were of a negatively accelerated, descending pulse form with a generally ascending base frequency.

Effects of alcohol and morphine on these ultrasonic cries have been assessed. Morphine strikingly reduces such ultrasonic vocalizations, while alcohol effects involve dose x time interactions, with reduced persistence at higher doses. The consistent differences between the effects of these compounds on ultrasonic vocalizations, as opposed to their effects on other antipredator defensive responses suggest that such vocalizations may be physiologically as well as functionally different from other antipredator defensive behaviors.

Supported by MH42803, AA06220, RR03061 and RR01825.

344.4

THE PRESENCE OF A SOCIAL COMPANION ALTERS ISOLATION RESPONSES OF 3-DAY OLD RAT PUPS. **S.E. Carden & M.A. Hofer. Columbia Univ. & N.Y.S. Psychiatric Inst., NY, NY 10032**

Three-day old rat pups, isolated in a novel environment, emit ultrasonic calls thought to be predominantly under thermal control (Allin & Banks, Dev.Psychobio., 1971). In 10- and 14-day old pups, the presence of a single anesthetized littermate reduces the rate of ultrasonic vocalization (Carden & Hofer, Behav.Neurosci., 1990; Hofer & Shair, Dev.Psychobio., 1978). We now find that the crying of 3-day old pups is also decreased by the presence of an anesthetized littermate. To determine if this quieting is a function of the body heat of the companion, in a second study, the companion's axillary temperature was lowered to 22°C. The presence of this cool companion also reduced the number of ultrasonic vocalizations. A third study showed that isolation calls were not diminished by the presence of a room temperature, plastic surrogate. Studies on opioid mediation of the companion effect are currently being conducted.

344.5

EFFECTS OF 8-OH-DPAT ON ROUGH-AND-TUMBLE PLAY AND FEEDING IN JUVENILE RATS. S.M. Siviy and L.A. Kerrigan*. Dept. of Psychology, Gettysburg College, Gettysburg, PA 17325.

Serotonin 1A ($5HT_{1A}$) receptors are thought to be involved in a variety of behaviors. To further study the behavioral function of $5HT_{1A}$ receptors in juvenile rats, the effects of the specific $5HT_{1A}$ agonist 8-OH-DPAT (DPAT) on rough-and-tumble play were assessed. Rats (30-45 days old) were housed individually and given a daily 5 minute opportunity to play. Male and female rats were injected with either saline or one of three doses of DPAT (0.125, 0.25, 0.5 mg/kg) 60 minutes prior to a play session. Frequency of pinning was used to assess levels of play. At 0.5 mg/kg, DPAT significantly reduced pinning by 13%. A higher dose (1.0 mg/kg) reduced pinning by 83%. Given that this dose also resulted in a marked "5HT syndrome", the behavioral specificity of this reduction is questionable. The effects of DPAT on feeding were also assessed in satiated juvenile and adult male rats. DPAT (0.1-0.5 mg/kg) had no effect on feeding in juveniles while increasing intake in adults. Since DPAT-induced feeding is thought to be mediated through pre-synaptic autoreceptors, juvenile rats may be differentially sensitive to the presynaptic effects of DPAT. Whether this will be a factor in understanding the extent to which $5HT_{1A}$ receptors are involved in the expression of mammalian playfulness remains to be determined.

344.6

BUSPIRONE EFFECTS ON SOCIAL EMOTIONS IN RATS AND CHICKS. L. Normansell, M. Euken*, M. Porter*, M. Nemunaitis* and D. Pippel*. Dept. of Psychology, Muskingum College, New Concord, OH 43762.

Buspirone (BUS), a 5HT1A agonist, has emotional effects in chicks, including increasing separation-induced distress vocalization (DV) and reducing the latency to jump from an elevated platform to rejoin the flock [Soc Neurosci Abstr 16: 599, 1990]. Following bilateral lesions of the archistriatum which reduced DVs by over 50%, the vocalization-enhancing effect of BUS remained intact. BUS did not change the tendency of control chicks to attempt to escape from the testing arena but dramatically reduced (by almost 70%) that tendency in lesioned chicks.

Chicks were also tested for vocalization in the presence of a large model owl. While controls continued to DV in the presence of this stimulus, BUS-treated chicks attenuated their calling.

Play in juvenile rats was observed following BUS administration. Play was reduced when testing occurred in white lighting rather than red, but this suppression was not attenuated by BUS. Rather, at the highest dose tested (2mg/kg), the number of pins and dorsal contacts were suppressed in the red light condition, perhaps indicating non-specific sedative effects rather than anxiolytic ones. Following chronic administration (5mg/kg, twice/day for 7 days) no anxiolyic effects were noted when animals were tested on an elevated plus maze.

In summary, BUS appears to have more robust emotional effects in chicks than it does in rats. Whether these effects reflect true anxiolytic activity will require more subtle analysis of brain emotional systems.

344.7

MELATONIN MODULATION OF SEPARATION DISTRESS, SOCIAL PLAY AND AFFECT. Eric Nelson*, Jaak Panksepp, and Satoshi Ikemoto, Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403

Melatonin has been implicated in the control of a large number of behavioral processes as well as in the genesis of psychiatric disorders ranging from autism to depression. In the following work we analyzed the effects of melatonin on isolation-induced distress vocalizations (DVs) of domestic chicks, rough and tumble social play of juvenile rats and place-preference conditioning in adult long-Evans rats.

DVs were reduced in a dose dependently by 0.1, 0.5 and 1.0 mg/kg of melatonin. The effects lasted about an hour and were accompanied by drowsiness. Central melatonin (1, 5 & 10 ug, icv) exhibited similar, albeit more modest and shorter-lasting effects. Tolerance to repeated melatonin injections and cross tolerance to morphine was not evident. Naltrexone pre-treatment did not block the effects of melatonin, but chronic naltrexone facilitated melatonin effects. Pinealectomy reduced DVs, but did not change sensitivity to melatonin or effects of darkness.

Melatonin doses up to 1.0 mg/kg had no clear effects on play in rats, and pairing of 0.1 and 1.0 mg/kg of melatonin with distinct environments did not yield either place preferences or place aversions. The data suggest that melatonin reduces separation distress without effecting several other affective behaviors.

344.8

BEHAVIORAL CHARACTERISTICS AND NATURAL KILLER CELL ACTIVITY IN TRANSGENIC TGFα MICE. L.A. Hilakivi-Clarke, P.K. Arora*, M. Burgess*, R. Clarke*, R.B. Dickson* and M.E. Lippman*. Lombardi Cancer Research Center, Georgetown Univ., Washington, DC 20007, and Lab. Neuroscience, NIDDK, Bethesda, MD 20892

Psychosocial factors have been widely implicated in tumorigenesis. Specifically, the ability to cope with stress and certain behavioral characteristics may contribute to an increased risk of developing a cancer. We examined the behavioral patterns associated with stress in apparently healthy 2-3 -month-old male transgenic mice overexpressing the gene encoding human transforming growth factor α (TGFα) (Jhappan et al., Cell 61:1137-1146, 1990). These animals show a high incidence of liver carcinomas at the age of 10-15 months. It was found that the TGFa mice spent a significantly longer time immobile (Mann-Whitney U test; $U=15$, $n=10+10$, $p<.02$) in Porsolt's swim test, a model of depressive behavior, and an elevated time showing aggressive behavior ($U=19$, $n=10+10$, $p<.02$) in the resident-intruder test of aggression, as compared with control CD1 mice. The transgenic mice did not differ from the controls in the plusmaze test of anxiety or in respect to voluntary alcohol intake. To investigate the possible biological mechanisms mediating these behavioral changes, we measured the natural killer (NK) cell activity in the spleen. This data showed that TGFα mice exhibited 25% lower NK cell activity than the controls (two-way ANOVA; $F(1,23)=8.26$, $p<.01$). We are in the process of assessing the levels of steroid hormones from the blood in these animals.

The preliminary data indicate that transgenic male mice overexpressing TGFα exhibit behaviors characteristic of an impaired ability to cope with stress and increased aggressivity. These changes may be associated with reduced NK cell activity.

344.9

EFFECTS OF MEDIAL HYPOTHALAMIC STIMULATION UPON MASSETER EMG ACTIVITY IN THE CAT. S. Weiner*, S.N.V. Rao*, S. Dunn*, L. Laemle and A. Siegel. Dept. of Prosthodontics, NJ Dental School and Dept. of Neuroscience, NJ Medical School, Newark, NJ 07103.

Little is presently known concerning the neural substrates for jaw muscle hyperactivity. Therefore, we utilized the mean power frequency (MPF) of the masseter muscle following electrical stimulation of the medial hypothalamus (MH) as a model of centrally mediated muscle hyperactivity in the cat. EMG recordings were made from the masseter muscle with bipolar surface electrodes prior to, during, and immediately after electrical stimulation (0.2-0.6mA) of MH at which affective defense behavior could be elicited. Recordings were also made at rest and during forceful biting activity unrelated to MH stimulation. EMG data were collected in 1 sec intervals with a sampling rate of 2048 Hz and then converted into digital form. Fast Fourier Transforms were calculated and the MPF were determined. During MH stimulation, MPF were significantly higher than MPF observed at rest ($p<.01$). Immediately after stimulation, MPF were significantly lower than at rest ($p<.01$). Since MH stimulation and forceful biting both produced equally elevated levels of MPF, it is argued that MH stimulation can serve as a useful model for the study of centrally mediated jaw muscle hyperactivity.

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344.10

EFFECTS OF AMPHETAMINE AND SULPIRIDE ON EXPLORATORY BEHAVIORS IN RATS WITH FORNIX KNIFE-CUTS. T.L. Steele and M. Williams*, Dept. of Psych., Univ. of Wis. Oshkosh, Oshkosh, WI 54901.

Rats with lesions of the hippocampus often display deficits in learning and memory. These rats are also frequently stereotypic, hyperactive, and inattentive (1), behaviors which oppose exploration and learning. Similar behaviors are noted in rats given d-amphetamine (AMPH, 2), suggesting a role for dopamine (DA) in the modulation of exploratory behaviors. It was therefore predicted that enhancement of DA activity would potentiate exploration deficits whereas blockade of DA would attenuate these deficits in rats with fornix knife-cuts.

In Study 1, rats with sham (S) or fornix knife-cuts (F) were given 0, 1.5, or 3.0 mg/kg AMPH and placed in a large arena comprised of multiple familiar and novel features. In Study 2, food-deprived S and F rats were given 0, 30, or 60 mg/kg sulpiride and placed in a modified arena which also consisted of familiar and novel features. Rats were observed for 10 min per day for 10 days in each study.

Relative to F rats, S rats displayed more dispersed exploration, devoted more time to novel aspects of the arena, and exhibited different patterns of exploration. AMPH significantly reduced exploration. Both knife-cuts and AMPH produced responses reminiscent of behavioral traps. Although sulpiride altered certain aspects of exploration, the overall effects were not robust.

These results demonstrate that the hippocampus (via the fornix) influences exploration, potentially through modulation of mesolimbic DA activity. The role of sulpiride in reducing exploration deficits, however, remains speculative.

(1) Steele, T.L. & Devenport, L.D. (1986). Neurosci. Abs., 12, 743.
(2) Steele, T.L. & Devenport, L.D. (1989). Neurosci. Abs., 15, 1252.

BIOLOGICAL RHYTHMS AND SLEEP VII

345.1

CHRONIC RO 15-1788 TREATMENT INCREASES REM SLEEP IN RATS. S.D. O'Connor, M. Urbancic, T.J. Marczynski and M. Radulovacki. Dept of Pharmacology, University of Illinois, Chicago, IL 60612

Ro 15-1788, a benzodiazepine (BDZ) receptor antagonist, was shown to have BDZ agonist properties which manifested in increased sleep when the drug was administered to dogs and humans. In the present study, we examined the effect of chronic administration of Ro 15-1788 on sleep in rats. Ro 15-1788 (3.6 mg/kg/day in drinking water for 14 days) increased rapid eye movement (REM) and total sleep during a standard six hour EEG recording, with the increases in total sleep time due entirely to increases in REM. EEG recordings were obtained on days 0, 1, 3, 7, 10, 14, as well as 24 and 72 hours following drug withdrawal. Enhanced REM sleep reached significance on day 7 of continuous drug treatment and remained significantly increased on days 10 and 14, as well as at 24 and 72 hours following drug withdrawal. Ro 15-1788 may exert its hypnotic effect either through the adenosinergic system involving inhibition of adenosine re-uptake, as was suggested for the mechanism of hypnotic action of BDZ's, or through the ability of BDZs to increase REM sleep via the disinhibition of the cholinergic system.

345.2

REM SLEEP SUPPRESSION INDUCED BY SEROTONIN UPTAKE BLOCKADE: EVIDENCE FOR A TYPE 2 RECEPTOR MECHANISM. R.J. Ross, S.I. Peymer*, L.D. Sanford*, W.A. Ball and A.R. Morrison. V.A.M.C. and Depts. of Psychiatry and Animal Biology, Univ. of Penna. Schools of Med. and Vet. Med., Phila., PA 19104.

Acute serotonin (5HT) uptake blockade by sertraline (SER) suppresses REM sleep (REMS) in the cat. To investigate the underlying serotonergic receptor mechanism(s), the effect of the relatively specific 5HT-2 antagonist ketanserin (KET) was investigated. Five cats were implanted with EEG, EOG, EMG, and lateral geniculate electrodes. Cats underwent 6-hr. polysomnographic testing under the following 5 conditions: 1) placebo capsule plus 3 cc saline, i.p.; 2) SER (1.0 mg/kg, p.o.) plus 3 cc saline, i.p.; 3-4) SER (1.0 mg/kg, p.o.) plus KET (either 1.0 mg/kg or 3.0 mg/kg, i.p.); and 5) placebo capsule plus KET (3.0 mg/kg, i.p.). Study condition order was counter-balanced, and all trials were separated by 1 week. KET co-administration reduced the inhibitory effect of SER on REMS% (REMS time/total sleep time), which increased from 4.0 (cond. 2) to 10.7 (cond. 3) and then 14.1 (cond. 4); yet REMS% remained lower than the placebo level (25.0). KET alone (cond. 5) increased REMS% to 31.5. Putative level of 5HT-2 receptor activation (cond. 2 > 3 > 4 > 1 > 5) correlated with REMS% ($r = -0.94$; $p < 0.01$). Thus, a 5HT-2 mechanism may partially explain SER's REMS suppressant effect. That 5HT-2 receptors are located primarily in the cortex suggests that some serotonergic modulation of REMS may occur rostral to the pons, where REMS control otherwise is thought to reside. Supported by VA Med. Res. Serv. and MH42903.

345.3

THE ROLE OF THE M1 MUSCARINIC RECEPTOR SUBTYPE IN REM SLEEP. R.K. Zoltoski, M.D. Shalauta*, and J.C. Gillin. Dept. of Psychiatry, San Diego VAMC and University of CA, San Diego, La Jolla, CA 92093.

Microinjections of carbachol (carb) into the peri-α-locus coereleus (α-LC) of cats induce rapid eye movement (REM) sleep. This response is blocked by pretreatment with atropine; however, the specific cholinergic receptors mediating the carb induced effects are not fully known. To study this, cats were surgically implanted with sleep recording electrodes and bilateral cannulas targeted for the α-LC region. Pirenzepine (a specific antagonist of the M1 receptor) (pir, 5μg) or saline (sal, 0.1μl) was administered bilaterally 15 minutes prior to the administration of either carb (0.4μg) or sal (0.1μl) in a randomized, repeated measures fashion. REM sleep was defined as presence of low frequency EEG, theta, PGO spikes, and atonia. Administration of sal and carb decreased latency to a dissociated REM state, characterized by all components of REM sleep, except theta waves; it also decreased slow wave sleep. Pir blocked this carb-induced effect, with the exception of the decreased latency of PGO spikes. Pir alone did not change the states measured. We hypothesize that the action of carb is mediated in part by the M1 muscarinic subtype. More studies involving different doses as well as other drugs are necessary to understand which cholinergic receptors (M1,M2, nicotinic) mediate REM sleep.

345.4

EVOCATION OF POSTURAL ATONIA AND RESPIRATORY DEPRESSION BY PONTINE CARBACHOL IN DECEREBRATE RATS. O. Taguchi*, L. Kubin, S. Manaker, and A.I. Pack*. Center for Sleep and Respiratory Neurobiology and Department of Animal Biology, University of Pennsylvania, Philadelphia, PA 19104

Pontine microinjections of carbachol in decerebrate cats produce a prolonged postural atonia similar to that occurring during rapid eye movement (REM) sleep (Morales, J Neurophysiol 57:1118, 1987) and respiratory depression (Kimura, J Appl Physiol 60:2280, 1990). This novel, acute preparation may be very useful for electrophysiological studies of the neural mechanisms activated in REM sleep and narcolepsy. However, for neurochemical and *in vitro* studies, development of a corresponding acute rat model would be attractive. To verify the viability of such studies in rats, carbachol (25-50 nl; 10 mM) was microinjected unilaterally into the pons of Sprague-Dawley rats decerebrated at a precollicular level. The EMG of dorsal neck and intercostal muscles was recorded. Eleven injections performed in 8 rats resulted, within 3 min, in a profound depression of neck and intercostal muscle tonic activity and a slowing of the respiratory rate (to ~74% of control). The episodes lasted 8-28 min (mean: 15 min) and terminated abruptly with the return of all parameters to pre-injection levels. The injection sites were localized within the dorsal regions of oral and caudal pontine reticular nuclei. Thus, as in cats, pontine carbachol in decerebrate rats produces postural atonia and respiratory depression. In cats, however, the episodes last over 1 h and terminate gradually. Still, the episodes in rats are substantially longer than individual periods of natural REM sleep. Their abrupt termination suggests that mechanisms beyond the injection site determine the duration of the atonia. (SCOR HL-42236)

345.5

REM AND SLOW WAVE SLEEP-LIKE STATES IN THE WHOLE GUINEA PIG BRAIN MAINTAINED IN VITRO. M. Moga, D. Paré, M. deCurtis and R. Llinás. Dept. Physiology and Biophysics, New York Univ. Med. Ctr., NY, NY 10016.

As a prelude to studies of cellular mechanisms underlying different sleep states, we examined whether REM and slow wave sleep-like states can be pharmacologically induced in the adult whole guinea pig brain maintained in vitro (31-33°C). Bipolar electrodes were positioned in specific thalamic nuclei and their respective cortical projection fields under physiological control, i.e., by stimulation of their specific prethalamic afferents. In addition, a suction electrode was placed on cranial nerve III. Focal ECG recordings revealed the presence of spontaneous (6-10Hz) spindles occurring every 4-6sec throughout the neocortex in synchrony with spindles recorded from the corresponding thalamic nucleus. Muscimol injections (2-8µg) into the lateral hypothalamus produced cortical slow wave activity (1-4Hz) of high amplitude (1 mV). Microinjections of carbachol (4-8µg) into the pontine tegmentum produced two types of PGO waves in the lateral geniculate nucleus and occipital cortex: isolated PGO waves (100-200msec) and bursts of 3-6 waves per 1-2sec. Following carbachol injections, cortical spindles decreased in amplitude and were replaced by ECG desynchronization. Animals pretreated with p-chlorophenylalanine (PCPA, a serotonin synthesis inhibitor, 35mg/150mg for 3 days) showed spontaneous PGO wave activity in the dorsolateral pons, occipital cortex and lateral geniculate nucleus, and bursting in the oculomotor nerve. In PCPA-muscimol treated animals, both PGO waves and cortical slow waves were present simultaneously, suggesting some state dissociation.

345.6

AUDITORY STIMULATION ENHANCES REM SLEEP IN YOUNG AND OLD FISCHER 344 RATS. **G. Arankowsky*, W.S. Stone and P.E. Gold, Department of Psychology, U. Virginia, Charlottesville, VA, 22903.**

As in other mammals, aged rats exhibit profound deficits in sleep patterns, particularly including deficits in rapid eye movement (REM) sleep.Auditory stimulation (AS) applied during (REM) sleep enhances the duration of REM sleep in cats (Drucker - Colin et al, Br.Res 278 : 308 - 312, 1983). The present experiment investigated whether AS would enhance REM sleep in young (6 month - old) and aged (22 month - old) rats. Two 4-hr baseline sleep records were obtained for each rat prior to AS tests. In young rats, sleep stages were monitored during the AS test sessions (4 hrs) to allow administration of AS (2 KHz, 75 dB, 100 msec each 20 sec throughout each REM sleep bout) during REM sleep. Because the duration of REM sleep bouts is quite short in aged rats, AS could not be administered during these bouts. Therefore, old rats received AS on a fixed schedule -- 10 min of AS (as above) alternating with 15 min quiet. In young rats, AS enhanced REM sleep bout duration (101 ± 6 vs. 179 ± 10 sec, P < 0.001) and enhanced total REM sleep time (23 ± 2 vs. 27 ± 2 min) but reduced the number of REM sleep periods (14 ± 0.8 vs. 10 ± 0.6). Baseline REM sleep measures were substantially depressed in old rats. In the old rats, AS enhanced total REM sleep time (14 ± 3 vs 21 ± 6 min, P < 0.05) and enhanced the number of REM sleep periods (13 ± 3 vs. 16 ± 2, P < 0.05), but did not significantly increase the duration of REM sleep bouts (70 ± 7 vs. 74 ± 3 sec). Thus, AS is an effective manipulation with which to augment REM sleep in young rats.AS also augments REM sleep measures in aged rats, but does not restore them to levels seen in young rats. [Supported by NIA (AG 07648), NSF (BNS - 9012239), and by DGAPA and Fogarty postdoctoral Fellowships to GA].

345.7

ELECTROLYTIC LESION OF LOCUS COERULEUS (LC) AUGMENTS PARADOXICAL SLEEP (PS) IN FEMALE RATS, BUT NOT MALES. J. Fang, S. W. Yang* and W. Fishbein. Dept. of Psychology, The City College & Graduate School, CUNY, New York 10031

In previous NS meetings we reported that (1) male rats have more PS than females and (2) this sexual dimorphism is influenced by neonatal manipulation of sex steroids. The present experiment is the first to examine the brain mechanism(s) involved in this phenomena. Since the LC is larger and embodies more neurons in females than males, we examined whether the inhibitory influence of the LC on PS may be different between the sexes.

Under anaesthesia we bilaterally lesioned the LC in both male (ME, n=7) and female (FE, n=8) adult (105-130 days) Sprague-Dawley rats by passing 1.0 mA direct current through stereotaxically placed electrodes. In all respects the control males (MC, n=9) and females (FC, n=8) were identically treated, with the exception that they were not lesioned. After 11 days recovery from lesion and sleep electrode implantation, sleep/wakefulness cycles were continuously recorded for 4 days. Verification of the lesions was determined by tyrosine hydroxylase stained brain sections using the immunohistochemistry technique.

Results: (1) Total sleep time and SWS are indistinguishable in all groups; (2) MC animals spend 4.08 min/hr in PS, FC 2.82 min/hr, reconfirming ($p<0.02$) our previous findings; (3) LC lesions increase PS significantly in females (4.45 min/hr; FE vs FC, $p<0.001$) but not males (ME, 4.27 min/hr). Partialled analyses shows that the PS increase is mostly found in the day time.

The results strongly suggest that the sex difference of PS in rats is mediated by the LC and its neuron projections.

345.8

REM=SLEEP DEPRIVATION DIMINISHES NEUROTRANSMIT= TER LEVELS IN THE HYPOTHALAMUS AND FRONTAL CORTEX OF RATS: T.Porkka-Heiskanen,D.Stenberg and T.Taira*. Department of Physiology, University of Helsinki, Siltavuorenpenger 20 J, 00170 Helsinki, Finland.

Adult male rats were deprived of REM-sleep for 72 hours. The deprivation group was kept on small platforms (diameter 6.5 cm) in a water bath, and the control group on large platforms (11 cm). In addition one group was kept in a cage that was not filled with water. The deprivations ended in the morning and the rats were decapitated either directly or having been allowed to sleep for 5 hours.Frontal cortex and hypothalamus were rapidly dissected, frozen and stored in -80 C until the assays. Monoamines were measured by HPLC with EC-detection.
In the anterior part of the hypothalamus the serotonin and noradrenaline concentrations of the REMs deprived rats were lower than those of the control groups. The dopamine concentrations of the deprived rats were lower in the posterior part of the hypothalamus. In the frontal cortex serotonin concentrations of deprived rats were lower than those of the controls. There was no recovery of the monoamine concentrations during the rebound sleep.

345.9

PGO-WAVE SUPPRESSION DURING REM-SLEEP IN MONOCULARLY DEPRIVED KITTENS INCREASES LGN RELAY CELL PLASTICITY. J.P. Shaffery, G.A. Marks, S.G. Speciale and H.P. Roffwarg. U.T. Southwestern Medical School, Dallas, Texas 75235.

An ontogenetic function for REM-sleep has been advanced proposing that the large amounts of endogenous CNS activation, which characterizes this state, is a necessary component of normal CNS development and maturation. Monocular deprivation (MD) studies have demonstrated that visual experience during a "critical period" of development strongly influences cellular plasticity in LGN. We have previously observed that restricting REM-sleep during this "critical period" in MD kittens exaggerates the effects of MD alone on cell sizes in LGN. In addition to tonic activation of the EEG, a cardinal feature of REM-sleep is the periodic appearance of PGO-waves. Prior to the "critical period", bilateral pontomesencephalic lesions of ascending pathways, which carry brainstem-generated PGO-waves, have been shown to affect both cellular growth and number in LGN. In our study, these lesions, which spare the tonic aspects of REM-sleep, were performed during the "critical period" on kittens that had been monocularly deprived for one week and allowed a post-lesion survival of an additional week. To date, three MD kittens have received bilateral lesions removing PGO-waves. In one animal, PGO-waves could not be recorded, so no lesion was made. In a preliminary analysis, LGN cell size data from the three lesioned kittens were compared to data from the non-lesioned kitten pooled with data from four other MD-only controls from another study. In the LGN ipsilateral to the occluded eye, interlamina cell size differences in lesioned kittens were significantly larger ($p<0.05$) than in controls. This new evidence supports our ontogenetic hypothesis of REM-sleep function during CNS development.

345.10

RELATIVE AMPLITUDE OF ELICITED PGO WAVES IS CORRELATED WITH BEHAVIORAL ORIENTING A.R. Morrison, L.D. Sanford*, W.A. Ball, and R.J. Ross. Depts. of Anim. Biol. and Psychiatry, Univ. of Penna. and VAMC, Phila, PA 19104

During REM sleep the brain exhibits electrophysiological signs (low voltage, high frequency EEG; hippocampal theta) indicative of alerting in waking (W), and cats with lesions in the dorsolateral pons that eliminate atonia during REM (REM-A) may exhibit orienting-like behaviors in REM-A. Pontogeniculo-occipital waves (PGO) occur spontaneously in REM, and PGO-like waves may be elicited (PGO_E) in the lateral geniculate body by auditory stimuli (S) during W, non-REM (NREM), and REM. To determine the relationship between PGO_E and overt behavioral orienting (OR), we presented S to 12 cats in W, NREM and REM on two separate days one week apart. On each test day a minimum of five blocks of 40 (or more) S (tones or white noise, 90 msec, 90-100 dB, 1 or 4 kHz, 2 sec ISI, 20 min between blocks) were presented in W. Then, S were presented throughout a REM or NREM episode and in a final block in W. OR did not occur in REM or NREM. OR was seen most often during the initial trials in each W block on each test day. Mean PGO_E amplitude (AMP) was higher in REM than in NREM ($p < .006$) and W ($p < .001$). PGO_E of smaller amplitude persisted after OR had habituated. However, the largest PGO_E AMP occurred on the first one or two trials of each block in W, and they were comparable to PGO_E AMP in REM. That high-AMP PGO_E in W are closely associated with OR in W suggests that the relative AMP of PGO signals more than the mere sensory registration of S. With as little as a 20 min pause between S, renewal of S presentations appears to reintegrate OR and large AMP PGO_E. Because high-AMP PGO occur spontaneously in REM and PGO_E of similar AMP occur during OR in W, the spontaneous PGO of REM may mark the endogenous activation of mechanisms underlying OR that are typically triggered by S in W. Renewal of large amplitude PGO_E may hinge on the recruitment of activity of a larger number of neurons, the activity of which precedes spontaneous PGO at varying latencies in REM. Supported by MH42903, MH18825 and the VA Med. Res. Serv.

345.11

ELICITED AND SPONTANEOUS WAVEFORM ACTIVITY IN LATERAL GENICULATE BODY AND THALAMIC CENTRAL LATERAL NUCLEUS ACROSS BEHAVIORAL STATES. L.D. Sanford*, A.R. Morrison, W.A. Ball, R.J. Ross and G.M. Mann. Depts. of Anim. Biol. and Psychiatry, Univ. of Penna. and VAMC, Phila., PA 19104

Ponto-geniculo-occipital waves (PGO) in the lateral geniculate body (LGB) and PGO-like waves in the thalamic central lateral nucleus (CL) occur spontaneously during rapid eye movement sleep (REM). Loud auditory stimuli elicit PGO-like waves in LGB (PGO_E) and CL (CL_E) in waking (W). Both may reflect activity in mechanisms involved in alerting. To determine whether changes in behavioral state would affect patterns of responses in PGO_E and CL_E, we presented long series of tones to cats in W, REM and non-REM (NREM). We also recorded spontaneous activity in CL and LGB in all states. Seven cats were presented with four blocks of 40 tones (100 dB, 2 sec ISI, 4 Khz) in W, followed by tones presented throughout REM or NREM, and a final 40 tones in W. REM and NREM were tested on separate days one week apart in a counterbalanced order. PGO_E magnitude (MAG; combined measure of amplitude and occurrence) was higher in REM than in W ($p < .014$), yet was not significantly greater in NREM than in W. CL_E MAG was not significantly greater in REM or NREM than in W. Each REM and NREM period was divided into quartiles. PGO_E MAG decreased linearly across quartiles in REM ($p < .024$) with a similar, but non-significant pattern of decline in NREM. CL_E MAG did not decrease across quartiles in either sleep state. Just as CL_E did not mimic the pattern of PGO_E, neither did spontaneous CL waves mimic the characteristic pattern of PGO in sleep. Spontaneous waves during REM in CL occurred less frequently and generally appeared as single waves rather than bursts. The dissimilarity between patterns of spontaneous PGO and CL waves and the difference in responsivity of elicited waves to tones in REM suggest that mechanisms producing each may have a different, perhaps functionally related, role in alerting. Supported by MH42903, MH18825 and the VA Med. Res. Serv.

345.12

PHARMACOLOGICAL STUDY OF BASAL FOREBRAIN NEURONS IN GUINEA PIG BRAIN SLICES. A. Khateb, M. Serafin, B.E. Jones, A. Alonso and M. Mühlethaler, *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and *Montreal Neurological Institute, McGill University, Canada H3A 2B4*

Providing a major innervation to the cerebral cortex, cholinergic neurons lie within the basal forebrain (BF) in amongst other cells, including GABAergic neurons. Collectively these cells sit in the path of ascending fiber systems originating in part within the brainstem and including noradrenergic, serotoninergic, and also cholinergic fibers. Extending from preliminary results that revealed a class of BFn that exhibit oscillatory properties with low threshold calcium spikes, the potential influence of the chemically specific afferent input upon this class of cells and its properties were further investigated in the guinea pig slice by pharmacological means. Cells were recorded within the region of the horizontal limb of the diagonal band and substantia innominata, where choline acetyl transferase (ChAT)-immunoreactive neurons have been localized, and filled with biocytin in order to study their morphological and histochemical identity. The cells were strongly depolarized and excited by noradrenaline. In contrast, they were consistently hyperpolarized and inhibited by serotonin. The responses of these neurons to cholinergic agonists demonstrated that they are depolarized and excited by nicotine but hyperpolarized by muscarine. These results indicate that certain BFn may be strongly influenced by brainstem afferents, notably excited by noradrenergic input from the locus coeruleus yet inhibited by serotoninergic input from the midbrain raphe. Although extrinsic cholinergic input may excite BFn via a nicotinic receptor, it or intrinsic cholinergic processes may inhibit these cells, which if cholinergic may involve a muscarinic autoreceptor. (Supported by the Swiss NSF and Canadian MRC)

345.13

EXCITATION OF BASAL FOREBRAIN NEURONS BY PEDUNCULOPONTINE STIMULATION. R. Szymusiak, S. Morairty and D. McGinty. VAMC Sepulveda, Depts. Anat. & Cell Biol., Neurosci., and Psychology, UCLA.

A prominent group of cholinergic neurons is located in the pedunculopontine tegmentum (PPT). PPT neurons project to several thalamic nuclei, exert excitatory effects on thalamic relay neurons, and are thought to contribute tonic arousal influences during EEG activated states such as waking and REM sleep. PPT neurons also project to the magnocellular basal forebrain (BF), an extrathalamic area implicated in the control of cortical activation, and, therefore, might be expected to exert excitatory effects on some BF cell types.

Adult cats were implanted with PPT stimulating electrodes and microwires for chronic BF unit recordings. Approximately 35% of BF units exhibiting spontaneous waking discharge responded to PPT stimulation with evoked excitation. Latency to onset averaged 4.9 ± 0.4 ms and mean latency to peak response was 7.3 ± 1.1 ms ($n=14$). Each PPT pulse evoked an average of 1.4 ± 0.2 action potentials and the mean duration of excitatory responses was 8.7 ± 1.3 ms. Latency and duration values suggest that evoked responses were predominately multisynaptic. PPT stimulation also enhanced responses evoked in some BF neurons by stimulation of other brain sites (e.g., medial thalamus). This increase in synaptic responsiveness persisted for about 10 ms after single PPT pulses, and for as long as 25 ms following brief high frequency trains (300 Hz, 3 pulses). BF cells exhibiting PPT-evoked responses had high spontaneous discharge rates in waking (17.1 ± 2.6 Hz) and REM sleep (14.6 ± 3.2 Hz) compared to slow-wave sleep (6.7 ± 1.5 Hz).

345.14

INVESTIGATING THE MECHANISMS OF AUDITORY PONTO-GENICULO-OCCIPITAL (PGO) WAVES IN THE CAT. G.A. Marks, J.P. Shaffery and H.P. Roffwarg. Department of Psychiatry, U.T. Southwestern Medical School, Dallas, Texas 75235.

PGO waves are gross field potentials that occur in association with REM sleep. Loci from which PGO waves can be recorded span diverse neurophysiological systems. Of interest to us has been the REM sleep-related phasic activity uncovered in the auditory system, specifically the cochlear nucleus (CN).

After the procedures of Hu et al [1], we have chosen to study "PGO-like" waves recorded in the acute reserpinized cat under urethane anesthesia. Reserpine was administered (5mg/kg, p.o.) and followed 18 hours later by urethane (1.0g/kg, IP). The cerebellum was partially aspirated to uncover the dorsal medullary surface, which thereafter was continuously bathed in warmed artificial cerebrospinal fluid. Electrode placement into the CN was achieved under direct visual guidance.

Preliminary data reveal that reserpine PGO waves can be recorded from the dorsal CN (DCN) with a bipolar electrode (200u). Reserpine's action, during most of the procedure, results in the continuous rhythmic appearance of waves at a rate of about 40/min. Examined simultaneously, waves in the lateral geniculate nucleus had the same pattern as in the DCN. Several single units were recorded in the anterior ventral CN (AVCN). Cells are identified by their response to tone burst. To date, we have found only a weak association between a few AVCN units' discharges and occurrence of PGO waves.

We plan to study unit activity in the DCN, which may give rise to the field potential activity and to study the transmitter-receptor interactions underlying PGO phenomena.

1. Brain Research 1988, 473:394-397.

345.15

APPLIED HEAT LOADS EVOKE DELAYED ENHANCEMENT OF SLOW WAVE SLEEP IN RATS. S. Morairty*, R. Szymusiak and D. McGinty. VAMC Sepulveda, Depts. of Neurosci., Anat. & Cell Biol., and Psychol., UCLA.

There are strong positive correlations between slow wave sleep (SWS) and brain or body cooling. Further, increasing brain or body temperature has powerful sleep-promoting effects. In humans, late afternoon heat loads give rise to a delayed increase in SWS during the subsequent nocturnal sleep period. These data have suggested the hypotheses that SWS is a homeostatic process involved in brain and body cooling and is controlled by thermoregulatory mechanisms. However, there are no previous studies examining the delayed effects of heat loads on subsequent SWS in experimental animals.

Male Sprague-Dawley rats were implanted with thermocouples into the medial preoptic hypothalamus and with standard cortical EEG and neck EMG electrodes. During the last 4 hours of the light period, ambient temperature was increased to keep brain temperature (T_{br}) between 39.5 - 40.5 °C. Under the control condition, rats were limited to the hourly sleep averages recorded during the corresponding heating period.

During the first 4 hours of the dark period following heating, SWS was increased and T_{br} was reduced compared to both baseline and control conditions. Paradoxical sleep was also enhanced but less consistently and to a lesser extent. These results support the hypothesis of a mechanism that can store information about heat loads during wakefulness. Such a 'memory' could induce delayed compensation for heat loads through sleep-related cooling.

345.16

CEREBRAL METABOLISM AND PROTEIN SYNTHESIS DURING SLEEP IN THE RAT. P. Ramm. Dept. of Psychology, Brock Univ., St. Catharines, Ontario, Canada, L2S 3A1

Slow wave sleep (SWS) has been shown to conserve cerebral energy expenditure, and this conservation may favor or reflect alterations in any number of fundamental biochemical processes. Most prevalent are suggestions that sleep states favor the synthesis of cerebral proteins. We use quantitative autoradiography to measure regional cerebral metabolism and protein synthesis during sleep and wakefulness. We have previously reported that, in sleep-deprived animals (48-72 hr platform deprivation), SWS is linked to lower rates of cerebral metabolism (1) and higher rates of cerebral protein synthesis (2). We now report that, following very short deprivation, we continue to observe lower rates of metabolism, but fail to observe higher rates of protein synthesis during SWS.

Four days after surgery and following 3 hr of manual sleep deprivation, polygraphic and power spectral recording were initiated. Standard procedures for quantitative 14C-2-deoxyglucose or L-[1-14C]leucine autoradiography were performed during a 45 min incubation period, while the freely moving rats engaged in sleep/wake behavior. Autoradiographs were then prepared and showed that animals exhibiting more SWS also exhibited significantly lower rates of cerebral metabolism and of cerebral protein synthesis ($r_{xy} = -0.63$, $p < 0.05$).

These data converge with reports of cerebral energy conservation during SWS. However, they contrast with our previous observation of higher rates of protein synthesis associated with SWS in sleep-deprived rats. The present animals did not show the very deep SWS patterns seen following more extensive deprivation. A difference in sleep need may underlie the contradiction between synthesis rates observed in deprived and non-deprived animals during SWS.

1. Ramm P. and Frost B.J. *Brain. Res.* 365: 112-124, 1986.
2. Ramm, P. and Smith, C.T. *Physiol. Beh.* 48: 749-753, 1990.

345.17

SOMNOGENIC AND PYROGENIC ACTIVITY OF TUMOR NECROSIS FACTOR-ALPHA (TNFa), -BETA (TNFb) AND FRAGMENTS OF TNFa. L. Kapas[1], A.B. Cady[1], M.R. Opp[1], A.E. Postlethwaite[2], J.M. Seyer[3], J.M. Krueger[1]. Departments of Physiology and Biophysics[1], Medicine[2], and Biochemistry[3], University of Tennessee, Memphis, TN 38163.

It is hypothetized that fever and increased sleep during acute infections are caused by cytokines. In fact, exogenous administration of IL-1, IFNa2 and TNFa elicits fever and enhances non-rapid-eye-movement sleep (NREMS). We investigated the effects of TNFa, TNFb and nine TNFa fragments on sleep and brain temperature using a rabbit model previously described[1]. TNFa and TNFb elicited dose-dependent increases in NREMS and biphasic fevers. Four TNFa fragments with overlapping sequences ($TNFa_{10-36}$, $TNFa_{10-69}$, $TNFa_{31-45}$ and $TNFa_{31-68}$) were somnogenic with a threshold dose of 25 ug, two of them ($TNFa_{10-36}$ and $TNFa_{31-68}$) also elicited a monophasic fever. Two fragments ($TNFa_{46-65}$ and $TNFa_{69-100}$) induced monophasic fevers but not sleep. Three other peptides ($TNFa_{44-68}$, $TNFa_{101-135}$ and $TNFa_{140-157}$) lack both hypnogenic and pyrogenic activities. These results indicate that the TNFa molecule has multiple active centers. Hypnogenic peptides contained amino acid residues 31-36, which is an exposed loop region in the three-dimensional structure of TNFa known to be important for biologic activity. Similar results were previously obtained using IL-1b fragments[1]. Such findings suggest the possibility that posttranslational and/or postreceptor hydrolysis of cytokines could provide a degree of tissue-response specificity.

[1]Am. J. Physiol. 1990 259:R439-R446. Supported by: NS 25378

345.18

METABOLIC FUEL UTILIZATION DURING HIBERNATION AND AROUSAL. J. Dark and N.F. Ruby, Dept. of Psychology, UC-Berkeley, Berkeley CA 94720

The golden-mantled ground squirrel survives winter by hibernating; metabolic needs are reduced by decreasing body temperatures (T_b) to ~2-3°C. Hibernation consists of a series of individual torpor bouts 2-14 days long, punctuated by periodic arousals of <24 h. Energy during hibernation is derived from adipose tissue stores. This experiment evaluated the relative contribution of glucose and fatty acids during torpor and arousal using 2-deoxy-D-glucose (2DG) and mercaptoacetate (MA).

T_b was monitored telemetrically. ß-oxidation of fatty acids and glycolysis were disrupted by MA and 2DG, respectively, during hibernation and forced arousals. Animals received MA, 2DG, and saline in a randomized order. MA induced arousals, but 2DG did not. Animals treated with 2DG did not differ from those injected with saline. Treatment with either 2DG or MA during an arousal did not affect latency to rewarm. Concurrent treatment with 2DG and MA, however, significantly retarded the rewarming process.

These data suggest several conclusions: Torpor depends upon a specific metabolic fuel; fatty acids are necessary and glucose is neither necessary nor sufficient to sustain torpor. Mechanisms sensitive to fatty acid availability appear to function at the low T_b's of hibernation. Fatty acid availability may influence torpor duration. Rewarming depends upon the general availability of metabolic fuels, rather than a specific fuel.

345.19

MONOAMINE AND METABOLITE LEVELS IN CEREBROSPINAL FLUID OF HIBERNATING AND EUTHERMIC YELLOW-BELLIED MARMOTS. M.S.Reid*, F.L.Watson*, L.M.Romero, T.S.Kilduff, E.Mignot*, H.C.Heller*, W.C. Dement. Sleep Disorders Res. Ctr., Stanford Univ., Stanford, CA 94305.

The levels of monoamines and their metabolites in the cerebrospinal fluid (CSF) of yellow-bellied marmots, *Marmota flaviventris*, during euthermic and hibernating states were investigated. The concentrations of dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine and serotonin in CSF samples were assayed by HPLC-EC. General population studies showed that dopamine levels were decreased ($p<0.05$), while DOPAC ($p<0.001$) and HVA ($p<0.001$) levels were greatly increased in hibernating marmots. Norepinephrine ($p<0.05$) and serotonin ($p<0.01$) levels were also increased during hibernation. In paired population studies using a group of three marmots sampled during both euthermic and hibernating states the results were similar. These findings are consistent with previous studies showing a modulation of dopamine, DOPAC, HVA and 5HT levels in discrete brain regions of the hibernating ground squirrel, suggesting a role of monoaminergic neurotransmission in the regulation of hibernation. (Supported by The Upjohn Company)

345.20

EFFECTS ON SLEEP AND BRAIN TEMPERATURE OF BILATERAL ETHANOL MICROINJECTION TO THE PREOPTIC AREA OF RATS. S. R. Ticho, G. Lekovic*, M. Stojanovic* and M. Radulovacki. Dept. of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612.

We have examined the effects on sleep and brain temperature of bilateral intracerebral administraion of 0.55%, 2.75%, and 5.5% ethanol to the preoptic area of rats. 2.75% and 5.5% ethanol significantly increased total sleep by 18% and 24%, respectively, during the 6 hr polygraphic recording period. Analysis of the various sleep states revealed that the enhancement of total sleep was due primarily to an increase in deep slow-wave sleep but the 2.75% dose also significantly increased REM sleep by 89%. Recording of brain temperature for 3 hrs after ethanol microinjection at all three doses showed no significant differences from saline microinjection. These observations support the hypothesis that the preoptic area may be the site of action for the sedative effects of ethanol in rats.

BIOLOGICAL RHYTHMS AND SLEEP VIII

346.1

ELECTROENCEPHALOGRAPHIC CORRELATES OF AROUSAL IN THE PERSISTENT VEGETATIVE STATE. S.P. Raps*, N.R. Relkin*, D.R. Labar and F. Plum. Department of Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021.

Although behavioral evidence of sleep-wake cycles are a part of the diagnostic criteria for the persistent vegetative state (PVS), no prior studies have detailed the electrographic correlates of cyclic arousal or the sleep architecture for patients in this condition. Continuously acquired 24h video electroencephalography (EEG) was performed in a 35-year old man in PVS for four years after severe head trauma. Subsequent post-mortem studies documented extensive white matter shearing, marked atrophy of the tegmentum and basis pontis, mild axonal loss from the pontine reticular formation, but nearly complete preservation of cerebral cortex. Periods of relative EEG synchronization and desynchronization were scored and correlated well with four behaviorally-determined states of arousal. These included unusual alternation between two states of wakefulness, one with stereotyped posturing and another with paucity of movement. Behavioral and electrographic cycling between these states occurred with a strikingly regular periodicity. An abnormal sleep architecture was present. Slow wave sleep (SWS) stages 1-3 progressed appropriately with regard to depth but cycled rapidly and were of shortened duration compared with normal sleep. REM sleep was absent. These findings suggest that: (1) EEG is reflective of state of arousal in this patient with focal subcortical injury, (2) the selective presence of SWS without REM sleep is consistent with experimental studies which indicate that there are multiple generators for SWS in forebrain and brainstem, while REM sleep may be more dependent on lower brain stem mechanisms.

346.2

EFFECTS OF SLEEP DEPRIVATION ON PLASMA CRH LEVELS IN MAJOR DEPRESSION. C. Reist, E. DeMet, C.C. Chen*, A. Chhoeu*, A. Chicz-DeMet, Depts. Psychiatry, Long Beach VA Medical Center, Long Beach, CA 90822 and Univ. California Irvine, Irvine CA 92717

Corticotropin releasing hormone (CRH) has been the focus of much interest since early studies reported elevation of CSF CRH in patients with major depression. A number of observations suggest that CRH may be involved in coordinating endocrine and behavioral aspects associated with depression. To further understand this relationship plasma CRH levels were studied in 13 inpatient depressed subjects and 10 normal controls. The depressed subjects were treated with one night of total sleep deprivation (SD), an intervention that results in transient improvement in many depressed subjects. Baseline (8:30am) plasma CRH levels did not differ between groups while ACTH and cortisol were significantly elevated in the depressed group. Sleep deprivation resulted in significant reduction of Hamilton Depression (HAM-D) scores. Paired comparisons of pre vs post SD values revealed robust positive correlations between CRH vs ACTH, and CRH vs HAM-D as well as an inverse relationship between CRH and GH. The results confirm that SD is an effective temporary therapeutic treatment for depressive symptoms. This treatment also resulted in concomitant neuroendocrine responses although these changes were not tightly coupled to the clinical response. A notable exception to this observation was CRH levels which paralleled changes in depression ratings after SD ($r=0.82;p=0.025$). This finding suggests that plasma CRH may, within individuals, be related to mood state.

346.3

ABNORMAL SLEEP-WAKING RHYTHMS IN PROFOUNDLY RETARDED INDIVIDUALS

Ch. E. Olmstead, R. H. Chaney[‡] and C. Givens[‡].
Olmstead Assocs, Playa del Rey, CA 90296, UCLA/MRRC Gp, Lanterman Developmental Ctr, Pomona, CA 91769.

Disruptions of circadian rhythms are frequently seen in individuals with severe brain damage. 22.5% of 1082 residents of a state residential facility for the developmentally disabled showed disturbances of the sleep-waking cycle. We have carried out 24 hr behavioral observations in 41 individuals (Ages 21 to 64 years) to further characterize the rhythm disturbances.
Sleep-waking cycle disturbances ranged from mild to severe with total sleep times that ranged from 25 min to 10.25 hours distributed in from 2 to 18 bouts. Only 7 of the subjects showed normal periods of uninterrupted sleep during the night. The type of insomnia included sleep phase delay in 10 patients and paroxysmal arousals in seven. Extensive daytime sleep, up to 10 episodes, was seen in 7 patients. In general, those individuals whose probable hypothalamic damage was attributable to post-natal causes such as trauma or infection were the most likely to show near normal circadian variations, while those with prenatal etiologies were the most severely disrupted.

346.4

DELTA AMPLITUDE SHIFTS FOLLOWING 48 HOURS OF SLEEP DEPRIVATION IN HUMANS. H. Sing*, M. Thomas*, G. Belenky, N. Shepanek*, D. Thorne*, S. Balwinski*, Y. Shaham*, D. Penetar*, U. McCann, & D. Redmond*. Dept. of Behavioral Biology, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Spectral analysis has been used to delineate EEG frequency changes during sleep stages and to characterize changes in the awake EEG of subjects (Ss) deprived of 24 hours of sleep. We instrumented 6 Ss for EEG, EOG, and EMG to document wakefulness during a PET study of brain glucose metabolism after prolonged sleep loss and while performing a cognitive task. All Ss were awake in both the rested and sleep deprived conditions, with few microsleeps in either condition. Spectral analysis of the delta frequency band (.75-3.75 Hz) in .5 Hz intervals revealed a shift in relative delta amplitude in both the C3 and Oz leads. Relative amplitude was greater in the sleep deprived condition than rested for the first third of the delta band (.75-1.75 Hz). This finding was reversed for the last third of the band (2.5-3.5 Hz). The increase in relative amplitude in the lowest delta frequencies suggests that neurons may be firing more synchronously, similar to slow wave sleep, and that this may underlie the slowing in mental performance resulting from sleep loss and/or a need to sleep. As a physiological marker of sleepiness, the changes in the delta frequencies may be used to distinguish alert Ss from sleepy Ss.

346.5

MECHANISMS OF POST-ASYSTOLE CORONARY BLOOD FLOW INCREASE DURING SLEEP. L.W. Dickerson, R.L. Verrier. Department of Pharmacology, Georgetown University School of Medicine, Washington DC 20007.

Spontaneous asystoles have been observed during sleep in normal humans and canines. We have recently shown in sleeping dogs that episodes of asystole of 1.1-8.0 sec are followed by surges in coronary blood flow (CBF) averaging 31% but ranging up to 84%. This study examined whether the post-asystole increase in CBF was due to (a) primary neurogenic coronary vasodilation, or (b) reactive hyperemia in response to the CBF decline during asystole. Five chloralose-anesthetized dogs were instrumented to record lead II ECG, femoral artery blood pressure (BP), and left circumflex (LCX) coronary artery Doppler flow. The cervical vagi were sectioned and the distal end of the left vagus was stimulated at 10 Hz to produce asystoles of approximately 2 sec duration. Then, the pericoronary nerves of the LCX were damaged both mechanically and by formalin application. After a 20 min rest period, the vagus nerve was restimulated and the results were compared (means ± SEM).

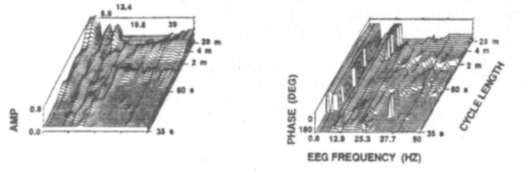
	% Change from Pre-Asystole Baseline	
	HR x BP	CBF
Normal	-30 ± 2	27 ± 6
Coronary denervation	-25 ± 2	-1 ± 4 (p<0.01)

Conclusions: (1) Vagus nerve excitation simulates the post-asystole increase in CBF observed during sleep, and (2) it appears to be due predominantly to primary vasodilatation and not cardiac metabolic factors since damaging the pericoronary nerves eliminates the CBF surge.

346.6

COHERENCE OF EEG SPECTRAL CHANGES AND VIGILANCE S. Makeig, M. Inlow and R. Galambos, Naval Health Research Center, San Diego, CA.

Ten male subjects participated in a total of 13 half-hour yes closed auditory vigilance tasks simulating passive sonar target detection. Targets were brief noisebursts peaking 6 dB above continuous background noise presented at a mean rate of 10 per minute. Button press responses to target bursts were recorded and fluctuations in vigilance estimated by fraction of targets responded to within a 32 s moving window. EEG was collected continuously from 13 scalp sites. The power spectrum of the local error rate measure was dominated by cycle lengths of 3-4 minutes and longer. Coherence between local error rate and EEG power in 81 frequency bins from 0.6-50 Hz was computed for each site and significance of coherence was determined by a Monte Carlo procedure. The figure plots coherency amplitude (square root coherence) at site Cz. 1) The EEG band structure is more marked in the coherence plane than in the mean EEG power spectrum. 2) A distinct band of significant coherence at high EEG frequencies for cycle lengths near 90 s per cycle is present at each scalp location. 3) At all scalp sites and for all cycle lengths, the coherence phase plane contains a sharp phase reversal near 6 Hz. 4) For almost all frequencies, EEG spectral changes neither lead nor lag performance changes.



INGESTIVE BEHAVIOR: SALT AND WATER

347.1

LICK RATE ANALYSIS OF SODIUM TASTE-DRIVE INTERACTIONS. P.A. Breslin, C.M. Zambito*, J.M. Kaplan, [1]A.C. Spector, and H.J. Grill. Psych. Dept., Univ. of Penn., Phila., PA 19104 and [1]Dept. of Psych., Univ. of Florida, Gainesville, FL 32611.

The physiological mechanisms responsible for sodium drive and sodium taste have received much attention. However, little is known about how these two factors combine to control sodium ingestive behavior. To measure this interaction we examined initial lick rates for different NaCl solutions in sodium replete and sodium deplete rats. In Experiment 1, the lick rates of six groups of rats, each consuming one of 6 NaCl solutions, were recorded at the beginning (first 3 min) of a NaCl meal (40 min), when post-ingestive influences of the test stimulus would be minimal. All six groups were offered the solutions while in the replete and deplete states in counter-balanced order. Depletion was accomplished by a 10 mg injection (ip) of the diuretic furosemide. All rats also received free access to water and Na* deficient chow in the home cage. Replete rats' initial lick rates increased nonmonotonically with concentration peaking at isotonicity, thus resembling NaCl ingestion concentration functions. The concentration lick rate function for the deplete rats were uniformly elevated, but retained the same shape as that of the replete rats. In Experiment 2 eight naive rats received access to all 6 NaCl solutions within a single session (40 min) in either the replete or deplete states. The solutions were repeatedly presented to the rats in a random order for brief 10 sec exposures with at least a 5 sec ITI. The replete and deplete lick rate functions strongly resembled those obtained in Experiment 1. When the deplete rats' lick rate functions were broken into the three drive states coinciding with the beginning, middle and end of the sessions, three lick rate concentration functions were derived. The functions shifted down over the session, but retained their shape. Overall, drive state dramatically affected the absolute lick rates, but the relative affective quality of the solutions, as revealed by the shape of lick rate concentration functions, was unaffected. This research was supported by NIMH program project #43787.

347.2

NACL INTAKE INCREASES DOPAC/DA IN ACCUMBENS AND OLFACTORY TUBERCLE AND 5HIAA/5-HT IN AMYGDALA IN SODIUM DEPLETED RATS. S.P. Frankmann, L. Broder*, J.H. Dokko*, D.V. Veltung* and G.P. Smith. Bourne Lab, NY Hosp.- Cornell Med. Ctr., White Plains, NY 10605.

Food intake following food deprivation increases central DA and 5-HT activity. To ask whether NaCl intake following sodium (Na+) depletion also increases central DA and 5-HT activity, male, Sprague-Dawley rats were given Na+ deficient diet overnight and brain regions were sampled at 24h after one of three treatments (n=16/ treat.): 1) vehicle injection, 2) Lasix (furosemide) injection (10 mg, sc) or 3) Lasix injection and 0.3M NaCl intake (for 9 min). DA, DOPAC, HVA, 5HT and 5HIAA were measured by HPLC-EC for amygdala (Amyg.), caudate, dorsal hindbrain, hypothalamus, nucleus accumbens (Acc.) and olfactory tubercle (OT).

Sodium depletion alone significantly increased 5HIAA/5-HT activity in the Acc. Ingestion of NaCl (x̄±SEM = 8.0±0.8 ml) after Na+ depletion produced increases of 5HIAA/5-HT in the Amyg. and DOPAC/DA in the Acc.

		Vehicle	Lasix	Lasix & 0.3M NaCl
DOPAC/DA:	OT	0.17±0.01	0.18±0.09	0.20±0.01*
	Acc.	0.25±0.02	0.28±0.02	0.33±0.03*
5HIAA/5-HT:	Acc.	0.76±0.03	0.88±0.03*	0.91±0.05*
	Amyg.	0.77±0.03	0.84±0.04	0.94±0.04*

* = p < .05 vs Vehicle.

The increases in 5-HT and DA metabolism suggest that Na+ depletion stimulates 5-HT terminals in the Acc. and that intake of NaCl during Na+ depletion stimulates 5-HT terminals in the Amyg. and DA terminals in the Acc. and OT. We hypothesize that 5-HT and DA activity associated with NaCl intake are involved in the sensory and/or hedonic processing of the effects of NaCl intake in the Na+ depleted rat.

Support: NIH RO1 DK39810 (SPF) & MH15444 & MH00149 (GPS).

347.3

BED NUCLEUS OF THE STRIA TERMINALIS LESIONS: REDUCTION OF YOHIMBINE- AND FUROSEMIDE-INDUCED SALT INGESTION. **A.M. Zardetto-Smith, T.G. Beltz and A.K. Johnson, Depts. of Psychology and Pharmacology, University of Iowa, Iowa City, IA 52242.**

Subcutaneous (sc) injection of the alpha-2 antagonist yohimbine (YOH) rapidly produces salt ingestion in sodium replete rats. Previously, we demonstrated that rats with bilateral lesions of the central nucleus of the amygdala (CeA) show a significant reduction in their intake of 2% NaCl in response to sc injection of YOH. Rats with CeA-lesions also demonstrated a significant decrease in their intake of 2% NaCl after furosemide depletion (Zardetto-Smith et al., Fed. Proc. 5:A1145, 1991). The bed nucleus of the stria terminalis (BST), particularly its lateral subdivision, is similar to the CeA in many aspects of its cyto- and chemoarchitecture, connections, and function. In this study, cumulative 3-hr intakes of 2% NaCl after sc injections of YOH (3 mg/kg) or ip furosemide-depletion (FD) were measured in male, Sprague-Dawley rats both before and after bilateral electrolytic lesions of the BST (n = 6) and compared to a group receiving sham lesions (n = 6). Prior to surgery, the groups drank equivalent amounts of 2% NaCl in response to YOH and FD treatments. After surgery, sham-lesion rats showed a slight increase in their intake of 2% NaCl following sc YOH. However, intake of 2% NaCl in response to YOH was significantly decreased post-BST lesion when compared to both pre-surgery intake of the group and post-surgery intake of the sham group. BST-lesioned rats also showed a significant decrease in their intake of 2% NaCl after FD, while intake of the sham group remained unchanged. Before and after surgery, the groups drank equivalent amounts of water in response to sc hypertonic saline. The results suggest the BST, like the CeA, may be an important integrative site for one or more afferent signals which mediate salt appetite. (Supported by NIH Grants HL44565 and HL14338).

347.4

ARTERIAL HYPOTENSION INCREASES THIRST, BUT NOT SALT APPETITE, TO ICV ANGIOTENSIN. R.L. Thunhorst and A.K. Johnson, Depts. of Psychology and Pharmacology, Univ. of Iowa, Iowa City, Iowa, 52242.

Drinking elicited by iv infusion of angiotensin II (AII) is enhanced when the concurrent pressor response is reduced. We tested if reduced blood pressure likewise would enhance drinking and salt ingestion elicited by icv infusion of AII. Rats received iv minoxidil (25 ug/kg/min) plus captopril (0.33 mg/min) to lower blood pressure and prevent endogenous AII formation. The resulting hypotension doubled water intakes compared with iv vehicle infusions (main effect) during 90 min of icv AII at 4 (3.8 vs 1.8 ml) and 16 (12.0 vs 6.2 ml) ng/hr. The increased intakes were maximal in the first 30 min of icv AII. Rats with access to both water and 0.3 M NaCl during 180 min of icv AII (16 ng/hr) responded to hypotension with increased water intakes in the first 30 min of icv AII (7.7 vs 4.4 ml), but not overall (14.4 vs 12.5 ml; interaction effect) compared to intakes during iv vehicle. Hypotension did not increase salt intakes (2.6 vs 3.2 ml). Overall, hypotension greatly reduced urine volumes, resulting in considerable fluid retention. The results indicate that AII and baroreceptor mechanisms interact in hypotensive states, enabling icv AII to elicit greater water intakes, but not greater salt intakes, in the face of fluid retention.

Supported by NIH grants HL44565 and HL14338.

347.5

CHORDA TYMPANI TRANSECTION ELIMINATES AMILORIDE-SENSITIVITY OF THE INGESTIVE RESPONSE TO NACL IN THE RAT. S.I. Sollars*, E.M. Taylor* and I.L. Bernstein. Department of Psychology, University of Washington, Seattle, WA. 98195.

Fungiform papillae on the anterior tongue are the site of gustatory receptors which are maximally responsive to NaCl stimulation. In the rat, this receptor population is innervated by the chorda tympani nerve (CT) which contains fibers displaying a relatively specific responsiveness to NaCl and which are blocked by the Na transport blocker amiloride. Although electrophysiological studies support a specific role for the CT in NaCl responsiveness, behavioral studies indicate that rats with bilateral transection of the CT are, at the most, only marginally affected in their NaCl consumption. To further characterize the role of the CT in responsiveness to NaCl, the present study examined whether behavioral expression of amiloride sensitivity is dependent upon the CT.

Wistar rats received bilateral CT transection (N=8) or sham surgery (N=8). After surgery they were adapted to a 23-hr water deprivation schedule and trained to drink from a lickometer which provided brief presentations of taste solutions. Individual licks were detected by a photocell and recorded by microcomputer. To assess amiloride-sensitivity animals licked for a 3% NaCl solutions for 3 minutes after either water or 100 μM amiloride hydrochloride exposure. In intact rats, licking for 3% NaCl was significantly increased by preexposure to amiloride ($p<.01$). In contrast, after bilateral CT transection, amiloride preexposure did not significantly affect licking for 3% NaCl. This suggests that, in the rat, the CT is the primary gustatory pathway conveying amiloride-sensitive NaCl signals.

347.6

DIFFERENTIAL EFFECTS OF PROLONGED SODIUM DEPRIVATION ON SALT APPETITE IN PREPUBESCENT AND SEXUALLY MATURE RAT PUPS. M.G. Scheidler, E.M. Stricker, J.G. Verbalis. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Adult male rats deprived of dietary sodium for 8 days express a robust salt appetite, as do ovariectomized but not intact female rats (see table). These and other results suggested that estrogen in some way suppresses the salt appetite in rats induced by extended sodium deprivation. To test this hypothesis in a physiologically intact model, 30-day-old prepubescent male and female rats were placed on 4 and 8 days of sodium deprivation. After 4 days of deprivation, the juvenile pups, like the intact adults, showed a modest saline intake that was comparable in the males and females. After 8 days of sodium deprivation, juvenile male and female pups both showed equivalently enhanced salt appetites, which on an intake-to-weight basis was similar to that of intact male rats. However, when 40-day-old sexually mature male and female rats, were tested under conditions of sodium deprivation, they behaved like their adult counterparts in that only males expressed robust saline intakes (see table).

Males	WATER	0.5 M NACL	**Females**	WATER	0.5 M NACL
Adult	19.6 ± 2.6	19.4 ± 1.9	Adult	10.9 ± 1.4	7.5 ± 1.2
Gonad-x	11.9 ± 1.4	19.7 ± 1.5	Ovar-x	8.3 ± 1.8	18.2 ± 1.9
30 day	10.7 ± 0.7	6.5 ± 1.4	30 day	9.6 ± 0.7	6.6 ± 2.8
40 day	14.7 ± 3.2	20.8 ± 2.7	40 day	9.4 ± 2.4	13.7 ± 1.5

These studies in prepubescent and sexually mature juvenile rats therefore provide additional evidence in support of an inhibitory role of estrogen on salt appetite under conditions in which salt appetite is especially pronounced.

347.7

A ROLE FOR THE SALIVARY GLANDS IN THIRST AND SODIUM APPETITE IN RATS. T.M. Nicholson, M.J. Fregly* and N.E. Rowland. Depts Psychology & Physiology, Univ of Florida, Gainesville, FL 32611-2065

Male Sprague-Dawley rats were either completely salivarectomized (SX) or were sham operated (SHM). Completeness of SX was verified at the end of the studies by a test of prandial drinking. Starting 1 mo postoperatively (mean body weights were similar), tests of water and NaCl intake were administered.

The SX rats drank comparable amounts of water to SHM after *SC* injection of angiotensin (ANG) II, but drank 50-80% less than SHM following *SC* injections of either ANG III, isoproterenol, serotonin, polyethylene glycol, or ramipril (an ANG converting enzyme inhibitor). Stimulated plasma renin activities were similar in both groups.

Studies on salt appetite also suggested reduced ANG responsiveness in SX. The spontaneous preference/aversion function for NaCl intake was unaffected by long term SX, but the expression of NaCl appetite by chronic treatment with ramipril was completely absent in SX rats. In contrast, the appetite to either acute depletion of sodium or chronic administration of DOCA (mineralocorticoid) was virtually normal in SX rats.

[*Supported by NSF BNS89-09439 and ONR N00014-88-J-1221*].

347.8

GENDER EFFECTS ON NACL PREFERENCE AND TASTE REACTIVITY O. Galaverna, J. Schulkin*, F.W. Flynn, M. Havens, J. Nardozzi*, P. Wilson* and R.R. Moore*. University of Pennsylvania, and University of Wyoming.

Previous studies have demonstrated that sexually mature Sprague-Dawley female virgin rats ingest greater amounts of hypertonic NaCl than age matched males when sodium replete; they also ingest greater amounts of the salt when sodium hungry. In the following study we inquired whether the female's response to NaCl is different across a range of concentrations of NaCl in both taste reactivity tests using oral-facial profile, and in intake tests. For the taste reactivity test, rats were fitted with chronic cannulae and infused in ascending order with either .03, .15, .3 or 1 molar NaCl. We also tested their ingestion of the NaCl in home cage intake tests where the NaCl was given along with water and food. In the taste reactivity test, we found that the females expressed greater oral-facial ingestive responses to the .15 and the .3 molar NaCl than the males did. And in the intake tests we found that the females ingested greater amounts of the NaCl at .15, .3 and at 1 molar concentrations. These results when taken together provide evidence that the basic taste reactivity to NaCl is different in females than males, and provides further evidence that the female's ingestion of NaCl is also greater. The enhanced avidity for NaCl, in the female over that of the male, may lie in the demands that she faces for sodium and other salts during pregnancy and lactation.

347.9

EFFECTS OF HYDRATIONAL STATUS AND LEARNING ON THE DRINKING ELICITED BY INJECTIONS OF MUSCIMOL INTO THE MEDIAN RAPHE NUCLEUS. M.R. Pitzer, T.R. Stratford & D. Wirtshafter. Dept. Psych., Univ. Ill. at Chicago, Box 4348, Chicago, Ill.

We have shown that microinjections of the GABA-A agonist muscimol into the median raphe nucleus (MR) lead to large increases in water intake by nondeprived rats. In the present study we examined whether this drinking behavior could be inhibited by overhydrating the subjects. Preloading naive animals with 40 ml/kg of water attenuated muscimol-induced drinking. In contrast, similar preloads were ineffective in animals experienced with drinking under muscimol. These findings suggest that while the initial occurrence of muscimol induced drinking is dependent on the hydrational status of the animals, the drinking response may become conditioned to the muscimol state and thus independent of hydrational factors.

Further support for a role of learned factors in muscimol-induced drinking is provided by another study in which we demonstrated that animals experienced with drinking under muscimol showed larger water intakes when tested with food and water present than did rats experienced only with feeding under muscimol.

347.10

EFFECTS OF DEPRIVATION AND PALATABILITY ON THE DRINKING BEHAVIOR INDUCED BY INJECTIONS OF MUSCIMOL INTO THE MEDIAN RAPHE NUCLEUS. I. Shim, T.R. Stratford & D. Wirtshafter. Dept. Psychol. Univ. Ill. at Chicago, Box 4348, Chicago, Il.

In previous experiments we have demonstrated that robust feeding and drinking can be produced in nondeprived animals by injections of the GABA-A agonist muscimol into the median raphe nucleus (MR). In the current study we examined the extent to which muscimol induced drinking can be altered by fluid deprivation or by changes in palatability of the fluids presented.

Nondeprived rats were trained to drink either a dilute or a concentrated sucrose solution and baseline intakes of the concentrated solution were higher than those of the dilute solution. Intra-MR injections of muscimol produced a significantly larger increase in the intakes of the concentrated than of the dilute solution, relative to baseline intakes. In an analogous experiment, muscimol produced equivalent increases, relative to baseline, in deprived and nondeprived rats. These results indicate that intra-MR muscimol interacts differently with different palatability and deprivation induced drinking .

347.11

INTRAGASTRIC HYPERTONIC NaCl ELICITS DRINKING WITHOUT SYSTEMIC DEHYDRATION IN RATS. F. S. Kraly and Y. M. Kim*. Dept. of Psychology, Colgate Univ., Hamilton, NY 13346.

Intragastric infusion of hypertonic NaCl increases plasma vasopressin without changing plasma osmolality in rats (Kwon et al., Am. J. Physiol. 259: E19, 1990). We examined whether such infusions elicit drinking without systemic dehydration. Adult Sprague-Dawley male rats were surgically prepared with a chronic gastric catheter. A test was initiated by a 2 ml (in 1 min) infusion of 300, 600 or 1200 mOsm NaCl. For rats (n=8) not deprived of food or water, 1200 mOsm NaCl decreased ($p<.01$) the latency to initiate drinking and increased ($p<.05$) 60-min water intake from 0.3 ± 0.2 ml on baseline (300 mOsm NaCl) to 2.3 ± 0.6 ml. For rats (n=5) food-deprived for 24 hr, 600 mOsm NaCl decreased ($p<.05$) the latency to initiate drinking without significant change in 60-min water intake; 1200 mOsm NaCl increased ($p<.05$) 60-min water intake from 0.4 ± 0.2 (300 mOsm baseline) to 6.6 ± 1.9 ml. Plasma osmolality and hematocrit (taken at the time drinking was initiated) were not affected by 600 or 1200 mOsm NaCl when compared to baseline (300 mOsm). Thus, intragastric hypertonic NaCl can elicit drinking in the absence of or prior to a change in systemic osmolality. These results are consistent with the hypothesis of an osmosensitive gastrointestinal and/or hepatic-portal mechanism for eliciting drinking in advance of systemic dehydration in the rat.

347.12

IBOTENIC ACID LESIONS OF THE DIAGONAL BAND OF BROCA RESULT IN EXAGGERATED POLYETHYLENE GLYCOL-INDUCED DRINKING BEHAVIOR. M. J. Sullivan, J. T. Cunningham, R. Nissen, A. M. Allen, E. Coderre and L. P. Renaud. Neuroscience Unit, Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9

Drinking behavior induced by peripheral angiotensin appears to be influenced by blood pressure. Robinson and Evered (1987) have demonstrated that normalization of blood pressure during angiotensin infusions results in increased water intake. Electrophysiological data from this laboratory has demonstrated that the diagonal band of Broca (DBB) mediates baroreflex supression of spontaneous activity of the vasopressinergic magnocellular neurons of the supraoptic nucleus. In this study, we examined the possibility that the DBB might also be involved in the regulation of water intake.

Ibotenic acid lesions of the vertical limb of the DBB were made in male Long Evans rats. Rats were injected with 0.5 µl of 5µg/µl ibotenic acid in phosphate buffered saline. A second group received control injections of 0.5µl the vehicle. Following a week of recovery, animals were tested for drinking responses to 0.9%, 4% and 6% saline (1ml/100 gm sc) and 40% polyethlene glycol (1ml/100 gm sc). Tests were carried out in home cages and were spaced a minimum of 48 h apart.

DBB lesioned rats drank significantly more than control rats in response to polyethylene gycol. The animals began drinking earlier and drank more in total volume over the course of five hours following the injection. No differences were seen in drinking responses to two doses of hypertonic saline.

The results suggest that the DBB plays a role in modulating drinking in response to hypovolemia.

347.13

DIFFERENTIAL EFFECT OF DuP 753 ON VARIOUS TYPES OF EXPERIMENTALLY-STIMULATED WATER INTAKE IN RATS. N.E. Rowland and M.J. Fregly*. Depts Psychology & Physiology, Univ Florida, Gainesville, FL 32611

When administered *SC*, DuP 753, an angiotensin (ANG) type 1 receptor (AT-1) antagonist inhibits water intake evoked by *SC* ANG II in rats. We now extend this finding to intake after *SC* ANG III, suggesting either that both of these ANG peptides engage one receptor or that DuP blocks two sites. In contrast, DUP 753 (up to 20 mg/kg *SC*) did not inhibit water intake after *SC* isoproterenol (ISO), serotonin, or polyethylene glycol. The dipsogenic action of the these agents thus may not depend upon the stimulation of AT-1 receptors accessed by SC-administered DuP 753. Alternatively, in the case of ISO, the plasma renin activity already enhanced by either ISO (10x basal) or DuP (24x basal), was further elevated (44x basal) by their combination, and the ANG II formed may overcome AT-1 blockade.

DuP 753 (*SC*) inhibited water intake after cerebroventricularly-administered (*ICV*) ANG II, suggesting that DuP 753 crosses the blood-brain barrier. DuP 753 (*ICV*) potently reduced water intake after *ICV* ANG II (10 ng): the 50% inhibitory dose was 10-100 ng. In contrast, DuP 753 (100 ug, *ICV*) did not inhibit ISO (25ug/kg, *SC*)-induced drinking.

Grant support: NSF BNS 89-09439, ONR N00014-889-1221.

347.14

EFFECTS OF AREA POSTREMA LESIONS ON SCHEDULE-INDUCED POLYDIPSIA IN RATS. K.-P. Ossenkopp and L. A. Eckel. Dept. Psychology, Univ. of Western Ontario, London, Ontario, CANADA N6A 5C2.

Food deprived rats develop excessive drinking when exposed to an intermittent schedule of food pellet delivery (schedule-induced polydipsia, SIP). In the present study acquisition of SIP was measured in male adult rats with area postrema lesions (APX) or sham lesions (APS). Following surgery the animals were given a 2 week rest period. Subsequently all rats were reduced to 85% of free-feeding body weight. During daily 1 hr conditioning sessions the rats were put in standard operant chambers and exposed to a fixed-time food presentation schedule of one 45 mg food pellet every 60 s. Water was freely available during the conditioning session and fluid intake levels were recorded every 15 min. Analysis of the results indicated that the APX rats showed significantly lower rates of SIP acquisition ($p<0.01$) than the control animals. When a 0.08 M NaCl solution was substituted for the water in the operant chamber, the APX rats drank more fluid than the APS animals. Substitution of a 0.08 M LiCl solution resulted in a large and rapid reduction in fluid intake in both groups, but the rats with area postrema lesions drank significantly ($p<0.05$) more of the LiCl solution than the sham lesioned rats.

(Supported by a grant from NSERC to KPO).

347.15

A DIPSOGENIC EFFECT OF EXOGENOUS ANGIOTENSIN-II IN ICR MICE IS PARTIALLY MASKED BY ITS PRESSOR ACTION. D.A. Czech. Dept. of Psychology, Marquette Univ., Milwaukee, WI 53233.

Several small animals, including mice, are reported to be relatively insensitive dipsogenically to peripheral Angiotensin-II (AII). This might be partly attributable to AII's pressor action interfering with a drinking system/response.

An initial dose of AII (200 or 600 μg/kg) or 0.15 M NaCl vehicle (VEH), plus 35 mg/kg captopril (CAP) was given sc; followed 15 min later by an injection of d,l-isoproterenol HCl (ISOP) (100 or 200 μg/kg) or VEH. Three additional groups first received a VEH injection, followed by ISOP (100 or 200 μg/kg) or VEH, as a control series for ISOP-induced drinking. Following the second injection, mice were given access to deionized water (no food present) and intake was monitored for up to 3 hr during the light phase of the L/D cycle. Cumulative intakes were evaluated with ANOVA and t-test procedures. Alpha was set at $p<0.05$.

Both AII and ISOP stimulated modest, but significant, drinking. For both agents, both latency to drink and amount consumed were quite variable. Further, mean intake in response to 600 μg/kg AII was significantly higher when mice were also given 100 μg/kg ISOP; 200 μg/kg ISOP did not significantly facilitate AII-induced drinking. ISOP did not significantly affect drinking to 200 μg/kg AII. These data suggest greater dipsogenic sensitivity to peripherally administered AII in the mouse than was previously reported, and also indicate that a dipsogenic effect of AII is partially masked by AII's pressor action.

DRUGS OF ABUSE—COCAINE: ANTAGONISTS AND SEROTONIN

348.1

THE EFFECTS OF SELECTIVE D1 AND D2 RECEPTOR ANTAGONISTS ON KETAMINE SELF-ADMINISTRATION IN RATS. C.B. Hubner and J.E. Moreton. University of Maryland School of Pharmacy, Baltimore, MD 21201

A role for dopamine in mediating the reinforcing effects of psychomotor stimulant drugs has been suggested from studies which find that dopamine antagonists increase rates of responding maintained by cocaine and amphetamine under fixed-ratio (FR) schedules of reinforcement. The purpose of the present study was to investigate whether dopamine plays a similar role in mediating the reinforcing effects of ketamine. The effects of the D1 antagonist, SCH 23390, and the D2 antagonist, spiperone, on rates of responding maintained by ketamine were examined. Sprague-Dawley rats, trained to self-administer intravenous ketamine (2.0 mg/kg/injection) on a FR 5 schedule, were pretreated 30 minutes prior to the session with s.c. 2.0 - 20.0 μg/kg SCH 23390 (n=6) or 2.0 - 40.0 μg/kg spiperone (n=6). At low to intermediate doses of either antagonist, there was no effect on response rate. Decreases in rates of responding were obtained at the highest doses tested. We have previously demonstrated that SCH 23390 and spiperone produce increased rates of responding maintained by cocaine under a FR 5 schedule, suggesting a reduction in the reinforcing effects of cocaine. Taken together, these data suggest that D1 and D2 receptors are not critical for mediating the reinforcing effects of ketamine. [Supported in part by DA05292 (CBH), BRS grant 2S07RR05770-10 (CBH, JEM) and DA03173 (JEM).]

348.2

ATTENUATION OF COCAINE SELF-ADMINISTRATION BY THE SELECTIVE D1 DOPAMINE RECEPTOR AGONIST A68930. J.E.G. Williams, P. Curzon, and D.R. Britton. Neuroscience Research, Dept. 47U, Abbott Laboratories, Abbott Park, IL 60064.

There is evidence that the reinforcing properties of cocaine are mediated in part by the actions of endogenous dopamine acting on D1 and D2 dopamine receptors. Acute blockade of D1 (or D2) receptors leads to a compensatory increase in the rate of cocaine self-administration (S/A)(Koob, et al., 1987). To more clearly assess the functional role of the D1 receptor in the maintenance of cocaine S/A, we tested the hypothesis that supplemental activation of the D1 receptor by pre-treatment with a potent D1 agonist (A68930 [1R, 3S] 1-aminomethyl-5,6-dihydroxyphenylisochroman HCL) would decrease the rate of cocaine S/A. Rats implanted with chronic intravenous catheters were trained to bar-press for cocaine (0.75 mg/kg/infusion) administered on an FR-5 schedule. Animals pre-treated with saline showed an average of from 17.2 to 20.2 infusions per 2 hour session. Pre-treatment with A68930 (0.32, 1.0, 1.8, and 2.5 umol/kg, sc) resulted in decreases in S/A by 7.0, 43.0, 32.0, and 65.0% respectively. While the sensitivity of individual animals to the effects of the agonist varied, all rats showed a consistent, significant decrease in S/A within this dose range. These data are not in themselves conclusive, but are consistent with the hypothesis that rats regulate levels of cocaine self-administration to achieve an optimal level of activation of the D1 receptors.

348.3

FOURPHIT INHIBITS COCAINE-INDUCED HYPERACTIVITY IN RATS. M.M.Schweri, B.R.de Costa* and K.C.Rice*. Mercer Univ. Sch. Med., Macon,GA 31207 and NIDDK,Bethesda,MD 20892.

Fourphit (4-isothiocyanato-1-[1-phenylcyclohexyl]piperidine; FPH), a phencyclidine derivative, irreversibly inhibits binding in vitro to the stimulant recognition site on the dopamine transporter, where cocaine (COC) is thought to exert its stimulant and reinforcing actions. The aim of this work was to determine whether FPH would inhibit the locomotor effect of COC in male Sprague-Dawley rats. Rats were acclimated overnight in individual cages in the activity monitors. After recording baseline activity for thirty min., rats were injected i.v. with 20 mg/kg of FPH (controls received vehicle [VEH]), and activity was monitored for 30 min. longer. Twenty-four hrs. later, both VEH and FPH-treated animals were challenged with 15 mg/kg COC·HCl (i.p.) and activity was recorded for 60 min. FPH significantly attenuated COC-induced hyperactivity (total, ambulatory, and nonambulatory) for the first 40 min. after injection of the stimulant, but not during the last 20 min. Surprisingly, no significant difference was found in binding to the stimulant recognition sites in striatal tissue of VEH and FPH-treated rats (measured using the [^{3}H]methylphenidate radioreceptor assay), whether determined one hr. after injection of FPH or at the conclusion of the experiment. These results suggest that FPH antagonizes COC-induced hyperactivity, probably at a site other than the dopamine uptake complex. (Supported by grants from NIDA [RO1#DA06305] and NINDS [R15#NS28584).

348.4

ROLE OF GLUTAMATERGIC TRANSMISSION IN MEDIATING THE LOCOMOTOR STIMULATION PRODUCED BY COCAINE AND DOPAMINE RECEPTOR AGONISTS. F.G. Kaddis,* N.J. Uretsky, L.J. Wallace. College of Pharmacy, The Ohio State University, Columbus, OH 43210.

The injection of DNQX, an antagonist of AMPA/kainate (A/K) receptors, into the nucleus accumbens (NA) has been shown to antagonize the locomotor stimulant actions of amphetamine. To study the role of A/K receptors in the locomotor stimulation induced by drugs that enhance DA transmission, the effect of DNQX was studied on the locomotor stimulant responses to cocaine and DA receptor agonists. Cocaine (20 mg/kg, i.p.) produced a 5 fold stimulation of locomotion, which was almost completely antagonized by DNQX (1 μg) in the NA. Similarly DNQX antagonized the stimulation produced by the co-administration of a D-1 and D-2 agonist. In a microdialysis study, cocaine (20 mg/kg, i.p.) did not significantly increase the extracellular levels of glutamate in urethane-anesthetized rats. However, infusion with high potassium CSF (80 mM) produced a 3.6 fold increase over basal levels. Thus, the activation of A/K receptors is necessary for the stimulant response to DA agonists, but is not associated with an increase in extracellular glutamate in anesthetized animals.

348.5

D-SERINE MODULATION OF PHENCYCLIDINE- AND DIZOCILPINE-INDUCED CHANGES IN ACTIVITY AND MOVEMENT PATTERNS IN RATS. V.D. Lehmann-Masten, M.P. Paulus and M.A. Geyer. UCSD Dept of Psychiatry, Lab of Biol Dynamics and Theoret Med, La Jolla, CA, 92093

The behavioral effects of d-serine on animals treated with either phencyclidine (PCP 5.0 mg/kg) or dizocilpine (DIZ 0.5 mg/kg) were assessed in the Behavioral Pattern Monitor (BPM). The BPM provides quantitative assessments of the animals' horizontal movements (crossovers) and investigatory responses, and also provides information about the geometrical patterns of movements. These patterns can be quantified by the spatial scaling exponent, **d**, describing the relationship between path length and measurement resolution. The contributions to the overall behavior of different geometrical patterns, such as long straight paths versus local circumscribed movements, were assessed by the spectrum of geometrical scaling exponents, **f(d)**. 36 male Sprague-Dawley rats were injected with d-serine (0, 100 or 300 mg/kg, i.p.), a strychnine-insensitive glycine agonist, and tested in the BPM. In addition, the effect of d-serine (100 or 300 mg/kg) on DIZ (0.5 mg/kg, i.p.) or PCP (5.0 mg/kg, s.c.) induced behavioral changes were assessed in the BPM. DIZ and PCP increased the number of crossovers, as previously reported, and changed the overall geometrical movement patterns. The overall geometrical path structure was characterized by increased local or circumscribed movements (increased **d**). The **f(d)** function revealed that this dramatic increase in highly local movements was also accompanied by a strong decrease in long distance-covering movements. Although d-serine pretreatment potentiated the PCP-induced increase in crossovers, it did not affect the DIZ-induced hyperactivity. D-serine pretreatment attenuated both the PCP- and DIZ-induced increase in the spatial scaling exponent, **d**. The **f(d)** function revealed that this attenuation was due to an effect on the highly local as well as on straight path patterns. These findings indicate that changes in patterns of activity induced by PCP and DIZ may be modulated by the glycine receptor associated with the NMDA channel complex.

348.6

THE CALCIUM ANTAGONIST ISRADIPINE INHIBITS COCAINE AND MORPHINE REINFORCING PROPERTIES IN RATS. W.Fratta, A. Kuzmin *, M.C. Martellotta* and G.L. Gessa*. "B.B. Brodie" Dept. of Neuroscience, Univ. of Cagliari, Cagliari, Italy 09124.

Considerable evidence indicates that dopamine is involved in the rewarding action of cocaine and morphine. It has been also shown that both drugs increase dopamine release via calcium dependent mechanism. The effect of the DHP calcium antagonist Isradipine on cocaine and morphine reinforcing properties was investigated by means of place preference and intravenous self-administration (SA) in rats. Isradipine inhibited both cocaine-and-morphine induced place preference at the dose of 1 to 2.5 mg/kg s.c. Pretreatment of the rats with Isradipine (1 to 5 mg/kg s.c.) before morphine or cocaine SA sessions, induced dose-dependent increase in the number of infusions of these drugs. This response pattern was very similar to the one observed when morphine or cocaine were substituted by saline in trained rats not pretreated with Isradipine. These data suggest that Isradipine suppresses the reinforcing properties of morphine and cocaine and may represent an effective pharmacotherapy for treatment of cocaine and heroin abuse.

348.7

ANTAGONISM OF THE STIMULANT ACTIONS OF COCAINE IN MICE BY A BENZODIAZEPINE (TRIAZOLAM), A 5-HT_3 ANTAGONIST (ICS 205-930) AND AN NMDA ANTAGONIST (MK-801). S. Williams, G. P. Vincent and J. Sepinwall. Neurobiology & Obesity Research, Hoffmann-La Roche Inc., Nutley, NJ 07110.

The ability of three different types of compounds to block stimulation of locomotor activity induced by cocaine (30 mg/kg i.p.) in mice was evaluated in the Digiscan apparatus. Test compounds were administered 25 min and cocaine 15 min, respectively, before the start of a 1 h test session. Triazolam (0.03 and 1.0 mg/kg p.o.), selected as an agent which depressed activity when given alone, blocked cocaine-induced stimulation on the four key parameters (horizontal activity, total distance, vertical activity, stereotypy counts). This antagonism may have been a consequence of the depressant effects of triazolam alone. ICS 205-930 (0.1 mg/kg i.p.), a selective 5-HT_3 antagonist, did not alter activity when given alone but did produce small, statistically significant decreases in the response to cocaine at some time intervals on three key variables. Interestingly, ICS 205-930 failed to antagonize the effect of cocaine on vertical activity. MK-801 (0.065 mg/kg i.p.), an NMDA antagonist, slightly increased locomotor activity when given alone; it antagonized cocaine on only one parameter, vertical activity. (Additional doses of MK-801 are currently being studied.) This methodology enables qualitative distinctions to be seen among compounds that can antagonize cocaine and should be useful in the search for a novel cocaine antagonist.

348.8

PHARMACOGENETIC ASSESSMENT OF THE USE OF CARBAMAZEPINE (CBZ) FOR THE TREATMENT OF COCAINE ADDICTION AND TOXICITY. R. J. Marley & S. R. Goldberg. NIDA-Addiction Res. Ctr., Box 5180, Baltimore, MD 21224.

CBZ is currently being tested in humans as a treatment for cocaine addiction and may be useful in the treatment of some aspects of cocaine-induced toxicity. We have previously shown that there are genetic differences among mouse strains in the development of increased susceptibility to the convulsant effects of cocaine following its repeated administration (cocaine kindling). The present studies evaluated genetic differences in the modulation of the convulsant and epileptogenic effects of cocaine by CBZ in BALB, C57 and SJL mice. The dietary administration of CBZ attenuated the development of cocaine kindling and suppressed the initial susceptibility to cocaine-induced seizures in all 3 strains, however, there were substantial differences among the strains in the degree to which CBZ inhibited cocaine-kindled seizures. Genetic differences in susceptibility to an acute, convulsant dose of cocaine were also observed following chronic CBZ alone, or in conjunction with cocaine kindling. The concurrent administration of CBZ and cocaine also had genotype-specific lethal effects. While little or no lethality was observed among C57 mice when administered either CBZ or cocaine alone, the concurrent administration of the two drugs resulted in 100% of the animals dying by the seventh day. No lethality was observed among BALB and SJL mice that received both drugs simultaneously. The pattern of genetic differences observed in these studies suggests that these strains may provide useful animal models for investigating the mechanisms underlying cocaine kindling and CBZ/cocaine interactions.

348.9

THE ROLE OF SEROTONIN IN MODULATING BASAL AND COCAINE-INDUCED INCREASES IN DOPAMINE OVERFLOW IN THE NUCLEUS ACCUMBENS. A. Pert and C.B. Hubner. NIMH/BPB, Bethesda, MD 20892.

Several studies have suggested that serotonin may influence the behavioral effects of psychomotor stimulants. Since many of these effects are thought to be mediated by dopaminergic systems, the present experiments were designed to investigate the role of serotonin and a serotonin antagonist in modulating extracellular dopamine levels and cocaine-induced increases in extracellular dopamine in the rat using the *in vivo* microdialysis procedure. When compared with vehicle, infusion of serotonin (5×10^{-6}, 10^{-5} and 5×10^{-5} M) through the dialysis probe produced a dose-dependent increase in extracellular dopamine concentrations in the nucleus accumbens. The largest effect (approximately 500% of control) was obtained at the highest concentration examined. The effect of serotonin produced by the intermediate concentration (10^{-5} M) was antagonized by pretreatment with the 5-HT3 receptor antagonist MDL 72222 (1.0 mg/kg, i.p.). Systemically administered cocaine (25 mg/kg, i.p.) also increased the levels of extracellular dopamine in the nucleus accumbens and in other experiments, stimulated locomotor activity. Ongoing studies are assessing whether MDL 72222 can antagonize these biochemical, as well as behavioral, effects of cocaine.

348.10

REGIONAL EFFECTS OF COCAINE ON SEROTONIN OVERFLOW FOLLOWING FOCAL APPLICATIONS. A. Zocchi, D.J. Fontana, and A. Pert. BPB, NIMH, Bethesda, MD 20892.

Cocaine prevents the neuronal reuptake of both serotonin (5-HT) and catecholamines. Alterations in dopaminergic (DA) functions by cocaine have been studied extensively and presumably underlie the major behavioral effects of this psychomotor stimulant. Less, however, is known about the actions of cocaine on 5-HT. The purpose of this study was to characterize the effects of focally applied cocaine on 5-HT overflow in rat forebrain structures with microdialysis procedures. A microbore reverse-phase HPLC-EC system was used in order to optimize detection of the relatively low levels of 5-HT in the extracellular space. Microdialysis probes were introduced into either the striatum, n. accumbens, or frontal cortex of chloral hydrate-anesthetized rats. Following stabilization of basal 5-HT levels (approximately 2 — 3 hours), various concentrations of cocaine (0.01, 0.1 and 1.0 mM) were introduced into the targetted brain structure in the perfusate. Focal applications of cocaine produced a concentration-dependent increase in serotonin in all three structures. The striatum, where the three concentrations of cocaine produced 5-HT levels of 226%, 553%, and 1042% of baseline, respectively, appeared to be the most sensitive to cocaine. Basal levels of 5-HT also differed among the three structures. Dialysate levels from the n. accumbens were approximately double those from the striatum, and frontal cortex levels were about half those in the striatum.

The effects of cocaine on 5-HT overflow in the striatum and n. accumbens are somewhat more modest than those found for DA in previous studies. Furthermore, cocaine appeared to have relatively little effect on DA in the frontal cortex, but in this study its effects on 5-HT were considerably more uniform across structures. This would suggest that 5-HT uptake mechanisms are more homogeneous throughout the brain.

348.11

COCAINE AND SEROTONIN: EFFECTS OF GEPIRONE ON BEHAVIORAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF ACUTE AND CHRONIC COCAINE. *J.M. Paris, N.E. Goeders[1] and K.A. Cunningham.* Dept Pharmacol Toxicol, Univ Texas Med Branch, Galveston, TX 77550; [1]Dept Pharmacol Therapeut, Louisiana State Univ Med Cent, Shreveport, LA 71330.

Cocaine (COC) is known to interact with the serotonin (5-HT) systems in the brain. Serotonergic drugs which may modify the effects of COC are actively being explored as potential treatments for the cocaine abuser. We examined the effects of the partial 5-HT_{1A} agonist and novel anxiolytic gepirone (GEP) on the behavioral and electrophysiological effects of COC. Male Sprague-Dawley rats (n=8/grp) were treated in automated test enclosures with saline (SAL) or GEP (7.5 mg/kg, ip) followed 15 min later by SAL or COC (10 mg/kg, ip) twice daily for 7 days (Days 1-7). On Day 8, the locomotor activity of rats was monitored after challenge with SAL or COC (10 mg/kg). Comparison of the activity between Day 1 and Day 7 indicated that SAL+COC rats developed behavioral sensitization. Interestingly, the activity of GEP+SAL animals was not different than that of SAL+ SAL rats on Days 1 or 7 while prolonged locomotor activity was displayed in GEP+COC vs SAL+COC rats on Day 7. On Day 8, diminished activity in response to COC was observed in GEP+COC vs SAL+COC rats suggesting that GEP pretreatment reduced the expression of COC sensitization. Furthermore, GEP+SAL rats demonstrated significantly lower activity when challenged with COC (10 mg/kg) as compared to SAL+SAL rats suggesting that pretreatment with GEP reduced the expression of the acute effects of COC. Additional rats treated with SAL or GEP (7.5 mg/kg, 2x/day, 7 days) were prepared for single-unit recordings of dorsal raphe 5-HT neurons. The inhibitory response of 5-HT cells to COC appeared to be unchanged in GEP vs. SAL-treated rats. Thus, GEP reduces the behavioral effects of COC in a manner possibly unrelated to GEP-induced changes in 5-HT autoreceptor sensitivity. *Supported by Bristol-Myers/Squibb, DA 05708, DA 06511 (KAC), DA 05381 (JMP).*

348.12

FLUOXETINE PRETREATMENT REDUCES BREAKING POINTS ON A PROGRESSIVE RATIO SCHEDULE REINFORCED BY INTRAVENOUS COCAINE IN THE RAT. N.R. Richardson and D.C.S. Roberts, Dept. of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Previous studies have reported reductions in rate of stimulant self-administration following pretreatment with the specific serotonin re-uptake inhibitor fluoxetine. Since changes in rate are difficult to interpret, we re-evaluated the effects of fluoxetine on cocaine self-administration using a Progressive Ratio (PR) schedule.

Male Wistar rats were implanted with intravenous cannulae and trained to respond on a PR schedule for cocaine (0.6 mg/inj). Pretreatment with fluoxetine (2.5, 5.0, 10.0 and 20.0 mg/kg IP) significantly decreased breaking points at the 5.0 and 20.0 mg/kg doses.

These results, together with data suggesting that rats will respond to higher breaking points for cocaine following lesions of ascending serotonergic fiber systems, support the hypothesis that serotonin plays an aversive role in stimulant reinforcement. (Supported by NIDA contract no. 271-90-7401).

348.13

SPECIFIC 5-HT_2 and 5-HT_3 RECEPTOR ANTAGONISTS FAIL TO ALTER BREAKING POINTS ON A PROGRESSIVE RATIO SCHEDULE REINFORCED BY INTRAVENOUS COCAINE IN THE RAT. S. Lacosta* and D.C.S. Roberts. Dept. of Psychology, Carleton Univ., Ottawa, Canada, K1S 5B6.

An important role for serotonin in cocaine reinforcement is suggested by the findings that rats, on a Progressive Ratio (PR) schedule, will respond to higher breaking points following lesions of 5-HT systems and lower breaking points following pretreatment with the 5-HT re-uptake inhibitor, fluoxetine.

In an attempt to determine which serotonin receptors are involved, we investigated the effects of pretreatment with ketanserin and MDL 72222 (a specific 5HT_2 & 5HT_3 antagonist, respectively) on cocaine self-administration.

Neither ketanserin (0.4 - 6.4 mg/kg, IP) nor MDL 72222 (7.5 - 120 ug/kg, IP) significantly altered breaking point values. These data suggest that neither 5HT_2 nor 5HT_3 specific receptors play a role in cocaine reinforcement and the specific receptors involved remain to be determined. (Supported by NIDA contract no. 271-90-7401).

348.14

COMPARISON OF EFFECTS OF COCAINE AND COCAETHYLENE ON DOPAMINE AND SEROTONIN IN VIVO, USING MICRODIALYSIS. C.W. Bradberry*, E. Murphy, J. Nobiletti, P. Jatlow, and R.H. Roth*. Depts. of Pharmacology, Psychiatry, and Laboratory Medicine, Yale Univ. Sch. Med., New Haven CT 06510.

Cocaethylene (CE) is an active metabolite of cocaine (COC), shown to be similar in potency to cocaine at inhibiting uptake of dopamine (DA) in vitro, and enhancing motor activity and extracellular DA in vivo. Initial reports suggested CE to be less effective at displacing 3H-paroxetine, (which binds to the 5-HT reuptake carrier) than cocaine. We performed in vivo measurements of extracellular 5-HT and DA to determine if CE differs from COC in its actions on 5-HT. Administration of 1 mg/kg i.v. COC and CE caused an indistinguishable increase in extracellular DA in the nucleus accumbens to 350-400% of basal levels. The actions on 5-HT were studied in the striatum using 5 mm microdialysis probes. Basal 5-HT values were approx. 2 fmol/ul (corrected for probe recovery). At 1 mg/kg i.v., COC caused a significant increase to 200% of basal levels, while EC caused no significant increase. To determine if larger doses of EC would be able to significantly increase 5-HT levels, 15 mg/kg i.p. was employed. With this dose, COC increased 5-HT levels to 350% of baseline, while EC caused a significant increase to 200% of basal levels. A portion of the microdialysates were used for determination of extracellular striatal levels of COC and EC following 15 mg/kg i.p. Supported in part by the State of CT, MH 14092, DA 04060, and DA 05119.

348.15

SEROTONIN MEDIATION OF CUE-INDUCED COCAINE CRAVING. S.L. Satel*, P.L. Delgado* and D.S. Charney. West Haven VAMC, Yale Univ Sch. of Med., New Haven, CT 06519

The aim of this study was to determine whether alteration in central serotonin, through the technique of tryptophan depletion, reliably influences cue-induced craving for cocaine.Twelve cocaine dependent males (DSM lll-R) without other Axis 1 diagnoses underwent two test days during their hospitalization on rehabilitation unit. All subjects had positive tox screens for cocaine at the time of admission and negative urines 24 hours prior to testing. Test days, one week apart, took place between weeks 3 and 4 of hospitalization. The design entailed a double-blind counterbalanced placebo-controlled administration of control or active tryptophan-depleting amino acid drink. Assessment of plasma TRP and subjective measures of mood, arousal and craving were administered baseline (0), +340 mins, +400 mins and +420 mins. Previous research has determined that the depleting drink results in a 60-80% decrease in plasma tryptophan at about 6 hours after ingestion and this correlates with a 60% decline in central 5HT levels. Thus, six hours after aa drink ingestion subjects were exposed to 15 minutes of cocaine cues (film of users, paraphernalia and guided imagery). Reported desire for cocaine assessed before and after cue-exposure represented the critical comparison. Mean subjective increase in reported cocaine craving following cue exposure was 20 mm on the depletion day and 55 on the control day ($p<.056$). Change in mood (at 400 mins. minus baseline) did not differ between test days. Data suggest that tryptophan depletion attenuates cue-induced desire for cocaine.

348.16

ON THE MECHANISMS BY WHICH 5-HT_3 RECEPTOR ANTAGONISTS INHIBIT COCAINE-INDUCED HYPERACTIVITY. **A.L. Svingos, R.E. Strecker, C.S. McNeish and R. Hitzemann, Departments of Psychiatry and Psychology, SUNY at Stony Brook, Stony Brook, NY 11794-8101 and Psychiatry Service, VAMC, Northport, NY 11768.**

In mice the 5-HT_3 receptor antagonists, zacopride and ICS 205-930, block the hyperactivity induced by an acute cocaine injection (Reith, 1990). We now report similar results in rats pretreated with (+)zacopride (0.03 mg/kg, i.p.), ICS 205-930 (0.1 mg/kg,i.p.), or MDL 72222 (1.0 mg/kg, i.p.) fifteen minutes before the challenge with (+)cocaine (10 mg/kg, i.p.). (+)Zacopride significantly inhibited (approximately 50%) the effects of cocaine at a dose of 10 μg/kg. 5-HT_3 antagonists may attenuate cocaine-induced behaviors through effects on dopamine transport or release (see e.g. Carboni et al. 1989). We investigated whether or not 5-HT_3 antagonists block the cocaine binding site on the DA transporter and/or effect the ability of DA to regulate this binding site. In well washed striatal membranes, neither zacopride nor ICS 205-930 (10^{-9} to 10^{-5}M) inhibited ^{3}H WIN 35,428 (3 x 10^{-9}M) binding. Furthermore, neither of these compounds affected the ability of DA to block WIN 35,428 binding. To determine if 5-HT is required for the 5-HT_3 antagonist effect, we are currently examining the interaction between cocaine and zacopride in rats pretreated with p-chlorophenylalanine (PCPA). We have also investigated, using *in vivo* microdialysis, the effect of zacopride on cocaine-induced release of DA from the nucleus accumbens. At both 30 and 100 μg/kg, zacopride did not block the 3-fold cocaine-increase of extracellular DA.

348.17

LONG-TERM FUNCTIONAL ALTERATIONS IN SEROTONERGIC SYSTEMS FOLLOWING IN UTERO EXPOSURE TO METHAMPHETAMINE G. Battaglia, T.M. Cabrera, F. Tung*, A.D. Levy, P.A. Rittenhouse, Q. Li*, J. Yracheta*, K. Kunimoto*, R.J. Handa, L.D. Van de Kar Department of Pharmacology & Dept. of Cell Biology and Anatomy, Loyola University Chicago, Maywood, IL 60153

In adult rats, methamphetamine(METH) produces biochemical alterations and degeneration of brain serotonin(5-HT) neurons. We hypothesized that since 5-HT is critical to the development of 5-HT neurons and target tissues during gestation, *in utero* METH would produce long-term changes in postnatal 5-HT systems. Pregnant Sprague-Dawley rats, administered either saline or METH(5 mg/kg s.c. b.i.d.) from gestational day 13 through 20, comprised 3 treatment groups(sal-inj./ad lib-fed; sal-inj./pair-fed; & METH-injected). All progeny were fostered to untreated lactating dams. Functional alterations were determined by measuring plasma hormone levels following 5-HT release induced by a single injection of 8 mg/kg PCA(p-chloroamphetamine). Marked attenuations in PCA-mediated increases in plasma renin activity (PRA; -68%) and plasma renin concentration (PRC; -54%) were observed in male progeny of METH-treated mothers on postnatal day(PD) 70. METH produced comparable reductions in PRC in PD28 female progeny. This METH treatment produced no neurodegeneration in the mothers as assessed by the lack of reduction in cortical 5-HT uptake sites. In contrast to the *in utero* data, no changes in PCA-mediated increases in either PRA or PRC were observed in adult rats 2 weeks post-treatment with neurotoxic doses of METH (20 mg/kg s.c., b.i.d for 4 days). These studies demonstrate the unique ability of *in utero* METH to produce long-term functional changes in progeny 5-HT systems. Additional studies should elucidate whether the functional deficits produced by *in utero* METH are due to (1) alterations within 5-HT neurons, (2) changes in 5-HT receptors or (3) changes in post-receptor components.

348.18

COCAINE-INDUCED ELEVATION OF PLASMA ACTH AND CORTICOSTERONE (CORT) IS MEDIATED BY SEROTONERGIC NEURONS. T.M. Cabrera, A.D. Levy, Q. Li*, J.E. Kerr*, P.A. Rittenhouse, G. Milonas*, G. Battaglia & L.D. Van de Kar. Dept. of Pharmacology, Loyola Univ. of Chicago, Maywood IL 60153.

The role of serotonin (5-HT) neurons in the mediation of cocaine-induced enhancement of ACTH and CORT was examined. Male Sprague-Dawley rats were pretreated with either (1) p-chlorophenylalanine (PCPA), a 5-HT depleting agent, (2) 5,7-dihydroxytryptamine (5,7-DHT), a 5-HT neurotoxin, (3) BMY 7378, a 5-HT_{1A} antagonist, (4) ritanserin, a 5-$HT_{2/1C}$ antagonist, or (5) their respective vehicles. The rats were then challenged with varying doses of cocaine (0-15 mg/kg, i.p.); 15 min later, the rats were decapitated, and trunk blood was collected for radioimmunoassay of plasma ACTH and CORT. Cocaine dose-dependently increased ACTH and CORT levels. These increases were prevented by PCPA, 5,7-DHT, and ritanserin but not by BMY 7378. These data suggest 5-$HT_{2/1C}$ mediation of the cocaine-induced elevations in ACTH and CORT.

To determine whether cocaine's effect on the hypothalamic-pituitary-adrenal axis is peripherally or centrally mediated, cocaine (0-1000 μg/kg) was administered into the lateral cerebral ventricles through chronically indwelling cannulae. Trunk blood was again collected 15 min post-cocaine injection and assayed for CORT and ACTH. ICV administration of cocaine significantly increased ACTH and CORT levels at doses 300 times less than the i.p. dose necessary to produce the same effects, suggesting that these actions are centrally mediated. In conclusion, our data strongly support the hypothesis that cocaine-induced stimulation of ACTH and CORT secretions are mediated largely by 5-HT neurons in brain, and furthermore that 5-HT_2 or 5-HT_{1C} receptors are responsible for these effects. (Supported by NIDA DA04865)

348.19

CHRONIC COCAINE INHIBITS THE SEROTONERGIC STIMULATION OF ACTH AND CORTICOSTERONE SECRETION. A.D. Levy, L.D. Van de Kar, A.M. Bonadonna*, P.A. Rittenhouse, J.E. Kerr*, L. Iyer*, G.B. Herbert*, M.C. Alvarez Sanz*, S.J. Lent*, M. Carnes. Dept. Pharmacology, Loyola Univ. Chicago, Maywood IL 60153 and Dept. Medicine, Univ. Wisconsin, Madison WI 53705.

To examine cocaine-induced changes in serotonergic function, the endocrine responses to the serotonin (5-HT) releaser p-chloroamphetamine (PCA), and 5-HT agonists RU 24969 and m-CPP were examined following repeated exposure to cocaine. In the initial experiments, the dose and duration of cocaine exposure were studied. Male Sprague-Dawley rats received cocaine (2x/day) in doses of 1-15 mg/kg for 30 days, or 15 mg/kg for 1-30 days, and were then administered PCA (8 mg/kg) 42 hr after the final cocaine dose. Rats were decapitated 1 hr following PCA and the blood was collected for radioimmunoassay of ACTH and corticosterone. In subsequent studies, rats received cocaine (15 mg/kg, 2x/day) for 7 days, and were challenged with RU 24969 (0.2-5 mg/kg) and m-CPP (1-20 mg/kg) 42 hr after chronic cocaine treatments. Blood was collected 30 min after 5-HT agonist challenges.

PCA markedly enhanced secretion of ACTH and corticosterone. The 15 mg/kg dose of cocaine for 30 days reduced the PCA-induced increase in corticosterone. Additionally, PCA-induced elevation of corticosterone was reduced by 1, 7, or 30 days of cocaine exposure (15 mg/kg), while PCA-induced enhancement of ACTH was inhibited only by 7 or 30 days of cocaine (15 mg/kg) pretreatments. Furthermore, the magnitude of the inhibitory effect of cocaine on PCA-induced ACTH release was more marked than for corticosterone. The 5-HT agonists RU 24969 and m-CPP also increase ACTH and corticosterone secretion. However, these increases were not reduced by 7 days of cocaine pretreatments. The data suggest that repeated cocaine alters function of 5-HT nerve terminals, but not postsynaptic 5-HT receptors. (Supported by DA04865 and MH45812).

348.20

THE EFFECTS OF SEROTONERGIC MANIPULATIONS ON COCAINE SELF-ADMINISTRATION IN RATS. R. Peltier and S. Schenk. Texas A&M Univ., Dept. Psychol., College Station, TX, 77843.

Recently, serotonin has been implicated as mediating the reinforcing properties of cocaine in that indirect agonists or specific neurotoxic lesions have been found to alter the rate of cocaine self-administration. The present experiment was an attempt to identify the specific serotonin receptor subtype that may be critical for this effect. Rats were trained to self-administer cocaine (0.5 mg/kg/infusion) and were then pretreated with the 5-HT1A agonist, 8-OHDPAT (0.125, 0.25 or 0.5 mg/kg, SC) or the 5-HT2 antagonist, ritanserin (0.01, 0.1 or 1.0 mg/kg, SC). Ritanserin had no effect on cocaine self-administration, while 8-OHDPAT produced a decrease in reinforced response rates. The effect of 8-OHDPAT (0.5 mg/kg) on the dose/response curve for cocaine self-administration was then assessed. A reduction of response rates was found for all doses of cocaine, although the effect was most pronounced when lower cocaine doses served as the reinforcer. Fluoxetine (10 mg/kg, IV) also reduced reinforced response rates for a low dose infusion of cocaine when the schedule was FR1. When an FR10 schedule of reinforcement was imposed, reinforced response rates for higher doses of cocaine were also reduced. Thus, under conditions that produce high rates of responding (low dose infusions or high ratio requirements/infusion) fluoxetine reduced responding. This effect may be due to effects at the 5-HT1A receptor, since 8-OHDPAT produced a similar dose/dependent effect on cocaine self-administration. However, given the dose and task dependency of the effects of these 5-HT agonists, it is likely that the suppression of responding reflects an effect that is not specific to the reinforcing impact of cocaine.

348.21

CHRONIC COCAINE MODULATION OF PREFRONTAL CORTEX CELLULAR ELECTROPHYSIOLOGIC RESPONSES. J.M. Lakoski and H.J. Moday*. Dept. of Pharmacol., Univ. Texas Medical Branch., Galveston, TX 77550.

Acute administration of cocaine inhibits neuronal responses mediated by monoaminergic systems, including serotonin (5-HT). Repeated cocaine administration has been demonstrated to decrease sensitivity to a subsequent cocaine challenge as recorded at the dorsal raphe nucleus (DRN) 5-HT autoreceptor. In these present studies, we have investigated the effects of chronic cocaine exposure on a terminal projection region of the DRN, the prefrontal cortex (PFC).

Adult male Sprague-Dawley rats received: (1) acute cocaine (15 mg/kg,i.p.) or saline (2) chronic cocaine (15 mg/kg, 2X daily, i.p.) or saline for 30 days. At 24 hr following the last injection, pyramidal cell responses were recorded *in vivo* (chloral hydrate) and iontophoretic responses to cocaine, 5-HT and GABA evaluated. No significant differences in sensitivity (IT_{50} values) were evident between acute cocaine, acute saline and chronic saline treated groups. Chronic cocaine significantly decreased responsiveness to cocaine and 5-HT (2-3X increase). Desensitization may underlie the cellular basis of cocaine's numerous behavioral effects mediated in the PFC.

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349.1

COCAETHYLENE FORMATION FOLLOWING SEQUENTIAL ADMINISTRATION OF COCAINE AND ETHANOL TO HUMANS: PHARMACOLOGICAL, PHYSIOLOGICAL, AND BEHAVIORAL STUDIES. E.F. McCance-Katz, L.H. Price, C.J. McDougle, G.J. Marek, T.R. Kosten and P. Jatlow, Yale University Depts. of Psychiatry and Laboratory Medicine, New Haven, CT 06519.

At least 50% of cocaine abusers in the U.S. are also concurrent ethanol abusers and simultaneous consumption is common. Cocaethylene (EC), the ethyl ester of benzoylecgonine, has been detected in urine and high concentrations have been measured in blood of individuals in association with concurrent cocaine and ethanol use. EC is equipotent to cocaine as an inhibitior of dopamine uptake and as a reinforcer in self-administration studies in primates. This study was undertaken to prospectively evaluate the interaction of cocaine and ethanol in humans. METHOD: Four cocaine-ethanol challenges were administered in a double-blind randomized sequence to each subject (n=4) over 8 days. The four test days included the following drug combinations: cocaine (2mg/kg)/ethanol (1g/kg), cocaine (2mg/kg)/ethanol placebo, cocaine placebo/ethanol (1g/kg), cocaine placebo/ethanol placebo. Cocaine hydrochloride powder was administered intranasally followed by ethanol administered orally. Physiological and subjective (visual analog scales, "High scale") measures and plasma cocaine, EC, and ethanol levels were assessed. RESULTS: EC formation following sequential administration of cocaine and ethanol was demonstrated. Peak concentrations of EC after single doses of cocaine and ethanol occurred at 90-120 minutes and were about one-sixth of the cocaine peak. Plasma concentrations of EC declined more slowly than cocaine, and by 8 hours concentrations of parent and metabolite were about equal. The plasma level of cocaine during combined cocaine/ethanol administration was significantly greater than that during cocaine/placebo administration ($p<.04$), but elimination constants for cocaine under the two conditions were approximately equal. Cocaine/ethanol and cocaine/placebo administration resulted in significant increases in heart rate as compared to ethanol and placebo administration. The combined use of cocaine and ethanol was associated with more intense subjective effects, which were prolonged over those of cocaine or ethanol consumption alone. CONCLUSION: This study confirms that EC is formed in humans following coadministration of cocaine and ethanol and may contribute to physiological and behavioral effects. During repeated self administration of cocaine and ethanol, as occurs during a binge, EC would accumulate. Higher cocaine levels detected during combined cocaine/ethanol administration may be the result of enhanced bioavailability of cocaine in the presence of ethanol.

349.2

EFFECTS OF INTRACEREBRAL COCAINE INFUSIONS ON I.V. COCAINE SELF-ADMINSTRATION. **G.H. Jones, S.E. Hemby, D.B. Neill & J.B. Justice, Jr. Depts. of Chemistry and Psychology, Emory University, Atlanta, GA 30322.**

The mesolimbic dopamine pathway, in particular, the ventral tegmental area, nucleus accumbens (NACC) and medial prefrontal cortex, have been strongly implicated in mediating the reinforcing effects of cocaine. This experiment was designed to assess the effects of infusing cocaine directly into discrete regions of this dopaminergic pathway on i.v. cocaine intake and the regularity of responding in the self-administration paradigm.

Rats were trained to self-administer cocaine i.v. in daily 3 hr FR-1 sessions. Each lever press delivered 0.25 mg cocaine HCl (in 0.1 ml saline) over a 4 sec period via a jugular catheter. Once stable responding had been achieved (a minimum of 10 days training) subjects were bilaterally infused with either 0.0, 12.5, 25, 50, or 100 $\mu g/\mu l$ cocaine on separate test days. For these intracerebral infusions cocaine was prepared in artificial CSF and infusions ($2 \times 1\ \mu l$) were carried out over a 1 min period with 1 min allowed for diffusion. A Latin-square design was used to determine the sequence of infusions. On each test day the subjects were allowed to self-administer cocaine for a minimum of 1 hr prior to infusion to achieve stable baseline responding. Following intracerebral infusions responding was monitored for at least 90 min.

There were clear dose-dependent effects of cocaine infusions into the NACC ($n=10$) on delaying the next lever press after infusion and on increasing the variability in the time interval between lever presses. In addition, all the NACC cocaine infusions significantly reduced i.v. cocaine intake in the 20 min period following infusion by about 50% (compared with the 20 min pre-infusion intake). This reduction in cocaine intake lasted for approximately 30 mins. CSF infusions into the NACC did not significantly reduce cocaine intake.

These effects of these NACC infusions will be compared with those following cocaine infusions into other regions of the mesolimbic dopamine pathway.

349.3

WITHDRAWAL FROM CHRONIC COCAINE ADMINISTRATION: EFFECTS ON BEHAVIOR AND BETA ADRENERGIC AND SEROTONERGIC BRAIN RECEPTORS IN RAT. E.A. Johnson, I.J. Goodman, Y.H. Shahan* and A.J. Azzaro. Dept.s of Behavioral Med./Psychiatry, Psychology, and Neurology. West Virginia Univ. Sch. of Med., Morgantown, WV 26506

The effects of withdrawal from chronic cocaine (COC) administration on behavior and on β adrenergic and 5-HT_2 receptors in frontal cortex and hippocampus were studied. Male Sprague-Dawly rats were administered cocaine (10 mg/kg, i.p.) daily for 15 days, then withdrawn from the drug. COC treated rats displayed a slower rate of adaptation to a novel open field at 24, 48, and 72 hours withdrawal, measured by exploratory behavior, compared to saline injected controls ($p < 0.05$). No difference between COC and saline controls was detected in the modified Porsolt swim test at any time. COC treated rats exhibited small, time dependent, differences in frontal cortex β ([^{3}H]CGP12177) and 5-HT_2 ([^{3}H] Ketanserin) receptor number compared to controls. No differences were detected in hippocampus. Cocaine withdrawal appears to be anxiogenic in rats. Supported by WVU Med. Corp. and NIH Biomed. Res. Grant No. 2S07RR05433

349.4

EFFECTS OF CHRONIC COCAINE AND WITHDRAWAL ON REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. E.A. Stein and S.A. Fuller* Dept. of Psychiatry, Medical College of Wisconsin, Milwaukee, WI

Chronic opiates have long been recognized to produce a behavioral and pharmacologic tolerance together with the development of physical dependence and a marked withdrawal syndrome. In contrast, the syndrome following chronic cocaine is less well understood. Withdrawal in man is characterized by a 'psychosis-like' paranoia. Sensitization has been reported in animals, manifest as an increase in locomotion and increased susceptibility to cocaine-induced seizures. Chronic cocaine also increases both cocaine and dopamine in the nucleus accumbens. The present study addresses the question of which neuroanatomic structures respond to and perhaps mediate the behavioral sensitization following chronic cocaine administration and which become activated during withdrawal. Rats were injected with either 0, 1.0 or 10.0 mg/kg cocaine IP twice a day for 14 d. On the day of rCBF determination, each treatment group received a challenge injection of either saline or 1.0 mg/kg cocaine 6-8 hrs after their last chronic dose. rCBF was determined using [^{14}C] Iodoantipyrene according to the method of Sakurada et al. Analysis of variance indicated no differences between the two chronic 1 mg/kg groups and the saline group. Additionally, the chronic 10 mg/kg group receiving the acute drug challenge also did not generally differ from saline. In contrast, increases in rCBF were seen in 18/63 areas in the 10 mg/kg group receiving acute saline compared to low dose and saline groups. Those areas activated by or during withdrawal include the nucleus accumbens, olfactory tubercle, bed nucleus, diagonal band, medial prefrontal cortex, septum and habenula. It thus appears that withdrawal from high but not low cocaine doses results in selective neuronal activation, with acute cocaine returning many regions to their baseline levels (Supported by grant DA 05012).

349.5

HEMODYNAMIC EFFECTS OF ACUTE COCAINE EXPOSURE IN CONSCIOUS LAMBS. F.M. Scalzo, J. Valentine* and B. Taylor*. Department of Pediatrics, University of Arkansas for Medical Sciences & Arkansas Children's Hospital, Little Rock, AR 72205.

Cocaine is known to have potent cardiovascular effects which include hypertension and tachycardia in adults. However, the adverse effects of cocaine exposure on cardiovascular function in newborns are not well understood. To identify potential adverse effects of cocaine exposure in newborns, we examined the hemodynamic effects of acute cocaine exposure in unanesthetized one month old lambs that were instrumented for measurement of aortic pressure (AP), left atrial and ventricular pressures, left circumflex coronary artery flow (CF) and heart rate (HR). Lambs were studied for 1 hour following saline or cocaine-HCl (3.0 or 6.0 mg/kg, i.v.) injection. At the 6.0 mg/kg dose the maximum increases for AP, CF and HR were 170%, 148%, and 160%, respectively, of baseline. The lamb thus appears to be a good model to study the cardiovascular effects of acute cocaine exposure in newborns and the results suggest that exposure to cocaine causes adverse hemodynamic effects in this animal model (supported in part by DA-06319 and HL-01822).

349.6

ZIF/268 EXPRESSION IN THE RAT BRAIN DURING COCAINE WITHDRAWAL. D.J. Ennulat, S.M. Babb*, S.E. Hyman and B.M. Cohen, Laboratory for Psychiatric Research, McLean Hospital and Harvard Medical School, 115 Mill Street, Belmont, MA 02178.

Changes in the expression of immediate early genes (IEGs) in response to drugs and other stimuli are generally characterized by rapid and transient kinetics. The present study describes long-term changes in the expression of the IEG zif/268 during cocaine withdrawal. Either 6 mg/kg cocaine or 0.9% saline (vehicle) was administered to male, Sprague Dawley rats (300-340g) by IV injection, via an implanted jugular cannula, 1 time daily (acute) or 3 times daily for 1 day, 2 days or 5 days (chronic). The chronically treated rats were allowed to withdraw from cocaine for 4 days, 3 days or 60 min, respectively. At the appropriate times three animals per group were sacrificed and the brains were rapidly removed and dissected into major regions of interest, based on changes in zif/268 expression during acute cocaine treatment, particularly amygdala (AM), frontal cortex (FC), and striatum (ST). Total RNA was extracted from the dissected brain regions and subjected to Northern analysis by hybridization with ^{32}P labeled probes for zif/268 and cyclophilin, as an internal reference. Following high stringency washes, the levels of cyclophilin and zif/268 were quantified by digitization of the resulting autoradiograms. After normalization to the amount of cyclophilin, the relative level of zif/268 was determined for the cocaine treated animals by comparison with the vehicle control. Acute cocaine treatment causes a rapid increase in the expression of zif/268 in the FC, ST and to a lesser extent in the AM. In this preliminary study, chronic treatment with cocaine resulted in a desensitization of the cocaine-induced increase in zif/268 expression. Also, withdrawal from continued cocaine treatment resulted in an apparent reduction in the expression of zif/268 below that found in vehicle controls. This observation may have implications in understanding the molecular and cellular changes that are associated with cocaine withdrawal.

349.7

ALTERATIONS IN DRUG METABOLISM RESULTING FROM PRIOR ADMINISTRATION OF DRUGS OF ABUSE. W.L. Backes*, C.S. Eyer*, G. Cawley*, and J.M. Moerschbaecher. Department of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

The purpose of this study was to determine if prior administration of different drugs of abuse could alter hepatic microsomal drug metabolism. Male Holtzman rats were pretreated with 3 daily i.p. injections of the following drugs: diazepam (1.8 mg/kg body weight), heroin (1 mg/kg), cocaine (18 mg/kg), and phencyclidine (PCP; 18 mg/kg). Rats were killed 24 hours after the final injection and liver microsomes were prepared. Drug-induced changes in the overall levels of cytochrome P-450, associated electron transport components, and a number of P-450-dependent reactions were examined to characterize the inductive response. PCP pretreatment produced a 30% increase in cytochrome b_5 levels, and a 55% increase in p-nitroanisole (pNA) demethylation. Heroin administration appeared to produce about a 30% decrease in 7-ethoxycoumarin-O-deethylation. Diazepam and cocaine were without effect in these initial experiments. Since cocaine has such a short biological half life, the effect of cocaine was reexamined using a different administration schedule. Cocaine was administered at time zero at a dose of 18 mg/kg body weight followed by injections at 12, 18, and 24 hrs. Microsomes were prepared at 36 hrs. Cocaine pretreatment resulted in a 45% increase in pNA demethylation, and a 35% increase in N,N-dimethylnitrosamine demethylation. These results clearly demonstrate that a number of drugs of abuse, particularly PCP and cocaine, can induce P-450-dependent activities. Such metabolic changes may have significant implications concerning the chronic abuse of these drugs. (Supported by NIEHS 04344 and DA03573).

349.8

RESPIRATORY PATTERNING FOLLOWING INTRAVENTRICULAR COCAINE ADMINISTRATION. C.A. Richard, R.K. Harper, H. Ni and R.M. Harper. Brain Research Institute and Dept. of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

Respiratory rate accelerates markedly in response to acute cocaine intoxication, a phenomenon which may contribute to respiratory failure associated with cocaine use in humans. This tachypnea develops almost immediately upon intravenous (IV) injection and can persist for 3-4 hours. We have quantified the diaphragmatic EMG response to IV cocaine by measuring total cycle time (TTot) as well as the inspiratory and expiratory phase durations (TI, TE, respectively; Richard et al. Pharmacol., Biochem, Behav., in press). To differentiate central and peripheral effects of cocaine, we unilaterally injected cocaine HCl into a lateral cerebral ventricle of adult cats. Animals were anesthetized and instrumented with EMG leads in the costal diaphragm. After 1-2 weeks of recovery baseline recordings were performed for 30-60 minutes in unanesthetized unrestrained animals. This was followed by an injection of 2.5 mg cocaine through a cannula placed in a lateral ventricle. TTot decreased by 45% at 10 min post-cocaine and the largest fall occurred at 60 min with a 59% reduction; however at 2 hr, TTot had increased to levels slightly higher than control (+3.56%). These changes were equally represented in the inspiratory and expiratory phase durations. TI/TE ratios tended to increase at 10 min but there were no significant differences from baseline over the duration of the intoxication. The TI/TTot ratio was significantly higher, however, at 10 min post-cocaine administration. The effects of ventricular administration are similar to those for IV injections except that recovery is much faster and a slight rebound in timing parameters occurs with intraventricular administrations. These results suggest that the primary respiratory effects of cocaine intoxication result from central actions on respiratory control systems rather than peripheral (e.g. diaphragmatic) actions. Supported by R01-DA04913.

349.9

EFFECTS OF MDMA ON ALCOHOL CONSUMPTION IN THE FAWN-HOODED RATS

A. H. Rezvani, C. J. Gordon*, D. B. Miller*, J. O'Callaghan*, P. L. Garges* and D. S. Janowsky. Skipper Bowles Center for Alcohol Studies, Chapel Hill, NC 27599 and The US EPA, Research Triangle Park, NC 27711

The Fawn-Hooded (FH) rats exhibit a genetically-controlled subsensitivity to serotonin (5-HT) stimulation. In addition, they show a significant preference for alcohol. It has been speculated that their alcohol preference is associated with their central 5-HT impairment. To further investigate the involvement of the serotonergic system in alcohol preference the following experiment was carried out. FH rats were given free access to water and 10% ethanol for at least 3 weeks. After establishing the baseline, rats were administered (sc) with either saline or 5 mg/kg of 3,4-methylenedioxymethamphetamine (MDMA), which promotes 5-HT release. Food, water and ethanol intake as well as body temperature were monitored during the course of the experiment. Our results show that MDMA, but not saline, significantly attenuated alcohol intake, increased water intake and body temperature without changing the food intake. The water and alcohol intake returned to the baseline the day after the treatment. These findings support the implication of a 5-HT deficiency hypothesis in alcohol-seeking behavior in a novel strain of alcohol preferring rats. (Supported in part by NCARA Grant# 9103 to AHR).

349.10

PARTIAL NORMALIZING OF MOVEMENT BY CAFFEINE IN NRTP-DAMAGED RATS. R.M. Chesire, M.C. Fratzke, & B.E. Digman Univ. Hawaii-Manoa, Honolulu, HI 96822.

Nucleus reticularis tegmenti pontis (NRTP) damage-induced increases in locomotion are not blocked by large doses of morphine, haloperidol or ethanol (1-4). Thus, these drugs exert their inhibitory effects partially via the intact NRTP (1-4). Recently, we have shown that amphetamine (5,6) or caffeine (7) can increase the locomotion of NRTP-damaged rats, and that amphetamine produces some normalizing of movement (6). Amphetamine-treated NRTP-damaged rats first slow their locomotion, then increase it, then become trapped in the same stereotypies displayed by amphetamine-treated unoperated rats (6). In effect, amphetamine reintegrates the NRTP-damaged animals' response to the drug. In this study, we report that 20 mg/kg i.p., caffeine normalized some postural/locomotor reactions of such rats. Caffeine produced a change from predominantly ventroflexed to predominantly lateral (normal) righting, and reduced excessive thigmotaxis in the open field. The results suggest that caffeine, like amphetamine (6) can exert reintegrative effects on locomotion/movement following NRTP damage, via their effects on intact compensatory neural systems.

349.11

CAFFEINE EXAGGERATES EXCESSIVE FORWARD LOCOMOTION IN NRTP-DAMAGED RATS. M.C. Fratzke, B.E.Digman & R.M. Chesire, Univ. Hawaii-Manoa, Honolulu, HI 96822.

Damage of the nucleus reticularis tegmenti pontis (NRTP) can produce uninhibited increases in forward locomotion that resemble Parkinsonian festination (1-4). Some of the major cataleptogenic or sedative hypnotic agents do not abolish such locomotion (4-6). However, amphetamine can decrease NRTP damage-induced locomotion after its initial excitatory effects, by instating the stereotypical motor behaviors often elicited by such stimulants in otherwise normal rats (7,8). In this report, we describe additional locomotor stimulation produced by 20 mg/kg, i.p. caffeine in already excessively kinetic NRTP-damaged rats. The results suggest that caffeine stimulates locomotion via effects on neural systems that do not depend on the NRTP inhibitory system needed for the expression of some motor effects of cataleptogens. Coupled with the finding that caffeine normalizes some aspects of movement in NRTP-damaged rats (9,10), the results imply that caffeine, a widely used and abused substance, may be of automedicative value (7-10) in some cases of pontine dysregulation.

349.12

COCAINE EFFECTS ON CNS NEUROTENSIN CONCENTRATIONS. S.T. Cain, D. Griff*, C.M. Joyner*, E.H. Ellinwood and C.B. Nemeroff. Depts. of Psychiatry and Pharmacology, Duke Univ. Med. Ctr. Durham, NC 27710

Neurotensin (NT) is an endogenous brain tridecapeptide which exhibits a variety of selective behavioral, neurochemical and physiological interactions with brain dopamine (DA) systems. (TIPS, 6, 1985, 201-205). In view, therefore, of the purported involvement of DA systems in cocaine-induced behavioral changes, we have initiated an investigation of the possibility that cocaine disrupts NT/DA homeostasis in the central nervous system (CNS). Adult male rats were used in all experiments. Using a sensitive and specific radioimmunoassay (RIA), the concentration of neurotensin-like immunoreactivity (NT-LI) was measured in the frontal cortex, nucleus accumbens, caudate nucleus, substantia nigra (SN), and ventral tegmental area 24 hours and 8 days following the conclusion of a 14 day regimen of 40 mg/kg cocaine/day administered as either 1 subcutaneous (sc) injection or continuously infused using minipumps. NT-LI was also measured 24 hours following one sc. injection of 40 mg/kg cocaine. Dopamine concentrations were measured using HPLC with electrochemical detection in nucleus accumbens and caudate nucleus 8 days following 14 days of chronic cocaine exposure.

Twenty-four hours following either an acute or 14 days of exposure to cocaine, NT-LI was significantly increased in the SN. Twenty-four hours following 14 days of once-daily, but not continuous exposure to cocaine, NT-LI was significantly increased in the frontal cortex. Acute, but not chronic exposure to cocaine, resulted in increased nucleus accumbens NT-LI. Eight days following chronic exposure to cocaine, no changes in NT-LI or DA concentration were observed in any brain region examined. These results provide evidence consistent with the early, but not residual involvement of NT systems in the behavioral and/or addictive properties of cocaine as previously suggested by Hanson et al. (Eur. J. Pharmacol. 160, 1989 23-30). Supported by NIDA DA-05303.

349.13

EFFECTS OF THE COCAINE METABOLITE BENZOYLECGONINE ON GLYCOSPHINGOLIPID SYNTHESIS BY NEUROBLASTOMA AND GLIOMA CELLS. *Y. Lin, S. Hickerson* and K.C. Leskawa.* Dept. Anatomical Sciences & Neurobiology, University of Louisville, Louisville, KY 40292

We have previously reported that following gestational exposure to cocaine total neutral glycosphingolipid (GSL) and ganglioside content of whole newborn rat brain approximately doubled (Jackson et al., Soc. Neurosci., 1989). To further pursue this, we have recently examined the effect of the major cocaine metabolite benzoylecgonine (BE) on GSL synthesis by neuroblastoma and glioma cells in culture using NG108-15 and C6 cells, respectively, as models. BE was added to complete growth media at varying concentrations (0 to 200 μM), cells exposed for 48 hr and GSL synthesis examined by adding ^{14}C-serine to the media during the last 8 hr. BE at very low concentrations (10 and 20 μM) significantly elevated the synthesis of both neutral GSLs and gangliosides by C6 cells. This stimulation was diminished, however, at BE concentrations higher than 50 μM. No elevation of either neutral GSL or ganglioside synthesis was observed with NG108-15 cells exposed to BE, and above 20 μM ganglioside synthesis was significantly reduced. Further examination demonstrated that the reductions observed at higher BE concentrations were not due to effects on glycolipid synthesis *per se*, but were due to cytotoxic effects. Diminished incorporation of ^{3}H-thymidine into DNA and loss of cells from the substratum was observed following exposure of cells to BE at 50 μM and higher.

These results suggest that the elevated neutral GSL and ganglioside content observed in whole newborn brain may be due to the cocaine metabolite benzoylecgonine affecting synthesis by glial cells, and also demonstrate a cytotoxic effect of this compound at low levels.

349.14

COCAINE ATTENUATES PHORBOL ESTER STIMULATED GnRH RELEASE FROM THE RAT HYPOTHALAMUS IN VITRO: ROLE OF Na^+ INFLUX. Thomas S. King, Inn Soo Kang*, Martin A. Javors and Robert S. Schenken*, Departments of CSB, OB-GYN and Psychiatry, University of Texas Health Science Center, San Antonio, TX 78284.

Phorbol esters, presumably acting to stimulate the activity of protein kinase C, have been shown to increase gonadotropin releasing hormone (GnRH) release from rat hypothalamus *in vitro* (*Brain Res. Bull. 15*: 657 - 659, 1985). Hypothalamic release of GnRH is dependent, in part, on fast Na^+ channel activation and Na^+ influx (*Neuroendocrinology 32*: 155 - 162, 1981). We therefore sought to determine whether Na^+ influx influences phorbol ester stimulated hypothalamic GnRH release *in vitro*. Hypothalami were collected from ovariectomized rats injected s.c. with 50 μg/kg of 17β - estradiol benzoate the two previous mornings. Sagittal sections of this block of CNS tissue comprising the preoptic area/anterior hypothalamus and mediobasal hypothalamus/median eminence were perfused at a rate of 6.18 ml/min with a modified Krebs-Ringer buffer (pH 7.4) using a programmable perfusion system. Perfusion results are as follows: 10 min pulses of phorbol 12-myristate 13-acetate (PMA) or phorbol 12,13-dibutyrate (PDBu) increased GnRH release in dose-response fashion (0.01 - 1μM). In contrast, the biologically inactive α-phorbol was without effect on GnRH release. PDBu-stimulated GnRH release was blocked by both tetrodotoxin and cocaine, known inhibitors of Na^+ influx. Cocaine attenuation of PMA-stimulated GnRH release could be partially restored by addition of monensin, a Na^+ ionophore. These results suggest that the effects of phorbol esters to stimulate hypothalamic GnRH release are, at least in part, mediated through Na^+ influx and that cocaine may directly affect hypothalamic GnRH release by blocking influx of this cation. (Supported by NIDA grant DA-06039 and by NIH grant HD-10102 [Neuroendocrine Core]).

GENETIC MODELS OF BEHAVIOR

350.1

HYPERACTIVITY INDUCED BY A SNAP-25 INCLUSIVE DELETION MUTATION IN COLOBOMA (*Cm*/+) MOUSE MUTANTS. E.J. Hess, H.A. Jinnah, and M.C. Wilson, Dept. of Neuropharm., Research Institute of Scripps Clinic, La Jolla, CA 92037 and U.C. San Diego Sch Med, La Jolla, CA 92093.

SNAP-25 is a neuron-specific synaptosomal associated protein which contributes to synapse formation. In characterizing SNAP-25 expression and function, we have identified the mouse mutant Coloboma (*Cm*/+) as carrying a chromosome 2 deletion mutation which includes the *Snap*-25 gene. Coloboma mice are neurological mutants whose phenotype includes opthalmic deformation and stereotypic head shaking. We have also observed that these mice were extremely hyperactive. To quantitate the hyperactivity exhibited by Coloboma mice, the spontaneous locomotor activity of control and Coloboma mice was recorded in photocell cages over twenty-four hours. Both mutant and control mice habituated within the first 150 min of the testing session and Coloboma mice appeared to have a normal circadian rhythm which paralleled control mouse activity. However, Coloboma mice were significantly more active than their control littermates ($p< 0.005$) with nocturnal activity counts exceeding three times those of controls. Coloboma mice exhibited enhanced locomotor activity with no difference observed between control and mutants in time spent quiescent, so these mutants are hyperactive while maintaining normal sleep/wake patterns. Although it is clear that the Coloboma deletion mutation includes *Snap*-25, the size of this deletion is unknown. To define exactly the gene(s) responsible for the hyperactivity, the extent of the deletion was assessed. We have determined that *Bmp*-2a and *Pax*-1, genes which reside near *Snap*-25 on mouse chromosome 2, do not appear to be deleted in the Coloboma mutation. These data suggest that a deletion of less than 5cM (the distance between *Bmp*-2a and *Pax*-1) which includes *Snap*-25 results in the extreme hyperactivity exhibited by Coloboma mice. Supported by PHS CA33730, NS23038 and an American Epilepsy Society Research Fellowship.

350.2

CENTRAL AUDITORY PROCESSING IN A PATIENT WITH SSADH DEFICIENCY. D.M. Daly, A. Hodson*, and K.M. Gibson*, Box 210855, Dallas, TX 75211, Univ Florida Jacksonville, FL, 32225, and BRF, Dallas, TX 75246

SSADH deficiency, an inborn error of GABA metabolism, forces SSA reduction in CNS through hydroxybutyric acid (HB). Reported cases have been hypotonic, and retarded with marked delays in speaking and in acquiring language. We tested central auditory processing [1] in one family.

A 9 yr old girl, referred at 4 yrs for complex partial seizures and profound delay in speech and language, was hypotonic, clumsy in fine movements, and unresponsive to painful stimuli. Audiometrics and AER were within normal limits; IQ(WISC)<50. Seizures were controlled with carbamazepine; methylphenidate (MPD) was added to offset increased daytime somnolence. Seizures remitted and she has been 2 yrs without medication. She has also endured episodes of prolonged sleep (>20h/da) associated with increased 4-HB in urine and CSF. An afflicted brother sleeps 10h/da; an unafflicted half brother may sleep 18hr/da if permitted.

During testing she spoke and pointed to respond. Classifications were occassionally well-defined, but in aggregate performance approached chance levels with no obvious consistency between pointed and spoken responses. Performance improved significantly ($p<0.0001$) following <5mg dose MPD sublingually: within 8 min she consistently distinguished each sound; within 10 min she spoke and pointed consistently; over next 30 min she returned to chance (confirmed with 2 observers and repeated with a second dose). While alert coordination improved; she spoke clearly and appeared free of gross auditory defect.

Whatever the relations between 4-HB and sleep, variations in vigilance can arise and be modified separately. Unlike controls with fluctuating vigilance, her initial unmedicated chance levels of performance seemed to arise from uniform responses to randomized stimuli.

[1] Daly et al, JNP 44:200-222 (1980). behavioral testing contributed by inventor who retains all proprietary rights and interests

350.3

GENETIC ANALYSIS OF CHROMOSOME 11 LOCI USING RFLPS IN INDIANA FAMILIES WITH BIPOLAR AFFECTIVE DISORDERS. D.K. Lahiri, S. Bye*, T. Foroud*, C. York*, P.M. Connealy and J.I. Nurnberger, Jr. Laboratory of Molecular Genetics, Institute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46202

The affective disorders are a common and heterogeneous group of illnesses that are characterized by abnormalities of mood, cognition and neurovegetative functions such as sleep, appetite and libido. Genetic factors appear to contribute to the development of both bipolar and unipolar depression. The mapping of genetic loci for these disorders has been difficult due to apparent genetic heterogeneity and nongenetic forms of the disorder. A suggestive linkage has been reported to 11p15, though extension of the family weakened the initial lod score significantly (Kelsoe et al., 1989); a translocation at 11q 22-23 has been reported in another family (Potkin et al., 1989).

We have used a molecular genetic approach to localize the gene for affective disorder in two clinically well characterized families from Indiana with over 60 members. Our linkage analysis involves the use of RFLP markers within a specific genomic area and then typing affected families with the polymorphic probe/enzyme combinations by Southern hybridization. The results are tested using the LOD score method to determine if a specific RFLP locus is closely linked to a disease gene locus that may transmit with the disease phenotype in several generations. We have chosen DNA probes (cDNA or genomic DNA) or synthetic oligomers with a PIC value of at least 0.34, distributed throughout chromosome 11 (11p 15.5 - 11q 23 q24). Our preliminary results have excluded linkage of a disease gene with the following loci: MUSC (*SacI*), SS6 (*TaqI*); we are testing linkage of HRAS (*SacI*), TH (*PstI*), pMCT128.1 (*MspI*), IGF2 (*Bam HI*), PTH and STMY (*TaqI*) loci. Results of RFLP analyses and lod scores using multiple polymorphic probes and restriction enzyme pairs will be presented.

Help from Dr. M.Hodes, Ms. D.Lawrence and C.Springer is gratefully acknowledged. Supported by the Indiana Department of Mental Health.

350.4

FIFTH MONTH FETAL-SIZE MARKERS IN SCHIZOPHRENIA: A DISCORDANT MZ TWIN STUDY, **H. S. Bracha, M.D., E. F. Torrey, M.D., L. B. Bigelow, M.D., K. Dykman, M.D.*, J. Mayfield, B.A.*; Clinical Brain Development Research Lab, Departments of Psychiatry and Neurology, University of Arkansas for Medical Sciences, and VAMC 116A1/NLR, North Little Rock, Arkansas 72114-1706**

Fingertip dermal cells migrate to form ridges during the second prenatal trimester, which is also a critical period of neural cell migration to the cortex. Since prenatal insults rarely affect both MZ twins to the same extent, we hypothesized that intrapair discrepancies between two MZ twins in the dermal ridge count may be markers of factors that might affect one fetus differentially during the second trimester. We examined discordant MZ twins to estimate the differences in the prenatal environmental interference disrupting fetal development in the second trimester. We measured the absolute differences in fingertip ridge count in 30 pairs of MZ twins recruited over a seven-year period [23 pairs discordant for schizophrenia and 7 normal pairs]. MZ twins discordant for schizophrenia had significantly higher intrapair discrepancy in ridge count than normal MZ twins; i.e. their fingerprints were significantly less "twin-like". This study suggests that second trimester prenatal disturbances in epigenesis in one twin in pairs discordant for schizophrenia may be related to the fact that only one of the two twins expresses his/her genetic predisposition toward schizophrenia. This study provides support for a "two-strike" etiology in schizophrenia - a genetic diathesis plus an unknown prenatal environmental stressor.

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350.5

INTERSTRAIN AGGRESSION IN WISTAR-KYOTO INBRED STRAINS: SHR, WKY, WKHA AND WKHT RATS. E.D.Hendley, W.G.Ohlsson* and R.E.Musty. Univ. Vermont, Burlington, VT 05405.

SHR and WKY rats have been selectively inbred for blood pressure: hypertension (HT) in SHR and normotension in WKY. These homozygous strains have also inadvertently been fixed with behavioral differences, including hyperactivity (HA) in SHR when compared with WKY. We developed WKHA and WKHT strains from a cross of SHR x WKY, and now WKHA express the HA trait and not HT, and WKHT express HT and not HA. These four strains were used in interstrain aggression studies to test whether aggression, reported in SHR, had cosegregated with the HA trait (if seen in SHR and WKHA and not in WKY and WKHT) or with the HT trait (if seen in WKHT and SHR and not in WKY and WKHA). We paired rats of different strains, but same age and sex, in an arena for 15 min, and counted aggressive acts according to Miczek (Psychopharmacologia 39:275, 1974). The most prevalent act observed was allogrooming (lick opponent's neck), and this was significantly higher in the strains with HT. In other dominance behaviors (attacks+upright postures+mounts) female WKHA and SHRs (HA trait) scored higher than all other groups. From this finding and others we concluded that the heritability of interstrain aggression is heterogeneously regulated in the rat genome, and that it is not a necessary cosegregant of either HT or HA. Supported by NIH RO1-NS26390.

350.6

ACTIVITY RESPONSES TO NOVELTY IN WISTAR RANDOMBRED AND WISTAR-KYOTO INBRED STRAINS. R.E.Musty and E.D. Hendley. Univ. Vermont, Burlington, VT 05405.

Hyperactivity and poor habituation to novel stimuli are behavioral characteristics which cosegregated with hypertension in the Spontaneously Hypertensive Rat (SHR), derived from the Wistar-Kyoto (WKY) strain of rat. Recombinant breeding of WKY and SHR followed by selective inbreeding to produce WKHA (hyperactive) and WKHT (hypertensive) strains has allowed us to study these characteristics of SHR separately. Randombred Wistar (WIS) and the aforementioned four strains were tested in an infra-red detector open field over three sessions: 1) with two identical objects in the field, 2) and 3) with one novel object replacing an object in 1). SHR and WKHA were more active than the other strains in session 1). Impaired habituation to stimulus change in 2) and 3) followed a similar pattern of strain differences. These data support the view that hyperactivity and attenuated response to novelty are cosegregated characteristics of WKHA. Supported by NIH RO1-NS26390.

350.7

HAD AND LAD RATS RESPOND DIFFERENTIALLY TO THE STIMULATING EFFECT BUT NOT THE DISCRIMINATIVE EFFECTS OF ETHANOL. E. C. Krimmer. Dept. of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

The drug discrimination paradigm (DD) was used to evaluate behavioral differences of rats selectively bred for differential ethanol drinking preferences. Seventh generation high alcohol drinking (HAD) and low alcohol drinking (LAD) rats were trained to discriminate between ethanol (0.5 g/kg, IP) and saline vehicle, following a 2 min presession interval (PI), using an FR-10 schedule of reinforcement. The HAD strain was more responsive than the LAD strain to the stimulating effects of ethanol as measured by total response rates. ED_{50} values of 0.239 and 0.244 g/kg for the HAD and LAD strains respectively do not reflect any difference in the discriminative effects of ethanol.

Response rates during DD indicated a dissociation of rate increasing effects and discriminative performance following ethanol. In addition to differential drinking preference these data suggest that selective breeding for the HAD and LAD animals also involves the stimulant action of ethanol but not on the discriminative effects. Supported by NIAAA Grant 8598.

350.8

SPATIAL LEARNING IN INBRED MOUSE STRAINS. C-A Gutekunst, AT Hartney*, HD Rees and RC Green Departments of Neurology and Psychology, Emory University School of Medicine, Atlanta, Georgia.

Strain-related differences in place learning ability were studied with a modified Morris water maze in the following strains: DBA/2, C3H/He, C57Bl/6J, El and ddY. DBA/2 mice are susceptible to audiogenic seizures after 2-3 weeks of age and El mice have generalized seizures in response to handling after 3 months of age.

At 7-9 weeks of age, males and females from the five strains underwent 32 training trials. DBA/2 and El mice without prior seizures, along with the non-epileptic ddY and C57Bl/6J mice all exhibited learning at rates that did not differ from one another (repeated measures ANOVA, p=0.14). The non-epileptic C3H/He mice were unable to learn the water maze task in the trials permitted (simple linear regression for latency across trials, p=0.38). In the C57Bl/6J strain only, female mice learned the task significantly faster than males (repeated measures ANOVA, p=0.02).

There was no difference in the learning rate between the El strain and its parent ddY strain prior to the development of seizures. These findings suggest that prior to the appearance of seizures in mice, genetic predisposition to epilepsy does not affect spatial learning ability. C3H mice are known to have poor visual acuity which may have contributed to their poor performance in this task. Sex differences in learning rates in the C57Bl/6J strain may be an example of sexually dimorphic learning behavior.

DEVELOPMENTAL DISORDERS OF THE NERVOUS SYSTEM I

351.1

FIBRILLARY TANGLES IN HEMIMEGALENCEPHALY AND ALZHEIMER'S DISEASE SHARE A COMMON ANTIGENIC DETERMINANT. T. Duong, M. DeRosa*, H.V. Vinters*, W. Peacock* and R.S. Fisher. UCLA Mental Retardation Research Center, Pathology Dept. and Neurosurgery Dept., Los Angeles, CA 90024.

Hemimegalencephaly is a rare congenital disorder characterized by unilateral hypertrophy of a cerebral hemisphere, errors of cortical gyration and lamination, and presence of meganeurons. The latter accumulate abnormal amounts of the medium (NF-M) and heavy (NF-H) neurofilaments, resulting in intracytoplasmic tangle-like formations (Duong et al., The Anatomical Record 229 no. 4, p. 24A, 1991). In this study, neocortical surgical samples were obtained from 3 hemimegalencephalic patients (3-, 15-, and 30-month) who had undergone hemispherectomy for the treatment of intractable seizures. Surgical samples from pediatric patients with intractable epilepsy but without hemimegalencephaly, and age-matched pediatric autopsy samples were also collected. Sections (40μm-thick) were processed free-floating for immunohistochemistry. The primary antibody used was SMI34, purchased from Sternberger Monoclonals, Inc., Baltimore, MD. SMI34 is specific for the phosphorylated forms of both NF-M and NF-H, and labels intracellular neurofibrillary tangles in Alzheimer's disease. We report that neurofilamentous tangles in the meganeurons typical of hemimegalencephaly are also immunoreactive to SMI34. The pattern of immunoreactivity differed between the 3 month-old and the older megalencephalic patients. In the 3 month-old, the immunoreactive meganeurons were usually surrounded by a non-immunoreactive zone. The intracytoplasmic labeling revealed tangle-like formations within the perikaryon extending into the proximal dendrites. In older patients, meganeurons were also strongly labeled but did not display the non-immunoreactive zone surrounding the cell body. Intracytoplasmic filamentous tangles usually displaced the nucleus and also extended into dendrites. **Thus, neurofilamentous tangles in hemimegalencephaly share a common antigenic determinant with Alzheimer's disease intracellular neurofibrillary tangles.**

351.2

HISTOLOGICAL OBSERVATIONS ON THE CENTRAL NERVOUS SYSTEM OF ATRANSFERRINEMIC MICE. J.R. Connor, S.L. Menzies*, and W.P. Bartlett. Dept. of Neuroscience & Anatomy, Penn State Unviersity, M.S. Hershey Medical Center, Hershey, PA 17033.

The role of transferrin in transporting iron and the importance of iron in neural development is well established. Tf is synthesized in the brain by oligodendrocytes and the choroid plexus. A strain of mice (ATf) which have an autosomal recessive defect in the Tf gene (Huggenvik et al., Blood 74:482-486, 1989) resulting in plasma levels of transferrin which are <1% of normal are investigated. The animals are maintained into adulthood by weekly injections of mouse serum. The adult mice (provided by Dr. Jerry Kaplan) were either immersed fixed in 10% buffered formalin or perfused through the ascending aorta with a mixture of 2% paraformaldehyde and 2% glutaraldehyde. A series of standard histological stains were performed on the brain and spinal cords. Grossly, the brain of ATf mice appears normal, but the spinal cord is smaller consistent with less myelin staining in the lateral columns. Histologically, in ATf mice the cerebral cortex and habenular nucleus have an increased cell density. In addition to a noticeable decrease in myelin in the spinal cord, larger than normal neurons are present in both the dorsal and ventral gray matter. These observations suggest locally synthesized Tf in the brain is important for normal neuronal and glial development.

351.3

EFFECT OF IN UTERO ALCOHOL EXPOSURE ON IRON REGULATION IN THE RAT BRAIN. A.J. Roskams, M.W. Miller, J.R. Connor. Dept. of Neuroscience and Anatomy, Penn State College of Medicine, Hershey, PA, 17033, and Dept. of Anatomy, UMDNJ, Rutgers University, NJ, 08854.

Iron (Fe) and iron binding proteins Transferrin (Tf) and Ferritin (Ft) and the Tf receptor (TfR) have been identified in the rat brain, and are associated with growth and differentiation. A rat model of Fetal Alcohol Syndrome (FAS) is used to examine alcohol's effects on iron regulation in the developing brain. Some CNS effects of in utero alcohol exposure are decreased brain weight, delayed myelination and neuronal development patterns. A developmental study of Tf, TfR, Ft is described in the cerebral cortex, the cerebellum/pons and the midbrain. Three groups are studied-offspring of mothers who are chow-fed, fed an isocaloric liquid diet or ethanol diet. Animals are taken at PND 1-3, 6-8, 11/12, 17/18, 23/24, and 60. Iron levels in ETOH cortex are severely depressed at all time points. Cortex Tf levels are equivalent in all three groups at birth. By PND 11/12, the ethanol group has higher Tf levels. At PND 11/12, TfR levels in the ethanol exposed groups are 2.5 times higher than in the control groups, but normalize by 18d. Ft in ETOH exposed group is higher than normals beginning PND 7/8. Similar effects are observed in the cerebellum & the Midbrain. We conclude that CNS iron regulatory systems are effected by in utero alcohol exposure.

351.4

THE INFLUENCE OF CHRONIC ETHANOL EXPOSURE IN THE CHICK EMBRYO ON THE EXPRESSION OF NEUROTROPHIC FACTORS AND CHOLINERGIC ACTIVITY. D.J.Swanson,* M. Paiva,* D.W. Walker and M.B. Heaton. University of Florida College of Medicine and V.A. Medical Center Gainesville, FL, 32610.

We have begun to develop an avian model for chronic prenatal ethanol exposure and its influence on neural development. Previously, our laboratory showed that chronic adult ethanol exposure in rats produces a reduction in neurotrophic activity in hippocampal extracts as determined in a cultured chick dorsal root ganglion (DRG) neuron bioassay (Walker et al., *Soc. Neurosci. Abst.* 16:823, 1990). In the present study chick embryos were exposed to either ethanol (approximately 30mg/day) or saline from embryonic day 4 (E4) until sacrifice and brain tissues were assayed for the relative abundance of neurotrophic activity or choline acetyltransferase (ChAT) activity. While ethanol exposure reduced brain tissue wet weight and ChAT activity of optic tectum and forebrain at E19, this treatment did not markedly influence overall development of these embryos compared to saline controls, as determined by morphometric measurements. Chronic alcohol treatment *in ovo* produced a significant reduction in neurotrophic activity in E16 forebrain extract (following exposure from E4-E13), as measured in our DRG bioassay. These results suggest that the production of neurotrophic factors and cholinergic enzymes may be appreciably altered following chronic prenatal ethanol exposure. Such alteration could underlie certain CNS anomalies seen in the fetal alcohol syndrome. Supported MH15737, NS20387, AA00200, and the Dept. of Veterans Affairs.

351.5

MORPHOLOGICAL BRAIN ASYMMETRIES IN MICE BORN ACALLOSAL AFTER GAMMA IRRADIATION EXPOSURE. E. M. Caparelli-Dáquer* and S. L. Schmidt. Instituto de Biologia, UERJ and Instituto de Biofísica, UFRJ.

In a strain of mice (BALB/cCF), in which about 20% of the animals are born with a very small corpus callosum (c.c.), we have suggested a relationship between the development of the c.c. and the direction of cerebral asymmetries. Here we report a study on morphological brain asymmetries in another model of early callosal damage (prenatal gamma irradiation). At E16 (E1, day of conception) 19 Swiss male mice were exposed to a ^{60}Co gamma source receiving a total dose of 2 Gy (the dose rate varied between 56 and 59 rads/min). At adulthood they were intracardially perfused with saline followed by formaline. Then the brains were photographed in the dorsal view, split through the sagittal fissure, photographed in the lateral and medial view and weighed. The sagittal areas of the c.c. measured in the medial view photographs allowed us to define a subgroup with a little callosal remnant (n=9; areas ranging from 0.22mm^2 to 0.58mm^2) and another with no measurable c.c. (n=10; acallosal subgroup). For each brain, an asymmetry score (A.S. = (R-L)/(R+L)) were calculated from 3 hemispheric measurement : 1-weight; 2-dorsal area and 3-lateral area. The means of the A.S. and of their absolute values (|A.S.|) evaluated respectively the directional asymmetries and non-directional asymmetries. Neither the total irradiated group nor the acallosal subgroup presented directional asymmetries. The callosal remnant subgroup displayed a tendency toward directional asymmetry. In the total group and in the 2 subgroups there was a significant non-directional asymmetry. The presence of non-directional asymmetries and the absence of directional asymmetries in this model of early callosal damage had been already observed in the BALB/cCF model. Taken together these data suggest that the absence of directional cerebral asymmetries may be assigned to callosal development and not to a feature pertaining to any of the 2 models.

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351.6

LACK OF ASYMMETRY IN CEREBRAL GLUCOSE METABOLISM IN NEONATAL AS COMPARED TO ADULT CEREBRAL HEMISPHERECTOMIZED CATS. D.A.Hovda, H.T.Chugani and J.R.Villablanca. Div. Neurosurg., Nuc. Med., Dept. Neurology, Psychiatry, and the Ment. Retard. Res. Cent. UCLA Sch. Med., Los Angeles, CA 90024

Following neonatal cerebral hemispherectomy (NH) adult cats exhibit greater behavioral recovery, more reinnervation from the remaining cerebral cortex and less degeneration compared to animals sustaining this lesion as adults (AH). The current study was designed to determine if these anatomical-behavioral differences are reflected metabolically in cerebral glucose utilization rates. Three neonatal (5-15 days of age) and 4 adult cats received a left cerebral hemispherectomy and cerebral glucose metabolism was studied using [^{14}C]-2-deoxy-D-glucose autoradiography as adults. Local cerebral metabolic rates for glucose (lCMRglu; ug/min/100gm) were measured in 48 regions bilaterally. Results indicated that on the intact side of the brain AH exhibited lCMRglc which were generally higher that either NH or intact controls (e.g., range of lCMRglc in the right cerebral cortex;AH=90.3-129.7;NH=71.5-101.2;Intacts=66.7-112.0). On the lesioned side lCMRglc values in regions not directly affected by degeneration or reinnervation were within the same range in all three groups (e.g. hypothalamus.;39.0-56.0). However, in AHs, nuclei ipsilateral to the lesion which, according to previous data, show more atrophy and less reinnervation compared to NH and controls exhibited lCMRglc which were much lower than the contralateral (intact) side (e.g. ventral posterior lateral thalamic n.; AH, ipsilateral = 76.8 contralateral = 107.0). Thus, overall, there was more left-right asymmetry in AH with lCMRglc being generally higher compared to both NH and intact controls with lCMRglc of NH being within the normal intact control range for most regions.(DOE, DE-AC03-SF, USPHS 5RO1-MH37916, 2PO1-NS15654).

351.7

INCREASED ^{3}H-FLUNITRAZEPAM BINDING IN VISUAL STRUCTURES OF ADULT CEREBRAL HEMISPHERECTOMIZED COMPARED TO NEONATALLY HEMISPHERECTOMIZED CATS. Youram Nassir*, Keith J Tatsukawa*, David Brown*, Ali Ebrahim*, Steven Chen*, David A Hovda, Jaime R Villablanca*, Harry T.Chugani. Depts. Neurology, MRRC, Neurosurgery, Pediatrics, UCLA School of Medicine, Los Angeles, CA.90024.

We have previously reported [Hovda & Villablanca Behav Brain Res 1990;37:119-32] that there is relative sparing of visual field perception in neonatal but not adult cerebral hemispherectomized cats. Since GABA-mediated inhibition has been demonstrated to play a role in plasticity within the visual system, we measured ^{3}H-flunitrazepam binding in visual structures of adult cats which had undergone left hemispherectomy either as kittens (NHEMI; N-2) or as adults (AHEMI; N-2) and compared values to controls (N-2). Following incubation of brain sections in 170 mM Tris-HCl buffer (Ph 7.4) containing 2 nM ^{3}H-flunitrazepam in the absence or presence of 1uM clonazepam (nonspecific binding), sections were exposed for three weeks. Autoradiograms were analyzed using a JAVA system. Results showed increased ^{3}H-flunitrazepam binding in the right lateral geniculate (600%), right visual cortex (370%), and right (281%) as well as left (257%) superior colliculus of AHEMI compared to NHEMI. Values for NHEMI were not different from controls. These results indicated that ^{3}H-flunitrazepam binding is affected differentially by the age at lesion and may reflect the degree of neural plasticity, or lack there of in the lesioned animals. (USPHS 5RO1-MH 37916)

351.8

GREATER NEUROLOGICAL IMPAIRMENTS IN PRENATAL VERSUS NEONATAL CORTICALLY-LESIONED KITTENS. J.R. Villablanca, J.B. Harrison, G.F. Jackson*, D.A. Hovda and C. Infante*, Mental Retardation Research Center, UCLA, Los Angeles, CA 90024

Our previous work showed fewer impairments in cats hemispherectomized as neonates than as adults; therefore, we expected even greater recovery after a prenatal lesion (see also Brain Res., 152:451, 1978). Kittens (N=8) received a unilateral (left) frontal cortical lesion prenatally (E 48.4 days mean age) and were tested as young adults (>7 months). Comparisons were with neonatally frontal-lesioned (N=4; P 5.5 days) and intact littermates. Comprehensive quantitative testing in prenatal-lesioned cats showed: left turning preference, mild right limb and eyelid paresis, decreased right face and right limbs tactile sensibility, defective right paw placing reactions, marked preference to use the left paw (food retrieval task), abnormal right paw attitude, and poor stereopsis (visual cliff test). Neonatal-lesioned cats either did not show some of the impairments or these were milder. Therefore, and since neurological impairments following this restricted lesion were more like those after neonatal hemispherectomy, we conclude that a unilateral brain lesion produces more impairments in the fetal versus the neonatal cat. This agrees with our anatomical results (Loopuijt et al., this meeting; Soc. Neurosci. Abst., 13:1116, 1987). Grants US PHS R01 NS-25780: P01HD-05958.

351.9

EFFECTS FROM EXPERIMENTALLY INDUCED HYDROCEPHALUS ON HIPPOCAMPUS: AN ULTRASTRUCTURAL STUDY. R.M. Kriebel, D.M. Cavanaugh, S.A. Cosmi and J.P. McAllister II. Depts. Anatomy, Phila Col Osteopath Med, Temple Univ Sch Med, Phila PA 19131.

The neurological deficits found in infantile hydrocephalus have most often been explained by pathological changes in cerebral neocortex. It has been the primary goal of our studies to provide a cellular basis for the residual neurological deficits observed even though surgical intervention may have relieved the effects of ventriculomegaly on the cerebrum. Previous studies have shown significant structural changes in subcortical nuclei, specifically in septal nuclei of the basal forebrain. The present studies were done to examine the ultrastructural of the hippocampal cortex, a primary target of septal projections. Kaolin injection induced hydrocephalus; aldehyde fixed brains were sectioned for electron microscopy. Preliminary cell counts show 70% of hippocampal pyramidal neurons in varying stages of degeneration from densification of cytoplasm to fully pyknotic. The plexiform laminae showed hydropic cellular degeneration and the number of synaptic contacts was decreased. The thickness of the hippocampus was not decreased and microscopically there was no indication of edematous extracellular space. In addition, the ependymal lining contiguous with the hippocampus was structurally intact, including surface cilia, intracellular junctions, and cell shape. The apparent structural integrity of the hippocampus led to the suggestion that the neuronal degeneration seen in the hippocampus may have resulted from the deficiency in basal forebrain innervation.

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351.10

EFFECTS OF MATERNAL DEPRIVATION ON NEURONAL POPULATIONS IN THE PRIMATE HIPPOCAMPUS. S.J. Siegel[1], P.R. Hof[1,2], W.G. Young[3], G.W. Kraemer[4*], W.T. McKinney[4], and J.H. Morrison[1,2]. [1]Fishberg Research Center for Neurobiology and [2]Dept. of Geriatrics, Mt. Sinai Sch. Med., New York, NY 10029, [3]Dept of Neuropharmacology, Scripps Clinic, La Jolla, CA 92037, [4]Dept. of Psychiatry, Univ. of Wisconsin Sch Med, Madison, WI 53792.

It has long been known that environmental manipulations during development have profound effects on the behavior of primates. However, many of the underlying brain regions involved in these behavioral abnormalities and the specific effects on these structures have yet to be elucidated. The long term effects of maternal deprivation on newborn monkeys include hypersexuality, inability to interact normally with peers and inappropriate responses to stress. Additionally the effects of hormones released during stress, glucocorticoids (GC), on neuronal populations in the hippocampal formation have been described. Increased GC have been shown to produce neuropathology in *CA3* while sparing *CA1* and dentate granule cells (Sapolsky, et al, *J.Neurosci.*, 1990:10). Conversely, adrenalectomy, leading to low levels of the hormone, produces a deficit in granule cells while sparing *CA1* and *CA3* (Sloviter et al, *Science*, 1989:243). We have examined the brains of 1.5-2 year old maternally deprived and matched control rhesus monkeys. Our hypothesis has been that maternal deprivation leads to chronic elevation of GC resulting in various forms of aberrant brain development. Preliminary data (n=6) suggests that there is a difference between groups in granule cell expression of a nonphosphorylated epitope of neurofilament protein recognized by the monoclonal antibody SMI32. Also there is a possible alteration of *CA3* dendritic pattern. Neuronal morphology (ie. spine counts of basal & apical dendrites within *CA1*, *CA3* and granule cells) will be examined using a confocal laser scanning microscope and computer assisted morphometric analysis following intracellular injection of Lucifer Yellow. Supported by the MacArthur Foundation & NIH AG06647.

351.11

QUANTITATIVE ANALYSIS OF TRANSMITTER-IDENTIFIED SYSTEMS IN THE MONKEY PARAVENTRICULAR NUCLEUS: EFFECTS OF DIFFERENTIAL REARING CONDITIONS. S.D. Ginsberg[1], P.R. Hof[1,2], W.G. Young[3], G.W. Kraemer[4*], W.T. McKinney[4], and J.H. Morrison[1,2]. [1]Fishberg Res Ctr for Neurobiology and [2]Dept of Geriatrics, Mt. Sinai Sch Med, New York, NY 10029, [3]Dept of Neuropharmacology, Scripps Clinic, La Jolla, CA 92037, [4]Dept of Psychiatry, Univ of Wisconsin Sch Med, Madison, WI 53792.

A robust monoaminergic input to the hypothalamus from brainstem nuclei has been reported in several species. The present study was undertaken to quantify putative noradrenergic (NA) terminals in the paraventricular nucleus (PVN) of socially deprived (SD) or socially reared (SR) rhesus monkeys. The subjects are sacrificed in pairs at 1-2 years of age. Presently, magnocellular (MC) and parvocellular (PC) subdivisions of the PVN in two SD and two SR have been quantified through serial sections on a laser scanning microscope (LSM) for dopamine-ß-hydroxylase (DBH) immunohistochemistry, using adjacent Nissl sections for cellular landmarks. At the time of submission, we are blind to the rearing condition of each subject. However, preliminary analysis has shown differences across the two groups in DBH stained boutons in both MC and PC divisions: mean group I MC, 5641±1076; mean group II MC, 8151±791; mean group I PC, 8161±6; mean group II PC, 10469±1779. The implication is that maternal and peer deprivation may permanently affect NA afferents to the hypothalamus. Additionally, discrete populations of cells in the PVN immunoreact with tyrosine hydroxylase (TH) and corticotropin-releasing factor (CRF) antisera. Preliminary cell counts have revealed no difference between rearing conditions, yet an interesting trend in rostrocaudal cell density has been identified. In all cases examined, a gaussian type distribution of both TH positive and CRF positive cell bodies has been noted. Cell density is greatest at intermediate levels, and systematically decreases in both the rostral and caudal poles. Early social deprivation may cause permanent alterations in brain structure which account for the pronounced behavioral aberrations observed in these subjects. Supported by the MacArthur Foundation, and NIH AG06647.

WEDNESDAY PM SYMPOSIA

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SYMPOSIUM. MOLECULAR BIOLOGY OF THE DOPAMINE SYSTEM: HETEROGENEITY OF THE DOPAMINE RECEPTORS. O. Civelli, Oregon Health Sciences University (Chairperson); M. Caron, Duke University; D. Sibley, NIH-NINCDS; J.C. Schwartz, INSERM-Paul Broca; H. Van Tol, University of Toronto; S. Watson, University of Michigan

The dopamine system is a predominant neuronal system which is the target of clinical treatment of psychomotor or affective disorders such as Parkinson's disease or schizophrenia. Classically, this system was thought to rely on the interaction of the neurotransmitter dopamine with two receptors the D_1 and D_2 dopamine receptors. The recent cloning of dopamine receptors has revealed that this family of receptors is more heterogenous than expected. It is now clear that several dopamine receptors exist that were not detected in previous pharmacological assays. In addition, the availability of dopamine receptor clones has led to a new way at studying the dopamine system. This symposium will unite leaders in the molecular biology of the dopamine receptors to discuss the present state of our knowledge of complexity of the dopamine receptors and of the regulation of their genes.

354

SYMPOSIUM. HOW CELLS KEEP TIME: THE MOLECULAR AND CELLULAR BASIS OF CIRCADIAN RHYTHMS. D.R. Liskowsky & L.L. Hall, OTA, U.S. Congress (Chairpersons); J.C. Hall, Brandeis U.; J.S. Takahashi, Northwestern U.; B. Rusak, McMaster U.; M. Menaker, U. of Virginia; F.W. Turek, Northwestern U.

In the last ten years, molecular biology, genetics, neural transplantation and neuropsychology have begun to unravel the mystery of how circadian rhythms are generated. The symposium will provide a forum for discussion of these exciting developments. Dr. Jeffrey Hall will discuss how specific genetic mutations alter the circadian pacemaker and influence other biological rhythms. Dr. Joseph Takahashi will review a vertebrate model system of the circadian pacemaker, the avian pineal. He will discuss data concerning the cellular regulation of circadian rhythms by light, second messengers, and macromolecular synthesis. Dr. Benjamin Rusak will discuss photic effects on gene expression and neural activity in the rodent supra-chiasmatic nucleus (SCN). The effects of light exposure on the expression of several immediate-early genes will be presented. Dr. Michael Menaker will discuss transplantation of SCN tissue in hamsters and the production of temporal chimeras. Their behavior and neuroanatomy will be discussed Dr. Fred Turek will present data from recent studies demonstrating that photic and non-photic stimuli can induce pronounced phase shifts in the rodent circadian clock. His presentation will focus on physiological mechanisms that mediate the phase shifting effects of these stimuli.

356.1

GABA$_A$ INHIBITION OF EXCITATORY AMINO ACID-INDUCED [^{3}H]DOPAMINE RELEASE FROM CULTURED MESENCEPHALIC CELLS. L. Chaudieu, H. Allaoua, R. Quirion and P. Boksa. Douglas Hosp. Res. Ctr., Dept Psychiatry, McGill Univ., Montreal, Quebec, Canada H4H 1R3.

The substantia nigra is known to contain the highest levels of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the mammalian brain. It has been shown that intranigral injections of GABA and GABAergic drugs decrease striatal dopamine (DA) release as measured by microdialysis. On the other hand, previous studies in our laboratory using cell cultures of fetal rat mesencephalon have established that NMDA, quisqualate (quis) and kainate stimulate [^{3}H]DA release from DA neurons. In an attempt to evaluate the relationship between the GABAergic and glutamatergic systems in the mesencephalon, we investigated the effect of GABAergic drugs on [^{3}H]DA release stimulated by excitatory amino acids (EAAs) from cultured mesencephalic cells. Muscimol (mus), a GABA$_A$ agonist, inhibited [^{3}H]DA release evoked by NMDA (IC_{50} for mus = 0.2 μM; 100% inhibition at 1 μM mus) and quis (65% inhibition at 100 μM mus) but not the response evoked by kainate. Baclofen (100 μM), a GABA$_B$ agonist, modified EAA responses only slightly. Muscimol inhibition of the NMDA response was reversed by bicuculline and picrotoxin and potentiated by 1 μM flunitrazepam. In the absence of Mg^{2+}, muscimol inhibited the NMDA response less effectively, suggesting that hyperpolarization leading to instatement of a voltage-dependent Mg^{2+} blockade may account for part of the muscimol effect. These results indicate that GABA modulates [^{3}H]DA release stimulated by NMDA and quis, but not kainate, via GABA$_A$ receptors. Supported by FRSQ.

356.2

EXCITATORY AMINO ACIDS CONTROL HIPPOCAMPAL FIELD POTENTIAL OSCILLATIONS. Schneiderman JH. Univ. of Toronto, Toronto, Ont., Can. M4Y 1J3.

Oscillating spontaneous field potentials (SFPs) in hippocampal slices become larger and slower in the presence of low doses of GABA blockers. These slow SFPs are due to synchronous synaptic potentials but the transmitters are unknown. We studied the role of excitatory amino acid (EAA) receptors in the production of SFPs since EAAs are important putative hippocampal transmitters.

The non-NMDA antagonist, CNQX (2-10 uM), completely blocked spontaneous activity in both ACSF and low concentrations of penicillin (50-300 IU/ml) as well as evoked responses in ACSF. The NMDA antagonist, APV (50-100 uM), partially suppressed SFPs in penicillin but had no effect on SFPs or evoked responses in ACSF. The late components of the evoked responses in penicillin were reduced, however, the early components were unaffected.

Non-NMDA receptors are critical for the production of SFPs. NMDA receptors participate only in the long-latency components of the SFPs and evoked responses in the presence of GABA antagonists. These delayed events are likely due to recurrent EPSPs.

356.3

THE CONTRIBUTION OF NMDA RECEPTORS TO EPSPS EVOKED BY SENSORY STIMULI IN THE VENTROBASAL THALAMUS (VB) *IN VIVO*. T.E. Salt and S.A. Eaton*. Institute of Ophthalmology, Judd Street, London WC1H 9QS, England.

In order to study the contribution of NMDA excitatory amino acid receptors to epsps evoked by somatosensory stimuli under physiological conditions, intracellular recordings were made from VB neurons in urethane-anaesthetised rats, and amino acid agonists and antagonists were applied by iontophoresis.

Stimulation of hair or vibrissa follicle afferents with a 10ms-duration air jet at 0.5Hz typically evoked an epsp which invariably gave rise to one or two action potentials. This sequence appeared to be curtailed by one or more ipsps. Application of the NMDA-receptor antagonist CPP, with iontophoretic currents adequate to antagonise NMA, selectively caused a reduction of the late component of the epsp in 14 of 15 neurons. Stimulation of 1000ms duration evoked a sequence of epsps and ipsps leading to trains of action potentials. This excitatory response was also CPP-sensitive. Application of the GABA$_A$-receptor antagonist SR95531 greatly reduced the stimulus-evoked ipsps and consequently revealed an enhanced sensory epsp. These previously occluded epsp components were very sensitive to antagonism by CPP.

These results indicate that NMDA receptors are involved in both low and high frequency sensory synaptic transmission to VB, and that this input is curtailed by stimulus-evoked GABA-ergic ipsps.

356.4

A STUDY OF THE CONTRIBUTION OF EXCITATORY AMINO ACIDS TO SYNAPTIC POTENTIALS IN THE CAT PREFRONTAL CORTEX IN VIVO. P.L. Herrling and T.E. Salt*, Sandoz Research Institute, CH-3000 Berne, Switzerland and Institute of Ophthalmology, Judd Street, London WC1H 9QS, U.K.

Agonists and antagonists of eaa receptors were applied by iontophoresis to 21 intracellulary recorded neurons in the prefrontal cortex of 13 halothane-anaesthetised cats during electrical stimulation of either the ipsilateral ventro-lateral thalamus (VL) or ipsilateral cerebral peduncle (PED). PED stimulation resulted in antidromic action potentials in 9 cells which were followed by a later latency depolarisation (local circuit epsp) in 5 cases. VL stimulation resulted in epsps or epsp-ipsp sequences in 18 cells. The selective AMPA receptor antagonist CNQX reversibly inhibited VL-evoked epsps in 6/6 cases. In several of these cells, AMPA-induced excitations were abolished by CNQX while NMDA-excitations were unaffected. The selective NMDA receptor antagonists D-AP7 or CPP partly reduced such epsps in 7/10 cells, predominantly affecting late epsp components. The NMDA antagonists produced larger reductions (60-80%) of PED-evoked epsps in 3/4 cells and also reduced ongoing activity in 6 neurons. Such effects occurred partly in parallel with the antagonism of iontophoretically applied NMDA. These results suggest a role for NMDA receptors in the generation of ongoing synaptic activity, local circuit epsps, and later components of thalamically-evoked epsps. AMPA receptors may be responsible for a major component of the early part of VL-evoked epsps.

356.5

CONFOCAL MICROSCOPIC IMAGING OF SUBCELLULAR CALCIUM CHANGES EVOKED IN CULTURED HIPPOCAMPAL CELLS BY NMDA. M. Segal and D. MANOR* Center for Neuroscience, The Weizmann Institute, Rehovot, Israel.

Changes in free intracellular calcium concentration ([Ca]i) in response to NMDA were measured in cultured rat hippocampal neurons loaded with the calcium dye Fluo-3 and exposed to argon-ion laser light in a Wild-Leitz confocal microscope. NMDA caused a glycine dependent, Mg^{+2} and 2-APV blocked rise of [Ca]i. A similar, but 2-APV, Mg^{+2} and glycine insensitive rise in [Ca]i could be evoked by kainate and quisqualate. The effects of NMDA were seen in the absence of [Na]o, but the recovery to normal [Ca]i following NMDA was slower than normal, indicating the involvement of the Na-Ca exchanger in the removal of excess [Ca]i. The rise in [Ca]i was first detected near the plasma membrane, and a wave of elevated [Ca]i could be seen, moving towards the center at a rate of 0.1 mm/sec. The level of [Ca]i was normally lower in the nucleus than in the cytoplasm, but following exposure to NMDA, nuclear [Ca]i was highest among all other cell compartments. Normal [Ca]o was necessery for the rise of [Ca]i following NMDA exposure, but the release of calcium from intracellular stores also contributed to this rise, as it was markedly reduced by dantrolene. It is suggested that activation of an NMDA receptor causes a rise in [Ca]i by a combined influx of calcium and its release from internal stores.

356.6

DIAZOXIDE REVERSIBLY BLOCKS GLUTAMATE DESENSITIZATION AND PROLONGS EXCITATORY POSTSYNAPTIC CURRENTS. K.A.Yamada & S.M.Rothman. Departments of Pediatrics, Neurology, and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Diazoxide (DZ) is an opener of ATP-sensitive potassium channels in pancreatic ß-cells and smooth muscle cells. Ben-Ari and colleagues have suggested that ATP-sensitive potassium channels might also be present on the presynaptic terminals of hippocampal neurons (*Brain Res*, **486**: 159-164). We recorded evoked synaptic currents between monosynaptically connected pairs of hippocampal neurons in dissociated cell culture, anticipating that DZ would reduce excitatory and inhibitory synaptic currents (epsc's and ipsc's) by a presynaptic mechanism. However, DZ reversibly enhanced the peak (200±71% of control,n=8) and prolonged the decay of epsc's (276±145% of control), while reducing the peak and prolonging the decay of ipsc's.

L-glutamate or quisqualate were exogenously applied by a rapid, whole-cell technique. Steady-state currents measured 700ms into a 750ms long application were reversibly increased to 380±170% (n=5) and 520±83% (n=5) of control respectively. DZ had no effect on currents from identical applications of NMDA, kainate, or GABA. DZ had no effect upon the glutamate reversal potential or upon passive or active membrane properties. Agonists and antagonists of ATP-sensitive potassium channels did not mimic or block DZ's effects.

We conclude that DZ reversibly inhibits rapid glutamate receptor desensitization, resulting in enhancement of the peak and prolongation of the decay of the epsc.

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356.7

GLUTAMATE-IMMUNOREACTIVITY IS ABOLISHED BY 6-DIAZO-5-OXO-L-NORLEUCINE. F. Conti, and A. Minelli*. Institute of Human Physiology, University of Ancona, Via Ranieri, I-60131 Ancona, Italy.

Phosphate activated glutaminase (PAG) provides most of the transmitter (releasable) pool of glutamate (Glu) in the CNS. In the present experiments we applied the PAG inhibitor 6-diazo-5-oxo-L-norleucine (DON) to the cerebral cortex of adult rats to test the hypothesis that Glu-immunoreactivity in cortical neurons might be a marker of Glu-ergic neurons.

DON (0,25-1 mM) was applied either intraparenchimally (0.2 µl) or topically (2 µl) to the parietal cortex of adult rats, and perfused transcardially with saline followed by 4% carbodiimide (in 0.1 M phosphate buffer, pH 7,4) and 4% paraformaldehyde (in the same buffer). Vibratome sections (30 µm-thick) were then processed for Glu-immunocytochemistry as previously described (*Conti et al., J. Neurosci., 7: 1887-1901, 1987*). Adjacent sections were used for Nissl staining, cytochrome oxidase (C.O.) histochemistry, and neuropeptide Y (NPY) immunocytochemistry. In other animals, saline was injected in the cerebral cortex, or an empty micropipette was advanced trought the cortical mantle.

Both intraparenchimal and topical application of DON abolished Glu-immunoreactivity ccompletely, the effect being more intense in the cases in which DON was applied topically. The extent of the effect was dose-dependent: 0.25 mM produced a narrow halo of inhibition (200-300 µm, both radially and tangentially); 0.5 mM produced inhibition in a wider cortical area (1mm radially; 2 mm tangentially), while 1 mM DON abolished Glu-immunoreactivity in all layers of a large region (about 2-3 mm wide). Controls showed that DON treatment did not change cytoarchitecture, neuronal morphology, C.O. activity and immunoreactivity to NPY, suggesting the effect is not aspecific.

Since PAG activity is mostly involved in the formation of releasable Glu, these results provide further support to the hypothesis that Glu-immunoreactivity in the cerebral cortex is related to the transmitter pool of Glu.

356.8

KYNURENIC ACID PRODUCTION IN THE RAT HIPPOCAMPUS: EFFECTS OF QUINOLINATE LESION. H.-Q. Wu, H. Baran, F. Du and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

The production of kynurenic acid (KYNA), an endogenous broad-spectrum antagonist of excitatory amino acid receptors, was studied with biochemical and immunohistochemical techniques in quinolinic acid (QUIN)-lesioned rat hippocampus. All analyses were carried out seven days after QUIN (120 nmol/1 µl). As compared to controls, the lesion caused a 91.8 ± 6.5% increase in KYNA tissue content and a 67.3 ± 4.3% increase in the activity of kynurenine aminotransferase (KAT), KYNA's biosynthetic enzyme. Immunohistochemical analysis using anti-KAT antibodies revealed a substantial increase in KAT-immunoreactivity, probably due to the proliferation of astrocytes, in the lesioned regions. Using in vivo microdialysis with fluorimetric KYNA detection, endogenous steady-state levels of extracellular KYNA in the normal hippocampus were found to be 90.4 ± 10.5 fmol/30 µl dialysate. QUIN lesions raised the basal extracellular KYNA to 212.7 ± 19.7 fmol/30 µl. Aminooxyacetic acid (-55% at 10 mM) and veratridine (-36% at 50 µM), administered through the dialysis probe, decreased extracellular KYNA levels. The effect of veratridine was abollished by the QUIN lesion. These data confirm the pivotal roles of neuronal activity and astrocytic KAT in the control of extracellular KYNA levels.

Supported by USPHS grant 16102.

356.9

KYNURENINE AMINOTRANSFERASES IN HUMAN BRAIN: CHARACTERIZATION USING ^{3}H-KYNURENINE AS A SUBSTRATE. W. Schmidt, [1]W.O. Whetsell, Jr., E. Okuno and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228 and [1]Vanderbilt Univ. Sch. Med., Nashville, TN 37232.

Using conventional spectrophotometric product analyses, two enzymes capable of synthesizing the neuroprotectant kynurenic acid have been identified in human brain tissue (Brain Res., 542:307, 1991). Partially purified preparations of both kynurenine aminotransferases (KAT I and KAT II) have now been used to further characterize their catalytic properties using physiological concentrations of kynurenine (KYN). With 2 µM (100 nCi) ^{3}H-KYN (1.9 Ci/mmol) as a substrate, both KAT I and KAT II were measurable using the assay conditions elaborated for rat brain KAT (Schmidt et al., J. Neurochem., in press). pH optima were 7.4 (KAT II) and 10.0 (KAT I), respectively. KAT I, but not KAT II, showed a pronounced preference for pyruvate compared to 2-oxo-glutarate, and was potently inhibited by glutamine (IC_{50} ~50 µM). Apparent K_m values for KYN at optimal pH were 9 µM (with 2-oxo-glutarate) and 670 µM (with pyruvate) for KAT I and 1.4 mM (with either cofactor) for KAT II. These data are in excellent agreement with those obtained previously using non-radioactive substrate. The novel, more sensitive radiometric KAT assay will therefore permit the study of KAT I and KAT II in small samples of normal and pathological human brain.

Supported by grants NS 16102, NS 28236 and MH 44211.

356.10

TRANSIENT INCREASE IN STRIATAL KYNURENATE SYNTHESIS FOLLOWING SYSTEMIC KAINATE ADMINISTRATION IN RATS. H. Baran, F. Du and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Because of the putative involvement of kynurenic acid (KYNA) in seizure processes (Neurosci. Lett., 48:273, 1984), its production from kynurenine (KYN) was assessed at various timepoints after the administration of the convulsant kainic acid (KA; 10 mg/kg, s.c.). KYNA synthesis was assessed by measuring kynurenine aminotransferase (KAT) activity in brain tissue homogenates, and by measuring KYNA in the medium of brain slices incubated with 50 µM KYN (J. Neurochem., 52:1629, 1989). No change in KAT activity was observed up to 2 days following KA treatment in the striatum (S), substantia nigra (SN), hippocampus (H) and piriform cortex (PC). At 7 days, KAT was increased by 66% (S), 99% (H) and 84% (PC) as compared to controls (no change in SN). At the same timepoint, striatal slices from KA-treated rats showed a 79% increase in KYNA production. Analysis after 1 month, performed in slices only, revealed large increases in H (+ 227%) and PC (+ 380%) but not in S. Immunohistochemical analysis of the striatum 7 days after KA did not show an obvious change in KAT-immunostaining. The persistent changes in H and PC are likely due to the well-documented neuronal loss and associated astrogliosis, whereas the transient increase in striatal KYNA production indicates a functional up-regulation of KAT activity in the absence of apparent neuropathological changes. Supported by grant NS 16102.

356.11

IMMUNOCYTOCHEMISTRY OF PUTATIVE ENDOGENOUS NMDA-LIKE ANTIGEN(S) IN THE RAT CNS. J.C.Woodley*, P. Ordronneau* and P. Petrusz. Curr. in Neurobiology, Dept. of Cell Biol. and Anat., Univ. of North Carolina, Chapel Hill, NC 27599.

An antiserum against the synthetic peptide glycyl-D-aspartate (GDA) was used to search for putative endogenous ligands specific for the NMDA subtype of glutamate (Glu) receptors. Results from immunocytochemistry and ELISA (Ordronneau et al., J. Histochem. Cytochem. 38:1033, 1990) showed that the antiserum recognized the non-endogenous compounds GDA, NMDA, and D-Asp, but it did not recognize L-Glu, L-Asp or other known NMDA receptor ligands. We report here that the anti-GDA serum labels a neuronal antigen in vibratome sections of rat CNS. This antigen shows a widespread and distinct distribution that includes intense immunostaining in the neocortex, ventral striatum, hippocampus, basal ganglia, as well as the cochlear, facial, and vestibular nuclei, but relatively light staining in portions of the cerebellum, thalamus, and olfactory bulb. This distribution is consistent with the putative role of this antigen as an endogenous NMDA receptor ligand. (Supported by APA 1 T32 MH18882-03, and NS 27679).

356.12

SEROTONIN (5-HT)$_{1A}$ BUT NOT 5-HT$_2$ RECEPTORS ARE ENRICHED ON CORTICAL NEURONES DESTROYED BY INTRASTRIATAL VOLKENSIN. P.T.Francis[*1], M.N.Pangalos[*1], R.C.A.Pearson[2], D.N.Middlemiss[*3], & D.M.Bowen[*1]. (SPON. Brain Research Association). [1]Inst. Neurol., London, UK, [2]Univ. Sheffield, UK and [3]Merck Sharp & Dohme Research Labs., Harlow, UK.

Following striatal injection of the retrogradely transported toxin volkensin, pyramidal neurones of lower cortical layers (forming the corticostriatal pathway) are destroyed. Pyramidal cells of the upper cortical layers together with GAD mRNA positive cells (considered to be interneurones) of all layers are preserved. To investigate receptor types affected cryostat sections from 6 rats receiving 2ng of volkensin by intrastriatal injection were incubated with [^{3}H]-8-OH-DPAT, for the 5-HT$_{1A}$, and [^{3}H]-ketanserin for the 5-HT$_2$ subtypes. Specific binding density was determined, by image analysis from autoradiograms, in upper and lower cortical layers ipsilateral and contralateral to the injection site. The binding of [^{3}H]-8-OH-DPAT was reduced by 23% on the side ipsilateral to the striatal lesion compared to contralateral (23.6 ± 7.6, mean ± SEM, fmol/mg tissue vs 30.4 ± 8.3, respectively, P=0.001 paired t-test) in lower layers but not in upper layers (22.7 ± 8.1 vs 25.9 ± 8.0, NS). The binding of [^{3}H]-ketanserin was unaffected (lower layers, ipsilateral, 27.7 ± 2.5; contralateral, 31.7 ± 3.5, NS and upper layers, ipsilateral, 32.2 ± 4.8; contralateral, 31.0 ± 4.4, NS). The lack of change in [^{3}H]-ketanserin binding may indicate that 5-HT$_2$ sites are on interneurones. The data (i) suggest glutamatergic pyramidal neurones which form the corticostriatal pathway are enriched in 5-HT$_{1A}$ receptors, (ii) aid the interpretation of receptor changes in autopsy brain and (iii) provide clues to PET ligands for monitering neuronal loss. Support by BRT, SERC, Wellcome Trust.

357.1

TEMPORAL RETINAL AXONS INITIALLY DO NOT MAKE DISTINCTIONS BETWEEN ROSTRAL AND CAUDAL COLLICULUS D.K. Simon and D.D.M. O'Leary Molecular Neurobiology Laboratory, The Salk Institute, La Jolla CA 92037

Theories on topographic map development have emphasized the guidance of growth cones of primary axons. In vitro studies of the developing chick retinotectal projection show that a glycoprotein of caudal tectal membranes is repulsive for the growth of axons from temporal retina (which is topographically matched with rostral tectum) and induces the collapse of temporal growth cones (Walter et al TINS 13, '90). Related findings are reported for the mouse retinocollicular projection (Godement & Bonhoeffer, Dev 106, '89). In perinatal rats, the retinocollicular projection is topographically diffuse, suggesting that, in vivo, rat retinal axons initially fail to make such regional distinctions in the superior colliculus (SC) (Simon & O'Leary, Dev Bio 137, '90). We have now directly addressed this issue with "exo utero" focal DiI injections in peripheral retina to label axons as they first extend across the SC. At E18-19, the labeled axons extend, without branching, across the rostral-caudal length of the SC. Qualitative examination reveals no differences in the targeting behavior of temporal versus nasal axons. Temporal axons show no tendency to change their trajectory to avoid caudal SC; some extend as far as the caudal SC edge. The similarity in temporal and nasal axon targeting along the rostral-caudal SC axis is confirmed by quantifying their distribution along this axis. Thus, the targeting of temporal and nasal axons are indistinguishable at this stage. Growth cone morphologies are also similar for temporal and nasal axons and, for both sets, the percentage of complex growth cones is higher in rostral versus caudal SC with similar ratios for temporal and nasal axons. These in vivo data suggest that, at this stage, the growth cones of primary retinal axons do not respond to cues that encode position along the rostral-caudal SC axis. Instead, the elaboration of topographically ordered connections depends on the extension of interstitial collateral branches by axons that overshoot their topographically correct positions, followed by the removal of mispositioned axon segments and branches. Supported by NEI EY07025.

357.2

A DEVELOPMENTAL MORPHOMETRIC AND VOLUMETRIC ANALYSIS OF AREA 18 PROJECTING LGNd NEURONS IN THE POSTNATAL CAT. L-A. Coleman and M.J. Friedlander, Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

Although the dendritic structure of neurons in the adult cat LGNd has been well characterized, relatively little is known about its development. Thus, we have studied how dendritic structure changes and how neighboring neurons of like-type interact and come to occupy defined space within the LGNd as it undergoes substantial volumetric changes during postnatal life. To minimize classification problems among immature LGNd neurons we have concentrated on the subset of geniculocortical relay neurons that project to area 18 of the primary visual cortex. Injections of fluorescently labelled latex microspheres were made into area 18 of the visual cortex in postnatal kittens aged 2 to 6.5 weeks and in adult cats. Following a 3 day survival, animals were perfused and the LGNd was sectioned at 400µm. Cortical injection sites were reconstructed. Density of labelled LGNd neurons was determined using confocal microscopy. Labelled cells were then injected intracellularly with Lucifer Yellow and HRP. Dendritic structure was analysed from a series of confocal images or from reconstructions of cells reacted for HRP. Labelled neurons were located in a discrete region with a consistent distribution (n=5 animals). In the caudal LGNd, labelled cells were located in the C laminae, while rostrally they were seen progressively more dorsal in both layers A and A1; a pattern described for adult cats (Geisert, J.Comp.Neurol. 190:793, 1980). The area 18 projecting cells were amongst the largest at all ages, their density decreased between 2 and 3 weeks postnatal, while dendritic morphology, even at 2 weeks, was consistent with adult Y-cell structure. However, features common to immature neurons, such as a greater density of dendritic spines, were seen in kittens.

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357.3

DEVELOPMENT OF EFFERENT PROJECTIONS IN RAT VISUAL CORTEX IN VIVO AND IN VITRO. J. Bolz, J. Kehrer, and N. Novak Friedrich-Miescher Labor der Max-Planck Gesellschaft, 7400 Tübingen, Germany.

Efferent cortical projections are highly specialized. For example, cortical output neurons within a single cortical layer, but with different projection sites, participate in different intrinsic circuits, and have different morphologies. In the visual cortex, cells in layer 5 which project to the superior colliculus (SC-cells) have a long apical dendrite and a dense basal dendritic field, whereas callosally projecting (CC-) cells have a short apical dendrite and relatively few basal dendrites. These characteristic features, however, are not present during early development. Using DiI labelling in fixed rat brains, we found that SC- and CC-cells in layer 5 had similar pyramidal cell morphology up to postnatal day 4 (P4). Both cell classes had few basal dendrites, and all apical dendrites reached layer 1. Between P5 and P8, the apical dendrites of SC-cells followed the fast growing cortex, but the apical dendrites of CC-cells did not extend at the same rate. Around P10, SC- and CC-cells had developed their target-specific morphology, and the distinct differences in the patterns of basal and apical dendrites were clearly expressed. To address the question as to how these neurons acquire their morphological characteristics, we studied cortical projection neurons in a slice culture system. When cortical slices from 0-2 day-old animals were co-cultured for 8-10 days with explants from the superior colliculus, the morphology of SC-cells *in vitro* was strikingly similar to SC-cells at the corresponding age *in vivo*. These results suggest that inputs from other cortical and subcortical areas are not required for the development of target-specific pyramidal cell morphology, since these inputs are not present in culture. Further experiments will examine whether the target plays a role in defining phenotypes of cortical projection neurons.

357.4

DEVELOPMENT OF AFFERENT PROJECTIONS IN RAT VISUAL CORTEX IN VIVO AND IN VITRO. M.Götz, N.Novak and J.Bolz; Friedrich-Miescher Labor der Max-Planck Gesellschaft, 7400 Tübingen, Germany.

Afferent fibers from the thalamus terminate with a high degree of specificity in the cortex: each nucleus of the thalamus projects to layer 4 of a distinct area in the sensory cortex. Thalamic fibers, however, reach the cortex early in development before their target cells have been generated, and accumulate beneath the cortical plate until layer 4 has been formed. We studied the invasion of thalamic fibers together with the development of glycosilated adhesion molecules in rat cortex by combining anterograde DiI-tracing with lectin-staining. The growth of thalamic fibers correlates closely with the distribution of lectin-binding glycoproteins. From embryonic day 16 (E16) until E19 most glycosilated molecules are located in the subplate and marginal zone, and thalamic fibers are restricted to the subplate zone. During further development the expression of lectin-binding molecules spreads from the deep to the superficial layers. Thalamic axons follow the front of lectin-binding molecules and thereby grow towards their target cells in layer 4. To examine the influence of the cortex on the ingrowth of thalamic fibers, we confronted thalamic explants in vitro with cortical slices of different developmental stages. Fibers from thalamic explants of all ages tested (E16-P1) invade a co-cultured postnatal cortical slice and terminate in their appropriate target layer 4. In contrast, the same thalamic fibers do not invade a cortical slice prepared at E16. Quantitative growth assays demonstrated the existence of growth-permissive, membrane-bound molecules in postnatal cortex, that are not present in embryonic cortex. The differential expression of these membrane-bound molecules in the cortex regulates the invasion of thalamic axons and allows thalamic ingrowth only after layer 4 has been established. Preliminary experiments indicate that such a mechanism might also be involved in the selection of cortical areas.

357.5

DEVELOPMENT OF FORWARD AND FEEDBACK CONNECTIONS IN RAT VISUAL CORTEX. A.Burkhalter and T.A.Coogan, Dept. of Neurosurgery, Washington University Sch. of Med., St. Louis, MO 63110.

The majority of feedforward projections from lower to higher areas of visual cortex originate from upper layers. By contrast, feedback projections arise preferentially form lower layers. In view of the inside-out development of cortical layers we hypothesized that ontogenetically older feedback projecting cells invade their targets before the younger forward projecting cells. To test this possibility forward projections from primary visual cortex (area 17) to the extrastriate area 18a were labeled by injecting Pha-L into area 17 of 1 to 10 day old rats and sacrifice after 2 days. Feedback projections to area 17 were visualized through anterograde tracing from area 18a.

At 3 days of age (P3) growth cone tipped forward and feedback fibers were confined to layers 1 and 6. Labeling in P4 animals included layers 1, 5 and 6. Forward and feedback fibers invaded the cortical plate (CP) at P5. At P5 projection columns emerged at topographically appropriate locations of areas 17 and 18a. Forward projecting fibers branched in outer layer 1, the bottom of the CP and at the layer 5/6 border. Feedback projecting fibers ramified in inner layer 1, in the outer half of the CP and layers 5 and 6. At P12 both projections appeared more branched but far less elaborate than in adults.

Contrary to our expectation these results indicate that in rat visual cortex forward and feedback projections develop simultaneously. In addition, both projections develop with great topographic and laminar specificity and there is no evidence for competitive interactions between forward and feedback fibers. NIH EY05935.

357.6

EXPERIMENTALLY INDUCED ESTABLISHMENT OF VISUAL TOPOGRAPHY IN AUDITORY THALAMUS. A.W. Roe, J. Hahm, and M. Sur, Dept. of Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139.

We have previously reported (Sur et al., '88) that following neonatal removal of primary retinal targets and deafferentation of the auditory thalamus (MGN) in ferrets, some retinal projections are induced to the MGN. The normal MGv (ventral or principal division of the MGN) contains a lamellar organization such that sound frequencies map in a mediolateral axis across lamellae while individual lamellae represent the same (iso) frequency. We have now investigated whether retinal projections map topographically in the MGv of "rewired" ferrets, and how the visual map relates to the lamellar organization of the nucleus.

We recorded extracellularly from visual units located in the MGv in 11 rewired ferrets and examined visual topography in the MGv by making grids of electrode penetrations spaced 250-500 um apart. Receptive fields of visually responsive units were plotted and characterised. A two dimensional visual map was established in the MGv. Low azimuthal representations were found medially and high azimuths laterally. Elevations were mapped in a dorsal-to-ventral as well as a rostral-to-caudal gradient. These data indicate that azimuthal representations map across lamellae (congruent with the axis normally representing sound frequency), and elevations map within "isofrequency" lamellae. In contrast to the visual map in the normal LGN but consistent with the frequency map in the normal MGv, lines of projection map *within*, and not across, lamellar/laminar planes.

Thus, retinal projections induced to non-visual thalamic targets can be topographically organized. In rewired ferrets, the azimuth and elevation axes of the visual map have a predictable relationship to the frequency and isofrequency axes of representation in the normal auditory thalamus, suggesting that the orientation of topographic axes of sensory maps are specified by cues present in the target.

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357.7

THALAMIC AXONS MAKE SYNAPTIC CONTACTS WITH SUBPLATE NEURONS IN CORTICAL DEVELOPMENT. K. Herrmann, A. Antonini and C.J. Shatz. Dept. Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, and Dept. Physiol, UCSF, San Francisco, CA 94143.

Early in development, ingrowing thalamocortical axons arrive within the telencephalon before their ultimate target neurons in the cortical plate (CP) have migrated into position. These axons accumulate or 'wait' in the subplate (SP), a zone beneath the CP, that contains the first born neurons of the cortex, the SP neurons. EM studies have demonstrated that SP neurons receive synaptic contacts of unknown origin, although physiological evidence suggests that some of them may derive from thalamic sources.

To conclusively establish that some of these synapses derive from the thalamus, the anterograde tracer PHAL (phaseolus vulgaris leucoagglutinin) was injected iontophoretically into discrete thalamic nuclei of neonatal (P3 to P13) ferrets, and the brains were prepared for EM. Light microscopical analysis confirmed that many PHAL-labeled axons, often tipped with growth cones, are present in the SP. At P8 in ferret, some of these thalamic axons had even invaded the CP. At the EM level, labeled processes were found presynaptic, mainly to mature dendritic spines or shafts of presumed SP neurons. PHAL-labeled synaptic contacts could be seen also in the CP. These ultrastructural observations provide strong evidence that thalamocortical axons can make synaptic contacts onto SP neurons. Moreover these results suggest that functional synaptic interactions with SP neurons could play a role in the development of the appropriate thalamocortical connections. Supported by NATO and Fight for Sight Prevent Blindness fellowships (KH), and grants from the Alzheimer's Association and NIH EY02858 (CJS).

357.8

THE INVOLVEMENT OF SUBPLATE NEURONS IN THE FORMATION OF OCULAR DOMINANCE COLUMNS IN LAYER 4 OF THE CAT'S VISUAL CORTEX. A.Ghosh and C.J.Shatz, Dept. of Neurobiology, Stanford University, Stanford, CA 94305

During development of the mammalian visual system, axon terminals from the lateral geniculate nucleus (LGN) are initially intermixed within layer 4 of cortex and gradually segregate to give rise to ocular dominance columns. Many experiments have demonstrated that neural activity is necessary for segregation, but there are likely to be additional factors since the process of segregation can be influenced only during the critical period. Here we have investigated whether subplate neurons play a role in maintaining an environment permissive for ocular segregation within layer 4 of the cat's visual cortex. Subplate neurons are present below the cortical plate in large numbers during development, and many send ascending axon collaterals into the cortical plate, particularly into layer 4, during neonatal life.

Subplate neurons were deleted by an injection of kainic acid into the white matter during the first postnatal week, two weeks before the onset of segregation. Such an injection appears to selectively ablate subplate neurons without directly affecting other cellular elements in the region (verified by cresyl violet staining, MAP2 and GFAP immunocytochemistry). At 8-10 weeks postnatal, after segregation is normally complete, the pattern of termination of LGN axons was revealed by transneuronal transport following an intraocular injection of [3H]proline. In the absence of subplate neurons LGN axons failed to segregate into ocular domonance columns, and instead remained diffusely spread within layer 4, as they are at birth. The lack of segregation is accompanied by marked changes in the cellular organization of cortical layer 4. These changes first appear weeks after the subplate lesion indicating that they are not a direct consequence of excitotoxic damage. Our observations suggest that subplate neurons may be required for interactions which lead to the formation of ocular dominance columns within layer 4 and possibly also for the differentiation and maintenance of layer 4 neurons. [Supported by NIH grants EY02858 (CJS) and HD07249 (AG).]

357.9

THALAMIC AND INTRACORTICAL PROJECTIONS ORIGINATE FROM SUBPLATE-1-IR AND GLUTAMATE-IR INVERTED PYRAMIDS IN THE WHITE MATTER OF DEVELOPING AND ADULT CAT NEOCORTEX. P.Wahle[1], K.Albus[1], J.R.Naegele[2], [1]MPI Biophysical Chemistry, 3400 Göttingen, FRG, and [2]Yale Medical School, New Haven, CT 06510, USA.

MAb SUBPLATE-1 was raised against immature cat neocortical white matter (Naegele, Barnstable, Wahle, PNAS 88:330-34,'91). SUBPLATE-1 is expressed by non-GABAergic neurons of an inverted pyramidal morphology with spiny dendrites (Wahle et al., Soc.Neurosci.Abstr.16:987,'90). The present study aims to determine the axonal projection pattern and the neurochemical phenotype of inverted pyramidal neurons in young (second postnatal week) and adult (one year and older) cats. DiI implants into cortical grey matter or thalamic nuclei of kittens label neurons of inverted pyramidal morphology in the cortical white matter. Rhodamine-conjugated latex beads injected into thalamus and neocortical grey matter of young kittens, and grey matter of adult cats were then used to label white matter cells by active retrograde transport. Immunohistochemistry with SUBPLATE-1 antibody and a glutamate antiserum (CHEMICON) was carried out. Many retrogradely labeled white matter neurons displayed glutamate - ir. In kittens some neurons retrogradely labeled from grey matter or thalamic injection sites also express the SUBPLATE-1 - antigen. When retrogradely labeled white matter cells were filled intracellularly with Lucifer Yellow, all thalamic projection neurons and the vast majority of intracortical projection neurons display a spiny, inverted pyramidal morphology. The results suggest, that glutamatergic pyramidal neurons of the white matter (subplate) project to the grey matter and the thalamus during postnatal life and in adulthood. The neuron type is thus very similar to the layer VI pyramidal neurons. It differs morphologically by its mainly inverted dendritic orientation and molecularly by the developmental expression of the SUBPLATE-1 antigen.

357.10

CALLOSAL PROJECTION CELLS IN RAT VISUAL CORTEX EARLY IN DEVELOPMENT ARE PREDOMINANTLY INHIBITORY, GABAERGIC NEURONS. F. Kimura and R.W. Baughman. Dept Neurobiology, Harvard Med School, Boston MA 02115.

The transcallosal projection in cortex is generally thought to contain exclusively excitatory cells. We have obtained evidence, however, that this pathway contains an important inhibitory component early in development. Fluorescent latex microspheres were injected into the visual cortex of 0-1 day-old Long-Evans rat pups. The contrateral visual cortex was enzymatically dissociated at day 5 and grown in culture for at least 10 days before recording. Dual whole-cell-patch recordings of membrane potentials were obtained from pairs of cells including both corticocallosal cells and unlabelled cells. Of 44 labelled cells tested, 25 (59%) produced monosynaptic IPSPs in follower cells, 8 elicited monosynaptic EPSPs, and 11 were not directly connected. The IPSPs were blocked with 1 μM bicuculline, persisted in 1 mM kynurenate, and reversed near E_{Cl^-}. Additionally we stained the cultures immunohistochemically with Gaba antiserum with the PAP technique. Of 41 labelled callosal cells, 27 (66%) showed Gaba-like immunoreactivity. Finally, we carried out the same experiments on neurons from a rat in which latex microspheres were injected on day 5, and enzymatic dissociation was done on day 6. Neither physiological nor immunohistochemical experiments showed existence of inhibitory neurons; none of 13 labelled neurons produced monosynaptic IPSPs, and none of 8 labelled neurons showed Gaba-like immunoreactivity. We conclude that there is a predominantly inhibitory transcallosal projection before about postnatal day 1, but that this is transient and is replaced by a completely excitatory projection by postnatal day 5. (EY03502 and Human Frontier Science Program)

357.11

DiI LABELED INTERHEMISPHERIC FIBERS DURING RAT POSTNATAL DEVELOPMENT SHOW TRANSITORY CORPUS CALLOSUM AXON TERMINAL DISTRIBUTIONS THROUGHOUT VISUAL CORTEX IN LAMINAE I-VI. A.J. Elberger and M.M. Hester*. Department of Anatomy and Neurobiology, Univ. Tennessee Memphis, Memphis, TN 38163.

DiI labeling of the corpus callosum (CC) in neonatal cat has revealed presumed CC axon terminals within laminae I-VI throughout visual cortex area 17 (Elberger, 1990 Soc. Neurosci. Abs.); this indicates a potential for extensive CC/visual cortex interactions during early development. To determine whether a potential for such interactions exists in other species, CC axons and presumed terminals (defined by size, configuration) were labeled by placing crystals of DiI in vitro in the mid-sagittal CC of normal rats 1-4 weeks old. The distributions of CC axons in visual cortex were compared at different developmental stages.

The results show that at 1 week, CC axons penetrate supra- and infragranular layers throughout areas 17, 18a and 18b; terminals are found in laminae I-VI. By 2 weeks there is a slight reduction in the number of CC axons in area 17, with terminals still found in laminae I-VI. By 3 weeks there are significantly less CC axons in areas 17, 18a and 18b. These are reduced further at 4 weeks; by this age, CC axons in area 17 are very scarce. Thus, many transitory CC axons have an opportunity to interact with all regions of developing visual cortex in rat, as in cat. Transitory CC axons may play a critical role in normal development of visual cortex.

Supported by EY08466 to AJE.

357.12

DEVELOPMENT OF TYPE I BENZODIAZEPINE RECEPTORS IN MONKEY STRIATE CORTEX. Hendrickson AE#, Shaw C†, Erickson A#*,and Richards G+*. #Biological Structure, U. Washington, Seattle WA 98195, †Ophthalmology, U. British Columbia, Vancouver Canada V6T1Z3, Canada, +Pharma Div. Preclinical Res., Hoffman-LaRoche, Basel, Switzerland.

The GABA-A receptor complex contains a benzodiazepine(BZ)-binding component which potentiates the action of GABA and can be labeled by [3H] flunitrazepam (3HFZ) for autoradiography. Studies using different types of BZ ligands have found two BZ receptors (types I and II) in immature rat brain and mainly type I in adult. Many studies using different methods have shown that the type I BZ receptor corresponds to the alpha1 subunit of the GABA-A receptor. We have previously shown that 3HFZ binding appears in macaque monkey striate cotex by fetal day (F) 72 (Shaw etal Soc. Neurosci. 1989) and rapidly reaches adult levels shortly after birth. To test whether monkey cortex also shows multiple BZ receptors during development we *a)* used the triazolopyridazine CL218872 (10^{-5}M) which is a specific ligand for the type I BZ receptor as a cold competitor for 3HFZ binding in fresh-frozen sections of striate cortex from F60 to adulthood, and *b)* immunocytochemically stained a similar developmental series of perfusion-fixed cortex with the alpha1 subunit-specific monoclonal antibody bd24.

CL218872 had little effect on 3HFZ binding until F152 (birth=F170) when it eliminated binding in layer 4C and reduced that in other layers. After birth CL218872 blocked almost all labeling in layer 4C to adulthood, but 3HFZ alone showed a prominent dark band over 4C. At 8-20wk layer 3 density also was markedly reduced by CL218872 while other layers were less affected. Little specific staining for bd24 was seen until F162 when layer 4C was lightly stained. By 1d layer 4C was much darker and by 3wk staining had spread throughout all layers with 4C remaining the heaviest. At 9wk staining is intense in 6, 4A, 3, 2 and 1 with 5 and 4B much lighter; 4C remains the most intensely stained layer into adulthood but in older animals layer 2/3 is also very dark.

These two methods produce complimentary results which show that the type I/alpha1/BZ receptor does not appear until late in gestation and then becomes predominant after birth, particularly in layers 4C and 2/3. The postnatal appearance and laminar location for the type I BZ receptor suggests that it could play a major role in visually-guided developmental interactions. (EY01208; MRC PG-29; B.C. Medical Services Fnd. Grant)

357.13

The Transient Columnar Expression of 5-HT1C Receptors in Developing Cat Visual Cortex is Dependent upon Normal Binocular Interaction. R. H. Dyck, F. Lepore† and M. S. Cynader; Dept Ophthalmology, Univ British Columbia, Vancouver, BC, V5Z 3N9, and †Dept Psychology, Univ Montreal, Montreal, PQ, H3C 3J7, Canada.

We have previously shown that, during postnatal development of the visual cortex in cats, serotonin receptor subtypes established unique temporal gradients of expression. In addition, 5-HT1C receptors, labelled with [^{3}H]-mesulergine, exhibited a columnar pattern of expression within area 17 between postnatal days (P) 30 and 90 (Dyck and Cynader, Soc Neurosci Abstr, 16, 987).

Here we determined the overall distribution of columns in developing cat visual cortex using autoradiographic methods to localize 5-HT1C receptors in tangential sections through opened and flattened cortex of P50 kittens. Furthermore, the relationship of 5-HT1C columns to afferent input was assessed in P50 kittens who had undergone either eyelid suture, enucleation, optic tract section, or lateral geniculate nucleus aspiration (all performed unilaterally) prior to P10.

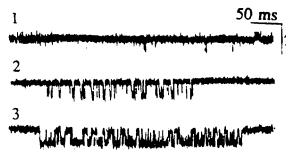
The distribution of 5-HT1C columns in area 17 was found to be periodic (at ~1 mm intervals), and organized in bands with a predominant orientation perpendicular to the 17 / 18 border. 5-HT1C columns were absent in visual cortex ipsilateral to the LGN aspiration; mostly absent ipsilateral to the optic tract section; virtually eliminated bilaterally following unilateral enucleation; and reduced following monocular deprivation. We conclude that the columnar expression of 5-HT1C receptors in visual cortex is dependent on binocular cortical inputs during development and suggest that these 5-HT1C sites are transient markers of ocular dominance bands in developing kitten striate cortex.

CALCIUM CHANNELS: PHYSIOLOGY AND PHARMACOLOGY III

358.1

MULTIPLE GATING MODES AND NORADRENERGIC MODULATION OF N-TYPE Ca^{2+} CHANNELS IN FROG SYMPATHETIC NEURONS. A.H. Delcour*, D. Lipscombe+ & R.W. Tsien, Dept. of Molecular and Cellular Physiology, Stanford Univ. and +Section of Physiology & Biophysics, Brown Univ.

Downmodulation of N-type Ca^{2+} channels by norepinephrine (NE) involves changes in the voltage- and time-dependence of gating. Cell-attached patch recordings (110 mM Ba) reveal multiple modes of N-type channel gating. In the low p_o mode (trace 1), p_o~0.05 at -10 mV, and voltage-dependent activation occurs over a relatively depolarized range of potentials. In this mode, openings are sometimes sufficiently resolved to show a larger unitary current amplitude than openings with higher p_o. Two additional patterns of gating with higher p_o (traces 2, 3) can be distinguished by consideration of mean open and closed times ($<t_o>$, $<t_c>$) for individual sweeps: high p_o sweeps (p_o~0.3) show shorter openings and longer closings while very high p_o sweeps (p_o~0.6) display longer openings and briefer closings. The negative correlation between $<t_o>$ and $<t_c>$ points to the existence of two gating pathways; consecutive sweeps tend to show the same pattern of gating, consistent with idea of modes. With 100 μM NE in the pipette, the pattern of gating shifts toward modes of lower p_o, but within each mode the open-closed kinetics remains essentially unchanged. In addition to these patterns of gating, we sometimes see an N-type "sub-conductance" (9 pS) like those in rat sympathetic neurons (Plummer et al., 1989). These smaller openings show N-type inactivation properties and ω-CgTx sensitivity, and activate at relatively negative potentials. NE modulation of the smaller openings is not readily apparent.



358.2

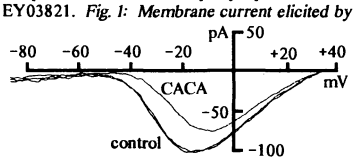
5-HT$_{1A}$ RECEPTOR ACTIVATION INCREASES K^+ CHANNEL OPENING AND SIMULTANEOUSLY INHIBITS Ca^{2+} CHANNELS: DEMONSTRATION IN THE SAME DORSAL RAPHE NEURON. N.J.Penington, J.S.Kelly and A.P.Fox* Univ. Chicago, Dept.Pharm/Phys, Chicago Il, 60637.& Dept. Pharmacol. Univ. Edinburgh U.K.

We have previously reported that 5-HT$_{1A}$ receptor activation dramatically inhibited Ca^{2+} currents in acutely isolated dorsal raphe neurons (Neuron 4, 751, 1990). Activation of the calcium current is greatly slowed by 5-HT. Occupation of the 5-HT$_{1A}$ receptor also induced an inwardly rectifying K^+ channel to open. The ED_{50} for both Ca^{2+} channel inhibition and K^+ channel activation was 30 nM 5-HT. Both effects are PTX sensitive when the cells were incubated at 35°C with the toxin (1μg/ml) for 12 hrs. The effect of 5-HT on both K^+ and Ca^{2+} currents was measured in isolation (consecutively) in the same cell and in other cells simultaneously. This was done by first using solutions appropriate for measuring I_K and then exchanging the external solution for one containing TEA, Ba, glucose and HEPES, a solution appropriate for measuring Ca^{2+} current in isolation. Both the inhibition of the Ca^{2+} current and the activation of 5-HT-induced K^+ current could be observed in the same neuron. The effect on K^+ currents but not on Ca^{2+} currents could be blocked by substituting Ba^{2+} for Ca^{2+}. Thus in these cells, 5-HT decreases neuronal excitability profoundly by a simultaneous inhibition of Ca^{2+} currents and potentiation of K^+ currents. We are investigating whether the same G-protein or two different G-proteins carry the signal from the 5-HT$_{1A}$ receptor to the Ca^{2+} and K^+ channels.

358.3

INHIBITION OF PRESYNAPTIC CALCIUM CURRENT VIA GABA$_C$ RECEPTORS. Gary Matthews, George Ayoub, and Ruth Heidelberger. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794-5230.

Type Mb1 bipolar neurons of goldfish retina have large synaptic terminals (10-12 μm diameter), which receive feedback synapses from GABAergic amacrine cells. Previously, we showed that GABA, but not the GABA$_B$ agonist baclofen, inhibits Ca current in these terminals (Heidelberger & Matthews, 1991, *Biophys. J., 51,* 83a). Here, we report that the GABA analog, *cis*-4-amino crotonic acid (CACA), an agonist for GABA receptors that are insensitive to bicuculline and baclofen (GABA$_C$ receptors; Drew *et al.*, 1984, *Neurosci. Lett., 52,* 317), mimics this action of GABA. Figure 1 shows that, like GABA, CACA shifted the activation of Ca current to more positive potentials, with peak suppression at -25 ± 1 mV (mean ± s.e.m., n=26); at more depolarized potentials, there was little effect on Ca current. The peak suppression of Ca current was 44.9 ± 4.2% (n=10) with 5 μM CACA and 25.7 ± 5.8% (n=3) with 0.5 μM. CACA did not activate GABA$_A$ Cl conductance in bipolar neurons, so it appears to be specific for the receptors mediating GABA's inhibition of Ca current. Together with the ineffectiveness of baclofen, this suggests a physiological role for GABA$_C$ receptors in modulation of presynaptic Ca current. Supported by NEI grant EY03821. *Fig. 1: Membrane current elicited by voltage ramp (100 mV/sec); no leak or capacitative current subtracted. Cs/ TEA pipette solution with ATP & GTP; $[Ca]_o$=2.5 mM. Control: before and after 5 μM CACA, applied by local superfusion.*



358.4

CALCIUM SIGNALS IN PRESYNAPTIC GLIAL CELLS CAUSED BY NERVE TERMINAL EXCITATION. B. Jahromi*, R. Robitaille and M.P. Charlton. MRC Group, Physiology Dept., Univ. of Toronto, Toronto, Canada M5S 1A8.

Glial cells are intimately associated with the nerve terminal at the frog neuromuscular junction. Each junction is covered by a few (2-5) glial cells whose fine processes wrap around the nerve terminal between active zones. While these cells are well positioned to influence the local ionic and biochemical environment of the presynaptic terminal and may thus influence synaptic transmission, little is known of their physiology.

We wondered whether these glial cells could respond to activity in the presynaptic terminal. Glial cells were loaded with the permeant Ca indicator fluo3-AM and were examined with a Biorad 600 confocal microscope using excitation at 488nm. The motor nerve was stimulated while images were obtained with the confocal microscope.

Trains of stimuli at 40 Hz for 30 sec caused a large Ca signal in the glial cells. Fluorescence from fluo3 increased in both the fine processes covering the nerve terminal and in the cell body which is several times the diameter of the terminal. The amplitude of the Ca signal depended on the frequency of nerve stimulation and the number of pulses given. The Ca signal did not occur in 0-Ca saline or in the presence of the Ca channel blocker Cd (50 μM) and is therefore not due entirely to internal release of Ca. The Ca signal does not depend on intact postsynaptic currents because these signals were obtained after application of α-bungarotoxin to block muscle contraction. Therefore, presynaptic glial cells are influenced by nerve terminal activity.

Supported by MRC Group (MPC) and MRC Centennial Fellowship (RR).

358.5

CALCIUM DEPENDENT REGULATION OF TRANSMITTER RELEASE OCCURS IN TERMINAL REGIONS OF SYMPATHETIC NEURONS. A.R. Wakade, S.V. Bhave, A.S. Bhave*, T.D. Wakade* & D.A. Przywara. Dept. of Pharmacology, Wayne State Univ. School of Medicine, Detroit, MI 48201.

We examined the functional aspects of cell bodies and terminal regions (neurites and growth cones) of chick sympathetic neurons in culture. Release of tritiated norepinephrine ([^{3}H]NE) and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were determined. Effects of various pharmacological agents on [^{3}H]NE release paralleled effects on $[Ca^{2+}]_i$ in terminal regions but not cell bodies. A new method is used to study localized uptake and release of [^{3}H]NE in somatic versus terminal regions of neurons. Somata had very low [^{3}H]NE uptake (0.23×10^{-6} cpm/mg protein) whereas neurites and terminals contained approximately 20 times more radioactivity. Excess K^+ and field stimulation evoked release of [^{3}H]NE was unaffected by removal of cell bodies. Muscarine (100 μM) activated phosphoinositide hydrolysis was measured to further asses the functional integrity of the two separated regions. The increase in [^{3}H]-inositol phosphates was more than 2 fold greater in cell bodies than terminals. These observations suggest that neurites plus growth cones are the prominent sites of uptake, storage and release of sympathetic transmitter. The data indicate that regulation of Ca^{2+} influx is distinct in the two regions and that $[Ca^{2+}]_i$ in the terminals only is related to [^{3}H]NE release.

358.6

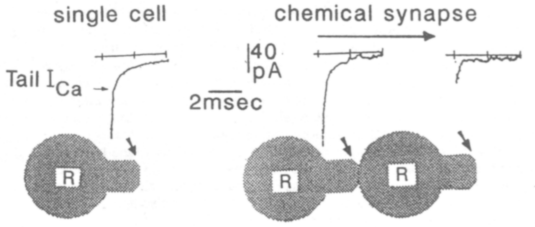
A LAMBERT-EATON MYASTHENIC SYNDROME ANTIGEN ASSOCIATED WITH PRESYNAPTIC CALCIUM CHANNELS M.J. Seagar*, T. Hoshino*, K. Leys*, C. Leveque*, B. Lang*, Y. Kasai*, P. David*, A. Omori*, O. El Far*, N. Martin-Moutot*, K. Sato*, E. Jover, J. Newsom-Davis* and M. Takahashi INSERM CJF 9016, Faculté de Médecine Nord, 13326 Marseille 15, France; Mitsubishi-Kasei Inst. of Life Sciences, Tokyo, Japan and Inst. of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford, UK

Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disease of the neuromuscular junction in which pathogenic autoantibodies downregulate presynaptic calcium channels resulting in reduced ACh release and muscle weakness. Antibodies have been detected in LEMS plasma that immunoprecipitate solubilized 125I-omega conotoxin (wCgTx) receptors from human neuroblastoma membranes (Sher et al. (1989) Lancet, 2, 640). We report that LEMS IgG immunoprecipitates calcium channels from rat brain synaptic membranes. The wCgTx receptor was partially purified (approximately 250 fold) by two chromatographic steps. The LEMS antigen was identified, in Western blots of purified channel proteins, as a 58kDa protein which is distinct from the 220kDa wCgTx binding polypeptide detected by photoaffinity labeling. A monoclonal antibody, produced by immunizing mice with synaptic membranes, shared properties with some LEMS IgG and is presently being used to characterize the calcium channel associated antigen.

358.7

SYNAPSE FORMATION INDUCES CHANGES IN THE DISTRIBUTION OF CALCIUM CURRENTS IN LEECH NEURONS IN CULTURE. Cooper, R.L., Fernandez de Miguel, F.*, and Adams, W.B. Dept. Pharmacology. Biocenter, Univ. of Basel, CH 4056, Switzerland

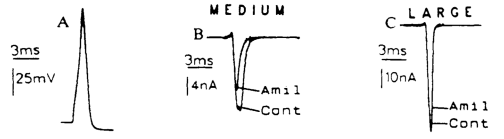
The distribution of Ca++ tail currents was measured by loose patch clamp in Retzius cells isolated from leech CNS. In single Retzius cells Ca++ currents were largest in the tips, smaller in the distal part of the soma and smallest in the mid region of the soma. When a chemical synapse was formed the tip of the postsynaptic cell showed a reduction in Ca++ current. Retzius cells that made electrical synapses or touched other cells without forming chemical synapses did not show this reduction in Ca++ current.



358.8

DURING ACTION POTENTIALS, T-TYPE Ca^{2+} CURRENTS CONTRIBUTE GREATLY TO Ca^{2+} ENTRY IN MEDIUM BUT NOT LARGE DIAMETER ACUTELY ISOLATED DRG NEURONS R.S. Scroggs and A.P. Fox* University of Chicago, Dept. Pharmacology/Physiology, Chicago, IL 60637

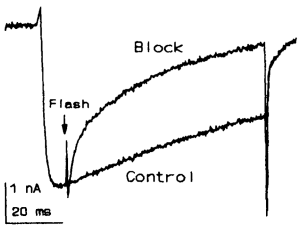
An action potential (AP) waveform (Fig. A) was used as a voltage command (holding potential -80mV, peak potential +20mV) to evoke Ca^{2+} currents in acutely isolated dorsal root ganglion (DRG) cells of medium (33-38μm) and large (42-50μm) diameter, using the whole cell patch clamp technique. As determined by blockade with 500μM amiloride, a large amount of current evoked by the AP protocol was conducted through T channels in medium diameter cells, which had large T currents, (Fig. B) but not in large diameter cells, which did not have T currents (Fig. C). Due to the slow de-activation kinetics of T channels, the entry of Ca^{2+} into medium diameter cells through T channels prolonged the duration of the current (Fig. B). Thus, Ca^{2+} entered medium diameter cells over a longer time period than was observed regarding large diameter cells (Fig. C). However, the total amount of charge per μm^2 surface area which entered medium diameter cells (5.4 $coul^{-15}$ $\pm$ 0.30 SE, N=10) was similar to that observed for large diameter cells (4.2 $coul^{-15}$ $\pm$ 0.68 SE, N=8). Differences in the duration of Ca^{2+} entry may alter diffusion patterns inside cells. Since DRG neurons with different diameter cell bodies may transmit different sensory modalities, the selective modulation of T channels might affect some sensory modalities more than others.



358.9

CALCIUM CHANNEL BLOCK BY PHOTO-RELEASED INTRACELLULAR CALCIUM. B.D. Johnson* and L. Byerly. Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-2520.

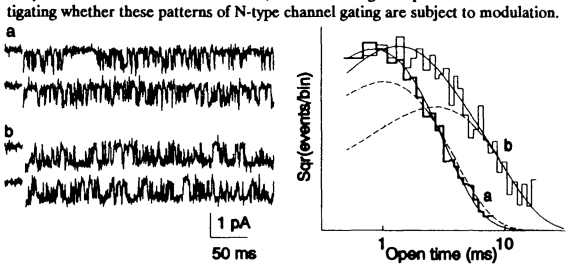
Intracellular Ca^{2+} has been shown to block Ca currents in many cells throughout the animal kingdom. One of the difficulties in studying this phenomenon of Ca^{2+}-dependent block has been to control intracellular Ca^{2+} over the cell's own buffering system. In the present study we have used the photolabile Ca^{2+} chelator DM-nitrophen to rapidly release Ca^{2+} on a millisecond time scale, near the Ca channels themselves. When neurons from the snail *Lymnaea stagnalis* were whole-cell patch-clamped with a pipette containing Ca^{2+}-loaded DM-nitrophen and exposed to a flash of UV light, 45 $\pm$ 16% (N=7) of the peak Ba^{2+} current was blocked. 52% of the block occurred with a τ of 3.5 ms while the remaining 48% had a τ of 27 ms (see figure). Recovery from block proceeded with a τ of 27 s and returned to within 11% of its original amplitude. Only 5% of this irreversible block could be attributed to the damaging effects of UV light alone. Both block and recovery follow the intracellular Ca^{2+} signal as measured optically and with Ca^{2+} electrodes. Further work will focus on the dose-response relation and the mechanism(s) involved.



358.10

MICROSCOPIC HETEROGENEITY OF N-TYPE CALCIUM CHANNEL GATING. A. R. Rittenhouse, M.R. Plummer, and P. Hess*. *Department of Cell & Molecular Physiology, Harvard Medical School, Boston, MA 02115.*

Single N-type Ca channels exhibit inactivating and noninactivating bursting patterns of activity (Plummer & Hess, 1991 *Biophysics J.*, 59: 537a). Within the non-inactivating bursts, we observed heterogeneous gating during cell-attached patch recordings of differentiated PC12 cells. Several distinct kinetic patterns can be observed; 2 such patterns from the same single N-type channel are shown below. The sweeps shown in **a**, have a low open probability (P_o) and the mean open times are fit by a single exponential (bold line). In contrast, the activity shown in **b** has a higher P_o and the mean open times are fit by the sum of 2 exponentials (thin line; the individual exponentials are shown as dashed curves), with the short open times similar to those of the low P_o bursts. The high and low P_o bursting patterns of activity also can be seen within individual, noninactivating sweeps. We are investigating whether these patterns of N-type channel gating are subject to modulation.



358.11

PTX-RESISTANT G_o EXPRESSED IN PTX-TREATED NG108-15 CELLS RESCUES THE $I_{Ca,V}$ INHIBITION BY NOREPINEPHRINE. F.Belardetti, M.Rifo*, L.Yun*, R.Taussig* and A.G.Gilman*. Dept. Pharmacology, UT Southwestern, Dallas TX 75235.

G proteins couple a class of membrane bound receptors to intracellular effectors; however, their specificity for particular cellular pathways is poorly understood. An useful model system to address the question of G protein specificity is the neuroblastoma-glioma cell line NG108-15, where a variety of neurotransmitter actions converge on the voltage-dependent Ca^{2+} current ($I_{Ca,V}$) to produce inhibition. In this cell line, the $I_{Ca,V}$ inhibition by L-enkephalin (L-EK) and norepinephrine (NE) is blocked by Pertussis Toxin (PTX); suggesting that these responses are mediated by one of the PTX sensitive G proteins, G_o, G_{i1}, G_{i2} or G_{i3}. To study the role of G_o in NG108-15, we have established a NG108-15 cell line stably expressing a PTX resistant mutant $G_{o\alpha}$ subunit and have demonstrated that following PTX treatment of these cells, the L-EK inhibition of $I_{C,aV}$ is still evident (Biophys.J. 59, 2, 2, 6a, 1991). We have used the same cell line to determine whether the α-adrenergic inhibition of $I_{Ca,V}$ occurs via the same pathway. In wild-type cells, NE reduced the $I_{Ca,V}$ to 76% of the control levels (n=6). In the same cell type after PTX (200 ng/ml/overnight), NE did not produce inhibition (n=5). When cells expressing the mutant G_o were employed, NE reduced the $I_{Ca,V}$ to 83% of control levels (n=5). After PTX treatment, NE still inhibited this current to 84% of control levels (n=7) in these cells. These experiments suggest that G_o might mediate the $I_{Ca,V}$ inhibition by both L-EK and NE in NG108-15 cells. We are currently using the same approach to determine whether the somatostatin and bradykinin inhibitions of $I_{Ca,V}$ occur via the same pathway.

358.12

Neuropeptide (NPY) Y1 and Y2 Receptors Mediate Opposite Actions on Nodose Neuronal Calcium Currents Via Pertussis Toxin-Sensitive Pathway(s). J.W. Wiley, R.A. Gross and R.L. Macdonald. Dept(s). Internal Medicine & Neurology, Univ. Michigan, Ann Arbor, MI.

Two different NPY receptor subtypes have been proposed, designated Y1 and Y2, based on bioassays and binding studies. [Pro34] NPY is a selective agonist for Y1 receptors while the C-terminal fragment 13-36 of NPY binds preferentially to Y2 receptors. Little is known about the electrophysiological actions of these agonists. We examined the effect of the Y1 and Y2 receptor agonists and native NPY on somal calcium currents in enzymatically dispersed postnatal rat vagal sensory (nodose) neurons using the whole cell version of the patch clamp technique. Voltage-gated calcium currents were recorded in media which blocked sodium and potassium currents. Nodose neurons exhibited three calcium current components, designated T, L and N which demonstrated distinct electrophysiological and pharmacological properties. NPY (0.1-100 nM) selective reduced the N current component in > 90% of responsive cells (52 out of 90 cells) while in < 10% of responsive cells a concentration-dependent increase in the N current component was observed. The Y2 agonist (0.1-100 nM) always decreased the N current component in 8/17 cells whereas the Y1 agonist (0.1-100 nM) uniformly increased the N current in 16/30 cells. Pretreatment with pertussis toxin (PTX; 100 ng/ml for 12 h), an inactivator of Gi/Go-type G-proteins, blocked the effect of the Y1-, Y2-agonists and NPY. In conclusion, nodose neurons express both Y1 and Y2 type NPY receptors; a Y1 agonist increased the N calcium current whereas a Y2 agonist reduced this current component. Both effects were mediated by PTX-sensitive mechanism(s).

TRANSPLANTATION: STRIATUM

359.1

FETAL NEURAL GRAFTING FOR THE TREATMENT OF HUNTINGTON'S DISEASE (HD) - REPORT OF THE FIRST CASE. I.Madrazo, R.E. Franco-Bourland*, C.Cuevas*, M.C.Aguilera*, F.Ostrosky-Solís*, N.Santiago*, H.Castrejón*, E.Magallón*, and G. Guizar-Sahagún*. Dept. Clin. Res. Neurol. Neurosurg., Ctr. Med. Siglo XXI, IMSS, 06725 México, D.F., México.

A neurosurgical procedure was designed and performed for the unilateral replacement of the striatum by homotopic homotransplantation of fetal striata in a patient with advanced HD, in accordance with the Mexican ethical regulations for human fetal brain grafting (Arch. Neurol. 47:1281,1990). The patient is a 37-year-old woman who first showed psychomotor alterations at the age of 28. In the last 3 years, she has shown significant continuous clinical deterioration. At the time of surgery, her scores of neurologic function on the various scales (S) were: Marsden-Quinn S/12 points; Shoulson-Fahn S/stage III; disability S/65%; and AIMS/26 points. Prior to surgery, the patient was started on immunosuppression. Surgery was performed on November 2, 1990. Both striata of a 13-week-old fetus were transplanted into multiple cavities made in the ventricular wall of the right caudate nucleus of the recipient. Her recovery from surgery was uneventful.

Three months postsurgery, she showed no further neurologic deterioration compared to her preoperative state, and no damaging effect of the surgery. Some subjective signs of improvement were apparent.

359.2

LONG AXONAL GROWTH IN ADULT RAT STRIATO-NIGRO-STRIATAL SYSTEM FROM GRAFTS OF HUMAN NEUROBLASTS
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One approach to study factors that influence axonal growth *in vivo* is that of intracerebral neural transplantation. For instance, it has been shown that axons can extend from embryonic tissue implants into the host brain, above all if the recipient is neonatal or if the graft is placed directly into, or close to, its normal, preferably denervated, target region in an adult CNS. Although axonal growth along the normal growth trajectories does occur also when embryonic tissue is placed into its homotypic site of an adult recipient, this growth is at the most for a few millimetres, and reaches only into nearby targets. In recent experiments, we analyzed cross-species grafts of human forebrain neuroblasts, placed into the adult excitotoxically lesioned rat striatum, using an antiserum, which stains human neurofilament-positive fibres, but does not recognize rat neurofilaments (Serotec Ltd., U.K.). Using this approach, we detected extensive human neurofilament-immunoreactive fibre outgrowth along the white matter of, e.g., the host internal capsule for up to 10-20 mm, and into several of the normal target regions (Nature 347, 556-558, 1990). In a follow-up study we have now used this cross-species grafting approach to further investigate questions related to axonal growth, such as tissue type specificity, growth trajectories and polarity.

In the rat model with a dopamine-denervated striatum, preliminary findings suggest an interesting ability of human mesencephalic cells to grow along the myelinated tracts towards and into the striatum, also when placed in the internal capsule and in the substantia nigra. Grafts placed inbetween the substantia nigra and the striatum show a striking polarity and grow towards their normal target region, i.e., the striatum.

In the model with excitotoxic lesions of the rat striatum, various tissue types have been implanted into the striatum. As described previously, forebrain tissue dissected from the ganglionic eminences extends axons for long distances along the internal capsule, whereas cerebellar neurons do not. We now report that also neocortical and spinal cord tissue grow well along the internal capsule bundles.

359.3

FETAL STRIATAL CROSS-SPECIES IMPLANTS AMELIORATE ABNORMAL MOVEMENTS IN A PRIMATE MODEL OF HUNTINGTON'S DISEASE.

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Recently, we developed an experimental model of Huntington's disease (HD) in primates using intrastriatal excitotoxin injections. After systemic apomorphine (1 mg/kg) a variety of abnormal movements analogous with the symptoms observed in HD patients, including dyskinesia, oro-facial dyskinesia, chorea and dystonia are observed. In the present study, the effects of neural implants on these motor symptoms were assessed. Comparison of the behavioral scores before lesion, 4 weeks after lesion and 10 weeks after grafting showed that implantation of cross-species fetal striatal cells into neuron depleted striatum of non-human primates reversed the abnormal movements observed in this animal model of HD. The abnormal movements returned after immunological rejection of the cross-species cells. In addition, in the case of transplantation with non-striatal foetal tissue (brain-stem cells) no recovery of motor function was observed. This study shows marked reductions of incidence of abnormal movements following transplantation in a primate model of HD and illustrates the potential of such an experimental approach to Huntington's disease

359.4

FETAL STRIATAL ALLOGRAFTS IN THE RHESUS MONKEY: AN ELECTRON MICROSCOPIC GOLGI STUDY.

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Dept of Neurosurgery, Univ. of Virginia, Charlottesville VA 22908

It is now well established that fetal neuronal transplants can lead to partial behavioral and biochemical recovery in rats with excitotoxically lesioned striata, a model of Huntington's disease. In the present study, we attempted to extend these studies into the primate. Primate fetal striatal neurons were transplanted into the ibotenic acid lesioned rhesus monkey striatum. Ten weeks after transplantation, the monkeys were transcardially perfused and graft tissue was Golgi impregnated and processed for electron microscopy. Large fetal striatal grafts contained numerous types of neurons at various degrees of differentiation. Medium-sized neurons with somatic diameters between 8 and 20 microns with occasional dendritic spines were characterized at the electron microscopic level by unindented nuclei, possibly representing immature medium spiny neurons. In addition, similar sized neurons displaying no dendritic spines contained indented nuclei, possibly representing the aspiny medium-sized neurons seen in the normal striatum. There were occasional neurons with somatic diameters between 20 and 40 microns which were characterized by indented nuclei and copious cytoplasm rich in organelles, representing the large neurons seen in the normal monkey striatum. The grafts contained developing dendrites, numerous growth cones, and mature synapses. In summary, the study demonstrated that ten weeks after transplantation fetal striatal allografts in the rhesus monkey contain neurons at various degrees of differentiation as assessed by Golgi impregnation and electron microscopy.

359.5

TRANSPLANTATION OF POLYMER ENCAPSULATED BOVINE ADRENAL CHROMAFFIN CELLS PREVENT QUINOLINIC ACID INDUCED LESIONS OF THE STRIATUM. P.R. Sanberg, D.F. Emerich, P.E. McDermott*, B.R. Frydel*, A.M. Bertino*, F.A. Kaplan, M.A. Palmatier, and L. Christenson. Cellular Transplants, Inc., Providence, RI 02906.

Excitotoxin-induced lesions of the striatum produce neural and behavioral deficits similar to Huntington's disease. It was demonstrated that certain cellular transplants can prevent striatal lesions induced by excitotoxins, possibly by release of trophic factors (Schumacker et al., *Soc. Neurosci. Abst.* 16:40, 1990). The present study examined whether xenograft adrenal chromaffin cell implants surrounded by an immunoisolatory polymer membrane reduce the neurodegenerative effects of the excitotoxin quinolinic acid (QA). Isolated bovine adrenal chromaffin (BAC) cells were encapsulated within a polymeric membrane, and cut into 4mm long capsules and their ends sealed. BAC cell-containing or empty capsules were then implanted stereotactically into the striatum of Sprague-Dawley rats. One week later the animals were intrastriatally injected with QA (225 nmol) or vehicle 0.8mm lateral to the implant. Body weights were recorded daily from the three experimental groups (BAC capsule/QA; empty capsule/QA; and QA alone). Thirty days after injection of QA the brains were histologically analyzed using cytochrome oxidase (metabolic activity) and Nissl stain. Both control groups lost weight following the QA lesion; however, the group implanted with BAC capsules had significantly less weight loss as a result of the QA lesion. Histologically, both control groups had ventricular dilation, neuronal loss and a complete lack of mitochondrial metabolic activity in the striatum. In contrast, the BAC implant group showed normal staining with cytochrome oxidase and relatively little neuronal loss. In conclusion, animals that received encapsulated BAC cell implants were protected from QA-induced damage in the striatum. Such a treatment may prevent progression of neuropathological damage in Huntington's disease.

359.6

INTRASTRIATAL AND INTRAVENTRICULAR FETAL STRIATAL TRANSPLANTS PROTECT AGAINST QA-INDUCED REDUCTION OF D_1 DOPAMINE RECEPTORS IN RAT STRIATUM. S.Y. Lu, P.R. Sanberg and A.B. Norman. Div. of Neuroscience, Dept. of Psychiatry, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267 and Cellular Transplant Inc. Providence, RI 02906

Neural transplants have been demonstrated to be able to reverse the behavioral deficits induced by excitotoxin lesions. The present study investigated the ability of fetal striatal transplants to prevent excitotoxin damage in the striatum. 3 groups of adult male rats received unilateral striatal transplants of 1) fetal striatal tissue (E-17) into the intact striatum (IS, n=8), 2) fetal striatal tissue into the lateral ventricle (ILV, n=8), or 3) sham transplant (vehicle only) into striatum (sham, n=9). 7 days after the transplantation, the IS and sham groups received a unilateral QA (100 nmol) lesion of the contralateral striatum. The ILV transplant group received QA in the ipsilateral striatum. 2 control groups consisted of 1) unilateral QA lesion only (QA, n=10), and 2) normal rats (NML, n=10). 21 days after the QA lesion, the B_{max} of [^{3}H] SCH23390 binding to dopamine D_1 receptors was measured in the striata. Compared with the normal control group (100%), the B_{max} of D_1 dopamine receptors for the QA, IS, ILV and sham groups were 40%, 64%, 85%, and 58% of control values, respectively. ANOVA and post-hoc comparison revealed that intraventricular striatal transplants provided full protection against QA-induced loss of D_1 dopamine receptor binding sites, whereas intrastriatal transplants only partially protected against the QA-induced lesion in the contralateral striatum. Sham transplants did not protect against the QA-induced damage. These results suggest that fetal transplants can prevent QA-induced neuropathological changes. The close proximity of the transplant to the lesion site may improve the protection effect.

359.7

TRANSPLANTS OF CULTURED ASTROCYTES ON EXCITOTOXIN-INDUCED LOCOMOTOR BEHAVIOR M. Giordano, S.Y. Lu, D.F. Emerich, S.K. Pixley, R.B. Norgren, M.N. Lehman and A.B. Norman. Div. of Neuroscience, Depts. Psychiatry and Anatomy. Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267 and Cellular Transplants, Inc. Providence, RI 02906

It has been reported that cultured astrocytes are able to reverse some behavioral deficits induced by lesions. The present study examined the effect of cultured astrocytes on locomotor deficits induced by bilateral kainic acid (KA) lesions of striatum.

Adult male rats (n=9) received bilateral striatal KA lesions. 10 weeks after the lesion, the rats received bilateral transplants of cultured astrocytes ($3x10^6$ cells) or fetal striatal tissue (E17) into the KA lesioned striata. The control group (n=7) received vehicle only. Abnormal locomotor behavior was observed in rats following the KA lesions. 5 weeks following striatal transplants, hyperlocomotor activity was reduced. 5 weeks following transplants of cultured astrocytes, abnormal rearing behavior was reduced. However, horizontal locomotor activity measures were increased after the astrocyte transplantation. Therefore, the astrocyte transplant produced differential effects on various locomotor measures. These effects of astrocyte transplants are dissimilar to the effects of fetal striatal transplants. Immunocytochemical analysis of the astrocyte transplant sites revealed heavy GFAP, OX-42 and laminin staining in the transplant areas. However, further study is necessary to firmly distinguish transplanted astrocytes from host reactive astrocytes.

359.8

GABA RELEASE IN THE RAT STRIATUM: A COMPARISON BETWEEN INTACT, LESIONED AND GRAFTED STRIATA USING INTRACEREBRAL MICRODIALYSIS. K. Campbell, C. Lundberg*, P. Kalén*, K. Wictorin*, R.M. Mandel and A. Björklund. Dept. Med. Cell Res., U of Lund, S-22362 Lund, Sweden.

In order to characterize GABA release from intrastriatal fetal striatal grafts intracerebral microdialysis coupled to high performance liquid chromatography (HPLC) with electrochemical detection was performed on intact, excitotoxic lesioned (ibotenate 14-18 µg) and grafted (cell suspensions of fetal striatal tissue, 7-10 d post-lesion) striata. Loop type dialysis probes were implanted into either the intact, lesioned (7-10 d post-lesion) or grafted (3 mos post-transplantation) striatum at least 12 hrs before experimental manipulations. Dialysis probes were perfused at a rate of 2 µl/min and samples collected every 15-20 mins.

Excitotoxic lesions of the striatum resulted in a 75% reduction of basal GABA levels (116 fmol/20 µl dialysate) when compared to its intact counterpart (469 fmol/20 µl). The addition of KCl (100 mM) to the perfusion fluid produced an increase from the baseline greater than 55-fold in the intact striatum, which was reduced by more than 80% when Ca++ was replaced by Mg++ (20 mM) and by at least 50% in the presence of 1 µM TTX. The KCl response was further reduced in the lesioned striatum amounting to about 3% of the intact striatum. On the second day of experimental manipulations the addition of nipecotic acid (Nip)(0.5 mM) resulted in a 4-fold increase from basal levels in the intact striatum again reduced in the lesioned striatum by 71%. Preliminary results from grafted animals indicate a normalization of the baseline (approx. 385 fmol/20 µl) and the KCl response (108-fold increase from basal levels). The Ca++ and TTX dependency of GABA release from striatal grafts is currently under investigation as well as a comparison between GABA release in the transplant and chronic lesions (3 mos post-lesion).

These results indicate that both basal and KCl-induced levels of extracellular striatal GABA are predominantly of neuronal origin (as much as 75 and 97%, respectively) and that intrastriatal transplants of fetal striatal tissue may completely or at least partially restore GABA release in neuronally depleted striata.

359.9

MESENCEPHALIC FETAL GRAFTS RECOVER MOTOR ACTIVITY BUT NOT INHIBITORY AVOIDANCE IN STRIATAL LESIONED RATS. A.L. Piña, C. E. Ormsby*, H. M. Corzo* and F. Bermúdez-Rattoni Instituto de Fisiología Celular, UNAM. México, D.F.

Studies in our laboratory have demonstrated that striatal but not mesencephalic fetal tissue grafts, promote recovery in the ability to acquire an inhibitory avoidance (IA) task in striatal lesioned rats. In the present study, we evaluated the effects of different post-mesencephalic graft times on the recovery of IA and motor activity (MA). One group of Wistar male rats was lesioned in the dorsal striatum and another remained as unoperated control. After eight days the groups were trained for IA task and measured for an "open field" (MA) test. One week later the lesioned group was divided into two groups, receiving mesencephalic grafts (15E). The groups were retrained and tested at different recovery times post grafts (15, 30 days). The brain tissues were analyzed for AChE-histochemistry and immunocytochemistry for choline-acetyltransferase (ChAT) and Tirosine hydroxilase (TH). Results 15 days post-graft showed that animals did not recover neither IA nor MA. However, tested 30 days post-graft the animals restored motor activity. Histological analyses showed that mesencephalic grafts have scarce AChE fibers and somas and strong TH immunoreactivity for somas, and a negative ChAT reactivity. These results suggest that specific tissue content is needed for differential recovery of motor activity and learning tasks.

359.10

TRANSPLANTATION OF EMBRYONIC OR ADULT EGF-GENERATED MOUSE NEUROSPHERES INTO ADULT RATS WITH CORTICAL OR STRIATAL LESIONS. S. Weiss, B.A. Reynolds, W. Tetzlaff, B. Kolb and I.Q. Whishaw. Neuroscience Research Group, Univ. of Calgary, Calgary, AB, Canada and Dept. of Psychology, Univ. of Lethbridge, Lethbridge, AB, Canada.

We have identified an EGF-responsive progenitor cell in the embryonic and adult mouse CNS, which will proliferate in vitro into a sphere (neurosphere) of undifferentiated cells that detach from the substrate after 5-7 days in vitro (DIV). At this stage, all the cells in the neurosphere contained nestin-like immunoreactivity, characteristic of undifferentiated neuroepithelial cells; these cells were negative for neuronal (NF, NSE) or glial (GFAP) cell markers. Two groups of rats received surgical lesions of the cortex or ibotenate lesions of the striatum. One week post-lesion, embryonic mouse cortical and embryonic or adult mouse striatal neurospheres were transplanted into the cortex and striatum, respectively, of the lesioned rats. Monoclonal antibody (M6), directed against a cell surface antigen located on mouse (but not rat) neurons, was used to identify mouse neurons in their rat hosts. Four weeks post-transplantation, M6-immunoreactive cells and fibres were detected in the lesion sites, but not in non-lesioned/non-transplanted regions of the rat CNS. These findings suggest that EGF-generated CNS progenitor cells differentiate into neurons when transplanted into lesioned adult CNS.

Supported by the Medical Research Council of Canada.

359.11

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF EMBRYONAL CARCINOMA-DERIVED NEURONS GRAFTED INTO LESIONED ADULT RAT STRIATUM. D. S. K. Magnuson, D. J. Morasutti, W. A. Staines, M. W. McBurney and K. C. Marshall. Depts. of Physiology, Medicine and Anatomy, University of Ottawa, Ottawa, Canada. K1H 8M5.

Multipotential P19 embryonal carcinoma (EC) cells terminally differentiate into neurons, glia, and smooth muscle cells following induction by Retinoic acid (RA). Neurons from RA-induced EC-cells (in vitro) have previously been shown to form synapses, and to possess neurotransmitter and morphological properties reminiscent of those found in rodent forebrain neurons. In this study we have characterized the development of voltage-sensitive and neurotransmitter responses in EC-derived neurons in vivo.

Intracellular recordings of RA-induced EC-cells grafted into ibotenic acid lesioned adult rat striata were made within a 400µm in vitro brain slice. Cells recorded from 5-7d. old grafts had very high membrane input resistances (500MΩ, Rin), were not capable of maintaining a resting potential (RMP), and displayed very little rectification. The majority of cells in grafts 8-30d. old displayed rectification during depolarizing current injection, were able to maintain RMPs, had progressively decreasing Rin's (down to150MΩ) and produced fast TTX-sensitive spikes. In younger grafts, spikes were small in amplitude, often more than 5msec in duration with negligible after-hyperpolarizations (AHPs). By 21-30days, most cells had progressed to produce "mature" action potentials of less than 2msec duration, overshooting 0mV in height, with large 10-20mV after-hyperpolarizations. Many cells in grafts older than 21 days displayed rectification during hyperpolarizing current injections. Sensitivity to L-glutamate and GABA was well developed in 8-30d. old grafts. Supported by the Canadian Centre of Excellence in Neural Regeneration and Functional Recovery.

GENE STRUCTURE AND FUNCTION II

360.1

AXONAL TRANSPORT OF PEPTIDE HORMONE ENCODING mRNAs IN THE HYPOTHALAMO-NEUROHYPOPHYSEAL TRACT OF THE RAT. E. Mohr*, S. Fehr* and D. Richter. Institut für Zellbiochemie und klinische Neurobiologie, Universität Hamburg, 2000 Hamburg, Germany.

Genes encoding vasopressin (VP) and oxytocin (OT) are highly expressed in hypothalamic magnocellular neurons which project to the posterior pituitary. The recent finding of VP and OT mRNAs in the neural lobe raises the question whether these transcripts are locally synthesized in pituicytes or, alternatively, axonally transported from the perikarya of magnocellular neurons to the nerve terminals. Several lines of evidence support the latter concept. 1. Northern blot and *in situ* hybridization analyses indicate that VP and OT mRNAs are also present in the neural stalk which connects the hypothalamus and posterior pituitary. 2. VP primary transcripts are absent in posterior pituitary cells as documented by RNase protection experiments using a labelled probe complementary to intron I as well as by polymerase chain reaction amplification using intron I and II sequences as forward and reverse primers, respectively. These sensitive assays detect respective primary transcripts in magnocellular neurons of the hypothalamus. 3. Nuclear run on experiments performed with isolated posterior pituitary cell nuclei failed to detect VP and OT mRNAs but not ß actin mRNA.

360.2

IDENTIFICATION OF MULTIPLE MEMBERS OF THE NEUROTRANSMITTER TRANSPORTER GENE FAMILY

M.M.Peek*[1], R.T. Fremeau, Jr[2], M.G.Caron[2], and R.D.Blakely[1]. Dept. of Anatomy and Cell Biology, Emory University School of Medicine[1], Atlanta GA, 30322, and Depts. of Neurobiology and Cell Biology, Duke University Medical Center[2], Durham NC 27110.

The cloning of a human Na+/norepinephrine transporter (NET) revealed significant amino acid identity between this carrier and the cloned rodent and human Na+/GABA transporters, suggesting the presence of a large family of related genes encoding other neurotransmitter reuptake proteins. To explore this possibility, we have utilized the polymerase chain reaction (PCR) with degenerate oligonucleotides synthesized from highly conserved regions of NET and GABA carriers to identify novel transporter sequences in rodent and human cDNA. These studies have identified 8 related species, each bearing significant amino acid identity with NET and GABA transporters. These fragments have been subsequently used as probes in high-stringency Northern and *in situ* hybridization experiments to determine both the size and cellular expression of endogenous mRNAs in the rodent CNS. Several clones selectively label neuronal populations with well characterized transmitter phenotypes. One clone selectively identifies soma in the serotonergic dorsal and median raphe, and thus is a strong candidate for the serotonin transporter. A second species exhibits a highly discrete expression pattern in the substantia nigra and ventral tegmental area, corresponding to the anticipated localization of dopamine transporter mRNA. A third clone, which hybridizes to a single 4kB mRNA on Northern blots, is expressed in cortical and hippocampal pyramidal cell layers as well as in the mitral cells of the olfactory bulb, consistent with the distribution of neuronal L-glutamate transporter mRNA. Other fragments hybridize with a more uniform and widespread cellular distribution, suggesting roles linked to intermediary metabolism and/or glial transport. Candidate clones corresponding to these initial isolates have been isolated from cDNA libraries and are presently being investigated. These data reveal the presence of multiple, related members of the neurotransmitter transporter gene family and provide new opportunities for the analysis of presynaptic regulation.

360.3

Cloning and characterization of the promoter region and gene encoding FLRFamide-like peptides in *C. elegans*. M. Rosoff and C. Li. Dept. of Biology, Boston University, Boston, MA 02215.

Approximately thirty neurons in *C. elegans* stain with an antibody specific for the RFamide moeity of the neuropeptide FMRFamide (Neurosci. Abst. 12:246). A cDNA clone whose putative translation product encodes eight potential neuropeptides was previously isolated (Neurosci. Abst. 16:305). Primer extension analysis shows that this cDNA is not full length. We are trying to isolate a full length clone from different cDNA libraries.

A λ1059 genomic library (courtesy of S. Emmons) was screened using the cDNA as a probe. Thirteen hybridizing clones were isolated and grouped into four overlapping classes by restriction mapping. A 3 kbp EcoRI fragment was subcloned from two separate overlapping lambda clones and sequenced. This fragment contains 300 bp upstream of the cDNA, the sequence corresponding to the entire cDNA, and a poly adenylation signal. We have recently isolated an additional 1.1 kbp genomic fragment upstream of the cDNA sequence. Constructs are being made to analyze the promoter region using the beta-galactosidase gene as a reporter in transgenic animals.

Concurrently, we have begun the purification of FMRFamide-like peptides from acetone extracts of mixed stage animals. Radioimmunoassays show the presence of FMRFamide-like peptides, and further characterization of the extracts by reverse phase HPLC reveals at least five peaks of FMRFamide-like immunoreactivity.

360.4

CLONING OF THE GENE ENCODING THE MITOCHONDRIAL BENZODIAZEPINE RECEPTOR. S.O. Casalotti, G. Pelaia*, D.R. Grayson, and K.E. Krueger. Fidia-Georgetown Institute for the Neurosciences, Georgetown University School of Medicine, Washington, D.C. 20007.

The mitochondrial benzodiazepine receptor (MBR) has been previously demonstrated to participate in steroid biosynthesis where an 18 kilodalton protein is responsible for the drug binding properties. The gene for this protein was isolated from a rat genomic library with the aim to correlate gene structure with functional domains and transcriptional regulation of MBR. Using the full length cDNA probe encoding the receptor mRNA six independent clones were identified, all of which showed identical internal restriction patterns with different amounts of flanking DNA indicating that each clone was derived from the same gene. Southern analysis of restriction digested rat genomic DNA confirmed the presence of a single copy of MBR per haploid genome. The sequence encoding the MBR protein is comprised of three exons where the two introns interrupt the coding sequence within potential transmembrane-spanning segments of the receptor. Approximately 3 kb has been sequenced including the flanking regions and introns. The putative 5' upstream sequence contains a CAAT box consensus sequence but other regulatory elements have not yet been identified.

360.5

CYCLIC AMP DEPENDENT REGULATION OF PROENKEPHALIN BY THE AP-1 RELATED PROTEIN JUN-D. L.A. Kobierski, H-M Chu, Y. Tan* and M.J. Comb*. Lab. of Molecular Neurobiology, Mass. General Hospital, Harvard Med. School, Charlestown, MA 02114.

Regulation of the opioid precursor, proenkephalin, by neural activity and intracellular signaling pathways is well documented and provides a model in the nervous system for exploring the molecular mechanisms of transsynaptic gene regulation. This gene is regulated by cAMP, phorbol ester and Ca++-dependent second messenger pathways through an enhancer consisting of two elements called CRE-1 and CRE-2. In a C6-glioma cell line (C6-D2) stimulated with forskolin and IBMX mRNA encoding endogenous proenkephalin and a proenkephalin/chloramphenicol acetyl transferase (CAT) gene, pENKAT-12, are rapidly activated. Experiments with the protein synthesis inhibitors anisomycin and cyclohexamide suggest that this activation is independent of new protein synthesis. To identify transcription factors that may mediate this effect we have cotransfected pENKAT-12 with plasmids that constitutively express different Jun or Fos-related proteins and a plasmid that produces the catalytic subunit of protein kinase A (cPKA) into F9 and C6-glioma cell lines. By measuring CAT activity we have determined that Jun-D activates expression from pENKAT-12 10-30 fold in a cPKA-dependent manner and that activation is dependent on the CRE-2 element. Gel mobility shift assays reveal that Jun-D specifically binds a probe containing the CRE-1 and CRE-2 elements. In addition we show that in C6-D2 cells Jun-D mRNA is pre-existing and not significantly stimulated by forskolin treatment. These data support a role for Jun-D as a pre-existing factor in cells that can mediate the rapid PKA-dependent activation of the proenkephalin gene.

360.6

Nerve Stimulation and Denervation Induce Differential Patterns of Immediate Early Gene mRNA Expression in Mouse Skeletal Muscle. S.R. Abu-Shakra, D.B. Drachman, A.J. Cole, J.M. Baraban & P.F. Worley. Dept. of Neurology, and Neuroscience, Johns Hopkins University, Baltimore MD 21205.

Many properties of skeletal muscle cells are closely regulated by motor nerves. For example, the acetylcholine receptor number, distribution and subunit composition is dependent on the state of neuromuscular synaptic transmission. However, little is known about the early regulatory events that occur in mature muscle cells in response to neuromuscular stimulation or denervation. We have examined the effects of motor nerve stimulation and denervation on the expression of 4 immediate early genes (IEGs):c-jun, jun-B, zif268, and nur77. Electrical stimulation of the sciatic nerve in a pattern that mimicked brisk intermittent exercise induced a marked rise in zif268 and c-jun mRNA levels within 30-45 minutes, while jun-B and nur77 mRNA levels remained unchanged. By contrast, surgical denervation resulted in a marked increase of nur77 and c-jun, a slight but definite rise in jun-B, and no consistent change in zif268 mRNA levels. Thus, neural stimulation and denervation lead to differential patterns of IEG expression. The selectivity of these patterns suggests that IEG expression may play an important role in regulating specific phenotypic changes in skeletal muscles that result from denervation, innervation, and various patterns of stimulation.

360.7

C-FOS AND C-JUN EXPRESSION IN THE RAT SUPERIOR CERVICAL GANGLION (SCG). J. Koistinaho[1], M. Pelto-Huikko[2], Åke Dagerlind[3], R. Roivainen[1] and T. Hökfelt[3]. Depts of [1]Public Health and [2]Biomedical Sciences, University of Tampere, 33101 Tampere, Finland and [3]Department of Histology and Neurobiology, Karolinska Institute,S10401 Stockholm, Sweden.

The expression of immediate-early genes c-fos and c-jun was studied in the rat SCG using in situ hybridization and immunohistochemistry. In the control rats, low levels of c-fos and c-jun mRNAs were present in the SCG, but colchicine treatment enhanced the labelling. Immunohistochemistry also revealed an increased number of neurons displaying Fos-like immunoreactivity (Fos-IR) after colchicine treatment. After nicotine injection (2 mg/kg) the levels of both c-fos and c-jun mRNA were rapidly increased (after 10-15 min) and remained elevated upto 60 min. Two hours after the nicotine injection 70-80% of the neurons showed Fos-IR and most of the neurons remained Fos-IR still at 5h after the injection. A dramatic increase in the expression of these genes was also observed two days after decentralization. Although immunohistochemistry showed some enhancement in Fos-IR of the neurons, the most intense immunolabelling was localized in satellite cells, which remained weakly Fos-positive even at 8 d postoperation. The finding that all these stimulations of the SCG increased also the level of tyrosine hydroxylase mRNA suggests that a higher functional activity is associated with the expression of c-fos and c-jun in the rat sympathetic neurons.

360.8

KAINATE-INDUCED INCREASES IN AP1 BINDING AND FRA IMMUNOREACTIVITY IN THE RAT HIPPOCAMPUS. **K. Pennypacker, L. Thai, R. Fannin*, and J.S.Hong. LMIN/NIEHS/NIH RTP, NC 27709.** **Kainate, a glutamate receptor agonist, causes seizures and induces the expression of c-fos and related antigens (FRA) in the rat hippocampus. Kainate treatment also increases the mRNA of dynorphin 3 hours after administration and enkephalin 24 hours after administration in the rat hippocampus, suggesting that FRA's binding to the AP1 element may enhance the expression of these neuropeptides. With the use of FRA-specific antibodies and AP1 DNA-binding studies, we have examined the relationships between the expression of FRA's and neuropeptides from 1.5 to 6.5 hours after kainate administration. Antibodies against the FRA's detected several proteins at the molecular weights of 60-70, 42, 40 and 35 KD. Weak FRA immunoreactivity was exhibited at 1.5 hours, while strongest immunoreactivity was observed at 6.5 hours after treatment, suggesting FRA expression was still increasing. Gelshift binding assays revealed that AP1 DNA binding increased with a time course identical to FRA immunoreactivity. In light of the extended time course required, the FRA's do not seem to be directly involved in dynorphin regulation, but still may be linked to enkephalin expression. Longer timepoints as well as DNA footprinting and AP1 crosslinking are being used to further examine whether neuropeptide expression is regulated by the FRA's.**

360.9

A PROTEIN COMPLEX DIFFERING FROM THE FOS/JUN COMPLEX BINDS AT AN AP-1 VARIANT SEQUENCE IN THE DYNORPHIN PROMOTER AND IS INDUCED IN SPINAL CORD BY PERIPHERAL INFLAMMATION. M.J. Iadarola, G. Mojdehi*, J. Gu*, C.L. Yeung*, D. Levens#* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR and #Laboratory of Pathology, NCI, NIH, Bethesda, MD 20892.

Previous studies demonstrating a co-expression of Fos protein and dynorphin in rat dorsal spinal cord after induction by peripheral inflammation suggest a possible role for Fos in dynorphin gene expression. However, the dynorphin promoter does not contain an AP-1 consensus sequence. TGAGTCA, which acts as a binding site for the Fos/Jun heterodimer. Using a 24 bp oligomer from the dynorphin promoter, we find that a protein-DNA complex forms between the variant dynorphin AP-1-like (DAP) sequence (TGCGTCA) and nuclear extracts of spinal cord or brain. The DAP complex appears to be composed of proteins in a unique combination since it was mostly resistant to further size modification or disruption when an anti-Fos antibody was added to a gel mobility shift assay. In contrast, an oligonucleotide containing an AP-1 consensus sequence from the Gibbon Ape Leukemia Virus enhancer (GALV) showed a complete further retardation when the anti-Fos antibody was added to the gel shift assay and was disrupted with an excess of antibody. Competition experiments using labeled GALV and labeled DAP sequence indicated that GALV produced a shallow displacement curve and was ~ 6-fold less effective than DAP in competing the variant complex when labeled DAP was the probe and vice versa, the DAP sequence was ~ 6-fold less effective when competing labeled GALV. These observations suggest that the DAP complex contains a protein that is distinct from Fos or Fos-related antigens. DAP complex formation was rapidly induced in spinal cord nuclear extracts from rats with an experimental peripheral inflammation. This treatment up-regulates expression of the genes for prodynorphin, proenkephalin and c-fos, suggesting a role for the variant complex in regulation of gene expression in spinal cord neurons.

360.10

cAMP DEPENDANT REGULATION OF THE cAMP-REGULATED ENHANCER CRE BY THE TRANSCRIPTION FACTOR ATF-1. R.P. Rehfuss*, K.M. Walton and R.H. Goodman*. Vollum Institute, Oregon Health Sciences University, Portland, OR, 97201.

Since the original isolation of the cAMP-dependent transcription factor CREB, several other factors have been cloned that are also able to bind to the cyclic AMP regulated enhancer (5'TGACGTCA3'). One of these factors, ATF-1, is approximately 70% identical to the CREB protein overall and is nearly 92% identical in the DNA binding and leucine zipper domains. Thus, ATF-1 and CREB may bind to the same target genes and probably can form heterodimers with each other. We have recently isolated both a full length ATF-1 clone and an alternatively spliced variant of ATF-1, termed ΔATF-1. Both ATF-1 and ΔATF-1 are present in a variety of tissues and cell lines and are capable of binding to the somatostatin CRE. Both ATF-1 and ΔATF-1 contain phosphorylation sites for protein kinase A and a domain, designated PDE 1, that can be phosphorylated by casein kinase II in vitro and appears to be essential for PK-A mediated transcriptional activation of CRE-containing genes. The ΔATF-1 isoform is missing a second domain, designated PDE 2, that is also required for CREB function. Transient transfection studies in F9 teratocarcinoma cells indicate that ATF-1 is as active as CREB in mediating cAMP-dependent transcriptional activation of a somatostatin CRE-CAT reporter gene. The ΔATF-1 isoform does not appear to respond to PK-A. We conclude that 1) CREB is not the only CRE-binding factor that can mediate transcriptional responsiveness to PK-A, 2) alternative RNA splicing can generate transcriptional factor isoforms with vastly different activities, 3) CREB, ATF-1, and ΔATF-1 are co-expressed in many tissues and cell lines, 4) the PDE 2 domains of CREB and ATF-1 are essential for PK-A mediated activity of these factors. We propose that ΔATF-1 may modulate ATF-1 and CREB activity.

361.1

DEVELOPMENTAL CHANGES IN *IN VIVO* RELEASE OF β-ENDORPHIN (β-END) FROM THE STALK-MEDIAN EMINENCE (S-ME) IN FEMALE RHESUS MONKEYS. E. Terasawa and S. Chongthammakun. Wisconsin Regional Primate Research Center, Univ. of Wisc., Madison, WI 53715.

Opiate peptides have been postulated to play a role in the control of LHRH release. In the present study, we have measured developmental changes in β-END release from the S-ME of female rhesus monkeys at prepubertal (n = 10), early pubertal (n = 8), and midpubertal (n = 11) ages using the push-pull perfusion method. Effects of ovariectomy (OVX) and effects of estradiol were also tested. β-END and LHRH in perfusates from the S-ME were measured by RIA. Results: 1) There were clear developmental increases in β-END release. β-END release in ovarian intact prepubertal monkeys was much smaller ($p<0.01$) than those in early pubertal and midpubertal monkeys. 2) This developmental increase in β-END was significantly correlated ($p < 0.01$) with pubertal increase in LHRH release. 3) However, the developmental increase in β-END occurred independent of estrogen. OVX did not alter β-END levels in monkeys at any developmental stage. In contrast, OVX resulted in the elevation of LHRH release in early and midpubertal monkeys, but not in prepubertal monkeys. 4) Similarly, while estrogen injection into OVX monkeys at the early and midpubertal stages resulted in suppression of LHRH release, estrogen did not cause any significant effects on β-END release. Estrogen suppressed neither LHRH nor β-END release in prepubertal monkeys. In summary: 1) since the increase in β-END release occurs with the increase in LHRH release during puberty, low levels of LHRH release in prepubertal monkeys are not likely due to tonic inhibition by β-END neurons and 2) the negative feedback effects of estrogen on LHRH release in pubertal monkeys are not mediated by β-END. It is concluded that β-END does not appear to be involved in the onset of puberty. (Supported by NIH grants 11355, 15433, & RR00167).

361.2

KEOXIFENE BLOCKS THE PERIPUBERTAL STIMULATION OF GALANIN GENE EXPRESSION IN FEMALE RATS. S.M. Gabriel and L.M. Kaplan, Dept. Psychiatry, Mount Sinai School of Medicine, New York, NY 10029 and Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA 02114.

Galanin is a regulatory peptide widespread in neuroendocrine tissues. In the anterior pituitary of the rat, galanin gene expression is potently enhanced by circulating estrogens. Sex differences in galanin immunoreactivity in the anterior pituitary and median eminence become apparent at puberty, coincident with elevated estrogen levels in females. We used northern blot hybridization to examine rat galanin mRNA in the hypothalamus and pituitary during the peripubertal period in rats. The induction of precocious puberty in 30 day-old female rats with pregnant mare serum gonadotropin *(PMSG, 10 IU, s.c., 1000h)* increased pituitary galanin mRNA more than ten-fold. This increase in galanin gene expression was blunted approximately 50% by simultaneous treatment with keoxifene *(LY156758, 100μg, twice daily, s.c.)*, a potent estrogen antagonist. Because our previous studies suggested little effect of estrogen on galanin gene expression in the whole hypothalamus, we examined hypothalamic fragments containing medial basal hypothalamus (MBH), peraventricular area (PVA), supraoptic area (SOA) and preoptic area (POA). Galanin gene expression in the PVA of 35 day-old female rats was two-fold higher than in males. In the immature female, galanin gene expression in the PVA increased significantly after treatment with PMSG. No sex differences or effect of PMSG were detected in the MBH, SOA, or POA. These studies indicate that estrogen stimulates galanin gene expression in a subset of hypothalamic neurons located in the PVA. Moreover, they demonstrate that the marked PMSG-induced increases in pituitary galanin gene expression are mediated by the induction of ovarian estrogen.

361.3

GALANIN NEURONS IN THE MONKEY ARCUATE REGION DO NOT CONTAIN OR INTERACT WITH GONADOTROPIN HORMONE RELEASING HORMONE NEURONS. P.C. Goldsmith, J.E. Boggan* and K.K. Thind. Reproductive Endocrinology Center, Univ. Calif., San Francisco, CA 94143.

Galanin (GAL), a 29 amino acid peptide named for its C-terminal glycine and N-terminal alanine, has been identified in neuronal subpopulations within the arcuate (ARC) nucleus of several species. In addition, GAL has been shown to affect gonadotropin secretion and to colocalize with gonadotropin-releasing hormone (GnRH) in rat preoptic area neurons. To investigate their interrelationship in primates, we examined GAL and GnRH coexistence and neuronal interactions in the hypothalamus of female cynomolgus monkeys. Neuroendocrine (NEU) neurons were retrogradely labeled by microinjection of tracer into the median eminence. After aldehyde perfusion, series of 40 um frontal vibratome sections at 500 um intervals were collected and immunostained for GAL or GnRH with PAP, or double immmunostained with PAP and colloidal gold. GAL fiber densities were highest in the anterior commissural nucleus and stria terminals, lower in the dorsal hypothalamic area, with trace amounts occurring in the medial preoptic area and periventricular nucleus. The 129 GAL neurons in a sample series of sections were confined to the posterior ARC (pARC) region. About 75% of these were NEU, similar to the 71% NEU GnRH neurons we previously reported in the ARC region (*J. Neuroendocrinol. 2:157, 1990*). Due to the presence of NEU GnRH neurons in this area, double immunostained sections of the pARC were serially thin sectioned and examined by electron microscopy. Colocalization of GAL and GnRH was not observed. GAL and GnRH elements came close together, but neither synapses, junctional specializations, nor interactions occurred. We conclude that GAL and GnRH neurons do not interact in the pARC region of the monkey hypothalamus, but might coexist or interact elsewhere, or affect gonadotropin secretion independently. (Supported by NIH HD10907).

361.4

HYPOTHALAMIC LESIONS THAT INDUCE FEMALE PRECOCIOUS PUBERTY ACTIVATE GLIAL EXPRESSION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR GENE: DIFFERENTIAL REGULATION OF ALTERNATIVELY SPLICED TRANSCRIPTS. M.P. JUNIER, D.F. HILL,* M.E. COSTA,* S.R. OJEDA. Div. Neurosci., OR Regional Primate Research Center, Beaverton, OR 97006.

We previously showed that lesions of the preoptic area-anterior hypothalamic area (POA-AHA) induce precocious puberty by activating the production of transforming growth factor alpha (TGFα) in reactive astrocytes surrounding the lesion site. TGFα was found to stimulate luteinizing hormone-releasing hormone (LHRH) release via interaction with EGF receptors (EGFR). In the present study, we determined whether expression of EGFR is affected by POA-AHA lesions. Using a cRNA probe complementary to the region where the EGFR mRNA diverges to originate an alternative transcript encoding a truncated and secreted form of EGFR, both transcripts were detected by ribonuclease protection assay in intact and lesioned POA-AHA. While levels of the intact EGFR increased 1.8-fold within a week after the lesion, no changes in the mRNA encoding the truncated form were detected. Immunoprecipitation of EGFR from hypothalamic solubilized homogenates followed by protein kinase-mediated autophosphorylation and SDS-PAGE showed that levels of the 170 kDa EGFR protein were also significantly increased by the lesion. EGFR mRNA and protein were localized by hybridization histochemistry and double labeling immunohistochemistry in a subset of reactive astrocytes around the site of the lesion. These results indicate that increased production of TGFα and up-regulation of EGFR expression in reactive astrocytes are concomitant events set in motion by POA-AHA injury. The increased availability of EGFR for ligand interaction in the face of unchanged expression of the truncated EGFR form may be critical for amplification of the TGFα stimulatory signal to occur. Supported by NIH Grants HD-25123 and RR-00163.

361.5

SEX DIFFERENCES IN SEASONAL REPRODUCTIVE TRANSITIONS IN SHEEP. R.J. Wood and D.L. Foster*. Reprod Sci Prog, Depts of Physiology, Obstetrics and Gynecology, and Biology, University of Michigan, Ann Arbor, MI 48109-0404.

Both the onset of puberty in the lamb and the annual resumption of reproductive activity in adult male and female sheep are characterized by increased secretion of LH due to reduced responsiveness to steroid inhibition. The mechanisms underlying these transitions are similar in males and females. However, the timing of puberty is sexually differentiated, for males undergo a reduction in sensitivity to steroid feedback at 10 weeks of age, whereas females remain highly responsive to steroid inhibition until 30 weeks (Claypool et al, *Endocrinology* 126:1206, 1990). This sex difference is determined by androgens *in utero* (Wood et al, *Endocrinology*, in press). The present study determined if a sex difference exists in the timing of seasonal transitions in adult males and females. We compared serum LH in gonadectomized, estradiol-treated males (n=8), females (n=6), and androgenized females (n=5) from blood samples collected twice weekly for one year. As determined by a sustained increase in LH to high levels, the onset and offset of reproductive activity in males, females and androgenized females was not different. Serum LH declined on February 1±4 days, and rose again on September 3±3 days. There was a transient increase in LH (May 20 to June 23) in males, but not in females or androgenized females. These data suggest that the timing of seasonal transitions in adult male and female sheep is generally similar, despite a marked sex difference in the timing of puberty. However, subtle sex differences in these adult seasonal transitions are not determined by androgens *in utero*. (Supported by USDA 89-37240-4561 & NIH-07048, -18258)

361.6

EXPRESSION OF cFOS IN GnRH CELLS OF THE EWE DURING INCREASED SECRETORY ACTIVITY. SM Moenter, FJ Karsch*, MN Lehman Reprod Sci Prog and Dept Physiol, Univ Michigan, Ann Arbor 48109; Dept Anat and Cell Biol, Univ Cincinnati, OH 45267.

The proto-oncogene cFos was used as a marker of neurosecretion to test the hypothesis that cFos expression in GnRH cells of the ewe is related to neurosecretory activity. GnRH and cFos expressing cells were identified and percent co-expression determined in 3 endocrine states: GnRH surge (high sustained release, n=8), ovariectomy (ovx, high pulsatile GnRH, n=6), luteal phase (low GnRH n=6). To induce the GnRH surge, a physiologic model for the estrous cycle was used; an estradiol rise to a late-follicular phase level was given after progesterone removal. Serum LH was measured as an indicator of GnRH release. Ewes were cranially perfused with 2% paraformaldehyde; nuclear cFos was immunostained using nickel-enhanced diaminobenzidine (DAB) (black) and GnRH with unenhanced DAB (brown). In the luteal phase, LH was basal; few cells expressed cFos; these were not GnRH cells. During the surge (sustained high GnRH release), 41±8% of GnRH cells expressed cFos. Despite high, but intermittent, release after ovx, GnRH cells did not express cFos. In addition to cFos in GnRH cells, many more non-GnRH cells expressed cFos during the surge than at other times, especially in the medial preoptic/anterior hypothalamic area and ventrolateral hypothalamus. We suggest cFos expression is increased by positive feedback (surge), whereas removal of negative feedback (ovx), has little effect despite increased GnRH release in both states. Our results are also consistent with the hypothesis that estradiol induces cFos sequentially in a chain of neurons, culminating with expression in GnRH cells. NIH-HD 18337 (FJK), HD 21968 (MNL)

361.7

ESTRADIOL RECEPTOR-IMMUNOREACTIVE CELLS EXPRESS cFOS DURING THE PREOVULATORY GnRH SURGE IN THE EWE. MN Lehman, SM Moenter, KZ Doll*, X Gu*, & FJ Karsch*. Dept Anat & Cell Biol, Univ Cincinnati, OH 45267; Reprod Sci Prog and Dept Physiol, Univ Michigan, Ann Arbor 48109.

Expression of cFos in the preoptic area and hypothalamus is increased during the estradiol-induced GnRH surge of the ewe, both within GnRH neurons and other preoptic and hypothalamic cells (Moenter et al., Neurosci. Abstr., 1991). Because the location of cFos-positive cells in surge animals overlaps with that of estradiol receptor-immunoreactive (ER-IR) cells, we tested the hypothesis that some cFos-positive cells might contain ER-IR. Ewes were cranially perfused during either the GnRH surge, the luteal phase, or shortly after ovariectomy (ovx) (see Moenter et al. abstract). A dual multiple-bridge immunofluorescent procedure was used to simultaneously visualize ER (monoclonal antibody H222) and cFos (polyclonal antibody, Oncogene Sciences Inc.). During the surge, approximately half of all cFos-positive cells of the medial preoptic/anterior hypothalamic area and ventrolateral hypothalamus also contained nuclear ER-IR. The same sections contained more ER-IR cells than cFos-positive cells; thus less than 50% of all ER-IR cells in these regions were cFos-positive. Similar total numbers of ER-IR cells were seen in ovx and luteal phase animals as in surge animals, but few if any ER-IR cells in ovx or luteal phase animals expressed cFos. The results indicate functional heterogenity among ER-IR cells, and suggest that estradiol may act either directly or indirectly upon a subset of ER-IR cells to induce cFos expression during positive feedback. NIH-HD 18337 (FJK), HD 21968 (MNL).

361.8

SERUM INHIBIN LEVELS DURING PHOTOPERIODICALLY CONTROLLED TESTICULAR REGRESSION IN THE GOLDEN HAMSTER. JD Kirby', AE Jetton, FW Turek and NB Schwartz. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

The golden hamster is a seasonally breeding species. The primary environmental cue regulating reproduction is photoperiod. Exposing male hamsters to short days (*e.g.*, 6:18 LD) results in testicular regression and spermatogenic quiescence. Transferring photically regressed males to long days leads to gonadal recrudescence. The importance of FSH in the initiation and maintenance of spermatogenesis in the hamster is well understood. However, the mechanisms underlying the seasonal changes in FSH secretion and gonadal function remain unresolved. Gonadal peptides, inhibin and activin, are known to be involved in the regulation of FSH secretion. To investigate what role inhibin might play in these photoperiodic changes, serum inhibin was measured by RIA throughout photically induced testicular regression. The antiserum used in this assay, kindly provided by Dr. C. Rivier and Dr. W. Vale of the Salk Institute, was raised against synthetic porcine inhibin alpha (1-26)-Gly-Tyr. Parallelism of hamster serum to the porcine standard was found. Two groups of male golden hamsters were transferred from 14:10 LD to 6:18 LD. The animals were bled by cardiac puncture under anesthesia and testes widths were measured on alternate weeks for 13 weeks. Serum inhibin levels and testes widths were analyzed by ANOVA. Testes widths and serum inhibin values were significantly lowered by 6 weeks after transfer from 14:10 LD to 6:18 LD (week 0, testes widths = 11.33 + 0.17 mm, inhibin = 2437.1 + 459.2 pg/ml; week 6, testes widths = 7.35 + 0.71 mm, inhibin = 658.8 + 91.7 pg/ml; $p<0.05$). This is the first use of inhibin as a variable in the study of photoperiodically induced changes within the hypothalamic-pituitary-gonadal axis in a rodent. These results demonstrate that daylength has a dramatic effect on circulating levels of inhibin. Supported by PHS P01 HD21921 and T32 HD07068.

361.9

SERUM INHIBIN LEVELS DURING SPONTANEOUS AND PHOTOPERIODICALLY STIMULATED GONADAL RECRUDESCENCE IN THE GOLDEN HAMSTER. AE Jetton, JD Kirby', NB Schwartz and FW Turek. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

Shifts in photoperiod control dramatic changes within the reproductive system of the golden hamster. Exposure to short daylengths causes testicular regression, and continued exposure to short daylengths eventually leads to photorefractoriness which is manifested as spontaneous recrudescence of the testes. Exposure of animals to long daylengths at any time following regression and prior to spontaneous recrudescence, can actively stimulate testicular recrudescence. Previous work in the golden hamster has shown that one of the initial changes within the hypothalamic-pituitary-gonadal axis in response to photostimulation is an early rise in serum FSH levels. To investigate what role inhibin, an FSH regulatory factor, might play in testicular recrudescence, serum inhibin was measured by RIA throughout spontaneous and photically stimulated testicular recrudescence. Four groups of males were exposed to 6:18 LD for 10 weeks; two groups remained on 6:18 LD for another 16 weeks and two groups were transferred to 14:10 LD for 14 weeks. The animals were bled by cardiac puncture under anesthesia and testes widths were measured on alternate weeks. Serum inhibin levels and testes widths were analyzed by ANOVA. Testes widths were significantly increased over 10 week values by 24 weeks of 6:18 LD ($p<0.05$); inhibin levels were not elevated by the end of the 15 week sampling period (25 weeks of 6:18 LD) ($p>0.05$). Testes widths were significantly increased by 3 weeks and inhibin levels were significantly increased by 12 weeks after transfer to 14:10 LD compared to 10 week 6:18 LD values ($p<0.05$). This is the first report of lowered circulating levels of inhibin during a time of known FSH stimulation; also, these studies indicate that a lack of circulating inhibin may play a permissive role in spontaneous gonadal recrudescence. Supported by PHS P01 HD21921.

361.10

USE OF cFOS AS A MARKER OF NEURONAL ACTIVATION REVEALS SEX DIFFERENCES IN RESPONSIVENESS TO NMA. M.S. Smith, W.-S. Lee, R. Abbud*, C.R.Pohl and G.E. Hoffman. Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

NMA stimulates LH secretion in both male and cycling female rats. To determine whether the patterns of neuronal activation in response to NMA are similar in females and males, we have used cFos expression as a marker of neuronal activation. NMA was administered systemically (iv, 40 mg/kg, 4 injections, 10 min apart) or intraventricularly (icv, 2 µg/2 µl, 4 injections, 10 min apart). In the females, similar results were observed in reponse to iv and icv injections of NMA; both stimulated LH secretion but did not induce cFos expression in LHRH neurons, although neurons surrounding LHRH cell bodies were activated. These same animals also had a high degree of cFos expression in the hippocampus and neocortex.

In the males, despite consistent stimulation of LH, the route of administration of NMA affected the pattern of neuronal activation. LHRH neurons expressed cFos in response to iv but not icv NMA treatment. As well, cFos expression in the hippocampus and neocortex was induced in response to iv but not icv NMA. Administration of pentobarbital before iv NMA blocked cFos expression in LHRH neurons and in the hippocampus and neocortex. The male appears to require sensory stimulation by NMA to activate the hippocampus and cortex. These results suggest that males and females may have different pathways sensitive to NMA, or that the steroid milieu may differentially alter the sensitivity to NMA stimulation. Supported by NIH grants HD14643 and HD12354 and the Samuel and Emma Winters Foundation.

361.11

HIPPOCAMPAL AND NEOCORTICAL REFRACTORINESS TO NMA STIMULATION IN THE LACTATING RAT AS REVEALED BY cFOS EXPRESSION: RECOVERY AFTER PUP REMOVAL AND BLOCKADE OF PROGESTERONE RECEPTORS. R. Abbud*, W.-S. Lee, G.E. Hoffman, and M.S. Smith. Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Lactating rats, unlike cycling rats, are refractory to NMA stimulation of LH secretion and cFos expression in the hippocampus and neocortex. To study the recovery of cFos activation in the hippocampus and neocortex in response to NMA, we examined lactating rats after pup removal, treatment with the progesterone antagonist, RU486 (5mg s.c. daily beginning on day 8 postpartum), or both. The responsiveness to four i.v. injections of NMA (40mg/kg) at 10 min intervals was examined during day 10 of lactation. LH responses and the degree of cFos expression in the postpartum groups were compared to the responses in diestrous rats. Control animals suckling 8 pups exhibited no LH stimulation or cFos expression in the hippocampus or neocortex in response to NMA. Treatment of the suckled animals with RU486 had no effect on the LH responses to NMA, but there was a significant degree of hippocampal and cortical activation, although still below diestrous levels. At 24 hr after pup removal, there was only a partial recovery of responsiveness to NMA stimulation. Full recovery was observed only in animals treated with RU486 which had their pups removed for 24 hours. In general, after pup removal, there was a good correlation between the recovery of the LH response to NMA and cFos expression in the hippocampus and neocortex. These results imply that the deficits in hippocampal and neocortical activation observed during lactation in response to NMA are mediated by the additive effects of suckling and progesterone. Support: HD14643 and the Samuel and Emma Winters Foundation.

361.12

GnRH INDUCES SLOW RHYTHMIC CHANGES OF MEMBRANE POTENTIAL IN RAT GONADOTROPES. Amy Tse & Bertil Hille. U of Washington, Seattle, WA 98195.

Dissociated pituitary gonadotropes identified by reverse hemolytic plaque assay are studied by whole-cell voltage clamp. Bath application of GnRH (>0.1 nM) induces a large, slowly oscillating current (frequency range: 0.06-0.3 Hz at 23°C), an effect that is competitively blocked by a specific GnRH antagonist. The oscillatory current is completely suppressed by apamin (>0.2 µM) and only half blocked by 5 mM TEA. The reversal potential shifts ~57 mV per 10-fold change of external [K]. With 5 mM BAPTA in the pipette, the GnRH-induced oscillations are largely suppressed. Hence GnRH is probably inducing oscillations of $[Ca^{2+}]_i$ which open SK K(Ca) channels in a rhythmic manner. The intermittent openings of K(Ca) channels leads to cyclic alternation of membrane hyperpolarizations (from -37.4±6.9 mV to -86.5±5 mV; S.D.; n = 16) and firing of action potentials. GnRH responses become irreversible with 100 µM internal GTPγS and abolished with 2 mM internal GDPβS. The response is mimicked by 20 µM internal IP_3 and abolished by heparin (1 mg/ml). Oscillations persist even in the absence of external Ca. Therefore, the intracellular pathway of this response involves the activation of a G protein which leads to increase in PI turnover and cyclic release of Ca^{2+} from an intracellular store. During the membrane oscillations, these cells also undergo exocytosis (presumably releasing gonadotropins) as evidenced by a rapid increase in membrane capacitance. (Supported by NS08174, HD12629 & the McKnight Foundation).

362.1

Vasoactive Intestinal Peptide (VIP) Enhances Protein Phosphorylation By Developing Sympathetic Neuroblasts.

Emanuel DiCicco-Bloom, Kuo Wu & Ira B. Black, Dept. Neurosci. & Cell Biol., UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ 08854

Recent studies suggest that neuropeptides play a regulatory role in early neuronal development. In particular, VIP has been found to stimulate mitosis, neurite outgrowth and survival in embryonic day 15.5 (E15.5) rat superior cervical ganglion (SCG) neuroblasts. Further, these diverse effects in vitro correlate with ontogenetic expression of VIP in SCG in vivo. Previous studies suggest that cAMP pathways are involved since peptide stimulates nucleoside levels and cAMP analogs reproduce VIP effects. The role of protein phosphorylation was examined in the present study.

To begin examining protein kinase activity, we employed a previously defined neuroblast culture system derived from E15.5 SCG. After 24h incubation, neuroblasts were exposed to VIP (10μM) or vehicle for 10min., in the presence of phosphodiesterase inhibitor, 500 μM IBMX. Following homogenization of cells in kinase buffer, samples were incubated with ^{32}P-ATP and protein kinase A substrate, histone H1, without and with cAMP for 3 min at 30° C and proteins were subjected to SDS-PAGE. Autoradiography revealed H1 phosphorylation in controls that was augmented by cAMP treatment, indicating ongoing and cAMP-responsive kinase activity in neuroblast homogenates. VIP exposure increased the H1 signal 2 to 5-fold. Moreover, addition of cAMP did not augment H1 phosphorylation in VIP-treated neuroblasts, suggesting that the peptide maximally increased phosphorylation. Our observations suggest that the multiple effects of VIP on mitosis, differentiation and survival involve a cascade of events including VIP ligand-receptor binding, cAMP induction and phosphorylation of endogenous protein substrates. (Support: NIH grant HD23315, Familial Dysautonomia and Juvenile Diabetes Fdns.)

362.2

Expression of Vasoactive Intestinal Peptide (VIP) And Receptor During Early Sympathetic Ontogeny: Potential Autocrine Role.

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While traditional models suggest that trophic agents are provided by targets, recent evidence suggests that neuronal generation and initial survival depends on local factors. Previously, we found that VIP stimulated mitosis, neurite outgrowth and survival in embryonic day 15.5 (E15.5) superior cervical ganglion (SCG) neurons. Moreover, peak expression of peptide in E15.5 SCG suggested that VIP of presynaptic or ganglionic origin plays a role in sympathetic ontogeny in vivo. The present data documenting expression of VIP and a putative receptor suggest an autocrine model.

To examine mechanisms, we employed a previously defined neuroblast culture system in which VIP stimulates mitosis (^{3}H-thymidine incorporation, ^{3}HdT Inc) and survival. To define receptor specificity, neuroblasts, grown in control or VIP (0.3μM)-containing medium, were exposed to a selective antagonist, [p-Chloro-D-Phe^6, Leu^{17}]-VIP (50μM). The antagonist completely blocked VIP-induced ^{3}HdT Inc, suggesting that the mitogenic signal was mediated via specific peptide receptors.

To characterize embryonic receptor, affinity cross-linking was performed. Mechanically dissociated SCG were incubated with ^{125}I VIP (10nM). Following cross-linking with bifunctional disuccinimidyl suberate (DSS), samples extracted with nonionic detergent were electrophoresed in 10% polyacrylamide gels. Autoradiography revealed a major 51 kD band not present in samples incubated with 1000-fold excess cold VIP or in the absence of DSS. Synaptic membranes from adult cerebral cortex exhibited a similar band, suggesting that E15.5 SCG and mature brain express a common VIP receptor.

To determine whether E15.5 SCG produce VIP, cultured ganglia were assayed for peptide content. After 4 days, when presynaptic terminals have degenerated, VIP increased 10-fold. While SCG production of VIP does not rule out presynaptic sources in vivo, the presence of peptide receptor and synthetic machinery in embryonic neurons supports an autocrine role for VIP in sympathetic ontogeny.

362.3

CNTF REGULATES NEUROBLAST MITOSIS, SURVIVAL, AND DIFFERENTIATION VIA MULTIPLE RESPONSE MECHANISMS.

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Neural ontogeny consists of an apparent sequence of events including proliferation, differentiation, and selective cell death. A panoply of signals regulate each of these processes; however, only a few factors are known to regulate neuronal development at all of these stages. One such molecule is Ciliary Neurotrophic Factor (CNTF), which has been shown to inhibit mitosis, enhance survival, and foster cholinergic transmitter differentiation in developing neurons. CNTF activities, however, have been examined in different cell types and animal species, hampering definition of underlying relationships. Using a rat sympathetic neuronal model, we have investigated CNTF bioactivities in a single population at various developmental stages.

We now report that CNTF (recombinant human, Synergen) inhibits neuronal mitosis in embryonic day 15.5 (E15.5) cultures in the presence of a number of previously defined mitogens including IGF, EGF, KCl and VIP. The factor decreases ^{3}H-thymidine incorporation as well as the percent of neuroblasts entering the cell cycle by ≥40%, with an EC_{50} of 10 pg/ml. In addition, CNTF promotes survival of E15.5 neurons independent of mitotic effects. However, the trophic effects require 100-fold greater doses (EC_{50}=1ng/ml), raising the possibility that CNTF simultaneously affects mitosis and survival via different receptors.

While CNTF does not stimulate cholinergic traits in the E15.5 cultures, choline acetyltransferase (CAT) activity is increased in cultures of neonatal sympathetic neurons. CAT-induction also requires the higher dose (EC_{50}=1ng/ml). In aggregate, our observations suggest that CNTF elicits diverse biological effects with differing dose-response relationships, suggesting multiple receptors and/or second messenger systems. Furthermore, the distinct ontogenetic actions of the factor may reflect selective developmental expression of different CNTF response mechanisms.

362.4

Characterization of receptors for BDNF and NT-3 in the central nervous system by affinity labeling

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Using heterologous cell lines expressing p145^{trkB}, the product of the trkB gene, we have shown that p145^{trkB} is a receptor for BDNF and NT-3 but not for NGF (Soppet et al. submitted). Incubation of p145^{trkB}-expressing cells with ^{125}I-labeled BDNF and NT-3, treated with the chemical crosslinking agent EDAC, and immunoprecipitated with specific anti-trkB antibodies reveals a single receptor complex of approximately 160 kDa. Viable cell suspensions were prepared from isolated rat hippocampus and subjected to the same affinity labeling and immunoprecipitation protocol. A crosslinked receptor complex of identical molecular size (160 kDa) was detected. However, if the immunoprecipitation step was omitted following crosslinking a more complex pattern of labeled products was revealed in the hippocampus. Three main receptor complexes of 160, 116 and 100 kDa, respectively, were found. Various brain regions showed discrete differences in the relative abundance of these complexes.

In addition, titration analysis studies suggest that the 160kDa species has a relatively higher affinity for both BDNF and NT-3 under the conditions of the crosslinking experiments. A developmental analysis indicates that BDNF and NT-3 binding activity reaches a peak of expression in early postnatal animals.

362.5

NEUROTROPHINS NGF, BDNF AND NT-3: ACTIONS ON RAT CHOLINERGIC AND DOPAMINERGIC NEURONS *IN VITRO* AND *IN VIVO*.

B. Knüsel, K.D. Beck, J.W. Winslow, L.E. Burton, K. Nikolics and F. Hefti. Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089 and Genentech, Inc., South San Francisco, CA 94080.

Brain-derived neurotrophic factor (BDNF) stimulates differentiation of brain cholinergic and dopaminergic neurons in culture, cells whose function, in the human brain, is reduced in Alzheimer's disease and Parkinson's disease, respectively. NGF is well known to affect the same basal forebrain cholinergic, but not the mesencephalic dopaminergic neurons. We now found that neurotrophin-3 (NT-3), though only at a concentration 1000 times higher than the required NGF concentration, stimulates cholinergic, but not dopaminergic differentiation in vitro. Additivity experiments, and comparison of the time course of stimulation, suggest action of NT-3, but not BDNF, on the NGF receptor. K-252b, a protein kinase inhibitor previously shown to inhibit NGF actions, abolished the effects of all three neurotrophins on cholinergic neurons and of BDNF on dopaminergic cells. In contrast, the actions of bFGF and IGF-I on these cells were not abolished, suggesting that K-252b selectively interferes with neurotrophin receptor mechanisms. We presently investigate if BDNF and NT-3, similar to NGF, increase cholinergic cell survival in the fimbria-fornix lesion model of cholinergic hypofunction.

362.6

EFFECTS OF BDNF AND NT-3 ON DOPAMINERGIC AND GABAERGIC NEURONS IN RAT VENTRAL MESENCEPHALIC CULTURES.

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We have examined the effect of neurotrophin-3 (NT-3) on the dopaminergic neurons of the ventral mesencephalon of E14 rat embryos in dissociated cell culture, under serum-free conditions. In addition, we have addressed the question of whether GABAergic neurons present in this system respond to BDNF or NT-3. Dopaminergic neuron viability was assessed by staining with antibodies to tyrosine hydroxylase (TH), and measurement of ^{3}H-dopamine (^{3}H-DA) uptake. Assay of ^{3}H-GABA uptake and measurement of glutamic acid decarboxylase (GAD) activity were used to monitor the survival and maturation of the GABAergic neurons. A single treatment with NT-3 at 10 ng/ml one day after placing the cells in culture resulted in a 1.8 fold increase in both the number of TH positive neurons and ^{3}H-DA uptake six days later. Cultures maintained for 7 days in the presence of BDNF showed a 2.7 fold increase in ^{3}H-GABA uptake activity when compared to control cultures. GAD activity in BDNF treated cells showed a 1.8 fold increase over that of control cultures. Similar experiments in cultures maintained in the presence or absence of NT-3 for 7 days, demonstrated an NT-3 induced 2.8 fold increase in ^{3}H-GABA uptake and a 2.3 fold rise in GAD activity. Thus, both BDNF and NT-3 affect dopaminergic and GABAergic neurons of the ventral mesencephalon.

362.7

BDNF mRNA EXPRESSION FOLLOWING DEAFFERENTATION OF THE HIPPOCAMPUS T. Denton, J. Day, M. Dugich-Djordjevic, P.A. Lapchak, C.E. Finch and F. Hefti University of Southern California, Andrus Gerontology Center, Dept. of Biological Sciences, Los Angeles, CA 90089

Brain Derived Neurotrophic Factor (BDNF) is a neurotrophic factor which has recently been cloned, and is available as a recombinant protein. BDNF has been shown, in vitro, to have trophic effects on the septal cholinergic neurons which degenerate in Alzheimer's Disease (AD). The highest concentration of BDNF mRNA in the brain is in the hippocampus, a target of the septal cholinergic projection. We are investigating changes in BDNF mRNA expression by in situ and Northern Blot hybridization in deafferenting lesions which model different aspects of the neurodegeneration which occurs in AD. Our results indicate that inhibition of the activity of the septal cholinergic pathway by either chronic administration of atropine (20 mg/kg/day for 10 days), or a partial sterotaxic knife lesion results in a significant down-regulation of BDNF expression (after 21 days). By contrast, a transection of the perforant pathway results in a marked increase of expression in the dentate, CA3 and CA4, with a peak at four days, followed by a return to control levels by fourteen days. These findings suggest a role for BDNF in the remodeling of the hippocampus in response to deafferenting lesions and by extension, in AD.

362.8

BDNF mRNA IS DECREASED IN HIPPOCAMPUS OF INDIVIDUALS WITH ALZHEIMER'S DISEASE H. S. Phillips, J. M. Hains, M. Armanini, G. R. Laramee*, [1]S. A. Johnson, and J. W. Winslow Dept. Dev. Biol., Genentech, S.S.F., CA 94080 and [1]Andrus Gerontology Ctr., USC, L.A. CA, 90089.

In recent years, the potential of nerve growth factor as a therapeutic agent for Alzheimer's disease (AD) has gained increasing attention. To compare expression of NGF and its homologs, BDNF and NT-3, in individuals with AD vs. non-demented control individuals, we conducted *in situ* hybridization on 15 samples of human postmortem hippocampus. Of the three members of the NGF family, only BDNF demonstrated a significant decrease in hybridization intensity in AD. Decreased BDNF hybridization in dentate gyrus was highly significant ($p<.001$), and could not be accounted for on the basis of differences in donor age or sex, sample autolysis times, or loss of cell density. BDNF hybridization was also significantly reduced in the pyramidal layer of Ammon's Horn. Normalization of neurotrophin hybridization signals to those obtained with a probe to NCAM revealed that BDNF/NCAM ratios decreased roughly two fold in dentate gyrus with AD, while NGF/NCAM and NT3/NCAM showed no trend towards decreasing in AD. Decreased abundance of BDNF mRNA in hippocampus of individuals with AD was verified in a second set of samples by ribonuclease protection assay. These results suggest the possibility that a deficiency in BDNF may contribute to the progression of cell death in AD.

362.9

BASIC FIBROBLAST GROWTH FACTOR MODULATION OF NEUROTRANSMITTER INFLUENCES IN CULTURED HIPPOCAMPAL NEURONS: POTENTIATION BY GANGLIOSIDE GM1. S.D. Skaper, A. Leon and L. Facci. Fidia Research Labs, 35031 Abano Terme, Italy.

Classical mitogenic growth factors like basic fibroblast growth factor (bFGF) can influence survival of CNS neurons. The ability of bFGF to affect responsiveness of hippocampal cells to the neurotransmitter glutamate was now studied. Cultures were generated from embryonic day 18 rat hippocampus, and contained mainly pyramidal neurons. After 4-5 days in a serum-free defined medium, glutamate ($\geq$ 100uM) became cytotoxic for these cells. Either 10ng/ml bFGF (Mattson et al., J. Neurosci. 9:3728, 1989) or 100uM ganglioside GM1 reduced this neuronal loss by 50-80% when added 24hr before glutamate. Low bFGF (0.3ng/ml) or GM1 (10uM) were ineffective. Combined incubation of these low levels of bFGF and GM1 for 24hr (but not 2hr) before glutamate addition raised the threshold of bFGF efficacy significantly. However, glutamate sensitivity of older ($\geq$ 2 weeks) cultures was unaffected by bFGF. Facilitation of trophic factor effects by gangliosides makes the latter compounds useful tools in the study of CNS plasticity and repair processes.

362.10

NUCLEAR AND CYTOPLASMIC LOCALIZATION OF BASIC FIBROBLAST GROWTH FACTOR IN ASTROCYTES AND CA2 HIPPOCAMPAL NEURONS. F.P. Eckenstein, W. R. Woodward, C. K. Meshul and R. Nishi, Oregon Health Sciences University, Portland, OR 97201

Fibroblast growth factors (FGFs) are known to stimulate mitogenesis in a variety of non-neuronal cell types and to support the survival *in vitro* of many neuronal cell types. The present study determined the distribution in the rat CNS of basic FGF (bFGF). Immunohistochemical analysis showed that bFGF-immunoreactivity was found predominantly in astrocytes throughout all regions of the CNS. In contrast, only a few neuronal populations were found to contain bFGF-immunoreactivity, most prominent among them, neurons in the CA2-area of hippocampus. This predominant localization of bFGF to astrocytes was confirmed by two other observations: 1) highly enriched cultures of astrocytes contained bFGF-immunoreactivity and -bioactivity, whereas highly enriched cultures of cerebral cortical neurons contained no detectable bFGF; and 2) neonatal rat cerebral cortex, which contains only a few differentiated astrocytes, also contained no detectable bFGF-immunoreactivity and only low amounts of bFGF-bioactivity. Light and electron microscopic analysis showed that bFGF immunoreactivity was present in the nucleus as well as the cytoplasm of astrocytes and CA2 neurons. Preparations of both nuclear and soluble fractions of brain extracts also contained bFGF-immunoreactivity and -bioactivity. These data suggest that bFGF might be involved in mediating astrocytic influences on the late postnatal maturation and plasticity in the CNS, and that the nuclear localization of bFGF within astrocytes may play an important role in the differentiation of these cells. In addition, bFGF may play a similar role in a few specific neuronal populations, such as CA2 hippocampal neurons.

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362.11

A CNTF-LIKE, CHAT AND VIP-INDUCING FACTOR IS PRESENT IN DEVELOPING RAT FOOTPAD. H.Rohrer*# and M.Sendtner. Max-Planck-Institute for Psychiatry, Dept. of Neurochemistry, 8033 Martinsried, and Max-Planck-Institute for Brain Research#, 6 Frankfurt/M. 71, FRG

The sympathetic neurons innervating sweat glands in the rat footpad acquire a cholinergic phenotype under the influence of the innervated target. Ciliary neurotrophic factor (CNTF) has been previously shown to stimulate *in vitro* the cholinergic differentiation of rat and chick sympathetic neurons.

We now demonstrate that a CNTF-like, ChAT and VIP-inducing factor is present in rat footpads during the period of target-dependent cholinergic differentiation. The CNTF-like factor increases about 6-fold between postnatal days 7 and 21 and is maintained at reduced levels in adult footpad. Antibodies against CNTF eliminate about 50% of ChAT-inducing activity in footpad homogenates. Thus a CNTF-like factor and an additional cholinergic factor(s), not recognized by an antiserum against CNTF, are candidates for inducing cholinergic properties *in vivo*. Immunohistological data and denervation experiments demonstrate, however, that the CNTF-like factor is localized in Schwann cells. These results suggest that the CNTF-like factor is a less likely candidate for the sweat gland-dependent cholinergic factor.

362.12

EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) AND EPIDERMAL GROWTH FACTOR (EGF) ON CELLS FROM DISSOCIATED CULTURES OF EMBRYONIC MIDBRAIN: A COMPARATIVE STUDY. D. Casper and M. Blum, Fishberg Research Center in Neurobiology, Mount Sinai School of Medicine, One Gustave Levy Place, New York, NY 10029.

EGF and bFGF are mitogens on epidermal and mesodermally derived tissue. It has also been shown that in the CNS each growth factor can be a glial mitogen, enhance neuronal survival, and stimulate neurite outgrowth in both separate and overlapping neuronal populations. We, and others have demonstrated EGF and bFGF to be mitogenic for astrocytes, and support the survival and maturation of dopaminergic neurons in cultured rat embryo mesencephalon. We also determined that bFGF was synthesized in the cultures by a nuclease protection assay for bFGF mRNA and by bFGF immunocytochemistry. In this study we investigate whether bFGF could mediate the neurotrophic and mitogenic effects of EGF, or whether their effects are distinct.

Dissociated cultures from 16 day rat embryo mesencephalon were established in defined medium. Dose response curves for EGF and bFGF were produced for cell proliferation by ^{3}H-thymidine incorporation into DNA and for dopamine neurons by ^{3}H-dopamine uptake. In order to block endogenous bFGF activity, antisense oligonucleotides to bFGF mRNA or neutralizing bFGF antibodies were added to control and EGF-treated cultures. Measurements of cell proliferation and dopamine uptake were made and FGF immunocytochemistry was performed. Preliminary results indicate that EGF and bFGF effects on dopamine uptake were additive.

Since mesencephalic cultures contain both the presumptive substantia nigra and ventral tegmental dopamine neurons which have distinct targets, it is possible that each cell population is differentially sensitive to growth factors. Alternatively, bFGF and EGF could act on overlapping populations of dopamine neurons by different mechanisms. This work has been supported by NRSA grant NS08600-02.

363.1

EFFECTS OF ANTI-β1 INTEGRIN ANTIBODIES ON AXONAL OUTGROWTH FROM EMBRYONIC RETINA *IN VITRO* AND *IN VIVO*. D.S. Sakaguchi and C.E. Holt. Program in Neurosci., Dept. Zool and Genetics, Iowa State Univ., Ames, IA 50011 and Dept. of Biol., UC San Diego, La Jolla, CA 92093.

The XR1 glial cell line, isolated from *Xenopus laevis* retinal neuroepithelium, deposits a proteinaceous extracellular matrix (ECM) with potent neurite outgrowth promoting activity. To investigate a potential role for the integrins as cellular receptors for these glial cell-derived ECM components, embryonic retinal explants were cultured in the presence of polyclonal antibodies (Abs) directed against the 140 kD chick β1 integrin subunit (from K. Yamada, NCI). The anti-β1 Abs strongly inhibited neurite outgrowth on the glial cell-derived ECM, although neurites grew freely across nonneuronal cells surrounding the explants. The Abs were also very effective at inhibiting outgrowth on purified laminin and ECL (entactin, collagen and laminin) substrates.

To examine the possible role of integrins *in vivo*, the presumptive optic tract on one side of the embryo was exposed to anti-β1 Abs through surgery or by direct injection of the Abs. Optic fibers from the contralateral eye followed a normal course through the Ab treated optic tract to the optic tectum revealing that the Ab does not block outgrowth. However, the Ab-treated projections tended to be delayed in their growth and to contain fewer fibers than preimmune Ab-treated controls suggesting that the anti-β1 Abs may subtly effect axon outgrowth *in vivo*, perhaps by acting on a subset of retinal axons. We conclude that while the integrins play a major role in mediating axon outgrowth on ECM substrates *in vitro*, other molecules in addition to integrins are probably important for outgrowth *in vivo*.

363.2

DEVELOPING SENSORY NEURONES HAVE DISTINCT GROWTH SUBSTRATUM REQUIREMENTS. T.E. Allsopp* and A.M. Davies, Dept. of Anatomy, St. George's Hospital Medical School, Tooting, London, SW17 ORE, U.K.

Sensory neurones of the vestibular (V), geniculate (G), petrosal (P) and nodose (N) cranial ganglia extend neurites at different rates *in vitro* (N>P>G>V) when cultured on EHS laminin. This relationship correlates with the *in vivo* growth characteristics and the respective ganglionic target distances; fastest growth occurs to the furthest targets. To initiate studies of how such growth rate differences could be regulated at the molecular level, growth promoting molecules present in the environment of the differentiating neurones were assessed for their ability to support axonal growth and reproduce the growth rate difference. Cells of all four ganglionic types express the axonal growth promoting glycoprotein G4 (L1/NILE/8D9) at the early stages of gangliogenesis (stage 17/18 Hamburger and Hamilton). Immunoaffinity isolated G4 fails to support the attachment and growth of vestibular neurones from stage 18 and 23 embryos, whereas many nodose neurones from the same stages attach and grow neurites on G4. Initiation of growth on G4 is delayed compared to growth on laminin. Initial analysis suggests that growth rate on G4 is slower than that on laminin, but is faster for the population of stage 23 compared with stage 18 neurones. These observations are consistent with the hypothesis that the cranial sensory neurones differ in their intrinsic regulation of axonal growth mechanisms. Also within a distinct neuronal population cells may use different growth strategies, in relation to their time of differentiation, to ensure that they can adequately compete for target derived trophic factors.

363.3

NEURITE OUTGROWTH ON IMMOBILIZED AXONIN-1 IS MEDIATED BY A HETEROPHILIC INTERACTION WITH L1(G4). T.B. Kuhn*, E.T. Stoeckli, M.A. Condrau*, F.G. Rathjen**, and P. Sonderegger. Biochemisches Institut und *Institut für Biomedizinische Technik, Universität Zürich, CH-8057 Zürich, Switzerland.**Zentrum für Molekulare Neurobiologie, D-2000 Hamburg,Germany.

The axon-associated cell adhesion molecule axonin-1 is expressed in two forms, one being glycophosphoinositol-anchored to the axonal membrane, and the other being released from the axons. When presented as a substratum for neuronal cultures, it strongly promotes neurite outgrowth. In this study, axonin-1 itself and L1(G4), which promotes neurite outgrowth in a similar fashion and is colocalized with axonin-1 in several nerve fiber tracts, were investigated with respect to a receptor function for axonin-1. Using fluorescent microspheres with covalently coupled axonin-1 or L1(G4) at their surface we showed that axonin-1 binds very specifically to L1(G4). At the sensitivity of this microspheres assay, axonin-1 does not interact with itself. Axonin-1-coated microspheres bound also to the axonal surface of cultured dorsal root ganglia neurons by interacting with L1(G4), as indicated by complete supression by monovalent anti-L1(G4) antibodies. The interaction between neuritic L1(G4) and immobilized axonin was found to mediate the promotion of neurite growth on axonin-1, as evidenced by virtually complete arrest of neurite outgrowth in the presence of anti-L1(G4) antibodies. Convincing evidence has recently been presented that neurite growth on L1(G4) is mediated by homophilic binding of neuritic L1(G4) (Lemmon, V., K.L. Farr, and C. Lagenaur. 1989. Neuron 2, 1597-1603). Thus, both L1(G4)- and axonin-1-expressing axons may serve as "substrate pathways" for the guidance of following axons expressing L1(G4) into their target area.

363.4

MESOSTRIATAL DOPAMINERGIC AXONS CONTAIN HIGH LEVELS OF NILE DURING EARLY DEVELOPMENT. C.W. Shults,+Δ T.A. Kimber,+* L. Alberti,°* and W.B. Stallcup.°* +Neurology Service, VA Med. Ctr. San Diego, CA 92161; ΔDept of Neurosci., UCSD, La Jolla, CA 92093; °La Jolla Cancer Res. Fndn., La Jolla, CA 92037

Our group has carried out a series of studies to identify specific cell types and molecules that control development of the mesostriatal dopaminergic (DA) system in the rat. In the present study fetal, postnatal, and adult brains were immunolabelled with both a murine monoclonal antibody that recognizes tyrosine hydroxylase (TH) and a rabbit polyclonal antiserum that recognizes the carboxyl terminus of NILE. At E14, 15, and 16, NILE-immunoreactive (NILE-IR) material outlined the surfaces of the TH-immunoreactive (TH-IR) neurons in the mesencephalon. At later ages, NILE-IR material could not be detected on the cell bodies of the DA neurons. At E14, TH-IR axons coursing from the mesencephalon rostrally were also NILE-IR. At ages E15, 16, and 18, the TH-IR axons had bundled into fascicles which contained high levels of NILE-IR material, but at later ages the amount of NILE-IR material on these axons was conspicuously reduced but still detectable. In the striatum of the adult rat NILE-IR material appeared to be diffusely distributed through the neuropil. Our observations suggest that NILE plays a role in defining the paths for growth of and fasciculation of mesostriatal DA axons.

363.5

GENETIC ANALYSIS OF GROWTH CONE GUIDANCE IN DROSOPHILA: FASCICLIN II FUNCTIONS AS A NEURONAL RECOGNITION MOLECULE. G. Grenningloh, J. Rehm*, and C.S. Goodman, Howard Hughes Medical Inst, Dept of Molecular and Cell Biology, U. of California, Berkeley, CA 94720

Fasciclin II, a neural cell adhesion molecule of the immunoglobulin superfamily, was previously characterized and cloned in grasshopper where it is expressed during embryogenesis on the surface of a subset of axon pathways including the MP1 fascicle (Harrelson and Goodman, 1988). Here we report on the cloning, characterization, and genetic analysis of the Drosophila fasciclin II homologue. Sequence analysis of cDNAs indicate that the gene generates multiple forms of the protein, including a PI-linked and a transmembrane form. Monoclonal and serum antibodies reveal that Drosophila fasciclin II is also expressed on the MP1 pathway. We have generated two classes of mutations in the *fas II* gene: a set of lethal, protein null alleles, and a set of viable, hypomorphic alleles. In *fas II* null mutant embryos, most axon pathways develop normally and the CNS displays no gross phenotype. However, the MP1 fascicle does not develop, the MP1 and vMP2 growth cones fail to recognize one another or other axons that normally join the MP1 pathway, and these growth cones stall and do not join any other neighboring pathway. Thus, fasciclin II functions as a neuronal recognition molecule for the MP1 axon pathway. The effects of removing this single recognition molecule are remarkably similar to the previously reported effects of removing single bundles of axons (e.g. papers by Raper, Bastiani, et al., 1983-1986), namely, in the absence of either the appropriate molecule or the appropriate pathway, individual growth cones do not extend along other neighboring axon pathways.

363.6

FASCICLIN 2 MEDIATES HOMOPHILIC ADHESION BETWEEN TRANSFECTED DROSOPHILA CELLS. A.L. Harrelson. Division of Biological Sciences, University of Missouri, Columbia, MO 65211.

Adhesion between cells is a critical process in the development of the nervous system. As growth cones grow out to their targets, they selectively fasciculate with specific axon bundles and cell surfaces in the local microenvironment. Fasciclin proteins are known to mediate some aspects of selective fasciculation in developing insect nervous system. The Fasciclin 2 (Fas2) protein is expressed on fasciculating longitudinal axons and is a member of the immunoglobulin gene superfamily. We constructed plasmid vectors which express full-length Fas2 protein in Drosophila S2 cells, under the control of a metallothionein-driven transcriptional promoter. The vector allows the expression of individual Fas2 domains in various combinations on the cell surface or secreted into the culture medium, or fused to different cytoplasmic domains.

Expression of Fas2 on the surface of normally non-adherent S2 cells causes a rapid, density-dependent increase in cell aggregation. Depending on the initial concentration of cells and the extent to which the promoter is induced, we observe increases in cell aggregates of between 2- to 50-fold. Monoclonal antibodies which bind to the immunoglobulin-related region of the protein show no effect on this aggregation. The role of the cytoplasmic domain and the specific extracellular domains which are necessary for adhesion are presently being investigated.

363.7

A ROLE FOR GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED MEMBRANE PROTEINS IN GUIDANCE OF PIONEER NEURON AXONS *IN VIVO*. Wesley S. Chang, Kyle Serikawa*, Karen Allen* and David Bentley. Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

Cell-surface proteins anchored to the plasma membrane via covalently attached glycosylphosphatidylinositol (GPI) have been implicated in cell-cell recognition events in numerous systems. Treatment of grasshopper embryos with phosphatidylinositol-specific phospholipase C (PI-PLC), an enzyme that cleaves GPI-anchored proteins from the cell surface, leads to reproducible disruptions of axonal guidance by pioneer neurons, as well as defects in pre-axonogenesis migration of guidepost neurons in the developing limb bud. Specifically, PI-PLC treatment induces (1) failure of Ti1 pioneer axons to make a highly stereotyped ventral turning response along an epithelial segment boundary, (2) loss of proximal growth orientation of Ti1 axons, resulting in looping, distal axonal growth, and (3) proximal migration of Cx1 guidepost neurons towards the central nervous system (CNS). These abnormal phenotypes occur with limited frequency in limb buds treated with PI-PLC, but are completely absent in control-treated embryos, indicating that the observed effects are specifically caused by the enzymatic treatment. These results suggest that GPI-anchored proteins located on the membrane of the neuronal growth cone and/or the epithelial substratum are involved in pathfinding events in the embryonic grasshopper limb bud.

363.8

SELECTIVE AXON FASCICULATION AND TRACT FORMATION OF MOLECULARLY DISTINCT PERIPHERAL NEURON SUB-POPULATIONS DURING LEECH EMBRYOGENESIS. J. Johansen, K.M. Johansen, D.M. Kopp†, and J. Jellies†. Department of Zoology & Genetics, Iowa State University, Ames, IA 50011 and †Neurobiology Research Center, University of Alabama, Birmingham, AL 35294.

A small population of axon tracts in the nerve roots and interganglionic connectives are formed by peripheral neurons projecting their axons into the CNS. Subpopulations of these neurons differentially express the antigens of two monoclonal antibodies, lan 3-2 and lan 4-2. These antigens are surface glycoproteins (McKay et al., Science 222:788), and at least the lan 3-2 antigens may be directly involved in axon tract formation since perturbation with Fab fragments of lan 3-2 disrupt normal fascicle formation (Zipser et al., Neuron 3:621). On Western blots lan 3-2 recognizes three protein bands with molecular weights of 130, 105, and 90 kD, whereas lan 4-2 recognizes only a single 130 kD band. Immunoprecipitation and 2D-gel analysis show that at least one 130 kD glycoprotein expresses both the lan 3-2 and 4-2 epitope, whereas the two lower molecular weight glycoproteins only carry the lan 3-2 epitope. Consequently, the two antibodies recognize different complements of surface glycoproteins. Using immunocytochemistry, light and electron microscopy we have studied the development of lan 3-2 and lan 4-2 positive axon fascicles during development in *Hirudo medicinalis*. Our results demonstrate that the lan 3-2 antibody is likely to recognize all the developing peripheral neurons, and that in day 10 embryos the central projections of their axons segregate into three separate axon fascicles. In contrast, the lan 4-2 antibody only recognizes a few peripheral neurons of distinct morphology. These neurons also send axons to the CNS; however, interestingly they limit their projections along only one of the lan 3-2 positive axon fascicles. Thus, more than one set of molecularly distinct guidance cues are likely to be involved in the normal fasciculation of peripheral neurons and these studies set the stage for comparative antibody perturbation to assess the potential role of the lan 3-2 and 4-2 antigens in this process. Supported by NIH grant NS 28857 to JJo and NIH grant NS 28603 to JJe.

363.9

THE *UNC-5* AXON GUIDANCE GENE OF *C. ELEGANS* ENCODES A PROTEIN WITH FEATURES OF A CELL ADHESION RECEPTOR. J. Culotti, A. Spence*, Y. Zhou*, C. Leung-Hagesteijn*, Medical Genetics, U. of Toronto, Ont., E. Hedgecock*, B. Stern*, Biology, Johns Hopkins U., Baltimore, MD

The *unc-5* and *unc-6* genes of *C. elegans* are required to guide dorsal migrations of neuron growth cones and mesodermal cells on the epidermis (Hedgecock et al.,Neuron4,61-85,1990). Genetically, *unc-5* and *unc-6* are interdependent. One possibility is that *unc-5* encodes a receptor for the *unc-6* protein, which we have shown to be a novel B2 laminin-like molecule. Several features of the predicted *unc-5* protein are consistent with this interpretation, including a single membrane-spanning helix, an extracellular Ig-like domain and 2 'WSX' domains found in different proteins that exhibit cell adhesion properties. The transmembrane domain has 2 cysteines at its cytoplasmic end followed by a short run of basic amino acids. A similar motif is found in certain receptors as well as the nerve growth associated protein GAP-43. In these proteins, the cysteines are palmitoylated. This modification may be required for linkage to G proteins. A 50 amino acid sequence within the predicted cytoplasmic domain is distantly related to the SH3 domains of several different proteins associated with the membrane cytoskeleton, including ones that bind to actin. Such a function for this domain in *unc-5* could provide a direct mechanical linkage to the actin-based motility system of migrating cells and growth cones.

363.10

MOLECULAR CORRELATES OF MECHANOSENSORY NEURAL CIRCUITRY IN THE NEMATODE CAENORHABDITIS ELEGANS. Shahid S. Siddiqui, Lab of Molec. Biol., Toyohashi Univ. of Technology, Toyohashi 441, JAPAN.

Formation of a functional neural circuitry may require both cell intrinsic and cell extrinsic factors. One way of studying these factors is to establish molecular correlates of neural networks that are amenable to a behavioral assay. Since the touch circuitry in C. elegans is well studied (M, Chalfie et al. 1985), we have examined the molecular correlates of the touch circuitry. Previously, we have shown immunocytochemical staining due to anti-alpha tubulin McAb (6-11B-1 G. Piperno), that stains the six touch cells (ALML, ALMR, AVM, PLML, PLMR, and PVM) and a single cell PVR, in the right lumbar ganglion. The role of PVR in the touch circuitry has not been known. We have begun laser ablation of precursor cells that give rise to the PVR cell in the embryo, and assayed the touch response of the manipulated animals by a gentle stroking with a hair. Our results show that ablation of PVR and its precursor cells in the embryo affects the response to a light touch in experimental animals (S. Siddiqui, K. Higashi & J. Miwa). These results suggest a role for PVR in the touch neural network of C. elegans in the tail region.

*Siddiqui, S. S. et al. J. Neuroscience 9, 2963-2972, 1989

Supported by grants from the Ministry of Education, Culture and Science, Japan, NEC Corpn., Japan, and TUT, Toyohashi.

ALZHEIMER'S DISEASE: AMYLOID I

364.1

TRANSLATIONAL REGULATION OF THE AMYLOID BETA PROTEIN PRECURSOR (APP) VIA RNA-BINDING PROTEINS: EFFECTS OF AN ALZHEIMER'S MUTATION. Rudolph E. Tanzi and Bradley T. Hyman, Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

A base substitution in exon 17 of the APP gene has been reported in affected individuals in familial Alzheimer's disease (FAD) pedigrees in the U.K., the U.S., and Japan. This mutation disrupts a putative regulatory stem-loop structure in the APP mRNA. The stem-loop occurs at the 3' end of region encoding the βA4 region of APP between nucleotides 1906 and 1924 (APP695 sequence) and matches a consensus sequence of iron-response elements (IRE) present in the mRNAs for ferritin and transferrin receptor. We are testing the possibility that the IRE stem-loop in APP may be a translational regulatory element which governs APP production, and which is destabilized by the Alzheimer's-associated base substitution in exon 17. Data will be presented on 1. attempts to characterize binding proteins for the APP IRE, 2. the effect of the Alzheimer's base-substitution on protein binding to the IRE, and 3. studies of translational regulation of APP via this IRE in wild type and mutated APP message.

364.2

THE CYTOSKELETAL ASSOCIATION OF THE ALZHEIMER'S AMYLOID PRECURSOR PROTEIN (APP) AND IT'S POSSIBLE FUNCTIONAL SIGNIFICANCE. L.M. Refolo, I.S. Wittenberg*, and N.K. Robakis. Dept.of Psychiatry, Mt. Sinai Med Center. N.Y. N.Y. 10029.

Recently, we reported data suggesting that APP is associated with the cytoskeleton of Type I astrocytes. Using a detergent extraction procedure to further investigate this phenomenon, we find that 50- 90 % of the total APP, detected in brain tissue and neural cell lines, is associated with the detergent-insoluble cytoskeleton. In addition, we find that cell surface and intracellular APP are anchored to the cytoskeleton. This association requires intact microtubules and is modulated by protein phosphorylation. Pulse-chase experiments indicate that newly synthesized APP rapidly associates with the detergent-insoluble cytoskeleton. Temperature-shift experiments indicate that the intracellular pool of cytoskeletal APP is located both in the ER and Golgi compartments. Brefeldin A, a protein transport blocker causes a dramatic shift in the amount of intracellular APP associated with the cytoskeleton. We posit that the cytoskeletal association of cell surface APP reflects specific protein interactions which may be required to fulfill it's function as an adhesion molecule. In addition we propose that the association of intracellular APP with the cytoskeleton is required for normal transport and post-translational processing. Altered cytoskeletal binding of APP might result in the abnormal transport of these proteins and could lead to aberrant proteolysis and generation of amyloidogenic fragments of APP.

364.3

THE ROLE OF HEPARIN IN β-AMYLOID PRECURSOR PROTEIN (APP) MEDIATED ADHESION

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The β-amyloid precursor protein (APP) is a glycoprotein consisting of multiple isoforms derived from a single gene by a process of alternative splicing. The membrane-bound forms of the protein have previously been implicated in the mediation of neural cell adhesion to a type IV collagen (CIV) component of the extracellular matrix. APP has been demonstrated to have a high heparin binding affinity, and the A4 polypeptide is also closely associated with heparan sulfate proteoglycans in the Alzheimer neuritic plaques.

The present study examined the role of heparin/heparan sulfate in the mediation of APP-mediated collagen adhesion. Excesses of both heparin and heparan sulfate, as well as treatment with heparinase, inhibited APP-CIV interaction. This interaction was independent of the presence of the Kunitz protease inhibitor domain of APP, and both soluble and membrane-bound forms of the protein were capable of binding CIV. These results suggest that APP collagen binding is mediated via a heparin bridge mechanism, and raises the question as the possible role of the heparin-binding characteristics of the protein in the genesis of neuritic plaques.

364.4

DISTRIBUTION AND CHARACTERIZATION OF AMYLOID β PROTEIN DEPOSITION IN NORMAL HUMAN AND ALZHEIMER'S DISEASED CEREBRAL CORTEX USING ^{125}I-βAP$_{1-40}$ AS THE RADIOLIGAND

P.W. Mantyh, M.E. Labenski, C.J. Allen, J.R. Ghilardi, D.C. Whitcomb, S.R. Vigna, H.V. Vintars, E.R. Stimson, C.E. Dahl, J.E. Maggio. Mol. Neurobio. Lab (151) VA Med. Cntr, Mpls., MN 55417; GI Div., VA Med. Cntr, Durham, N.C., 27705; Dept. Pathol., UCLA, LA, CA, 90024; Dept. of Biol. Chem. and Mol. Pharm., Harvard Med. School, Boston, MA 02115

Amyloid β protein, a 39-42 amino acid peptide, is a primary constituent of senile plaques and cerebrovascular deposits in Alzheimer's disease (AD). Recently it has been reported that amyloid β protein 1-40 (βAP_{1-40}) is neurotoxic to mature neurons in culture, suggesting a possible role for the peptide in the pathology in AD. To explore the sites at which βAP is deposited and what factors may enhance or inhibit this aggregation, we have developed an in vitro assay using ^{125}I-labeled βAP_{1-40} to characterize and localize the deposition of βAP in homogenates or thin sections of normal or AD human cortex. The deposition of the ligand to AD tissue was blocked by unlabelled βAP_{1-40} and the smaller fragment βAP_{25-35}-NH2, while βAP_{25-35}, substance P and neurokinins A and B were essentially inactive. Autoradiography suggests that the radioligand binds to nearly every senile plaque, but almost none of the degenerating neurons, stained with thioflavin S or anti-A4. There was essentially no binding of the radioligand to normal tissue. The present assay provides a novel method to assess agents which may affect amyloid deposition in AD.

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364.5

HIGH AFFINITY BINDING OF EXTRACELLULAR MATRIX PROTEINS TO THE BETA AMYLOID PRECURSOR PROTEINS.

R. Kisilevsky*, S. Narindrasorasak*, R.A. Altman, M.B. Fairbanks, P. Gonzalez-DeWhitt*, D.E. Lowery and B. Greenberg. Queen's University, Kingston, Ontario and The Upjohn Co., Kalamazoo, Michigan.

Extracellular matrix (ECM) proteins and the Alzheimer amyloid precursor (AAP) proteins are found as components of neuritic plaque and cerebrovascular amyloid. Binding studies were conducted between the basement membrane heparan sulphate proteoglycan (HSPG) or laminin and the 695, 751, and 770 AAPs. Quantitative analyses of binding data identified a single class of binding sites for the HSPG on AAP-695 (Kd = 9 x 10^{-10}M), -751 (Kd = 10 x 10^{-9}M), and -770 (Kd = 9 x 10^{-9}M). The "Kunitz" protease inhibitor domain in -751 and -770 may reduce the affinity of AAPs for HSPG through steric hindrance and/or conformational alterations. HSPG binding was inhibited by heparin and dextran sulphate but not by dermatan or chondroitin sulphate. HSPG protein cores, also bound to the AAPs with equally high affinity indicating that the binding site is constituted by the peptide chain rather than the carbohydrate moiety. Two classes of binding sites were identified for laminin with affinities an order of magnitude higher than HSPG. Characterization of these interactions suggest that binding between the AAPs and the ECM may be involved in the nucleation stages of Alzheimer's beta-amyloidogenesis.

364.6

C1q BINDING TO β-AMYLOID AND AMYLOID PRECURSOR PROTEIN (APP).

Joseph Rogers, James Schultz, Scott Webster, Libuse Brachova*, Pamela Ward#, and Ivan Lieberberg#. Institute for Biogerontology Research, Sun City, AZ 85372, and #Athena Neurosciences, Inc., South San Fransisco, CA 94080.

The full range of classical pathway complement proteins from C1q through the membrane attack complex (MAC, C5b-C9) have been demonstrated to be profusely present in Alzheimer's disease (AD), co-localized with β-amyloid containing pathology such as neuritic plaques. Normally, classical pathway inflammation is initiated by C1q binding to Ig. However, consistent AD specific Igs have proven difficult to find. As an alternative we have noted that the classical complement pathway can be initiated by many non-Ig substrates, and we have investigated the possibility that APP and/or β-amyloid might be such substrates. Slot blots of membranes onto which solutions of APP, β-amyloid, or control substances (e.g., BSA) had been fixed were incubated with C1q, washed, and assayed with anti-C1q antibody. C1q was not detected on BSA or other control membranes. A light band of C1q immunoreactivity was observed on APP membranes, and a heavier band on β-amyloid membranes. We next investigated whether this binding was sufficiently active to survive immunoprecipitation. Conditioned media from APP transfected and untransfected cells was incubated with physiological concentrations of C1q, immuno-precipitated with anti-C1q antibody, and run on SDS gels with anti-APP detection. APP co-precipitated with C1q in the transfected but not untransfected cell line. Under normal circumstances C1q-APP binding would not necessarily initiate inflammation. However, naked β-amyloid deposits, which are profuse in AD brain, might provide an escape from both fluid phase and cellular regulatory mechanisms for complement inactivation, leading ultimately to complement-mediated destruction of nearby neurons, neurites, and synapses.

Supported by NIA AGO-7367.

364.7

HUMAN ANTIBODIES REACTIVE WITH AMYLOID BETA PROTEIN IN NEURITIC PLAQUES AND CEREBROVASCULAR AMYLOID IN ALZHEIMER'S DISEASE (AD).

F. Gaskin and J. Finley* Dept. of Behavioral Medicine and Psychiatry, Univ. of Virginia Sch. of Med., Charlottesville, VA 22901

Immortalization of B cells from patients with AD, other neurodegenerative disorders, strokes and age-matched controls by Epstein-Barr virus provides cell lines secreting human monoclonal antibodies (Abs). These Abs may be representative of circulating serum Abs. This approach was undertaken by us to circumvent the difficulties in defining the specificities of serum auto-Abs unique for AD patients. These difficulties include the low concentrations of relevant Abs and the presence of other auto-Abs to cellular constituents in aged individuals. Our previous studies have identified unique anti-neurofibrillary tangle and anti-neural Abs. The nature of these reactive antigens have not been defined. In the present study, we have identified by immunofluorescence and double labeling with thioflavine S, three B cell lines from an AD patient which secrete IgM Abs reactive with neuritic plaques and blood vessels in AD temporal cortex. Absorption studies and dot blots indicate the reactive epitope is located in the region of 1-28 amino acids of the amyloid beta protein. These Abs represent the first auto-Abs against the amyloid beta protein in man. The pathological significance of these auto-reactive Abs remains to be determined. Supported by AG-06348 and the Eleanor Naylor Dana Trust (New York).

364.8

Mass spectrometric analysis of amyloid β/A4 protein of Alzheimer's disease (AD).

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We purified β/A4 protein from AD cortex according to a new protocol which involved 10% SDS extraction at the initial step. The Western blot with anti-synthetic peptide showed closely-spaced two bands at $M_r \sim 4,200$, indicating that β/A4 was not homogeneous. After digestion of β/A4 with *Achromobacter lyticus* protease 1 (specific for Lys-X), three produced fragments were separated by reverse-phase HPLC and subjected to sequence and plasma desorption mass spectrometric analyses. The N-terminal fragment gave two sequences, β/A4 1-16 and 3-16, without any N-terminal blocking. The C-terminal fragment consisted of two major and minor sequences, β/A4 28-40 and 28-43, respectively. From these observations we conclude that (1) β/A4 1-40 is a major species; (2) The N-terminus is not blocked. Thus, previous protocols for he purification of amyloid cores may have allowed β/A4 to be modified at its N-terminus during isolation. The presence of β/A4 1-43 suggests that larger molecules may have deposited before the cleavage at Val-40 occurs.

364.9

ALZHEIMER'S AMYLOID PRECURSOR PROTEIN CONTAINS A TETRAPEPTIDE SEQUENCE -RHDS- THAT PROMOTES CELL ADHESION. J. Ghiso,* A. Rostagno* and B. Frangione. Dept of Pathology, New York University Medical Center, New York, NY, 10016.

Amyloid β, the major constituent of the fibrils composing senile plaques and vascular amyloid deposits in AD and related disorders, is an abnormal internal degradation product of a larger precursor molecule (APP). The predicted multidomain structure of the four APP isoforms, generated by alternative splicing of a single gene located on chromosome 21, correlates with that of a cell-surface receptor. Two isoforms (APP_{751} and $_{770}$) contain an extra domain homologous to the Kunitz family of protease inhibitors. The secreted amino terminal fragment containing the KPI domain has been shown to be identical to nexin II, a serine protease inhibitor.

APP isoforms, nexin II and amyloid β contain the sequence RHDS, closely homologous to the conserved tetrapeptide RGDS widely distributed among cell adhesion proteins. Sequence analysis and secondary structure prediction indicate that, like the conformation described for RGDS within fibronectin, RHDS (positions 657-660 of APP_{751}) is located in a hydrophylic β-turn. Therefore, RHDS may well function as a cell-adhesion recognition signal and compete with RGDS for common receptors.

To test this hypothesis, synthetic peptides SP28 (amino acids 652-679), RHDS and RGDS were used to promote cell attachment and to block fibronectin-mediated adhesion in competitive inhibition experiments. Peptide SP28 (containing the RHDS segment) specifically mediated adhesion of U937 cells in a dose-dependent manner. Preincubation of the cells with SP28, RGDS and RHDS inhibited cell attachment to SP28-coated substrata. The isolated peptide RHDS in solution was able to inhibit the adhesion of U937 cells to fibronectin-coated microtiter plates, mimicking the classic RGDS binding to the $\alpha 5\beta 1$ integrin. The data indicate that through the interaction of the tetrapeptide RHDS with an integrin-like receptor, APP/nexin II may function as a cell-adhesion protein.

364.10

IDENTIFICATION OF A NUCLEAR FACTOR BINDING DOMAIN THAT IS REQUIRED FOR APP PROMOTER ACTIVITY.

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A manifestation of Alzheimer's disease is the presence of amyloid plaques in brains of afflicted individuals. The major component of amyloid plaques is the amyloid beta protein, which is a truncated form of the larger amyloid precursor proteins (APP). Several forms of APP have been identified that are derived from the same gene by differential splicing.

To investigate the regulation of the APP gene, the promoter was analyzed for its ability to direct cell type specific expression. The APP promoter and selected deletions were placed 5' to the reporter gene chloramphenicol acetyl transferase (CAT). The promoter deletions were transfected into different cell lines that showed variant levels of endogenous APP transcripts. The transient transfection assays showed that 96 base pairs 5' to the transcriptional start site are sufficient for full cell type specific promoter activity.

In order to identify trans-acting factors that bind to this region, a promoter fragment from position -96 to +49 was analyzed by band shift assay. The ^{32}P-end-labeled fragment was exposed to nuclear extracts from rat brain and a human retinoblastoma cell line. Prominent and identical band shifts were observed, indicating the binding of nuclear factors to this fragment. Mapping the binding domain by DNAse footprinting and methylation interference revealed a DNAse protected region covering about 20 base pairs on both strands. In addition, the methylation of two G residues on each strand within this DNAse protected domain interfered with factor binding. Work is currently in progress to define the role of this factor in promoter expression.

364.11

TRANSGENIC MOUSE STUDIES OF ALZHEIMER AMYLOID PRECURSOR (AAP) PROTEINS AND DERIVATIVES. B.D. Greenberg, S.M. Ali*, R.A. Altman, P.A. Gonzalez-DeWhitt*, D.E. Lowery, J.M. Colvin* and H.G. Polites*. Upjohn Laboratories, Kalamazoo, MI 49001.

We are attempting to generate a transgenic mouse model for Alzheimer-type amyloidogenesis based on overexpression of AAP proteins in appropriate brain regions. We have utilized several promoter systems to express the full length and various segments of the AAP-695. A C-terminally truncated derivative begins at the natural N-terminus (Leu_{18}) and extends to Val_{640}, functionally deleting the C-terminal 56 residues. An N-terminal truncation begins at Glu_{593} and extends to the natural C-terminus. One of the promoter systems is based on the metallothionein-growth hormone system of Swanson et al. (Nature 317:363 (1985)) with AAP replacing the rGH coding sequence described in that paper. The other promoter system will be described. In order to guide our efforts in mice, we have transfected human HeLa and mouse L cells with these transgenes to study expression, processing and inducibility. Two of our three transgene series are abundantly expressed and inducible in transfected cells. In addition, Northern and Western blotting analyses of AAP expression in extracts of transgenic mouse brains are ongoing, as are *in situ* hybridization and immunocytochemical analyses on brain sections. The status of these studies will also be reported.

364.12

STRUCTURAL ANALYSES OF BACULOVIRUS-DERIVED ALZHEIMER AMYLOID PRECURSOR (AAP) PROTEINS.

M.B. Fairbanks[1], M.D. Prairie*[1], J.M. Pasternack[2], H.A. Zurcher-Neely*[1], R.L. Heinrikson*[1], S. Narindrasorasak[3], R. Kisilevsky[3], W.C. Krueger*[1], S.G. Younkin[2] and B.D. Greenberg[1]. [1]Upjohn Laboratories, Kalamazoo, MI 49001; [2]Inst. Pathology, Case Western Reserve University, Cleveland, OH 44106; [3]Dept. Pathology, Queen's University, Kingston, Ontario.

In order to better understand the amyloidogenesis which accompanies Alzheimer's Disease (AD), we have employed the insect cell baculovirus expression system to produce quantities of the AAP proteins sufficient for biochemical and structural analyses. AAPs secreted into the conditioned medium extend from the natural amino-terminus (Leu_{18}) to several C-terminal cleavage sites within the β protein domain. Details of this C-terminal processing will be presented. Determination of AAP secondary structure utilizing circular dichroism (CD) reveal essentially identical spectra and extinction coefficients for secreted AAP-695, AAP-751 and AAP-770. Calculations derived from CD analyses show that these AAP proteins contain 32% α helix, 18% antiparallel β-sheet, 3% parallel β-sheet, 15% β-turn and 32% non-defined structure (random coil, etc.). Effects of metal ions, proteoglycans, glycosaminoglycans and extracellular matrix proteins on AAP secondary structure are underway. Relevance of these findings to AD amyloidogenesis will be discussed.

NEURAL-IMMUNE INTERACTIONS

365.1

TUMOR NECROSIS FACTOR MODULATES NOREPINEPHRINE SECRETION IN CULTURED SYMPATHETIC NEURONS. B. Soliven and J. Albert*. Dept. of Neurology and The Brain Research Institute, The University of Chicago, Chicago, IL 60637

Immune peptides/cytokines are known to be important in immunoregulatory mechanisms. These peptides also exert multiple effects on other cellular functions, including modulating hypothalamic neuronal firing rate and secretion of pituitary hormones as part of a complex immune-neural interaction. To investigate the possibility that immune peptides could alter the function of the sympathetic nervous system, we studied the effects of cytokines on the calcium-dependent release of ^{3}H-norepinephrine (^{3}H-NE) from cultured neonatal rat superior cervical ganglia (SCG) neurons. Incubation of SCG neurons with rat γ-interferon (100-300 U/ml)(n=8), recombinant human interleukin-1β (0.14 nM - 0.7 nM)(n=9), or recombinant human tumor necrosis factor-α (rhTNF) (1 nM)(n=15) for 24-48 hrs had no effect on the baseline release of ^{3}H-NE and the initial release evoked by 70 mM K^+, when compared to untreated cells (n=16). However, a repeat K^+-induced depolarization after 6 min resulted in a decrease of 31 $\pm$ 5.8%(n=11) in secretion in rhTNF-treated cells, but only 4.6 $\pm$ 5.5%(n=11) decrease in untreated cells ($p < .01$). The secretory response was restored in rhTNF-treated cells when the interval between the two K^+-induced depolarizations was increased to 10 min. We conclude that rhTNF prolongs the recovery from inactivation of secretion, thereby decreases the responsiveness of the sympathetic neurons to repetitive stimulation with high K^+ solution.

365.2

SURVIVABILITY OF MITOGENIC CELL SUSPENSION GRAFTS WITHIN THE CNS OF ATHYMIC HOST RATS. B. Baker, P. Ebert*, R. Broadwell. Div. Neurosurgery, Univ. MD, Balto., 21201.

The belief that graft survival in the CNS of immunoincompetent (athymic) hosts is absolute has been investigated using monoclonal antibodies against major histocompatibility complex (MHC) classes I and II, CD4, and CD8 antigens. Cell suspensions of PC12 or canine brain tumor cell line were prepared in culture, and 250K-500K cells were grafted into the caudate/putamen of homozygous athymic Buffalo, NIH, and ACI rats; animals were sacrificed 10 days post-grafting. Cell suspension grafts were evident within the parenchyma and/or on the pial surface of host brains and appeared healthy in sections stained with toluidine blue. Immunohistochemistry revealed populations of CD4+, CD8+, and MHC class II+ cells inhabiting the grafts. Differences in numbers of immunopositive cell types varied among the rat strains. The NIH nude strain always exhibited denser populations of the immunopositive cells in the two different tumor cell lines compared to Buffalo and ACI rat strains. Numbers of immunopositive cells in the two types of cell suspension grafts varied considerably. The data suggest that homozygous athymic rats can be capable of mounting an immune response against foreign antigen introduced to their CNS. Severity of the immune response may be related to the specific strain of athymic host and to the antigenicity of grafted cells. Supported by NIH/NINDS Grant #NS18030.

365.3

INTERLEUKIN 1-β (IL1-β) INCREASES SYNAPTIC INHIBITION IN RAT HIPPOCAMPAL PYRAMIDAL NEURONS (HPNs) *IN VITRO*. Marc L. Zeise*[1], Samuel G. Madamba and George Robert Siggins. Dept. of Neuropharmacology, Scripps Clinic and Research Foundation, La Jolla, CA, U. S. A., and [1]Clinical Neuropharmacology, Max-Planck-Institute for Psychiatry, 8000 München, Germany.

IL1-β is a cytokine polypeptide produced—mainly by macrophages—after viral infection, injury or antigenic challenge. Central neurons may be exposed to IL1-β when macrophages release cytokines after viral (e.g., HIV) infection. However, its effects on neuronal physiology are unknown. Thus, we examined the action of IL1-β on rat CA1 HPNs in a slice preparation, using standard intracellular current- and voltage-clamp methods. We superfused human recombinant IL1-β (280 nM) for 10 min, followed by washout. We stimulated the stratum radiatum during current or voltage steps, and measured voltage and current amplitudes before and after the synaptic stimulation, for calculations of conductances. In all cells, IL1-β enhanced postsynaptic inhibitory conductances 35 ms after synaptic stimulation (at the peak of the early GABAergic inhibition), by more than twofold (from 370 nS to 870 nS). At 80 ms after synaptic stimulation, average conductance increased from 110 to 410 nS, suggesting that synaptic inhibition was also considerably prolonged. The IL1-β effect was most pronounced about 1 hr after superfusion. Partial recovery occurred over the next hour. Resting membrane potential, input resistance, and amplitude and threshold of action potentials were unchanged. These results suggest that IL1-β can change interneuronal communication. The increase in GABAergic synaptic transmission in the hippocampus could lead to reduced memory or learning, as in AIDS-related dementia. (Supported by the USPHS (MH-47680) and the Deutsche Forschungsgemeinschaft.)

365.4

INTERLEUKIN-1 INDUCED GH SECRETION IS SUPPRESSED BY NEUTRALIZATION OF ENDOGENOUS GHRH USING GRF-ANTIBODIES. LC Payne *, F Obál, Jr.* and JM Krueger. University of Tennessee, Memphis

Growth hormone-releasing hormone (GHRH) promotes non-rapid eye movement sleep (NREMS) (1). Interleukin-1 (IL1) enhances NREMS (2) and alters growth hormone (GH) secretion via a hypothalamic mechanism (3), probably GHRH. Low doses of IL1 enhance GH release and NREMS, whereas high doses inhibit GH and NREMS in rats (4). To study the possible involvement of GHRH in the mediation of IL1-induced GH secretion, affinity-purified goat anti-rat-GRF-antibodies (GRF-Ab) (500 μg) were injected intravenously (iv) to neutralize endogenous GHRH in conjunction with ICV injected IL1 (1- to 25 ng). Plasma GH levels were assayed by RIA. GRF-Ab suppressed GH secretion induced by low doses of IL1. Further, GRF-Ab potentiated the suppression of GH by high (or large) doses of IL1. This suggests that IL1 induces GH release via a pathway involving GHRH. These data further prompt the speculation that the sleep-promoting actions of IL1 may be mediated via GHRH neurons projecting to the basal forebrain. Regardless of whether this speculation is correct, it is currently clear that GRF-Ab can block an IL1 action.

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Research supported by NIH: NS27250

365.5

LOCALIZATION OF TYPE I INTERLEUKIN-1 RECEPTOR MESSENGER RNA IN THE HYPOTHALAMIC-PITUITARY-ADRENAL AND GONADAL AXES. **E.T. Cunningham, Jr., E. Wada, D.E. Tracey, D.B. Carter, J.F. Battey and E.B. De Souza. Neurobiology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224 (E.T.C. Jr., E.B.D.S.); Laboratory of Neurochemistry, NINDS, Bethesda, MD 20892 (E.W., J.F.B.); and the Upjohn Company, Kalamazoo, MI 49007 (D.E.T, D.B.C.).**

The cytokine interleukin-1 (IL-1) has pronounced endocrine effects, including activation of the hypothalamic-pituitary-adrenal axis (HPA) and inhibition of the hypothalamic-pituitary-gonadal axis (HPG). In this study, we sought to identify areas in the brain, adrenal gland, and testis that might mediate these effects of IL-1. In situ hybridization was employed with ^{35}S-labeled antisense cRNA probes derived from a full-length murine T-cell Type I IL-1 receptor cDNA. With the exception of endothelial cells of post-capillary venules, the signal in the hypothalamus, including the paraventricular nucleus, was comparable to background. An intense signal was observed over the entire anterior lobe of the pituitary gland. The signal over the intermediate and posterior lobes was comparable to background. Within the testis, an intense signal was observed over virtually all interstitial cells, a majority of which are known to be of the Leydig type, and over epithelial cells of the epididymal ducts, particularly in the head region. The signal within the adrenal gland was comparable to background. These results, in conjunction with previous data demonstrating high levels of Type I IL-1 receptor mRNA in extrahypothalamic brain regions such as the hippocampus, support the notion that IL-1 may alter HPA and HPG activity at both central and peripheral sites.

365.6

SPECIFICITY OF INTERLEUKIN-1-MEDIATED INDUCTION OF TRANSFORMING GROWTH FACTOR-BETA (TGF-) IN GLIA. A. da Cunha, J. Jefferson*, F.S. Jannotta* and L. Vitkovic. Lab. of Immunoregulation, NIAID, NIH, Bethesda 20892.

We previously reported that interleukin-1 (IL-1) stimulated the expression of TGF-b in rat cortical astrocytes. The objective of this study was to determine the specificity and in vivo relevance of this observation. Near homogeneous populations of three glial cell types, astrocytes (A), oligodendrocytes (O) and microglia (M) derived from neonatal rat cortex and grown in primary cultures were exposed to IL-a and IL-1b (250 U/ml, 24h). TGF-b was detected immunocytochemically in cells and tissues and quantified with a biological assay in culture supernatants. IL-1a and b were assayed immunocytochemically in tissue sections of human frontal cortex and subcortical white matter. The induction of TGF-b was glial cell type-specific: (1) the amount induced differed for each cell type (O>M>A); (2) M secreted active and the other cells secreted latent TGF-b; (3) A and M were 2- to 3-fold more, whereas O were equally, responsive to IL-1b than IL-1a. IL-1b was expressed at a higher level than IL-1a in all tissues. TGF-b was not detected in brains (n=8) with low levels of IL-1, whereas, it was dramatically elevated in those (n=6) with high levels of IL-1. TGF-b immunoreactivity appeared to be localized in glia rather than neurons. These results indicate that elevation of IL-1 in brain parenchyma may lead to the induction of TGF-b in glia.

365.7

THE EFFECT OF NEUROPEPTIDES ON THE PROLIFERATION AND CYTOLYTIC ACTIVITY OF HSV-SPECIFIC CYTOLYTIC T LYMPHOCYTES. J.F. Sheridan, I.D. Plaza*, R. H. Bonneau* and R. Glaser. Dept. of Medical Microbiology & Immunology, and Oral Biology, Ohio State University, Columbus, OH 43210.

The presence of neurotransmitter receptors on immune cells suggests a potential role for neuropeptides in immune regulation. Furthermore, the possibility that cells of the immune system produce neurohormones and peptides may indicate a homeostatic role for these substances in the immune system. Although other studies have demonstrated a role for neuroendocrine products in immune function, none have examined their effects on a viral antigen-specific T cell response. The purpose of this study was to investigate the effects of the neuropeptides, VIP, SOM, and SP on the biological activity of an HSV-specific cytotoxic T cell (CTL) cell line. A murine-derived, HSV-specific, CD8$^+$ cell line, designated RAB-1, was generated from HSV-immune mice. This cell line proliferated only in response to MHC-restricted, HSV-specific antigenic stimulation; withholding antigen stimulation for 2 successive passages arrested these cells in the G_0/G_1 phase of the cell cycle. Various concentrations (10^{-6} to 10^{-12}M) of either VIP, SOM, or SP were added to G_0/G_1 arrested RAB-1 cells in the presence of HSV-specific stimulator cells. The kinetics of the proliferative response to antigenic stimulation was determined by [^{3}H]-thymidine incorporation at 24, 48, 72, and 96 hours following initiation of culture. All three neuropeptides were shown to suppress proliferation from 58% to 80% as compared to control cultures at 48 and 72 hours of culture. Further studies are in progress to examine the cytolytic activity of RAB-1 cells in the presence of these neuropeptides.

365.8

REGULATION OF MURINE T-LYMPHOCYTE FUNCTION BY ENDOGENOUS SEROTONIN J.L. Kut*, M.R.I. Young*, J.W. Crayton, L. van de Kar, and R.C. Arora Section on Biological Psychiatry and Research Service, VA Hines Hospital, Hines IL, 60141 and Departments of Pathology, Pharmacology, and Psychiatry, Loyola Stritch School of Medicine, Maywood, IL 60153.

Studies have suggested that serotonin may function as an immune modulator. On further exploration of this possibility, we found that pretreatment of mice with p-cholorphenylalanine (PCPA) reduced T-cell blastogenesis to Con-A by 74%. In contrast, pretreatment of mice with tryptophan slightly increased T-cell blastogenesis, but tryptophan pretreatment plus the addition of fenfluramine (Fen) *in vitro*, increased blastogenesis by 54%. Serotonin treatment of cultured cells at low doses increased T-cell blastogenesis, whereas high-dose serotonin produced a 40-65% inhibition of blastogenesis. Similarly, high-dose Fen inhibited T-cell responses. PCPA abolished this Fen inhibition of blastogenesis. Consequently, the Fen inhibitory effect may have been mediated via endogenous spleen-cell derived serotonin. Our results suggest that this endogenous pool of serotonin may have a regulatory effect on T-cell blastogenesis. Supported by VA Merit Review (M.R.I.Y.) and RAG (J.W.C.) grants.

365.9

SEX DIFFERENCES IN STRESS EFFECTS ON CLINICAL DISEASE COURSE IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE). Ann C. Griffin* and Caroline C. Whitacre, Department of Medical Microbiology & Immunology, The Ohio State University, College of Medicine, Columbus, OH 43210.

EAE, often studied as a model for multiple sclerosis, is induced by a single injection of myelin basic protein (MBP) and adjuvant. In the Lewis rat, EAE is a monophasic disease with symptoms of hindlimb paralysis and perivascular infiltrates confined to the CNS. EAE is known to be mediated by MBP-specific, CD4+ lymphocytes. Levine (1962) showed that restraint stress delayed the onset and decreased the severity of clinical signs of EAE induced by whole nervous tissue in female rats. We have found that restraint stress applied during EAE is capable of suppressing clinical signs of disease in both male and female rats. Female rats exhibit a more profound suppression of disease, and appear to be more stress-reactive than males. Supporting this proposal, we found that female rats exhibit significantly higher circadian levels of corticosterone, and markedly higher levels of corticosterone in response to a single, 30-minute stress period when compared to males. To determine where stress-induced immunomodulation may affect EAE, we compared several different immunological parameters involved in EAE in stressed and non-stressed rats of both sexes. There was no evidence of stress effects on the development of CD4-bearing cells or frequency of MBP-reactive lymphocytes in rats of either sex. We are continuing to investigate other immunologic mechanisms involved in EAE that may be influenced by stress-induced hormones, such as antigen processing and presentation, and T cell receptor usage. (USPHS grants NS 23561 & MH 44660)

365.10

GLUCOCORTICOID RECEPTOR GENE EXPRESSION IN THE THYMUS IS UNDER TRANSCRIPTIONAL CONTROL OF ESTROGENS. A. Peiffer, B. Marchetti, and N. Barden. Molecular Psychogenetics, CHUL, Québec, Canada and Dept.of Pharmacology, Catania Univ. Sch. of Med., 95125 Catania, Italy.

Suppression of thymus-dependent immune functions by glucocorticoids and presence of a sexual dimorphism in the immune response underline the important role of steroid hormones in the control of immune functions. The aim of the present study was to identify the expression of the glucocorticoid receptor (GR) gene in the thymus and to determine alterations in GR mRNA concentration after ovariectomy and treatment with gonadal hormones. Using a 2.2 kb cRNA probe, we have identified an approximately 6.7 kb GR transcript in the thymus, corresponding to that seen in the rat pituitary gland. Ovariectomy results in a marked increase of GR mRNA content in rat thymic tissue, an effect which can be completely reversed by administration of 17-B-estradiol. These results suggest that estrogens act at the level of gene transcription to modify GR mRNA level in the thymus. The negative regulation of thymic GR RNA by estrogens may represent an important mechanism by which sex hormones modulate glucocorticoid action in the thymus.

STRESS, HORMONES AND THE AUTONOMIC NERVOUS SYSTEM: NEUROTRANSMITTERS

366.1

ACUTE STRESS INCREASES ^{3}H-AMPA BINDING TO THE AMPA/QUISQUALATE RECEPTOR IN THE HIPPOCAMPUS AND THE INCREASE IS NOT GLUCOCORTICOID-DEPENDENT. T.J. Shors, G. Tocco, K.A. Patel, M. Baudry, and R.F. Thompson. Univiversity of Southern Calif., Dept. of Psychology and Neurosciences Program, LA, CA 90089.

To determine whether the binding properties of the glutamate receptors, NMDA and AMPA/ quisqualate, are altered in reponse to acute stress, rats (n=6) were restrained for one hour and exposed to 60, 1 sec, 1 mA tail-shocks. Rats were immediately sacrificed; brains were rapidly frozen and sectioned. Ligand receptor autoradiography was performed using ^{3}H-TCP and ^{3}H-AMPA to label NMDA and AMPA receptors, respectively. Relative to naive controls (n=6), stress significantly increased binding of AMPA, but not TCP, in CA1 radiatum and oriens, CA3 radiatum and dentate gyrus. To establish whether glucocorticoids were responsible for the increased AMPA binding in stressed rats (51µg/dl serum corticosterone), autoradiography was performed on brains from adrenalectomized rats (ADX), with (n=6) and without previous exposure to stress (n=6). The increase in AMPA binding was still evident in ADX rats exposed to inescapable shock and restraint despite the near absence of circulating corticosterone (<3µg/dl), thus indicating that the stress- induced increase in AMPA binding is not glucocorticoid-dependent.

[NIH(AG05500, AG05142) and McKnight Foundation]

366.2

5-HT1A-, BENZODIAZEPINE-RECEPTOR ANXIOLYTICS AND α-HELICAL CRF DIFFERENTIATE GLUCOCORTICOID AND BEHAVIORAL RESPONSES TO SOCIAL STRESS. K.A. Miczek, W. Tornatzky, G.F. Koob, C. Rivier. Dept. of Psychology, Tufts University, Medford, MA 02155; Neuroscience, Res. Inst. Scripps Clinic; Peptide Biology Lab, Salk Institute, La Jolla, CA 92037.

Profound neurochemical changes are triggered when an intruder reacts to an attacking opponent and begins to cope behaviorally and physiologically with the threat of attack. Adult male Long-Evans rats confronting an aggressive "resident" for 1 h behind a protective screen, engage in submission (i.e. supine posture and ultrasounds), defense and behavioral restraint, while showing large endocrine and cardiovascular activation. After 1-5 h behavioral and physiological resting responses are re-established. Diazepam, gepirone, clonidine and α-helical CRF leave the defensive postures in reaction to the conspecific threat unaltered, even at high motorically compromising doses. Ultrasonic distress calls remain unaltered by diazepam and clonidine, but are significantly reduced by gepirone. Only clonidine attenuated the cardiovascular stress response effectively. Comparisons of rats who are stressed for the first time with experienced rats reveal no adaptation and the same pharmacological profile. Diazepam, gepirone and clonidine attenuate at low doses the ACTH and corticosterone responses to social stress, but at high diazepam and gepirone doses the ACTH/corticosterone values of socially threatened rats are further elevated suggesting a dose-dependent inversion of the endocrine anti-stress effects to a stress-intensification.

366.3

HEMODYNAMIC STRESS ACTIVATES NORADRENERGIC LOCUS COERULEUS (LC) NEURONS OF UNANESTHETIZED RATS. R.J.Valentino, A.L. Curtis, G. Aston-Jones and G. Drolet. Dept Mental Health Sciences, Hahnemann University, Philadelphia, PA 19102.

The LC has been implicated in stress partly because it is activated by physiologic challenges. Hypotension (-55 mmHg) induced by nitroprusside infusion in halothane-anesthetized rats was temporally correlated with an 32% increase in LC discharge rate. This effect was completely prevented by local LC injection of a corticotropin-releasing factor (CRF) antagonist, suggesting mediation through local CRF release (Valentino et al, Brain Res. in press). Since anesthesia may impair cardiovascular reflexes and interfere with inputs to the LC, the effect of hemodynamic challenge on LC discharge of conscious rats was investigated. Rats were chronically implanted with LC recording electrodes, and catheters in the jugular vein and the femoral artery for nitroprusside administration and blood pressure recording, respectively. In conscious rat, the same dose of nitroprusside (0.33 µg/µl/min for 15 min) that caused prolonged LC activation of anesthetized rats was less efficacious and of shorter duration in increasing LC discharge rate (24%) and in producing hypotension (-25 mmHg). However, a larger dose of nitroprusside (2.5 µg/ µl/min for 15 min) that produced the same level of hypotension observed in anesthetized rats (-51 mmHg), resulted in a much greater increase (92%) in LC discharge rate. The present results indicate that: 1) Nitroprusside is a less potent cardiovascular challenge in conscious rats, presumably because anesthesia blunts hemodynamic reflexes; 2) The magnitude of LC activation elicited by nitroprusside is correlated to the magnitude of hypotension; and 3) Hypotension may be a more efficacious stimulus of LC activation in conscious than in anesthetized rats. PHS Grants MH 40008, MH 00840, MH 42796, NS 24698, NARSAD (ALC), FRSQ (G.D.).

366.4

LOCUS COERULEUS (LC) ACTIVATION BY DIFFERENT PHYSIOLOGIC CHALLENGES IS MEDIATED BY DISTINCT NEUROTRANSMITTERS. M.E. Page and R.J. Valentino. Dept. of Mental Health Sciences, Hahnemann Univ., Philadelphia, PA 19102.

The LC is activated by non-noxious physiologic stimuli such as hypotension and bladder distention (Svensson et al., 1987), and noxious stimuli, i.e., footshock. The present study investigated the neurotransmitter(s) mediating LC activation by hypotension and bladder distention. Halothane-anesthetized rats, implanted with either an i.v. cannula for nitroprusside administration or with a cannula in the bladder were prepared for LC recording and i.c.v. drug administration. Nitroprusside infusion increased LC spontaneous discharge rate by 32 ± 5% (n=8) and this was completely prevented by local LC injection of a corticotropin-releasing factor (CRF) antagonist, α helical CRF_{9-41} (150 ng), but not saline. The excitatory amino acid antagonist, kynurenic acid (0.5 µmol, i.c.v.) did not alter LC activation by hemodynamic stress, although it completely blocked activation of the same cells by footshock. These results indicate that LC activation by hemodynamic stress requires CRF release within the LC, but not excitatory amino acid neurotransmission. Injection of 0.5 ml saline into the bladder increased bladder pressure by 72 ± 9 mmHg (n=14) and this was associated with a 57 ± 7 % increase in LC discharge rate. In contrast to LC activation by nitroprusside, LC activation by bladder distention was not blocked by an effective antagonist dose of α helical CRF_{9-41}. However, kynurenic acid (0.5 µmol, i.c.v.) almost completely prevented this activation indicating that like footshock, bladder distention increases LC discharge by an excitatory amino acid mechanism. Taken together, the results demonstrate that LC activation by two physiologic challenges, hemodynamic and visceral, is mediated by separate pathways that utilize different neurotransmitters. PHS Grants MH 40008, MH 00840, and MH 42796.

366.5

CONDITIONED FEAR STRESS INCREASED DOPAMINE AND SEROTONIN METABOLISM IN THE RAT MEDIAL PREFRONTAL CORTEX. T.Inoue*, T.Koyama, T. Ohmori, and I.Yamashita*. Dep. of Psychiatry and Neurology, Hokkaido Univ. Sch. of Med., Sapporo, Japan.

The effects of electric footshock stress(EFS) and conditioned fear stress(CFS) on dopamine and serotonin metabolism in seven various brain regions of the rat were examined by measuring dihydroxyphenylacetic acid (DOPAC),homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA). EFS for 30 min increased DOPAC and HVA levels in all brain regions and increased 5-HIAA levels in the medial prefrontal cortex (mPFC), nucleus accumbens and amygdala. CFS (exposure to an environment paired previously with footshock) increased plasma corticosterone levels and defecation, and induced freezing behavior. CFS increased DOPAC levels in the mPFC, paraventricular nucleus of the hypothalamus and lateral hypothalamus, and increased 5-HIAA level in the mPFC. In contrast with EFS which increased dopamine and serotonin metabolism in several brain regions, increased metabolism of both dopamine and serotonin was especially shown in the mPFC after CFS regarded as psychological stress.

366.6

ROLE OF LOCUS COERULEUS AND SEROTONERGIC DRUG ACTIONS ON SCHEDULE-INDUCED POLYDIPSIA. C.-S. Tung*, C.-C. Lu*,C.-J. Tseng, Y.-P. Liu* and T.-H. Yin. Dept. of Physiology and Pharmacology, National Defense Medical Center, PO BOX 90048, Taiwan, ROC.

As one of the hungry animal behavior, schedule-induced polydipsia (SIP), poses a general buffering property to reduce the heightened arousal produced by schedule of intermittent feeding paradigm, it provides an opportunity to study the regulation of central nervous system in stress coping reactions. We are interested in determining the functions of locus coeruleus (LC) and pharmacological actions of serotonergic 5 HT_2 analogues on this behavior. Levels of water intake, licking, and bar presses per minute were recorded as an index of SIP activities after rats exerted one hour performance of a fixed-interval one-minute operant pellet conditioning. Our results showed SIP was attenuated by 5 HT_2 agonist, DOI (0.1; 0.5; 1 mg/kg I.P.), and activated by 5 HT_2 antagonist, RIT (2.5 mg/kg I.P.). After bilateral LC lesions, SIP was attenuated and the activating effect of RIT was abolished. In addition, SIP was found decreased in a persistent way after bilateral LC lesions followed by bilateral ventral tegmental area lesions. Neurotoxin DSP-4 also induces an inhibitory action on SIP potency. We concluded that LC is involved in central control of SIP, and the modulating effects of 5-HT_2 receptors on SIP depend upon an integrity of LC functions.

366.7

PLASMA DOPA RESPONSES IN STRESS: DEPENDENCE ON SYMPATHONEURAL ACTIVITY AND TYROSINE HYDROXYLATION

R.Kvetnansky, I.Armando*, V.K.Weise*, K.Fukuhara*, A.Deka-Starosta*, I.J.Kopin and D.S.Goldstein*, Clinical Neuroscience Branch, NINDS, NIH, Bethesda, MD 20892.

Sources of dopa in plasma and the meaning of plasma dopa levels in terms of sympathoneural function have been unclear. This study comprehensively examined plasma concentration of DOPA, the catecholamines norepinephrine (NE), epinephrine, dopamine and the catechol metabolites during immobilization stress in conscious rats. Animals were pretreated with chlorisondamine to block ganglionic neurotransmission or α-methyl-para-tyrosine to inhibit tyrosine hydroxylation, and blood was obtained either via indwelling arterial cannula or by decapitation. Immobilization produced rapid, sustained increases in plasma levels of DOPA, catecholamines and catecholamine metabolites. Chlorisondamine decreased baseline plasma DOPA and NE levels and abolished increases in plasma DOPA and NE levels during immobilization. α-methyl-para-tyrosine administration produced sustained decreases in plasma DOPA levels and markedly attenuated immobilization-induced increases in plasma DOPA levels. Similar results were seen when blood was obtained by decapitation as when obtained by indwelling arterial cannula. Bilateral adrenalectomy augmented baseline plasma levels of DOPA and NE and also augmented DOPA and NE responses during immobilization. The results indicate that during immobilization stress, increased post-ganglionic sympathoneural outflow stimulates the synthesis of DOPA in sympathetic neurones and evokes release of DOPA into circulation.

366.8

SELECTIVE INDUCTION OF TYROSINE HYDROXYLASE (TOH) IN THE SYMPATHETIC GANGLIA AND ADRENAL GLAND OF RATS IN RESPONSE TO DIFFERENT STRESS. B. K. Kiran* and I. H. Ulus. Dept. of Pharmacology, Uludağ Univ. Medical School, Bursa, Turkey.

The increase in TOH activity in the individual sympathetic ganglion (SG) and in adrenal gland (AG) utilized as an index for increased impulse flow from the central nervous system to the sympatho-adrenal system under various stress situations. Rats were exposed to different stress for four days and they were killed on 5th day. AG and twentyfour SG were dissected for measurement of their TOH activity. Forced immobilization (6 hours/daily) increased TOH activity primarily in the lumbar (L_{1-6}) and the sacral (S_{1-3}) SG, and in AG. Immobilization failed to alter TOH activity in the thoracic SG. Exposure to hypercapnic environment (20% O_2+25% CO_2+55% N_2, 5 hours/daily) increased TOH activity primarily in the thoracic (T_{1-10}) SG, and in the first two lumbar ($L_{1,2}$) SG. When animals kept at cold environment (4°C;64 hours), TOH activity increased in, primarily, AG, and in the coeliac, L_2 and the cervical inferior SG. Hypoglycemia, induced by 2-deoxy-D-glucose (600 mg/kg, i.p.) administration, increased TOH activity only in AG. These data indicate that the increase in impulse flow from central nervous system to the sympathetic system and AG in response to stress is not uniform. Clearly, the response is showing very high regional selectivity depending upon the type of stress situation.

MODULATION OF NEUROTRANSMITTER RECEPTORS

367.1

FURTHER IN VIVO EVIDENCE FOR MULTIPLE SIGMA RECEPTOR SITES. Smriti Iyengar, S.J. Mick, V.M. Dilworth, N.M. Gray, T.S. Rao and P.C. Contreras. Searle Research and Development, G. D. Searle and Co., St. Louis, MO 63198.

The effects of three ligands having potent sigma receptor affinity, ifenprodil, opipramol and BMY 14802, were evaluated on four in vivo neurochemical parameters, ACTH release, prolactin release and dopamine metabolism in rat and NMDA-dependent cGMP increase in mouse cerebellum. All three ligands increased dopamine metabolism in A9 and A10 regions, increased prolactin release and ACTH release and inhibited NMDA receptor dependent cGMP increases. While these effects differed from those observed with other non-benzomorphan ligands, (+) 3PPP and (-) butaclamol, they were similar to those observed with benzomorphan sigma ligands, (+) NANM and (+) pentazocine (Iyengar et al., Neuropharmacol.in press). However, the increases in ACTH and dopamine metabolism caused by BMY 14802, ifenprodil and opipramol were not CPP-reversible, unlike the benzomorphan ligands. The three ligands also did not reverse the effects of (+) 3PPP, (-) butaclamol or (+) pentazocine, precluding the possibility that they were antagonists at sigma sites. In toto, the effects of various sigma ligands tested in the four in vivo parameters to date, fall into three separate categories, with BMY 14802, ifenprodil and opipramol behaving like functional NMDA antagonists. Taken together with the observation that these ligands also exhibit neuroprotective properties (abstract, Contreras et al., this meeting), unlike (+) 3PPP and (-) butaclamol, we postulate that these ligands mediate antiischemic activity via a unique sigma site. This site appears to be different from the site(s) mediating the effects of benzomorphan ligands or other non-benzomorphan sigma ligands like (+) 3PPP. In conclusion, the present data further supports the concept of multiple sigma sites.

367.2

CHOLESTEROL AND PREGNENOLONE FORMATION IN INTACT GLIOMA C6 CELLS: REGULATION BY MITOCONDRIAL BENZODIAZEPINE RECEPTOR LIGANDS (MBR). P. Guarneri, V. Papadopoulos*, B. Pan* and E. Costa. Fidia-Georgetown Institute for the Neurosciences and Dept. of Anatomy & Cell Biology, Georgetown University School of Medicine, Washington, D.C. 20007

Neurosteroids regulate GABA action on $GABA_A$ receptors. They act as positive (tetrahydroprogesterone) or negative (pregnenolone sulfate and dehydroepiandrosterone sulfate) allosteric modulators of $GABA_A$ receptors. In brain, the first step of neurosteroid biosynthesis occurs in glial cell mitochondria under the regulation of MBR. A novel system has been developed to study the regulation of steroid biosynthesis in glioma C6-2B cells. Cultures maintained in serum-free media were incubated with ^{3}H-Mevalonolactone (MVA) in the presence of various concentrations of cold MVA (0-100 nmoles) and in a time-related manner were analyzed for the formation of ^{3}H-cholesterol and ^{3}H-pregnenolone. Mevinolin (10 uM) was used as a specific inhibitor of 3-hydroxy-3-methylglutaryl Coenzyme A reductase to decrease endogenous MVA. In order to quantify pregnenolone synthesis, cells were preincubated for 30 min with the pregnenolone metabolism inhibitors trilostane and SU-10603. Steroids were extracted from media and cell cultures and then separated through Sep-Pak Silica cartridges and Si 60 Lichrosorb HPLC column. Under these conditions, the formation of cholesterol and pregnenolone was found to be dependent on the amount of substrate (MVA) added and occurred within seconds. Preincubation of the cultures with 4'-Chlorodiazepam (10^{-7} M), a specific PBR ligand, significantly increased the rate of cholesterol and pregnenolone synthesis.

367.3

PHOSPHORYLATION OF MITOCHONDRIAL BENZODIAZEPINE RECEPTORS. M.E. Whalin*, V. Papadopoulos*, E. Costa, and K.E. Krueger. Fidia-Georgetown Institute for the Neurosciences and the Dept. of Anatomy & Cell Biology, Georgetown University School of Medicine, Washington, D.C. 20007.

The mitochondrial benzodiazepine receptor (MBR) appears to be active in steroid biosynthesis stimulated by ACTH and other polypeptide tropic hormones. Since the action of these hormones in the acute stimulation of steroidogenesis is tranduced via activation of adenylate cyclase and cyclic AMP accumulation it was of interest to ascertain whether MBR is a substrate for protein kinase A activity. Using mitochondrial fractions from rat adrenals or the rat glioma C6 cell line, MBRs located in the outer mitochondrial membrane are phosphorylated in the presence of protein kinase A catalytic subunit. Phosphorylation is blocked by antibodies directed against the carboxyl terminal of MBR. Furthermore, a phosphorylated MBR adduct can be purified from mitochondrial membranes following digitonin solubilization and fractionation by hydroxylapatite and reverse-phase high pressure liquid chromatography. These findings demonstrate that MBR is a substrate for phosphorylation and suggest that phosphorylation/dephosphorylation of this protein might be involved in the regulation of steroidogenesis by ACTH which is apparently mediated by DBI, an intracellular protein.

367.4

ACETYLCHOLINE RECEPTOR ASSEMBLY IS STIMULATED BY cAMP DEPENDENT PHOSPHORYLATION OF ITS γ SUBUNIT W. N. Green*, A. F. Ross* and T. Claudio Department of Cellular & Molecular Physiology, Yale University School of Medicine, New Haven, CT 06510.

Different combinations of *Torpedo* acetylcholine receptor (AChR) subunits stably expressed in mouse fibroblasts were used to establish a role for phosphorylation in AChR biogenesis. When cell lines expressing fully functional AChR complexes ($\alpha_2\beta\gamma\delta$) were labeled with ^{32}P, only γ and δ subunits were phosphorylated. Forskolin, which causes a 2- to 3-fold increase in AChR expression by stimulating subunit assembly, increased unassembled γ phosphorylation, but had little effect on unassembled δ phosphorylation. The forskolin effect was rapid, significantly preceding its effect on expression. Of the two phosphorylated subunits, only γ was necessary for the cAMP stimulation of AChR expression. This was demonstrated by treating cell lines expressing $\alpha\beta\gamma$ or $\alpha\beta\delta$ complexes with forskolin and observing increased expression of only $\alpha\beta\gamma$ complexes. Forskolin also up-regulated the expression of $\alpha\gamma$ complexes, but not $\alpha\delta$ complexes in cells expressing just these subunits. We conclude that the cAMP-induced increase in expression of cell surface AChRs is due to phosphorylation of unassembled γ subunits which leads to increased efficiency of assembly of all four subunits, and in turn, an increase in AChR cell surface expression.

367.5

SELECTIVE DOWN-REGULATION OF D2 DOPAMINE RECEPTOR mRNA IN MOUSE STRIATUM FOLLOWING CONTINUOUS INFUSION WITH THE D2 DOPAMINE AGONIST QUINPIROLE. J.F. Chen, L.W. Zhou, and B. Weiss. Div. of Neuropsychopharmacology, Dept. of Pharmacology, Medical College of Pennsylvania/EPPI, Philadelphia, PA 19129.

Abnormal activity of dopamine receptors has been implicated in the development and treatment of schizophrenia and certain motor disorders. Accordingly, the pharmacological modulation of dopamine receptors at the level of gene expression may add substantially to our understanding of the molecular mechanisms underlying the pathophysiology and treatment of these diseases. In the present study, we examined the effect of continuously infusing the specific D2 dopamine receptor agonist quinpirole on the D2 dopamine receptor mRNA in mouse striatum and compared these molecular events with behavioral effects induced by this drug. Male mice were treated with quinpirole (8 umole/kg/hr) for 6 days via implanted Alzet minipumps. Stereotyped behaviors were measured during the course of treatment. The animals were sacrificed, total RNA from striatum was isolated, and D2 receptor mRNA was determined by Northern analysis using a synthetic radiolabeled oligonucleotide probe complementary to the D2 receptor mRNA. The results, which support previous findings from our laboratory, showed that continuously infusing quinpirole initially produced a stereotyped behavior which disappeared with 24 hr and remained low for the 6 days of infusion. At this time there was a significant reduction in the D2 receptor mRNA in striatum. Infusing the D1 agonist SKF38393 had little effect on the D2 mRNA. These results suggest that the down-regulation of stereotyped behavior induced by quinpirole is associated with a reduction in D2 dopamine receptor mRNA in corpus striatum. (Supported by MH42148).

367.6

DIFFERENTIAL MODULATION OF DOPAMINE RECEPTOR SUBTYPES BY PROTEIN KINASES C AND A. Ya Fang Liu, Olivier Civelli, and Paul R. Albert. Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Canada H3G-1Y6, and Vollum Institute for Advanced Biomedical Research, Portland, USA 97201.

The human dopamine-D1, -D2L, and -D2S receptors were expressed in mouse fibroblast Ltk- cells to compare their relative sensitivities to protein kinases C and A. The D1 receptor stimulated cAMP accumulation (10-fold), while both D2 subtypes inhibited cAMP. Each subtype, including D1, induced an increase of [Ca++]i via enhancement of phospholipase C activity in L cells. The rank order of potency of dopamine for the action on [Ca++]i was D2L>D2S>D1. D1-induced increases in PI turnover and [Ca++]i were enhanced (at least 2-fold) by prior activation (10'-3h) of protein kinase A with forskolin or 8-Br-cAMP, which did not alter the D2 receptor-mediated actions. In contrast, activation of protein kinase C completely abolished all actions of D1, D2S, and D2L on [Ca++]i, and inhibited D1-induced cAMP accumulation, but did not alter D2S/D2L-induced inhibition of cAMP. We conclude that the dopamine-D1 and D2 receptors induce multiple signal transduction pathways in L cells which are differentially modulated by protein kinases C and A.

367.7

NEURONAL EXPRESSION OF THE β-ADRENERGIC RECEPTOR KINASE. J.L. Arriza[1*], R.B. Simerly[2], T.M. Dawson[3], S.H. Snyder[3], M. Caron[1], and R.J. Lefkowitz[1*]. [1]Howard Hughes Med. Inst., Duke Univ. Med. Center, Durham, NC 27710; [2]Div. of Neurosci., Oregon Regional Primate Research Center, Beaverton, OR 97006; [3]Dept. of Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD 21205.

The β-adrenergic receptor kinase (βARK) phosphorylates the agonist-occupied form of the β-adrenergic receptor and other G-protein coupled receptors to regulate receptor function in the paradigm of desensitization. The dependence of this kinase activity on receptor occupancy suggests it may be of special importance in the context of synaptic transmission. To examine this hypothesis the rat homologues of two closely related βARK genes were characterized by molecular cloning and sequencing. The two gene products, βARK1 and βARK2, share 90% amino acid identity and both were demonstrated to phosphorylate purified β-adrenergic receptor. These kinase genes are predominantly expressed in the central nervous system; moreover, *in situ* hybridization histochemistry indicates that this expression is largely neuronal and that the βARK isoforms are to a great extent co-distributed. Although βARK1 mRNA is generally more abundant throughout the central nervous system, in a few regions, such as the substantia nigra and taenia tecta, cellular levels of βARK2 mRNA appear to be significantly higher. Antibodies to βARK1 and βARK2 were developed to determine their protein distribution by Western blot analyses and by immunohistochemistry. Immunoreactivity for both βARK proteins was found predominantly in neurons. This staining was present in cell bodies and fibers and, most interestingly, was present at the synapse. These data support the model in which βARK promotes the rapid desensitization of target neurotransmitter receptor systems and thus contributes to short-term synaptic plasticity.

367.8

MODULATION OF STRIATAL OPIOID BINDING SITES BY THE WEAVER GENE. Sandra E. Loughlin and Frances M. Leslie. Departments of Anatomy and Neurobiology and of Pharmacology, University of California, Irvine, California 92717.

The weaver mutant mouse undergoes a spontaneous, early postnatal loss of dopaminergic nigro-striatal neurons. This results in a near total loss of dopaminergic terminals in the striatum by the third postnatal week (Roffler-Tarlov, et al, 1984). Previous studies have suggested that a large percentage of opioid receptors are located on dopaminergic terminals. In the present study, striatal opioid receptor subtypes were examined in normal (B6CBA-$A^{w\text{-}J}$/A background, +/+) mice and in animals heterozygous (+/wv) and homozygous (wv/wv) for the weaver gene. Animals were sacrificed and their brains processed for autoradiographic localization of mu, delta and kappa opioid receptors using [^{3}H] DAGO, [^{3}H] DADLE (in the presence of D-PRO^4 morphiceptin) and [^{3}H]diprenorphine (in the presence of D-PRO^4 morphiceptin and DSLET), respectively. Nonspecific binding was determined with levallorphan. Data were analysed by quantitative densitometry.

In the weaver mouse, mu opioid receptors in patch and matrix compartments of the adult striatum were not significantly changed relative to nonmutant controls. Delta receptors were also unchanged. In contrast, kappa receptors were significantly decreased in the weaver striatum ($p < 0.001$). Opioid binding density in nucleus accumbens was unchanged in weaver with respect to control. The changes associated with early postnatal, genetically programmed cell loss differ from those we have observed following 6-OHDA lesion (Smith, et al, 1991). These data suggest that only a small percentage of striatal opioid receptors are located on dopaminergic terminals and that striatal opioid receptor subtypes are differentially modulated by loss of dopaminergic innervation.

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367.9

THE *MAS* ONCOGENE/ANGIOTENSIN RECEPTOR mRNA IS UPREGULATED BY SEIZURE. K.A. Martin and S. Hockfield. Sect. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The goal of the present study was to identify and characterize structural effector genes that are regulated by neuronal activity in the adult CNS. Specifically, we have analyzed the influence of seizure activity on the expression of the *mas* oncogene/angiotensin receptor mRNA in the adult rat brain. This gene was selected because of its oncogenic properties and its localization to hippocampal neurons, a population of cells that demonstrate activity-dependent changes in molecular, morphological, and physiological properties.

As a first step in determining if the *mas* oncogene is regulated by activity we have analyzed its transcription by *in situ* hybridization histochemistry and RNase protection assay, following pentylenetetrazole-induced seizures. *In situ* hybridization analysis revealed a consistent increase in *mas* expression in both the dentate gyrus and CA fields of the hippocampus. No increase was detectable in any other brain regions. *Mas* mRNA levels were not elevated 1h post seizure, were increased at 2, 4, and 6 h and had returned to baseline levels by 24h following seizure. No change in the level of neurofilament mRNA was observed over this time course. The effect of activity on *mas* mRNA levels was confirmed and quantified by RNase protection analyses; a significant increase was observed 6h post seizure (mean increase=64%, t_4=3.288, $p<0.03$).

The observation that transcription of the *mas* oncogene/angiotensin receptor can be regulated by neuronal activity suggests that *mas* could have an important function in producing changes in cellular phenotype in response to neuronal activity. [Supported by the NSF.]

CEREBELLUM I

368.1

CAFFEINE SENSITIVE CALCIUM STORES IN CULTURED CEREBELLAR PURKINJE NEURONS. J.R. Brorson, D. Bleakman, S.J. Gibbons, and R.J. Miller. Department of Physiological and Pharmacological Sciences, University of Chicago, Chicago IL 60637.

Cerebellar Purkinje neurons contain high levels of the inositol 1,4,5-trisphosphate (IP_3) receptor and the ryanodine receptor, which are each thought to be associated with intracellular Ca^{2+} stores. We have examined the properties of such stores in cultured rat cerebellar neurons taken from 16 day rat embryos. In this system, about one half of the neurons could be identified as Purkinje cells as indicated by staining for the Ca^{2+} binding protein calbindin D-28k, as well as for the IP_3 and ryanodine receptors. In double immunofluorescent staining, the IP_3 and ryanodine receptors primarily colocalized with staining for calbindin. Caffeine (10mM) released Ca^{2+} from intracellular stores in about one half of the cultured neurons. This effect could be repeated only if the stores were reloaded by a depolarization-induced elevation in $[Ca^{2+}]_i$. Ca^{2+} release by caffeine was abolished by prolonged application of ryanodine (10μM). Furthermore, we demonstrated that the caffeine sensitive Ca^{2+} stores could regulate electrophysiological events in some cells, altering patterns of spontaneous action potential firing and modulating the size of afterhyperpolarizations following evoked action potentials.

368.2

ACTIVITY INDUCED EXPRESSION OF CEREBELLAR CGRP BINDING SITES. A. ROSINA*, S. MORARA*, L. PROVINI*[1] and G. FORLONI[2] (SPON:ENA). Ist. Fisiol. Centri Nervosi CNR, [1]Ist. Fisiol. Gen. Chim. Biol. Fac. Farm., [2]Ist. M. Negri, Milano, Italy.

High affinity binding sites for calcitonin gene-related peptide (CGRP) are present in the molecular layer of the adult cerebellum, while CGRP-like immunoreactivity can be detected in the olivocerebellar system, only at neonatal stages. We have hypothesized that the expression of CGRP binding sites can be modulated by climbing fiber activity.

Adult rats were treated with harmaline, a drug known to increase the firing rate of olivary neurons, at tremorogenic doses (25 mg/Kg, every 3h, for 12 to 36h). Quantitative autoradiography was used to assess the density variations of CGRP binding sites. The CGRP recognition site was labelled using I125-CGRP (human -CGRP).

Rats treated for 12h and allowed to survive 24h after the treatment showed a significant increase (+13%, $p<0.05$) of specific CGRP binding sites in the molecular layer. Further increases were recorded after longer treatments (e.g. 33%, $p<0.01$, after 36h). These results give evidence that the activation of climbing fibers induced by harmaline is able to modulate the expression of cerebellar CGRP binding sites.

368.3

PURKINJE CELL HETEROGENEITY IN FOUR CEREBELLAR MUTATIONS REVEALED BY ZEBRIN I EXPRESSION. C. Sotelo and M. Wassef*, INSERM U. 106, 75013 Paris (France)

The differential expression of zebrin I, an adult marker of Purkinje cell (PC) heterogeneity (Hawkes et al. 1985, Brain Res. 333 : 359), has been immunohistochemically studied in four cerebellar mutant mice : weaver (wv), reeler (rl), meander tail (mea) and staggerer (sg). Despite the severe cortical disorganization which characterizes the cortex of these granuloprival cerebella, the pattern of zebrin I expression in wv, rl and mea is normal, whereas it is abnormal in sg. Thus, for example in the anterior lobe, the only cortical region affected in mea, the absence of granule cells (wv and mea) does not prevent the normal expression of zebrin I into three sagittal bands of clustered PCs: one medial and two symmetric lateral bands. In rl, zebrin I parcellation is also shared by the bulk of PCs remaining in the white matter (central mass). In sg -a mutation affecting only PCs- the pattern of zebrin I expression is abnormal because none of the 20% remaining PCs is zebrin I+. These observations indicate that PC heterogeneity resulting in cortical parcellation is an intrinsic property of developing PCs.

(The authors are grateful to Dr R. Hawkes for the gift of the mab Q113).

368.4

THE ORGANIZATION OF FIBRES WITHIN THE RAT BASIS PEDUNCULI M. Glickstein, I. Hans, C. Legg, B. Mercier, M. Ramna-Rayan and E. Vaudano, Department of Anatomy, University College London, Gower Street, London WC1E 6BT.

Small volumes of wheatgerm agglutinin HRP were injected into discrete areas of the cerebral cortex of rats. After two days survival time the brains were processed to reveal the location and extent of labelled fibres within the peduncles. In rats, unlike primates, the basis pedunculi fibres arise from the entire cerebral cortex. There is an orderly organization of fibres within the peduncles. Fibres originating from cells in the frontal cortex maintain a position in the ventromedial part of the peduncles. Those from the occipital and temporal cortex travel in the most dorsolateral portion. Somatosensory fibres are between these two. There is a high correlation between the volume of the peduncles which contains efferent fibres and the relative density of corticopontine cells in different regions of cortex. The organization makes it possible to study the behavioral effects of lesions which interrupt selectively the input to the cerebellum from different cortical areas.

368.5

CHARACTERIZATION OF THE GOLDFISH VESTIBULO-CEREBELLUM. A.M. Pastor*, R.R. de la Cruz*, J.I. Simpson and R. Baker. Dept. of Physiol. and Biophys., NYU Med. Ctr., New York, NY 10016.

The afferent and efferent organization of the goldfish vestibulocerebellum was examined by means of intra- and extracellular injection of biocytin. Axons of vestibular neurons originating either from the semicircular canals or the descending and anterior octavus nuclei terminated ipsilaterally in the eminentia granularis pars lateralis as claw-like boutons. Cerebellar injections comprising the caudal half of the corpus cerebelli revealed two main cerebellofugal targets, namely the ipsilateral vestibular nuclei and the mesencephalic perioculomotor region. No terminals were seen in the oculomotor complex. Parallel fiber bundles originating from the injection site extended transversally to the contralateral vestibulo-cerebellum. The ipsilateral nuclei of the accessory optic system and contralateral inferior olive also sent afferents to the vestibulo-cerebellum. Purkinje cells, physiologically identified by their visually evoked climbing fiber response, terminated in the descending vestibular nucleus. Single unit recordings of Purkinje cells during optokinetic stimulation demonstrated reciprocally related simple/complex spike responses that were greatest for whole field rotation about axes orthogonal to the semicircular canal planes. Two types of Purkinje cell discharge patterns were seen during visual-vestibular interactions about the vertical axis. In one case, Purkinje cells coded the vectorial addition of head and eye velocity, with a slight saccadic and eye position sensitivity. The second type of Purkinje cell showed a discharge pattern related solely to eye movements. These data establish a structural and functional basis for further investigating cerebellar regulation of the goldfish vestibulo-ocular reflex.

368.6

INACTIVATION OF THE DEEP CEREBELLAR NUCLEI AFFECTS VISUO-MOTOR ADAPTATION IN A VISUALLY-GUIDED TRACKING TASK.

RC MIALL*, GK KERR, JF STEIN* & J PHILLIPS. University Lab. of Physiology, Parks Road, Oxford OX1 3PT, U.K.

The cerebellum receives a large amount of visual input and is involved in motor coordination. It is not yet clear to what extent it contributes to transformation between visual and motor coordinate frames, or to recalibrating these signals for different tasks. We therefore tested a monkey's ability to perform a visually guided tracking task and to adapt to changes in the task's visual or motor aspects during reversible inactivation of the dentate or interposed nuclei.

The monkey used a joystick to follow a target displayed on a computer monitor. The motor aspects of the task were altered by increasing joystick gain, so that the arm excursion was reduced while visual inputs remained unchanged (GAIN). Alternatively, visual aspects were altered by increasing the excursion of the visual display of both target and cursor while the required arm movement remained unchanged (SCALE). Hence in both tasks the vision-to-motor ratio increased to 150% of normal; in the GAIN task the required movement changed whereas in the SCALE task it did not.

Infusion of lignocaine (7.5%, 4µl/min, <12 mins) into dentate nucleus impaired the monkey's adaptation to the GAIN change, while adaptation to the SCALE change was unaffected. Infusion into the anterior interposed nucleus affected both tasks, but the SCALE change was more severely affected. These results suggest that the process of transforming visual signals into motor responses is assisted by the cerebellum, and that visual and motor aspects of this process may be separate.

368.7

HEMICEREBELLECTOMY INDUCES SPATIAL MEMORY DEFICITS IN RATS. M. Molinari, L. Petrosini+*, M.E. Dell'Anna*, S. Giannetti*. Inst. of Neurology, Catholic University, Rome and +Dept. of Psychology, University of Rome "La Sapienza", Rome.

The present study was aimed at investigating the effects of cerebellar lesions on spatial memory abilities in rats. Rats were hemicerebellectomized (Hcbed) on the right side after deep anaesthesia at two developmental stages, namely at birth (postnatal day two) or in adulthood. Spatial memory abilities have been tested in adult animals by means of maze tests performed in water in order to overcome locomotory deficits due to the cerebellar lesion. In fact, while Hcbed rats present an impaired locomotion they are very skillful in swimming. Therefore, spatial abilities were test in a water T maze and in a Morris water maze. In both experimental groups, although in a different degree, clear deficits in solving the spatial tasks, that could not be related to the motor deficits, were present. The present report indicates that Hcb in rats affects spatial memory abilities.

368.8

A CHOLINERGIC PATHWAY TO THE DORSAL CAP OF THE INFERIOR OLIVE OF THE RAT. N.H. Barmack, A. Burleigh*, P. Errico* and M. Fagerson*, RS Dow Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97209.

The inferior olive is divided into several subnuclei that receive specific sensory information. For example, The caudal dorsal cap of the medial accessory subdivision of the inferior olive receives horizontal optokinetic information from the nucleus of the optic tract (NOT). The immediately subjacent β nucleus receives vertical vestibular information mediated by a GABAergic pathway from the descending and medial vestibular nuclei (DVN, MVN). None of the transmitters to the dorsal cap have been identified.

Using choline acetyltransferase (ChAT) immunohistochemistry, we have identified a cholinergic pathway that terminates in the dorsal cap. Following a microinjection of HRP into the dorsal cap, we observed HRP positive, but no ChAT positive cells in the NOT. However, both ChAT and HRP-labeled neurons were observed in the MVN and DVN as well as the nucleus prepositus hypoglossi (NPH). We made lesions in the vestibular nuclei by ibotenic acid injection (100-400 nl) to destroy unilaterally the presumed cells of origin of this pathway. Lesions in the caudal-medial aspect of the MVN and NPH caused an ipsilateral loss of ChAT staining in the dorsal cap. Lesions placed more laterally within the MVN or DVN failed to cause this ipsilateral loss of ChAT staining. We also injected iontophoretically the orthograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) into the vestibular complex. PHA-L injections into the NPH and MVN, but not the DVN labeled terminals within the dorsal cap. Injections in the MVN and DVN labeled the β nucleus.

In sum, the dorsal cap receives a cholinergic pathway originating from the NPH-MVN. Physiological studies will reveal how this cholinergic pathway interacts with optokinetic inputs mediated by a different transmitter to these same dorsal cap neurons.

368.9

THE ROLE OF SUBTHRESHOLD OSCILLATIONS IN SYNCHRONIZING NEURONAL ELECTRICAL ACTIVITY. I. Lampl* and Y. Yarom. Dept. of Neurobiology, Life Science Institute, Hebrew University. Jerusalem ISRAEL.

In our first description of membrane potential oscillations in olivary neurons it was pointed out that these subthreshold oscillations occur simultaneously and in phase in a large number of neurons. It has therefore been postulated that this phenomenon gives rise to the synchronized suprathreshold activity so characteristic of complex spike activity recorded from cerebellar Purkinje cells. In the present study the role of such subthreshold activity in synchronization of olivary activity has been studied by analyzing the integrative process that sums synaptic potentials with subthreshold sinusoidal membrane oscillations.

The work was carried out on *in vitro* olivary neurons utilizing the coronal brainstem slice technique. A gated sinusoidal current source was used to elicit subthreshold sinusoidal oscillations of the membrane potential with a peak to peak amplitude of 5-10mV and frequencies of 3-9 Hz. Bipolar metal electrodes were placed at the slice midline and used for stimulating presynaptic axons. The responses to such stimuli were characterized as short latency (1msec), fast rise time (2 msec) synaptic potentials followed by a slower wave (100 msec rise time). The maximum amplitude was about 10mV. At a given stimulus intensity the responses were reliably reproducible. The synaptic potentials were evoked at different phase relations during the sinusoidal oscillations.

A non-linear summation of the sine wave and the synaptic potential was found to occur in olivary neurons; when the synaptic potential was elicited at the trough of the sine wave or during the rising phase, a superlinear summation occurred. Furthermore, the maximum amplitude of the response occurred at the peak of the sine wave regardless of the exact time of stimulation. On the other hand, when the synaptic potentials were evoked during the falling phase of the wave, a less then linear summation occurred.

These results demonstrate the prominent role of the subthreshold activity in determination of the precise timing of the output signals, thereby synchronizing the electrical activity in the olivary nucleus.

368.10

ALTERATION OF COMPLEX SPIKE SYNCHRONICITY BY MICRO-INJECTION OF TEA AND 5-HT INTO THE INFERIOR OLIVE STUDIED WITH MULTIPLE ELECTRODE RECORDINGS. I. Sugihara, E. J. Lang, and R. Llinás. Dept of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, N.Y. 10016.

Shunting of dendro-dendritic electrotonic coupling in the olivary glomeruli by the GABAergic cerebellar nucleo-olivary projection has been proposed to underlie the spatial and temporal modification of synchronization of inferior olive (IO) neurons. In previous studies, blockade of this GABAergic system either by microinjection of picrotoxin into IO or cerebellar nuclear lesion resulted in a significant increase of the synchrony in complex spike (CS) activity throughout a given cerebellar lobule. To further investigate whether the spatial pattern of synchrony is modulated only by the GABAergic afferents to the olivary glomeruli, the excitability of IO neuron was altered by injection of K channel blockers to the IO. The excitability change was determined by multiple electrode recordings of the spontaneous CS activity from crus 2a Purkinje cells of adult anesthetized rats. Microinjection of TEA (20 mM, 1 µl) increased the average firing frequency of CS 1.5 - 4 times, but the rostro-caudal banding pattern of Purkinje cell CS activity stayed unchanged. This implies that neither the excitability nor the input resistance of the neuron specifically affect the spatial pattern of synchronicity. Microinjection of 5-HT (1 mM, 1-2µl), however, produced moderate increases (two fold) in the CS synchrony throughout the lobule without changing the average CS firing frequency. Since 5-HT terminals are known to distribute mainly to distal dendrites (King et al., 1984), which give rise to most of the spines forming the olivary glomeruli (Ruigrok et al., 1990), and since 5-HT decreases K conductance in IO neurons (Llinás and Yarom, 1986), the effect of 5-HT may be considered to be an enhancer of electrotonic coupling through the olivary glomeruli.

368.11

MULTIELECTRODE RECORDINGS OF COMPLEX SPIKE ACTIVITY AND THEIR RELATIONSHIP TO SPONTANEOUS AND CORTICALLY EVOKED VIBRISSAL MOVEMENTS IN THE RAT. E. J. Lang, I. Sugihara, J. P. Welsh and R. Llinas. Dept of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, N.Y. 10016.

The function of the inferior olive (IO) is hypothesized to be that of a timing element important in the coordination of movements. Consistent with this idea, complex spikes (CS) have been found to occur ≈10 msec prior to onset of lip and tongue movements (Fukuda et al: Soc Neurosci Abst, 1987). The present experiments examined the relationship between vibrissal movements and the CS of Crus IIa Purkinje cells (PC). The onset of vibrissal movements was detected with a photosensor while simultaneous multielectrode recordings of CS were made. CS activity was found to increase ≈10 msec prior to the onset of vibrissal movements and remained high until ≈15 msec after onset.

The synchronous discharge of CS prior to movement onset may act to facilitate on going activity in other CNS areas, leading to the proper execution of a movement. To investigate this hypothesis, electrical stimulation of the vibrissal region of the motor cortex was used to evoke movements of the vibrissa. The amplitudes of the evoked movements were measured before and after microinjection of drugs into the IO. Microinjections (1μL of 1 mg/ml) of harmaline or picrotoxin into the IO resulted in a 4.5 and 6.6 -fold increase, respectively, in the amplitudes of the evoked movements. Subsequent injection of 4% lidocaine reduced the amplitude approximately to control values. In cerebellectomized rats, microinjections of harmaline or picrotoxin had no effect on movement amplitude. These experiments suggest that the synchronicity of CS may combine with activity from motor cortex to help determine the amplitude of a movement. Supported by NIH grant NS13742.

368.12

MULTIELECTRODE RECORDING OF PURKINJE CELL COMPLEX SPIKES DURING CONDITIONED TONGUE PROTRUSION IN THE AWAKE RAT. J.P. Welsh, I. Sugihara, E.J. Lang, and R. Llinás, New York Univ. Med. Ctr., New York, NY 10016.

Rats were trained to protrude their tongue 7 mm in response to a 750-ms, 2-kHz tone stimulus. Training was accomplished via a 2-stage procedure consisting of 6 d of autoshaping followed by 70 d of operant conditioning with sucrose solution as the reinforcer. Increasing lengths of tongue protrusion were shaped by successive approximation during the operant phase. Tongue and mouth movements were simultaneously monitored by infrared photodetectors. Mouth opening and tongue protrusion were found to occur in a highly coordinated and stereotypic fashion, with both behaviors demonstrating a robust 7-8 Hz rhythm. Complex spikes (CSs) within cerebellar folium Crus IIa were recorded from trained rats chronically prepared for the simultaneous recording of up to 40 Purkinje cells. In the majority of Purkinje cells, the probability of CSs increased approximately 200 ms after tones that elicited tongue protrusion but not after tones that did not elicit tongue protrusion. The maximal probability of CSs occurred 75 ms before maximal tongue protrusion - approximately the time of mouth opening. The results support the hypothesis (Llinás & Sasaki, Eur. J. Neurosci., 1990) that the olivo-cerebellar system operates in real-time to ensure the proper coordination of movement. (Supp. NIH NS13742 and NS08844)

368.13

FUNCTIONAL GEOMETRY OF PURKINJE CELL POPULATION RESPONSES AS REVEALED BY NEUROCOMPUTER ANALYSIS OF MULTI-UNIT RECORDINGS. Pellionisz, A.J. and Bloedel, J.R., NASA Ames Research Center, VRF 242-3, CA 94035 and Barrow Neurological Institute, 350 West Thomas Rd, Phoenix, AZ 85013

Approximately a decade ago a geometrically framed hypothesis was formulated portraying sensorimotor function as a process requiring generalized coordinate transformations that utilize non-Cartesian vectors expressed in frames of reference that are intrinsic to biological systems. The goal of this study was to experimentally determine the geometric, non-Euclidean properties characterizing the population responses of neurons in specific CNS nuclei and to relate those properties to the geometrical theory. For this purpose responses of up to 10 simultaneously recorded Purkinje cells were investigated during perturbed and unperturbed locomotion in acutely decerebrated cats.

The population responses were analyzed based on the method for calculating the non-Cartesian axes from the cross-correlogram of the neural activity of n neurons, a procedure that also yields the matrix characterizing the covariant metric tensor *(AJP, '88 in Cotterill, RM. "Comp. Sim. in Brain Sci.", Cambridge UP)*. Using our transputer-based neurocomputer, the Moore-Penrose generalized inverse was used to derive the contravariant metric tensor characterizing the geometry of the population response. Responses were recorded to intermittent perturbations of the forelimb as well as during the acquisition and performance of the movement conditioned to avoid an obstacle.

The results indicate that the geometry describing the population responses is expressed in non-Cartesian coordinates and that the characteristics of this geometry are comparable to characteristics of the locomotor movement. Furthermore the data reveal that the geometry of the responses is modified when the characteristics of the movement are changed either by perturbing the swing phase of the ipsilateral forelimb or by changing the treadmill speed. The initial findings suggest that the derived geometry of the population responses may be the basis for reconstructing physical invariants such as movement direction and distance. If so, this theoretical-experimental method is a candidate for deciphering the neural code internally representing invariants of the external world. *Supported by NASA DDF-T4967 (AJP) and NS21958 (JRB)*

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS I

369.1

GLIA CULTURED FROM HYPEREXCITABLE HUMAN EPILEPTOGENIC FOCI EXHIBIT MARKED INCREASES IN $CA^{2+}I$ OSCILLATIONS AND INTERCELLULAR CA^{2+} WAVES. A. H. Cornell-Bell, M. Gunel*, P.G. Thomas*, M.L. Brines*, D.D. Spencer, N. de Lanerolle*, Cell Biology, Internal Medicine, Neurosurgery, Yale Univ Sch. of Med., New Haven, CT 06510.

Tissues surgically removed from epileptogenic foci of patients with intractable epilepsy were grown in 10% FBS-DMEM-F12. Cells were obtained from: tumor (low grade glioma) and adjacent hyperexcitable and normal cortex (judged by electrical recordings). Cultures were stained for neurofilaments, GFAP, tetanus toxin, A2B5, and neuron-specific enolase. After 4-6 weeks astrocyte-enriched coverslips were loaded with the Ca^{2+} indicator Fluo-3AM and time-lapse images were collected using a Bio-Rad MRC 600 confocal scanning laser microscope. Normal cortical astrocytes exhibit dynamic, spontaneous $Ca^{2+}i$ oscillations which are more frequent than those previously studied from rat hippocampal astrocytes (Cornell-Bell and Finkbeiner, 1991). Extensive waves and oscillations (Period= 2.8 ± 0.1sec) develop following superfusion of 100μM glutamate at an elevated frequency over baseline (period = 5.1 sec ±0.3 sec). Astrocytes from hyperexcitable focus exhibit spontaneous $Ca^{2+}i$ oscillations and waves with an accelerated frequency (period= 0.94 ± .09 sec). Following glutamate this frequency is accelerated slightly (period = 0.85 ± 0.1 sec). In dramatic contrast cells derived from tumor show few spontaneous oscillations (period =4.4 ± 0.08 sec) and following glutamate respond with an initial spike and an attenuated $Ca^{2+}i$ response (period = 6.0 ± 0.08 sec). Inherent differences in glutamate-initiated responses persist in these cells after extended culturing suggesting the astrocyte may play an active role in development or maintenance of the seizure focus.

369.2

PRIMARY CULTURES OBTAINED FROM ADULT HUMAN EPILEPTIC BRAIN CONTAIN DIVERSE NEURONS AND GLIA N. de Lanerolle, M. Gunel*, D. D. Spencer, Paul Q. Trombley, and M. L. Brines, Yale University School of Medicine, New Haven, CT 06510

The controlled environment during brain resection for treatment of intractable seizures offers a unique potential to examine fresh tissue obtained from a variety of normal and hyperexcitable brain regions. We wondered whether viable primary cultures could be established from this adult tissue, and whether the surviving cells exhibit any unique characteristics. To study this possibility, tissue was obtained from the operating field on ice, dissociated by mild enzymatic/mechanical disruption, plated onto coated coverslips, and maintained in either serum-enriched or defined media. At several days in vitro the cultures exhibited a varied population of cells, including many of neuronal morphology. Immunohistochemical staining for specific cell markers confirmed that a mixed population of neurons and glia were present, and patch-clamp recording documented intact neuronal activity. This neuronal pool was further characterized by immunohistochemical staining for NPY, somatostatin, and GABA; NPY was found to be widespread. These primary cultures appear to express functional receptors and exhibited striking responsiveness to activation of the protein kinase A second messenger system by dcAMP. We conclude that primary cultures are readily established from surgical specimens with a surprisingly complex and functional population of cells, including neurons. The cells are viable and responsive for at least several weeks in vitro and are thus potentially important for study of normal and pathologic physiology.

369.3

WHOLE-CELL RECORDINGS FROM HUMAN DENTATE GRANULE CELLS IN SLICES PREPARED FROM EPILEPTIC HIPPOCAMPAL SPECIMENS. M. Isokawa and M.F. Levesque. Brain Research Institute and Dept. of Neurology and Neurosurgery, CHS, UCLA, Los Angeles, CA 90024-1761.

Passive membrane properties and synaptic responses of human dentate granule cells were studied using patch electrodes and whole-cell recording from slices (500 μm) of surgically-resected epileptic hippocampus. In current clamp mode, the input resistance was 165-199 MΩ, and time-dependent rectification was observed with high levels of hyperpolarizing current pulses. Action potentials with amplitudes of 89-104 mV were accompanied by depolarizing afterpotentials. Excitatory and inhibitory PSPs (5.4-16.0 mV in amp., 99.5-402 msec in dur.) were generated by perforant path stimulation. Spontaneously-occurring prepotentials were also present. In voltage clamp mode, perforant path stimulation produced inward currents with amplitudes of 42-54 pA and durations of 70-135 msec at a holding potential (Vm) of -65 mV. The currents revealed dual peaks at more negative Vm. When bicuculline (10μM) was added to the external solution, the inward currents were increased to 67-163 pA with durations of 128-178 msec (Vm = -45 to -30 mV). This suggests the presence of $GABA_A$ receptor-mediated IPSCs possibly masking a large EPSC component especially at low levels of negative Vm. Further studies are in progress to provide a detailed description of excitatory and inhibitory synaptic currents in human epileptic hippocampal neurons. Supported by NIH Grant NS02808.

369.4

CONTROL OF EPILEPTIFORM BURST RATE BY THE ACTION OF THE ANTICONVULSANT 4-HYDROXY-4-PHENYLHEXAMIDE ON THE AFTERHYPERPOLARIZATION OF THE CA3 HIPPOCAMPAL CELLS.
M.F. Pacheco, A.M. Flores* and Z.J. Aguirre*. *CUIB Universidad de Colima, Apdo. Post. 199, Colima, Col. 28000 Mexico.*

The goal of the present study was to explore the mechanisms of action of the anticonvulsant 4-hydroxy-4-phenylhexamide (HPH), which has been recently evaluated pharmacologically (Meza-Toledo et al., Arzneim-Forsch./Drug Res. 40:1289-1291, 1990). Epileptiform activity was induced on guinea-pig hippocampal slices by a 15 min perfusion of 10 μM bicuculine (final concentration on normal Kreb's saline solution), and extracellular recordings were made with an AC-coupled electrometer with glass microelectrodes (2 M NaCl, 5-10 Mohms) positioned on the pyramidal stratum of the CA3 region. The effects of HPH (10 - 500 μM on normal solution, for 6 min) on burst frequency, coastline burst index, and the epileptiform burst afterhyperpolarization (AHP) were analyzed by computer programs from digitized records. HPH showed a long lasting (1-2 hr) inhibitory action on the burst frequency which was dose-dependent up to 200 μM of HPH, at which concentration the epileptiform activity was totally blocked. Such effects were correlated with an increase in the amplitude of the AHP, without a change in the number and amplitude of the spikes per burst. These results suggest that HPH might exert an anticonvulsant action by exerting a control on the epileptiform burst rate through an increase on the AHP.
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369.5

FLUNARIZINE BLOCKS KCL-INDUCED Ca^{2+} INFLUX INTO CEREBELLAR GRANULE CELLS THROUGH AN EFFECT ON GLUTAMATE-GATED Ca^{2+} CHANNELS. G. A. Skeen, H. H. Wolf, E. A. Swinyard, and H. S. White. Anticonvulsant Drug Development Program, Dept. of Pharmacol. & Toxicol., Univ. of Utah, S.L.C., UT 84108

The Ca^{2+} channel blocker flunarizine is a diphenylpiperazine with proven efficacy as an add-on anticonvulsant in a number of clinical trials involving therapy-resistant epileptic patients. The anticonvulsant mechanism of action of flunarizine is thought to be related to its ability to block the T-type voltage-sensitive Ca^{2+} channel (VSCC; Wang et al., J.P.E.T., 254:1006-1011, 1990). The present investigation was initiated in order to assess the effect of flunarizine on KCl-induced Ca^{2+} influx into primary mouse cerebellar granule cells (GC's). Granule cells grown on 25 mm aclar coverslips and maintained in culture for 7-9 days were loaded with indo-1 (5 μM) for 20 min. Depolarization of GC's with elevated KCl (55 mM) resulted in a marked increase in the free $[Ca^{2+}]i$. This effect of KCl was dependent on the presence of extracellular Ca^{2+} and was blocked by flunarizine in a concentration-dependent and irreversible manner. In contrast, this effect of KCl was not affected by pretreatment with the L-, N-, or T-type channel blockers nimodipine, w-conotoxin, or ethosuximide, respectively. The lack of effect with these classical VSCC blockers is consistent with the conclusion of Jalonen et al., (Br. Res., 535:33-38, 1990) that GC's do not possess VSCC's. Subsequent studies demonstrated that glutamate (1 mM) induces a $[Ca^{2+}]_i$ transient comparable to that of KCl and that flunarizine blocks glutamate-induced $[Ca^{2+}]_i$ transients at concentrations similar to those which block KCl-induced $[Ca^{2+}]_i$ transients. Further studies demonstrated that flunarizine blocks $[Ca^{2+}]_i$ transients induced by the glutamate agonists NMDA (100 μM), quisqualic acid (100 μM) and kainic acid (100 μM). These results suggest that the KCl-induced $[Ca^{2+}]_i$ is mediated by glutamate which is released from GC's upon depolarization with elevated K^+, and that flunarizine blocks glutamate's effects. Supported by NIH Contract NO1-NS9-2328 (EAS & HHW) and NIH Grant 2RO1-NS22200 (HSW) from the NINDS.

369.6

7-CHLOROKYNURENIC ACID ANTAGONIZES THE ANTICONVULSANT ACTIVITY OF D-CYCLOSERINE IN MAXIMAL ELECTROSHOCK SEIZURES. S.L. Peterson. Dept. of Medical Pharmacology and Toxicology, Texas A&M University, College Station, Texas 77843.

D-Cycloserine is a partial agonist of strychnine-insensitive glycine receptors that freely passes the blood-brain barrier. The purpose of this study was to characterize the anticonvulsant activity of D-cycloserine in maximal electroshock seizures (MES).

MES was induced in male Wistar rats by passing a 60 Hz, 150 mA and 0.2 second duration current through saline-soaked corneal electrodes. Seizure severity was quantified by tonic hindlimb extension (THE).

The calculated ED_{50} for D-cycloserine (i.p., 1 hour) was 154 mg/kg (Litchfield and Wilcoxon analysis). None of the eleven rats treated with 250 mg/kg L-cycloserine was protected from THE. Intracerebroventricular administration of 100 μM 7-chlorokynurenic acid (specific strychnine-insensitive glycine receptor antagonist) reversed the anticonvulsant activity induced by 300 mg/kg D-cycloserine ($P < 0.05$, maximum likelihood logistic regression analysis).

The stereospecific activity and antagonism by 7-chlorokynurenic acid are evidence that D-cycloserine may act at the strychnine-insensitive glycine receptor to inhibit MES. (Supported by NIH grant 24566).

369.7

INCREASED GLUCOSE METABOLISM ASSOCIATED WITH "INTERICTAL" EEG SPIKING IN THE BICUCULLINE METHIODIDE MODEL IN RAT. A. Handforth and D.M. Treiman*. DVAMC West Los Angeles and Dept. of Neurology, UCLA, Los Angeles, CA 90024.

There is considerable debate regarding what are "interictal" vs. "ictal" epileptiform discharges on EEG and much uncertainty about neural mechanisms of the interictal-ictal transition. Increased local cerebral glucose utilization has been employed as an indicator of "ictal" activity. We studied the effect of bicuculline methiodide (BM) on EEG and cerebral glucose metabolism using ^{14}C-2-deoxyglucose (2DG) in 29 adult male Sprague-Dawley rats. 4 epidural screw electrodes were surgically implanted over cerebral cortex in each animal. 2-4 weeks later 135-270 mg/kg BM was given i.p., followed by 25 μCi 2DG i.v. EEG was recorded continuously. 10 min after 2DG the brain was removed and sections applied to x-ray film. 5 BM animals had normal EEGs; 2DG revealed mild cortical injury under the epidural electrodes but no hypermetabolism. Of 12 BM animals demonstrating focal "interictal spiking" on EEG but no convulsive activity, 7 had focal hypermetabolism in the area of the EEG spikes. Of 12 animals with convulsions and generalized forebrain hypermetabolism, 9 had more intense hypermetabolism under one or more epidural electrodes. These data show that increased glucose utilization can occur with interictal spiking. This model also provides a useful paradigm to study seizure initiation and spread.

369.8

MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF INTRACTABLE EPILEPSY IN CHILDHOOD
A.C onnelly, JH Cross, DG Gadian, GD Jackson, FJ Kirkham, and F Vargha-Khadem
Institute of Child Health and Hospital for Sick Children, Gt.Ormond St.,London

Abnormalities of the hippocampus and temporal lobe are common in intractable focal epilepsy. Magnetic resonance imaging (MRI) has allowed reliable assessment of the mesial structures, particularly the hippocampus. If 1H magnetic resonance spectroscopy can monitor neuronal loss or damage through changes in the N-acetylaspartate (NAA) signal, then a combined MRI / MRS examination could contribute significantly to the localisation of epileptic foci and to our understanding of the relationship between neuronal damage and abnormal function.

MRI and MRS were carried out on 10 fully evaluated children with intractable complex partial seizures. In 8 cases, imaging showed hippocampal or temporal abnormalities, and in 7 of these MRS showed reduced NAA / Creatine ratios. There was definite evidence of bilateral abnormalities in 3 cases. MRS showed good correlation with neuropsychological profiles with regard to side of abnormality and unilateral vs bilateral involvement. Our results suggest that combined MRI and MRS could help significantly in patient management, in particular when surgical intervention is being considered.

369.9

RELATION BETWEEN GLUCOSE METABOLISM AND BLOOD FLOW IN HUMAN EPILEPTIC FOCI. WH Theodore, WD Gaillard*, P Herscovitch
Clinical Epilepsy Section, NINDS, and Nuclear Medicine Department, NIH, Bethesda MD 20892

We used positron emission tomography (PET) with 18-F-2-deoxyglucose and 15-O water to study glucose metabolism (CMRglc) and blood flow (CBF) in 10 patients with partial seizures. PET was performed on the Scanditronix PC2048-15B (resolution 6.5 mm; fifteen simultaneous slices). Patients were positioned with a thermoplastic headholder and laser guide. CBF (ml/100gm/min.) measured immediately before CMRglc (mg /100gm /min), without moving the patient, and EEG recorded continuously. CMRglc and CBF were measured in 192 6 mm regions on a standard template grouped into 26 anatomic areas on 7 slices.

Mean inferior lateral temporal (ILT) CMRglc was 6.96 ± 1.56 ipsilateral, and 7.50 ± 1.99 contralateral to the epileptic focus ($p = .11$), and CBF 43.56 ± 10.53 versus 43.14 ± 8.98. Inferior mesial temporal (IMT) CMRglc was 5.58 ± 1.31 versus 6.06 ± 1.3 ($p = .049$). IMT CBF was 37.4 ± 97.24 versus 42.08 ± 6.99 ($p = .0036$). The ratio of CMRglc to CBF was lower ipsilateral than contralateral to the epileptic focus In ILT (.146 ± .023 vs .158 ± .027: $p = .0054$) but not IMT. In thalamus, there was a trend toward lower ipsilateral CMRglc / CBF (.138 ± .02 vs .147 ± .026: $p = .052$).

We found greater depression of CMRglc and CBF in mesial than lateral temporal cortex ipsilateral to epileptic foci, in contrast to previous reports. The difference in results may be explained by our higher spatial resolution scanner. Our data suggests that CMRglc may be relatively more depressed than CBF ipsilateral to epileptic foci in temporal cortex and thalamus: substrate supply may be normal, while use is impaired.

369.10

HUMAN PERINATAL BRAIN DAMAGE, CORTICAL REORGANIZATION, AND DEVELOPMENTAL EPILEPSY: A GOLGI STUDY. M. Marín-Padilla, Dept. Pathology, Dartmouth Medical School, Hanover, NH 03756.

Hypoxic, ischemic, and/or hemorrhagic damage to the developing cerebral cortex could occurs in prematurely born infants after prolonged neonatal asphyxia due to lungs immaturity. While severe damage often resulted in demise, less severe injuries are compatible with life and could eventually result in a variety of neurological disorders (e.g. cerebral palsy, epilepsy, mental retardation, dyslexia, blindness, minimal cortical dysfunction, and poor school performance). Neither the neuronal alterations caused by this type of injury or the degree of cortical reorganization around damaged areas have been adequately documented. For several years, I have been studying the cortex of premature infants that survive (days, weeks, month, or even years) this type of injury using the rapid Golgi method. Data is presented to illustrate that still developing neurons around damaged areas undergo a progressive reorganization in response to the anomalous local circuitry caused by the injury. Surviving neurons around the lesion, free of normal developmental constrains, respond to local circuitry demands acquiring new and often anomalous morphologic features. It is proposed that these acquired neuronal anomalies are the actual underlying cause for the developmental neurological disorders observed in these children rather than the original cortical lesion itself.

Supported by Grant # NS-22897, NIH.

369.11

ALTERED KYNURENINE METABOLISM IN A MODEL OF CHRONIC EPILEPSY: IMMUNOHISTOCHEMICAL ANALYSIS. F. Du, E.W. Lothman[1], E.H. Bertram[1], J.M. Williamson[1] and R. Schwarcz. Md. Psych. Res. Ctr., Baltimore, MD 21228 and [1]Dept. Neurology, Univ. Virginia, Charlottesville, VA 22908.

The cellular localization of three enzymes critical for the metabolism of kynurenic and quinolinic acid, kynurenine aminotransferase (KAT), 3-hydroxyanthranilic acid oxygenase (3HAO), and quinolinic acid phosphoribosyltransferase (QPRT), was studied in a rat model of epilepsy with acute status epilepticus and chronic seizures (Epil. Res., 3:107, 1989). One month after status, immunoreactivities (-i) of all three enzymes were found to be reorganized in brain areas activated during status and later displaying neuronal damage. In the hippocampus, a large number of proliferated, mostly hypertrophic, glial cells exhibiting KAT-, 3HAO-, and QPRT-i were detected in all layers of CA1 where pyramidal neurons degenerated. Substantial increases in labelling were also consistently observed in several thalamic nuclei. Altered patterns of KAT-i, 3HAO-i, and QPRT-i were noticed on both sides of all brains examined, though they were less obvious on the stimulated side. In addition, extremely dense immunoreactivities occurred around severe lesions in the piriform cortex, usually contralateral to the stimulated side. These changes might reflect altered metabolism of kynurenic acid and quinolinic acid, and indicate their possible role in epileptogenesis.

Supported by USPHS grants NS 16102 and 25605.

369.12

REVERSAL OF EPILEPTIC STATE BY PATTERNED ELECTRICAL STIMULATION SUGGESTED BY TRION MODEL CALCULATIONS Xiaodan Leng*, John V. McGrann* and Gordon L. Shaw, Center for Neurobiology of Learning and Memory, Univ. of California, Irvine, CA 92717

The trion model (Shaw et al. (1985) PNAS 82, 2364) is based on the Mountcastle columnar organizational principle of cortex. A trion represents an idealized minicolumn with three levels of firing activity and is highly structured in time and in spatial connections. A network of trions has a large repertoire of quasi-stable, periodic spatial-temporal firing patterns, MPs, which can be excited and each MP can be readily enhanced by a Hebb learning rule. A particular MP present in most repertoires has all trions firing together in synchrony, which we identify as the "Epileptic" MP, EMP. In trion model simulations, the EMP can be enhanced via the Hebb rule after electrical stimulation so that an epileptic focus with after discharge (about 3-6 Hz) is formed and spontaneous firing of the EMP occurs (as in kindling). Following this, by using two or more closely-spaced stimulating electrodes out of phase, other MPs are enhanced eliminating the dominance of the EMP.

CELL LINEAGE III

370.1

IN SITU ANALYSIS OF CRANIAL NEURAL CREST CELLS IN MICE. George N. Serbedzjia, Scott E. Fraser and Marianne Bronner-Fraser. Developmental Biology Center, UC Irvine, Irvine, CA 92627

Analysis of mouse cranial neural crest cell migration and differentiation has been hampered both by the lack of a reliable cell marker and by the difficulty of manipulating the embryo *in vivo* at the stage of neural crest cell migration. In order to study cranial neural crest cell migration in the mouse, premigratory neural crest cells were labelled by injecting DiI into the amniotic cavity. Embryos were labelled on embryonic day 8 and maintained *exo utero* in the mother for an additional 1-5 days. Hindbrain-level neural crest cells migrated ventrolaterally along three subectodermal pathways extending from the dorsal portion of the neural tube to the distal portion of each of the three branchial arches. The midbrain-level neural crest cells migrated ventrolaterally through the mesenchyme overlying the mesencephalon. Forebrain-level neural crest cells migrated ventrally through the mesenchyme between the eye and the diencephalon.

By labelling embryos at different stages of development, the duration of neural crest cell emigration from the neural folds was observed. Rostral hindbrain-level neural crest cells ceased emigrating by the 11 somite stage. neural crest cell emigration from the midbrain and caudal hindbrain regions ended by the 14 somite stage. Neural crest cells in the forebrain continued to emigrate until the 16 somite stage. In each region, cranial neural crest cells populated their derivatives in a ventral-to-dorsal order.

This overall pattern resembles that previously observed in chick embryos , however, some differences exist in the trajectories of the individual pathways.

370.2

THE DIFFERENTIATION OF NEURONAL STEM CELLS INTO LIMBIC NEURONS, *IN VITRO*. R. Ferri and P. Levitt. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Previous transplantation studies have identified a critical period for the committment of stem cells to express a specific limbic cortical phenotype (Barbe and Levitt, 1991, J. Neuroscience 11: 519-533). At embryonic day 12 (E12), multipotential precursor cells are uncommitted and can respond to environmental cues to produce neurons of different phenotypes but by E14 are unable to change their fate. We have developed an *in vitro* assay system to study the factors that regulate stem cell differentiation. Neuroblasts from presumptive perirhinal (limbic) and sensorimotor (non-limbic) cortices are dissected from E12 rats, dissociated, and plated in either a defined medium or glial conditioned medium. Neuronal differentiation is determined immunocytochemically by the expression of MAP2, and the phenotypic specificity is defined by the ability of the neuron to express the Limbic System Associated Membrane Protein (LAMP), a neuronal cell surface glycoprotein specific for limbic structures. Preliminary data identifying the percentage of MAP2-positive cells that are also LAMP-positive shows that LAMP expression first becomes evident after 2 days in culture, corresponding to the appropriate time when LAMP is first localized *in vivo* in the cerebral wall. Cultured limbic and nonlimbic neuroblasts will differentiate into neurons phenotypically identical to their respective *in vivo* counterparts, suggesting that the stem cells maintain an ability to differentiate into an appropriate phenotype in the absence of their complex *in vivo* milieu. Manipulations of this system will allow us to identify potential epigenetic factors regulating stem cell differentiation and leading to the formation of a distinct functional system. (Supported by NIMH Grant MH45507).

370.3

ORIGINS AND FATE OF RADIAL GLIA IN CHICK OPTIC TECTUM. G.E. Gray and J.R. Sanes. Department of Anatomy and Neurobiology, Washington University Medical School, St. Louis, MO 63110.

To continue our analysis of cell lineage in chick optic tectum (PNAS 85:7356, 1988; PNAS 87:458, 1990; Neuron 6:211, 1991), we have used retrovirus-mediated gene transfer to study the origin and fate of radial glia (RG). Retroviral infection labels progenitors with a heritable marker (lacZ) that is later detected histochemically in the infected cells' progeny. In these studies, retrovirus was injected on embryonic days (E) 3-8, and RG were identified morphologically and immunohistochemically (see accompanying abstract by Herman et al.) in clones analyzed on E10-20. Our main results are: 1) Clonal relatives of RG include neurons and astrocytes, but generally not other RG. Thus, these three cell types arise from a common precursor in chick optic tectum. In contrast, RG and neurons are thought to have separate progenitors in mammalian cortex. 2) Infection at any time between E3 and E8 labels RG. Thus, RG continue to be generated after the peak of neurogenesis (~E5), and after radial migration of neuroblasts has commenced (~E6). 3) Clones marked as late as E8 can contain both neurons and RG, suggesting that these lineages do not diverge. 4) During radial migration, neuroblasts are frequently in apparent contact with processes of clonally related RG. Thus, RG may guide the migration of their clonal relatives. 5) The number of labelled RG declines between E15 and hatching (E20), during which time many labelled astrocytes and "intermediate forms" appear. Thus, as in mammals, some tectal RG may transform into astrocytes. These results provide the first clonal analysis of RG lineage in any species, and suggest novel relationships between RG and neurons. (Supported by NIH.)

370.4

RETROVIRUS-MEDIATED INTRODUCTION OF ANTISENSE INTEGRIN ß1 HALTS RADIAL MIGRATION IN CHICK OPTIC TECTUM IN VIVO. D.S. Galileo*, J. Majors[1]* and J.R. Sanes. Departments of Anatomy and Neurobiology and of [1]Biochemistry, Washington University Medical School, St. Louis, MO 63110.

We have been using a recombinant retrovirus, LZ10, to study lineage and migration of cells in chick optic tectum (see accompanying abstract by Gray and Sanes). LZ10 inserts the lacZ gene into progenitors; its product is later detected histochemically in progeny of infected cells. Most cells in marked clones migrate along radial glia from the ventricular zone (VZ) to their laminar destinations. Here, we test the hypothesis that integrins, which are present in developing tectum and mediate cell movements elsewhere, are involved in radial migrations in tectum. We constructed a vector in which LZ10 was modified to contain an antisense sequence for the chicken ß1 integrin subunit. This virus (LZ15) or LZ10 was injected into embryonic day (E)3 tecta and the resulting clones were compared. At E6, when the tectum was still almost entirely a VZ, both LZ10- and LZ15-marked clones consisted of radially stacked cells extending across its width. By E7.5, a tectal plate (TP) had appeared and many LZ10-marked cells had entered it. In contrast, cells in LZ15-marked clones remained radially arrayed but accumulated in the VZ and failed to enter the TP. At E9, the TP had thickened considerably, and most cells in LZ10 clones (68%) lay within it. In contrast, only 22% of cells in LZ15 clones were found in the TP; most remained in the VZ. Furthermore, whereas LZ10- and LZ15-marked clones contained the same mean number of cells on E6 and E7.5, LZ15 clones contained ~50% less cells than LZ10 clones by E9. These results suggest that integrin-dependent interactions are not required for the initial stacking of cells in the VZ, but are required for migration along radial glia into the TP. In addition, cells that fail to migrate may die. (Supported by NIH.)

370.5

DEVELOPMENTALLY REGULATED AND DOMAIN-SPECIFIC ANTIGENS OF RADIAL GLIA IN CHICK OPTIC TECTUM. J.P. Herman, J.C. Victor* and J.R. Sanes. Department of Anatomy and Neurobiology, Washington University Medical School, St. Louis, MO 63110.

Radial glia (RG) are believed to guide migration of embryonic neurons to their laminar destinations. As part of a study of RG development and function (see accompanying abstracts by Gray & Sanes and Galileo et al.), we have generated and characterized monoclonal antibodies to developmentally-regulated and lamina-specific antigens of RG in chick optic tectum. They fall into three groups of two antibodies each: a) H5 and a previously generated antibody, R5 (Drager et al., J. Neurosci. 4:2025, 1984), stain the processes but not the cell bodies of RG in a uniform manner between embryonic day (E)5 and hatching (E20). In vitro, both reveal a dense, intracellular filamentous meshwork in tectal non-neuronal cells. On Western blots, both recognize a protein of ~51 kd. b) H28 and H29 stain RG somata and processes between E7 and E14, but not later. Moreover, H28 and H29 staining is markedly more intense in the ventricular and intermediate zones than in the laminae of the forming tectal plate. H28 and H29 recognize an intracellular epitope in cultured cells, and immunoblot a protein of ~35 kD. c) Finally, H2 and H27 recognize antigens concentrated in the most superficial processes and endfeet of RG in late (E14-E20) embryos. H27 also stains ganglion cells in E10-E14 tecta, and recognizes proteins of ~29 and ~170 kD on Western blots. Thus, antibodies H5 and R5 are good markers of RG at all stages, whereas the others define at least two antigens that are developmentally regulated and localized to discrete domains. Together, these antibodies can be used to study temporal and spatial specializations of RG that may be important to their function. (Supported by NIH, NATO, and CNRS.)

370.6

A MODEL FOR CEREBELLAR PURKINJE CELL LINEAGE SUPPORTS THE HYPOTHESIS THAT PURKINJE CELLS ARE DESCENDED FROM A SMALL NUMBER OF PROGENITOR CELLS. M.W. Vogel. MD Psychiatric Research Center, Baltimore, MD 21228.

To test the hypothesis that Purkinje cells (PCs) are descended from a small number of progenitor cells, a computer program was devised that simulates the numerical distribution of wild type PCs in +/*Lc* ↔ wild type chimeras. The model assumes that the PC population is clonal and both the total number of clones and the number of PCs per clone can be varied. The predicted number of total PCs is compared with the total number of PCs in wild type mice. In the simulations, the mean number of PCs per clone was varied from 2 to 15,000 and the range of PC number variation was varied between 1% to 50% of the mean. Frequency histograms show that the predicted distribution of wild type PCs is multimodal, but the number of peaks and their steepness depends on the number of clones and the mean number and range of PCs per clone. The wild type PC distribution becomes more uniformly distributed as clone size decreases or the range of variability of PCs per clone increases.

In 3 different wild type mouse strains analyzed in +/*Lc* chimeras, 8/10, 10/12, and 11/12 data points meet the criteria for a quantal model of PC lineage: i.e. they fall within 3 to 3.5% of an integral multiple of a quantal PC clone size (10,400, 9,200 or 7,900 PCs/clone, respectively). Monte Carlo simulations of this data show that the frequency of matching the experimental data is low for small clone sizes (e.g. for 1 to 4 PCs/clone, $p < .02$), but increases as the number of PCs per clone increases (e.g for 10,000 ± 1,000 PCs/clone, $p > .1$). However, the frequency of obtaining similar results decreases for all clone sizes as the range of PCs per clone increases (e.g. for 10,000 ± 5,000 PCs/clone, $p < .02$). The results of the Monte Carlo simulations indicate that it is more likely that the wild type PCs in +/*Lc* chimeras are descended from a small number of progenitor cells each of which generates similar numbers of PCs in their descendent clone.

Research support provided by NARSAD.

370.7

A NUCLEUS OF MOUSE HYPOTHALAMUS ORIGINATES FROM IDENTIFIED PRECURSORS IN THE NEURAL TUBE. G. Teitelman and T.A. Milner, SUNY Health Sci. Ctr., Brooklyn, NY and Cornell Univ. Med. Coll., New York, NY.

To investigate the cell lineage relationships of hypothalamic cells, we examined transgenic mice (RIP-Tag) that harbor a hybrid insulin gene comprised of the 5' flanking region of the rat insulin II gene linked to the sequences encoding an oncogene, the simian virus 40 (SV40) large T antigen (Tag). Ectopic expression of the transgene was seen in cells of the hypothalamus of embryos and adults. This study used immunocytochemical techniques to determine the phenotype of Tag$^+$ cells of brain. In embryos, Tag$^+$ cells first appeared at day 10 of development (E10) in the ventricular layer of the diencephalon. From E11 to E14 abundant expression of Tag was seen in both ventricular and mantle layers while at E16 only the mantle layer contained Tag$^+$ cells. In adults, Tag$^+$ cells were found in the arcuate nucleus of the hypothalamus. At E12 and E14, immature Tag$^+$ cells contained the neuronal markers β-endorphin (BE) and tyrosine hydroxylase (TH) respectively. However, TH and BE neurons of adults did not express the oncogene. In adults, most or all the Tag$^+$ cells contained the glial cell marker GFAP and had the ultrastructural morphology of astrocytes. This suggested that Tag$^+$ precursors gave rise to both TH and BE neurons and astrocytes. However, while neurons contained Tag transiently during differentiation, expression of the oncogene in glial cells was permanent. We conclude that neurons and glial cells of the arcuate nucleus originate from a subset of precursor cells that become specified prior to migration. (Supported by HL18974).

370.8

ISOLATION OF ROD PRECURSORS FOR CELL CULTURE. J.K. Knight and P.A. Raymond. Neuroscience Program and Department of Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI 48109.

Rod precursor (RP) cells, located in the outer nuclear layer of the mature fish retina, normally give rise exclusively to rod photoreceptors. Previous experiments in our lab suggested that RPs can give rise to other cell types depending on their microenvironment. The goal of the present study is to develop an *in vitro* system for determining the effect of environment on the fate of RP cells.

Mitotic activity of RPs was enhanced by multiple punctures of the eye. After 15d, ^{3}H-thymidine (T) or bromodeoxyuridine (BrdU) was injected intraocularly, and fish were sacrificed the next day. Retinas were isolated, cut into pieces ~ 2 mm^2, treated with papain and mechanically dissociated. Cells were layered onto Percoll density gradients (1.017-1.141 g/ml) and centrifuged to achieve separation of cell types. Enriched cell fractions were collected and either fixed in 4% paraformaldehyde or placed in hanging drop culture in M199 medium with 10% fetal calf serum and 1% lineoleic acid at 27 C.

To determine which cell fraction contained the RPs, DNA was precipitated from ^{3}H-T labelled retinal cells and counted in a scintillation counter. The cell fraction at 1.12 g/ml showed, on average, 8 fold higher cpm than any other fraction. To determine whether the dividing cells were RPs, we fixed cells from BrdU-injected retinas and immunostained them with anti-BrdU and NN (a monoclonal antibody which labels microglia and endothelial cells in goldfish retina); RPs are BrdU+/NN-. The 1.12 g/ml cell fractions contained both BrdU+/NN+ and BrdU+/NN- cells, indicating that some, but not all, of the dividing cells were rod precursors. Preliminary experiments demonstrated that these cells proliferated for up to 7 days in culture, as evidenced by incorporation of BrdU added to the culture medium for 16 h. Future experiments will attempt to further enrich the population of isolated RPs with the eventual goal of determining factors that regulate their differentiated fate. Supported by NIH T32EY07022 and R01EY04318.

370.9

CLONAL ORIGIN OF THE TADPOLE RETINA AND LINEAGE CHANGES AFTER ABLATION OF A MAJOR PROGENITOR. S. Huang and S. A. Moody, Dept. Anatomy & Cell Biology, Univ. Virginia, Charlottesville, VA 22908

The clonal origin of Xenopus tadpole retina from 32-cell embryos was quantitatively assessed by lineage tracing at st 42-44. It is derived from 4 dorsal (D) and 1 ventral (V) animal blastomeres: D111, 56.7%; D112, 18.5%; D121, 12.2%; D122, 12.0%; and V121, 0.6%. All dorsal blastomeres contributed to both retina, but the majority of their progeny were ipsilateral. The clones from each blastomere usually formed radial columns across all layers and always included more than one cell type. Descendants from each were distributed in a mosaic in all regions of the retina, however D111 and D112 contributed more to anterior retina, while the rest contributed more to posterior retina. Thus, blastomere origin restricts neither cell type nor the location of clones within the retina.

After ablation of both D111, which are the major progenitors, 60% of the tadpoles developed normal appearing eyes, but they contained a significantly smaller number of retinal cells (4968 vs. 9600) and a smaller retinal volume ($5.88x10^6$ μm^3 vs. $7.59x10^6$ μm^3). The surrounding blastomeres changed their retinal lineages to effect a partial compensation. Three responses were observed: 1) V111 and V122, which normally do not contribute to the retina, were induced to produce novel progeny, i.e., 16.2% and 0.2% of the retinal cells, respectively. 2) V121 and D121 drastically increased their contribution to the retina (to 55.2% and 26.2%, respectively). 3) In contrast, D112 and D122 decreased their retinal contribution (to 0.3% and 1.9%, respectively). These results demonstrate that retinal lineages are not determined during early cleavage stages. However, these lineages also are not uniformly plastic and regulatory, as demonstrated by the fact that different blastomeres have different responses to the ablation and that complete compensation is not accomplished. Supported by NS23158.

370.10

CELLULAR INTERACTIONS THAT REGULATE CELL FATE DURING GROWTH AND REGENERATION OF THE GOLDFISH RETINA. J.E. Braisted & P.A. Raymond. Dept. Anat. & Cell Biol., Univ. Mich., Ann Arbor, MI 48109.

We recently demonstrated (Braisted & Raymond, 1991, Invest Opthalmol Vis Sci Suppl 32:923) that dopaminergic interplexiform cells (DA IPCs) in the inner nuclear layer (INL) of the juvenile goldfish retina are permanently destroyed by intraocular injection of 6-hydroxydopamine (6OHDA), 0.14-0.29 µg/µl, estimated intraocular concentration. However, after exposure to 0.58 µg/µl, >30% of the cells in both the INL and the outer nuclear layer (ONL) are destroyed, and DA IPCs as well as other neurons in the INL and ONL regenerate. We suggested that damage to the ONL stimulated a regenerative response in the population of mitotic rod precursors in the ONL. In order to directly test this hypothesis, we specifically ablated DA IPCs, then asked whether they regenerated if rods, but no other neurons, were also ablated.

Right eyes of fish were injected with 6OHDA (0.14 µg/µl) on two consecutive days. This paradigm destroys DA IPCs without causing non-specific damage that leads to their regeneration. After 24 hours, tunicamycin was injected intraocularly (0.005 µg/µl) to destroy rods. One to 2 weeks later, rod nuclei were counted in 3 µm methacrylate sections. A 45-50% reduction in rod density was observed in ventral retina, with less damage in dorsal retina. Retinas isolated 6-7 weeks later were prepared as whole mounts and processed for immunofluorescence with antibodies to tyrosine hydroxylase (TH). Many TH-immunoreactive (TH+) cell bodies and processes were observed in control retinas (left eyes), but TH+ cells in experimental retinas were found only in the most peripheral retina, which represents new growth from the germinal zone. No regenerated TH+ cells were found in central retina.

These preliminary data suggest that rod precursors in the ONL do not alter their normal pathway of development to replace DA IPCs in the INL when damage to the ONL is limited to destruction of rods. Experiments in progress will attempt to destroy both cones and rods to further elucidate the cellular interactions involved in regulating rod precursor fate. Supported by EY04318.

CELL LINEAGE: GENETIC AND BIOCHEMICAL MARKERS

371.1

DEVELOPMENTALLY EXPRESSED ANTIGENS IN THE VENTRAL NERVE CORD OF THE MOTH, MANDUCA SEXTA. S.A. Monsma and R. Booker Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In response to hormonal changes during the final larval instar, holometabolous insects such as Manduca undergo drastic changes in behavior and physiology which lead to the adult form. In the ventral nerve cord (VNC) many immature neurons (IN cells) begin to differentiate while other cells degenerate. We have used nerve cords from wandering animals (day W2) to generate monoclonal antibodies (MAbs) against antigens expressed during this period. MAb 16H7 stains the peripheral somata of nearly all functional larval neurons as well as all nerves and connectives of fifth instar and wandering larvae; no immature IN cells are stained. On the first day of pupation (day P0), almost all surviving IN cells begin to stain and the staining of larval neurons intensifies. By the day of adult eclosion only large neurons stain reliably. Staining of the nerves and connectives remains unchanged. MAb 15B11 stains the cell bodies of many neurons of fifth instar and wandering larvae. In the thoracic ganglia 72 paired neurons are stained, while in the abdominal ganglia only 16 paired neurons are stained. 8 of these 16 neurons correspond to identified neurosecretory cells which contain the cardioacceleratory peptides or the peptide hormone bursicon. At day P0, almost all larval neurons and a few IN cells begin to stain. By the day of adult eclosion only a few neurons are stained. MAb 15G4 stains a pair of bilateral cells in each abdominal ganglion. Staining is present in all stages from fourth instar through pharate adult, with the strongest signal apparent on day P0. These cells appear to be a subset of the identified bursicon-containing cells. MAb 13F10 stains a pair of medial cells in the suboesophogeal ganglion, a bilateral cell in T1 and T2, and a pair of bilateral cells in T3. Projections of the thoracic cells out the median nerve are also stained Staining is weak in fourth and fifth instar larvae, becomes strongest by day W2 and was undiscernible by day P0 and after. MAb 5G7 stains 4 pairs of large bilateral brain cells in fifth instar larvae. Staining was absent in fourth instar larvae nor after day W2. These cells appear to be a subset of the Group IIa neurosecretory brain cells.

371.2

NEW MARKERS FOR STUDYING NEURAL DEVELOPMENT IN CHICK EMBRYOS. Z. Korade, W.P. Donohoe and E. Frank. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Developmental studies of the nervous system are facilitated by early markers of distinct classes of neurons and glia. In our continuing efforts to generate such markers, we have used cyclophosphamide to suppress the immune response to embryonic chick trigeminal sensory ganglia followed by immunization with membranes from E16 lumbosacral dorsal root ganglia (DRGs). Although this protocol has not yielded DRG-specific antibodies to date, several markers with striking developmental staining patterns have been isolated. Two are described here.

Antibody 4D8 primarily labels Schwann cells located throughout the peripheral nervous system. This staining first appears at E8, significantly later than staining with another Schwann cell marker (1E8) we have isolated. The most obvious contrast to 1E8 staining, however, is 4D8's intense labeling of the notochord from E4 until its disappearance. Although staining of Schwann cells and notochord is also characteristic of HNK-1, the patterns of HNK-1 and 4D8 labeling are quite distinct. A second difference from 1E8 is that although both markers initially stain satellite cells in DRGs, 4D8 staining persists through E21 while 1E8 staining does not.

Antibody 10D11 selectively labels the neural tube from E1 (stage 10). From the outset, the dorsal region of the tube (the neural crest) is unstained, and this lack of staining persists until well after crest cells have migrated away. As development proceeds though E4, the brightest staining is located imediately adjacent to the central canal, but cell-surface labeling is present throughout the forebrain and midbrain except at the dorsal edge of the tube. Staining intensity diminishes caudally, being confined primarily to the dorsolateral region of the neural tube. The outer circumference of the notochord is also stained, as with HNK-1. From E8, radial non-neuronal cells throughout the spinal cord are labeled with 10D11; the detailed pattern of staining is different at different levels of the neuraxis. Outside the CNS, 10D11 stains many satellite cells but no neurons in the sensory and sympathetic ganglia beinning on E12. Supported by NS24373 to EF.

371.3

MUTATIONS THAT DISRUPT THE DEVELOPMENT OF CRANIAL NEURAL CREST CELLS IN THE ZEBRAFISH. T.F. Schilling*, C. Walker* and C.B. Kimmel. Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Zebrafish cranial neural crest cells form sensory neurons, pigment cells and cartilage in the branchial arches. To learn the activities of genes that specify crest cell fates we have generated mutations that block the development of branchial arches and have abnormal pigmentation; the cellular bases of these defects were analyzed using cell labeling and transplantation techniques.

In two "chinless" mutations, *chw-1* and *chw-2*, cartilage and pigment cells were absent or abnormally patterned, while sensory neurons developed in their normal locations. The mutant phenotypes appeared after crest cells migrated to form branchial arch primordia suggesting that the mutations disrupt late steps in differentiation. Further, *chw-1* crest cells failed to develop normally when orthotopically transplanted into wild-type hosts, suggesting that this mutation acts autonomously in the neural crest cell lineage. A third mutation, "shorttail" *stl-1*, disrupted formation of the branchial arch primordia. Its phenotype appeared earlier, before crest migration, as a thinning of the CNS and a shortened tail bud. Older*stl-1* embryos lacked large groups of interneurons in the hindbrain suggesting that regions of the neuroepithelium, including neural tube as well as crest, were deleted. Similar to*chw -1* and *chw-2*, this mutation disrupted cranial cartilage and pigment cell development while sensory neurons appeared unaffected.

We suggest that all three mutations affect genes involved in the development of the cranial neural crest, and probably in particular crest sublineages. Supported by NIH 5T32GM07 and HD22486.

371.4

CHARACTERIZATION OF FOUR HOMEOBOX GENES EXPRESSED IN ZEBRAFISH NEURAL CREST. M. Ekker*, M.-A. Akimenko*, R. Bremiller* and M. Westerfield. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

To study genes that are involved in the specification of neural crest cell fate in zebrafish, we have isolated three genes with homeodomains related to the mouse *Hox-7* gene, which is expressed in limb bud and neural crest cell derivatives. A fourth gene, cDNA10, with a unique homeobox was isolated in the same screen. Analysis of genomic and cDNA clones reveals that the three genes, *hox-7a*, *hox-7b* and *hox-7c*, encode similar homeodomains with more than 90% conservation at the amino acid level. Furthermore, the homeodomain of *hox-7a* is identical to that of the mouse *Hox-7* gene. However, there is little additional sequence similarity among the three genes or with related genes in other vertebrates except for the amino acid residues immediately outside the homeodomain and a short peptide located at the amino-terminal end of the protein. *In situ* hybridization of probes specific for each of the four genes to sections of zebrafish embryos demonstrates that they are expressed in regions where neural crest or crest derivatives differentiate, including the dorsal part of the neural tube (*hox-7b &7c*), pectoral fins (*hox-7b &7c*), caudal fins (*hox-7a &7c*), heart (*hox-7c*), jaw (cDNA10) and other brain structures (*hox-7c* & cDNA10). Expression in jaw and fin mutants is presently being analysed. Supported by HD22486.

371.5

A NOTCH HOMOLOG IN THE LEECH. B.J.Norris and D.A.Weisblat. Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720

We are interested in studying decision between the neural/epidermal fates made by ectodermal cells in early development of the leech. One approach to studying this process is to look for homologs to gene products that are involved in this decision in other organisms. One such gene product is the Drosophila neurogenic gene Notch. This gene is particularly advantageous as a Notch homolog has recently been isolated from Xenopus (Xotch - Coffman, et al., Science 249:1438-1441).

We have used the polymerase chain reaction (PCR) to isolate part of a putative homolog to Notch, from genomic DNA of Helobdella triserialis. Oligos were designed to complement sequences (within the cdc10/SWI6 region of the gene) that are highly conserved between Notch and Xotch. A 180 base pair fragment was amplified, cloned and sequenced, yielding a conceptual amino acid sequence that is 60% identical to Notch and 53% identical to Xotch. This PCR fragment was then used to isolate genomic DNA clones from Helobdella. A 1.4 kilobase fragment containing the PCR sequence was subcloned and sequenced, revealing additional putative coding sequence with strong homology to Notch and Xotch, as well as putative introns.

Preliminary nonradioactive in situ hybridizations show widespread transcript distribution in the leech embryo.

371.6

THE EFFECT OF NMDA ON THE EXPRESSION OF A NEW POU/HOMEOBOX GENE IN CEREBELLAR GRANULE NEURONS. Robert F. Bulleit. Dept. of Pharmacology, University of Maryland School of Medicine, Baltimore, MD 21201

POU/Homeobox genes encode transcriptional regulatory proteins involved in determining cellular phenotypes. Those expressed in the CNS are likely to be involved in specifying CNS cellular phenotypes. We are interested in identifying POU/Homeobox gene expressed in the mammalian CNS and also determining if their expression is modulated by NMDA. It has been suggested that activation of the NMDA subtype of glutamate receptor is required for certain aspects of CNS development. Thus, POU/Homeobox genes, who's expression is regulated by NMDA, may play key roles in these developmental processes. We have begun by using the polymerase chain reaction (PCR) to amplify and clone cDNAs encoding POU/homeodomain proteins expressed in the adult mouse cerebellum. Several POU/homeobox sequences were identified using this strategy. One of these sequences appears to be a new member of the POU/homeobox gene family and has been termed MBP-1 (mouse brain POU sequence 1). A probe generated from this sequence has been used to screen a mouse brain cDNA library in an effort to obtain cDNA clones encoding the entire MBP-1 transcript. Several positive clones have been identified. Clone MBP-1A contains the longest insert (~5.0kb). Probe synthesized from this clone has been used for northern blot analysis of RNA obtained from cultured cerebellar granule neurons. Granule neurons were cultured in serum free meium in the presence or absence of 50μM NMDA, plus or minus 100μM APV (an antagonist at the NMDA subtype of glutamate receptor) or 10μM CNQX (a non-NMDA glutamate receptor antagonist). The results indicate that treatment with NMDA increases the level of MBP-1 transcripts. APV blocks this increase while CNQX has no effect.

371.7

CLONING OF A CHICK HOMOLOG OF A *DROSOPHILA* PRONEURAL GENE J. Helms†‡, J.Patrick§, and G.D. Maxwell†. Neuroscience Program†, Departments of Anatomy† and Periodontology‡, University of Connecticut Health Center, Farmington, CT 06030, and Division of Neuroscience§, Baylor College of Medicine, Houston, TX 77030

Molecular mechanisms that control cell determination and differentiation are often conserved through evolution, suggesting that genes which are required for specifying neuronal cell fates in *Drosophila* may have structural and functional homologs in vertebrate nervous systems. The Drosophila gene, *daughterless* (*da*), has a role in two developmental processes: sex determination and neurogenesis. *da* encodes a protein with a helix-loop-helix (HLH) motif. *da* and other HLH proteins are capable of forming homo- and heterodimers that exhibit sequence-specific DNA binding; these complexes may regulate the transcription of genes initiating neuronal precursor development. A chick brain cDNA library (generously provided by B. Ranscht) was screened by polymerase chain reaction using fully degenerate oligonucleotide primers corresponding to a conserved region of *da*. Five clones contained sequences that suggested they were members of the HLH gene family. The clone most homologous to the *da* HLH was used as a probe to re-screen the library. A 2.7 kb clone was isolated and sequenced, confirming that the clone contains a HLH region sharing 93% homology at the amino acid level with *da*. The cellular distribution of the expressed HLH gene will be characterized. This work was supported by NIH DE00157 (JH), NS13546 (JP) & NS16115 (GDM).

371.8

CHARACTERIZATION OF NOVEL cDNAs HYBRIDIZING TO mRNAs EXPRESSED IN THE DEVELOPING QUAIL RETINA. L. Bidou*, P. Crisanti* and Pessac B. Centre de Biologie Cellulaire, CNRS, 67 rue Maurice Günsbourg, 94205 Ivry sur Seine cedex, France.

The goal of this study is to isolate gene products that might be involved in neuronal quiescence and differentiation onset. We have constructed an "autosubtracted" cDNA library of mRNAs isolated from quiescent cells of a quail embryo neuroretina (NR) cell clone (K2), immortalized by a ts mutant of Rous sarcoma virus and whose proliferation stops after transfer to 41,5°C (Soc. Neurosci. Abstr. Vol. 15 part 2 p. 1129 1989).

Among the twenty recombinants which hybridize only with probes prepared from quiescent cells, we have selected four distinct clones which recognize quail mRNAs during development and after hatching. The nucleotide sequence of these partial cDNAs has no homology to sequences in the databanks. Clones p65, p62 and p64 appear specific for NR. The p65 mRNA is strongly expressed as three distinct bands of 4, 5.8 and 7 kb in post-hatching NR ; p65 shows a diffuse NR signal from ED 7, and after hatching is heavily transcribed in all nuclear layers and particularly in photoreceptors ; p65 is only observed in K2 cells 6 h after transfer to 41,5°C. p62 clone hybridizes to a 7 kb mRNA in ED 14 NR and to an additional band after hatching ; it is strongly expressed in nuclear layers and photoreceptors, with the most intense signal in ganglion cell somas. Clones p46 and p64 recognize multiple mRNAs in a development-dependent pattern ; distinct p46 and p64 mRNAs are specifically expressed in K2 cells after shift to 41,5°C. These novel cDNAs might play a role at multiple steps in NR development.

371.9

A NOVEL SEQUENCE EXPRESSED BY ACTIVATED T-CELLS AND NEURONS. J.A. Cohen, M. Arai, E. Luning Prak, M.B. Prystowsky. Departments of Neurology and Pathology, Univ. of Penn., Philadelphia, PA 19104

By differential hybridization screening, we isolated a cDNA clone (F5) of a 3.6 kb mRNA induced by interleukin-2 (IL2) in a mouse helper T-cell clone (L2 cells). Northern blotting demonstrated modest F5 expression in adult mouse lymphoid tissues and, surprisingly, high levels in brain, spinal cord and eye. Expression was undetectable in other tissues including sciatic nerve. In cerebrum expression was undetectable up to 2 weeks after birth with a marked increase to adult levels on postnatal day 21. *In situ* hybridization histochemistry demonstrated high levels of F5 mRNA in large neurons in neocortex, hippocampus, diencephalon, brainstem, cerebellum, and spinal cord. Double-labelling studies confirmed that expression was restricted to neurons. Sequence analysis demonstrated a 1125 bp open reading frame encoding a 42 kDa protein with multiple potential phosphorylation sites and a nuclear translocation sequence. The F5 coding region hybridized under stringent conditions to DNA from a variety of species demonstrating substantial evolutionary conservation. However, neither the nucleic acid nor protein sequences showed homology to any previously reported sequence. F5 is a previously unreported gene, possibly encoding a nuclear regulatory protein. The function of the F5 protein and the mechanisms regulating its expression in neurons, whether by IL2 or by mechanisms distinct from those in T-cells, should be of great interest.

372.1

INTRINSIC PROPERTIES OF *HELISOMA* GROWTH CONES IN THE PRESENCE AND ABSENCE OF CONDITIONING FACTORS. D.L. Kania and C.S. Cohan Dept. of Anatomical Sciences, SUNY at Buffalo, Buffalo, N.Y. 14214

Previous studies investigated the cytoskeletal rearrangements at the cut end of an axon in response to conditioning factors. This study compares behavioral properties of growth cones of identified neurons in the presence and absence of conditioning factors.

We have developed an *in vitro* preparation for studying the initial effects of conditioning factors on regenerative outgrowth. Neurons B19 and B5, from the mollusc, *Helisoma trivolvis*, plated in the absence of conditioning factors form growth cones at the end of attached axon stumps. These growth cones exhibit filopodial and lamellipodial movements but do not advance across the substrate. Growth cones of neuron B19 treated with serotonin in the absence of conditioning factors lose their filopodia while growth cones of B5 do not, similar to their response in the presence of conditioning factors.

The addition of conditioning factors to the medium promotes neurite extension from approximately 50-75% of the growth cones of both attached and isolated pieces of axon. The rate of extension of the newly formed neurites was found to be comparable with rates reported from cells plated directly into conditioned medium.

Intracellular calcium levels are known to be important in the regulation of neurite outgrowth. Therefore, the effects of conditioning factors on intracellular calcium levels of the growth cones are being investigated. Preliminary results suggest that the presence of conditioning factors in the medium are related to low-level oscillations of intracellular calcium in the axon and growth cone. The magnitude of these oscillations appears to increase in isolated axons exposed to conditioning factors. Isolated axons initiate outgrowth quicker than attached axons in response to conditioning factors.

These results suggest that while neurite extension is dependent on conditioning factors, formation and intrinsic behavioral characteristics of growth cones are not. The effects of conditioning factors may be caused by changes in intracellular calcium.

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372.2

CALCIUM CHANGES ASSOCIATED WITH ALTERATIONS IN GROWTH CONE MOVEMENTS OF DEPOLARIZED HELISOMA NEURONS. C.S. Cohan. Dept. of Anatomical Sciences, SUNY Buffalo, Buffalo, New York 14214.

It was previously shown that depolarization of outgrowing Helisoma neurons with high levels of K^+ results in time dependent changes in growth cone movements. In the presence of the depolarizing stimulus, neurite elongation is suppressed for a period of 45 min followed by transient recovery of elongation. In the present experiments, the calcium changes induced by the depolarization and which underlie the suppression and spontaneous recovery of elongation were studied.

Identified neurons B4 and B19 were isolated from *Helisoma* buccal ganglia and plated into culture dishes containing a neurite promoting conditioned medium. Outgrowing neurons were injected with the acid form of Fura-2 and viewed with a cooled ccd after brief exposures to 340nm and 380nm illumination. Neurons were depolarized by KCl (5, 10, and 25 mM) added to the dish after control calcium values were obtained. Calcium changes in growth cones were monitored at 15 min intervals.

Depolarization caused a dose dependent increase in calcium levels in growth cones of B4 and B19. Calcium levels in growth cones of B4 changed by 90% and 141% for depolarizations with 5 and 10mM K^+ and were significantly higher compared to growth cones of B19 which changed by 19% and 88%. This is consistent with the greater suppresion of outgrowth in B4 than B19 during depolarization. However, treatment with 25mM K^+ did not produce a further increase in calcium levels even though it caused a greater suppression or even retraction of growth. The recovery phase of outgrowth was associated with a small but significant decrease in calcium levels that did not reach control levels. These data indicate that the differential effects of depolarization on neurite elongation of B4 and B19 can be explained by the magnitude of calcium changes in their growth cones. However, the sustained elevation of calcium levels during the recovery period suggests that other factors may be involved in the recovery of neurite elongation.

(Supported by NIH grant NS25789)

372.3

PROTEIN SYNTHESIS IN ISOLATED NEURONAL GROWTH CONES IS ALTERED BY CHANGES IN INTRACELLULAR CALCIUM LEVELS. L. Davis, P. Dou, and S.B. Kater. Program in Neuronal Growth and Development and the Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

This study investigates the ways in which neuronal growth cones act independently from the cell body when growth cones encounter signals in the extracellular environment. We are focusing on protein synthesis within growth cones which could enable the rapid, local synthesis of protein components of the cellular machinery necessary in the formulation of responses to stimuli. We recently demonstrated that protein synthesis occurs in isolated growth cones of the snail Helisoma (Davis, L. and S.B. Kater (1990)*Soc. Neurosci. Abstr.* **16**: 961) and are now exploring second messenger regulation of this process. Cultures containing growth cones isolated from buccal ganglion neuron B5 were pre-treated with the calcium ionophore 4-bromo-A23187 (1μM) for 5 minutes prior to pulse-labeling with 3H-leucine for 15 minutes in the presence of 4-bromo-A23187 (1μM) to raise intracellular calcium levels. Quantitative analyses of autoradiographs revealed that the number of silver grains (indicating the presence of 3H-proteins) over isolated growth cones was significantly increased ($p< 0.01$) in A23187 treated cultures as compared with controls. These experiments demonstrate that protein synthesis may be modulated by alterations in the intracellular calcium concentration; thus, protein synthesis in growth cones may be regulated by environmental cues that alter calcium levels. Supported by NIH postdoctoral training grant NS08445 to LD.

372.4

SMALL ELECTRIC FIELDS ALTER NEURONAL GROWTH CONE MORPHOLOGY, NAVIGATION AND CAUSE HIGHLY LOCALIZED INTRACELLULAR CALCIUM CHANGES R.W. Davenport, S.B. Kater Program in Neuronal Growth and Development, Department of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523.

Previous investigators demonstrated the ability of small electric fields to alter predictably cell motility, direction of neurite outgrowth and growth cone morphology. We similarly demonstrate in identified, cultured neurons of the pond snail *Helisoma* the ability of electric fields to alter neuronal growth cone navigation (they turn toward a cathode source) and filopodia elongation (filopodia lengthen on the cathode oriented side of the growth cone). The mechanism(s) of these cellular responses remain unknown. Previous studies implicate calcium influx and/or membrane receptor redistribution as the causal mechanism to field-induced growth cone orientation. Using Fura-2 calcium imaging techniques, we provide the first direct evidence correlating a change in $[Ca]_i$ with field-induced growth cone orientation. With small, focally applied electric fields an ≈7 fold rise in $[Ca]_i$ occurs rapidly within about one minute and remains elevated ≈2 fold above rest levels as long as the electric field is sustained. This change in $[Ca]_i$ is blocked in $0[Ca]_o$/EGTA containing media. Focally applied fields show a rapid, local change in $[Ca]_i$ on the cathode side of the growth cone. The increased $[Ca]_i$ spreads across the growth cone as a wave from the cathode to anode side, creating a brief gradient (≈2-5 min.) of $[Ca]_i$ across the growth cone. This calcium wave and gradient may be responsible for the directional changes of growth cone morphology and navigation.

372.5

REGULATION OF NEURONAL GROWTH CONE FILOPODIA BY CHANGES IN INTRACELLULAR CALCIUM LEVELS. V. Rehder and S.B. Kater, Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523

Filopodia on neuronal growth cones, historically have been regarded as sensory probes of their environment and more recently as important elements for steering growth cones towards their targets. Since the area encountered by a growth cone logically depends on the area spanned by the filopodia, an active change in filopodial length or number could result in the 'exploration' of different sample sizes of the environment at different times during development. The present study tests the regulatory role of intracellular calcium levels on filopodia. Changes in [Ca]i were directly correlated with growth cone filopodial behavior in an identified neuron from the snail *Helisoma*. Calcium influx into the cytoplasm was experimentally increased, and the [Ca]i monitored using the fluorescent calcium indicator fura-2. A rise in [Ca]i causes two distinct, concentration-dependent effects separable by their different time courses: within the first 10 minutes filopodia undergo significant elongation, while the second phase is characterized by a massive loss of filopodia in a calcium-dependent fashion. The degree of filopodial loss correlates well with the transient peak values of [Ca]i reached during each treatment. Filopodial numbers were gradually restored to pretreatment levels within two hours during continuous stimulation. A transient change of as little as 30-50 nM reliably alters filopodial disposition. This indicates that even small changes in intrinsic calcium homeostatic properties or extrinsic signals which alter calcium levels intracellularly can act as regulators of the environmental area sampled by an elongating growth cone.

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372.6

INTERACTIONS AMONG EXTERNAL CALCIUM ION CONCENTRATIONS, INTRACELLULAR FREE CALCIUM ION CONCENTRATIONS, AND NEURITE GROWTH IN CULTURES OF PRIMARY AND TRANSFORMED NEURONS. J. Frank*, C. Ferguson*, L. Cabell-Kluch*, D. Shugarts*, T. Audesirk and G. Audesirk. Biology Dept., U. Colorado at Denver, Denver, CO 80217-3364.

Two primary neuronal types (embryonic rat hippocampal neurons and chick brain neurons) and two transformed neuronal types (rat B50 neuroblastoma cells of CNS origin and mouse N1E-115 neuroblastoma cells of PNS origin) were cultured in media in which calcium concentrations were varied from approximately 0.1 to 7.2 mM (0.1 to 4x normal). Both primary neuronal types showed maximum neurite initiation in media with normal calcium levels of 1.8 mM and decreased initiation in media with either higher or lower calcium concentrations. In both transformed cell types, neurite initiation was not affected by altered external calcium concentrations. Intracellular calcium measurements using fura-2 were made on rat hippocampal neurons and N1E-115 neuroblastoma cells. In hippocampal neurons, intracellular free calcium ion concentrations varied monotonically with altered external calcium. In N1E-115 cells, intracellular calcium was not affected by altered external calcium. These results indicate that (1) neurons differ in their ability to regulate intracellular calcium in the face of varied external calcium; (2) transformed neurons may have superior calcium-regulatory abilities; (3) intracellular calcium concentrations modulate neurite initiation.

372.7

CNS MYELIN NEURITE GROWTH INHIBITOR NI-35 CAUSES A LARGE TRANSIENT RISE IN INTRACELLULAR CALCIUM WHICH PRECEDES GROWTH ARREST AND COLLAPSE OF RAT DRG GROWTH CONES. M.F.Schmidt[1], C.E.Bandtlow*[2], T.D.Hassinger[1], M.E.Schwab[2] and S.B.Kater[1]. (1)Dept. Anatomy and Neurobiology, Colorado State University, Fort Collins, CO and (2) Brain Research Institute, University Zurich, 8029 Zurich, Switzerland.

Two proteins present in oligodendrocyte membranes and myelin exert potent inhibitory effects on growing neurites. Incorporation of one of these inhibitory proteins (NI-35) into liposomes was used to study the mechanism by which this protein mediates its inhibitory effect on neurite growth. Addition of these liposomes to cultures of dissociated rat DRG neurons causes 75% of the observed growth cones to arrest and subsequently to collapse within 60 minutes. This effect was specifically abolished by the neutralizing antibody IN-1. The collapse was significantly reduced in the presence of a cocktail of dihydropyridine calcium channel blockers. Addition of the calcium ionophore A23187 caused DRG growth cones to collapse. In order to further study the possible role of calcium in the mechanism of action of NI-35, DRG cultures were loaded with the calcium indicator Fura-2. The mean $[Ca^{2+}]_i$ in advancing growth cones was 158±8 nM. Addition of NI-35 liposomes caused a large ($\bar{x}$=737±73 nM) transient (5-10 minutes) rise in intracellular calcium which preceded growth cone collapse. As in the behavioral studies, the rise in calcium could be prevented when NI-35 liposomes were preincubated with the specific antibody IN-1. These results indicate an important role of calcium in the neurite growth inhibition produced by NI-35.

372.8

DEPOLARIZATION-INDUCED CHANGES IN INTRACELLULAR CALCIUM IN GROWTH CONES OF ACUTELY DISSOCIATED ADULT RAT SENSORY NEURONS. D.L.Eng and J.D.Kocsis, Dept. of Neurology and Sect. of Neurobiol., Yale Med. Sch., New Haven, CT 06510; and VAMC, West Haven, CT 06516

Adult rat dorsal root ganglion (DRG) neurons obtained in dissociated cell culture were used as a model of regenerating neurons to study intracellular calcium $[Ca^{2+}]_i$ changes during regeneration. DRG were dissected free of the epineurial sheath and treated in collagenase and papain followed by trituration with a siliconized glass pipette in a BSA/trypsin inhibitor cell media. The DRG cell suspension was plated on poly-ornithine/laminin coated glass coverslips and maintained in DMEM/F12,10%FCS,100 u/ml penn/strep cell media. Within hours after dissociation the neurons gave rise to neurites with prominent growth cones, as seen with Hoffmann modulation contrast optics,Lucifer yellow dye injection and with antineurofilament immunostaining. The neurons were viable as indicated by stable resting and action potentials. In order to study depolarization-dependent changes in intracellular Ca^{2+}, the neurons were loaded with the Ca^{2+} fluorescent dye, Fluo-3AM (cell permeant), and examined with a laser scanning confocal microscope or with an epifluorescent system while under continuous perfusion with normal Krebs solution. When the neurons were depolarized with brief bath applications of substituted Krebs containing 62 mM KCl, all three classes of DRG neurons (small, medium, and large) generated intracellular Ca^{2+} signals. The Ca^{2+} signals were prominent in cell bodies, neurites and growth cones, and they were nearly eliminated by the addition of divalent cations suggesting that transmembrane Ca^{2+} flux was primarily responsible for initiating the signals. These results indicate that soon after dissociation adult sensory neurons give rise to growth cones and neurite extension. Depolarization of these neurons leads to transmembrane fluxes of Ca^{2+} into cell bodies, neurites, and growth cones. Supported by the VA and the NMSS.

372.9

$GABA_A$ RECEPTOR-MEDIATED CONDUCTANCES OF ADULT DORSAL ROOT GANGLION NEURONS INCREASE WITH TIME IN CULTURE. R.B. Bhisitkul, D.L. Eng, and J.D. Kocsis, Dept. of Neurology and Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510; and VAMC, West Haven, CT 06516

Adult rat dorsal root ganglion (DRG) neurons of the lumbar (*L4* and *L5*) spinal cord were dissociated and maintained in culture up to 7-8 days. Single electrode current and voltage clamp recordings were obtained from large diameter GABA-sensitive neurons at 0-24 hours (Group 1) and at 7-8 days (Group 2). Cell body diameter and neurite extension were characterized from neurons injected with the fluorescent dye Lucifer Yellow. Group 1 cultures are characterized by an abundance of DRG neurons with few primary neurites and a paucity of fibroblasts and Schwann cells. These acutely dissociated neurons (Group 1) have deep resting potentials, large amplitude action potentials, a prominent inward rectification to applied hyperpolarizing pulses, and a distinct depolarization and inward current to applied GABA. After a week in culture (Group 2) there was massive proliferation of surrounding nonneuronal cells. Resting potential and action potential amplitude of Group 2 neurons were not statistically different from Group 1. However, there was a marked reduction in input resistance, a doubling of action potential width, an increase in the peak depolarization and current induced by bath application of GABA over a range of concentrations, a positive shift in the equilibrium potential for the GABA response and an increase in GABA-mediated conductance. The correlation of these electrophysiological changes with proliferation of surrounding nonneuronal cells supports the proposal that nonneuronal elements or their products may have a trophic influence with respect to specific physiological properties of neurons. Supported by the VA.

372.10

TRANSGENIC STRATEGIES TO INVESTIGATE CEREBELLAR DEVELOPMENT K. Schilling, J. Oberdick*, R.J. Smeyne, M.H. Dickinson, and J.I. Morgan Dept. Neurosciences, Roche Institute of Molecular Biology, 340 Kingsland Street, Nutley, NJ 07110

A quest of developmental neurobiology is to delineate the molecular entities and mechanisms that orchestrate the ontogeny of the nervous system. To approach this question, we have combined transgenic mouse strategies with a primary culture system that allows cerebellar neurons to be grown under controlled conditions. The normal morphogenesis of Purkinje cell dendrites correlates with the maturation of their electrical properties and increasing cytoplasmic calcium levels. Indeed, electrical activity appears to direct dendritic growth since agents that interfere with elctrical activity and/or synaptic communication compromise normal dendrogenesis. Using cells derived from animals carrying a L7βGal transgene it can be shown that this Purkinje cell-specific gene product is also regulated by electrical activity in culture; indicating that activity can also influence gene expression. Using mice carrying a fos-lacZ fusion transgene, we have analyzed the stimuli that induce c-fos and characterized the responsive cell types. Combining these results we suggest that depolarization and/or synaptic activation of developing Purkinje neurons triggers a sequence of events that includes changes in cytoplasmic calcium levels and the expression of immediate-early genes. Since many cellular immediate-early genes encode transcription factors, they may provide a mechanistic link between neuronal excitation and the transactivation of L7 as well as subsequent changes in Purkinje cell morphology. We are currently using the culture system to further address this hypothesis.

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372.11

HIGH DOSE NMDA INDUCES RETRACTION AND LOW DOSE NMDA INDUCES ELONGATION OF RAT RETINAL GANGLION CELL (RGC) NEURITES. J. Offermann, Kunihiko Uchida* & Stuart A. Lipton. Dept. of Neurology, Children's Hosp & Progr in Neuroscience, Harvard Med Sch, Boston.

We have used real-time, computer-enhanced video microscopy to visualize the effect of varying doses of NMDA (10-100 μM) on identified rat RGCs in vitro. Cultures were produced from 1 to 4 day-old LE rats as described previously (Leifer, Lipton et al., *Science* 1984;224:303; Cheng & Lipton, *Soc. Neurosci. Abstr.* 1989;15:650). Experiments on neurites ≥1 cell diameter in length were performed 4-9 hrs after cell plating. In a low [Mg] bath, high dose NMDA (100 μM) produced *retraction* in 43 of 73 RGC neurites (~60%). This process was, however, reversed in 1 mM external Mg, which resulted in *elongation* in 16 of 20 RGCs. With 10 mM EGTA and no added Ca/Mg in the bath, NMDA produced neurite retraction in only 2 of 7 RGCs (29%), suggesting that $[Ca]_o$ may be an important, albeit not mandatory, factor in retraction. In order to block retraction, we used a receptor antagonist (APV; 100 μM) or a channel blocker (MK-801; 12 μM). The combination of NMDA and MK-801 prevented retraction in 9/9 RGCs, with *elongation* actually being observed in 3 of these cells. With APV, NMDA induced retraction in only 3 of 8 RGCs (38%). In contrast to high dose, low dose NMDA (10-20 μM) produced neurite *elongation* in 24 of 24 RGCs tested. As a control, 5 of 15 RGCs exhibited spontaneous neurite elongation. In 3 of 3 RGCs, neurite elongation in the presence of low dose NMDA occurred despite 10 mM EGTA and no added Ca/Mg in the bath, suggesting that external Ca may not be necessary for elongation under these conditions.

372.12

Effects of Membrane Depolarization on Neurite Outgrowth and Microtubule Polymerization Equilibria in Axons and Dendrites of Sympathetic Neurons in Culture. Charles H. Keith, Department of Zoology, University of Georgia, Athens GA.

In a number of neurons in culture, depolarization, which elevates $[Ca^{++}]_i$, is correlated with a cessation of neurite outgrowth. We have hypothesized that this effect on neurite extension occurs by means of an effect on microtubule polymerization equilibria, and have developed methods to measure polymer/tubulin ratios locally in cells. These methods are based on video densitometry of microinjected rhodamine-tubulin before and after extraction of free dimer.

We find that depolarization of rat sympathetic neurons has effects dependent on the substrate on which the cells are grown. Neurites of cells grown on laminin in the presence of serum - conditions that favor dendrite growth from these cells - retract and depress their microtubule polymerization ratio on depolarization in high K^+. By contrast, neurites grown on collagen in the absence of serum - conditions that promote the development of unipolar, axon-bearing neurons - continue growing and have unaffected polymerization ratios on depolarization. We feel that these differences between axons and dendrites are related to the differences in their microtubule-associated proteins, and may be significant in sculpting axonal and dendritic arbors during development.

372.13

INVOLVEMENT OF $GABA_A$ RECEPTORS IN THE OUTGROWTH OF CULTURED RAT HIPPOCAMPAL NEURONS

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Recent studies in our laboratory have shown a depolarizing effect of GABA in slices of the hippocampus taken from rat neonates (Ben-Ari et al., J. Physiol. 416 : 303 (1989)). In slices of the neocortex the GABA -induced depolarization is associated with a transient rise in intracellular calcium concentration (Yuste and Katz, Neuron 6 : 333 (1991)). Given the multiplicity of Ca^{++} dependent biochemical mechanisms this cation is able to regulate neuritic outgrowth, and growth cone behavior.

We have explored the involvement of $GABA_A$ receptors in the development of dissociated rat hippocampal neurons. Cells were cultured from embryonic day 18-19 hippocampal tissue in a chemically -defined medium on a polylysine substratum coated with fetal calf serum. Pharmacological agents were added three hours after seeding the cells. After a culture period of two to four days cells were silver stained. For each experimental situation about 200 neurons were examined and various parameters were monitored to probe their morphology i.e. number of neurites, bifurcation points and length of processes. When cultures were exposed to 200 µM bicuculline noticeable changes were observed as compared to control cultures or cultures grown in the presence of GABA (10^{-5}M) : i) the number of neurites emanating from the cell soma was lower, ii) the neurites were less ramified, iii) the neuritic length was reduced.

These observations (see also Michler, Int. J. Dev. Neurosci. 8 : 463 (1990)) suggest that the activation of $GABA_A$ receptors during the early development of the rat hippocampus might sculpture neuronal architecture as it has been shown for other neurotransmitters.

NERVE GROWTH FACTOR IV

373.1

EARLY EMBRYONIC QUAIL DORSAL ROOT GANGLIA (DRG) EXPRESS HIGH AFFINITY NERVE GROWTH FACTOR (NGF) RECEPTORS.

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We have previously shown that ^{125}I-NGF binding to DRG, *in situ*, is first seen at embryonic day 3.5 (E3.5; stage 23) in cryostat sections of quail. High affinity NGF receptors appear to be present, because specific binding was seen at a low ^{125}I-NGF concentration (2 ng/ml; 80 pM). These results are surprising since other labs have shown that DRG are unresponsive to NGF prior to E5, with respect to neurite outgrowth and neuronal survival. This is presumably due to an absence of high affinity receptors. We have used two independent methods to confirm the presence of high affinity NGF receptors at this early stage. First, binding was done as above, but control sections were done with a relatively low concentration of nonradioactive NGF (200 ng/ml;8000 pM), in addition to ^{125}I-NGF. Under these conditions, 80% of ^{125}I-NGF bound to high affinity receptors will be displaced while that bound to low affinity receptors will be unaffected. Examination of radioautographs revealed that there was a substantial decrease in the amount of binding under these control conditions. The second method involved determining whether the high molecular weight ^{125}I-NGF-receptor complex, which corresponds to the high affinity NGF receptor, is present. Dissociated E3.5 DRG cell suspensions were exposed to ^{125}I-NGF (20 ng/ml; 800 pM; 2 hr; 4°C), followed by crosslinking. Radioautographs of gels revealed the presence of both the high and low molecular weight complexes. These studies have confirmed the presence of high affinity NGF receptors in E3.5 DRG of quail. The nature of the response(s) mediated by high affinity NGF receptors at this early stage remains to be elucidated. Supported by a grant from the NSF (BNS-8896101).

373.2

DEVELOPMENTAL PATTERN OF NERVE GROWTH FACTOR RECEPTOR EXPRESSION IN THE SENSORY EPITHELIUM OF THE INNER EAR OF QUAIL AND MOUSE.

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A previous study demonstrated the presence of specific NGF receptors on the epithelium of the primordium of the inner ear (otic vesicle, OV) of 72 hr quail embryos (Bernd and Represa, 1989). The present study uses quantitative and radioautographic techniques to define NGF receptor expression throughout development, using both quail and mouse. OV and sensory epithelium obtained from quail and mouse embryos exhibited different levels of specific ^{125}I-NGF binding during development. Maximal levels were achieved at mid-gestation (E7 quail; E14 mouse) in the sensory epithelium of the vestibular labyrinth, and somewhat later in the cochlear duct (E10 quail; E16 mouse). By E13 (quail) or E18 (mouse), ^{125}I-NGF binding was minimal in both vestibular and auditory epithelia. Light microscopic radioautography demonstrated, in both quail and mouse, that NGF receptors were concentrated in areas containing differentiating sensory hair cells, as well as nerve endings. Likewise, areas of OV known to contain presumptive sensory cells bound ^{125}I-NGF. The apparent presence of NGF receptors on developing hair cells or nerve endings suggests that differentiation of the sensory cell population may be affected by NGF. Supported by grants from the Deafness Research Foundation (PB) and NIH (TRV; DC00088).

373.3

NGF TREATMENT IN AGED RATS INCREASES MUSCARINIC INDUCED DOPAMINE RELEASE IN STRIATUM AND IMPROVES SELECTED ASPECTS OF MOTOR PERFORMANCE BUT NOT MAZE LEARNING.

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Emerging evidence supports the potential therapeutic benefits of nerve growth factor (NGF) in treating age-related neurodegenerative disorders. We used 24-mo old male F-344 rats to assess the effects of NGF (0.3 µg/day) delivered intraventricularly by osmotic pump (Alza) compared to implanted age-matched controls and untreated young controls (3 mo). About 3 wk after treatment was initiated, rats were tested in a battery of motor tasks, including open field, inclined screen, wire suspension, rotorod, that had proven to be age sensitive (A. Markowska *et al. Neurobiol. Aging*, 10:31, 1989). Motor assessment was followed by one-way avoidance training in a straight runway and then training in a 14-unit T-maze that has also provided robust evidence of age-related performance declines in rodents (D. Ingram, *Neurobiol.Aging*, 9:475,1988). Comparing 3-mo old rats to aged controls, significant performance declines were noted across age in all tasks except the open-field test. NGF treatment improved performance only in the inclined screen test, but impaired performance in the maze task. Rats were sacrificed 1 day after completing behavioral testing, or about 4 wk after NGF treatment. Analysis of oxotremorine enhancement of K^+-evoked release of dopamine (K^+-ERDA) using perifused striatal slices obtained from these groups indicated greater enhancement of K^+-ERDA in the NGF-treated and young controls than in aged controls. Analysis of choline acetyltransferase activity (ChAT) revealed an age-related decline (32%) in this marker of cholinergic function in striatum of control rats. NGF treatment in aged rats stimulated ChAT activity to levels observed in young controls.

373.4

SURVIVAL OF ISOLATED SEPTAL CHOLINERGIC NEURONS FROM RAT EMBRYOS IN CULTURE WITHOUT NERVE GROWTH FACTOR.

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Nerve growth factor (NGF) enhances cholinergic properties of some septal neurons, but it is not known whether NGF is essential for their survival. We had previously found that cells cultured from embryonic rat septum produce NGF (or NGF-like activity) in amounts sufficient to support the survival of co-cultured sympathetic ganglion neurons. To analyze the trophic requirements of septal cholinergic neurons, we studied the survival of isolated septal cells, thus removing the source(s) of NGF-like activity produced by other cells. Septal cells from day 14 embryonic rats were cultured at very low density (1 to 5 cells per culture well) in a defined medium together with an NGF-free serum fraction that promotes the survival of central neurons.

We found that many acetylcholinesterase (AChE)-positive septal neurons survived for more than 20 days isolated in cell culture. (Co-cultured dorsal-root ganglion or sympathetic ganglion neurons died within 48 hours.) Some septal progenitor cells occasionally divided in culture, giving rise to clones of AChE-positive cells. After 15 days in culture, 40-50% of the cells were intensely AChE-positive, and about the same percentage stained with antibody 192 against NGF-receptor. Addition of NGF or anti-NGF antibodies did not affect septal neuron survival, cell proliferation or number of AChE-positive neurons.

We conclude that under these culture conditions the survival of many septal neurons expressing AChE and/or NGF receptors does not depend on NGF or the presence of other cells.

373.5

EXPRESSION OF THE NGF RECEPTOR GENE BY CEREBELLAR PURKINJE NEURONS IN CULTURE IS REGULATED BY DEPOLARIZING INFLUENCES. S. Cohen-Cory, R.C. Elliott, C.F. Dreyfus and I.B. Black. Lab. of Neurobiol., Rockefeller Univ., New York, NY 10021, and Dept. of Neurosci and Cell Biol. UMDNJ-Robert Wood Johnson Med Sch., Piscataway, NJ.

Increasing evidence suggests that NGF plays an important role during cerebellar ontogeny. Purkinje cells express both low- and high-affinity NGF receptors during development, suggesting responsiveness to the trophic agent. Indeed, previous work indicates that afferent neurotransmitters and NGF interact to regulate Purkinje cell survival and morphogenesis (J. Neurosci. 11: 462-471,1991).

To investigate mechanisms by which afferent neurotransmitters and trophic factors interact, regulation of NGF receptor gene expression by depolarizing influences was studied in dissociated cerebellar cell culture. Purkinje cells were identified immunocytochemically with anti-Calbindin D28K antibodies (a gift of Dr. S. Christakos) and were shown to express NGF-R immunoreactivity. Sensitive receptor cross-linking, and ribonuclease protection assays were employed to examine NGF receptor protein, and messenger RNA levels respectively. Exposure of neurons to pharmacologic depolarizing agents, or to the excitatory neurotransmitter aspartate substantially increased both NGF receptor mRNA and protein expression. In addition, expression of the NGF gene by cerebellar glia in culture was studied. Our results suggest that in the cerebellum, impulse activity increases Purkinje cell responsiveness to NGF by regulating NGF receptor expression, while glia may act as a potential local source of NGF synthesis. (Supported by NINDS, NICHD, March of Dimes and McKnight Fellowships).

373.6

UP-REGULATION OF NGF RECEPTOR BY BDNF AND NGF IN C6 GLIOMA CELLS.
P.E. Spoerri, L. Petrelli*, A. Negro[1], L. Facci, R. Dal Toso, A. Leon and S.D. Skaper. Fidia Research Laboratories and [1]Advanced Technology Division, Fidia S.p.A., 35031 Abano Terme, Italy.

The neuronotrophic proteins BDNF and NGF are structurally related, sharing about 50% amino acid sequence homology (Leibrock, J. et al. Nature 341:149, 1989) and the same low affinity receptor (Rodriguez - Tébar, A. et al. Neuron 4:487, 1990). In this study we investigated NGF receptor (NGF-R) immunoreactivity in cultured C6 rat glioma cells treated with mouse NGF (50ng/ml) or recombinant rat BDNF (10ng/ml). The cells were exposed to the anti-NGF-R antibody 192-IgG, followed by IgG conjugated with RITC or colloidal gold. Untreated C6 cells exhibited occasional fluorescence or gold label. Cells treated with NGF or BDNF showed significantly augmented fluorescence or gold labeling on all surfaces. We are currently examining if this up-regulation of low-affinity NGF-R by NGF and BDNF is reflected by a corresponding increase in gene transcripts for this NGF-R protein.

373.7

CORTICAL NEURONS REGULATE NERVE GROWTH FACTOR SECRETION FROM CO-CULTURED ASTROGLIA.
B.C. Wise, B. Tang*, and X. Vige. FGIN, Georgetown University, Washington, D.C. 20007.

Neonatal rat cortical astrocytes and neurons in primary culture synthesize and secrete nerve growth factor (NGF). The content of NGF mRNA in cortical neurons is comparable to that in astrocytes, although the ratio of secreted to cellular NGF is about 2 for neurons and 75 for astrocytes. Co-culturing neurons with astrocytes for 7 days decreased NGF secretion to 30% of the level seen in astroglia alone and to 70% of that seen in neurons alone. The neuronal inhibition on co-culture NGF secretion was dependent on time, the number of neurons plated, and was partially reversible upon selectively decreasing the number of neurons by glutamate (0.5 mM for 24 hrs) treatment. Interleukin-1 (IL-1, 10 U/ml) and the phorbol ester TPA (100 nM) stimulated by about 4- and 7-fold, respectively, NGF secretion from astrocytes while having only small effects on NGF content in neurons. The magnitude of the IL-1 stimulated NGF secretion was decreased by 65% in the co-cultures suggesting a down-regulation of astroglial responsiveness induced by the neurons. Addition of neuronal cell membranes, cytosol or neuronal conditioned medium failed to change NGF secretion from astrocytes. Thus, astroglial NGF secretion including secretion stimulated by IL-1 and TPA may be regulated by neurons.

373.8

Nerve Growth Factor (NGF) Modifies Solitary Striatal Neurons In Culture.
P.W. Coates and M.S. Walker*. Department of Cell Biology & Anatomy, Texas Tech University HSC-School of Medicine, Lubbock, TX 79430.

Central cholinergic neurons degenerate in Alzheimer's disease. Neurons from cholinergic regions other than basal forebrain, such as striatum, can be affected. NGF may reverse this process. It is difficult to adequately assess direct effects of NGF on such neurons in vivo or in vitro, since contact with other neurons and/or glia may provide NGF. In vitro, neurons typically reaggregate and make extensive connecting neurite networks and also contact glial cells. Thus, effects of cell contact and/or endogenous paracrine factors cannot be distinguished from putative effects of exogenous NGF. Our lab is using a culture model (three-dimensional hydrated collagen lattice) in which neurons easily grow and differentiate as single non-contacting cells, to determine whether such neurons from different cholinergic (and non-cholinergic) regions of fetal rat brain are independently able to respond to NGF. For this project, neurons from the striatum were used. To determine whether solitary striatal neurons express cholinergic properties without 'instructions' provided by cell contact, immunocytochemistry was used to localize the enzyme choline acetyltransferase. To determine whether there was a dose-dependent change in growth and differentiation or in survival, the neurons were cultured with and without 2.5 S NGF (10, 50 and 100 ng/ml). Quantitative measurements were obtained using image analysis. Data was analyzed using a non-parametric analysis of variance. There were significant ($p < 0.05$) dose-dependent increases in growth and differentiation by 48 hr. However, survival was not promoted by any dose of NGF. Results suggest that striatal neurons are intrinsically NGF-responsive with respect to growth and differentiation but not survival. Synaptic or other cell contact-mediated mechanisms are not required for the response. Axons, dendrites and complexity of receptive fields appear to be neuronal structures specifically modified by NGF. Supported by INS-TTU and NS 20802.

373.9

NGF-LIKE IMMUNOREACTIVITY IN RESECTED HUMAN NEOCORTEX.
K.A. Crutcher and J. Weingartner*. Dept. of Neurosurgery, Univ. of Cincinnati, Cincinnati, OH 45267.

Nerve growth factor-like immunoreactivity (NGF-LI) has been demonstrated in the CNS of rodents and non-human primates. However, there is little information on the presence of NGF-LI in human brain tissue, although NGF mRNA and NGF receptor mRNA levels have been reported. A monoclonal antibody (27/21, Boerhinger) raised against purified mouse NGF has also been reported to reliably detect human recombinant NGF (Heinrich and Meyer, '88; Söderström et al. '91). We sought to determine whether NGF-LI can be detected in human brain using a 2-site ELISA. The monoclonal antibody (1G3) was raised against mouse NGF and kindly provided by Dr. William Mobley (UCSF). The polyclonal antibody was raised in a goat immunized with purified mouse NGF (provided by Dr. Mobley) and prepared by Hazelton Research Products, Inc. (Denver, PA). Samples were obtained from temporal or frontal lobe resections undertaken for the treatment of epilepsy (provided through Dr. Hwa-Shain Yeh and Dr. Michael Privitera, UC Med. Ctr.). The tissue was frozen on dry ice and stored at -70°C. The samples were thawed and homogenized at a 1:10 dilution in buffer containing 1% Tween. The ELISA was carried out as described previously using mouse NGF as the standard (Saffran et al., '89). Recoveries were assessed by adding 25 pg of mouse NGF to parallel samples. The average concentration of NGF-LI in these samples (n=19) was .33 ng/gm +/- .13 (s.d.) with an average recovery of 61%. These results indicate that NGF-LI can be detected in fresh tissue samples of human neocortex and provide a basis for further studies of NGF-LI in human neurodegenerative disorders, including Alzheimer's disease. (Supported by the Samuel A. Blank Research Fund of the ADRDA.)

373.10

A NOVEL POPULATION OF NGF RECEPTOR-IR CORTICAL NEURONS IN ADVANCED AGE AND ALZHEIMER'S DISEASE.
E.J. Mufson, G.A. Higgins, and J.H. Kordower, Dept. Neurological Sci., Rush Presbyterian Med.Ctr., Chicago Ill. 60612, and NIA, Bethesda MD.

In our ongoing evaluation of degenerative and regenerative processes associated with aging and dementia, we observed a novel population of cortical neurons which express the receptor and mRNA for nerve growth factor (NGF). These neurons are found preferentially within the temporal neocortex in very old (e.g 98 year old) normal patients. Furthermore, NGF receptor-immunoreactive (NGFR-ir) cortical neurons were consistently observed in Alzheimer's disease (AD) patients (n=12) of all ages. In contrast, such neurons were rarely seen in normal patients below 80 years of age (n=12). These neurons were scattered throughout all cortical lamina and morphologically appeared interneuronal displaying small somata with extensive dendritic arbors. These cells appeared healthy, failing to counterstain for thioflavin-S. This is in contrast to the extensive neuronal loss and morphological degeneration seen within the AD basal forebrain. A few NGFR-ir cortical neurons were also observed within the amygdaloid complex. In contrast, virtually no such staining was seen in the AD hippocampus. Interestingly, the NGFR-ir neurons seen in cortex appeared morphologically similar to those we have recently observed in the embryonic human cortex (see adjacent abstract). These data suggest that advanced aging and AD initiate an expression of NGF receptors upon select cortical neurons which is not seen in normal adulthood. This novel synthesis of the NGF receptors may be a re-expression of a normal developmental pattern. (Support: AHAF and AG 09468)

373.11

NGF RECEPTOR-IR IN THE DEVELOPING HUMAN BRAIN J.H. Kordower, H.K. Le*, and E.J. Mufson Dept. Neurological Sci., Rush Presbyterian Med.Ctr., Dept. Anatomy & Cell Biology, Univ. Illinois School of Medicine., Chicago Ill. 60612 USA.

NGF receptor-immunoreactivity (NGFR-ir) was examined in embryonic human material (n=5; 10-26 weeks of gestation (E)) and compared to acetylcholinesterase (AChE) and diaphorase (NADPH-D) staining. Numerous NGFR-ir neurons were observed at E16 weeks within the basal forebrain (BF). Similar AChE staining was seen at E16 while NADPH-D containing BF neurons were not yet observed. At E16, NGFR-ir fibers were traced within the external capsule to layers V and VI of the cerebral cortex with beaded processes in layers II and III. At E16, NGFR-ir neurons were seen in the substantia nigra and a band of NGFR-ir was observed coursing the length of the ventral brainstem. At E19, numerous NGFR-ir neurons were observed in cortex which was enhanced at E21. Numerous NGFR-ir and AChE-containing neurons also were seen in both segments of the globus pallidus (GP) at E21. A few NGFR-ir neurons were first seen in the striatum at E21. This pattern was dissociated from AChE-containing neurons which were more numerous at this time and were also evident in the younger specimens. At E24 we observed an apparent reduction in NGFR-ir cortical cells within the infragranular layers, ir neurons were restricted to the external GP and the stained neurons in substantia nigra were replaced by fibers. In striatum at E24, we found more NGFR-ir neurons, as well as a patch-matrix pattern similar AChE and NADPH-D stained sections. Similar staining patterns, including the persistence of NGFR-ir cortical neurons, were observed at E26 weeks.

373.12

DIFFERENTIAL DISTRIBUTION AND CELLULAR COMPARTMENTALIZATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR C Wetmore[1], Y Cao*[2], RF Pettersson*[2], L Olson[1]

[1]Dept. of Histology & Neurobiology, Karolinska Institute, Box 60400, and [2]Ludwig Institute for Cancer Research, Stockholm Branch, Box 60202, 10401 Stockholm, Sweden.

Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor (NGF) family of trophic factors, has been shown to affect the growth of distinct, yet somewhat overlapping populations of neurons which are known to be stimulated by NGF. Specifically, while all three family members affect the growth of neural crest sensory neurons, only BDNF has been shown to stimulate the growth of dopaminergic neurons from substantia nigra, and to increase the number and uptake of GABA-containing neurons in basal forebrain cultures.

In the present study, antibodies were raised against synthetic peptide sequences contained within pro- and mature forms of BDNF. These antibodies have been used used to localize BDNF to cells which synthesize the protein, as well as cells in which BDNF mRNA has not been detected. Specifically, at the subcellular level, these antibodies recognize nuclei as well as cytoplasm of the same populations of neurons in hippocampus and cortex which contain BDNF mRNA, which give evidence of differential subcellular compart-mentalization and transport of the BDNF. BDNF can also be detected in cytoplasmic granules in the perikarya of cholinergic neurons known to project to regions enriched in BDNF-synthesizing neurons. We propose that, among other places, BDNF is synthesized in hippocampal and cortical pyramidal cells and that one form of the protein enters the nucleus and may directly influence transcription factors, while another form is directed to other non-nuclear compartments.

373.13

BDNF AND NT-3 ARE EXPRESSED IN SENSORY NEURONS AND MOTONEURONS DURING MOUSE EMBRYOGENESIS. L.C. Schecterson and M. Bothwell. Dept. of Physiology and Biophysics SJ-40, University of Washington, Seattle, WA 98195

The survival of sympathetic neurons, primary afferent sensory neurons and motoneurons during development is dependent upon the presence of neurotrophins and neurotrophic factors produced by the target tissues of these neurons. To identify specific cells expressing neurotrophins, *in situ* hybridization was performed on several stages of developing mouse embryos using radiolabeled NGF, BDNF and NT-3 cRNAs as probes. The results demonstrated that sympathetic neurons, sensory neurons and motoneurons also express these neurotrophins. BDNF mRNA was detected in most neurons in trigeminal ganglia and dorsal root ganglia (DRG), beginning at E15.5, and in some cells at E17.5, P1 and P3, whereas NT-3 mRNA expression was present in a fewer number of cells compared to BDNF in the DRG at E15.5. Expression of NT-3 was not detected in DRG neurons at P1 and P3. In contrast, NT-3 mRNA expression was abundant in motoneurons in the spinal cord from early stages (E 8.5) through birth. Sympathetic ganglia contained both BDNF and NT-3 mRNAs at E17.5 and P1. Expression of BDNF and NT-3 mRNAs in the developing mouse appears to be confined specifically to neurons and may be involved in either regulation of neuron-Schwann cell interactions, or in autocrine or paracrine regulaton of cell survival and neuronal maturation. Alternatively, these factors may be used by the targets of these neurons.

373.14

LOW-AFFINITY NERVE GROWTH FACTOR RECEPTOR EXPRESSION IN REGENERATING AND NON-REGENERATING ADULT RAT SPINAL MOTONEURONS M. Rende*, T. Hagg, S. Varon, Dept. Biol., UCSD, La Jolla, Calif.

Somatic motoneurons (SMN) of the rat spinal cord express low-affinity NGF receptors (LNGFR) and its mRNA during developmental stages characterized by axonal growth. In the adult, only 3% of the SMN are LNGFR immunoreactive (LNGFR-IR). The potential correlation between reexpression of LNGFR-IR in adult lumbar SMN induced by sciatic nerve injury and motor axon regeneration was investigated. We established a time-course of the number of LNGFR-IR SMN following a cut lesion with resection of the distal stump (no regeneration), a crush lesion (followed by regeneration), and implantation of a silicone chamber between the cut nerve stumps (regeneration delayed relative to crush). With a cut lesion alone, the number of LNGFR-IR SMN rapidly increased to a maximum between day 1 and 7 and returned to baseline levels by day 30. After a crush lesion approximately the same number of LNGFR-IR SMN appeared with the same time-course but the progressive disappearance was delayed, so that at 30 days the most caudal neurons (which are last to reach their muscle targets), were still LNGFR-IR. With a chamber, LNGFR-IR SMN appeared 2 days later, but their disappearance was delayed even further, so that the number at 30 days was still maximal and at 60 days still above baseline. Thus, the development of the "LNGFR" response in injured motoneurons is relatively independent of the outcome of regeneration, i.e. may be regulated by a common signal. In contrast, the response is variably sustained in correlation with the duration of the axonal outgrowth process, suggesting that the LNGFR has an important function during axonal outgrowth. Support: NINCDS NS-16349.

373.15

INDUCTION OF NERVE GROWTH FACTOR (NGF) GENE EXPRESSION BY NOREPINEPHRINE (NE) IS PARTIALLY MEDIATED THROUGH N-METHYL-D-ASPARTATE (NMDA) RECEPTORS. F. M. Sessler, B.J. Gwag, R.D. Mouradian, B.D. Waterhouse, and J.E. Springer Depts. of Neurology, and Physiology and Biophysics, Hahnemann University, Philadelphia, Pa 19102.

The presence and actions of NGF in the central nervous system (CNS) support its role as a growth factor involved in neuronal plasticity. The mechanism(s) by which this neurotrophic factor is regulated is still unclear. We demonstrated in our companion study that NMDA infusions increase hippocampal NGF mRNA expression in a time-dependent fashion (Gwag et al). NMDA receptor stimulation is involved in a number of neuronal events including the induction of long term potentiation, which can be facilitated by NE. Therefore, we used RNA protection assays to determine whether NGF mRNA expression in cortex or hippocampus is altered in the presence of NE. In addition, we tested whether the effects of NE can be modified by pretreatment of the brain sections with the NMDA antagonist, 2-amino-5-phosphonovaleric acid (AP-5). Cortical and hippocampal tissue slices (300 microns) were incubated for 1 hr. at 35°C in oxygenated artificial cerebrospinal fluid (ACSF). NMDA and NE were applied at various concentrations for brief intervals and slices then allowed to incubate in ACSF for two more hours. Some sections were incubated in the presence of AP-5 prior to the application of NMDA or NE. The sections were then frozen on dry ice, total RNA isolated, and the levels of NGF mRNA determined using the RNA protection assay procedure. The application of NMDA or NE increased NGF mRNA. Interestingly, the effects of NE were partially blocked by pretreating the slices with AP-5, suggesting an indirect effect of NE. The results of these studies indicate that NGF may be under the control of both NMDA and NE neurotransmitter systems, and that these two systems may interact to further regulate the expression of this neurotrophic factor. Supported by PHS grant AG-08969 (JES), NINDS KO4 NS01233 and the Klingenstein Foundation (BDW), and BRSG 2-SO7-RR07241 (FMS).

373.16

N-METHYL-D-ASPARTATE (NMDA) REGULATION OF NERVE GROWTH FACTOR (NGF) GENE EXPRESSION. B.J. Gwag, F.M. Sessler, R.D. Mouradian B.D. Waterhouse and J.E. Springer, Depts. of Neurology, and Physiology and Biophysics, Hahnemann University, Philadelphia, PA 19102-1192

NGF is a neurotrophic agent that may be associated with plasticity occurring in basal forebrain cholinergic neurons and their cholinergic projection fields (e.g. hippocampus and neocortex). However, it is unclear how NGF may be regulated in the CNS. Recent studies show that neuronal production of NGF mRNA is increased in hippocampus and cortex following limbic seizures generated by electrolytic lesion or kainic acid. Based on the anatomical distribution and function of NGF in the CNS we have suggested that NGF may also play a role in plasticity associated with learning and memory. At the present time, long term potentiation is one candidate for information storage in the mammalian brain and requires the activation of NMDA receptors for induction. We used in situ hybridization and RNA protection assays to investigate whether NMDA can modify NGF mRNA expression. Radiolabeled antisense NGF mRNA probes were transcribed from a 771bp cDNA (a gift from S. Whittemore) and hybridized to brain sections of rats which were sacrificed at 15 min, 4, and 24 hours following hippocampal injection of NMDA. NGF mRNA levels were dramatically increased in the stratum granulosum at 4 hours but unchanged at 15 min and 24 hrs after NMDA treatment. When 2-amino-5-phosphonovaleric acid (AP-5), an NMDA antagonist, was infused into lateral ventricle 30 min prior to NMDA administration, the NMDA-induced increase of NGF mRNA was blocked. These results were confirmed using the RNA protection assay. This study demonstrates that NMDA receptor activation can alter the expression of NGF mRNA in hippocampus. Supported by PHS grant AG-08969, the Alzhemier's Disease Foundation (JES), NINDS KO4 NS01233 and the Klingenstein Foundation (BDW) and BRSG 2-SO7-RR07241 (FMS).

373.17

ALTERED EXPRESSION OF NGF AND NGF-RECEPTOR IN INJURED TEETH: EFFECTS OF PRIOR DENERVATION OR GLUCOCORTICOID PRE-TREATMENT. M.R. Byers, E.F. Wheeler, M. Bothwell. Anesthesiology, Bio. Structure, Physiology and Biophysics. Univ. of Washington, Seattle, WA, USA 98195

Profuse sprouting of sensory nerve fibers occurs in adult tooth pulp by 1-4 days following injury. A possible role for nerve growth factor (NGF) in that response is suggested by the present experiments. Adult rats (n=13) were anesthetized and injured by drilling cavities halfway into molar dentin. A subsequent similar operation allowed comparison of 2 different injury times (6 hr, 1 d, 2 d or 5 d) with intact controls in each jaw. Formaldehyde-fixed tissues were decalcified and prepared for in situ hybridization using ^{35}S-UTP-labeled sense and antisense riboprobes for NGF and NGFR. Digital analysis of autoradiograms showed that pulpal fibroblasts co-expressed NGF and NGFR mRNAs in intact teeth. By 6 hrs after injury, NGF expression had increased 2-10x compared to adjacent intact controls (mean ± SD, 4.5 ± 2.6), but NGFR expression decreased close to background levels. By 2-5 days NGF mRNA expression had also decreased below normal. The inverse shifts in fibroblastic NGF and NGFR expression at 6 hrs after injury were not inhibited by prior denervation (n=3) or pre-treatment with dexamethasone (n=5). Thus the synthesis of NGF by pulpal fibroblasts during the first day after tooth injury must be regulated by endogenous mechanisms that do not require nerve fibers or arachidonic acid metabolites. The local increase in NGF in injured tooth pulp precedes neural sprouting and may stimulate it. Supported by NIH Grants DE05159, HL43397, NS23343.

373.18

NGF DOES NOT IMPROVE RECOVERY OF VISION AFTER GRADED OPTIC NERVE CRUSH.

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Nerve Growth Factor (NGF) is known to have neuroprotective effects after injury, enhancing survival of different cell types in the CNS, including retinal ganglion cells (Carmignoto et al., J.Neurosci.9, 1989, 1263). To assess whether NGF administration also improves recovery of vision following trauma, we have now used the graded crush of the rat optic nerve as a model (Duvdevani et al. Rest. Neurol. Neurosci. 2,1990, 31).

In our behavioral task rats had to learn to orient towards visual stimuli presented in all sectors of the visual field. Two groups of rats which sustained a unilateral crush of the optic nerve received intraocular injections of either NGF (2.6µg in 1µl, n=12) or saline (1µl, n=12) on postoperative days 0, 2 and 4. Two sham operated groups were treated accordingly (n=4, n=3). Following crush we observed an initial deficit in the rats' orienting performance, followed by near complete recovery of vision within 2 weeks. However, NGF did not affect the rate or extent of recovery.

It is conceivable that the amount of NGF, the mode or the duration of NGF-administration was not optimal. In addition, while NGF may enhance cell survival after mild optic nerve crush, a study currently under investigation, this may be of no functional benefit.

We thank FIDIA Research Laboratories for kindly providing NGF.

373.19

PARTIAL INHIBITION OF NGF-INDUCED CHICK NEURONAL SURVIVAL BY ANTIBODIES TO PEPTIDES OF NGF. R.A. Murphy, A. Acheson, J. Haskins, E. Reklow, and R. Hodges. Dept. of Anatomy and Cell Biology and Dept. of Biochemistry. Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Antibodies raised in rabbits against synthetic peptides of mouse NGF were tested against salivary NGF in chick neuron survival assays. Antibodies to residues 23-35, 59-67, and 69-79 recognized mouse NGF on Western blots, and antibodies to 23-35 and 59-67, but not 69-79, reacted with NGF in ELISAs. Conversely, antibodies to intact NGF recognized peptides 23-35 and 59-67, but not 69-79, and a monoclonal antibody (MC 27/21, Boehringer Mannheim) that blocks NGF biological activity recognized only peptide 23-35. The antipeptide antibodies did not alter the elution position of NGF on gel filtration columns, indicating a lower affinity than antibodies to the intact protein.

Under conditions where antibodies to NGF and MC 27/21 blocked NGF-induced survival of E 10 sensory neurons, antibodies to peptides 23-35 and 69-79 were ineffective; however, antipeptide 59-67 inhibited survival by approximately 85%. Conversely, tested in combination with NGF at a 1000 molar excess, peptide 23-35 inhibited survival by 54% and peptide 59-67, by 79%. Peptide 69-79 had no effect. On E10 sympathetic neurons, treatment either with antibodies to peptide 59-67 or with peptide 59-67 together with NGF inhibited survival by 45%. Results suggest that residues 23-35 and 59-67 play important roles in the survival promoting activity of NGF on chick neurons.

373.20

ANTIBODIES AGAINST NGF PEPTIDES INHIBIT THE BIOLOGICAL ACTIVITY OF FIBROBLAST-DERIVED NGF AND BDNF. A. Acheson, R.F. Alderson+, R. Hodges*, M. Russell+* and R.A. Murphy. Univ. of Alberta, Dept. of Anatomy & Cell Biology, Edmonton, AB and +Regeneron Pharmaceuticals, Inc., Tarrytown, NY.

NGF is a target-derived trophic factor, and a variety of cell types secrete NGF in culture. Mouse L929 fibroblasts (L cells) secrete into conditioned medium (CM) molecules with both NGF- and BDNF-like biological activities, and express both NGF and BDNF mRNA. We have recently shown that polyclonal antibodies raised against intact salivary NGF recognize recombinant human BDNF on Western blots and inhibit both NGF- and BDNF-like biological activities present in L cell CM, as well as 65% of recombinant BDNF's survival-promoting activity *in vitro*. We have now raised antibodies against 3 peptides derived from the mouse NGF sequence and examined their ability to block L cell CM-mediated survival of E10 chick DRG neurons. L cell CM promotes the survival of 91% of DRG neurons over 3 days *in vitro*. Both anti-NGF and anti-peptide C (amino acids 69-79 of mNGF) substantially block the activity of L cell CM (-86% and -71%, respectively), whereas antibodies against peptides A (23-35) and B (59-67) were less effective (-43% and -28%, respectively). When tested on Western blots, all 3 anti-peptide antibodies recognized recombinant human BDNF, with anti-peptide C being the most effective. All 3 anti-peptide antibodies also recognized mouse salivary NGF on Western blots, with anti-peptide A being the most effective, but only anti-peptide B inhibited NGF-mediated survival (see R.A. Murphy et al., Neurosci. Abstr. 1991). Anti-peptide antibodies also inhibited the biological activity of BDNF on E8 DRG neurons (-34%, 51% and 40% for anti-peptides A-C respectively). Results suggest that NGF and BDNF, molecules with 50% amino acid identity, may also share functionally-important epitopes.

NON-NEURONAL CELLS I

374.1

NGF REGULATES GLIAL CELL NUMBER IN SEPTAL DISSOCIATED CULTURES. M. Yokoyama, I.B. Black, and C.F. Dreyfus. Cornell Univ. Med. College and Dept. Neurosci. and Cell Biol. UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, N.J.

In previous studies, we have found that low affinity NGF binding sites are associated with flat non-neuronal cells dissociated from embryonic septum and grown for 7 days. These cells were also labelled immunohistochemically using a monoclonal antibody to the NGF receptor, 192 IgG, suggesting that low affinity NGF receptors are associated with a non-neuronal population (Bernd et al., 1988).

To define the potential effect of NGF on these non-neuronal cells, septal regions from embryonic day 17 rats were dissociated and grown in fully-defined medium either in the presence or absence of NGF. As an additional control, one group of cultures was grown in the presence of cytochrome C, a compound similar in structure and physicochemical properties to the trophic agent. To define actions of NGF on the astrocyte subpopulation in these cultures, glial fibrillary acidic protein (GFAP) was used as a marker. After 7 days, NGF elicited a dramatic 9-fold increase in the number of GFAP-positive cells over control cultures or cytochrome C-treated groups.

Our results suggest that NGF increases astrocyte cell number directly or indirectly. Moreover, in related studies, we have demonstrated that during development or after a lesion, proliferating glia express the NGF gene *in vivo* (Lu et al., 1991). These data suggest that astrocytic NGF, acting in an autocrine or paracrine fashion, may regulate astrocyte cell numbers during development, or in response to injury. Presently we are investigating the mechanisms through which NGF may exert its effects. (Support: NINDS, NICHD, McKnight Foundation, JDF and March of Dimes)

374.2

RECEPTORS FOR CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN ASTROCYTE CULTURES FROM NEONATAL RAT BRAIN. M. Reddington, P. Lazar*, G. Ralvich*, W. Streit and G.W. Kreutzberg. Dept. of Neuromorphology, Max Planck Institute for Psychiatry, Martinsried, F.R. Germany.

Axotomy of the rat facial nerve leads to hypertrophy of astrocytes and to the proliferation of microglial cells in the facial nucleus. Since the concentration of the neuropeptide CGRP increases rapidly after axotomy in the injured motoneurons it has been suggested that CGRP might play a role in neuron-glia interaction following nerve injury (Streit et al., Neurosci. Lett. 101, 143-148, 1989). This possibility was examined using 9-14 day-old cultures of astrocytes obtained from neonatal rat brain. Addition of CGRP in the range 10 - 1000 nM increased the concentration of cyclic AMP by up to 20-fold in astrocyte cultures. This action was inhibited in the presence of equimolar concentrations of the CGRP receptor antagonist CGRP(8-37). Calcitonin stimulated cyclic AMP accumulation only at micromolar concentrations. Ligand binding studies using ^{125}I-Tyr-CGRP showed membranes derived from astrocyte cultures to have a single class of high affinity binding sites (Kd 0.3 nM). ^{125}I-Tyr-CGRP was displaced by unlabeled CGRP and by CGRP(8-37) but not by calcitonin. The properties of these CGRP binding sites were identical with those in membranes derived from adult rat brain. These data show that astrocytes bear functional CGRP receptors linked to adenylate cyclase and support a role for CGRP in neuron-astrocyte interaction.

374.3

CULTURED GLIAL CELLS FROM THE OLFACTORY BULB OF THE ADULT RAT. A. Ramón-Cueto* and M. Nieto-Sampedro. Neural Plasticity Lab., Cajal Institute, Madrid 28002, Spain

Cultures of olfactory nerve and glomerular layers (ONGL) of the adult rat olfactory bulb contained three types of cell, distinguished by both morphological and immunohistochemical criteria. One cell type was multipolar and GFAP-positive; a second type had fried egg-like morphology, reacted with antibody to ED1 and had all the features of the monocyte-microglia lineage. The third type had elongated, fusiform morphology, was GFAP and ED1-negative, laminin-positive and may be capillary endothelial cells. Trypsinization (3 min, 37 °C) of these primary cultures detached multipolar and bipolar cells only. When secondary cultures of the detached cells were set up on a glass substrate (PLL-treated labtek multiwell chambers) bipolar cells did not attach, leaving a pure culture of multipolar cells These cells were capable of enfolding axons, were GFAP- and myelin basic protein (MBP) positive and may be the so-called ensheathing cells or Blanes glia. We have identified at least three immunologically distinct ensheathing cell populations, based on immunoreactivity with anti-NGF receptor and anti-fibronectin. The three populations were multipolar, GFAP, MBP-positive. However, one of them (about 42%) was NGFR-positive, fibronectin-negative. The other population (41 %) was NGFR-negative and fibronectin-positive. The remainder cells (about 17 %) were negative for both antibodies. The cultured ONGL cells have morpholoby, behavior and immunological properties very similar to those of Schwann cells. Ensheathing cells surround olfactory axons and their Schwann-like properties could account for the permissivity of the olfactory bulb to axonal growth.

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374.4

IMPROVED YIELDS IN SCHWANN CELL CULTURES FROM ADULT RAT SCIATIC NERVES A.D. Ansselin*, D.F. Davey and S.D. Corbeil*, Microsurgery Research Centre, Sydney and Department of Physiology, University of Sydney, 2006, NSW Australia.

Schwann cell cultures from adult rat peripheral nerves are difficult to obtain because of the abundance of connective tissue and myelin. It has been shown that both neurons and Schwann cells are conditioned by a nerve lesion, speeding up Schwann cell proliferation and neuronal regeneration. This effect is most pronounced in the first three days after applying the lesion (Sjoberg & Kanje, *Brain Res.* 529:79-84, 1990). In this study, we have attempted to improve the yield in the culture of Schwann cells obtained from adult rat sciatic nerve by applying a conditioning lesion 1 to 10 days (inclusive) prior to isolating the cells. The left sciatic nerve in young adult Wistar rats was exposed, severed at the sciatic notch and deflected. At the appropriate survival time, the severed (conditioned) and unoperated (control) sciatic nerves (20mm each side) were excised using sterile techniques, and processed for tissue culture according to the method described by Scarpini *et al.* (*Exp. Neurol.* 102:167-176, 1988). The cells from each nerve segment were counted prior to plating on a single laminin coated well in 6-multiwell plates (Linbro). Cell counts indicate that severing the nerve prior to dissociation for culture increases the yield of Schwann cells 48h after lesioning, and the best yields were obtained at day 5 and day 6. Furthermore, 24h after plating, more Schwann cells isolated from the lesioned nerve than from control nerve were attached to the laminin substratum.

374.5

ACTIVATION OF STROMELYSIN IS REQUIRED FOR AUTOCRINE INHIBITION OF SCHWANN CELL PROLIFERATION.
D. Muir and S. Varon. Dept. of Biology, University of California, San Diego.

Cultures of isolated rat sciatic nerve Schwann cells (SCs) proliferate very slowly in serum-supplemented medium. A recent report (Muir et al., 1990. J. Cell Biol. 109: 2663) described that SC conditioned medium (CM) contains several distinct forms of antiproliferative activity and concluded that SC proliferation in vitro is under negative autocrine control. This work included the isolation and partial characterization of a 55 kD neural antiproliferative protein (NAP) found in SC and Schwannoma CMs. In the present study we provide evidence that the 55 kD NAP possesses metalloprotease activity and copurifies with stromelysin immunoreactivity. The SC-derived protease shares many properties with stromelysin isolated from other sources including the ability to cleave the serum and extracellular matrix protein fibronectin (FN). Furthermore, limited proteolysis of FN by the SC-derived protease generates a FN fragment which itself expresses a potent antiproliferative activity for SCs. The antiproliferative FN fragment corresponds to the 29 kD N-terminal region of the FN molecule which is found as an active component in SC CM. Both the NAP / stromelysin and the N-terminal fragment of FN can completely and reversibly inhibit proliferation by mitogen-stimulated SCs while both are similarly ineffective at inhibiting proliferation by transformed SC lines. In addition, although both normal and transformed SC types secrete the proform of stromelysin, in contrast to normal SCs, transformed cultures do not produce activated stromelysin and thus can not generate the antiproliferative fragment of FN. These results suggest that SC -derived stromelysin, once activated, cleaves FN and generates a cryptic antiproliferative activity which maintains normal SC quiescence in vitro.

374.6

CONTROL OF GAP-43 EXPRESSION IN SCHWANN CELLS.
H.J.S. Stewart, R. Mirsky and K.R. Jessen. (Spon: Brain Res Association). Department of Anatomy, University College London, WC1E 6BT, U.K.

It is now apparent that GAP-43 expression is not confined to periods of axonal elongation or indeed to neurones. Recently we have established that GAP-43 is expressed by Schwann cells in vitro and non-myelin-forming Schwann cells in vivo. This study examined the control of Schwann cell GAP-43 expression in the context of other non-myelin-forming Schwann cell phenotypic markers. In particular we studied the role of cAMP, a molecule thought to be of fundamental importance in Schwann cell biology on GAP-43 expression in vitro. Using techniques including immunoblotting and immunocyto-chemistry we show that Schwann cell GAP-43 expression is rapidly downregulated by cAMP and is upregulated by increasing cell density. In addition we show that the density dependence of GAP-43 expression is mediated by a Schwann cell secreted factor. Thus the control of GAP-43 expression is obviously complex and quite different from that of other non-myelin-forming Schwann cell markers. The involvement of cAMP in GAP-43 expression however, emphasizes the importance of this molecule in Schwann cell development.

374.7

CELL POPULATION KINETICS IN THE SUBEPENDYMAL LAYER OF THE CONTROL AND IRRADIATED MOUSE BRAIN. N.B. Manley, K.A. Frankel*, M.H. Phillips*, J.I. Fabrikant*, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

The subependymal layer in the 4 week old $CB6F_1$ mouse brain is a "mixed" cell population proliferating with a moderately high tritiated thymidine labeling index (17.35%--26.35%), but a low mitotic index (0.5%--1.5%). Mitotic figures are seen in all three morphologically different types of cells which are in sequential stages of proliferation, differentiation and migration. The 3 types of cells are: (1) cells with small dark nucleus (SD); (2) cells with small light nucleus (SL); and (3) cells with large light nucleus (LL). Using high resolution autoradiography, we find varying grain-count decrements with time after a single pulse of tritiated thymidine. This suggests that there are varying cell cycle times among these subpopulations.

Percent -labeled mitoses curves (PLM) were derived by a best fit to the experimental data and by the computer- generated modified Barrett-Steel model. Partial brain irradiation with helium (230 MeV/amu) was confined to one cortex of the brain (0.25 x 1.5 cm along the sagittal axis). The unirradiated hemi-brain in the irradiated animal served as an internal control. Unirradiated controls were also used to compare the effects of helium-ion irradiation on the PLM curves.

Analyses of PLM curves obtained one week after irradiation with 10 Gy He and 25 Gy He indicate that this layer is made up of subpopulations with varying cell cycle times and phase durations. There is significantly more cell damage following 25 Gy He irradiation as compared to 10 Gy He.

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374.8

DOUBLE IMMUNOPEROXIDASE (IP) SIMULTANEOUSLY DETECTS IODODEOXYURIDINE (IUDR) AND BROMODEOXYURIDINE (BUDR) IN GLIA. P.E. McKeever, J.M. Rowe*, S.J. Genik*, P.W. McLaughlin*, W.R. Mancini* and W.D. Ensminger*. Pathology Department and Upjohn Center, Univ. Michigan, Ann Arbor, MI 48109-0602.

An IP/alkaline phosphatase (AP) method provides IUDR and BUDR labeling indices (LI) on certain cells (Asai A., et al, J. Neurosurg. 73:254, 1990; Miller MA, et al, J. Histochem. Cytochem. 39:407, 1991). However, other tissues contain endogenous AP difficult to block, and the buffer for IP is incompatible with AP. These factors diminish the flexibility of IP/AP. To alleviate this, we developed a double IP method for IUDR and BUDR. All standard rinses were with phosphate buffered saline (PBS). After DNA denaturation and blocking endogenous peroxidase, sections were incubated 20 min in normal horse serum, followed by 30 min in Br-3 monoclonal antibody (MAb) specific for BUDR (1:1000 in PBS with 1% BSA). Sections were incubated with biotinylated horse anti-mouse IgG for 30 min, and stained with avidin-biotinylated horseradish peroxidase complex using diaminobenzidine (DAB) substrate. After acid elution of immunoglobulins, these sections were incubated with B-44 MAb which binds both BUDR and IUDR (1:10 in PBS-BSA). This was followed by indirect staining with peroxidase conjugated goat anti-mouse IgG using 3-amino-9-ethylcarbazole (AEC) substrate. The resulting red reaction product was distinctive from both brown DAB reaction product and blue hematoxylin, demonstrating a 67% growth fraction and 8% LI in a human glioma xenograft treated with IUDR and pulsed with BUDR. This was much higher than non-neoplastic glia. Since hematoxylin is the effective counterstain for halopyrimidine LI, DAB/AEC provides visually compatible double labeling when AP cannot be used.

374.9

CHARACTERIZATION OF A NOVEL GLIAL CELL ADHESION MOLECULE, G-CAM. M.H. Irwin, T.P. Murphy, and E.E. Geisert, Jr. Department of Cell Biology, Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, Alabama 35294.

Using a mAb, designated AMP1, a novel cell adhesion molecule present in rat astrocyte-astrocyte and astrocyte-oligodendrocyte contacts in culture was identified. In confluent monolayers of cultured neonatal astrocytes, AMP1 showed a discrete labeling of cell-cell contacts. The antibody does not stain neurons cultured from embryonic rat cortex nor does it reorganize under neurons plated on monolayers of astrocytes. When cultures of astrocytes were placed in Hank's balanced salts with 100 μg/ml of AMP1 the astrocyte-astrocyte contacts were severely disrupted. This was not observed in parallel cultures treated with an irrelevant mAb of the same isotype as AMP1. By immunoblot analysis, AMP1 recognized a 106 kDa protein in samples of reduced astrocytic or brain proteins. Immunoblots of 2-D gels indicated the protein has an apparent pI of 6.1. We have tentatively termed this molecule "glial cell adhesion molecule" (G-CAM), and present data demonstrating that G-CAM is distinct from the known cell adhesion molecules present on astrocytes: N-CAM, N-cadherin, or members of the β1 integrin family. Supported by The Spinal Cord Society, The Whitehall Foundation, Inc., and PHS grant NS23613.

374.10

EXPRESSION OF A NOVEL GLIAL CELL ADHESION MOLECULE, G-CAM, IN CNS DEVELOPMENT AND WOUND HEALING. T.P. Murphy, M.H. Irwin, and E.E. Geisert, Jr. Department of Cell Biology, Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, Alabama 35294.

Using the mAb AMP1 we have identified a 106 kDa protein that is involved in glial cell-cell adhesion (G-CAM). By immunoblot analysis, G-CAM expression was detected at low levels in E18 rat brain, gradually increasing to adult levels in the maturing rat CNS. At E18, AMP1 immunoreactivity was restricted to the choroid plexus and ependyma. Later in development, the labeling was observed throughout the brain, and was more intense in areas enriched in glial cells. One of the dynamic processes involving glia in the adult brain is the response to injury. To begin to define the role of G-CAM in the process of CNS wound healing, chronic CNS gliotic scars were labeled with AMP1 and examined by confocal microscopy. In the region of the gliotic scar there was a 100-fold increase in relative fluorescence intensity as compared to surrounding normal tissue, indicating that G-CAM is expressed at high levels in the immediate vicinity of the gliotic scar. Unlike N-CAM, N-cadherin and β1 integrins, G-CAM is glial-specific, indicating that it may play a specialized role in the selective stabilization of glial interactions during the development and wound healing in the CNS. Supported by The Spinal Cord Society, The Whitehall Foundation, Inc., and PHS grant NS23613.

374.11

IMMUNOCYTOCHEMICAL DETECTION OF A NOVEL ASTROCYTE SURFACE MOLECULE IN THE TRANSECTED ADULT RAT OPTIC NERVE (ON). B. Mittal, A. Ajemian* and S. David. Centre for Research in Neuroscience, MGHRI, McGill University, Montreal, Canada, H3G 1A4.

Neurite outgrowth on astrocytes is mediated by interactions involving adhesion molecules such as laminin, N-cadherin, N-CAM and L1. We have previously characterized a novel astrocyte surface molecule using a monoclonal antibody (mAb) designated as 1A1 (Neurosci. Abst., 16:315, 1990). Monovalent fragments of this mAb were shown to inhibit neurite growth from rat cerebellar neurons on astrocytes. Immunoaffinity chromatography using detergent extracts of I^{125}-labeled astrocyte surface proteins, revealed by SDS-PAGE a major band migrating at ≈ 150 kDa under both reducing and non-reducing conditions.

We now report using an indirect immunofluorescence technique that 1A1-immunoreactivity is increased in the degenerating adult rat ON. Enhanced 1A1 staining is seen on astrocytes at the site of injury 10 days after transection, and continues to spread distally along the degenerating ON with time. Weak staining is observed in normal ONs. The role of this neurite growth-promoting molecule in injury-induced axonal sprouting and in the ability of degenerated ON tissue sections to support neurite growth *in vitro* (Neuron, 5:463, 1990) is not yet known. *(Supported by The Canadian MRC and The Rick Hansen Man in Motion Legacy Fund).*

374.12

IMMATURE HAMSTERS INCREASE LEVELS OF VIMENTIN AND GLIAL FIBRILLARY ACIDIC PROTEIN AFTER CORTICOSPINAL AXOTOMY.

S. A. Mikucki, L. Singh* and M. M. Oblinger, Dept. of Cell Biology and Anatomy, Chicago Medical School, North Chicago, IL 60064.

Astrocytes are one of the few cells which express two different intermediate filament (IF) proteins during development. Vimentin, a Type III IF is expressed in high levels in developing astrocytes where as mature astrocytes express glial fibrillary acidic protein (GFAP), another Type III IF. Recently, it has been observed that axotomizing injury to the mature central nervous system (CNS) results in increases in both vimentin and GFAP mRNAs. However, there is little information available regarding the molecular response of developing astrocytes during Wallerian degeneration in immature animals. Can immature astrocytes upregulate both vimentin and GFAP expression prior to the time in which the GFAP gene is normally induced? In the present study, we used *in situ* hybridization and immunological methods to examine whether developing astrocytes alter their normal developmental program of IF expression and increase GFAP and/or vimentin levels during Wallerian degeneration. The corticospinal tract of postnatal day 8 hamsters was unilaterally transected in the medulla just rostral to the pyramidal decussation and animals were allowed to survive for 2, 7 and 14 days after injury. Histological sections of brain stem were hybridized to a ^{35}S-labeled cDNA probe to GFAP mRNA and subjected to autoradiography. The results from *in situ* hybridization experiments revealed that developing astrocytes have the potential for altering their normal developmental program of GFAP expression after injury. We observed substantial increases in GFAP mRNA in the degenerating part of the corticospinal tract compared to the contralateral control as early as 2 days after injury; increase was still present at 14 days. Double-label immunfluorescence experiments examining GFAP and vimentin protein levels during Wallerian degeneration revealed pronounced increases in GFAP levels at 2 days with a less pronounced increase at 7 and 14 days after injury. Increases in vimentin levels were not as dramatic as those for GFAP and were much more confined to the degenerating tract. We believe these results are the first to demonstrate an altered developmental program of GFAP expression after CNS injury in immature hamsters.

374.13

GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) AND ITS MRNA ARE DRAMATICALLY UP-REGULATED AFTER INTENSE NEURONAL ACTIVITY E. R. Torre , E. Lothman and O. Steward. Depts. of Neuroscience, Neurosurgery, and Neurology Univ. of Virginia Health Sciences Center. Charlottesville VA 22908.

Intense neuronal activity produced by electrically-induced seizures leads to dramatic increases in the m RNA for GFAP (Steward et al; J.Neurosci.**10**, 2373; Proc.Natl.Acad.Sci., in press). The present study evaluates whether the activity-induced up-regulation of GFAP mRNA leads to glial hypertrophy similar to that which occurs after lesions. Since increased synthesis of GFA protein is considered a marker for "reactive astrocytes", we evaluated the changes in GFA protein and its mRNA at different times after the induction of kindled seizures in adult rats.

Twelve seizures were induced over the course of 1 day via indwelling electrodes implanted in the CA3 region of the hippocampus. Messenger RNA for GFAP was evaluated by *in situ* and dot blot hybridization techniques. GFA protein in each hippocampus was quantified in western blots and dot blots using a rabbit antibody against GFAP and goat anti rabbit labeled with I^{125}.

Kindled seizures led to 4-5 fold increases in GFAP mRNA as measured by quantitative *in situ* hybridization. These increases were evident near the site of stimulation as well in distant structures in which seizures occured as a result of synaptic activation (the contralateral hippocampus). GFAP mRNA levels were maximal 24 h after stimulation and returned to baseline by 4 days post stimulation. GFA protein levels also increased bilaterally in the hippocampus following kindled seizures, reaching their peak 1 day after the peak in GFAP mRNA levels (at 48 hours poststimulation). However, GFA protein levels remained high for at least 4 d poststimulation. This is similar to the time course of increases in GFA protein levels after lesions of the entorhinal cortex. Our results suggest that a reactive astroglial response similar to that which occurs after lesions can be elicited by intense neuronal activity.

Supported by NIH grant NS12333 and NSF grant BNS8818766 to O.S.

374.14

REGIONAL DISTRIBUTION OF HUMAN BRAIN GFAP AND ChAT. Karson, C.*, Griffin, W.S.T.,** Casanova, M.*, Kleinman, J.E*. Department of Psychiatry & Behavioral Sci.* & Pediatrics**, UAMS, Little Rock, AR 72202. Clinical Brain Disorders Branch, Wash, D.C. 20032*

GFAP, the structural protein of astrocytes and ChAT, a key enzyme in cholinergic neurotransmission, were measured in multiple regions of human brain using Western immunoblot,(N=53, age=34 ± 13 years, M/F=44/9). GFAP concentrations were higher in most rostral structures in contrast with ChAT concentrations (Table). Occipital cortex had GFAP and ChAT concentrations similar to cerebellum and pontine tegmentum.

[1]	FP	LSTG	TH	OC	CER	PT
GFAP (ng/ug protein)						
	2.73±	2.40±	2.96±	1.33±	1.75±	1.40±
	0.78	0.99	1.33	0.83	1.14	0.45
	(N=8)	(N=8)	(N=8)	(N=12)	(N=11)	(N=22)
ChAT (ng/ug protein)						
	.046±	0.048±	0.067±	0.077±	0.068±	0.089±
	.011	.012	.028	.040	.026	.040
	(N=8)	(N=8)	(N=8)	(N=12)	(N=12)	(N=22)

[1] FP=Frontal pole. LSTG=Left superior temporal gyrus. TH=Thalamus. OC=Occipital cortex. CER=Cerebellum. PT=Pontine tegmentum.

SUMMARY. GFAP and ChAT are inversely distributed in human brain which may reflect neuronal density and/or vulnerability to injury of the given brain region.

374.15

HIPPOCAMPAL 5HT INNERVATION DENSITY INFLUENCES THE EXPRESSION OF GFAP AND S-100 DETECTED BY IMMUNOCYTOCHEMISTRY. J.A. Schroer and J.H. Haring. Dept. Anat. & Neurobiol., St. Louis Univ., St. Louis. MO 63104.

Astrocytes possess receptors for 5HT that may have a role in CNS development (Whitaker-Azmitia et al., NY Acad. Sci. 600:315, 1990). A recent in vitro study demonstrated the regulation of astrocyte GFAP expression by 5HT (LePrince et al., Dev. Brain Res. 51:295,1990). The purpose of this study was to determine whether altering hippocampal 5HT innervation would elicit changes in GFAP and S-100 immunocytochemistry.

Adult, male Sprague-Dawley rats received injections of 5,7-DHT (3ug in 20nl saline) or saline alone in the median raphe nucleus (MRN). After 2 or 6 weeks, rats were perfused with 4% paraformaldehyde in PBS and frozen or vibratome sections cut through the hippocampus. Sections were processed for GFAP or S-100 immunocytochemistry and analyzed stereologically using a cycloid test system (Braendgaard & Gundersen, J. Neurosci. Meth.18:39, 1986). MRN lesions resulted in a significant decrease in hippocampal 5HT content and uptake by 2 weeks followed by a return of these parameters to low normal levels by 6 weeks. At 2 weeks, GFAP expression was significantly higher than normal but S-100 was decreased. These changes were most evident in the hilar region where GFAP increased 62% while S-100 fell to 46% of control. The increase in 5HT at 6 weeks was accompanied by a decrease in GFAP and an increase in S-100. These results are consistent with those of in vitro studies. Support: NS25752 and DE07734.

374.16

KERATINS AND GFAP ARE DIFFERENTIALLY DISTRIBUTED IN THE NEUROEPITHELIUM OF THE EMBRYONIC ZEBRAFISH. R. C. Marcus and S. S. Easter Jr. Dept of Biology, University of Michigan, Ann Arbor, MI 48109.

In the embryonic zebrafish, the early axon tracts develop between 16-18h and enlarge thereafter. Application of dil to the ventricles of embryos between 15-72h reveal that axons traverse a cellular environment containing radial cells that extend from ventricular to sub-pial surfaces of the brain. last year we reported GFAP expression in a population of these radial cells (SNA 16, 139.6). We now report that a second intermediate filament (IF) type, keratin, is also expressed in radial cells of the neuroepithelium. Frozen sections from unfixed embryos at 30, 38,48 and 72h were labeled with anti-keratin antibodies (anti bands 3 and 7; Maggs and Scholes, J. Neurosci. 10, 1600). At all time points, radial cells in the hindbrain and spinal cord were labeled in their entirety. Cells located at the ventral midline, in the region of the floorplate, labelled most intensely. By 38h, keratins labelled the optic nerve, consistent with the observation that keratins, and not GFAP, are expressed in the glial cells of the optic nerve of adult fish. Our results demonstrate that IFs define different classes of cells in the embryonic neuroepithelium. SDS page and western blot analysis are underway to further characterize IF expression in these different cell types. Supported by R01-EY-00168 and T32-EY-007022.

374.17

HEME OXYGENASE IS A HEAT SHOCK PROTEIN AND PEST PROTEIN IN RAT ASTROGLIAL CELLS. B.E.Dwyer*, R.N.Nishimura, J.deVellis and T.Yoshida*. Molecular Neurobiology Lab, Sepulveda VAMC, Sepulveda, CA 91343.

The induction and synthesis of heme oxygenase was studied in rat forebrain astrocytes and in spontaneously transformed rat astroglial cells (ATs). Unstimulated astrocytes contained significant amounts of immunostainable heme oxygenase whereas it was undetectable in ATs. Heme oxygenase was inducible in both cell types by heat shock and by submicromolar amounts of H_2O_2. Increased synthesis correlated with elevated levels of heme oxygenase mRNA suggesting that transcription was a major site of regulation under stress conditions. Inhibition of RNA synthesis with actinomycin D or protein synthesis with cycloheximide resulted in the rapid loss of immunostainable heme oxygenase in astrocytes. Analysis of the primary structure of heme oxygenase suggest that it is a PEST protein, proteins which are targeted for rapid turnover.

Supported by the research service of the Department of Veterans Affairs

374.18

INTERLEUKIN-1ß REGULATES PROENKEPHALIN GENE EXPRESSION IN ASTROCYTES IN VITRO. A. Negro, A. Tavella*, L. Facci#, L. Callegaro* and S.D. Skaper#. Advanced Technology Division and #Fidia Research Laboratories - FIDIA S.p.A., 35031 Abano Terme, Italy.

Astrocytes possess receptors for neurotransmitters and neuromodulators functionally coupled with some aspect of intracellular metabolism. Activation of cell surface receptors on astrocytes can also regulate proenkephalin mRNA levels (Melner et al., EMBO J. 9: 791, 1990). Because astroglial cells are biologically responsive to the cytokine interleukin-1ß (IL-1ß), we examined the possible influence of these molecules on the expression of opioid genes in type-I astrocytes cultured from neonatal rat brain. Proenkephalin mRNA expression was enhanced several-fold by IL-1ß in a dose-dependent manner, but not by IL-2, γ-IFN, glutamate or carbacol. IL-1ß also regulated a proenkephalin-chloramphenicol acetyltransferase fusion gene transiently transfected into astrocytes. These effects of IL-1ß were not likely due to a cyclic AMP-inducible DNA element, as IL-1ß not induce the conversion of a stellate astrocyte morphology characteristic of cyclic AMP-elevating agents such as isoproterenol. These results suggest that enhanced proenkephalin gene expression in astrocytes by IL-1ß may be important in neuroimmune interactions and following brain injury.

NON-NEURONAL CELLS II

375.1

POSTNATAL ANGIOGENESIS AND MICROGLIAL DIFFERENTIATION IN THE RAT. S.D. Hurley* and W.J. Streit. Dept. of Neuroscience, Univ. Florida, Gainesville, FL 32610.

In the adult rat CNS microglial cells are often seen in close association with small blood vessels, and these are referred to as **perivascular microglia**. Theoretically, both microglia and endothelial cells are of mesodermal origin, which led us to examine the morphological relationship between endothelial and microglial cells during postnatal development. Blood vessels and microglia were visualized in brain sections using lectin staining. Microglial-endothelial contacts were far more extensive in young animals (P0-P4) than in older ones, and immature microglia demonstrated an intimate association with growing vessels. The immature microglia associated with the developing vasculature generally had ramified processes, however, ameboid microglia in the corpus callosum were also seen to form close vascular contacts. We saw a positive correlation between the stage of vascularization and the extent of microglial branching in gray matter, i.e. more developed vascular trees were accompanied by highly branched microglial cells making extensive vascular contacts. In contrast, the corpus callosum showed a lesser degree of vascularization, fewer microglia-endothelial associations, and was predominated by ameboid microglia until P13. Generally, gray matter regions were rapidly populated with large numbers of ramified microglial cells, except for the mitral cell layer in the olfactory bulb. A conspicuous absence of microglia in this zone during the early postnatal stages coincided with a lack of vessels. Our study shows a positive correlation between CNS vascular development and the differentiation of microglia, however, the anatomical evidence argues against a differentiation of blood monocytes into microglia, as we observed microglia in areas where blood vessels were absent.

375.2

DISTRIBUTION OF HIGH AFFINITY BINDING SITES FOR [^{125}I] VASCULAR ENDOTHELIAL CELL GROWTH FACTOR (VEGF) IN DEVELOPING AND ADULT RAT BRAIN AND SPINAL CORD L.B. Jakeman, N. Ferrara*, J. Winer* and C.A. Altar; Genentech, Inc.; South San Francisco, CA 94080

While several factors contribute to the complex regulation of neovascularization in the central nervous system (CNS), many of these factors have several different actions on vascular and non-vascular cells. In contrast, VEGF is a recently described mitogenic growth factor that is specific for vascular endothelial cells *in vitro* and stimulates angiogenesis *in vivo*. To determine if VEGF might play a physiological role in the CNS, the *in vitro* binding of [^{125}I] VEGF was examined by autoradiography in brain and spinal cord sections from adult and newborn rats.

Biologically active [^{125}I] VEGF bound to a single class of sites in the adult CNS. The binding was saturable and of high affinity (K_d = 34.0 ± 1.0 pM) and low capacity (B_{max} = 1.9 - 6.0 fmol/mg protein). The binding was specific for VEGF, as it was displaced by 75 - 90% with rhVEGF (IC_{50} for 80 pM [^{125}I]rh VEGF = 59 ± 3 pM) but not with 100 nM PDGF, EGF or basic FGF. In newborn and adult brain, the localization of specific binding sites was consistent with the distribution of large and small vessels. Binding was greater in gray matter regions than in the white matter of the corpus callosum or spinal cord tracts. The binding in adult brain and spinal cord was colocalized with vascular endothelial cells identified by factor-VIII-like immunoreactivity, but no binding was associated with ventricular ependymal cells or choroid epithelia. Although a role for VEGF in the proliferation or repair of vessels of the CNS remains to be determined, these findings strongly suggest that both developing and adult CNS vascular endothelial cells may respond to endogenous or exogenous VEGF.

375.3

EPINEPHRINE AND NOREPINEPHRINE EFFECTS ON SPLEEN LYMPHOCYTE BLASTOGENESIS. N. Azad*, N.V. Emanuele*, N. La Paglia*, M.R. Young*, J. W. Crayton and A.M. Lawrence*. Research, Psychiatry (Biological Psychiatry), and Medical Services, Department of Veterans Affairs, Edward Hines Jr. Hospital, Hines, IL 60141; Departments of Medicine, Biochemistry, and Pathology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153.

Despite considerable interest in the effects of catecholamine systems on immune function, there is little direct evidence on effects of epinephrine (E) or norepinephrine (NE) upon conventional measures of lymphocyte blastogenesis. Spleen lymphocytes from 70-80 day-old male Sprague-Dawley rats were prepared on a histopaque gradient. They were cultured for 72 hrs at a concentration of one million cells per ml. in microtiter wells in sub-optimal concentration of Concanavalin-A (Con-A) and with varying concentrations of epinephrine (10^{-5} M to 10^{-8} M) and norepinephrine (10^{-4} M to 10^{-8} M). At 10^{-5} M, epinephrine enhanced Con-A-stimulated blastogenesis by 64% ± 10. This stimulation was blocked completely by prazosin (10^{-5} M), suggesting a specific α-1 receptor mediated response. In contrast, norepinephrine 10^{-4} M produced a significant inhibition of Con-A-stimulated blastogenesis by 80% ± 5.2. These findings support the role of catecholamines in immune system function particularly at higher concentrations and indicate a direct and differential effect. This work was supported by the Department of Veterans Affairs Medical Research Service, NIH Grant #RO1-DK-39361, and by the Claire and Leonard Tow Foundation.

375.4

DEGRADATION OF IMMOBILIZED FIBRONECTIN-GELATIN SUBSTRATES BY LPS AND IL-1 STIMULATED MICROGLIA. J. Keri*, W.-T. Chen*, W. Monsky*, J. Yao*, C. Colton, and D. Gilbert. Georgetown Univ. Sch. of Med., Washington, DC 20007, and NINDS, NIH, Bethesda, MD 20892.

Microglia share anatomical, biochemical and functional similarity to other tissue macrophages. We examined extracellular matrix degradation under unstimulated and stimulated conditions. A primary culture of microglia was obtained from neonatal rat cerebral cortices. Microglia were plated on immobilized rhodamine-fibronectin-gelatin substrates. After adhering, the microglia were treated with a range of concentrations of lipopolysaccharide (LPS) and interleukin-1 (IL-1) diluted in DMEM. After 48 hours, the cells were stained with trypan blue to identify dead cells and then by the microglia-specific lectin, GS I-FITC. Cells were fixed and studied by light and fluorescent microscopy. Proteolytic activity was assessed by observing the number and size of fluorescent negative areas in the rhodamine-linked fibronectin associated directly with microglia. Our results showed LPS (1.0 ug/ml) treatment, and IL-1 (10.0 U/ml) treatment yielded the highest percentage of fibronectin-degrading cells with 20% and 22% respectively. The percentage of untreated cells degrading was 2%. Thus LPS and IL-1 can stimulate microglia to degrade their extracellular matrix substratum.

375.5

ISOLATION AND CHARACTERIZATION OF HUMAN FETAL MICROGLIA IN CULTURE. F.Gremo,V.Sogos*,S.Torelli*,M.G.Ennas*,A.Meloni*, G.M.Lauro*. Dept. of Cytomorphol., Inst. of Obst. & Gynecol. Medical School, CAGLIARI; Dept. Dev. Cell Biol.,ROMA,Italy

We have isolated microglial cells from a culture of a 15-week-old human fetal brain. In brief, cells were dissociated after trypsinization and grown in DMEM + 10% fetal calf serum (FCS) in polylysine-pretreated dishes. After 30 days, cells were frozen in 90%FCS + 10% DMSO in liquid nitrogen. At different intervals, cells were defrosted, cultured as above for 1 week and frozen again. With this method, we obtained cell cultures at different "cell passages". Then, cells were characterized with monoclonal antibodies against the macrophage markers M1 and M5, against Fc receptor for IgG, vimentin, glial-fibrillar-acidic protein (GFA), neurofilaments (NF), galactocerebroside (GC),$alpha_1$-chimotrypsin, $alpha_1$-trypsin,lysozime, CD4. Different tests for phagocytosis were also used. Results showed positivity for all the markers listed above, except GFA,NF,GC, CD4. Cells could actively proliferate, were stained with fluorescent RCA lectin,had phagocytic activity. Morphologically, they were either round-shaped with thin, long processes or large,flat and firmly attached to the substratum. All these results show the presence of highly purified microglia cultures.

375.6

EFFECTS OF BLOCKING PERINEURONAL MICROGLIAL PROLIFERATION FOLLOWING PERIPHERAL AXOTOMY.

M. Svensson and H. Aldskogius*. Karolinska Institutet, Dept. of Anatomy, S-10401 Stockholm, Sweden

Peripheral axotomy causes a number of structural and metabolic changes in the neurons as well as in non-neuronal cells. Axotomized neurons become chromatolytic, lose presynaptic terminals and retract their dendrites. In parallel with these events, microglial cells proliferate and migrate towards the injured neurons and astroglial cells hypertrophy. We have investigated some aspects of the functional significance of the microglial cell response by blocking their proliferation following hypoglossal nerve transection, using intracisternal (cisterna magna) infusion of cytosine-arabinoside via a subcutaneous osmotic minipump. The synaptic density on the axotomized motorneurons is reduced to the same level following blockade of the microglial reaction compared to nerve transection in control animals. Glial fibrillary acidic protein-immunoreactivity in astrocytes is not increased following nerve transection in combination with microglial blockade. Axotomy-induced nerve cell death seems to be reduced in the microglial free environment. However, the regeneration and reinnervation capacity of these motorneurons seems unaltered after blocking the microglial cell reaction. These observations suggest that: 1)microglial cells are not crucial for synaptic detachment 2)do not support regeneration 3)astrocytes may be activated by microglial cells 4) microglial cells impair survival capacity of neurons

375.7

BONE-MARROW CHIMERAS REVEAL AN ORIGIN OF CELLS FOUND IN ADULT MOUSE BRAINS AFTER INJURY, C.M. Morshead and D. van der Kooy. Neurobiology Research Group, Department of Anatomy, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Injury to adult nervous tissue results in a gliotic response which is typified by the accumulation of many darkly staining cells within the tissue near the site of injury. Using an adult mouse model in which kainic acid is injected into the striatum, we see gliosis throughout the ipsilateral forebrain extending from the lateral edge of the lateral ventricle, through the striatum and into the cortex. The response is most pronounced at the kainic acid injection site. We have used this model to ask about the type and origin of the cells that respond to injury. In earlier studies we found that there are two separate populations of cells constituting the response: exogenous blood-borne cells that come into the brain through the broken blood-brain-barrier and endogenous glial cells, probably microglia. We were able to manipulate these two populations separately using irradiation to eliminate the bone marrow precursors. This resulted in a selective loss of cells only at the injection site. The gliosis in the surrounding tissue remained unchanged. In order to further test our hypothesis we have made bone-marrow chimeras by injecting bone marrow cells, carrying a B-globin transgene marker, into mice that were lethally irradiated to destroy the endogenous haemopoetic population. Using the same kainic acid model described above, we have found transgenically marked blood-borne cells primarily at the site of the lesion. This supports our hypothesis that some of the large population of cells in the immediate vicinity of brain lesions are haemopoetic cells.

375.8

MACROPHAGE/MICROGLIAL CELLS IN THE XENOPUS VISUAL SYSTEM. I.A.Goodbrand*,M.A.Wilson* and R.M.Gaze. ICAPB, Division of Biological Sciences, Edinburgh University, Edinburgh EH9 3JT, Scotland, U.K.

A proportion of Xenopus optic axons regenerate after injury. We have studied the macrophage response to optic nerve injury, using a monoclonal antibody, 5F4. This recognises macrophage/microglial cells. $5F4^+$cells are phagocytic: they ingest pigment granules from injured melanocytes at the site of the nerve crush and Indian ink introduced through lesions of the brain. These cells appear in large numbers after nerve injury, along the course of degenerating optic axons. Increased $5F4^+$cell activity is found in the nerve 2h postlesion (PL). The response peaks 3-7 days PL, before the retinotectal projection is restored. Three weeks PL, as the brain response diminishes, $5F4^+$cells are found deep in the tectum and in some cases seem to pass through the ependyma into the ventricle. Six weeks PL, $5F4^+$cells have been seen engulfing degenerating retinal ganglion cells. The rapid course of the $5F4^+$response to optic nerve injury may assist in the production of an environment conducive to axonal regeneration in Xenopus.

375.9

LAMPREY GLIAL CELLS OF THE BRAIN AND SPINAL CORD CONTAIN KERATIN SE Merrick*, SJ Pleasure, D Pijak*, DI Lurie*, ME Selzer, and VM-Y Lee*. Dept of Path and Instit of Neurosci, Univ. of PA, Phila., PA 19104.

The lamprey is exceptional in its capacity to undergo extensive regeneration following spinal cord transection. During this process glial scarring appears not to inhibit axonal regeneration. Since we have been unable to detect a GFAP homologue (a major component of the astroglial scar in mammals) in the lamprey CNS we embarked on studies to identify the intermediate filament (IF) proteins of lamprey glial cells as an initial step to assess the environment of glial cells in regeneration. To this end, monoclonal antibodies (mAbs) were raised to lamprey spinal cord cytoskeletal extracts and these mAbs were characterized using Western blotting and immunocytochemistry. On 2-D Western blots of spinal cord cytoskeletal extracts, five of the mAbs detected three major IF polypeptides in the MW range 45-60 kD. Further studies were conducted to determine the relationship between the lamprey glial specific antigen with other mammalian IF proteins. We found that anti-cytokeratin 8 antibody recognized two of the three polypeptides. In addition several of the glial-specific mAbs reacted with human cytokeratin 8 and 18 on Western blots. The immunocytochemical staining patterns of the glial-specific mAbs were also examined and were found to be indistinguishable on lamprey spinal cord sections. However, on brain sections two distinct patterns were observed. A subset of the mAbs only stained select glial fibers in the brain whereas others stained almost all glial cells in the brain. Interestingly, the former group of mAbs only recognized the two lower MW polypeptides on 2-D Western blots, but the latter group of mAbs recognized all three major IF polypeptides. This correlation is supported by the observation that the highest MW IF polypeptide has an increased level of expression in the brain relative to the spinal cord. Thus, the glial cells of lamprey spinal cord and brain express homologues of simple epithelia cytokeratins, but the brain IF expression pattern may be more complex than the spinal cord.

375.10

The response of non-neuronal cells to optic nerve injury in goldfish. J. Wang, J. Chako*, W. Battisti, T.C. Eckenrode* and M. Murray. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Goldfish optic nerve axons, unlike mammalian axons, readily regenerate after axotomy. Since a permissive environment provided by non-neuronal cells associated with the optic nerve is likely to contribute to successful regeneration, it is important to characterize their normal distribution and responses to optic nerve crush.The intact optic nerve is composed of fascicles of myelinated axons. Astrocytes, are the dominant non-neuronal cell within the fascicles. Granule-laden macrophages, identified by positive staining with an antibody to complement receptor II (OX-42), are present in large numbers surrounding the nerve and between but never within the fascicles. The goldfish nerve differs from mammalian optic nerves by the presence of this resident macrophage population.Within one hour following optic nerve crush, the activated macrophages show increased immunostaining. Astrocytes also become activated; many astrocyte nuclei acquire complex and tortuous shapes with unusually prominent clumping of chromatin. Within the first week, some astrocytes have become phagocytic, while others are associated with regenerating axons. Macrophages retain an interfascicular location but are now increased in number. The early (1hr.) activation of both macrophages and astrocytes following optic nerve crush is likely to be associated with the changes in synthesis of secreted molecules which has been shown to accompany the regenerative process in goldfish optic nerve. Supported by ASRI 91-016-1 and NS 16556.

375.11

ISOLATION AND SEQUENCE ANALYSIS OF TWO INTERMEDIATE FILAMENT cDNA CLONES FROM FISH OPTIC NERVE. I. Cohen*, Y. Shani*, E. Blaugrund* and M. Schwartz. Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

The fish central nervous system (CNS), unlike its mammalian counterpart, is endowed with a high capacity of regeneration. This phenomenon has been attributed, at least in part, to the nature of the cells surrounding CNS nerves and their response to injury. Characterization of the structural proteins in nonneuronal cells of intact and injured nerves, might give an insight to their role in regeneration. Our model of choice is the fish optic nerve. In the present study, we isolated cDNA clones encoding fish intermediate filaments. mRNA was isolated from carp (*Cyprinus carpio*) optic nerves and analyzed by Northern blot analysis with a mouse GFAP probe. Two transcripts of approximately 2.1 and 2.3 kb, were found. A λgt-10 library, prepared from regenerating carp optic nerve mRNA, was screened by the mouse GFAP probe. Sequencing analysis revealed that the 2.1 kb cDNA transcript is the fish vimentin, which shows 91% and 97% homology in the respective rod domain of mouse and chicken vimentin. This high homology emphasizes the evolutionary conservation of intermediate filaments among species. A partial sequence of another clone revealed that the 2.3 kb transcript is identical to fish keratin 8.

DEVELOPMENT AND REGENERATION OF MOTOR SYSTEMS I

376.1

CALCIUM BINDING PROTEINS ARE NOT INVOLVED IN NEUROGENESIS IN THE AVIAN SONG SYSTEM

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Telencephalic nuclei of the vocal motor system of song birds show synaptic plasticity during vocal learning (Rausch and Scheich, 1982). One specific aspect of the functional organization of this system is the abundance of neurons containing the calcium-binding proteins parvalbumin and calbindin. Such neurons in the song system of zebra finches, are characterized by a high metabolic and electrical activity (Braun et al. 1985) and partly overlap with GABAergic neurons (Zuschratter et al., 1987). In the present study we investigated the relationship between these calcium-controlled neurons and neurogenesis, which was observed in vocal motor nuclei during the critical period of song learning (Alvarez-Buylla et al. 1988; Nordeen and Nordeen 1990). Zebra finches of different ages (20-30/40-50/50-60/adult) were injected with ^{3}H-thymidine for 10 days. Birds were sacrified as adults and vibratome sections of the brains were treated immunocytochemically with antisera against parvalbumin and calbindin D28K and afterwards processed for autoradiography. Although we found a good correlation between the sensitive phase of song learning and cell mitosis in all telencephalic vocal motor nuclei, immuno-positive neurons did not show any ^{3}H-thymidine reaction product.

These results indicate that parvalbumin-IR and calbindin-IR neurons are born early in ontogenesis and represent stable elements within the plastic song system.

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376.2

DEVELOPMENT OF THE NIGRO-TECTAL PATHWAY IN THE RAT: A TRACING WITH A FLUORESCENT CARBOCYANINE DYE. Thomas J. Mahalik and Andrew Carrier'. U. of Colo. Health Sci. Ctr. Denver, Colorado 80262.

Neurons in both the zona reticulata and the zona compacta are mostly born between fetal day 14 and fetal day 16. The nigral dopaminergic neurons begin to send their axons to the caudate nucleus shortly after they arrive in the zona compacta. Little is known however, about the development of the pathway between the neurons in the zona reticulata, and the tectum. The purpose of the present study was to chart the development of the nigro-tectal pathway with the carbocyanine dye, DiI.

Fetuses were obtained at embryonic days E13, E14, E15, E18 and E19. Fetuses were emersion fixed in 4% paraformaldehyde and DiI crystals were applied either to the tectal plate, or to the ventral mesencephalon, and then were incubated in 4% paraformaldehyde for 10 to 30 days. The brains were sectioned in the sagittal plane on a cryostat. When DiI was applied to the tectum, retrogradely labeled ventral mesencephalic cell bodies were present as early as E15. No retrogradely labeled cells were present in the VM of E13 fetuses. In older fetuses, retrograde labeling of VM cell bodies after tectal application of DiI was even more robust.

376.3

UNILATERAL PERINATAL HYPOXIC-ISCHEMIC STRIATAL INJURY IN RAT RESULTS IN A DECREASED NUMBER OF IPSILATERAL NIGRAL DOPAMINERGIC NEURONS. *RE Burke and N Kenyon*. Department of Neurology, Columbia University, NY, NY, 10032.*

The striatum is relatively vulnerable to hypoxic-ischemic (H-I) injury during development, and yet little is known of the lasting alterations in its neurochemical anatomy. In a unilateral rodent model, we and others have shown that biochemical and morphological markers of striatal dopaminergic (DA) fibers are preserved, consistent with the hypothesis of an excitotoxic, axon-sparing lesion. However, there has been no study of effects on the development of DA neuron cell bodies in the substantia nigra (SN). We have studied these neurons by using the immunoperoxidase technique for tyrosine hydroxylase(TH), and counting neurons in serial sections. We find that unilateral *striatal* H-I injury results in a significant decrease in the number of TH-positive SN neurons (Experimental(E): 598±27; Control(C)(contralateral SN): 696±12; p=.003, N=8). Nissl and immunoperoxidase staining for glial fibrillary acidic protein on adjacent SN sections *excluded direct H-I injury to the nigral area.* There was no difference in the number of neurons between E and C sides in animals subjected to H-I but without striatal injury, nor between right and left of Normals. In the rostro-caudal dimension, neuron loss was greatest anteriorly in Paxinos-Watson plane 4.2, where it was 30%. Neuron loss was also marked in the ventral tier of SN TH-positive neurons; those in SN reticulata were reduced by 50%. This neuron loss may be indirectly due to striatal injury, by loss of trophic support from that nucleus. NINDS NS26836, United Cerebral Palsy, Parkinson's Disease Foundation.

376.4

REINNERVATION OF RAT TIBIALIS ANTERIOR MUSCLE BY TIBIAL NERVE AFTER PROLONGED DENERVATION OR AXOTOMY. S. Fu* and T. Gordon. Dept. of Pharmacology, Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada, T6G 2S2.

To determine the role of intramuscular nerve sheaths in the success of nerve regeneration and muscle reinnervation, we sutured the axotomized tibial nerve (TIBn) to the distal stump of the cut common peroneal nerve (CPn, nerve-nerve; n-n) or to the denervated tibialis anterior (TA) muscle (nerve-muscle; n-m) and determined the number and size of motor units (MUs) in the reinnervated TA 6 months later. In addition, we investigated whether prolonged axotomy, or denervation, influences reinnervation by delaying the suture of TIBn to the CPn, or TA muscle, respectively, for 2-6 weeks. Muscles showed an average of 60% recovery of force after n-n and 10% after n-m suture, irrespective of the time of axotomy or denervation. Correspondingly, there was a larger number of MUs in the former muscles, 88 ± 6.4 (S.E.M.) compared to 11 ± 3.1 after n-m suture. Analysis of glycogen depleted MUs showed that the number of fibers increased up to 3 times after n-m suture permitting more muscle recovery than predicted from the number of MUs, but this was inadequate to compensate for poor regeneration. Spatial analysis and examination of intramuscular nerve branches (Ag/ChE stain) indicates that proximal branching of regenerating nerves is considerably less extensive after n-m suture. These data indicate 1) that intramuscular nerve sheaths provide a permissive environment for nerve regeneration as evident from the larger number of reinnervated MUs and more extensive proximal branching after n-n compared to n-m suture, however 2) since MUs increase in size (n-m), the muscle surface can support terminal branching and 3) prolonging axotomy and denervation does not alter the success of reinnervation. Supported by MRC and MDAC.

376.5

MYELINATED AXONS IN MUSCLE NERVES ARE UNAFFECTED BY CHANGES INDUCED IN THE MUSCLE BY TENECTOMY. B. A. Dirks* and M. E. DeSantis, Univ. of Washington School. of Medicine, Seattle, WA.

A piece of the triceps surae tendon was resected (tenectomy) unilaterally in rats. Nerve branches to the lateral head of the gastrocnemius and soleus (LG-S) and the medial head of the gastrocnemius (MG) muscles were examined to see whether tenectomy influenced the number and size of myelinated nerve fibers which innervated those muscles. Analysis of variance tests were done on the following variables for myelinated fibers: total number, proportion of fibers of small diameter, and mean diameters and standard deviations for the groups of smaller and larger fibers. In one group of rats, the nerve to muscle remained intact, and tenectomy was produced at various postnatal ages (6-70 days) when myelination of axons was in progress. Examination of their nerves, 7 to 18 weeks later revealed no statistically significant differences for nerve fibers when tenectomized and the sham-operated nerves were compared. The 6 day old operated rats showed a slight shift, consistent for both muscle nerves, toward smaller diameters for those myelinated fibers innervating tenectomized muscle. In a second paradigm, regenerated fibers were examined 18 weeks after tenectomy and axonotmesis had been done in adult rats. There were no statistically significant differences in the number or any of the size variables of remyelinated fibers when comparing the experimental (tenectomy and axonotmesis) and control (axonotmesis only) nerves. We conclude that tenectomy of a rodent's hind limb muscles after the first postnatal week exerted no adverse effect on the process of myelination of axons which innervate the altered peripheral target tissue.

376.6

HINDLIMB SUSPENSION FOR SHORT PERIODS IMPAIRS MOTOR DEVELOPMENT IN NEONATAL RATS. Kerry D. Walton, Daniel Lieberman, Mathew Begin and Rodolfo R. Llinás, Dept. of Physiology & Biophysics, NYU Medical Center, 550 First Ave., New York, NY 10016

We have identified a 5-day "critical period" for motor development, P 8 to P13 (Walton et al, IBRO abst., 1991). The motor system of neonatal rats was perturbed by unloading the hindlimbs (HL) via suspension of the pups by their tails for 20 hrs each day. Suspension was implemented from P2 to P7 (n=5), from P8 to P13 (n=6) or from P14-P19 (n=3). Swimming was used to measure motor development at all ages since a) it is present at birth, b) can be quantified, and c) does not stress antigravity over other muscles. A motion analysis program was used to determine HL joint angles during walking from P13. Suspended and littermate (LM) pups were videotaped daily from P2 to P21 and again at 2-5 months. Suspended animals were clearly distinguished from LMs. In every way (time spent swimming, speed, balance, stoke phase, and "style") swimming in the P8 to P13 suspended pups were significantly more impaired than the P2 to P7 and P14-19 group. S pups walked slower than LM and walked on their toes. During the stance phase on P13 the ankle joint subtended an angle of 180 to 160° in S pups compared to 180 to 100° in LMs. Recovery began immediately post-suspension, joint angle values approaching controls after 6 days. After 5 months S animals were still clearly distinguished from LM. Similar deficits were not seen in animals suspended P2-P7 or P14-19. Animals suspended for only a 20-24 hour period between P8-13 also showed significant deficits in balance, swimming and locomotion. Our results suggest that P8 to P13 is a critical period for motor development. However, the motor system is more adaptable than sensory systems. These data indicate that lack the of appropriate use (including both motor and sensory aspects) of hindlimb and back musculature for even 20 hours during a 5-day period critical period severely impairs motor development and that the resulting deficit is largely, but not completely compensated. Supported by NASA and The Hirschl Trust.

376.7

REFLEX RECRUITMENT IN SELF-REINNERVATED MEDIAL GASTROCNEMIUS OF THE DECEREBRATE CAT. T.C. Cope and B.D. Clark Dept. Physiol & Biophys, Hahnemann Univ., Phila. PA 19102

It is unclear how recruitment is organized following the reconstitution of motor units after nerve section. Pairwise analyses of motor unit properties and recruitment were performed as described by Clark and Cope (1990; Neurosci Abstr. 16: 888) on 5 cats whose medial gastrocnemius (MG) nerve had been cut and resutured >18 mos. previously. Only 4 out of 113 tested units could be recruited by MG stretch. In tests of 63 pairs of units recruited using electrical stimulation of the caudal cutaneous sural (CCS) nerve, the size principle correctly predicted recruitment order of units ranked by conduction velocity (CV; 36/52 pairs), maximum isometric force (48/62 pairs) and twitch contraction time (35/40 pairs). These frequencies are not significantly different from control pairs recruited with CCS stimulation or MG stretch (G-test of independence), except for CV, which was significantly lower than control recruitment by stretch. In an additional cat whose MG nerve was crushed, not cut, both stretch and CCS nerve stimulation recruited motor units consistent with the size principle at frequencies approaching controls. We conclude that following self-reinnervation, the size principle of recruitment still holds. (Supported by NIH NS21023)

376.8

INTRA- AND INTERLIMB COORDINATION DURING MOTILITY IN CHICK EMBRYOS. S.H. Chambers and N.S. Bradley. School of Physical and Occupational Therapy, McGill Univ., Montreal, PQ, Canada H3G 1Y5.

Recent studies indicate coordinated movement can occur early in development, but these studies also identify considerable variability during spontaneous motility. We are now exploring the characteristics of this variability as a means to better understand the development of motor control in the chick. Here we report preliminary kinematic data on synchronous wing and leg movements *in ovo* at embryonic day 9.

The entire portion of continuous movement (33-42s) for each of 3 episodes (100-200s in duration) were video recorded and computer analyzed (sample rate 30Hz) to obtain joint excursions for the shoulder, elbow, hip and knee. Linear trend analyses suggest that elbow flexion/extension is closely timed with shoulder extension/flexion ($r^2 \approx 0.7$) over 8-9 consecutive cycles. Hip flexion/extension is closely timed with knee flexion/extension ($r^2 \approx 0.7$) over 6 consecutive cycles. While wing and leg movements begin and end coincidently, cycle periods for the wing are typically shorter (3s) than for the leg (4s) and coincident timing of wing/leg cycles was found only briefly in each episode.

Initial analyses suggest that intralimb coordination is relatively reliable over an episode of spontaneous motility but that interlimb coordination may either be unstable, more complex than a simple 1:1 correspondence, or limited to chance. Further analyses will examine these possibilities. This work was supported by the McGill Faculties of Medicine and Graduate Studies, FCAR and NSERC.

376.9

DEVELOPMENT OF L-DOPA-INDUCED AIR-STEPPING IN PREWEANLING RATS: COORDINATION WITHIN AND BETWEEN LIMBS.
D.J. Stehouwer, A.E. Sickles* and C. Van Hartesveldt. Dept. of Psychology, University of Florida, Gainesville, FL 32611.

Coordination of air-stepping elicited by subcutaneous injections of l-DOPA (25 to 100 mg/kg) was studied in rats from the day of birth through 20 days of age. Results revealed a nearly linear increase in the rate of stepping from about 1.5 steps/sec at day 0 to nearly 5 steps/sec at day 20, independent of dose of l-DOPA. This increase was found to result from decreases in the duration of both retraction and protraction phases of the step cycle, but the decrease in the retraction phase was greater. The duration of retraction dropped from 60% of the step cycle on day 0 to about 45% on day 15. There was an ontogenetic increase in the amplitude of movement at the wrist, knee, and ankle joints, a slight decline in the amplitude of movement at the elbow, and little change in amplitude at either the shoulder or hip. The timing of movements at joints within each limb also changed with age. The shoulder increasingly led forelimb movements throughout development, followed by the elbow and then the wrist. Hindlimb movements were led by the hip, followed by the ankle and finally the knee, which increasingly lagged behind the other two joints of the hindlimb. At all ages, diagonal limbs moved in phase with each other and in antiphase with the contralateral limb of the same girdle during diagonal progression. However, other gaits became more prevalent between 10 and 20 days of age.

376.10

Corticospinal Plasticity Following Neonatal Hemipyramidotomy
B.R. Clark[1], E. Theriault[2], and D.L. Tolbert[3]. Program in Physical Therapy, Sch. of Medicine, Washington Univ. St. Louis, MO[1], Depts. of Surgery (Neurosurgery) and Pediatrics, Playfair Neuroscience Unit, Univ. Toronto. Toronto, Ontario[2], Depts. Anatomy and Neurobiology and Surgery (Neurosurgery), Sch. of Medicine, St. Louis Univ., St. Louis, MO[3].

The development of corticospinal (CS) projections in cats is characterized by the temporally protracted postnatal growth of CS axons into the spinal cord and bilateral terminations in the intermediate gray that subsequently become refined to an adult-like, mainly contralateral input by seven postnatal weeks. We have shown previously that early neonatal pyramidotomy causes redirected growth of ipsilateral late developing CS axons along aberrant pathways. We now report on the development and maturation of CS projections from the cortex opposite the lesion. Partial deafferentation of CS input to one side of the spinal intermediate gray results in the persistence of normally transient CS projections from the contralateral side. Densitometric image analysis suggests that WGA-HRP labeled CS input from the primary sensorimotor area of the opposite hemisphere is equivalent in the intermediate gray bilaterally in the cervical and lumbar enlargements. These data indicate that neonatal unilateral pyramidotomy induces the persistence of normally transient CS projections. Supported by NIH grant NS 20227 and the Canadian Paraplegic Associaton.

376.11

MODULATION OF LOCOMOTION THROUGH A PERIPHERAL NERVE GRAFT ACROSS A COMPLETE CHRONIC SPINAL CORD TRANSECTION IN THE ADULT RAT. N.B. Reese, E. Garcia-Rill, J.D. Houle and R.D. Skinner, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Stimulation of the mesencephalic locomotor region (MLR) in the decerebrate rat has been found to induce controlled, four-limb locomotion. This preparation was employed on rats which had received a spinal cord transection and had the spinal cord reconnected by a peripheral nerve autograft (PNG). Testing 2-3 months later showed that MLR stimulation induced forelimb but not hindlimb stepping. However, in 4/6 cases, tail pinch interrupted MLR stimulation-induced forelimb alternation, while pinna pinch, a) interrupted MLR stimulation-induced forelimb alternation, and b) induced EMG changes in hindlimb muscles. These effects disappeared once the PNG was cut. Labeling of the cut mid portion of the PNGs using DiI revealed significant numbers of labeled axons entering the spinal cord through both ends of the PNG in those animals which showed the above effects. These results suggest that non-specific information which can modulate locomotion may be flowing through some PNGs across a complete, chronic spinal cord transection in the adult rat.

Supported by a grant from the UAMS Neuroscience Coordinating Committee.

376.12

POSTNATAL DEVELOPMENT OF SYNAPTIC RESPONSES, MEMBRANE PROPERTIES AND MORPHOLOGY OF RAT NEOSTRIATAL NEURONS *IN VIVO* F. Trent and J.M. Tepper. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ, USA 07102.

In vivo intracellular recordings from neostriatal (NS) neurons were obtained in urethane-anesthetized Sprague-Dawley rat pups from birth (PD1) to PD40 and in adult males using biocytin-filled micropipettes. Most NS neurons <PD11 were not spontaneously active, but even in the youngest animals, cortical stimulation elicited an initial EPSP whose mean onset latency significantly decreased with postnatal development, but whose maximum amplitude and duration were similar to those observed in adults. In some cases cortical stimulation evoked an initial IPSP that decayed within 1-3 minutes to reveal the usual initial EPSP. Virtually all of the NS neurons recorded in neonates <PD20 failed to show the long-lasting disfacilitation succeeding the initial EPSP as well as the late rebound excitation characteristic of adult NS neurons. The fast anomalous rectification seen in mature NS neurons was almost always absent in neonates <PD11, and the proportion of anomalously rectifying neurons increased with age through PD30-40. The above differences in synaptic and membrane properties between neonatal and adult NS neurons are similar to those recently reported for fetal neostriatal neurons grafted to the kainate-lesioned neostriatum (Xu et al., *J. Neurophys.* 65:477, 1991) suggesting that the tonic excitatory input exerted by cortical afferents may be decreased or absent in neonates and contribute in part to the observed differences. Examination of filled NS neurons revealed almost all of them them to be medium spiny cells as indicated by somatic and axonal morphology although the density of dendritic spines was greatly reduced and did not attain adult levels until the end of the 4th postnatal week. Local axon collaterals were present in the youngest labeled neurons examined (PD11) which failed to show the long-lasting disfacilitation succeeding the cortically-evoked EPSP. These observations suggest that NS neurons undergo a prolonged postnatal maturation *in vivo* but precede that of the cortex as indicated by the temporal disparity in the development of anomalous rectification and the later appearance of cortically-evoked disfacilitation. Supported by MH45286 and Rutgers University Research Council.

DEVELOPMENT AND REGENERATION OF MOTOR SYSTEMS II

377.1

EMERGENT CONTROL OF MANUAL AND VOCAL-MOTOR ACTIVITY IN REFERENCE TO HUMAN LANGUAGE. J. L. Locke, K. E. Bekken*, D. Wein* and L. McMinn-Larson*. Neurolinguistics Lab., Mass. Gen. Hosp., BOSTON, MA 02108

Vocal control is essential to development of spoken language. Typically, command centers in the left cerebral hemisphere are primarily responsible for speech. We report studies of human infants' development and control of vocal behavior in relation to manual activity under differing conditions of audibility. Sixty normally developing infants in equal numbers were seen prior to onset of canonical syllable production and at two intervals following onset of babbling. In experimental trials, audible or inaudible rattles were placed in left or right hands equally often. Analysis of manual activity revealed that audibility enhanced duration and stereotypy of right-handed activity in the youngest and vocally least developed subjects. The magnitude of this dextral effect declined significantly with age and vocal experience. These and other results suggest that the left cerebral hemisphere is initially dominant for audible hand activity, then relinquishes this control with the emergence of a species-specific maturational program according to which the left hemisphere assumes command of audible vocal activity several months prior to the demonstrable onset of spoken language.

377.2

TEMPORAL PATTERNS OF VARIABILITY IN TRAJECTORY, VELOCITY, AND ACCELERATION OF MOVEMENT.
S.R.Gutman*, G.L.Gottlieb, M.L.Latash. Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612

Standard deviations (SD) of angle, velocity, and acceleration of reaching movement demonstrate non-monotonic time profiles which differ in the number of maxima. The angle SD has one maximum, while the velocity and acceleration SDs show two and three peaks respectively (assuming movements without overshoots). We suggest an explanation for these findings based on random modulation of an "inner" time scale used by the motor control system for planning the movement. In the general case, SD for any kinematic variable f(t) can be expressed as

$$SD(f(t)) \sim |t\, f'(\zeta_t)|\, SD(\zeta),$$

where $SD(\zeta)$ is the standard deviation of time scale factor ζ.
This leads to the following general properties of the time profile of SD(f(t)):

1) it has the same number of extrema as the derivative of f(t),
2) these extrema are shifted to the right along the time axis.

The presence of other sources of movement variability will distort this form of SD(t). It was found that the formula satisfies SD(t) for reaching movements. A particular case of the general expression for SD(t) was investigated with a help of kinematic model of reaching movement with an exponential trajectory (S.Gutman, G.Gottlieb (1990), Abstr. of 1st World Congr.of Biomech., v.1, p.190):

$$x(t)=D(1-\exp(-t^3/\tau_{mov}),$$

where D is the movement distance, τ_{mov} is the movement inner time constant. The patterns of SD functions for trajectory, velocity, and acceleration demonstrate a close resemblance to the experimentally observed patterns.
The study was supported by NIH grants AR 33189 and NS 15630.

377.3

MOVEMENTS OF NEONATAL RATS: RANDOM OR ANTECEDENTS? C.R. Almli and E.A. Strauss*. Develop. Neuropsychobiol. Lab., Washington University Medical School, St. Louis, MO 63110.

Cyclic motility patterns are ubiquitous for embryos of a variety of species, and they persist for some unknown time even after birth in rats and humans. The role played by this type of motility pattern in behavioral ontogeny is not understood, i.e., movements displayed may be random or they may be antecedents of species-specific behaviors. Neonatal rats were studied to determine if movements were random or patterned.

Newborn rats were video-taped and movements of individual body segments and "complex" movements were scored with computer during "bursts" of activity.

Results indicate that movements displayed by neonatal rats during cyclic motility may have both random (i.e., movements showed no obvious inter-relations) and antecedent (i.e., movements appear homotypical with later behaviors) components. (Conducted under NIH Guide for Care and Use of Laboratory Animals).

377.4

THE ORIGINS OF SUPRASPINAL PROJECTIONS TO LUMBAR AND CERVICAL LEVELS OF THE SPINAL CORD AT DIFFERENT STAGES OF DEVELOPMENT IN THE GRAY SHORT-TAILED BRAZILIAN OPOSSUM, MONODELPHIS DOMESTICA. X.M. Wang*, X.M. Xu and G.F. Martin. Dept. of Cell Biol., Neurobiol. and Anat., The Ohio State Univ., Columbus, OH 43210.

We have employed the retrograde transport of Fast Blue (FB) to study the origins of supraspinal projections to lumbar and cervical levels of the spinal cord at different stages of development in the Brazilian short-tailed opossum, Monodelphis domestica. Monodelphis was chosen for study because its young are born in a very immature stage, 15 days after conception. When injections were made into the lumbar cord at postnatal day (PD) 1, brainstem neurons were most intensely labeled within the reticular formation of the pons and the presumptive coeruleus complex. Neurons were also labeled in the bulbar reticular formation and the vestibular nuclei, however. By PD 2, additional labeling was present within the caudal raphe and, by PD 3, within the interstitial nucleus of Cajal as well as the spinal and mesencephalic trigeminal nuclei. The red nucleus and hypothalamus were not labeled with certainty until PD 5. By PD 10, labeled neurons were present in most of the brainstem areas labeled by comparable injections in adult animals. By at least PD 7, cervical injections of FB labeled neurons in all hypothalamic and brainstem areas labeled by lumbar injections and by PD 15-17 additional labeling was present within the neocortex, the amygdala, the superior colliculus and the deep cerebellar nuclei. The growth of supraspinal axons into the spinal cord of Monodelphis follows the same general sequence as in the North American opossum, Didelphis virginiana, but specifics of the developmental time table are different. (Supported by NS-25095 and NS-10165).

377.5

MOTOR BEHAVIOR IN DEVELOPING GRAY SHORT-TAILED OPOSSUMS: THE EFFECTS OF TEMPERATURE. Barbara H. Fadem, Hector O. Cordero*, Alex M. Mercado*, Jeffrey C. Pan* and Scott R. Robinson*, Department of Psychiatry, UMDNJ-New Jersey Med. School, Newark 07103 and SUNY at Binghamton, NY 13902.

Gray short-tailed opossums are useful models for studying fetal behavior since they are born at a stage neurally equivalent to gestational day 14 in rats. Neural development does not reach the level seen in newborn rats until about postnatal day (pd) 16 ("pseudobirth"). In this study, motor behavior in gray opossums was observed on the day of birth and at selected ages over the first month of postnatal life at three different environmental temperatures. It was found that frequency of forelimb movements was highest in the "prenatal" age group (pd 4-8), while curling trunk movements occurred most frequently at the age of pseudobirth (pd 12-16). Hindlimb movements first occurred between pd 12 and pd 16. Significantly more head, forelimb and curling movements were seen at the lowest temperature (25°C) than at either of the higher temperatures (37°C and 45°C); this temperature effect was more pronounced in younger animals.

These findings are discussed with respect to the ectothermic nature of the newborn marsupial and intrinsic vs. extrinsic control of fetal motor behavior.

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377.6

THE ONTOGENIC DEVELOPMENT OF LOCOMOTOR BEHAVIORS IN THE OPOSSUM, MONODELPHIS DOMESTICA. A COMPARISON WITH OTHER MAMMALS. G. Cassidy, J.-F. Pflieger* and T. Cabana. Dépt. de Sciences biologiques, Université de Montréal, C.P. 6128, Succ. "A", Montréal, Canada, H3C 3J7.

The Brazilian opossum Monodelphis domestica is born in a more immature state than placental mammals. At birth, the forelimbs (FL) are capable of alternate, rhythmic movements which enable the newborn to climb on the mother's belly and reach a nipple. The hindlimbs (HL) are merely more developed than buds. The development of locomotor behaviors was studied in the opossum from birth until 37 days postnatal (PND), when locomotion resembles the adult pattern. The FL segments and joints are clearly visible at birth but all those of the HL do not externally appear well formed until about PND 15. The FL begin to support weight at about PND 13 and the HL a few days later. Pivoting is first observed at PND 15 and foreward progression on a surface at about PND 20, but FL and HL movements are not well coordinated. Full support of the body weight is observed at about PND 33 and the mature locomotor pattern is attained a few days later. Mature quadrupedal locomotion follows the appearance of a number of sensorimotor reflexes such as grasp, body righting on a surface anf foreward hopping (Cassidy et al, Soc. Neurosci. 1990). These observations on the opossum are compared with our observations on the Mongolian gerbil (Cassidy et al, IBRO 1991) and those of others on the rat and the cat.

377.7

DEVELOPMENT OF NEURONAL NETWORKS IN THE LOBSTER STOMATOGASTRIC NERVOUS SYSTEM. B. Casasnovas*, I. Cournil, P. Meyrand* and M. Moulins. Lab. de Neurobiologie et Physiologie Comparées, Univ. de Bordeaux, CNRS, Arcachon, 3312 FRANCE.

The stomatogastric nervous system (STNS) of adult Crustacea contains several neuronal networks which are now well known in term of synaptic wiring and cellular properties. They, therefore, constitute a very good model for the study of the ontogeny of rhythm generation of the central nervous system.

Using histological and electrophysiological techniques, we have studied the embryonic development of the stomatogastric ganglion (STG) of the STNS in the lobster (*Homarus gammarus*). According to the percentage scale (fertilization 0%; hatching 100%) of Hulley & Beltz (in: *frontiers in crustacean neurobiology*, 1990), we find that the STG is morphologically developed very early, at 20% development. It contains 24-26 neurons, the main nerves are already established at this stage, and the stomodeal muscles are innervated by STG motoneurons. Morever, the ganglion spontaneously displays a single rhythmic motor pattern which controls movement of the entire stomodeum. In contrast to the adult animal, not one of the individual STG neurons we have studied displayed intrinsic oscillatory properties; rather the embryonic motor pattern appears to be organised via an excitatory synaptic drive. An immunohistochemical investigation has shown that aminergic (Dopamine and Serotonin) STG inputs known in the adult STNS are not yet present in the embryo, while several peptidergic inputs (FMRFamide, Proctolin) are detectable. The role of these inputs in the ontogeny of the STG neuronal networks is being investigated.

377.8

GENERATION OF RESPIRATORY AND LOCOMOTOR PATTERNS BY FETAL RAT BRAIN STEM SPINAL-CORD PREPARATION. J.J. Greer, J.C. Smith & J.L. Feldman, Systems Neurobiology Laboratory, Department of Kinesiology, UCLA, Los Angeles, CA 90024-1527.

The *in vitro* neonatal brain stem-spinal cord preparation is a powerful model for studying the neural mechanisms generating respiratory and locomotor patterns. We have now isolated the brain stem-spinal cord from fetal rats and begun to study developmental processes in these motor pattern generating networks. Fetal rats (E14-E21) were removed via cesarian section and the brain stem-spinal cords isolated and maintained *in vitro* in oxygenated mock cerebral spinal fluid solution. In some preparations, the ribcage or hindlimbs were left attached for EMG recordings of respiratory and locomotor activity, respectively. Cranial nerve (IX, X, XII) and spinal ventral root (C1-C6, T1-T5, L1-L5) activities were recorded with suction electrodes. Brain stem respiratory neurons located in the ventrolateral medulla were recorded with extracellular electrodes. Cranial, spinal motoneurons and afferent fibers were labelled *in vitro* with HRP in fetuses at different stages of development.

Fetal brain stem-spinal cord preparations generate rhythmic respiratory motoneuron and medullary neuron activity beginning early in the last trimester (E14), before complete morphological development of the motoneurons. Rhythmic limb motoneuron activity can be chemically activated (dopamine, NMDA or acetylcholine) in the fetal spinal cord as well as the isolated hemisected cervical and lumbar segments. Coordinated locomotor patterns were generated by late in the last trimester. With the brain stem intact, the respiratory and locomotor rhythmic activity can show synchronization. These results suggest that major components of the respiratory and locomotor pattern generating circuitry are assembled and functional in the last trimester. Supported by American Lung Association, NIH grants HL40959, HL02204, & NS27941.

377.9

SLICE CULTURES OF EARLY POSTNATAL RAT SPINAL CORD
J. Zimmer. PharmaBiotec, Institute of Neurobiology, University of Aarhus, DK-8000 Aarhus C, Denmark.

Early postnatal (P0-P4) rat spinal cord was removed in toto, cut in transverse 300 μm thick slices and grown as slice cultures ad modum Gähwiler, either alone or in cocultures with slices of corresponding postnatal hippocampal tissue. After 3-6 weeks the slice cultures were stained histochemically for acetylcholinesterase (AChE) to visualize cholinergic fibers, or immunocytochemically for calcitonin gene related peptide (CGRP).

The spinal cord tissue survived well both in single culture and in coculture. Two types of outgrowing AChE-positive fibers were observed. One fiber type, observed in virtually all cultures, displayed irregular branching and fasciculating patterns, but a fairly limited extension onto the coverslip except when innervating adjacent hippocampal tissue. The other type of AChE-positive fibers was found in a subpopulation of cultures containing large motoneuron-like, AChE-positive cell bodies. These fibers grew for long distances on the coverslips with characteristic sharp bends and narrow loops, with little branching. They also entered the hippocampal slices, but without apparent preference for these. In parallel sets of cultures, large motoneuron-like cell bodies and fibers with a distribution similar to the last type of AChE-positive fibers were found to be CGRP-immunoreactive. CGRP-immunoreactive, extensively growing fibers arising from large cells were also found in a few cultures containing what appeared as mistakingly included dorsal root ganglion cells.

Further studies of spinal cord slice cultures, including human fetal tissue, may help establish a test system for spinal cord injury and repair.

377.10

CLONAL HYBRID CELLS DERIVED FROM MOUSE SPINAL CORD MOTOR NEURONS.
E.F. Salazar-Grueso, S. Kim* & H. Kim*. Brain Research Institute, Department of Neurology, Univ. of Chicago, Chicago, IL 60637.

Investigations of motor neurons have in general been hampered because of the difficulty in their isolation and culture. This makes biochemical, physiological, and genetic studies seeking to define motor neuron-specific functions challenging. A potential solution to this problem is the production of clonal neural hybrid cell lines that are able to express characteristic that can typify motor neurons. Using this strategy, we have been able to immortalize embryonic mouse spinal cord motor neurons by somatic cell fusion to mouse neuroblastoma cells. We fused an aminopterin-sensitive and neomycin-resistant mouse neuroblastoma cell line (N18NEO) to isolated embryonic mouse spinal cord motor neurons. Several hybrid cell lines expressing high levels of choline acetyltransferase (Chat) enzyme activity were obtained. These cell lines were cloned twice by limiting dilution and clones expressing high levels of Chat enzyme activity were isolated. The hybrid nature of the cloned cells was confirmed by determining glucose phosphate isomerase allozymes and chromosome counts. The hybrid cells but not the neuroblastoma cell line also express high and medium neurofilament proteins, developmental characteristics typical of differentiated neurons. The availability of these embryonic clonal hybrid cells will make possible molecular, biochemical, physiologic studies that are aimed at defining motor neuron-specific properties.

377.11

EARLY STAGES IN THE DEVELOPMENT OF MOTOR NEURONS
A.Y. Chiu and E. Chen* Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

In order to identify early events in the differentiation of motor neurons, the expression of several developmentally regulated, neuronal molecules was investigated by immunohistochemistry on consecutive sections of cervical spinal cord. Within the embryonic rat spinal cord, motor neurons undergo their terminal mitosis on embryonic days 10 and 11 (E10 - E11), and acquire detectable levels of choline acetyltransferase (ChAT), by E11.5 (Phelps *et al.*, J. Comp. Neurol. 273:459). Staining with antibodies to the intermediate-size neurofilament protein revealed motor neurons extending processes out the ventral root as early as E10.5. Monoclonal antibodies to two epitopes on the cell adhesive molecule, NCAM, bound to myotomes on E10.5, and began to recognize motor neurons by E11. Two other markers of developing neurons, the growth-associated protein, GAP-43, and the surface glycoprotein, TAG-1, were also detected on young motor neurons by E11.5. Thus, within one and a half days after their final mitosis, motor neurons have acquired a number of cytoskeletal, enzymatic and cell surface components that distinguish them from other developing cells within the spinal cord. Not all of the newly acquired molecules continue to be expressed by motor neurons. E12.5 motor neurons lost immunoreactivity for TAG-1, followed a gradual reduction of NCAM and GAP-43 epitopes a day later. By E16, only neurofilament and ChAT immunoreactivity (Phelps et al., J. Comp. Neurol. 307:1) persisted in motor neurons within the embryonic spinal cord. The transient expression of NCAM, GAP-43 and TAG-1 epitopes coincides with the period of vigourous axonal growth, and declines when motor neurons reach their targets. Loss of these antigens may signal the onset of synapse formation and a new phase in the differentiation of motor neurons. *This work was supported by HD26810 and NS18858.*

377.12

ANDROGEN INDUCES MUSCLE FIBER TYPE CONVERSION IN DENERVATED LARYNGEAL MUSCLE OF *X. LAEVIS*.
M. L. Marin, M. L. Tobias, D. B. Kelley, Dept. Biol. Sci., Columbia Univ., NY, NY 10027

Laryngeal muscle is the final effector for sexually dimorphic vocalizations in *Xenopus laevis* frogs. Adult male larynges produce rapid (~60Hz) trills whereas adult female larynges produce trills at a much slower rate (~6Hz). Fiber twitch type, assessed with ATPase fiber histochemistry, correlates well with these behavioral observations. Adult male laryngeal muscle contains only fast twitch fibers while adult female laryngeal muscle contains predominantly slow twitch fibers. Juvenile laryngeal muscle contains equal numbers of slow and fast twitch fibers. In response to androgen treatment, juvenile laryngeal fibers are converted from slow to fast twitch. To determine if androgen induced fiber type conversion is nerve dependent, juvenile male and female larynges were unilaterally denervated. At the time of denervation, half the animals received a testosterone pellet while the other half received no pellet. All animals were reared an additional five weeks and laryngeal muscle fibers typed using ATPase histochemistry. Following androgen treatment, larynges of both sexes contain only fast twitch muscle fibers. There was no difference in fiber type composition between the innervated and denervated sides. Denervation alone does not cause selective loss of slow twitch fibers since denervated, untreated laryngeal muscle contains both slow and fast twitch fibers. We conclude that androgen acts directly at the level of the muscle fiber to regulate myosin ATPase expression. Supported by NS 23684

377.13

SELECTIVE DEATH OF AXIAL MUSCLE PRIMORDIA.
K.W.Tosney. Biol. Dept., Univ. of Michigan, Ann Arbor, 48109.

I have discovered that the primordia of lateral body wall muscles are initially present in each segment of the chick embryo but have different, segment-specific fates. In thoracic segments, these primordia become mature at the lateral edge of the dermamyotome by exhibiting three changes during stages 21-23. First the dermatome cells surround the lateral edges of the myotomes, then the number of myotubes increases, and then the primordia invade the lateral body wall. In contrast, in limb segments, the dermatome cells die and are phagocytosed during stages 21-23; when the dermatome cells die, the myotubes do not increase in number and the primordia do not undergo further morphogenesis. Because the death of the dermatome cells in limb segments curtails the addition of myotubes and maturation of the primordia, I suggest that the dermatome cells rather than the myotome cells are primarily responsible for the patterned development of axial muscle primordia.

What interactions control the differential fate of these primordia in thoracic and limb segments? Two embryonic surgeries identify interactions that can alter the segment-specific survival or death of the dermatome cells. When the proximal portion of a limb is deleted, the dermatome cells do not die; instead, they surround the myotome and myotubes increase in number. In contrast, when a limb is transplanted to thoracic segments, the dermatome cells die and the muscle primordia fail to mature. These results suggest that local interactions control the differential fate of axial muscle primordia and do so by supporting the survival of dermatome cells or by causing dermatome cells to die.

Supported by NIH grant NS-21308.

377.14

ELECTROPHYSIOLOGICAL CHANGES THAT ACCOMPANY TRANS-SYNAPTICALLY INDUCED ATROPHY IN GRASSHOPPER THORACIC MUSCLES.
L.L.Rankin & E.A. Arbas. ARLDN, Univ. Ariz., Tucson AZ 85721.

When grasshoppers, *Barytettix psolus*, shed a hindlimb by autotomy, certain undamaged, fully innervated thoracic muscles atrophy severely. Previous experiments indicate that: 1) severing of the leg nerve during autotomy trans-synaptically induces muscle atrophy, and 2) undamaged motorneurons (Mns) to affected muscles persist in the CNS despite atrophy of their targets. We are comparing the electrophysiological properties of the normal and atrophying neuromuscular system to uncover the basis of trans-synaptically induced atrophy.

Intracellular recordings from one of the affected muscles, tergotrochanteral muscle #133 b,c, show that a decline in resting membrane potential (e.g., from a mean of -73 mV to between -10 and -50 mV) occurs in some fibers by day 5 post-autotomy and in a majority of fibers by day 12. Junction potentials evoked in normal muscle by stimulating the motor nerve fall into at least 4 classes: 1) large, fast, non-overshooting ejp's (55-70 mV amplitude, 2-5 ms rise time), 2) smaller, intermediate ejp's (10-50 mV, 3-10 ms), 3) small, slow ejp's (2-10 mV, 8-18 ms), and 4) small, slow ijp's of variable amplitude that were based on a decreased conductance. These classes could be discriminated by differences in threshold or by fatiguing the large, fast potentials with repetitive stimulus trains. Beginning 5-6 days after autotomy, many muscle cells were depolarized and showed smaller and slower ejp's, although some large, fast ejp's could still be evoked. The occurrence and character of the ijp's seems unaltered up to 10 days post-autotomy. It also becomes easier to discriminate thresholds of the different classes of synaptic potentials at increasing times post-autotomy. Current studies are focused on discriminating which of these effects are due to changes in the physiology of muscle cells or Mns and determining their cause. Supported by NIH grants NS-07309 & the Ctr. for Insect Science.

377.15

ABNORMAL THORACIC MUSCLE ORGANIZATION IN *bendless DROSOPHILA*. R.S. Edgecomb, T.H. Yuan* and A.M. Schneiderman. Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

A small group of mutations isolated in *Drosophila* alters genes involved in specifying individual synaptic connections between neurons. The recessive *bendless* (*ben*) mutation disrupts the synaptic connection between two neurons in the escape-response pathway of the adult: the giant fiber neuron (GF) and the motoneuron innervating the tergotrochanteral (jump) muscle (TTM) (Thomas & Wyman, 1984, J Neurosci 4:530). Stimulation of this pathway normally results in an escape response with characteristic motoneuron latencies and following frequencies. In *ben* flies the TTM motoneuron responds with a longer latency and fails at a lower frequency after stimulation of the GF. We report here that *ben* also affects the development of the TTM.

In wild-type flies the origin of the TTM is invariant, attaching posterior to the intrascutal suture near the lateral margin of the scutum. In contrast, a small percentage (<25%) of homozygous *ben* flies exhibit variable abnormalities in the dorsal attachment of the TTM to the scutum. Its origin may be displaced anteriorly and/or medially. In female flies hemizygous for *ben*, the origin of the TTM is aberrant in over 66% of animals examined. In some cases the muscle is absent; in others it diverges and attaches at two separate sites on the scutum. The pattern of muscle fiber organization is also altered. We conclude that in addition to its role in giant fiber pathway development, the product of the *bendless* gene is important for proper development of the TTM. (NSF BNS-90-09833, NIH 5-T32-NS07303)

377.16

METAMORPHOSIS OF UNPAIRED MEDIAN NEURONS IN MANDUCA. H.J.Pfluger and R.B.Levine. ARL Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721 and Free Univ., Koenigin-Luise-Str. 28-30, D-1000 Berlin 33, FRG.

Unpaired median neurons modulate muscle contraction in insects. We followed the fate of identified unpaired neurons in the abdomen of *Manduca*, where changes in muscles during metamorphosis suggest a modified behavioral role for these neurons. Ni^{++}/Co^{++} backfills of peripheral nerves and intracellular Co^{++} fills reveal two ventral unpaired neurons with bilateral axons in each abdominal ganglion in larvae, pupae, and adults. In larvae, the two cells project out distinct branches of the dorsal segmental nerve to non-overlapping sets of muscles. Despite the loss of some muscles and the development of others, both neurons survive metamorphosis and project to similar regions of the body wall in larvae and adults. A comparison of the dendritic structures at different stages reveals a subtle regression of fine processes at the end of larval life, followed by the elaboration of a new dendritic tree during adult development. The adult dendrites possess terminal branches with numerous varicosities, and in general were more complex than in larvae. The dendritic complexity appeared to increase for the first several days following adult emergence, even though this period is associated with the degeneration of some major body-wall muscles. Future studies will explore the function of these putative neuromodulatory cells, and how it is modified during metamorphosis. (supported by NSF and NIH grants to RBL; and a DFG travel grant to HJP.)

REGENERATION: TISSUE CORRELATES

378.1

EMBRYONIC RETINAL ABLATION AND POST-METAMORPHIC OPTIC NERVE CRUSH: RELATIONSHIP BETWEEN HEALING, EXTRA CELL DIVISION AND RESULTANT VISUOTECTAL PROJECTIONS DURING REGENERATION. L. Wunsh Underwood, P. Nelson*, E. Noelke* and C. F. Ide. Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

We examined relationships between healing, extra cell division and resultant visuotectal pattern formation observed during Xenopus laevis retinal and optic nerve regeneration. Dorsal (D), nasal (N) and nasoventral (NV) 1/3 sized eye fragments were surgically created in stage 32 embryos and analyzed. Dorsal fragments either showed little cell displacement and no associated extra cell division, or showed massive cell displacements ventrally with extra cell division. Neither healing type showed duplicated projections which is predicted by the polar coordinate model. Nasal fragments always showed cell displacements ventrally with associated extra cell division, and over 90% formed duplicated projections, also predicted by the polar coordinate model. In additional studies in adult animals (NV fragments), the optic nerves of eyes with duplicated projections were crushed and allowed to regenerate for one year. Duplicated projections were restored, indicating that developmental and maturational factors are probably not responsible for duplicative pattern formation; rather, information intrinsic to the eye, possibly created during healing interactions underlies pattern duplication.

378.2

ANATOMICAL ANALYSIS OF RETINOTOPIC REFINEMENT IN A COMPRESSED RETINOTECTAL PROJECTION IN GOLDFISH WITH AND WITHOUT TTX ACTIVITY BLOCKADE. M.D. Olson and R.L. Meyer, Developmental and Cell Biology, University of California, Irvine, California 92717

When the optic nerve is crushed and the posterior half of the tectum is removed, electrophysiological analysis has shown regenerating optic fibers eventually form a complete, compressed retinotopic projection onto the remaining half tectum. In this study, we followed the progression of retinotopic refinement in half tectum, using 2nl "spot" injections of WGA-HRP into the retina. In some animals, activity was eliminated by repeated intraocular injections of TTX. Spot injections were placed such that the label would be in the posterior 1/4 of the normal tectum. At one month, label was dispersed over most of the anterior tectal remnant in both control and TTX animals. At two months, label in control animals had condensed into small clusters in the posterior end of the half tectum. At the same time, label in TTX animals had moved posteriorly, showing substantial condensation but remaining more diffuse than with activity.

These results suggest that compression may proceed directly from an early diffuse and nonretinotopic projection rather than from an orderly uncompressed projection. They also show substantial compression under TTX blockade indicating compression is generated by activity independent mechanisms.

Supported by NIH grant 9R01 EY06746.

378.3

HYPOTHALAMO-HYPOPHYSEAL TRACTS TRANSECTED IN THE LATERAL RETROCHIASMATIC AREA DEVELOP NEUROVASCULAR CONTACT ZONES. J. Carithers and H.-D. Dellmann*, Dept. of Vet. Anatomy, Iowa State Univ., Ames, IA 50011.

Neurosecretory axons regenerate into neural lobe, optic nerve or sciatic nerve transplanted into lateral retrochiasmatic lesions of the magnocellular neurosecretory tract. Now we report the establishment of neurohemal contact zones in association with explanted neural lobe, optic nerve or sciatic nerves placed into the tract at the site of lateral retrochiasmatic lesions 15 d earlier.

Regions with increased microvascularity and abundant profiles immunoreactive for neurophysin lie adjacent to all three types of grafts. The organization of such areas resembles that of neural lobe, however, their capillaries are not fenestrated. Numerous neurosecretory axons are present, and palisades of axon terminals abut the perivascular basal laminae. Glial cells partially ensheath regenerating neurosecretory axons, and some glial processes extend between terminals to the neurovascular contact zone. Terminals with many microvesicles and few neurosecretory granules provide morphological evidence of hormone release. Neurohemal contact zones that clearly incorporate grafted tissue occur only in grafted neural lobe explants, in which neurosecretory terminals are associated with fenestrated capillaries and pituicytes. Very limited development of neural lobe-like regions is occasionally seen near lesions without grafts.

Supported in part by NSF BNS 8919729.

378.4

REGENERATION OF TRANSMISSION FOLLOWING LESIONS TO THE CNS OF THE NEONATAL OPOSSUM, *Monodelphis domestica*, ISOLATED AND MAINTAINED IN CULTURE. S.K.A.Woodward, J.M.Treherne, Z.M.Varga, J.M.Ritchie and J.G.Nicholls. Pharmacology Dept., Biocenter, Basel, Switzerland.

Little information is available about the ability of embryonic and neonatal mammalian CNS to regenerate after injury. Since development is still occurring and no myelin is present in the immature spinal cord it seemed that the conditions for regeneration or new growth across a lesion might be more favorable than in adults. The isolated CNS of the new-born opossum, which survives well in tissue culture, provides an accessible system for such studies.

The brain and spinal cords of three-day-old opossums were dissected and the spinal cords crushed in the lower cervical region, abolishing all through conduction. The preparations were maintained in tissue culture for up to six days. The ability of the cords to conduct action potentials was monitored during this time. Between three and four days after the lesion was made, transmission through the spinal cord was re-established. Carbocyanine dyes, HRP and silver stain are now being used to analyse changes occurring at the crush site.

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378.5

PLASTICITY OF THE CATECHOLAMINERGIC SYSTEM IN THE ADULT RAT CEREBRAL CORTEX AS A RESULT OF CHOLINERGIC PATHWAY LESIONS. S. A. Welner and Z. C. Koty*. Douglas Hospital Research Centre, McGill University, Department of Psychiatry, Montreal, Quebec, Canada H4H 1R3.

It is well established that the CNS of developing organisms is capable of extensive growth and plasticity; until as recently as the last 2 decades, however, the notion that adult mammalian neurons were plastic in nature was usually not considered. The latter has recently been shown in the septo-hippocampal system; denervation of this pathway results in sprouting of sympathetic fibers into the hippocampus. In the present study, we show that similar plasticity appears to be present in adult mammalian neurons of the cerebral cortex; lesions of the cholinergic pathway from the nucleus basalis magnocellularis (NBM) to the cerebral cortex results in an elevation of cerebral cortical levels of tyrosine hydroxyase (TOH), the rate limiting enzyme in catecholamine synthesis. The experimental group consisted of male Sprague-Dawley rats, lesioned bilaterally in the NBM with 0.12M quisqualic acid and was compared to an unoperated control group. Four months following lesion, TOH activity in the fronto-parietal area of cortex was measured. Results show an approximate three-fold elevation of TOH levels in the lesioned cortex compared to the intact cortex. These results are interesting with respect to plastic changes that may occur in disease states where cortical cholinergic function is disrupted, such as with Alzheimer's disease, and may contribute to the symptomatology of the disorders. (Supported by the Alzheimer Society of Canada)

378.6

MORPHOLOGICAL CHANGES IN THE TROCHLEAR NERVE FOLLOWING AXOTOMY. P. Iannuzzelli,[1] M. Murray,[1] R. Baker[2] and E.H. Murphy.[1] Dept. of Anatomy and Neurobiology,[1] Medical College of PA, Philadelphia, PA 19129, and Dept. of Physiology and Biophysics,[2] NYU Med. Center, New York, NY 10016.

In the adult cat, following axotomy of the trochlear nerve (TN), surviving trochlear motor neurons regenerate and re-innervate their target by 4 weeks post-axotomy. We used quantitative electron microscopy to examine cross sections of the regenerating TN distal to the transection. There are approximately 1,000 myelinated axons in the normal TN and the same number just distal to the lesion site in the long term (>6 mos.) regenerated nerve. At 4 weeks post-axotomy, as many as 4,000 regenerative sprouts were present in the distal nerve stump, indicating that regenerating axons branch. Most cells in the distal nerve stump showed typical Schwann cell morphology, although occasionally, cells morphologically resembling astrocytes were seen. Schwann cells appear to be associated either with regenerating axons or degenerating debris. Just distal to the lesion site Schwann cells associated with regenerating axons usually contain only one axon profile. However, the number of regenerating axons per Schwann cell increases in more distal parts of the nerve stump. Our data suggests that following axotomy regenerating axons branch as they grow distally. In addition, it appears that there are at least two distinct functional classes of Schwann cells; those associated with regenerating axons and those associated with degenerating debris, and that these functions are not overlapping. (Supported by NIH-NS24707)

378.7

CONTRALATERAL EARLY BLINK REFLEX IN PATIENTS WITH FACIAL NERVE PALSY: EVIDENCE FOR SYNAPTIC REORGANIZATION IN THE FACIAL NUCLEUS DURING REGENERATION. R. Töpper*, W. Nacimiento, K. Podoll*, M. B. Graeber, E. Möbius*, J. Noth and G. W. Kreutzberg. Dept. of Neurology, Alfried Krupp-Hospital, 4300 Essen, F.R.G. and Max-Planck-Institute of Psychiatry, Dept. of Neuromorphology, 8033 Martinsried, F.R.G.

50 patients with Bell's palsy and 30 patients with etiologically different symptomatic peripheral facial nerve palsy were studied by means of electrically evoked blink reflexes 1-23 days after onset of paresis. Their results were compared with a normal control group of 30 healthy subjects. In a significant number of patients (64% in Bell's palsy and 53% in symptomatic facial nerve palsy) a contralateral early blink reflex response (R1) could be elicited upon stimulation of the normal side as compared to 13% in the control group. It is suggested that this result may be explained by synaptic reorganization of the facial nucleus leading to functional unmasking of pre-existing crossed trigemino-facial reflex pathways during regeneration. This view is in line with previous experimental data in animals on the time course of structural changes in the facial nucleus after lesioning of the ipsilateral facial nerve.

378.8

PARTIALLY LESIONED RAT NIGROSTRIATAL SYSTEM - A MODEL FOR EARLY PARKINSON'S DISEASE. F.S. Kim*, K. Steece-Collier, T.J. Collier, D.L. Felten, S.Y. Felten, J.R. Sladek, Jr. Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

Rat models of Parkinson's disease have been characterized by complete or near complete unilateral destruction of the nigrostriatal pathway using the neurotoxin 6-hydroxy-dopamine (6-OHDA). We are developing and characterizing a hemi-parkinsonian rat model, using unilateral injection of 6-OHDA into the medial forebrain bundle, in which there is partial destruction of the nigrostriatal system. The behavior of such animals was characterized by ipsiversive rotation following an amphetamine injection, but no rotation following an apomorphine injection, in contrast to the contraversive rotation observed in the completely lesioned models. The mean striatal dopamine (DA) content of the partially lesioned animals, as measured with HPLC, was approximately 50% of the control side versus 20% in the completely lesioned animals. The nigral DA cell count using tyrosine hydroxylase immunocytochemistry showed similar results. The degree of cell loss of the A8 and A9 DA cell groups, rather than the A10 cell group correlated better with the observed behavior. This model better estimates the condition of the DA system in clinical Parkinson's disease and can be used to study factors influencing the progression of nigral cell death and the potential for homotopic regeneration in the illness. Supported by PO 1 NS 24032.

378.9

SPARSE REGENERATION AFTER AXOTOMY OF CHOLINERGIC PROJECTIONS FROM PONTINE TEGMENTUM TO ANTERIOR THALAMUS. T.W. Farris and L.L. Butcher. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

To assess the regenerative capabilities of projection neurons of the cholinergic pontine tegmental complex (CPTC), assessed previously for cortical projections from the cholinergic basal nuclear complex (CBNC), we performed unilateral Scouten wire knife-cut axotomies of a cholinergic pathway arising from the laterodorsal tegmental nucleus and projecting to anteroventral and anteromedial thalamic nuclei in 8 week old female rats. Brain tissue was collected at 4, 7 and 14 days post-axotomy and was processed histochemically for acetylcholinesterase (AChE); immunohistochemically for choline acetyltransferase and glial fibrillary acidic protein; and for Nissl substance. Computerized densitometry performed via light microscopy showed, in all groups, the expected accumulation and depletion of AChE fiber density proximal and distal, respectively, to the thalamic knife-cut. However, unlike our findings for the CBNC projections to medial cortex, there was little recovery from axotomy-induced depletion of AChE-positive fiber density in the CPTC thalamic target regions at these time points. No AChE-positive fibers were observed to cross the cut. These data (1) indicate that CPTC neurons are less morphologically plastic than CBNC neurons; (2) provide evidence of a correlation between the regenerative and degenerative capabilities of these cholinergic neuronal populations as the CBNC degenerates in Alzheimer's disease and expresses nerve growth factor receptor whereas the CPTC does neither appreciably; and (3) demonstrate that the assessment of axotomy effects via AChE-fiber densitometry can be extended reliably to a non-cortical region as a model for testing mammalian CNS cholinergic regeneration *in vivo*. [Support: NIH NS 10928 to LLB]

378.10

BEHAVIORAL RECOVERY OF THE GOLDFISH STARTLE RESPONSE AFTER SPINAL CORD CRUSH S.J. Zottoli and M.M. Freemer*. Dept. of Biology, Williams College, Williamstown, MA 01267.

The goldfish central nervous system is capable of functional regeneration after spinal cord injury. Specifically, damage to the spinal cord may be followed by the recovery of swimming, feeding, equilibrium and startle responses (Neurosci. Abst.15: 333). We have used a high speed, digital imaging system (i.e., an image is collected every 2 ms) with computer display and analysis (Eaton et al., J. Neurosci. 8: 2758) to compare recovered startle responses with those of sham-operated controls. Fish were anesthetized, their whole spinal cord was crushed at the spinomedullary level, and they were tested for the return of startle responses by computer activation of a solenoid which moved the test tank upward. Of the 25 fish that survived to 170 postoperative days, 12 had regained startle responses. Significant differences in a number of movement parameters existed between startle responses of these experimental fish and sham-operated controls. The differences included: lower frequency of response, longer latency from stimulus presentation to initiation of the response, and smaller body angle and distance traveled by the center of mass 70 ms after the initiation of the response. The recovered startle responses were lost in 5 fish on re-crush of the spinal cord.

The return of the startle response in goldfish is due to regeneration of the central nervous system. We speculate that the longer response latency and the reduced movement of recovered startle responses may compromise the animal's ability to escape predators. Thus, the use of the term "functional regeneration" may be inappropriate in this case. Supported by NSF grant BNS-8809445.

378.11

REGENERATION OF AXOTOMIZED NEURONS AFTER SPINAL CORD LESIONS IN NEWBORN RATS: A QUANTITATIVE DOUBLE-LABELING STUDY. B.S. Bregman and H. Bernstein-Goral. Dept. Anatomy and Cell Biology, Georgetown Univ. Sch. Med., Washington, D.C. 20007

Transplants (TP) of fetal spinal cord tissue support the survival and growth of immature axotomized brainstem-spinal neurons. Both late-developing and regenerating neurons contribute to this transplant induced anatomical plasticity (Bregman and Bernstein-Goral, 1991, Exp. Neurol. 112: 49-63). The current study was designed to 1) determine the magnitude of the regenerative response and 2) test the hypothesis that the long distance growth beyond the site of a neonatal lesion plus transplant is by late-developing neurons, whereas regenerating neurons project to spinal cord levels immediately caudal to the transplant. We used temporally spaced retrograde tracing with the florescent dyes fast blue (FB) and diamidino yellow (DY) to address this issue. Spinal projecting neurons were axotomized and labeled with FB at 2 dpn. The source of FB was removed and a spinal cord TP was placed into the lesion site. 3-6 wks later DY was injected into the spinal cord bilaterally either <5mm or 10-15mm caudal to the TP. Cell counts show that 28% of the neurons in the red nucleus, 32% of the neurons in the locus coeruleus and 37% of the neurons in the raphe nuclei regenerated within 5mm caudal to the TP. Furthermore, after DY injections up to 15 mm caudal to the TP, a substantial population of regenerating neurons (7-9%) was identified in each of the brainstem nuclei examined. Thus, the hypothesis that the long distance growth beyond the transplant is solely by late-developing neurons was not supported. Rather, both regenerating neurons and late-developing neurons extended axons long distances caudal to the lesion site. Surprisingly, the proportion of regenerating neurons was similar in each of the 3 brainstem-spinal nuclei examined. Supported by NIH grants NS 19259, NS 27054 and NS 01356 to BSB.

378.12

CHANGES IN VENTRAL HORN SYNAPSES IN SALAMANDER SPINAL CORD FOLLOWING THORACIC TRANSECTION WITH CORRELATIONS TO BEHAVIOR. L.K. Garner, M.C. Anderson, J. *Ayers and B.M. Davis. Dept. of Anatomy & Neurobiology, Univ. of Kentucky, Med. Ctr., Lexington, KY 40536. * Marine Sci. Inst., Northeastern Univ. Nahant, MA 01908.

To determine if newt spinal circuitry produced by regeneration was the same as that produced by embryogenesis, four groups of adult newts (*Notophthalmus viridescens*) were processed and analyzed for EM: 1) unoperated newts (containing spinal circuitry produced by normal development), 2) acute thoracic transected newts (7-21d post-lesion), 3) regenerated newts (exhibiting various degrees of function), and 4) retransected newts (recovered newts re-lesioned at original site). The number of synapses/1002μm and the average area of the boutonal profile was measured in lumbar ventral horn at the level of spinal nerve 16-18. Only boutons that contained synaptic specializations (active zones and vesicle accumulation) were included. Analysis of normal newts (n=4) revealed 4.6 ± 0.7 synapses/1002μm (average bouton area = 1.5 ± 0.4μm). Following thoracic transection the number of synapses decreases up to 33% with no change in bouton size. To date, two newts 3mo post-transection have been analyzed. In newt #1, the size and number of boutons were normal (4.6 syn/1002μm, ave. size =1.1μm). However, newt #2 had only 1.1 syn/1002μm, but average bouton size was twice normal (2.4 μm). We are now performing kinematic analysis to relate these changes to recovery of function. Supported by NS25617 to BMD.

378.13

CHANGES IN VENTRAL HORN NEURON SIZE AND INNERVATION DURING TAIL REGENERATION IN THE LIZARD *ANOLIS CAROLINENSIS*. M.T. Duffy, B.M. Davis, D.R. Liebich* and S.B. Simpson, Jr. Dept of Biological Sciences, University of Illinois at Chicago, 60680; Anatomy & Neurobiology, Chandler Medical Center, University of Kentucky, Lexington, 40536.

We have previously shown that ventral horn (VH) neuron somata in normal tail spinal cord rostral to large regenerated tails (≥ 6 cm) are significantly hypertrophied compared to normal (329.7 ± 38 μ^2 vs 154.8 ± 24 μ^2) and receive more axosomatic contacts (18.35 ± 1.6 vs 9.04 ± 0.8). We also wished to know if changes in VH neuron size were correlated with changes in synaptic input. For these experiments we chose lizards autotomized two months previously in which regenerating tail segments were ≤ 10 mm (mean length = 8 mm). Preliminary data indicates that mean VH neuron size (n=6) in these animals is greater than in normal tailed lizards (302 ± 36 μ^2 vs 154.8 ± 24 μ^2; $p < .01$) and is equal to that of neurons rostral to large mature regenerated tails. The number of synaptic bouton contacts on these somata is slightly increased over normal (12.5 ± 1.6 vs 9.04 ± 0.8; p=.13) but significantly decreased when compared to larger mature regenerates ($p < .01$).

These results suggest that changes in neuron size and innervation may be independent of one another in Anolis lizards. However, these results might also indicate that changes in neuron size and central innervation are not complete at two months post-autotomy. Further studies of intermediate stages of regeneration will be needed to show when such changes are complete.

378.14

DEVELOPMENT OF REGENERATED DORSAL ROOTS WITHIN FETAL SPINAL CORD TRANSPLANTS.

Y.ITOH[1], T.SUGAWARA*[1], M.KOWADA*[1], A.TESSLER[2]. [1]Dept. of Neurosurg., Akita Univ., Akita 010, Japan, and [2]Philadelphia VA Med. Ctr. and Dept. of Anat. and Neurobiol., The Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

Adult rat dorsal roots regenerate into transplants of fetal spinal cord (FSC) and form synapses, but the time course of regenerated axon growth within transplants is unknown. In this study we used calcitonin gene-related peptide (CGRP) immunohistochemistry to label regenerated dorsal roots in FSC transplants and stereological analysis to evaluate the development of transplant neuropil. Transplants of E14 spinal cord were introduced into a hemisection cavity aspirated in the lumbar enlargement of adult Sprague-Dawley rats, and the adjacent L4/L5 dorsal root was cut and juxtaposed to the transplant. Sagittal vibratome sections were prepared for CGRP immunohistochemistry after survivals of 14 days to over 1 year. The area fraction of transplant neuropil occupied by regenerated CGRP-containing myelinated axons and axon terminals increases for the first 3 months and then persists unchanged for over 1 year. The area fraction occupied by regenerated CGRP-containing unmyelinated axons does not change through 1 year, nor does the area fraction occupied by perikarya and dendrites within the transplant. Myelinated dorsal roots therefore grow for several months within the transplants, and the innervation established by regenerated axons increases over the same period and is permanent. Supported by VA Medical Research Service, NIH grant NS24707, and USAMRDC grant 51930002.

378.15

GROWTH OF PROCESSES ACROSS SPINAL CORD LESIONS IN XENOPUS CNS EXPLANTS. D.L.Norris, M.S.Beattie, and J.C.Bresnahan. Neuroscience Program and Dept. CBNA, The Ohio State Univ., Columbus, OH 43210.

Brainstem/spinal cord explants were dissected out of Xenopus tadpoles (stage 50-51) following MS-222 anesthesia. Explants were severed at the brainstem-spinal cord junction and placed into collagen gels with the cut ends of the pieces in apposition. Explant cultures were maintained in dilute L-15 culture medium, pH 7.8, at 24°. Cultures were fixed in 4% paraformaldehyde after 8 days. Crystals of DiI were placed into the spinal cord caudal to the lesion to back-label brainstem neurons whose axons had grown across the lesion. DiI was allowed to diffuse for 3 (n=3) or 12 (n=8) days. Explants were examined using confocal laser microscopy. Several small, round neurons with processes extending into the lesion site could be seen within most brainstem explants; 3 explants also contained larger labeled cells with laterally-oriented dendrites. These results complement previous studies demonstrating growth of descending processes across spinal cord transections in Xenopus tadpoles (Beattie et al., 1990), and suggest that explant cultures can be used to study Xenopus CNS regenerative processes. (NS-10165)

378.16

SPINAL MOTONEURON MORPHOLOGY AND RETROGRADE AXONAL TRANSPORT AFTER SCIATIC NERVE TRANSECTION/REPAIR L. Lipworth*, N.H. Evans* and C.M. Bowe, Dept. Clinical Neurosciences, Brown Univ., Providence, R I 02912

Morphological changes and enhanced retrograde labelling with HRP are reported in spinal motoneurons (SMNs) examined at 10-12 months after nerve crush. In the present study, the morphology and the retrograde labelling characteristics of rat SMNs were evaluated at 10 months following unilateral, sciatic nerve transection and repair with a guidance channel. Distinct retrograde labelling agents were employed to identify SMNs innervating muscles in the sciatic nerve distribution and SMNs of origin for regenerated axons. Two weeks prior to sacrifice, Fluorogold was applied bilaterally to the muscles of interest; 2 days before sacrifice, regenerated and control sciatic nerves were transected distal to the guidance channel and exposed to HRP. No morphological abnormalities were seen in the ipsilateral SMNs. However, compared to the contralateral side, the total number of ipsilateral SMNs was reduced by 17% and the number of double-labelled SMNs was decreased by 33%. There was an increase in the number of ipsilateral SMNs exhibiting only fluorescent labelling. Lengthening the time interval between HRP application and sacrifice resulted in a reduced discrepancy in HRP labelling between the two sides and an increased deposition of HRP-reaction product in the ipsilateral, but not the contralateral, cells. In contrast to the nerve crush lesion model, axonal regeneration after nerve transection/repair is not associated with clear morphological changes in SMNs but retrograde axonal transport mechanisms appear to be compromised.

378.17

CUTANEOUS, MUSCULAR AND ARTICULAR NERVE FIBRE REGENERATION AFTER RAT SCIATIC NERVE LESIONS. C. Hildebrand, and B. Povlsen*. Dept of Cell Biology, and Dept of Hand and Plastic Surgery, Faculty of Health Sciences, S-581 85 Linköping, Sweden.

In young adult rats the right sciatic nerve was subjected to a crush lesion 5 mm below the tendon of the internal obturator muscle. In other animals the nerve was divided. The cut ends were rejoined using 9-0 nylon sutures. Some rats served as normal controls. After 3 months survival all animals were perfused with glutaraldehyde. Specimens from the sural nerve (SN), the lateral gastrocnemius nerve (LGN) and the posterior articular nerve of the knee joint (PAN) were processed for electron microscopy (EM). For each nerve all myelinated (MA) and unmyelinated (UA) axons were counted on EM prints (x 2100) from thin cross-sections. Following a crush lesion the SN shows a 15% increase in the proportion of MA but a 25% decrease in the proportion of UA. In the LGN both MA and UA increase by 30-35%. The PAN, on the other hand, presents a 60% increase of MA, and the proportion of UAP more than doubles. In the divided and sutured SN the MA increase 40% while the UA decrease 40%. In the LGN the MA and the UA increase 180% and 83% respectively. The PAN shows a 30% increase of the MA but the proportion of UA does not change. These results show that the outcome of axonal regeneration is markedly different in functionally different branches of a mixed major nerve trunk.

378.18

GLIAL RESPONSE TO DORSAL ROOT LESION IN THE IRRADIATED SPINAL CORD T. J. Sims and S.A. Gilmore Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR. 72205-7199.

Exposure of the lumbar spinal cord in early postnatal rats to x-rays induces a marked reduction in the normal glial populations in the irradiated region. The present study was undertaken to determine what effects this reduction of glia has on the glial scar formation that follows root injury in the normal spinal cord. Rat pups were irradiated on postnatal day 3 with a single 4000R dose of x-rays over the lumbar spinal cord. At 20 days following irradiation the L4 dorsal root was lesioned by crushing once and then freezing and thawing twice. Non-irradiated littermates were subjected to the same lesioning procedures and served as controls. Between 60 and 90 days following the lesion, the rats were perfused with fixative and the L4 spinal cord segments were prepared for ultrastructural examination. There was a distinct difference in the extent of the astrocyte response between the irradiated and the non-irradiated rats following dorsal root injury. In the non-irradiated rats, a thick astrocytic scar formed over the dorsal horn. The scar was composed of multiple layers of astrocyte processes with numerous gap junctions interposed. This astrocytic response was not confined to the surface of the spinal cord but extended into regions normally considered as PNS. In irradiated rats, astrocytes formed a glia limitans with an irregular contour and did not develop a thick astrocytic scar. Gap junctions occurred less frequently than in non-irradiated spinal cord. The presence of many small diameter axons just below the surface of the cord, along with larger myelinated axons interposed between processes of the glia limitans, is consistent with an earlier report that axons are capable of regrowing into the irradiated spinal cord (Sims and Gilmore, 1990). Mechanisms involved in conversion of the irradiated spinal cord to a permissive substrate for axonal regrowth are only partly understood; the reduction in the astrocyte population and the absence of scar formation no doubt play important roles. Supported by PVA Grant 745 from the Spinal Cord Research Foundation and NIH Grant NS 04761.

378.19

POSTINJURY DISAPPEARANCE OF ASTROCYTES AND ARREST OF AXONAL GROWTH MAY BE INTERCONNECTED. E. Blaugrund*, R. Duvdevani*, V. Lavie and M. Schwartz. Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

An injury to the mammalian central nervous system results in loss of function, due to its inability to regenerate. It has been postulated that adult mammalian central neurons have the ability to regenerate, but the surrounding glial cells are either inhibitory or nonsupportive for their growth. In this study, we closely examined the spatial and temporal relationship among injured/growing axons, astrocytes and phagocytic cells after crush of the adult rat optic nerve. This was carried out by immunohistology at the light microscope, using anti-GFAP and ED-1 antibodies for identifying astrocytes and macrophages, respectively. Transmission electron microscopy was used for estimating the number of these cells and for evaluating abortive axonal growth. Two days after the crush, a massive death of astrocytes occurred at the crush site. After a week, the crush site was very poor in astrocytes and filled with macrophages, while the proximal and distal areas contained many astrocytes and few macrophages. In the proximal site, growth cones observed at this stage were seen in close proximity to both astrocytes and phagocytes, while in the crush site area, which contained only few astrocytes and many phagocytes, almost no growth cones could be observed. We, therefore, suggest that disappearance of astrocytes from the crush site and the arrest of abortive axonal growth may be interconnected.

378.20

CULTURED SCHWANN CELLS WILL MYELINATE CNS AXONS WHEN IMPLANTED INTO THE BRAINS OF ADULT RATS. C.T. Montgomery* and J.A. Robson. Department of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210.

Schwann cells play an important role in axonal regeneration in the PNS and they can support axonal growth in the CNS of adult animals. The present study examines the morphological relationships between CNS axons and implanted Schwann cells. Cultured Schwann cells were transferred to tubes made of polycarbonate film. Each tube was then implanted into the brain of an adult rat in a manner that supports growth of CNS axons (J. Neurosci. Meth. 32:135-141, 1990). Six months later the tubes were fixed and prepared for electron microscopy.

The tubes are filled with tissue including a central, dense structure that resembles a small peripheral nerve. This central "nerve" is surrounded by a perineurial sheath and is filled with myelin figures. The perineurial cells have a prominent basal lamina and form tight junctions at their appositions. The core of these nerves is divided into small compartments by cells resembling the perineurial cells. Each compartment contains longitudinally oriented bundles of collagen and most contain axons of different sizes. All axons are surrounded by Schwann cells. Some axons are myelinated and others are unmyelinated. Unlike normal peripheral nerves some small axons ($<1\mu m$) are heavily myelinated. (Supported by EY03490 and NATO 0235/87)

378.21

EFFECT OF NEURAL TRANSECTION ON REFLEX RESPONSES IN CRAYFISH. J. Hernández-Falcón, V. Campos-Loza-da*, and B. Fuentes-Pardo. Depto. Fisiología, Fac. Medicina, UNAM. México, D. F. Apdo. Postal 70-250. MEXICO.

The aim of this work was to obtain physiological evidence about neural regeneration mechanisms in crayfish after the transection of the abdominal neural chain. Here, both tail flip reflex and righting reflex (complex fast responses involving a wide group of muscles) were measured before and after the neural cord transection at the 1st, 4th or 6th level of the abdominal chain. Immediately after transection a sustained tail flexion was observed resulting, at the long-term, in uropod destruction. Shortly after transection, the tail flip reflex was diminished or even abolished; 45 days after, a slight recovery could be detected. About 20-30 days after the neural transection, righting reflex latency increased; from this moment on it decreased approaching initial values These results suggest that neural transection results in neural degeneration followed by a physiological recovery involving a neural regeneration.

378.22

RECRUITMENT OF RETICULOSPINAL NEURONS DURING SWIMMING COMMAND MICROSTIMULATION IN LAMPREY. S. Jordan and J. Ayers Marine Science Center, Northeastern Univ., East Point, Nahant, MA 01908

We have developed procedures for the analysis of functional activity of giant Müller reticulospinal neurons (RNs) to examine their recruitment patterns in response to electrical microstimulation of brainstem locomotor centers in normal and recovering spinally transected specimens. Specimens are prepared for *in vivo* recording by implantation of fine wire electrodes in the spinal canal. Spontaneous activity was recorded from specimens in a large tray which were video-taped from above while the RN activity was recorded with a VCR. We perform correlated acquisition of undulatory movement and electrophysiological signals from video media and subject it to image motion analysis and signal processing. 3-D Cluster analysis of the proximal and distal spike amplitudes and inter-electrode latencies of RN action potentials (APs) allows segmentation of the APs of individual RNs, determine their discharge patterns and directly relate them to features of ongoing behavior. Specimens are then prepared for focal brainstem microstimulation An anterior isthmic cluster (PLR) and an anterior medial tract (MLR) are stimulated at varying strengths and patterns using a double barreled glass microelectrode to evoke undulatory behavor. The undulations and RN responses were recorded as above for spontaneous behavior. RN APs are subsequently identified by focal stimulation of their cell bodies in the brainstem and correlated cluster analysis. Our results indicate that: (1) Although evoked swimming closely approximates normal swimming, the evoked RN populations are overlapping subsets of the normal population, (2) Increasingly intense microstimuli have diverse effects on different elements within the subsets. (3) In regenerated cords where recovered RN activity is observed PLR stimulation evokes a similar subset to that observed spontaneously. Supported by NSF Grant DIR-8917532

378.23

CHARACTERIZATION OF AXONAL REGENERATION IN THE ABDOMINAL GANGLION OF *APLYSIA*. J.F.Hamilton and S.M.Fredman. Department of Physiology, Meharry Medical College, Nashville, TN 37208.

The ability of neurons in the abdominal ganglion of *Aplysia* to regenerate their axons following branchial nerve crush was studied in 21 ganglia using retrograde staining and intracellular injection of nickel-lysine. Regeneration, as evidenced by retrogradely stained neurons, was first observed by postlesion day 15. The number of stained neurons in ganglia with crushes increased thereafter until postlesion day 33. The number of stained neurons in the abdominal ganglia of experimental animals was always less than that of controls (66% at postlesion day 56). More axonal regeneration was seen in the hemiganglion ipsilateral to the branchial nerve. In control abdominal ganglia, neurons with somata in the 50-100 μm range comprise the majority (51%) of cells in the ganglia, while the larger cells (200μm-500μm) in the ganglia are only 4%. The regeneration of cells in the 50-100μm range was first noted at post lesion day 15, but the number of cells in this range never returned to control levels (115 ± 2 cells in control v. 73 ± 17 cells at post lesion day 56). While regeneration of 200 μm-500μm cells did not begin until postlesion day 28, the number regenerating approached control levels by post lesion day 42 (Control 9.3 ± 3.5 cells v. 4 ± 1.7 cells at postlesion day 42). Neuron R2 (200-500 μm) was shown by intracellular dye injection and antidromic action potentials to have an axon in the branchial nerve in all control ganglia and in all experimental ganglia allowed to regenerate for greater than 32 days. This study suggests that in *Aplysia*, complete axonal regeneration does not occur in the adult CNS and that there may be intrinsic differences in the ability of neurons to regrow axons. (Supported by NINDS Grant NS28199 to SMF. JFH is a American Psychological Association Fellow).

MEMBRANE COMPOSITION: CELL SURFACE MACROMOLECULES II

379.1

REGULATION OF SYNAPTOSOMAL MEMBRANE CHOLESTEROL. A.M. Rao* and W.G. Wood. VA Medical Center, GRECC, and Dept. of Pharmacology, Univ. of Minnesota, School of Medicine, Minneapolis, MN 55417.

The kinetics of cholesterol transport have been well established in non-neuronal membranes. There are few if any studies on cholesterol transport in neuronal membranes. The purpose of the experiments reported here was to determine the kinetics of cholesterol transport in synaptosomes and how such transport may be regulated. Synaptosomes were isolated from brain of C57BL mice. Cholesterol transport was accomplished using radiolabeled small unilamellar vesicles incubated with synaptosomes. The $t_{1/2}$ of cholesterol exchange was 9 hr between SUV and synaptosomes. The amount of the exchangeable pool of cholesterol was approximately 40% in synaptosomes. Both the $t_{1/2}$ of exchange and the exchangeable pool were altered by phosphatidylserine (PS) and sphingomyelin (SM). PS facilitated exchange and SM slowed exchange and reduced the exchangeable pool. Membrane cholesterol is not uniformally distributed but consists of cholesterol poor and rich domains. Synaptic plasma membrane (SPM) cholesterol has recently been shown to be asymmetrically distributed between the exofacial and cytofacial membrane leaflets. PS a cytofacial phospholipid and SM an exofacial phospholipid may be involved in the asymmetric regulation of SPM cholesterol. Supported in part by the Dept. of V.A. and NIAAA 07292 (WGW).

379.2

PALMITOYLATION OF THE α-SUBUNIT OF Go, A MAJOR NEURAL G PROTEIN. M. Parenti, C. Newman*#, G. Milligan*## and A. I. Magee*#. Dept. Pharmacol., Sch. Med., Univ. Milan, Milan, Italy, #Lab. Eukaryotic Mol. Genet., NIMR, Mill Hill, London and ##Dept. Biochem., Univ. Glasgow, Glasgow, U.K.

Go is an heterotrimeric G protein mostly found in neural tissues and presumably involved in transmembrane signalling. In order to perform its function Go must associate with the cytoplasmic face of the plasma membrane. This association may be partly mediated by the βγ-subunits and the amide linkage of myristic acid to the N-terminus of the α-subunit. We now report that Goα is post-translationally modified by the linkage of an additional fatty acid, palmitic acid. Palmitoylation, as well as myristoylation, of Goα were detected in two neural cell lines such as PC 12 and neuroblastoma x glioma NG 108-15 hybrid cells differentiated with dibutyryl cyclicAMP. These cells were metabolically labelled with tritiated fatty acids and the protein was immunoprecipitated with polyclonal antisera raised against a purified brain Gi/Go preparation or specific peptide sequences. The attachment of palmitic acid, via a thioester bond, did not occur on the cysteine residue four amino acids from the C-terminus, since palmitoylation of Goα could be detected even by in vivo pertussis toxin-catalyzed ADP-ribosylation of this site in differentiated NG 108-15 cells.

379.3

FATTY-ACID COMPOSITION OF RAT ASTROCYTES AND NEURONS IN PRIMARY CULTURE: EFFECTS OF EXOGENOUS LINOLEIC ACID. M.G. Murphy and Z. Byczko*. Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia Canada B3H 4H7

We have analyzed the phospholipid fatty-acid compositions of rat-brain astrocytes and neurons that were cultured for 9 days in the absence or presence of exogenous linoleic acid (LA, 35μM). The mol% of the major saturates (16:0, 18:0) and monoenes (16:1, 18:1) were similar in both cell types; the monoene content of both was reduced significantly by exposure to LA. The mol% of the major polyunsaturates (PUFA) were:

	Astrocytes		Neurons	
Fatty acid	-LA	+LA	-LA	+LA
18:2ω6	1.1	7.5	1.3	4.2
20:3ω6	0.3	1.1	0.2	1.0
20:3ω9	0.9	0.2	0.5	0.2
20:4ω6	7.7	13.3	5.9	5.8
22:4ω6	1.4	3.1	2.8	2.9
22:5ω6	1.1	1.2	1.3	1.2
22:5ω3	0.8	1.5	0.5	0.4
22:6ω3	2.9	2.7	6.0	4.1

Whether discrepancies in the metabolism of PUFA in these cells are related to cell-type-specific functions is not known. (Supported by the MRC)

379.4

A MONOCLONAL ANTIBODY THAT RECOGNIZES PHOSPHATIDYLINOSITOL-LINKED PRESYNAPTIC ACHE S. Wright and P.D. Kushner ALS Research Foundation, Pacific Presbyterian Medical Center, San Francisco, CA 94115

Tor 23 is a monoclonal antibody derived from the cholinergic synaptosome preparation of the *Torpedo* ray that has been shown to delineate the external surface of a precise subset of neurons in the rat CNS. This subset has both motor system and cholinergic overlaps. Previously we reported that *Tor* 23 recognized *Torpedo* presynaptic acetylcholinesterase (AChE). With other AChE antibodies, we confirm the identification of the *Tor* 23 antigen in the *Torpedo* as AChE. Furthermore, phosphatidylinositol specific phospholipase-C treatment of the membrane liberates the AChE identified by *Tor* 23. The epitope of the AChE molecule identified by *Tor* 23 is not sensitive to lipase treatment, lipid extraction, or treatments with endoglycosidases F or H; the epitope is sensitive to trypsin, N-glycanase, and Triton X-100. These results indicate that *Tor* 23 identifies a specific structural conformation of the presynaptic AChE molecule and suggests that this conformation plays a significant role in determining the uniqueness of this form.

379.5

SELECTIVE ACETYLCHOLINESTERASE mRNA ACCUMULATION AT THE AVIAN NEUROMUSCULAR JUNCTION; RELATIONSHIP TO NUCLEAR DOMAINS. R.K. Lee*, B.J. Jasmin, and R.L. Rotundo. Dept. of Cell Biol. & Anatomy, U. of Miami School of Medicine, Miami, FL 33101.

Acetylcholinesterase (AChE) exists as a family of oligomeric forms encoded by a single gene. In skeletal muscle fibers the asymmetric form is concentrated at the neuromuscular junction. The molecular mechanisms underlying such selective accumulation of AChE molecules are unknown, but may include transcriptional as well as translational and post-translational mechanisms. To determine the relative abundance of AChE mRNA in junctional versus extrajunctional regions of quail PLD muscle fibers we developed a sensitive polymerase chain reaction-based (PCR) assay using primers designed to specifically amplify the asymmetric AChE form transcript. The 5' (AC1) and 3' (AC3) primers are located in exon 1 of the common coding region and exon 3a encoding the asymmetric carboxyl terminus, respectively, and amplify a 352 nt target region confirmed by restriction enzyme mapping. Neuromuscular junctions, identified by AChE histochemistry, and extrajunctional regions were microdissected, the mRNA reverse transcribed and amplified, and the products analyzed by gel electrophoresis. Results consistently show higher levels of AChE mRNA in innervated regions thus suggesting compartmentalization of AChE gene expression. These data support the hypothesis that transcription of genes encoding synaptic proteins is spatially regulated and confined to nuclei within the subsynaptic sarcoplasm. Supported by grants from the NIH and MDA to R.L.R..

379.6

PRODUCTION OF DIACYLGLYCEROL BY EXOGENOUS PHOSPHOLIPASE C STIMULATES CTP:PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE ACTIVITY AND PHOSPHATIDYLCHOLINE SYNTHESIS IN HUMAN NEUROBLASTOMA CELLS. B.E. Slack, J. Breu* and R.J. Wurtman, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge MA 02139.

The involvement of endogenous diacylglycerol (DAG) production in the stimulation of phosphatidylcholine (PtdCho) synthesis by exogenous phospholipase C (PLC) was examined in LA-N-2 neuroblastoma cells. PLC treatment (0.1 U/ml) of intact cells stimulated CTP:phosphocholine cytidylyltransferase activity significantly more effectively than did maximal concentrations (1 mM) of sn-1,2-dioctanoylglycerol (diC_8). The effects of PLC and oleic acid were additive, but those of PLC and diC_8 were not, suggesting that diC_8 and PLC might act via a common mechanism. The time course of activation of cytidylyltransferase by PLC paralleled that of [^{3}H]DAG production in cells prelabeled with [^{3}H]oleic acid. DAG mass was similarly increased. Significant elevations in [^{3}H]oleic acid were not detected until time points after those at which cytidylyltransferase was maximally activated. No significant reduction in total or [^{3}H]PtdCho was elicited by 0.1 U/ml PLC; but higher concentrations (0.5 U/ml) significantly reduced total PtdCho content. The stimulation of cytidylyltransferase activity by PLC or by diC_8 was associated with enhanced incorporation of [methyl-^{14}C]choline into PtdCho. The effect of diC_8 was largely due to increased formation of [^{14}C]diC_8PtdCho. The results indicate that the generation of DAG by exogenous PLC activates cytidylyltransferase activity in neuronal cells under conditions in which membrane PtdCho content is not measureably reduced.

379.7

Electrical Stimulation of Rat Striatum Decreases the Phospholipid Levels and the Levels of Choline Containing Metabolites. S.A. Farber, & R. J. Wurtman. Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

Cholinergic neurons are especially vulnerable in certain neurologic disorders, including Alzheimer's disease (AD). They also have the unique property of utilizing choline (Ch) for two purposes, i.e. synthesis of both the neurotransmitter acetylcholine (ACh) and membrane phospholipids (PL). We previously showed that prolonged stimulation of rat brain slices rich in cholinergic neurons was associated with sustained ACH release, but caused a progressive decrease in membrane PL. Present studies were designed to determine whether the reduction in PL (which can be prevented by adding sufficient Ch to the medium) reflected decreased synthesis or accelerated degradation. We observed that electrical stimulation, in the absence of choline, decreased levels of phosphocholine (PCh) by 19% (n=4, $p<.01$), and levels of ^{14}C-PCh in tissue by 27% (n=3,$p<.01$). Tissue levels of the PC metabolite glycerophosphocholine (GPCh) declined by 42% (n=4, $p<.01$). This could reflect slowed degradation of PL or, more likely, accelerated harvesting of Ch from GPCh. Prolonged stimulation of the tissue also caused depletion of ACh which varied with stimulation time and current (at 75 mA there was a 44 % reduction, n=4, $p<.05$). Supported by NIMH grant NH-28783.

379.8

CHANGES OF RAT STRIATAL MEMBRANE MORPHOLOGY AND STEROID CONTENT DURING THE ESTROUS CYCLE. M. Morissette[1,2], L.M. Garcia-Segura [3], A. Bélanger[2*], and T.Di Paolo[1]. [1]Dept. Mol. Endocrinol., CHUL Res. Centre, G1V 4G2 and [2]Sch. Pharm., Laval Uni., G1K 7P4, Québec. Canada, [3]Instituto Cajal, C.S.I.C., Dr Arce 37, 28002, Madrid, Spain.

The membrane effects of sexual steroid hormones on the rat striatum during the estrous cycle was evaluated by freeze-fracture methodology. In addition, the steroid content was simultaneously investigated in the serum, striatum and in the rest of the brain. As previously described, serum 17ß-estradiol (E2) concentrations peak in proestrus while progesterone (PROG) was high in diestrus and proestrus PM (PPM). Interestingly, we found a similar E2 and PROG pattern in the striatum and the rest of the brain. E2 and PROG concentrations in the striatum and the rest of the brain were significantly correlated with serum levels. In a freeze-fracture study, a significant difference in the content of intramembrane protein particles (IMP) in dendritic shafts was found during the estrous cycle. The numerical density of large (>10nm) IMPs was increased in diestrus I and II and in PPM compared to estrus, proestrus AM and ovariectomized (OVX) rats. In contrast, the numerical density of small (<10nm) IMPs was decreased in cycling animals compared to OVX rats and showed a fall in PPM and then a progressive increase in the following days to reach a peak in proestrus AM. None of the above mentioned changes were observed in the membranes of dendritic spines. We observed, for the first time, that both, striatum E2 and PROG levels, fluctuated during the estrous cycle with a pattern similar to serum levels and this was associated with changes of IMP number of striatal plasma membrane suggesting that these steroids could play a direct action in this brain structure. Supported by the MRC of Canada and DGICYT of Spain.

379.9

CALCIUM AFFECTS EXOGENOUS GANGLIOSIDE BINDING TO CORTICAL SLICES UNDER RESTING AND STIMULATING CONDITIONS. L. R. Wolf* and L. N. Irwin, Dept. Biol., Simmons Col., Boston, MA 02115, and Dept. Biol. Sci., Univ. of Texas at El Paso, El Paso, TX 79668.

Gangliosides and calcium ions bind reversibly under physiological conditions. The possibility that this interaction could have functional consequences was studied by measuring ganglioside turnover in cortical slices of brain tissue perfused with varying concentrations of calcium and potassium. Mildly depolarizing levels of K+, or a reduction of [Ca++] in the perfusing solution, increased release of exogenously incorporated gangliosides. In cortical slices from chronically hypocalcemic rats, more exogenous gangliosides were bound under low [Ca++] than under normal [Ca++] perfusion. We speculate that brain cells adapt to chronic hypocalcemia by altering the nature of calcium-ganglioside interactions in the plasma membrane. These results provide direct support for the historical notion of a functional interaction between gangliosides and calcium ions related to the excitatory state of brain cells. [Supported by NSF #BNS8819801 to L.I.]

379.10

STUDIES ON SIALYLTRANSFERASE ACTIVITY (SAT-1) IN DEVELOPING MUSCLE AND BRAIN. *L.D. Cambron and K.C. Leskawa*. **Dept. Anatomical Sciences & Neurobiology, University of Louisville, Louisville, KY 40292**

Others have suggested that cell surface glycosyltransferases may play a role in development of brain, and this has been demonstrated in studies of cell interactions in nonneural systems. We have recently found that addition of UDP-dialdehyde also blocks myoblast membrane fusion, implicating a role for surface glycosyltransferases in myogenesis. Upon direct assay of glycosyltransferases in myoblasts exposed to UDP-dialdehyde, several activities were found to be inhibited, including SAT-1. This was curious because the normal donor for this reaction is a CMP-sugar and not a UDP-sugar.

To pursue this we analyzed SAT-1 activity, which catalyzes the formation of ganglioside GM3 from lactosylceramide and CMP-sialic acid, using microsomal preparations from homogenates of 14 day chick embryos. Various compounds were examined for inhibitory actions (0 to 1000 μM). It was found that both UDP-dialdehyde and CMP-dialdehyde inhibited SAT-1 activity, with CMP-dialdehyde being only slightly more effective at low concentrations (50 and 100 μM). A similar situation was observed with the addition of nucleotides alone (CMP and UDP), each resulting in approximately 50% inhibition with CMP being only slightly more effective. Addition of UDP-Gal and UDP-GalNAc also resulted in approximately 25% inhibition of SAT-1 activity.

Taken together, these results suggest: (1) a direct role for membrane GSLs in myoblast membrane fusion; (2) the involvement of surface glycosyltransferases, including SAT-1; and (3) that the active site in SAT-1 may not be very selective for the nucleotide portion of the donor nucleotide sugar. Supported by NIH grant NS21057

379.11

DIFFERENTIAL DISTRIBUTION OF mRNA for Na, K-ATPase SUBUNIT ISOFORMS IN THE RAT CEREBELLUM. W.R. Anderson* and W.L. Stahl. VA Medical Center and Univ. of Washington Sch. Med., Seattle, WA 98108

The Na, K-ATPase is an integral membrane protein composed of α and β subunits, with multiple isoforms of each subunit. Utilizing in situ hybridization techniques we have attempted to identify the cellular distribution of subunit mRNA within neural tissue. With synthetic oligonucleotide probes (Filuk et al., Neurosci. Res. Comm. 5: 155-162, 1989) directed against mRNA for the α1, α2, α3, β1, and β2 subunit isoforms for the Na, K-ATPase we have examined adult rat cerebellum. Our results from brain sections show differences in distribution between subunit isoforms. In cerebellum labelling with all probes was restricted to neuronal cell layers with none seen in fiber tracts. The α1 probe labelled more heavily in the cerebellum than elsewhere in the brain and was heaviest in the granule cell layer. The α2 probe labelled at comparable levels in the cerebellum and throughout the rest of the brain. In the cerebellum the most intense labelling was associated with the Purkinje cell layer. The α3 probe showed a pattern of distribution similar to that of the α2 probe. The β1 probe showed high levels of labelling in the cerebellum that were roughly similar to those in the rest of the brain. The highest levels were also associated with the Purkinje cell layer. The β2 probe showed much higher levels of labelling in the cerebellum than elsewhere in brain. Labelling was low in the Purkinje cell layer. The most striking finding is the high level of labelling of α1 and β2 mRNA in cerebellum compared to other brain structures. (Supported in part by the VA and NIH.)

379.12

UBIQUITOUS CELLULAR AND REGIONAL DISTRIBUTION OF (NA,K)-ATPase α2 ISOFORM mRNA IN THE RAT NERVOUS SYSTEM. D.J.Fink, V.Hieber, G.Siegel and M.Mata. Department of Neurology, University of Michigan, and GRECC VAMC, Ann Arbor, MI 48105

We have used an RNA probe directed against the unique 3′ untranslated region of (Na,K)-ATPase α2 cDNA to study the localization and expression of the mRNA for this isoform of the catalytic subunit of (Na,K)-ATPase at the cellular level in the nervous system of the rat.

Within the brain, α2 mRNA was found diffusely in all brain regions. Unlike mRNA for α1 and α3 isoforms, α2 mRNA was abundant generally in glia as well as neurons, and was found in the meninges. In the cerebellum α2 mRNA was found in nearly all cell types including Bergmann glia. Label was sparse, however, in Purkinje cells.

In the spinal cord α2 mRNA was found in all cells including neurons, astrocytes of the gray matter, and oligodendroglial cells of the long tracts of spinal cord. In the dorsal root ganglia (DRG) both neurons and satellite cells contained α2 mRNA, and in the peripheral nerve Schwann cells contained α2 mRNA.

These results demonstrate a nearly ubiquitous regional and cellular distribution for the α2 isoform mRNA in the nervous system, and suggest that α2 may be the principal glial and Schwann cell isoform of (Na,K)-ATPase. In addition, these results demonstrate α2 mRNA to be present in variable amounts in most neuronal cell types.

379.13

PERIPHERAL BENZODIAZEPINE RECEPTOR LIGANDS ACTIVATE DNA POLYMERASE γ ACTIVITY OF CULTURED GLIOMA CELLS.
T. Shiraishi, K. L. Black and D. P. Becker. Division of Neurosurgery and Brain Research Institute, Univ. of California, Los Angeles, CA 90024.

We have previously reported that ligands for peripheral benzodiazepine receptor increase the number of mitochondria and dividing mitochondria of cultured glioma cells. To further elucidate the effect on mitochondrial proliferation, DNA polymerase α (replicates nuclear DNA), β (repairs nuclear DNA) and γ (replicates mitochondrial DNA) activities were determined.

C6 rat glioma cells were incubated in medium with 10% calf serum (S+ group) or serum free medium alone (S- group), or serum free medium plus 10 nM of either PK11195 or Ro5-4864 (both peripheral type ligands; PBL group), or clonazepam (central type ligand; CBL group) for from 3 to 72 hr. The homogenates from crude cell extracts were incubated with the polymerase α,β and γ assay mixture and radioactivity was counted in liquid scintillation counter.

In S+ and PBL group, polymerase α activity increased within 24 hr to approximately 220 and 20% of initial activity, respectively. Polymerase β activity remained nearly constant in each group. In PBL group, polymerase γ activity increased to reach a maximum (approximately five folds of the initial value) after 3 and 6 hours and then decreased again, while in other group no major changes with time were observed.

These findings suggested that peripheral benzodiazepine receptor might activate DNA polymerase γ activity and be involved in mitochondrial DNA duplication.

379.14

Expression of a novel T-cell molecule, 4-1BB, in the brain. B. S. Kwon,* C. F. Pu,* Z. Zhou,* K. Kim,* Y-J. Kim,* and F. C. Zhou. Department of Anatomy and Department of Microbiology and Immunology, and Walther Oncology Center, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, IN 46202.

The cDNA clone 4-1BB was isolated from T-lymphocytes by a modified differential screening procedure. The potential 4-1BB protein (4-1BBP) contains features seen in known receptor proteins and a region of amino acids similar to those in the nerve growth factor receptor, Drosophila gene *sina*, and Dictyostelium gene DG17. We have obtained polyclonal antibodies against a hydrophilic region of 4-1BBP and examined its distribution in the brain. The specific expression pattern of 4-1BBP in the brain was identified in the gray matter where neuronal cell bodies, dendrites, and fiber terminals reside but was almost entirely absent in the white matter where axonal fibers dwell. A peculiar rosette pattern was observed in a granular layer of cerebellum and to be scattered in the stria terminalis. The staining pattern strongly resembled the receptor/nerve terminals in the brain and in the peripheral nervous system. This study suggests that the 4-1BBP is a novel receptor which may be associated with brain function and is another example of a cell surface molecule which is found in both the immune and nervous systems.

379.15

IDENTIFICATION AND LOCALIZATION OF A NEW MEMBRANE TYROSINE KINASE RECEPTOR IN THE AVIAN NERVOUS SYSTEM: E. B. Pasquale*Φ, T. J. Deerinck*ψ, S. J. Singer*Σ and M. H. Ellisman ψ. Departments of Neurosciences Ψ, and Biology Σ, University of Calif., San Diego; La Jolla Cancer Research Foundation Φ, La Jolla, Calif.

It is now clear that protein tyrosine kinases play very crucial roles in the cascade of events involved in signal transduction during embryonic development. However, tyrosine kinases are also expressed at high levels in non-proliferating cells, such as neurons and platelets. Among adult tissues, the brain contains the highest levels of protein tyrosine kinase activity, comparable to those found in embryonic tissues. By screening a chicken embryo expression library with antibodies to phosphotyrosine a novel membrane-bound tyrosine kinase receptor of the Eph family (Hirai, et al., Science 238:1717, 1987), Cek5, was identified and its c-DNA sequenced (E.B.P., to be published). Cek5 is developmentally regulated and predominantly expressed in chicken embryonic and adult brain. The abundant expression of Cek 5 in embryonic tissues and in the adult CNS strongly suggest that its expression is important for the function of many cell types. In order to determine the precise cellular distribution of Cek5 in the CNS specific antibodies were made to a synthetic peptide and a Cek5-β galactosidase fusion protein. Although specific immunoreactivity was detected in many regions of the 1 day postnatal and 17 day embryonic chicken CNS, the highest concentration of Cek5 was found in the molecular layer of the cerebellum. High resolution light and electron microscopic studies demonstrate this immunoreactivity to be associated with the axons of mature granule cells, (parallel fibers) and with the cell bodies of immature granule cells (before migration to the granular layer). Some immunoreactivity is also observed in post-migratory granule cells at embryonic day 17, but not at postnatal day 1. Although the molecular layer of the brain is particularly rich in synapses, Cek5 appears concentrated in the non-synaptic portions of the parallel fiber axons. This distribution of Cek5 suggests a role for tyrosine phosphorylation either in the regulation of the function of parallel fibers or in their associations with neighboring cells.

379.16

EXPRESSION OF TWO GLUCOSE TRANSPORTER ISOFORMS, GLUT1 AND GLUT3, IN CULTURED CEREBELLAR NEURONS. F.Maher, T.Davies* and I.A.Simpson*. NIDDK, NIH, Bethesda, MD 20892.

Expression of different isoforms of the facilitative glucose transporter was investigated in primary cultures of rat cerebellar granule neurons. During neuron development from day 2 to day 8 in vitro uptake of ^{3}H-2-deoxyglucose increased from 250±67 to 910±99 pmol/min/10^6 cells. Expression of mRNAs for GLUT1 (2.8kb) and GLUT3 (4kb) was detected by Northern blot analysis. Both transcripts increased to peak expression at day 4, followed by a decline. Western blotting showed that expression of the glucose transporter isoforms GLUT1 (45kD) and GLUT3 (45kD) increased by 10-15-fold from day 2 to day 8. Transporter isoforms GLUT2 and GLUT4 were not expressed in neurons. The concentration of glucose in the medium did not markedly alter the rate of 2-deoxyglucose uptake or the levels of GLUT1 or GLUT3. Immunofluorescence staining showed localization of GLUT3 at the neuronal plasma membrane whereas GLUT1 appeared both at the plasma membrane and in the cytoplasm. GFAP$^+$-astrocytes were GLUT3-negative and GLUT1-positive in extended 16 day cultures. In this neuron culture system GLUT3 appears to be more abundant than GLUT1 suggesting a predominant role for the GLUT3 isoform in neuronal glucose transport.

379.17

MEMBRANE AND CYTOSKELETAL LOCALIZATION OF DYSTROPHIN IN CULTURED RAT MYOTUBES. G. M. Dmytrenko, D. W. Pumplin* and R. J. Bloch*. Departments of Neurology, Anatomy and Physiology, University of Maryland School of Medicine, Baltimore 21201

Dystrophin's location and its interactions with the cytoskeleton and cell membrane were examined in primary cultures of neonatal rat myotubes. Fixed and permeabilized intact myotubes displayed specific anti-dystrophin labeling along the sarcolemma. Substrate-associated membrane (SAM) containing acetylcholine receptor (AChR) clusters was isolated from myotubes by detergent extraction or physical shear. Immunofluorescence microscopy demonstrated that anti-dystrophin label in SAM devoid of AChR was diffusely punctate. In AChR clusters, dystrophin label was present within AChR rich domains to a variable extent, but was usually absent from adjacent AChR-poor domains. At the ultrastructural level, immunogold labeling of sheared myotubes demonstrated that dystrophin was present in a meshwork closely applied to the inner surface of the cell membrane of AChR domains. Overlying actin filaments that attached to this meshwork were not labeled. When intact myotubes were exposed to media containing inhibitors of energy metabolism or lacking calcium, AChR cluster organization was disrupted and dystrophin label appeared diffusely punctate throughout myotube SAM. Exposing SAM to buffers of low ionic strength or high pH also disrupted AChR organization, but did not remove dystrophin label. Chymotrypsin treatment of SAM removed dystrophin label completely. We compared these results with the extraction profiles for other cytoskeletal components of AChR clusters, namely actin, *B*-spectrin, and the 58 K and 43 K proteins. Dystrophin was more resistant to extraction than any of these cytoskeletal elements. Thus, dystrophin is spatially related to the cytoskeletal network associated with AChR organization and may interact with this network. However, dystrophin is tightly bound to the cell membrane by mechanisms independent of the other proteins known to be within this network.

Supported by an NIH grant to GMD, and NIH and MDA grants to DWP and RJB.

379.18

REGULATION OF SARCOLEMMAL DYSTROPHIN IN THE CARDIAC AND SKELETAL MUSCLES OF CHF-146 STRAIN DYSTROPHIC HAMSTERS (DH) BY DILTIAZEM THERAPY. *S.K. Bhattacharya, T.A. Adamec*, P.L. Johnson, D.L. Lovett*, R.K. Handa, and M.P. Gupta*. Surgical Research Lab, University of Tennessee, Memphis, TN 38163.

Membrane-mediated chronic cellular degeneration plays a fundamental pathogenetic role in hereditary muscular dystrophy (HMD). We have shown that Diltiazem (DTZM) (80 mg/kg BW/d, ip.) reduced membrane-mediated necrosis and histopathology in the heart (HT) and rectus femoris (RF) muscle of DH (*J. Neurol. Sci.*, 8:238, 1990). Dystrophin (DYST), a sarcolemmal bound protein (400 kD), is shown to be missing in Duchenne muscular dystrophy (DMD) and Becker's MD (BMD), mdx-mice, xmd-dogs (*Nature*, 333:861, 1988), and in DH (*FASEB*, 5(5):A1053, 1991). However, the specificity of DYST and its association with HMD remains ill-defined. DYST anomalies have not been found in other dystrophic mice and chickens, and in most forms of human muscular dystrophies, except in DMD and BMD. Using immunohistochemical techniques and NCL-DYS1/DYS2 monoclonal antibodies from Novocastra Lab (Newcastle upon Tyne, UK), we studied DYST regulation in DH and normal hamsters by chronic ip. DTZM therapy. *In situ* frozen HT, RF, and gastrocnemius (GN) biopsies were used. **We conclude that** DYST staining is significantly reduced and discontinuous in the cardiac and skeletal muscles of DH, although less severely than seen in patients with DMD or BMD. Concomitant with numerous salutary effects, improved morphology and longevity, ip. DTZM therapy also enhances DYST staining in the myocardium, rectus femoris, and gastrocnemius muscles of DH. (Supported by NIH Grant #AR-38540)

379.19

A DEFECTIVE HSV VECTOR TARGETS A LYMPHOCYTE PROTEIN TO NEURONAL AND GLIAL PLASMA MEMBRANES. R.J. Wyborski[1], P.D. Olivo*[2], J.M. Nerbonne[3], S. Gallamore*[1], D. Gottlieb[1], Departments of [1]Anatomy & Neurobiology, [2]Internal Medicine, [3]Molecular Biology & Pharmacology, Washington University, St. Louis MO 63110

The plasma membrane plays a fundamental role in the cell biology of neurons and glia. By expressing membrane proteins coded by cloned DNA the composition and function of the plasma membrane can be manipulated to gain greater understanding of the roles of proteins in physiological function. We have used a defective HSV vector to target the lymphocyte cell surface protein CD8 to the surface of cultured cortical neurons and glial cells. A defective Herpes was constructed in which a cDNA coding for the entire length of CD8 was inserted downstream of the Herpes alpha 4 promoter. Stocks of the defective virus were prepared using the ICP6 delta helper virus. Cultures of dispersed rat visual cortical neurons grown on astrocytes were infected with vector. After infection (24-48 hrs) living cells were stained with a monoclonal antibody to CD8. Stained cells were detected in cultures infected with the vector but not in uninfected cells or cells infected with HSV alone. Glial cells were intensely stained throughout the plasma membrane. Neurons were intensely stained with both somata and processes carrying the antigen. These results demonstrate that neurons and glia can synthesize the lymphocyte protein CD8 and that they have the necessary molecular machinery to transport it to the cell surface. They also show that it is possible to identify living neurons and glia expressing genes introduced by a herpes vector by including a membrane protein marker in the vector.

LONG-TERM POTENTIATION: PHYSIOLOGY AND PHARMACOLOGY II

380.1

INPUT SPECIFIC INDUCTION AND PRECLUSION OF NMDA RECEPTOR INDEPENDENT LTP IN AREA CA1 OF RAT HIPPOCAMPUS. L. Grover & T. Teyler. Neurobiol. Dept., N.E. Ohio Univ. Col. of Med., Rootstown, OH 44272.

A frequency dependent, NMDA receptor independent component of LTP (non-$NMDA_R$ LTP) can be induced in area CA1 (Grover & Teyler, Nature, 1990). It seemed possible that non-$NMDA_R$ LTP might differ in some characteristics from NMDA receptor dependent LTP ($NMDA_R$ LTP). We therefore asked if non-$NMDA_R$ LTP would show input specific induction, as previously shown for $NMDA_R$ LTP. In the presence of APV (to block $NMDA_R$ LTP) we stimulated 2 independent afferent inputs. Tetanization of one input induced non-$NMDA_R$ LTP only in that input, with the second input showing no LTP. However, non-$NMDA_R$ LTP induction in one input precluded non-$NMDA_R$ LTP when the second input was subsequently tetanized, but did not preclude $NMDA_R$ LTP (induced after APV washout). Preclusion of non-$NMDA_R$ LTP, but not $NMDA_R$ LTP, may reflect differences in spatial distribution of post-synaptic Ca^{2+} influx. Supported by NIH grant NS28698.

380.2

VISUAL CORTICAL SYNAPTIC PLASTICITY IN THE KITTEN: A STUDY USING ANTAGONISTS IN VITRO. T.P. Hicks and K.-I. Ito*. Dept. of Psychology, UNCG, Greensboro, N.C. 27412-5001.

In order better to understand the synaptic mechanisms at work underlying long-term changes (LTE, LTD) in synaptic transmission in neocortex, we recorded responses from layers II/III before and after tetanic stimulation of the white matter in tissue slices from young kittens and assessed the effects of the amino acid antagonists, AP5, DNQX, BMI, 2-OH-Sac, D-Ser and 7-Cl-Kyn on different components of these evoked potentials. A survey of stimulation parameters in our system showed 10 Hz, 2 min to be optimal for generating LTE. Evoked responses were separated by computer calculation into antidromic and synaptic components, following data collection during perfusion with Ca^{2+}-free media. The data show complex and strikingly different effects of each of these selective antagonists on different components of the synaptic responses. All blockers interfered to greater or lesser extents with some aspect of the responses; the data with drugs acting on the glycine site show that D-serine enhances, but 7-Cl-Kyn doesn't suppress, low-frequency synaptic transmission - one of several basic observations at variance with what is seen in hipocampus.

380.3

INDUCTION OF LONG-TERM POTENTIATION (LTP) WITHOUT NMDA RECEPTOR INVOLVEMENT IN VISUAL CORTEX OF JUVENILE AND ADULT RATS. V.A.Aroniadou, L.M.Grover, and T.J.Teyler. Neurobio. Dept. NE Ohio Col. Med., Rootstown, OH 44272.

In slices of rat visual cortex, white matter stimulation elicited a two-component field potential in layer III, with peak latencies 5-8ms (EPSP1) and 12-19ms (EPSP2). AP5 applied to the bath or at the recording site, caused a reduction/blockade (reversible) of EPSP2. EPSP2 may be generated polysynaptically via an input arising from infragranular layers. Bath applied DNQX revealed a DNQX-resistant/AP5-blocked component (EPSP1b) with onset latency of EPSP1, and peak at 10-23ms. Compared to juveniles, adults showed reduced sensitivity of EPSP2 to AP5 and lower relative amplitude of EPSP1b. In juvenile cortex tetanic stimulation of the white matter in normal medium induced LTP of EPSP1 (173-449%). In AP5, LTP of EPSP1 (136-200%) was induced only when EPSP2 was not blocked by AP5 (37% of slices); no change or depression (LTD) was observed in the remaining slices. In adult cortex, LTP of EPSP1 was induced following tetanus either in normal medium (139-208%), or in AP5 (137-270%). When maximally potentiated, under any of the above conditions, EPSP1 was unaffected by bath applied AP5. In some slices EPSP2 also expressed LTP. Following tetanus in DNQX, the isolated NMDA receptor-mediated component (EPSP1b) expressed LTP or LTD; the direction of change appeared to relate to both age and stimulus intensity. In visual cortex of either juvenile or adult rats LTP can be induced without reduction of inhibition or participation of NMDA receptors. These results suggest that reduced expression of NMDA receptor activity in adult neocortex may be accompanied by enhanced efficiency of other LTP inducing mechanisms. Supported by NS28698.

380.4

AN NMDA ANTAGONIST, CPP, BLOCKS ASSOCIATIVE AND NON-ASSOCIATIVE LONG-TERM DEPRESSION IN THE DENTATE GYRUS. B. R. CHRISTIE AND W. C. ABRAHAM, Department of Psychology and the Neuroscience Research Centre, University of Otago, Dunedin, New Zealand.

The present study evaluated associative and non-associative synaptic interactions between the medial (MPP) and lateral (LPP) perforant path projections to the ipsilateral dentate gyrus (DG) in barbiturate anaesthetized rats. Associative interactions were investigated using 5 Hz bursts of short 100 Hz trains delivered to one path in association with single pulses, administered to the other path, spaced equidistantly between the bursts. Non-associative interactions were studied using the conditioning trains or the single pulses alone. Data are presented as the mean ± SEM percent change from baseline at 30 minutes post-tetanus. Application of the single pulses as a 5 Hz train produced a homosynaptic EPSP increase (LTP)when delivered to either pathway (MPP:9.9±3.3%; LPP:8.7±3.8%), but did not induce heterosynaptic depression (LTD). In the non-associative paradigm, MPP tetanization produced LTP of the MPP responses (26.1±4.8%) and LTD in the LPP (-15.9±4.2%), while LPP tetanization produced LTP in the LPP (35.2±14.9%) and to a lesser extent, LTD in the MPP (-3.9±3.4%).The associative paradigm also produced LTP of the conditioned pathway (MPP:18.5±2.9%; LPP:47.8±6.6%) and similar LTD to that seen in the non-associative condition (LPP:-18.4±4.2%; MPP:-4.7±8.6%). No significant differences were found between the effects produced by the associative and the non-associative paradigms, and both were found to produce equivalent depression in previously tetanized pathways (Associative: MPP:-16.5±6.7%; LPP:-23.3±4.7%; Non-Associative: MPP:-15.7±6.8%; LPP:-17.4±4.5%). Both associative and non-associative LTP and LTD were blocked by the competitive NMDA antagonist CPP (10 mg/kg; administered i.p. 2hrs prior to tetanization). These data suggest that associatively and non-associatively induced synaptic depression in the dentate gyrus involve similar mechanisms, including NMDA receptor activation.

380.5

AN INCREASE IN THE AFFINITY OF THE AMPA RECEPTOR FOR AGONISTS IS ASSOCIATED WITH INCREASED SYNAPTIC RESPONSES IN CA1 NEURONS. **K. Shahi and M. Baudry, Neuroscience Program, USC, Los Angeles, CA.**

A number of manipulations has been shown to modify the affinity of ligands for the AMPA subtype of glutamate receptors and it has been proposed that the long-term potentiation (LTP) of synaptic transmission observed in the CA1 region of the hippocampus is due to a change in receptor properties. It is therefore of critical importance to determine whether changes in binding properties of agonists for the AMPA receptors are accompanied by modifications of the physiological responses elicited by agonists. It has been shown that the chaotropic ion SCN- produces a large and reversible increase in the affinity of agonists for the AMPA receptors. We determined the effect of iontophoretically applied SCN- on the characteristics of synaptic potentials recorded extracellularly in the dendrites and cell body layer of CA1 in the hippocampal slice preparation. Picrotoxin was included to eliminate possible effects of SCN- on GABA currents. Brief applications of SCN- produced a rapid and reversible increase in the slope and amplitude of EPSPs as well as an increase in population spike amplitude; these effects were absent when NaCl was substituted for NaSCN. Antidromic responses and paired pulse facilitation remained unchanged during SCN- application. These results suggest that an increase in the affinity of the AMPA receptor for glutamate is accompanied by increased physiological responses elicited by glutamate and provides further support for the hypothesis that LTP is due to a change in receptor properties.
(Supported by Grant BNS 96284 from NSF).

380.6

HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) IS ASSOCIATED WITH INCREASED [^{3}H]-AMPA BINDING IN RATS. S. Maren, G. Tocco, T. J. Shors, M. Baudry, and R. F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

The nature of synaptic modifications underlying long-term potentiation (LTP) is a vigorously debated issue; evidence for both presynaptic and postsynaptic modifications has been reported. In the following we report an increase in [^{3}H]-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) binding following the induction of LTP in perforant path-granule cell synapses of anesthetized rats. Male Long-Evans rats were implanted stereotaxically with a bipolar stimulating electrode in the perforant path and a recording electrode in the hilus of the dentate gyrus. Animals received either low-frequency stimulation (200 pulses at 1 Hz; *n*=5), low intensity theta burst stimulation (TBS; 10 40 ms 400 Hz bursts at 5 Hz, intensity sufficient to elicit a 1 mV population spike [PS]; *n*=6), or high intensity TBS (intensity twice that which elicited a maximal PS; *n*=5). One hour following stimulation the rats were sacrificed and their brains rapidly dissected and frozen. Quantitative autoradiography of [^{3}H]-AMPA and [^{3}H]-*N*-{1-[thienyl]cyclohexyl}piperidine (TCP) binding to 10 µm coronal sections was used to examine AMPA and *N*-methyl-D-aspartate (NMDA) receptors, respectively. Bilateral increases in [^{3}H]-AMPA binding were observed in CA_1 oriens and radiatum, CA_3 radiatum, dentate gyrus, hilus, and cerebral cortex in animals exhibiting LTP (low intensity TBS group). Pretreatment of rats with the noncompetitive NMDA antagonist ketamine (30 mg/kg; *n*=3) eliminated both the increases in [^{3}H]-AMPA binding and LTP. No significant changes in [^{3}H]-TCP binding were observed in any treatment group. These results indicate that *in vivo* LTP is associated with a selective modification of the binding properties of AMPA, but not NMDA receptors in the rat hippocampus. Supported by NIH(AG05142, AG05500) and the McKnight Foundation to RFT and NSF(96284) to MB.

380.7

LTP, BUT NOT PRE-SYNAPTIC FACILITATION, CHANGES THE EFFECTS OF A DRUG THAT ENHANCES AMPA RECEPTOR CURRENTS. U. Staubli[1], P. Xiao*, M. Kessler, J. Ambros-Ingerson* and G. Lynch. [1]McGill University, Dept. Psychology, Montreal, QC H3A 1B1, and University of California, Center for the Neurobiology of Learning and Memory, Irvine, CA 92717.

We recently reported that the response enhancing action of the nootropic compound aniracetam is reduced in synapses expressing long-term potentiation (Staubli et al., Psychobiol. 18: 377, 1990). Here we present data from hippocampal slices showing that the percent facilitation produced by aniracetam remains constant in the presence of treatments known to augment response size by increasing transmitter release (i.e., changes in extracellular calcium, 4-aminopyridine and paired pulse facilitation) but is reduced following induction of LTP. Aniracetam displayed a regional variation in its effect: it had a significantly smaller facilitating action in CA3-CA3 synapses than it did in CA3-CA1 or perforant path-dentate gyrus synapses. The drug did not affect NMDA receptor mediated synaptic responses but did cause a small (5-10%) and statistically significant decrease in [^{3}H]AMPA binding. Other nootropics did not produce aniracetam-like effects.

These observations indicate that aniracetam produces a selective facilitation of AMPA receptors which, given its small effect on binding, is probably due to an enhancement of channel conductance. These conclusions are in accord with the original results obtained by Ito et al. (J.Physiol. 424: 533, 1990). The finding that LTP, but not pre-synaptic manipulations that increase the size of field EPSPs, alters the actions of aniracetam strongly suggests that LTP is also due to a receptor change. A mathematical model of synaptic transmission and dendritic currents has been used to identify the types of receptor changes that reproduce the observed interaction between aniracetam and LTP. (Supp. by AFSOR #89-0383 and ONR #N-00014-89-J-3179).

380.8

Long-term potentiation (LTP) induced by metabotropic receptor activation coupled with subthreshold tetanus

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The involvement of metabotropic receptors in LTP was investigated in rat CA1 slices. Bath-application of the metabotropic receptor agonist *trans*-(+-)-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD, 50 µM) caused a transient depression of the field EPSP. However, *trans*-ACPD produced LTP when it was coupled with a weak tetanus (50 Hz, 0.25-0.5 s) (12/15 slices). Weak tetanus itself was subthreshold for LTP, producing only short-term potentiation (STP). Coupling a weak tetanus with -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 5 µM, n=6) or N-methyl-D-aspartate (NMDA, 10 µM, n=4) did not produce LTP. Furthermore, although STP was also induced by combined application of AMPA and NMDA, coapplication of *trans*-ACPD (50 µM) with these agents could not produce LTP. From these results, we conclude that this form of LTP (LTP_M) is induced by activation of metabotropic receptors and coupled weak tetanization.

STP was blocked by D(-)-2-amino-5-phosphonopentanoic acid (AP5, 50 µM). In this condition, LTP_M was not induced (n=4). Similarly, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 µM) blocked LTP_M (n=2). LTP_M was also observed intracellularly without changes in input resistance and spike threshold (n=5).

Metabotropic receptor activation results in release of Ca2+ from the internal store. Our results suggest that the elevation of (Ca2+)i by metabotropic receptor activation is additive to a rise of (Ca2+)i following NMDA receptor activation and induces LTP_M. Whether this is a common feature with conventional, tetanus-induced LTP is under current investigation.

380.9

LONG-LASTING MODULATION OF SYNAPTIC TRANSMISSION BY A SELECTIVE METABOTROPIC GLUTAMATE RECEPTOR AGONIST IN DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS *IN VITRO*.

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We have previously demonstrated (Zheng and Gallagher, Soc. Neurosci. Abstr. 16, 653.) that induction of long-term potentiation in the DLSN was not blocked by the selective NMDA receptor antagonist APV, but is blocked by a putative metabotropic glutamate receptor antagonist L-2-amino-4-phosphonobutyrate (APB).

In the present study, we applied *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), a selective agonist of metabotropic glutamate receptors to DLSN neurons recorded intracellularly from rat forebrain coronal slices. Transient application of ACPD induced a concentration dependent depolarization followed by a hyperpolarization. At higher concentrations, ACPD elicited an oscillation of membrane potentials and burst firing. Superfusion of 20μM (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD), the active enantiomer of ACPD, produced an additional action, namely, it initiated a slow afterdepolarization probably through the unmasking of a calcium-activated non-selective cation current. Superfusion of 1S,3R-ACPD (20-40μM) for a period of 8-10 min resulted in a long-lasting potentiation of synaptic transmission subsequent to washout. EPSP amplitudes were increased to 150% of control following a 30-50 min washout of 1S,3R-ACPD. However, at higher concentrations, 1S,3R-ACPD caused a long-lasting depression of synaptic transmission; EPSP amplitudes were reduced to 60% of control even after a 60 min washout period.

These results demonstrate that activation of metabotropic glutamate receptors can produce a concentration-dependent dual modulatory action upon both the firing pattern and synaptic transmission in rat DLSN neurons. Such actions may play an important role in neuronal plasticity at this synapse.

380.10

EFFECT OF AGING AND METABOTROPIC GLUTAMATE RECEPTOR ANTAGONISTS ON CA1 LTP

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In 30 day old postnatal rats (P30), the metabotropic glutamate receptor antagonist, 2-amino-3-phosphonopropionate (AP3), blocks LTP in the CA1 hippocampal region when administered for 5 min immediately after delivery of the electrical tetanus. Similarly, 1μM NMDA also blocks LTP when administered posttetanically. The NMDA-mediated inhibition is reversed by coapplication of trans-amino-1,3-cyclopentanedicarboxylic acid (ACPD). We have studied developmental aspects of metabotropic receptor involvement in CA1 LTP by examining the effects of AP3, AP4 and NMDA in hippocampal slices prepared from 15, 30, 60 and 90 day old albino rats.

A single 1 sec, 100 Hz tetanus delivered to the Schaffer collateral pathway induces stable LTP ($\geq$ 20% increase in EPSP slope) lasting 60 min or longer in 86% of slices from P15-P30 animals (N=14), compared to 17% of P90 slices (N=6). Additionally, the potentiation produced in P90 slices decreases from 20 to 60 min after the tetanus. While posttetanic application of 1μM NMDA blocked LTP in all slices from P15-P30 animals (N=11), there was no inhibition in slices from P60-P90 animals. Similarly, 100μM AP3 and 100μM AP4 inhibited LTP in P15-P30 slices but were less effective in P60 slices.

These results suggest that aging affects the generation of CA1 LTP, even in slices from relatively young rats. Additionally, antagonists of metabotropic glutamate receptors are more effective blockers of LTP in slices from 15-30 day old postnatal animals.

380.11

TRIAZOLAM AND DIAZEPAM BLOCK LTP IN RAT HIPPOCAMPAL AND PIRIFORM CORTEX SLICES.

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The present experiments tested for possible links between long-term potentiation (LTP) and the amnestic action of benzodiazepines. The effects of diazepam and triazolam, two benzodiazepines with a different pharmacological and clinical properties, were tested on LTP in slices of hippocampus and piriform cortex. Infusion of 50 μM of both drugs blocked LTP but via different mechanisms. Diazepam reduced the increase in burst responses that occurs during the theta pattern stimulation used to induce the potentiation effect; the drug thus appears to interfere with the initial triggering events for LTP. This may reflect the known action of diazepam on GABA mediated inhibitory potentials. Triazolam did not alter burst responses in hippocampus or the development of LTP in piriform cortex; however, the LTP that resulted decayed back to baseline in 15-30 minutes. We suggest that the effects of triazolam on plasticity are mediated by its actions as an antagonist of the platelet activating factor receptor, since non-benzodiazepine blockers of the receptor also prevent the induction of LTP (del Cerro et al, Behav. Neural Biol, 1990, **54**, 213-217). These results provide a plausible explanation for the amnestic effects of benzodiazepines and further support the role of LTP in memory encoding in animals and humans.

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380.12

INDUCTION OF LONG-TERM DEPRESSION UNDER BICUCULLINE AT EC-DG SYNAPSES BY COMMISSURAL CONDITIONING.

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Long-term potentiation (LTP) of EC-DG responses concomitantly induces long-term depression (LTD) of inactive commissural synapses in the dentate gyrus. We find a symmetric effect: activation of the commissural pathway induces LTD of EC-DG responses (Zhang & Levy, in prep.). As reported by many authors using the paired-pulse technique, commissural stimulation can depress the EC-DG responses by a GABAergic mechanism. Therefore, using a bicuculline-containing recording electrode, to block GABAergic inhibition, we examined the effect of conditioning the commissural pathway on the EC-DG responses. The experiment used 15 urethane-anaesthetized albino rats. There was 1 bicuculline-containing recording electrode in the left DG, and there were 3 stimulating electrodes (one in the right anterior DG symmetric with the recording electrode, one in the right posterior DG and one in the left angular bundle). In each of the 15 rats, the first conditioning of the commissural pathway (13 anterior placements, 2 posterior placements) induced LTD of EC-DG responses. In 9 of the 15 animals the initial EC-DG responses showed two or more population spikes (multispikes) in response to a single test activation. Such multispikes indicate the effectiveness of the bicuculline treatment. In each of these 9 rats, activation of the anterior commissural pathway induced LTD of the EC-DG responses. The decreased EC-DG slope (4.51$\pm$1.23%, n=9, $p<0.01$) is the same change seen without bicuculline. In 8 of these 9 rats, there was also LTD of the EC-DG response p-spike amplitude. The results imply that this kind of LTD does not depend on GABA-A synaptic activation.

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380.13

MODULATION OF THE HIPPOCAMPAL COMMISSURAL-CA3 LONG-TERM POTENTIATION BY SEROTONIN

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Synaptic transmission in the hippocampus is modulated by subcortical afferents. This study investigates the modulatory effect of serotonin (5-HT) on long-term potentiation (LTP) induction in the hippocampus. The commissural/associational (C/A) input to CA3 was measured by extracellular field potentials in rat hippocampal slices. The intensity of the high frequency stimulation (HFS) was adjusted to yield population EPSPs of about 1 mV. Following induction of LTP (142 $\pm$ 9% of pre-tetanus pEPSP slopes measured at 1 hour post-tetanus, n=6), perfusion for 30-45 minutes with 1 μM 5-HT had little or no effect on synaptic transmission (96 $\pm$ 3% of the pre-perfusion pEPSP slopes, n=7). HFS in the presence of 5-HT, however, did not produce any further LTP (108 $\pm$ 8%, n=7), whereas HFS performed after prolonged wash-out of 5-HT did induce LTP (123 $\pm$ 12%, n=6). In a second set of experiments $GABA_A$ transmission was blocked by application of 10 μM picrotoxin and 10 μM bicuculline. 5-HT perfusion, following control LTP (166 $\pm$ 13%, n=8), induced a small but significant decrease in synaptic transmission (89 $\pm$ 4%, n=7). HFS in the presence of 5-HT did not induce further LTP (101$\pm$6%, n=7), whereas after prolonged wash-out HFS induced significant potentiation (116 $\pm$ 4%, n=5). In other experiments 5-HT perfusion before inducing LTP caused a small decrease in synaptic transmission (90$\pm$5%, n=5). LTP was blocked by 5-HT (106$\pm$6%, n=5), but LTP was observed after wash-out (119 $\pm$ 3%, n=4). Perfusion with the 5-HT antagonist methysergide (1 μM) did not affect normal synaptic transmission (95$\pm$2%, n=4), but did block the effect of 5-HT on the induction of LTP (139 $\pm$ 12%, n=4). We conclude that the activation of serotonergic receptors blocks the induction of LTP at the C/A-CA3 synapse. (MH44754)

380.14

ANTAGONISTS OF THE PLATELET-ACTIVATING FACTOR (PAF) RECEPTOR BLOCK LONG-TERM POTENTIATION IN HIPPOCAMPAL SLICES.

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Platelet-activating factor (PAF) is a naturally occurring alkyl-ether phospholipid with a variety of biological activities. In particular, PAF regulates calcium mobilization by interacting with a specific receptor and through this mechanism controls shape change and aggregation in platelets and secretion in several cell types. Recently, binding sites for PAF have been identified in synaptosome fractions from brain (Marchesselli *et al.*, J.Biol.Chem. 265:9140, 1990) and PAF was found to increase calcium influx in cultured neuronal cells (Kornecki and Ehrlich, Science 2:1792, 1988). These findings suggest that PAF plays a role in neuronal function.

Long-term potentiation (LTP) involves calcium activated processes and produces changes in synapse morphology. The similarity to the processes controlled by PAF in platelets prompted us to test the effect of antagonists and agonists of the PAF receptor on the formation of LTP in field CA1 of rat hippocampal slices. The antagonist trans-BTD (trans-2,5-bis-(3,4,5-trimethoxyphenyl)-1,3-dioxolane) at concentrations of 8-16 μM blocked LTP while the same concentration of a stereo-isomer (cis-BTD) with low affinity for PAF receptors was without effect. The blockade of LTP by trans-BTD was partially reversed by simultaneous application of the non-metabolizable receptor agonist carbamyl-PAF. Trans-BTD at the concentration sufficient to block LTP did not change paired-pulse facilitation, burst responses during the induction of LTP and NMDA receptor mediated responses. It thus appears that trans-BTD interferes with LTP at some step after induction and initial expression. CV3988, an antagonist structurally related to PAF also attenuated LTP. These results suggest that activation of PAF receptors contributes to the stabilization of LTP, possibly via an effect on intracellular calcium levels.

380.15

PLATELET-ACTIVATING FACTOR AUGMENTS EXCITATORY SYNAPTIC TRANSMISSION IN CULTURED RAT HIPPOCAMPAL NEURONS. G.D. Clark[1], L.T. Happel[2], C.F. Zorumski[3] & N.G. Bazan[4]. [1,2]Depts. of Neurol., [2]Neurosurg., [2]Physiol. [4]Ophthal. & [1,2,4]Neurosci., LSU Med. Ctr., New Orleans, LA 70112. [3]Depts. of Psych., Anat. & Neurobiol., Wash. Univ., St. Louis, MO 63110.

A platelet-activating factor (PAF) antagonist has been shown to block the formation of long-term potentiation (LTP) in the hippocampus. Additionally, PAF binds to distinct brain microsomal and synaptosomal sites. We have tested the hypothesis that PAF increases synaptic release of excitatory transmitter in cultured rat postnatal hippocampal neurons. Perfusion of 1μM methyl carbamyl PAF, a nonhydrolizable PAF, significantly augmented evoked excitatory synaptic currents in a reversible manner. 1μM BN-52021, a ginkolide with antagonist activity at the synaptosomal PAF binding site, blocked this effect of 1μM methyl carbamyl PAF. No effect of PAF was seen on evoked inhibitory currents. Spontaneous miniature excitatory postsynaptic currents markedly increased in frequency, but not amplitude, during perfusion of 1μM methyl carbamyl PAF. These results indicate that PAF probably acts by a presynaptic mechanism and at a specific binding site to augment excitatory synaptic transmission.

380.16

EFFECT OF EXOGENOUS GM1 GANGLIOSIDE ON LTP IN RAT HIPPOCAMPAL SLICES PERFUSED WITH DIFFERENT CONCENTRATIONS OF CALCIUM. H.-M. Hwang, J.-T. Wang*, and T. H. Chiu*. Dept. of Anatomy, Chang Gung Medical College, Taoyuan, Taiwan, R.O.C.

Exogenous application of GM1 ganglioside was found to enhance synaptic transmission in the hippocampus. The present study examined its effect on long-term potentiation (LTP) of rat hippocampal slices perfused with different concentrations of calcium. LTP magnitude was measured as an increase in population spike amplitude, recorded from CA1 pyramidal cells, over the baseline after tetanic stimulation on Schaffer collateral by 3 trains of 100 Hz at an interval of 1 min. In perfusion with 2.5 mM calcium, GM1-treated slices, 50 uM for 1 hr, appeared to be more potentiated than untreated ones. At 5.0 mM calcium, field potentials in a fast decay were recorded. No LTP or only post-tetanic potentiation was induced. GM1 seemed not only to stabilize evoked responses but also to reinstate LTP. At 1.0 mM calcium, LTP could not be induced and retrieved by GM1 treatment. Once calcium was raised to 2.5 mM, LTP could be recovered without further tetanic stimulation. Thus, exogenous GM1 might incorporate into synaptic membrane to participate LTP induction.

380.17

THE NITRIC OXIDE SYNTHASE INHIBITOR *N*ω-NITRO-L-ARGININE REDUCES THE MAGNITUDE OF LONG-TERM POTENTIATION IN THE DENTATE GYRUS BUT NOT IN AREA CA1 OF THE HIPPOCAMPUS *IN VITRO*. M.L. Errington*, Y.-G. Li*, H. Matthies **, J.H. Williams* and T.V.P. Bliss. Division of Neurophysiology and Neuropharmacology, National Institute for Medical Research, Mill Hill, London, NW7 1AA, UK and * Inst. of Pharmacology and Toxicology, Medical Academy, Leipziger Str. 44, 3090 Magdeburg, Germany.

NMDA releases nitric oxide from hippocampal slices (East et al., Neurosci. Lett. 123, 17, 1991), raising the possibility that nitric oxide (NO) may act as an early retrograde messenger in long-term potentiation (LTP). The enzyme NO synthase, which promotes the conversion of L-arginine to NO and citrulline, is differentially distributed in the hippocampus, with higher levels in the dentate gyrus than in the hippocampus proper (Snyder et al., TIPS 12, 125, 1991). We have investigated the effect of the NO synthase inhibitor *N*ω-Nitro-L-arginine (NARG) on the induction of LTP in the dentate gyrus and area CA1 of rat hippocampal slices. Stimulating electrodes (S1 and S2) were placed to activate separate but converging pathways, and the population EPSP and spike recorded with extracellular electrodes. LTP was induced by 3 trains of 4 bursts of 5 pulses at 100 Hz, burst interval 200 msec, train interval 1.5 min. Pathway S1 was tetanized during perfusion with control medium; pathway S2 was tetanized after perfusion had been switched to a medium containing NARG. NARG did not affect the amplitude of evoked potentials in either region at the concentrations used. In area CA1, the magnitude of LTP was not affected by NARG at concentrations of 100μM (n=3) or 200μM (n=6). However, in experiments on dentate minislices (perfused with medium containing 4mM Ca2+, 3.5mM Mg2+ and 100μM picrotoxin) the magnitude of LTP of both population EPSP and population spike was consistently less in the pathway (S2) tetanized in the presence of 50μM NARG (mean percentage increase (±SEM) measured 10-20 min after the tetani to S1 and S2 was 77.2 (16.1) and 13.4 (6.2) respectively for the EPSP (n=9; $p<0.005$, 2-tailed t-test) and 166.0 (38.5) and 57.7 (13.2) for the population spike (n=7, $p<0.05$)). These results suggest that in the dentate gyrus, in contrast to area CA1, NO is necessary for the full expression of LTP.

380.18

LTP AND VACCINIA VIRUS INFECTION OF SELECTED TARGETS IN THE HIPPOCAMPAL SLICE. D.L. Pettit & R. Malinow, Neuroscience Program and Dept. of Physiology & Biophysics, University of Iowa.

LTP is initiated at the postsynaptic membrane in the CA1 region of the hippocampus, but the location of the long-term modifications underlying LTP remains unclear. We are developing a system for introducing biochemical agents into selected pre or postsynaptic targets. Introduction of agents like pseudosubstrate protein kinase inhibitors into presynaptic cells would allow us to test the contribution of kinase activity to these long-term modifications.

We are using a vaccinia virus to infect specific targets in the rat hippocampal slice. This virus carries a gene for beta-galactosidase inserted next to early and late vaccinia promoters (generously provided by Dr. Bernard Moss). The virus is introduced into the extracellular space of the CA3 region by multiple picospritzer injections. Following incubation, the slices were fixed in paraformaldehyde and stained for the presence of beta-galactosidase with X-gal. We see staining, as early as 3 1/2 hours after infection and as late as 8 hours, localized to the injection site.

To test whether slices infected with virus are still capable of LTP we infected the CA3 and CA4 regions of slices and incubated at least 3 1/2 hours. Stimulating electrodes were placed in the CA3-CA4 cell body layer, at the site of injection, and the subiculum, stimulating fibers that would be unlikely to be infected. Recordings were made from CA1 cells with the whole-cell voltage clamp technique. We were able to induce LTP in both pathways for up to 10 hours after infection.

SODIUM CHANNELS: MOLECULAR BIOLOGY

381.1

EXPRESSION AND CHARACTERIZATION OF UNLINKED CHIMERIC SODIUM CHANNELS. M.M. Stephan, J.F. Potts, W. M. James and W.S. Agnew. Dept. of Cell. and Mole. Physiology, Yale Univ. School of Medicine, New Haven, CT 06510.

Sodium channel isoforms vary in their primary structure and pharmacological properties, and may vary in their sensitivity to modulation by second messenger systems. Not all isoforms are readily expressed in frog oocytes; the eel electroplax channel is not functional in this system, while the rat skeletal muscle channel is. Unlinked chimeras have been used to explore these observations. Partial channel constructs encoding only domains I and II or domains III and IV were prepared from μI and eel cDNA's and used to make mRNA (cRNA). Co-injection of eel cRNA encoding domains I and II with μI cRNA encoding domains III and IV gave rise to typical sodium currents. Expression of the inverse combination, μI domains I and II with eel domains III and IV, did not result in detectable sodium current. If failure to express the eel channel is due to interactions with oocyte regulatory enzymes such as protein kinases, our results may localize the site of this effect to domains III and IV. These results demonstrate the feasibility of using interspecies chimeras, linked or unlinked, to localize regions responsible for functional attributes such as sensitivity to toxins, drugs, or neuromodulatory factors.

381.2

FUNCTIONAL EXPRESSION OF THE μI SODIUM CHANNEL IN A TRANSFORMED HUMAN KIDNEY CELL LINE. IDENTIFICATION AND ISOLATION OF BIOSYNTHETIC INTERMEDIATES. C. Ukomadu and W.S. Agnew, Yale University School of medicine, New Haven, CT 06510.

We describe a method for efficient transient expression of the rat skeletal muscle sodium channel, μI in a mammalian cell line; 40-60% of cells express currents of upto 10 nA and are reactive immunocytochemically (Ukomadu et al, *Biophys.J*, 59,69a(1991); Ukomadu et al; submitted). The cells are suitable for biochemical characterization of sodium channel biosynthesis and functional characterization. Pulse chase experiments suggest the presence of at least 3 distinguishable forms of the channel protein enroute to the cell surface. A core peptide of Mr~210 kDa is seen when co-translational N-linked glycosylation is inhibited with tunicamycin.This product is insensitive to Endo H, Endo F, and Neuramidase. A second product which is sensitive to Endo H but not neuraminidase is the predominant form in the cell. It has an Mr~220-240 KDa, and appears to be retained in the ER until degradation some 10 hours later. Adsorption of cell lysates to wheat germ agglutinin (WGA) shows the presence of a third form of Mr~250 KDa which may represent the cell surface form. A majority of the synthesized protein is associated with intracellular membranes and we may now explore whether the intracellular forms are electrophysiologically and/or pharmacologically functional.

381.3

MOLECULAR CLONING AND CHROMOSOMAL LOCALIZATION OF HUMAN BRAIN SODIUM CHANNEL SUBTYPES. C.M. Lu, J. Han[1*], T.A. Rado[2*] & G.B. Brown, Department. of Psychiatry and Behavioral Neurobiology, [1]Laboratory of Medical Genetics & [2]Division of Hematology/Oncology, University of Alabama at Birmingham, Birmingham, AL 35924.

We have previously reported the cloning and physical mapping of a human brain sodium channel gene segment (Proc. Natl. Acad. Sci. USA 88, pp 335-339, 1991). The reported 212bp clone shares 86%, 91%, and 86% homology with rat brain sodium channel subtypes I, II and III, and is located on chromosome 2q22-q23 by chromosome microdissection PCR (CMPCR). Anchored PCR has been used to synthesize four additional 0.9kb clones starting with the 212bp sequence. Analysis of these clones suggests that three of them represent a single segment of the human brain sodium channel gene (equivalent to amino acid 1709 to 2005 in rat brain sodium channel II). The sequence of these clones shares 77% 85% and 82% homology with rat brain sodium channel subtypes I, II and III respectively. The sequence of the fourth clone shows 73%, 75% and 72% homology to rat brain sodium channel I, II and III, indicating the possible existence of at least one other human brain sodium channel subtype. When a pair of human sodium channel specific primers was used in a PCR sib selection experiment for screening a human brain cDNA library, a 1.3kb clone was isolated which shares 90%, 85% and 85% homology with rat brain sodium channel subtypes I, II and III respectively (equivalent to amino acid 1518 to 1940 in rat brain sodium channel I). Based on the degree of sequence homology with rat brain sodium channel genes, we named the consensus 0.9kb clone as human brain sodium channel II (HBSC II) and the 1.3kb clone as human brain sodium channel I (HBSC I). Homology between HBSC II and I is 87.5%. Using HBSC I specific primers for CMPCR, the gene was mapped to the same locus as HBSC II. This report is the first evidence for the existence of at least two and possibly three sodium channel subtypes in human brain tissue.

381.4

MOLECULAR CLONING, SEQUENCE ANALYSIS AND CHROMOSOMAL LOCALIZATION OF GENES ENCODING VOLTAGE GATED SODIUM CHANNELS FROM HUMAN BRAIN CORTEX. D.H. Ware,[†*] S.C. Lee,[†*] C.M.I. Ahmed,[†*] C.B. Wagner- McPherson,[‡*] G.A. Evans,[‡] and M. Montal.[†] [†]Biology and Physics, University of California, San Diego; [‡]The Salk Institute for Biological Studies, La Jolla, CA 92037 USA

Several different cDNAs encoding human brain sodium channel proteins were isolated. Overlapping cDNAs were used to determine the sequence of a full length clone denoted class 1. The cDNA begins 145 bp upstream from the start of the open reading frame and ends approximately 2000 bp 3' to the termination codon. The complete primary structure was deduced from sequence analysis of the cDNA. The sequence defines an open reading frame that encodes a 2005 amino acid protein which exhibits 80%, 80% and 77% nucleotide sequence homology with type I, II, and III rat sodium channels, respectively. Homology between human and rat genes is even higher, 82%, 97% and 86%, and all of the 52 amino acid substitutions found in the human gene are located in variable regions of the rat brain II gene. A 1.6 kb fragment of the 3' noncoding region maps the corresponding structual gene to human chromosome 2.

A cDNA for the second class begins at amino acid 1100 when compared to rat brain II and contains approximately 800 bp of 3' noncoding region. Deduced amino acid sequence shows 95%, 94% and 93% homology with respect to type I, II, and III rat brain genes. To map the corresponding structual gene, primers to 3' noncoding region were synthesized and the polymerase chain reaction was used to amplify the gene in human-hamster somatic cell hybrids. The class 2 cDNA was localized to human chromosome 2. A genomic cosmid clone was isolated corresponding to the 3' end of the class 2 cDNA and used to localize the class 2 cDNA to 2q23-q24 by *in situ* suppression hybridization with human metaphase chromosomes.

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381.5

Na^+ CHANNEL ISOTYPES IN RAT DORSAL ROOT GANGLION NEURONS IDENTIFIED BY ELECTROPHYSIOLOGICAL AND MOLECULAR BIOLOGICAL TECHNIQUES. Caffrey J.M., Brown* L.D., Emanuel* J.G.R., Eng D.L., Waxman S.G. and J.D. Kocsis. Dept. of Neurology, Yale Univ.Sch.Med., New Haven, CT 06510; V.A. Med.Cntr., W. Haven CT. 16516

Expression of Na^+ currents with distinctive kinetic and pharmacological properties can be correlated with defined size classes of rat dorsal root ganglion (DRG) neurons. Neurons are enzymatically isolated from early post-natal (days 1-3, 7-9, 14-16) and at adult animals and maintained in short-term (1-4days) primary culture. A single TTX-sensitive Na^+ current (V_h=-87.5mV; 6msec > τ_h > 0.4msec) is detectable in outside-out patches excised from large cells ("A-type": adult diameter>50μm). Rapidly-inactivating, TTX-sensitive Na^+ current (V_h=-85mV; 0.7msec > τ_h > 0.3msec) and slowly-activating and -inactivating, TTX-resistant Na^+ current (V_h=-45mV; 24msec > τ_h > 1msec) are always expressed in small cells ("C-type" neurons: adult diameter<30μm), measured in whole-cell current and excised patches. Neurons of intermediate size either express the single current type found in large neurons or the two found in small neurons. To further define these channels, Na^+ channel mRNA is visualized in cultured DRG neurons by *in situ* hybridization histochemistry using PCR-derived, non-radioactively-labeled probes. "Generic" Na^+ channel probe is ≥90% homologous with brain forms I, II, and III; "form-specific" probes are ≤73% homologous with one another. Correlations between message and channel expression are being established. V.A.Med.Ctr. supported.

381.6

FUNCTIONAL EXPRESSION AND AMINO ACID SEQUENCE OF THE TETRODOTOXIN-INSENSITIVE HUMAN CARDIAC MUSCLE SODIUM CHANNEL. R.G. Kallen, L. Chen, M.E. Gellens, A.L. George, Jr., M. Chahine, R. Horn, R.L. Barchi *Dept. of Biochemistry and Biophysics and Medicine and Mahoney Institute for Neurological Sciences, U of Penn School of Medicine, Phila., PA 10104-6059; Neurosci. Dept., Roche Institute, Nutley, NJ 07110*

One rat voltage-sensitive sodium channel subtype (SkM2) is tetrodotoxin-insensitive (TTX-I) and constitutes the predominant mRNA present in adult heart and immature skeletal muscle (Kallen *et al.* Neuron 4, 233-242 (1990). SkM2 also constitutes up to 40% of the sodium channel mRNA in denervated skeletal muscle. Using the rat SkM2 probe we have identified overlapping cDNA clones from an adult human heart cDNA library and determined the complete nucleotide sequence for the short 5'-UT (150bp), the 6kb coding region and almost all of the 3'UT (2.5kb). The human heart sodium channel (HH1) compared to those of rat SkM1 and SkM2 revealed approximately 72% and 92% overall amino acid sequence homology, respectively.

	% HOMOLOGY OF HH1								
Subtype	N-ter	D1	ID1-2	D2	ID2-3	D3	ID3-4	D4	C-ter
SkM1	57	70	21	79	28	80	86	86	45
SkM2	95	97	85	96	83	87	100	98	87

Antisense RNA transcribed from the 3'-UT region of HH1 constituted a subtype-specific probe for Northern blots and hybridizes to a ~9.5kb transcript in human atrium and ventricle RNA but no transcripts were detected in human skeletal muscle, brain or myometrium RNA. Sense transcripts from a full-length cDNA clone were expressed in X. laevis oocytes. Macroscopic currents were recorded both with a 2-microelectrode voltage clamp and with macro-patches in the outside-out configuration. The currents had apparently normal kinetics of activation and inactivation, and were tetrodotoxin-insensitive (10μM caused ~57% reduction of the peak current at -10mV). Single channel currents in outside-out patches (150mM Na^+, 1.5mM Ca^{2+}, 22°C) had a conductance of ~22pS, by comparison with 10pS for rat SkM2 and 32pS for rat SkM1.

381.7

EXPRESSION OF THE GENE FOR THE RAT BRAIN SODIUM CHANNEL IIA ALPHA SUBUNIT IN CHINESE HAMSTER OVARY (CHO) CELLS. P.H. Lalik*, D.S. Krafte and R.B. Ciccarelli. Depts. of Molecular Biology and Cardiovascular Pharmacology, Sterling Research Group, 81 Columbia Turnpike, Rensselaer, NY, 12144.

In order to carry out protein structure-function studies with ion channels, we constructed a plasmid for inducible expression of the rat brain sodium channel IIa alpha subunit gene in mammalian cells. A 6 kB SalI fragment was removed from pVA2580 (A.L. Goldin, U.C. Irvine) and was inserted into pMAMneo (Clonetech). The resulting plasmid, pPHL110, contained the sodium channel gene under transcriptional control of the MMTV LTR. Transfection of pPHL110 in CHO cells followed by induction with dexamethasone resulted in transient expression of sodium channel mRNA as determined by PCR analysis. Sodium channel function was confirmed by a whole cell patch clamp assay. Isolation of inducible CHO cell lines stably expressing the gene was then attempted. Following transfection, 50 cell lines resistant to G418 were selected and were screened by immunoblot assay with an antibody (R. Levinson, U.Colorado) specific to the sp19 region of the sodium channel. Sodium channel gene expression was also analyzed in these cell lines by PCR analysis of mRNA. Evaluation of sodium channel function is being determined by a patch clamp assay. Positive results with both the immunoblot assay and the PCR assay indicate that the sodium channel gene was successfully incorporated into several of these cell lines.

381.8

IDENTIFICATION OF A PUTATIVE NA^+ CHANNEL cDNA IN THE JELLYFISH *CYANEA CAPILLATA*. M.A. Holman, R.M. Greenberg, W.A.S. Bonert* and P.A.V. Anderson. Whitney Laboratory, University of Florida, 9505 Ocean Shore Blvd., St. Augustine, FL 32086.

Cyanea capillata is a coelenterate whose neurons contain Na^+ channels with unusual pharmacological properties. The predominant inward current in these cells is a fast, transient current that is carried exclusively by Na^+, but is completely insensitive to standard Na^+ channel blockers such as TTX, STX, and BTX. However, this current is blocked by common Ca^{++} channel blockers such as Cd^{++}, Nicardipine, verapamil, and W7.

The purpose of this study is to examine these channels at the molecular level. Poly A^+ RNA was extracted from neuron-enriched tissue and a cDNA library constructed. Primers were designed from highly conserved regions in the last transmembrane segment of domains III and IV of known Na^+ channels. These primers were used for PCR amplification of sequences from the cDNA library. A putative Na^+ channel cDNA has been identified and partially sequenced. The conceptual translation thus far shows open reading frames of over 100 amino acids with extensive similarity to known Na^+ channels. The amplified sequence will be used as a homologous probe to obtain the full length cDNA. The complete sequence of this clone, along with expression of its cRNA in *Xenopus* oocytes, will allow a more complete understanding of the novel properties of this Na^+ channel.

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381.9

MOLECULAR CHARACTERIZATION OF A RAT GLIAL Na CHANNEL ISOFORM. G.Dos Santos*, S.Gautron*, A.Koulakoff*, D.Pinto-Henrique*, P.Ascher° and Y.Berwald-Netter*. Collège de France, 75231 Paris; ° Ecole Normale Supérieure, 75005 Paris, France.

Astroglial cells and Schwann cells (Sch cs) from rats and mice express in culture voltage-gated Na channels (Na Ch) that are relatively insensitive to TTX (Rev.: Berwald-Netter et al., in: Astrocytes, Acad. Press 1986). To explore whether the glial channels correspond to any of the 3 rat brain Na Ch isoforms identified by Numa et al. (1986,1988) we performed molecular hybridizations of mRNA extracts from cultured rat astroglia or neurons with the isoform-specific cDNA probes and with a highly conserved "common" probe. All were ~ 400 nt long; all hybridized to 9-9.5 kb neuronal mRNA; only the common probe gave a hybridization signal with glial mRNA, at 7.5 kb. This probe was thus used to screen a glial cDNA library. About 5 kb of contiguous glial cDNA were cloned and sequenced. Its nt and deduced amino acid composition are remarkably similar to rat brain and muscle Na Ch in overall structure. It spans 3 out of 4 Na Ch domains (1st 5' domain is yet to be cloned) plus a 3' non-coding region. The sequence homology of putative transmembrane segments varies from 36% to 91% and indicates a gene product unambiguously distinct from the previously defined brain and muscle Na Ch isoforms. A "glial Na Ch" probe was generated by subcloning a 480 nt 3' segment and used to explore the tissue distribution of the corresponding mRNA. By RNAse protection assay an identical sequence was detected in rat brain and cultured Sch cs. By Northern blot the RNA identified is in all cases 7.5 kb. It is present in a fairly high amount in DRGs and cardiac muscle and in low amount in fetal skeletal muscle. In situ hybridization in cultures of rat brain cells showed labeling of astroglia but not neurons and in cultures of DRGs of both neurons and Sch cs. Altogether the data are consistent with the "glial" channel being a new TTX-insensitive Na Ch isoform.

381.10

DEVELOPMENTALLY REGULATED ALTERNATIVE RNA SPLICING OF THE RAT BRAIN SODIUM CHANNEL MRNA R. Sarao, S. K. Gupta, V. J. Auld and R. J. Dunn. Centre for Research in Neuroscience, Montreal General Hospital, McGill University, 1650 Cedar Avenue, Montreal, Quebec M3G 1A4

Four distinct cDNAs encoding the α-subunit of the sodium channel have been isolated from the mammalian brain. Two rat cDNAs, named RII and RIIA, are almost identical throughout the coding regions, with a divergence of 36 nucleotides (or 2%) over a coding region of 6 kb. A cluster of 20 divergent bases, in a short 90 nucleotide segment within the homology domain I, results in silent mutations in the two encoded proteins except for position 564, which specifies Asn 209 in the RII and Asp 209 in the RIIA sequences respectively. We now demonstrate that this 90 nucleotide segment is encoded twice in the RII/RIIA genomic sequence. The mutually exclusive splicing event that gives rise to the two cDNAs is developmentally regulated, with RII mRNAs being abundant at birth, and RIIA mRNAs gradually replacing RII mRNAs as post-natal development proceeds. The two mRNAs also appear to have different regional distributions in the developing rat brain. These results suggest that the maturation dependent splicing observed in the RII/RIIA sodium channel may serve to modulate channel properties in a significant manner during neuronal development.

381.11

[^{14}C]-GUANIDINE INFLUX AND [^{3}H]-BATRACHOTOXININ BINDING IN CHO CELLS EXPRESSING THE ALPHA SUBUNIT OF TYPE IIA Na$^+$ CHANNELS. D.K. Boyd, R.D. Schwarz, D. Dooley,[1]J. West* and [1]W.A. Catterall. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI. and [1]Dept. of Pharmacology, University of Washington, Seattle, WA.

Recently, the alpha subunit of Type IIA Na$^+$ channels has been cloned and stably expressed in high numbers using CHO (C81-11) cells (Scheuer, et al., Biophys. J. 59:262a, 1991). Electrophysiological experiments have shown that these cells have Na$^+$ currents of 5-20nA with characteristics similar to those of native Na$^+$ channels. To further characterize these cells, measurements of ion flux and receptor binding were performed. Using [^{14}C]-guanidine as the tracer, it was found that there was a time and concentration-dependent stimulation of influx produced by veratridine which was potentiated by alpha scorpion venom. Tetrodotoxin (TTX) fully reversed this stimulation. Representative anti-convulsants, local anesthetics, and antiarrythmics also blocked ion flux with varying potencies. The cells were also characterized in a assay measuring [^{3}H]-batrachotoxinin (BTX) receptor binding. A comparison of results obtained in [^{3}H]-BTX binding and those from ion flux showed a reasonable correlation between the two assays. Thus, understanding the function and pharmacology of Type IIA Na$^+$ channels will be greatly facilitated by the use of C81-11 cells.

381.12

MUTATION OF A SINGLE PHOSPHORYLATION SITE BLOCKS THE ACTION OF PROTEIN KINASE C ON RAT BRAIN TYPE IIA SODIUM CHANNELS. J. W. West*, R. Numann*, T. Scheuer and W. A. Catterall. Dept. of Pharmacology, U. of Washington, Seattle, WA 98195

Diacylglycerol activators of protein kinase C reduce sodium current and slow the inactivation of rat brain type IIA sodium channels[1]. A predicted cytoplasmic loop between homologous domains III and IV has been implicated in the inactivation process. This loop contains a consensus sequence for phosphorylation by protein kinase C. We have mutated S1506A in this site by oligonucleotide-directed mutagenesis to render it unavailable for phosphorylation by the kinase. Plasmids containing the control and mutant Rat brain type IIA sodium channel α subunit cDNAs have been stably expressed in Chinese Hamster Ovary (CHO) cells by calcium phosphate-mediated transfection. Both control and mutant cell lines stably express large sodium currents (>2 nA) when examined by whole cell voltage clamp. Effects of the diacylglycerol activator OAG (1-oleoyl-2-acetyl-sn-glycerol) were examined by recording macroscopic currents in cell attached patches. In the control cell line 25 μM OAG caused a large reduction in sodium current amplitude as well as slowing its inactivation. In two independent clonal isolates of stably expressing mutant cell lines sodium current was unaffected by the activator, even at concentrations as high as 100 μM. It appears that phosphorylation of SER1506 is essential for sodium channel modulation by protein kinase C.

[1] Numann et. al. Biophys J. 59:262a,1991.

381.13

PROTEIN KINASE A MODULATES SODIUM CHANNEL FUNCTION IN *XENOPUS* OOCYTES. R.D. Smith and A.L. Goldin. Dept of Microbiology & Molecular Genetics, U. California, Irvine, CA 92717.

The rat brain Na$^+$ channel is a substrate for protein kinase A (PKA) *in vivo*, allowing for the possibility of functional modulation by PKA phosphorylation. To test this possibility, we have examined the effects of PKA activation on Na$^+$ currents resulting from expression of the rat IIA clone in *Xenopus* oocytes using two electrode voltage clamping. PKA was activated by increased cAMP levels resulting from either isoproterenol stimulation of a co-expressed β_2-adrenergic receptor, or perfusion with dibutyryl cAMP. Whole cell Na$^+$ currents were increased by 20-50% within fifteen minutes following PKA activation by either of these procedures. The current increases were blocked by Protein Kinase Inhibitor, a specific inhibitor of PKA, indicating that the increase was due to activation of PKA. The current-voltage relationship was not significantly altered by PKA induction. When uninduced oocytes were injected with phosphatase, Na$^+$ currents were decreased by about 60%, suggesting that basal Na$^+$ current levels were dependent on phosphorylation. To determine if the modulation by PKA was mediated by direct phosphorylation of the Na$^+$ channel α subunit, five PKA consensus sites located within the intracellular linker region between domains I and II of the α subunit were modified by site-directed mutagenesis. A mutant altered at all five sites expressed currents that were dramatically decreased in amplitude compared to wild-type, without a shift in the current-voltage relationship. Alterations at individual PKA sites resulted in variable effects on current amplitude, depending on which of the sites were modified. Data for all of the mutants with altered PKA consensus sites will be presented.

381.14

THE SODIUM CHANNEL III-IV LINKER MAY SUBSTITUTE FOR THE AMINO TERMINAL "INACTIVATION BALL" IN THE *SHAKER* H4 POTASSIUM CHANNEL. D.E. Patton and A.L. Goldin. Dept of Microbiology & Molecular Genetics, U. California, Irvine, CA 92717.

The short cytoplasmic linker that connects domains III and IV of the voltage-gated sodium channel is thought to be involved in the fast inactivation process. At present, the mechanism by which this linker region is involved in the inactivation process is unclear. The amino terminal region of the *Shaker* B potassium channel has been shown to mediate the fast inactivation process in potassium channels in a manner similar to the "ball and chain" mechanism proposed by Armstrong and Bezanilla (J. Gen. Physiol., 1977, 70:567). To test if the III-IV linker of sodium channels and the amino terminus of *Shaker* potassium channels serve similar functions, a chimeric ion channel was constructed with the III-IV linker of the rat IIA sodium channel substituting for the first 11 amino acids of the *Shaker* H4 (ShH4) potassium channel (ShH4Δ11NaIII-IV). For comparison, a mutant ShH4 potassium channel with the first 11 amino acids deleted was also constructed (ShH4Δ11). Macroscopic currents through these channels expressed in *Xenopus* oocytes were characterized using two microelectrode voltage clamp techniques. The ShH4Δ11NaIII-IV channel inactivated more rapidly than the ShH4Δ11 channel, although still more slowly than ShH4. In addition, depolarizations to potentials more positive than +10 mV induced a fast phase of inactivation in ShH4Δ11NaIII-IV that was not present in ShH4Δ11. These results suggest that the III-IV linker of the rat IIA sodium channel and the amino terminal "ball" region of the ShH4 potassium channel may serve similar functions in the inactivation process.

381.15

SODIUM CHANNEL STRUCTURE/FUNCTION: EFFECTS OF NEGATIVE CHARGE MUTATIONS ON BLOCK BY TTX. K.J. Kontis and A.L. Goldin. Dept of Microbiology & Molecular Genetics, U. California, Irvine, CA, 92717.

Sodium channels have structural similarities to other voltage-gated channels, but they are highly selective for sodium and are specifically blocked by externally applied tetrodotoxin (TTX). The site for TTX binding is thought to be in close proximity to the actual pore, since chemical modifications of the channel which affect TTX binding also reduce inward current (Sigworth et al., 1980, Nature 283:293). Stühmer et al. (FEBS Lett., 1989, 259:213) have shown that neutralization of a negatively charged amino acid at position 387 in the α subunit results in resistance to TTX and reduced inward current. We have used site-directed mutagenesis to investigate the role in TTX binding and ion permeation of negatively charged amino acids between the proposed transmembrane segments S5 and S6 in domain II. We have neutralized the negatively charged amino acids at positions 927, 942 and 945 of the rat IIA sodium channel α subunit, and have analyzed these mutants in *Xenopus* oocytes using two electrode voltage clamping. The D927N mutation resulted in sodium channels with TTX sensitivity equivalent to wild-type. The E942Q and E945Q mutations resulted in channels which were inhibited by TTX with an apparent K_D higher than 10 μM, compared to 18 nM for the wild-type channel and D927N. In all three cases the gating characteristics of the mutants were not significantly different from wild-type. However, macroscopic currents resulting from injection of RNA from mutant E942Q were approximately five-fold lower than those resulting from injection of equivalent amounts of wild-type RNA. The unitary conductance and selectivity of these mutants are currently being analyzed, along with the effects of additional mutations in the S5-S6 region.

SODIUM CHANNELS: PHYSIOLOGY AND PHARMACOLOGY

382.1

VOLTAGE-DEPENDENT CURRENTS IN *STERNOPYGUS* ELECTROCYTES VARY WITH ACTION POTENTIAL DURATION. M.B. Ferrari and H.H. Zakon. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

In *Sternopygus macrurus*, electrocyte action potential (AP) waveform and duration are under the control of steroid hormones. Since electrocyte AP durations can range from 4 to 14 ms, electrocytes can serve as a model system in which to study the biophysical basis of steroid-mediated changes in cell excitability.

A two-microelectrode current- and voltage-clamp setup was used to examine the APs and ionic currents of *Sternopygus* electrocytes in a semi-intact preparation. Using various voltage clamp protocols from a holding potential of -80 mV, we observed a TTX-sensitive sodium current, a delayed outward current, a transient outward current, an inward rectifier current, and a large linear leakage. The sodium and delayed outward currents had the largest relative magnitudes, with peak sodium ranging from 171 to 900 nA near 0 mV. Sodium current characteristics were similar to those described for vertebrate skeletal muscle. The delayed outward current displayed activation kinetics similar to a delayed rectifier potassium current and did not inactivate. The transient outward current was seen in only a few cells and was of small magnitude. The inward rectifier current shows a steep voltage-sensitive inactivation around -60 mV and accounts for the 2-5 fold increase in input resistance observed during current clamp depolarizations.

Electrocyte AP durations ranged from 5.1 to 13.5 ms with a corresponding change in the relative contributions of the described currents. Cells with long duration APs had much slower sodium inactivation kinetics and had little or no delayed outward current. Conversely, cells with short duration APs had faster sodium inactivation kinetics and larger delayed outward currents. These observations suggest that sodium current kinetics and delayed outward current magnitude are significant factors in determining electrocyte AP duration and waveform. Supported by NIH and ONR.

382.2

NOVEL INACTIVATION CHARACTERISTICS OF SODIUM CURRENT IN GUINEA-PIG CELIAC GANGLION NEURONS. S.L. Purnyn*, S.R. Knoper and D. L. Kreulen, Dept. Pharmacology, University of Arizona, Tucson, AZ USA.

The characteristics of the fast sodium current were determined in dissociated guinea-pig celiac ganglion neurons after 1-4 days in primary culture. Whole cell currents were measured at 22°C. Fast inward current was reversibly blocked by tetrodotoxin (KD: 8.7 $\pm$ 2.1 nM). Sodium current activation and inactivation processes were fit to modified Boltzmann equations. Steady-state activation and inactivation parameters were similar to those described for other types of neurons. Activation parameters for the half activation potential (V_h) and slope factor (K) were -31 mV and 6.0, respectively. Half inactivation potential and inactivation slope factor were -65 mV and 7.2, respectively. Unlike other neurons, including mammalian sympathetic neurons, the time constant of inactivation depended on the direction of approach to the conditioning potential. The removal of inactivation evaluated with a 2-impulse protocol resulted in a time constant of inactivation of 55$\pm$5 ms whereas inactivation evaluated with a single impulse protocol resulted in an inactivation time constant of 218$\pm$21 ms. (V_m: -60 mV). These differences are statistically significant at $p<0.01$ (group t-test). These studies demonstrate some unique kinetic characteristics of sodium currents in neurons from the guinea pig celiac ganglion and suggest that the Hodgkin-Huxley model may have to be modified to fit these characteristics completely. Support: HL27781, Az.Dis. Cont. Res.Comm.

382.3

SLOW SODIUM CONDUCTANCE IN IONIC CONTROL OF CONDUCTION IN MYELINATED AXON: A COMPUTATIONAL MODEL. F. Pongracz, S.G. Waxman, G.M. Shepherd and J.D. Kocsis, Sect. of Neurobiol. and Dept. of Neurology, Yale Medical Sch., New Haven, CT 06510 and Neuroscience Research Center, VAMC, West Haven, CT 06516

Myelinated axons may express fast and slow Na^+ and K^+ channels with distinct membrane localization. To study the functional role of slow Na^+ channels, we developed a computational model of a myelinated axon with 16 nodes containing a variety of kinetically distinct Na^+ and K^+ channels. Nodal, paranodal, and internodal regions have been represented with different electrical properties. The periaxonal space has been represented by a serial shunt path and voltage- and concentration-dependent K^+ leak through the paranodal loops and internodal myelin.

The model predicts that the depolarizing afterpotential and burst generating properties of the axons are regulated by a small slow Na^+ current. During blockade of the fast K^+ current, this slow Na^+ component of the nodal and paranodal currents abruptly increased concurrent with impulse burst activity. In accordance with experimental data, the model predicts that increased activation of the slow Na^+ current can activate a prolonged K^+ current. The model also showed that a heterogeneous distribution of slow Na^+ current within the nodal and paranodal regions could elicit spike burst activity which could propagate in a retrograde direction.

These results suggest a highly sensitive ionic control of transmission in myelinated axons, presumably mediated by activation of fast and slow components of Na^+ current, together with fast and slow K^+ conductance, which may modulate the conduction properties and excitability of myelinated axons and their terminals. Supported in part by the NIH, VA and the NMSS.

382.4

CALCULATIONS OF QUANTUM TUNNELING BETWEEN CLOSED AND OPEN STATES OF SODIUM CHANNELS. P. J. Marshall*, C. C. Chancey*, and S. A. George. Physics Department and Neuroscience Program, Amherst College, Amherst MA 01002.

Transitions between states of ion channels have previously been considered in terms of classical statistical mechanics. However, transitions in many systems, including some organic molecules, are known to occur by quantum mechanical tunneling. We estimated times for sodium channel activation by tunneling, using the structural models of Catterall and Guy. We calculated coulomb interactions between the S4 α-helix and negative charges on nearest neighbor helices, and included longer range interactions by adding a background electric field. Periodic pairing of charges between the S4 and adjacent helices in the model causes the resting and depolarized states of the channel to correspond to local minima in the S4 potential energy curve. Harmonic potentials closely fit the deviations from each local minimum. Tunneling rates between bound states of each harmonic potential were calculated using a WKB approximation. At 37°C, for an interhelix axial spacing of 10 Å, and with sufficient electric field to account for the voltage dependence of activation, a tunneling time of 1 msec was computed for a single S4 segment. When more than a single S4 helix was included, tunneling times remained within the range of observed transition times. Thus quantum tunneling is possible in this channel. If tunneling turns out to be prevalent in sodium channel transitions, the framework for understanding neural function will have to expand to include quantum concepts.

382.5

VENOM FROM CONUS TEXTILE ACTIVATES A TETRODOTOXIN-SENSITIVE INWARD CURRENT IN BAG CELL NEURONS OF *APLYSIA*. G.F. Wilson, T.E. Fisher, W.J. Joiner*, B.M. Olivera[2], and L.K. Kaczmarek. Dept.of Pharmacology and Sect. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510 and [2]Dept. of Biology, University of Utah, Salt Lake City, UT 84112.

A crude preparation of venom from the marine snail Conus textile (CtTx) elicits a spontaneous afterdischarge when applied extracellularly to bag cell neuron (BCN) clusters of *Aplysia*. In BCNs that have been isolated and maintained in primary culture, CtTx causes a long-lasting depolarization averaging 33 mV in normal seawater (n=12). This effect appears to be due to CtTx activation of a voltage-dependent inward current carried primarily by Na^+ ions, since the depolarization disappears entirely when N-methyl-D-glucamine replaces Na^+ in the bath (n=4) and since both depolarization and current are blocked by TTX (n=8). Although the effective TTX concentration is similar to that required for block of *Aplysia* voltage-dependent sodium currents (100 μM) which reverse at ~ +50 mV, the TTX-sensitive, CtTx-activated inward current reverses nearer to 0 mV and the cation permeability (Na > TEA > Tris > N-methyl-D-glucamine) of the response appears more similar to that of Ca-activated non-specific cation channels [Partridge and Swandulla (1988) TINS 11, 69-72] than that of traditional sodium channels. Indeed, the CtTx-induced depolarization is partially eliminated when intracellular Ca^{2+} is buffered to low levels by including 20 mM EGTA, 4.14 mM Ca^{2+} in patch pipettes (n=8). In addition, in excised outside/out patches, we have characterized a ~ 30 pS channel similar to the CtTx-activated current in its reversal potential and ionic selectivity. Preliminary evidence suggests that this channel could be the target of the toxin.

382.6

ALLOSTERIC EFFECTS ON [^{3}H]BATRACHOTOXININ-A ORTHO-AZIDOBENZOATE PHOTOAFFINITY LABELING OF THE RAT BRAIN SODIUM CHANNEL. T.L. Casebolt and G.B.Brown. Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35294.

Neurotoxins have classically been used to identify and characterize voltage-sensitive sodium channels. The characteristic selectivity, site-specificity, and high affinity binding of neurotoxins have allowed the dissection of both channel function and structure. Site 1, which is bound externally by tetrodotoxin (TTX) and saxitoxin (STX), and site 3, which is selective for alpha-polypeptide neurotoxins from scorpion and sea anemone, interact with the batrachotoxin (BTX) binding site on the sodium channel as measured by [^{3}H]batrachotoxinin-A 20-alpha-benzoate ([^{3}H] BTX-B) binding. Using a photolabile derivative of BTX, [^{3}H]batrachotoxinin-A ortho-azidobenzoate ([^{3}H] BTX-OAB), the specific labeling of lipid and protein components of the BTX binding site can be quantified. In general, the binding behavior of [^{3}H] BTX-OAB parallels the binding behavior of [^{3}H]BTX-B and requires the addition of scorpion toxin (ScTx) to enhance ligand binding. To investigate the allosteric effects of site 1 neurotoxins on specific covalent lipid and protein binding, we measured photoaffinity labeling of [^{3}H] BTX-OAB in the presence of ScTx at room temperature and at 37°C in the presence and absence of 1 uM TTX. Addition of TTX increased the specific incorporation of [^{3}H]BTX-OAB into protein components from less than 1% to as much as 15%, with a corresponding drop in lipid incorporation. Increasing incubation temperature from 25°C to 37°C had a similar but less marked effect. Specific protein labeling was localized on a 270 Kdal protein, presumably the alpha subunit of the sodium channel protein. These results support the proposal that the BTX binding site is located at the lipid-protein interface such that treatments which induce conformational changes in the sodium channel protein (i.e. addition of TTX) or altered membrane fluidity (such as increased incubation temperatures) can result in a redistribution of BTX binding sites in the protein and lipid domains of voltage-sensitive sodium channels.

382.7

EFFECTS OF ALKALOID TOXINS ON THE ION CONDUCTING PROPERTIES OF PURIFIED SODIUM CHANNELS. H.C.Wartenberg[1*], A. Hernandez[1*], B.W. Urban[2], and D.S. Duch[1*], [1] Cornell University Medical College, New York, N.Y.; and [2]Univ. Bonn, F.R. of Germany.

As previously reported (Duch et al., 1991, Biophys. J., 59:259a), grayanotoxin-modified (GTX) eel electroplax sodium channels in planar bilayers have single channel conductances of 15-16 pS (500mM NaCl), smaller than batrachotoxin-modified (BTX) channels, but larger than in the presence of veratridine (VTD). However, while the BTX and VTD-modified channels have linear and symmetrical I-V relations, GTX-modified channels are symmetrical and superlinear, especially at potentials greater than +/-100mV. A comparison of the conductance-ion concentration relationships of the three toxins in symmetrical sodium solutions indicated similar shaped rectangular hyperbolas for all three toxins, with similar half-saturating concentrations. In addition, GTX-modified channels also show pre-opening and pre-closing bursts of 40-50 pS conductance before a significant fraction of open-closed transitions, which were voltage-dependent and not observed with BTX or VTD. These bursts may represent toxin binding and unbinding events.

382.8

ALLETHRIN EFFECTS ON TETRODOTOXIN RESISTANT SODIUM CHANNELS IN RAT DORSAL ROOT GANGLION CELLS. K. S. Ginsburg and T. Narahashi, Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611

To study the mechanism of action of allethrin, a pyrethroid insecticide, on Na^+ channels, we recorded whole cell TTX resistant Na^+ currents from newborn rat DRG cells, at 11, 18, and 27°C. Tail currents recorded with allethrin (10 μM) present decayed biexponentially. Both time constants (100 μsec and 4 msec at 18°) increased as temperature decreased. The amplitude of the slow phase developed with a time constant of about 200 sec on application of allethrin and declined at a similar rate on washout. With pulses of increasing duration, the amplitude of the slow phase first increased, then decreased. The amplitude ratio of the slow phase to the fast phase increased with pulse duration. The pulse duration giving maximum amplitude decreased with increasing temperature and was 3 msec at 18°. However, the maximum slow phase conductance, expressed as a fraction of the peak conductance during the depolarization, did not change with temperature. Thus, changes in the extent of allethrin modified conductance may not explain the temperature dependence of allethrin toxicity. Supported by NS14143.

382.9

EFFECTS OF SCORPION VENOM, SEA ANEMONE TOXIN, AND CHLORPROMAZINE ON TETRODOTOXIN-SENSITIVE AND TETRODOTOXIN-RESISTANT SODIUM CHANNELS. M.-L. Roy and T. Narahashi, Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Dorsal root ganglion neurons acutely dissociated from 3-10-day-old rats express TTX-sensitive (TTX-S, K_d~1 nM) and TTX-resistant (TTX-R, K_d~100 μM) sodium channels in varying proportions. These current types differ in their activation and inactivation kinetics (Roy and Narahashi, Neurosci. Abstr. 16: 181, 1990), as well as their responses to divalent cations and lidocaine (Roy and Narahashi, Biophys. J. 59: 263a, 1991). Scorpion venom and sea anemone toxin, but not chlorpromazine, have now been found to exert differential actions on these two channel types. Currents were recorded using the whole cell patch clamp technique. Leiurus quinquestriatus scorpion venom and sea anemone toxin ATX-II (1-100 nM) each inhibited TTX-S current inactivation, but failed to affect TTX-R current. This may indicate that the TTX-R and TTX-S channels differ in two distinct neurotoxin receptor sites. Tonic block (K_d~100 nM) and use-dependent block of TTX-R and TTX-S channels by chlorpromazine were comparable, indicating that this drug's site of action is conserved in both channel types. The comparison of TTX-S and TTX-R sodium channel properties is of importance in discerning the role of these channels in CNS development and drug action. Supported by NIH grants RO1 NS14144 and F31 MH09839.

382.10

INHIBITION OF SODIUM CHANNEL CURRENT BY THE ANTICONVULSANT U-54494A IN MOUSE NEUROBLASTOMA CELLS. Yu Zhu and Wha Bin Im. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001

The benzamide U-54494A has been reported to act as a non-analgesic anticonvulsant in intact animal studies (VonVoigtlander *et al.* J. Pharm. Exp. Ther. 243:542, 1987). To better understand the mechanism of its anticonvulsant action, the effects of U-54494A on the voltage-gated sodium channel current were investigated by using the whole-cell patch clamp technique in mouse neuroblastoma cells (NIE-115). U-54494A tonically blocked the TTX-sensitive Na current with an IC_{50} of 72 μM and a t½ of 2.2 min. Its block of the Na channel was fully reversible. U-54494A shifted the steady-state inactivation of the Na channel 3mV to the more hyperpolarized potential, but had no effect on the steady-state activation of the channel. The drug did not alter the kinetics of the channel activation and inactivation, but appeared to interact with the open and inactivated states of the channel, as evidenced by the use-dependent block even with a very short depolarization (<1 ms) and the slow recovery from the steady-state inactivation (τ=2.7 s). Our results suggest that block of the Na channel by U-54494A, especially the use-dependent block, is an important pharmacological basis in the treatment of certain types of seizures during which the excitability of neurons is excessive.

382.11

TETRODOTOXIN-SENSITIVE SODIUM CURRENTS IN FLATWORM NEURONS. Kevin L. Blair and Peter A.V. Anderson. Whitney Laboratory, University of Florida, St. Augustine, FL 32086.

Platyhelminthes (the flatworms), the simplest organisms to exhibit cephalization and discrete central and peripheral nervous systems, are believed to express tetrodotoxin (TTX)-sensitive and insensitive Na^+ channels in their cephalic neurons. Voltage-gated ionic currents in neurons dispersed from the brains of the flatworm *Bdelloura candida* were characterized using the whole-cell voltage-clamp technique. Cells ranging from 15 to 30 microns in diameter were studied and found to possess two inward Na^+ currents. The more prominent current was a fast-activating, fast-inactivating current with a threshold voltage of -25 mV. It reached peak current at 0 to +5 mV and reversed at +55 to +65 mV in a normal Na^+ and Ca^{2+} sea water (E_{Na} = +58 mV) . This current was half inactivated (V_h) by a 50 ms pre-pulse to -30 mV. A non-inactivating current, roughly 10% of the amplitude of the inactivating current, was also present and exhibited similar voltage sensitivities, except that it was not inactivated by pre-pulses up to 500 ms in duration. This non-inactivating current was never observed to "run-down".

When the $[Na^+]$ of the bath was decreased, the peak amplitude of both the inactivating and non-inactivating currents decreased and their reversal potentials shifted to the left with E_{Na}, indicating that Na^+ was the dominant carrier ion. Furthermore, both of these currents were blocked by TTX, with IC_{50}'s of 30 n*M*. Thus, contrary to previous reports, flatworms appear to express only tetrodotoxin-sensitive Na^+ channels in their cephalic neurons. Supported by NSF grant BNS 88-05885.

382.12

ELEVATED EXPRESSION OF TYPE II SODIUM CHANNELS IN HYPOMYELINATED TRACTS OF *Shiverer* MICE. R.E. Westenbroek[1], J.L. Noebels[2], and W.A. Catterall[1]. [1]Department of Pharmacology, University of Washington, Seattle, WA 98195, and [2]Developmental Neurogenetics Laboratory, Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

The voltage sensitive sodium channel is a transmembrane protein responsible for the rising phase of the action potential in electrically excitable tissue. In the *Shiverer* mouse, a deletion at the *Shiverer* locus (chr 18) results in the absence of myelin basic protein gene expression and development of hyperexcitability. It has recently been demonstrated using [^{3}H]-saxitoxin binding methods that the large caliber fiber pathways in the brain of the *Shiverer* mutant exhibit a relatively high density of sodium channels as compared to normal mice (Noebels et al., *Soc. Neurosci. Abstrs.* **15**:1365, 1989). Affinity-purified anti-peptide antibodies which distinguish between the α subunits of rat brain sodium channel subtypes I, II, and III and an antibody against synthetic peptide SP20 which recognizes the α subunit of all three subtypes, were used in combination with indirect peroxidase-antiperoxidase technique to investigate the distribution of the three sodium channel subtypes in normal and *Shiverer* mice. Staining with anti-SP20 was similar in normal and mutant mice except for relatively dense staining of hypomyelinated fiber tracts in the mutant which is absent in normal mice. This difference in staining was most evident in the corpus callosum, internal capsule, fimbria, fornix and cerebellum. Subtype-specific antibodies revealed that Type I is expressed in cell bodies of projection neurons throughout the brain, Type II is expressed mainly in axons and Type III is expressed in scattered neurons in the cortex and other nuclei in the normal mouse brain as previously observed in rat brain (Westenbroek et al., *Neuron* 3:695-704, 1989). In *Shiverer* mice, the pattern of expression remained unchanged for Types I and III. However, there was intense staining for Type II in hypomyelinated fiber tracts including corpus callosum, internal capsule, fimbria, fornix and cerebellum which was not observed in normal mice. These results provide further support for our previous conclusion that Type II sodium channels are preferentially localized in axons of brain neurons and suggest that up regulation of the number of Type II channels in hypomyelinated fiber tracts may contribute to the hyperexcitable phenotype of the adult *Shiverer* mouse.

382.13

[^{3}H]PD 85,639 IS A HIGH AFFINITY LIGAND FOR RAT BRAIN SODIUM CHANNEL. W.J. Thomsen*[1], S.J. Hays[2], R.D. Schwarz[2], J.L. Hicks*[2], and W.A. Catterall[1], 1) Dept. of Pharmacology, Univ. Wash., Seattle, WA, 98195; 2) Parke Davis Research Div., Warner Lambert Co., Ann Arbor, Mi 48105

PD 85,639 (N-[3-(2,6-dimethyl-1-piperazinyl)propyl]-α-phenylbenzeneacetamide) is structurally related to local anesthetics and blocks veratridine-stimulated ^{14}C-guanidine influx in brain slices. In this study, the binding of [^{3}H] PD 85,639 to both rat brain synaptosomes and purified sodium channel (NaCh) reconstituted into phospholipid vesicles has been characterized.

Specific binding of [^{3}H] PD 85,639 to both synaptosomes and reconstituted NaCh is optimal at pH 9.0 (75-90% of total binding). At this pH, the uncharged form predominates. PD 85,639 competition for [^{3}H] PD 85,639 binding to synaptosomes is best fit by a two site model with EC_{50} values of 40nM (40%) and 14uM (60%). Batrachotoxin and α-LqTx V both decrease binding to synaptosomes with EC_{50} values similar to their K_d values for neurotoxin receptors 3 and 4, respectively. Both neurotoxins also inhibit [^{3}H] PD 85,639 binding to reconstituted NaCh. These data provide evidence that PD 85,639 binds specifically to NaCh. The drugs etidocaine, tetracaine, prilocaine, tocainide, bupivacaine, mepivacaine and verapamil also compete for [^{3}H] PD 85,639 binding to both synaptosomes and reconstituted vesicles. Etidocaine, tetracaine, and verapamil competition for binding to synaptosomes is best fit by a two site model in which there is a high affinity (1-2nM) and a lower affinity (10-50uM) component of displacement. In contrast, competition curves for the other local anesthetics are best fit by a one site model with EC_{50} values between 40-150uM.

The time course of [^{3}H] PD 85,639 binding to synaptosomes is monoexponential and rapid with a $t_{1/2}$ of 1 minute. With incubations longer than 30 minutes, binding decreases to 50-70% of that observed at 2 minutes. Association of [^{3}H] PD 85,639 with reconstituted NaCh is slower with a $t_{1/2}$ of 8 minutes and remains stable up to 3 hours of incubation. Dissociation of [^{3}H] PD 85,639 from synaptosomes and reconstituted NaCh initiated by 100uM PD 85,639 is biexponential consisting of a fast ($t_{1/2}$=0.6 min, 60%) and a slow ($t_{1/2}$=46 min, 40%) component of decay. Dissociation of [^{3}H] PD 85,639 from synaptosomes initiated by 100 uM tetracaine has identical kinetics. The rate of dissociation of ligand from synaptosomes is not altered by the inclusion of 100uM tetracaine in addition to 100uM PD 85,639 suggesting that both drugs bind to the same binding site.

These results indicate that [^{3}H] PD 85,639 is a useful high affinity ligand to further study the NaCh. It will be of interest to further establish that this compound interacts with the local anesthetic receptor and whether the different binding sites for PD 85,639 correspond to different states or different isoforms of the NaCh.

382.14

PD 85639: A POTENT INHIBITOR OF NA^+ INFLUX INTO RAT NEOCORTICAL SLICES. S. J. Hays*, R.D. Schwarz, D.K. Boyd, L. Coughenour, D. Dooley, D. Rock, C. Taylor, M. Vartanian, and W. Moos. Parke-Davis Pharm. Res Div., Warner-Lambert Co., Ann Arbor, MI.

Binding to specific sites associated with Na^+ channels appears to be involved in the pharmacological action of anticonvulsant, local anesthetic, and antiarrythmic agents. As part of a search to identify Na^+ channel blockers, PD 85639, N-[3-(2,6-dimethyl-1-piperidinyl)propyl]-α-phenylbenzeneacetamide, was tested for its ability to inhibit veratridine-stimulated influx of ^{14}C-guanidine into rat neocortical slices. It was found to be a potent influx inhibitor (IC_{50} = 2nM) with the inhibition curve possessing both high (nM) and low (uM) affinity components. PD 85639 was also able to potently inhibit ^{3}H-batrachotoxinin binding with an IC_{50} = 142nM. In contrast, PD 85639 (1 and 10uM) failed to inhibit electrically-evoked [^{3}H]-NE release from rat neocortical slices, whereas tetrodotoxin (TTX)was active at 1uM. Electrophysiologically, the compound (10uM and above) reduced sodium-dependent action potentials in cultured rat spinal cord neurons and in rat sciatic nerve. Block was partly use-dependent with slow recovery. Depolarizations from veratridine were reduced and shifted to higher veratridine concentration by 10uM. Thus, PD 85639 is a more potent blocker of veratridine-induced ion flux than of sodium channel-dependent physiolgical responses. In vivo, PD 85639 failed to cause anticonvulsant effects at doses up to lethality (10mg/kg, IV). Thus, PD 85639 represents a new and potent tool in the investigation of Na^+ channel structure and function.

ION CHANNELS: MODULATION AND REGULATION II

383.1

METABOTROPIC GLUTAMATE RECEPTOR MODULATES HIGH-THRESHOLD CALCIUM CURRENT OF NEURONS ACUTELY ISOLATED FROM RAT NEOCORTEX. R.J. Sayer, P. C. Schwindt, and W. E. Crill. Dept. of Physiology & Biophysics, Univ. of Washington Sch. of Med., Seattle, WA 98195.

In addition to their rapid ionotropic actions, excitatory amino acids can affect neuronal excitability over a slower time course. We investigated the effects of quisqualate on the high-threshold Ca^{2+} current of neocortical neurons. Slices of dorsal frontoparietal neocortex from 14-28 day old rats were treated with papain and triturated. Whole-cell recordings were made at 20-23°C and the recording pipette solution included 10 mM EGTA. Ca^{2+} currents, evoked by steps from -60 mV to -20 or -10 mV, were reduced by glutamate and quisqualate. Quisqualate (20 μM) applied by puffer pipette to 16 neurons reduced the peak Ca^{2+} current by a mean of 31% (range 11-50%). Suppression of the current took 3-5 min to fully develop and was readily reversible by washing. AMPA (100 μM) was ineffective (*n*=4). In the presence of CNQX (50 μM) and DL-APV (100 μM), sequential applications of quisqualate (20 μM) and the metabotropic glutamate receptor agonist *trans*-ACPD (300 μM) produced comparable reductions (*n*=5, mean decrease 33% and 29% respectively). We conclude that quisqualate is acting via the metabotropic glutamate receptor. Qualitatively similar, but smaller, responses have been reported for cultured hippocampal neurons (Lester & Jahr, 1990, Neuron 4: 741-749). Supported by NINDS grants NS16792 & NS20482.

383.2

TEMPERATURE DEPENDENT BLOCK OF NMDA RESPONSES BY TTX, STX AND PHENYTOIN. M.J. McLean and A.W. Wamil. Dept. of Neurology, Vanderbilt Univ. Med. Ctr., Nashville, TN 37212

We studied the effects of tetrodotoxin (TTX), saxitoxin (STX), and phenytoin (PT) - three sodium channel blockers - on the responses of mouse spinal cord neurons in cell culture to NMDA at different temperatures with intracellular electrophysiological techniques. Neurons were quiescent in 7 mM Mg^{++}-containing phosphate buffer. Pressure application of 10^{-5}-10^{-3} M NMDA (3 sec) elicited depolarizing waves with action potential firing. STX, TTX, and PT blocked responses to serially applied NMDA in use- and concentration-dependent manner at 37°C. At 33°C, block by TTX ($1.5x10^{-8}$ M) and STX (10^{-8} M) did not occur. Increased PT concentrations (≥ten times) were required to produce use-dependent block at the reduced temperature, with or without TTX. Thus, NMDA block by toxins was temperature dependent and concentration-dependence of block by PT was shifted to the right by cooling.

383.3

SUSTAINED POTENTIATION OF NMDA RECEPTOR MEDIATED-GLUTAMATE RESPONSES THROUGH ACTIVATION OF PROTEIN KINASE C BY A μ-OPIOID. Li Chen[1] and Li-Yen Mae Huang[1,2] , Marine Biomedical Institute[1] and Department of Physiology and Biophysics[2], University of Texas Medical Branch, Galveston, TX. 77550

The μ-opioid receptor agonist, D-Ala[2]-MePhe[4]-Gly-ol[5]-enkephalin (DAGO), was found to decrease glutamate release and to potentiate the glutamate-activated currents in trigeminal neurons (Chen & Huang, Neurosci Abstr. 16:60, 1990). We have studied the postsynaptic modulation of NMDA-activated responses by DAGO in greater detail. All the experiments were performed on spinal trigeminal neurons in thin medullary slices using patch recording technique. DAGO caused a sustained increase in glutamate-activated currents that were mediated by NMDA receptors. To determine which second messenger system might mediate the sustained actions of DAGO, we studied the effects of the intracellularly applied protein kinase A (PKA) and protein kinase C (PKC) on NMDA-activated responses. PKA had no effect on NMDA-activated currents. On the other hand, PKC mimicked the effect of DAGO; a specific PKC inhibitor interrupted the sustained potentiation produced by DAGO. These results suggested that PKC plays a key role in mediating the action of the μ-opioid peptide. Supported by grants: NS23061 and RCDA NS01050.

383.4

CALCIUM-DEPENDENT INACTIVATION OF NMDA CHANNELS. P.Legendre†, C. Rosenmund & G. Westbrook. †INSERM U261, Paris, France and Vollum Institute, Oregon Health Sciences Univ., Portland, OR.

Calcium is permeant through N-methyl-D-asparate (NMDA) channels and can also lead to inactivation of the whole-cell current. We examined Ca-dependent inactivation of NMDA channels using whole-cell voltage clamp and cell-attached recording on cultured hippocampal neurons. An ATP regeneration solution was included in the patch pipette to retard current 'rundown'. In normal extracellular Ca (1-2 mM), 10 μM glycine and no added Mg, macroscopic currents evoked by 15 second applications of NMDA (10-100 μM) inactivated slowly following an initial peak. At -50 mV in cells buffered to $[Ca]i < 10^{-8}$ M with 10 mM EGTA, the inactivation time constant was ≈4 sec. Inactivation could be removed by holding the membrane potentials at +40 mV or by reducing the extracellular Ca (≤0.2 mM), suggesting that inactivation resulted from transmembrane calcium influx. The fraction of the peak current that inactivated was dependent on the extracellular calcium concentration, reaching a maximal inhibition of 43% at 1.3 mM [Ca]o. Further increases in [Ca]o accelerated the inactivation time constant without altering the maximal inactivation. Inactivation was significantly slower using BAPTA compared to EGTA, suggesting that the measured inactivation time constant reflects primarily the rate of [Ca]i buffering.

Inactivation was present following substitution of Ba or Sr for Ca; and was not blocked by intracellular perfusion with phosphatase inhibitors including fluoride, okadaic acid or calmidazolium. Raising intracellular Ca via KCl-induced depolarization also resulted in decrease in channel activity in cell-attached patches, suggesting that Ca-dependent inactivation occurs in intact cells and can be triggered by calcium entry through nearby voltage-gated calcium channels. This mechanism could play a role in down regulation of postsynaptic calcium entry during sustained synaptic activity and may reflect a direct action of Ca on the NMDA channel. Supported by INSERM, USPHS and the McKnight Foundation.

383.5

RUNDOWN OF NMDA CURRENTS: DEPENDENCE ON ATP AND CALCIUM. C.Rosenmund, P.Legendre† & G.L.Westbrook. †INSERM, U261, Paris; Vollum Institute, Oregon Health Sciences University, Portland, OR.

Progressive loss of activity can occur during whole-cell recording of both voltage- and liganded-gated ion channels. In the case of voltage-dependent Ca channels and GABA channels, this can been reversed by adding ATP to the patch pipette, an effect attributed to phosphorylation of the channel or a modulatory protein. Rundown of NMDA currents also occurs, and has been reported to be reversed with ATP, but insensitive to [Ca]i (MacDonald et al., *J.Physiol.* 414,17,1989). However increases in [Ca]i partially inactivate NMDA currents (see Legendre et al., adjacent poster), thus we examined whether ATP-dependent rundown of NMDA channels is also Ca-dependent. Whole-cell recordings were made on cultured rat hippocampal neurons. Intracellular dialysis was maximized using 1-2 MΩ pipettes while continuously monitoring the access resistance. Timed pulses of NMDA (10 μM, 0.3-3sec, 2/minute) were delivered using flow pipes controlled by a piezoelectric device. Glycine was 10 μM with no added Mg. 'ATP' solutions contained ATP 4, Mg 4-6, phosphocreatine 20, creatine kinase 50 U/ml. [Ca]i was 20 nM (BAPTA 2.4, Ca 0.4).

In normal solutions (2 mM Ca) without ATP, NMDA currents decreased to 40-60% of control over 30 minutes. Rundown was reversible, use-dependent, and was reduced by lowering [Ca]o or increasing the intracellular buffer. Essentially no desensitization or rundown was seen in Ca-free solutions. Dialysis with ATP prevented rundown in Ca-containing solutions, but the addition of alkaline phosphatase (100 μg/ml) or kinase inhibitors (staurosporine ,1 μM or calmidazolium,10-20 μM) to the patch solution did not reverse the effect of ATP, suggesting a mechanism other than phosphorylation. However, vanadate (50 μM) or reduction of extracellular sodium induced rapid rundown. It is plausible that ATP maintains NMDA responses by reducing Ca accumulation via a membrane ATPase or the Na/Ca exchanger. Supported by USPHS and the McKnight Foundation.

383.6

CONCANAVALIN-A AND INSULIN ACT VIA DIFFERENT MECHANISMS TO AFFECT GLUTAMATE RESPONSES IN *APLYSIA* NEURONS. P.S.Katz and I.B.Levitan. Department of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

The plant lectin concanavalin A (Con A) causes neurons from the marine mollusc, *Aplysia californica*, to respond to glutamate application with an inward cation current that reverses near zero mV (Kehoe, *Nature*, 274: 866-9, 1978). We investigated the mechanism underlying this effect. Responses of neurons in primary cell culture to pressure-applied glutamate were recorded with two electrode voltage clamp. In each of over 70 trials, application of Con A (50μg/ml) to the bath caused the inward current response to glutamate. However, when the interior of the cell was perfused with a whole cell patch electrode *prior* to Con A application, 5 of 8 cells did not develop this inward current response to glutamate. When the neuron was perfused *after* the establishment of the inward current response, 17 of 26 cells partially or completely lost the response. Thus, the induction of this response by Con A is dependent upon a diffusible substance in the cell.

Con A is known to bind to insulin receptors and insulin-like peptides are present in *Aplysia* (Van Minnen and Schallig, *Cell Tiss.Res.*, 260: 381-6, 1990). However, bovine insulin (Sigma) did not mimic or antagonize the effect of Con A, but instead acted in a dose-dependent fashion to block another glutamate-evoked current, carried by Cl^-.

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383.7

MODULATION OF ELECTRICAL SYNAPSES BETWEEN CULTURED *APLYSIA* NEURONS BY IMMUNOGLOBULIN. M. P. Wilson*, G. M. Carrow and I. B. Levitan. Dept. of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Electrical synapses form between *Aplysia* neurons in primary cell culture with high frequency and characteristic synaptic strength. We show that the strength of electrical synapses, measured as the junctional conductance between the cells, is much reduced when the neuron pairs are grown in the presence of nanomolar concentrations of an immunoglobulin fraction (IG) prepared from rabbit serum. By contrast, IG-depleted serum does not affect synaptic strength. Monovalent Fab fragments derived from the IG fraction similarly decrease the strength of electrical synapses. Furthermore Fab fragments reversibly block the modulation of synaptic strength by concanavalin A, a plant lectin that increases the junctional conductance between weakly coupled pairs of *Aplysia* neurons (Carrow and Levitan, J. Neurosci. 9(1989)3657-3664). SDS-polyacrylamide gel electrophoresis analysis demonstrates that more than 98% of the protein in the IG fraction is immunoglobulin and that no IG remains in the depleted serum. The results suggest that the inhibition of electrical coupling by mammalian serum reported in other cell culture systems is likely due to IG. These actions of IG on formation and modulation of electrical synapses may provide clues about molecular mechanisms of synaptic plasticity. Supported by NS25366 to I.B.L.

383.8

PHOSPHORYLATION SHIFTS UNITARY CONDUCTANCE OF CONNEXIN43 GAP JUNCTION CHANNELS. A.P.Moreno, G.I. Fishman*, J. Saez, E.L. Hertzberg and D.C. Spray A. Einstein College of Medicine, Bronx, NY 10461

The gap junction proteins found between oligodendrocytes and between certain neurons (connexin32) and between astrocytes (connexin43) are phosphoproteins. Phosphorylating agents display various actions on gap junctional conductance (g_j) in different tissues. To explore the effects of these treatments on a known connexin, we transfected a communication-deficient cell line (SKHep1, derived from a highly metastatic human hepatoma) with cDNA encoding human connexin43 (hCx43). Clones of stable transfectants were isolated in which Northern and Western blots and g_j measurements verified abundant connexin43 expression. Treatment with the phosphorylating agents TPA (a tumor promoting phorbol ester that activates kinase c), 8 Br-cAMP (a membrane permeant cAMP analogue that activates kinase a), forskolin (which activates adenylyl cyclase), and okadaic acid (which inhibits phosphatases and increased hCx43 phosphorylation) or with staurosporine (which inhibits kinase activation and decreased hCx43 phosphorylation) did not appreciably change g_j between pairs of these cells. All these agents, however, had marked effects on unitary junctional conductance (γ_j). hCx43-transfected cells exhibit two γ_j values, 60 and 90 pS; phosphorylating agents favored the 60 pS and less voltage dependent state, while staurosporine favored the 90 pS and more voltage dependent state. Mutants expressing truncated hCx43 (phosphorylation sites removed) exhibited voltage dependence that was similar to the parental hCx43 and single values of γ_j. We conclude that phosphorylation of hCx43 reduces the unitary conductance of gap junction channels and reduces their voltage dependence and that these phosphorylation sites are not required for insertion or assembly of hCx43 into functional channels.

383.9

REDUCTION OF [Ca^{2+}] NEAR THE PLASMA MEMBRANE FOLLOWING INFLUX IN AtT-20 PITUITARY CELLS. S.J. Korn and R. Horn. Neurosciences Dept., Roche Inst. of Molec. Biol., Nutley, N.J 07110.

The duration of calcium-dependent chloride currents ($I_{Cl(Ca)}$) in voltage clamped AtT-20 pituitary cells was used as an indirect measure of the timecourse of [Ca^{2+}] reduction near the plasma membrane following Ca^{2+} influx through plasma membrane Ca^{2+} channels. Increasing extracellular pH (pH_o) from 7.3 to 8.0 reversibly prolonged $I_{Cl(Ca)}$ tail currents in perforated patch recordings from cells bathed in Na^+-free solutions. This prolongation was prevented in standard whole cell recordings when the pipet solution contained 0.5 mM EGTA. The prolongation was not due to alteration of intracellular pH, since it still occurred when intracellular pH was buffered with 80 mM HEPES. The prolongation occurred by a mechanism other than by a direct action on Ca^{2+} channels, since tail currents were prolonged when pH_o was changed rapidly during the tail current, long after all Ca^{2+} channels were closed. The prolongation of $I_{Cl(Ca)}$ was not due to a direct action on Cl^- channels, since changing to pH_o 8 did not prolong Cl^- tail currents when intracellular [Ca^{2+}] was fixed. Raising pH_o did, however, prolong depolarization-evoked [Ca^{2+}] transients, measured directly with the Ca^{2+} indicator dye, fura-2. Taken together, these data demonstrate the presence of a Na^+-independent, pH_o-sensitive mechanism for reduction of $[Ca^{2+}]_i$ following influx through Ca^{2+} channels. We suggest that this mechanism is the plasma membrane Ca^{2+} ATPase.

383.10

LIMITATIONS IMPOSED UPON CHANNEL ACTIVATION BY LOCAL Ca^{++} CONCENTRATION: A MODELLING STUDY. L.D. Partridge, D. Swandulla* & T.H. Müller*. Dept. of Physiology, Univ. of New Mexico, Albuquerque, NM 87131 & Dept. of Membrane Biophysics, Max-Planck-Institute, D-3400 Göttingen, FRG.

The rapid rise in intracellular Ca^{++} concentration that occurs during activity of many neurons is responsible for the subsequent activation of potassium, chloride, and non-specific cation (CAN) channels. Details of Ca^{++} binding to these channels is not well understood. We have modelled the local environment of CAN channels with data from molluscan neurons in an attempt to determine some of the conditions for direct channel activation that result from the punctate delivery of Ca^{++} into the cell through voltage-activated calcium channels.

Ca^{++} concentration in the vicinity of an open calcium channel was calculated as a function of distance and time assuming diffusion from a point source into a semi-infinite space that contains a fixed Ca^{++} buffer. Interaction of Ca^{++} with CAN channels was modelled by using either a Hill equation or a concerted allosteric interaction. In the model, calcium and CAN channels were placed in a hexagonal array at either their measured average inter-channel spacing or clustered more closely together than this distance. Whole-cell CAN current was calculated as a function of inter-channel spacing and the K_D, Hill coefficient, or allosteric constant of Ca^{++}'s interaction with the CAN channel and then compared with the CAN currents measured in bursting *Helix* neurons. The necessity for channel clustering was a very sensitive function of K_D, n, or the allosteric constant with clustering required for expected values of these constants. These predictions are consistent with our patch clamp studies where infrequent patches that contained CAN channels each had an average of 3.4 channels.

383.11

KINETIC DIFFERENCES BETWEEN THE ONSET AND RECOVERY OF Ca^{2+}-DEPENDENT CALCIUM CHANNEL INACTIVATION. M.W. Fryer and R.S. Zucker. Dept. of Mol. & Cell Biology, Univ. of Calif., Berkeley, CA 94720.

We have previously shown that a rapid drop in $[Ca^{2+}]_i$ during a long depolarizing pulse did not cause a step increase in calcium current (I_{Ca}) but merely reduced the subsequent rate of inactivation (Fryer & Zucker, Soc. Neurosci. Abs. 16:1171, 1990). In this study we have investigated the onset kinetics of Ca^{2+}-dependent inactivation of I_{Ca} by suddenly increasing $[Ca^{2+}]_i$ with caged Ca^{2+}. Identified neurons from the dorsal surface of the Aplysia abdominal ganglion were pressure injected with 60% Ca^{2+}-loaded DM-Nitrophen to a final concentration of approximately 2-5mM. A brief, intense light flash given during a voltage step from -40mV to -5mV induced a large (60%), rapid, step-like decrease in I_{Ca} that could be well fitted by the sum of two exponentials (τ_1=3.4ms, τ_2=72.3ms). Similar rapid effects were seen using Ba^{2+} as the charge carrier. A rapid $[Ca^{2+}]_i$ drop elicited by photolysis of the caged BAPTA derivative Diazo-4 had no effect on I_{Ba}. A flash given 20ms before the voltage step typically reduced the peak I_{Ca} by 50% but had little effect on the normalized decay of I_{Ca}. The results show that the onset kinetics of I_{Ca} inactivation induced by photoreleased Ca^{2+} are fast and the recovery kinetics of inactivation seen after rapid $[Ca^{2+}]_i$ buffering are slow. The much slower rate limiting step for recovery might reflect the re-phosphorylation rate of inactivated calcium channels. Supported by an NH&MRC C.J. Martin Fellowship to MF and NIH Grant NS 15114.

383.12

INHIBITION OF ODOR-SENSITIVE CYCLIC NUCLEOTIDE GATED CHANNELS BY INTRACELLULAR Ca^{2+}: A POSSIBLE MECHANISM FOR SENSORY ADAPTATION. F. Zufall*, S. Firestein, H.F. Gerardo* and G.M. Shepherd. Section of Neurobiology, Yale University School of Medicine, 333 Cedar St., New Haven, CT, 06510 and Physiological Institute Technical University, D-8000 Munich 40 Germany.

We have previously described an odor-sensitive cation channel in salamander olfactory receptor neurons which can also be gated by the cyclic nucleotides cAMP and cGMP. This ion channels is believed to mediate olfactory transduction in vertebrates.

Here we report that intracellular calcium directly regulates the activity of this channel. With 100 nM Ca^{2+} present on both sides of the membrane, a saturating concentration of cAMP (0.1 mM) applied to an inside-out patch elicited strong channel activation with an open probability of about 0.6. When the intracellular Ca^{2+} concentration was raised to 3mM, channel activity markedly decreased, to an open probability of 0.09. This effect was reversible and did not require ATP. The IC_{50} value for the Ca^{2+} mediated channel inhibition was approximately 1mM. The decrease in the mean open probability was correlated with an increase in channel closed time; neither open time nor channel amplitude were affected in 3mM Ca^{2+}. Other non-desensitizing channel properties, such as cyclic nucleotide sensitivity, were also unaffected by Ca^{2+}. This effect of odor-sensitive channel inhibition by intracellular Ca^{2+} provides a mechanism which may contribute to sensory adaptation in olfaction.

A cyclic nucleotide gated channel has also been identified in isolated rat olfactory receptor neurons and preliminary analysis suggests that it possesses similar properties to those found in amphibia.

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383.13

SINGLE CHANNEL STUDY OF A Ca-ACTIVATED K CURRENT ASSOCIATED WITH RAS-INDUCED TRANSFORMATION. Y. Huang*, D. McDonald* and S. Rane. Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Ras oncogene transformation of fibroblast cell lines correlates with constitutive appearance of a Ca-activated K current recorded by whole-cell patch clamp. Little or no K current is available for activation in confluent normal cells (Amer.J.Physiol. **260**: C104-C112, 1991). Passage of normal cells and maintenance in fresh serum greatly increases activateable current (after 2 days), coincident with an increase in rate of cell division. Also, proliferation is inhibited by TEA in the growth medium at concentrations which also block the K current.

To better understand regulation of the Ca-activated K current and its role in cell proliferation we have characterized it at the single channel level. More than 85% of inside-out patches from ras-transformed cells contain a channel class which: is highly-selective for K over Na ($P_{Na}:P_K << 0.02$); requires ≥ 0.1 µm intracellular free Ca for activation; shows an increase in opening probability up to 10 µm free Ca; and, displays little or no voltage-dependence. These characteristics are consistent with those of the whole-cell, Ca-activated K current. The channel is inwardly rectifying in symmetric 150 mM K (33/17 pS at Em -60/60 mV, respectively). In outside-outside patches, replacement of extracellular 150 mM K with 37 mM K results in a more linear I-V curve, consistent with the linear appearance of whole-cell Ca-activated ramp currents in asymmetric K. Therefore, the physiology of the whole-cell and single channel currents from ras-transformed cells are identical. Pharmacological identity of these currents is being tested by applying blockers for the whole-cell current to outside-out patches containing the K channel. The selective appearance of the channel with ras transformation or mitogenic stimulation suggests a modulatory role for activated ras protein. This idea will be tested by application of purified protein to confluent, nontransformed cells in both whole-cell and single channel recording configurations.

383.14

PROSTAGLANDIN E_2 SELECTIVELY INHIBITS CALCIUM-ACTIVATED POTASSIUM CHANNELS IN RAT BRAIN SYNAPTOSOMES. J. Ren* and C.G. Benishin, Department of Physiology, Faculty of Medicine, University of Alberta, Edmonton, Alberta CANADA, T6G 2H7.

This study was designed to determine whether prostaglandin E_2 (PGE_2) is capable of affecting certain nerve ending potassium channels, and its subcellular mechanism. ^{86}Rb was used to quantitate nerve ending potassium channel activities as described previously (Benishin et al, Mol. Pharmacol. 34:152, 1988). Synaptosomes were pretreated for 30 min with PGE_2 (1 uM) before the efflux study was initiated. The results showed that PGE_2 could inhibit the calcium-activated component of ^{86}Rb efflux (Ca-K) in a dose-related manner, but had no effect on the resting or voltage stimulated components. This inhibition could be reversed by the protein kinase C inhibitor H7 (10 uM). PGE_2 did not inhibit synaptosomal ^{45}Ca uptake suggesting that the action of PGE_2 is not secondary to inhibition of voltage-gated calcium channels. These results suggest that PGE_2 modulates nerve ending potassium channels, and that protein kinase C may be involved.

384.1

AN ASSAY FOR NICOTINIC RECEPTOR FUNCTION IN MOUSE BRAIN BY MEASUREMENT OF ION FLUX. M.J. Marks, S.R. Grady* and A.C. Collins Institute for Behavioral Genetics, University of Colorado, Boulder, CO.

A widely applicable, convenient, and sensitive biochemical assay for nicotinic receptor function in the central nervous system has been difficult to establish. Since nicotinic receptors are ligand gated-ion channels, an assay using nicotine-induced ^{86}Rb efflux to measure receptor activation has been developed. Synaptosomes were prepared from mouse midbrain (thalamus and mesencephalon) by Percoll gradient centrifugation. The synaptosomal fraction prepared in this manner was enriched in [^{3}H]nicotine binding and could actively accumulate ^{86}Rb by a ouabain-sensitive process. The rate of ^{86}Rb release from the tissue was monitored by superfusion. ^{86}Rb efflux was stimulated by nicotine in a time- and concentration-dependent fashion. Using a 2 min stimulus period the EC_{50} for nicotine was about 1 μM. The nicotine-induced increase in ion efflux was completely inhibited by 10 μM mecamylamine. The extent of mecamylamine inhibition was dependent upon time of exposure to the antagonist. The results indicate that functional nicotinic receptors in mouse brain can be measured using a simple in vitro assay system.
Supported by DA 03194 and DA-00116.

384.2

NICOTINE INDUCES C-FOS EXPRESSION IN RAT BRAIN AND DIMINISHES THE RESPONSES TO METRAZOLE. B. Sharp,* S. Beyer,* K. McAllen,* S. Nicol and S Matta. Endocrine-Neuroscience Res. Lab., Dept. of Medicine, Hennepin Cnty. Med. Ctr. and U. of Minnesota, Mpls., MN 55415

c-Fos mRNA and protein are rapidly expressed in the CNS in response to a variety of acute neuronal stimuli, including metrazole. The capacity of nicotine to induce c-fos mRNA expression and to modify the response to metrazole was determined using total RNA extracted from 6 regions of rat brain (pyriform, C8 and cerebellar cortex and hippocampus, dentate gyrus and medial habenula). c-Fos was detected after hybridization of dot blots or Northern transfers and quantified by computerized videodensitometry. One half hour after a single dose of nicotine (0.5-2.0 mg/kg b.w.), c-fos mRNA levels significantly increased by approximately 2-8 fold ; the magnitudes were dose and region-dependant. The hippocampus, dentate gyrus and medial habenula were most sensitive and responsive. c-Fos mRNA levels remained elevated for 1h and then receded toward baseline by 2h. A single pre-treatment dose of nicotine (2 mg/kg) failed to alter the c-fos response to metrazole (40 mg/kg) delivered 2h later. Three doses of nicotine, administered 2h apart followed by metrazole 2h later, appeared to reduce the c-fos response by 50% only in the hippocampus ($p<0.05$). In contrast, after injecting nicotine 3x/d for 10d, the response to metrazole was significantly attenuated in all 6 brain regions and the hippocampus appeared to be most affected (decreased by 66-90%). In summary, nicotine-induced c-fos expression is dose and region dependent, and chronic exposure to nicotine desensitizes the c-fos response to another ion channel-dependant (gaba receptor) neuronal stimulant, metrazole.

384.3

FUNCTIONAL STUDIES OF NEURONAL NICOTINIC RECEPTORS IN CHICK BRAIN SLICES. L.L. McMahon, W.R. Weaver* and V.A. Chiappinelli. Depart. of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis, MO 63104.

We have recently identified a kappa-bungarotoxin-insensitive neuronal nicotinic response in the mesencephalic lateral spiriform nucleus (SPL) of the newly hatched chick (Sorenson, E.M. and Chiappinelli, V.A., Neuron 5:307, 1990). Using conventional intracellular and whole cell patch-clamp recording techniques, we have further examined the functional properties of nicotinic receptors in SPL neurons. Electrical stimulation of a cholinergic fiber tract located laterally to the SPL (Sorenson, E.M., et. al., J. Comp. Neurol. 281:641, 1989) via an ultrasmall bipolar stimulating electrode elicited subthreshold and superthreshold responses from SPL neurons with a latency of 3-5 msec. These responses were blocked in the presence of 100μM $CdCl_2$, demonstrating their synaptic nature. In addition, the nicotinic antagonists d-tubocurarine (50μM) and dihydro-β-erythroidine (100μM) substantially blocked these responses, indicating that they were mediated by nicotinic receptors. Muscarinic receptors were continuously blocked during all of these experiments by the presence of 1μM atropine in the perfusion buffer. Nicotinic responses to the exogenously applied agonist carbachol were detected as early as 14 days of incubation. The chick SPL is thus a good model system for the pharmacological and developmental examination of central neuronal nicotinic acetylcholine receptors. Supported by NIH Grant NS17574 to VAC.

384.4

AN UNUSUAL CHOLINERGIC RESPONSE IN COCHLEAR HAIR CELLS. P.A. Fuchs and B.W. Murrow, Dept. Physiology, U. Colorado Med. School, Denver, CO 80262

Sensory receptor (hair) cells of the cochlea are subject to efferent neuronal regulation. Outer hair cells of mammals and birds are thought to be inhibited by the release of ACh from the efferent neurons. We have isolated outer hair cells (short hair cells - SHCs) from the chick's cochlea and examined their responses to cholinergic agonists under voltage-clamp. Both ACh and carbachol elicited outward currents from SHCs voltage clamped at -40 mV. The reversal potential of the outward current varied with changing K concentration and the instantaneous I-V relation was "N-shaped". That is, the K current grew with increasing driving force, up to a maximum near -10 mV (E_K at -80 mV). At increasingly positive membrane potentials the K current diminished, virtually disappearing at +20 mV. A similar I-V relation is displayed by Ca-dependent K current activated by voltage-gated Ca channels in these cells. Indeed, in many of the responses to ACh a small inward current could be seen to precede the larger K current, and this early inward current was more easily seen when the K current was reduced by replacement of internal K with Cs. These observations suggest that ACh might elicit K current in chick hair cells by causing Ca influx. In keeping with this suggestion, the response to ACh was dependent on external Ca and could not be elicited when external Ca was replaced with Mg.

ACh and carbachol had similar effects, however, neither nicotine nor muscarine (100 μM) produced any current on cells that responded well to 100 μM ACh. Curare (3 μM), atropine (3 μM), and tetra-ethyl ammonium (1 mM) all blocked the ACh response. Purified samples of 1 μM α- or k-bungarotoxin (Biotoxin) completely and reversibly blocked the response to 100 μM ACh. Supported by NIDCD DC00276.

384.5

RAPID ENHANCEMENT OF ACETYLCHOLINE (ACh) RESPONSES IN CHICK CILIARY GANGLION NEURONS. D.Gurantz, A.T. Harootunian*, R.Y. Tsien, V.E. Dionne*, J.F. Margiotta*. Dept. of Pharmacology, UCSD, La Jolla, CA 92093 and *Dept. of Physiology & Biophysics, Mt. Sinai. New York, NY 10029

Incubation of ciliary ganglion neurons in 8Br-cAMP and IBMX for 6h results in a 2-fold increase in ACh-induced conductance. When cAMP is applied intracellularly, a 50-70% increase in the ACh response is observed after only 2-5 min. Here we examined the effect of two agents, bovine serum albumin (BSA) and vasoactive intestinal peptide (VIP). BSA but not globulin or ovalbumin induced a dose dependent increase in the ACh response that was maximal (3.5-fold) with 10 mg/ml BSA within 5 min. The BSA and cAMP effects were not additive, suggesting that their signals ultimately converge on a common downstream site. Using a new cAMP imaging technique (Adams et al., Nature 349:694, 1991) we found no increase in cAMP levels in the neurons following treatment with BSA for up to 30 min. VIP, a cAMP-elevating neuropeptide present in avian ciliary ganglia, did cause a significant increase in cAMP within 1 min (18/19 cells). VIP treatment also rapidly increased the ACh-induced conductance by ~40% ($p<0.004$, $n=34$), similar to that seen previously following intracellular injection of cAMP. The cAMP-dependent and independent modulation of ACh responsiveness suggests that ciliary ganglion neurons may use a variety of first messengers and signaling pathways in modulating synaptic input. Supported by NIH NS20962, NS27177, NS24417 and HHMI.

384.6

FUNCTIONAL DESENSITIZATION OF DOPAMINE RELEASE FOLLOWING STIMULATION BY L-NICOTINE IN MOUSE STRIATAL SYNAPTOSOMES. S.R. GRADY, M.J. MARKS, AND A.C. COLLINS Institute for Behavioral Genetics, University of Colorado, Boulder, CO.

It has been proposed that upregulation of nicotinic receptor binding sites in response to chronic nicotine treatment could be caused by chronic receptor desensitization (Marks et al., 1983, J. Pharm. Exp. Ther. 226:817-825). To investigate whether mouse brain receptors can functionally desensitize, experiments were conducted using a continuous perfusion method of measuring release of previously loaded ^{3}H-dopamine from mouse striatal synaptosomes. Using various time intervals of L-nicotine stimulation, it was found that the maximum dopamine release obtainable in 5 min was 3 units above baseline (where 1 unit is 1 min of unstimulated baseline release) or 0.6 units/min, while a 1 min stimulation could release 1.4 units indicating that desensitization does occur. Other experiments indicated that at least 12-15 units could be released by nicotine under the proper conditions; therefore it is unlikely that the apparent desensitization was caused solely by depletion. Initial rate calculations from data obtained with 0.2 to 1 min stimulations indicate a maximum theoretical rate in the absence of desensitization of 10 units/min with an EC_{50} of 2.5 uM. Using 5-10 min exposures, desensitization appeared to be 1st order with a half-time of about 1 min and an EC_{50} of about 1 uM. Apparent first order recovery from desensitization had a half-time of about 10 min. The results indicate that dopamine release is desensitized by nicotine at concentrations similar to those that stimulate release. Supported by DA-03194, DA-00116, and AA-06391.

384.7

NOVEL MODULATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS BY CALCIUM. M. Amador*, S. Vernino*, C. Luetje, J. Patrick and J.A. Dani, Department of Molecular Physiology and Biophysics and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Nicotinic acetylcholine receptors (nAChRs) expressed in Xenopus oocytes were studied using two-electrode voltage clamp. The current through muscle nAChRs decreases when the extracellular calcium concentration is elevated. This decrease is consistent with the high affinity but low conductance of the muscle nAChR channel for Ca^{2+}. Ca^{2+} occupies the channel and decreases the single-channel currents that underlie the ACh-induced macroscopic currents. The current through neuronal nAChRs, however, increases dramatically as $[Ca^{2+}]_o$ is increased and decreases as $[Ca^{2+}]_o$ is decreased. Ba^{2+} and Mg^{2+} do not substitute for Ca^{2+}. Increasing $[Ba^{2+}]_o$ or $[Mg^{2+}]_o$ decreases the current through both neuronal and muscle nAChRs. All 6 α ß-subunit combinations that form functional neuronal nAChR channels in oocytes are modulated by calcium.

To verify the results, neuronal nAChRs were examined in bovine chromaffin cells and PC-12 cells. Ca^{2+}-enhancement of ACh-induced currents is seen even when excised patches or whole cells are internally dialyzed with simple electrolyte solutions containing calcium chelators. Onset of and recovery from calcium enhancement are rapid and repeatable for as long as the giga-ohm seal lasts. The calcium modulation is seen over a wide range of voltage. Taken together these results indicate that a Ca^{2+}-dependent intracellular cascade is not a likely mechanism for the modulation of neuronal nAChRs. Supported by MDA, Whitaker Foundation, BRSG, and NIH.

384.8

HIGH CALCIUM PERMEABILITY OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS. S. Vernino*, M. Amador*, D.S. Zatechka*, K. Flood*, C. Luetje, J. Patrick and J.A. Dani, Department of Molecular Physiology and Biophysics and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Three different sets of experiments indicate that the neuronal nicotinic acetylcholine receptor (nAChR) channel has a higher Ca^{2+} permeability than the muscle nAChR. In the first set of experiments, nAChRs expressed in *Xenopus* oocytes were studied using two-electrode voltage clamp. Macroscopic currents through neuronal nAChR channels expressed in oocytes cause a Ca^{2+}-dependent activation of an endogenous Cl^- conductance. These results indicate that Ca^{2+} is entering the oocyte through neuronal nAChRs, and then, the Ca^{2+} activates the Cl^- conductance. Under the same experimental conditions, currents through muscle nAChRs did not activate this Ca^{2+}-dependent Cl^- conductance. In a second set of experiments, oocytes and cultured cells were used to measure reversal potentials as $[Ca^{2+}]_o$ increased from 1.8 to 18 mM. The magnitude of these reversal potential shifts indicate that the Ca^{2+} permeability of the neuronal nAChR is intermediate between the muscle nAChR and the NMDA-subtype of glutamate receptor. In a third set of experiments, simultaneous current measurements and optical measurements of $[Ca^{2+}]_i$ with fura-2 indicate that the neuronal nAChR of bovine chromaffin cells conducts Ca^{2+} well. A large fluorescent fura-2 signal is seen when neuronal nAChRs are activated by the nicotinic agonist, DMPP, in the presence of atropine. Supported by MDA, Whitaker Foundation, BRSG, and NIH.

384.9

NEURONAL NICOTINIC RECEPTOR BINDING IN RAT BRAIN: A COMPARISON OF THREE RADIOLIGANDS D.J. Anderson and S.P. Arneric, Neuroscience, Pharmaceutical Discovery Division, ABBOTT Laboratories, Abbott Park, IL 60064-3500.

Neuronal nicotinic receptors (nAChRs) have been defined with the nicotinic agonists, [3H](-)nicotine ([3H]-NIC) and [3H]methyl-carbamylcholine ([3H]-MCC). These ligands are plagued with problems of stability and reproducibilty. Recently, a new tritiated ligand, [3H](-)cytisine ([3H]-CYT), has become available (Pabreza, et al., *Mol. Pharmacol.*, **39**:9-12, 1991). CYT is a very high (Ki = 0.1 nM) affinity agonist that is very selective for the nicotinic receptor. Bmax and Kd values were determined for [3H]-CYT, [3H]-MCC, and [3H]-NIC from Scatchard analysis using a brain membrane enriched fraction prepared from Sprague-Dawley rats. Subsequently, the affinities of 11 reference compounds were determined against each ligand from concentration-inhibition curves. Correlations of affinities for each ligand were calculated to probe possible relationships between populations of receptor subtypes. To validate selectivity for nAChRs cholinergic receptors, the same series was tested against [3H]-QNB, a muscarinic receptor antagonist. Scatchard analysis indicated a one-site model for the three nicotinic ligands. Respective Kd values for [3H]-CYT, [3H]-MCC, and [3H]-NIC were 0.13 nM, 1.0 nM, and 0.75 nM, while Bmax values were 126, 66, and 113 fmol/mg protein. The rank order potencies of Ki values against all three ligands were (ranging from 0.13 to 200,000 nM): CYT> (-)NIC, lobeline, MCC> DMPP, (+)NIC,> carbachol, arecoline> > THA, oxotremorine> > heptyl-physostigmine. Correlations between the three nicotinic ligands revealed close agreement of profiles with all slopes very near to 1.0 and all r^2 values >0.95. In contrast, most of the reference compounds had weak affinity for [3H]-QNB (Ki s ranging from 1000-300,000 nM). Remarkably, CYT was 3,000,000-fold selective for the nAChR. These data suggest that [3H]-CYT, [3H]-MCC, and [3H]-NIC bind to the same population of nAChRs in rat whole membrane preparations.

384.10

METHYLLYCACONITINE (MLA) IS A POTENT ANTAGONIST OF NICOTINIC ACETYLCHOLINE RECEPTORS (AChR) ON RAT HIPPOCAMPAL NEURONS: SINGLE CHANNEL STUDIES. E.F.R. Pereira[1,2], S. Wonnacott[3] and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exptl. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201; [2]Lab. Mol. Pharmacol. II, IBCCF, Brazil 21944; [3]Dept. Biochem., Univ. Bath, Bath, England BA2 7AY.

Recent biochemical studies have shown that an alkaloid extracted from seeds of the *D. brownii*, MLA, is a potent competitive blocker of α-BGT binding to AChR in mammalian brain (*FEBS Lett.*, **226**:357, 1990). In addition, preliminary studies demonstrated that nicotinic agonists activate single channel and whole-cell currents in hippocampal pyramidal cells (*FEBS Lett.*, **222**:63, 1987; *Eur. J. Pharmacol.*, **191**:505, 1990). Here, we evaluated AnTX-activated single channel currents recorded from outside-out patches excised from fetal rat hippocampal neurons grown in culture for 20-30 days. The effects of MLA on these currents were analyzed. All solutions contained TTX (0.1 μM), atropine (1 μM) and APV (50 μM), and were delivered via a glass mini-pipe connected to a perfusion system. AnTX (1 μM) activated single channel currents that appeared mainly as brief, isolated events. The most predominant conductance was 22 pS. The channel open times were fitted to a single exponential function whereas the closed and burst times showed double exponential distributions. Open times appeared to be voltage dependent such that they increased with membrane hyperpolarization. MLA (1-100 fM) had no effect on open times of AnTX-activated single channel currents. However, MLA significantly reduced the frequency of channel openings. This effect was concentration-dependent, and in the presence of 1 pM MLA practically no channel activation was detected. Such an effect was completely reversed after wash-out. Therefore, our eletrophysiological results confirm that MLA is an important probe for AChRs present in the CNS. Support: U.S. Army Med. Res. & Devel. Comm. Contr. DAMD17-88-C-8119 & USPHS NS25296. CAPES Fellow, Brazil (EFRP).

384.11

METHYLLYCACONITINE (MLA) A POWERFUL ANTAGONIST OF NICOTINIC ACETYLCHOLINE RECEPTORS. D. Bertrand, S. Bertrand*, M. Ballivet and S. Wonnacott. Dpt of Physiology, Medical School,1 Rue Michel Servet, 1211 Geneva 4, Switzerland.

The natural insecticide MLA from the plant *Delphinium brownii* is an antagonist of cholinergic transmission in invertebrates. We have investigated the effects of MLA on avian neuronal nicotinic acetylcholine receptors (nAChRs). MLA was assayed on α4/nα1, α3/nα1 and α7 receptors expressed in *Xenopus* oocytes following nuclear injection with chicken cDNAs. Application of MLA alone had no effects on the holding current in any cells we tested. However, when MLA was applied simultaneously with acetylcholine (ACh) a reduction of the response was observed. Receptors formed with α3/nα1 are more sensitive to MLA than α4/nα1. Application, in perfusion, of 10 μM MLA on α3/nα1 abolished currents evoked by ACh (1 μM). The onset and recovery from MLA was fast and indistinguishable from the perfusion time. The homo-oligomeric nAChR formed by α7 was extremely sensitive to MLA, much more so than α3/nα1 or muscle ($\alpha\beta\gamma\delta$) nAChR. α7 receptors expressed in *Xenopus* oocytes were measured by ^{125}I-α-bungarotoxin binding to individual oocytes: there was excellent correlation between toxin binding and ACh-induced currents. Toxin binding was totally displaced by 1 μM MLA.

384.12

NON-COMPETITIVE INHIBITORS ALTER NICOTINIC ACETYLCHOLINE RECEPTOR AFFINITY FOR AGONIST M.W. Stephens, S. Wonnacott & P. Whiting♦ Dept. Biochem., Univ. Bath, Bath BA2 7AY UK ♦Merck, Sharp & Dohme, Harlow CM20 2QR UK

The anti-convulsant MK801, and mecamylamine, a ganglionic antagonist, are believed to operate by physically blocking the ionophore of NMDA and nicotinic acetylcholine (nACh) receptors respectively. (-)[^{3}H]-Nicotine, which labels the α4β2 subtype of neuronal nAChR, binds to a detergent (Triton X-100) extract of rat brain membranes with parameters K_d=12±2nM, B_{max}=90±15 fmol/mg protein (n=13). MK801 (500μM), shown to crossreact with nAChR channels, reduces affinity for [^{3}H]nicotine (K_d=30±4nM; n=3) by a decrease in association rate (the dissociation rate remains unchanged); the number of sites (B_{max}) is comparable to control. Mecamylamine (60-600μM) induces a curved scatchard plot, characteristic of positive cooperativity. DHBE, a competitive antagonist, reduces B_{max} in a predictable fashion, whilst K_d is unaltered. Thus, non-competitive inhibitors can influence agonist binding, but may operate by differing molecular mechanisms.

Functional correlates have been explored by ion flux assay, using a stably transfected mouse cell line expressing avian α4 and β2 subunits.

(Supported by SERC CASE studentship to MWS)

384.13

EFFECT OF NONCOMPETITIVE BLOCKING DRUGS ON LIGAND BINDING TO NEURONAL NICOTINIC RECEPTOR . B.A. Dodson and L.M. Braswell*. Dept. of Anesthesia, UCSF, San Francisco, CA 94143-0648

This study examines the effect of chlorpromazine, haloperidol, pentazocine, d-tubocurarine, diazepam, pentobarbital and diphenylhydantoin (DPH) on high affinity postsynaptic nicotinic, cholinergic binding to synaptosomes prepared from freshly dissected rat cerebral cortex. The binding of L-^{3}H-nicotine (15 nM) to neuronal nicotinic acetylcholine receptors (nAChR) (200 µL 5% brain homogenate, 250-280 µg protein) with and without drug (10^{-9} to 10^{-3} M) was determined by centrifugation assay. Experiments were performed in tripicate in TRIS/HEPES buffer, pH 7.4 at 0°C. Nonspecific binding was defined as that occuring in the presence of excess (1 ml) L-nicotine with specific binding defined as the difference between total and nonspecific binding (~45-50% of total binding). With the exception of DPH, all of the drugs tested inhibited L-^{3}H-nicotine binding in a concentration-dependent, saturable fashion consistent with a mass action mechanism with EC_{50}s for half maximal inhibitions similar to that of the competitive antagonist d-tubocurarine (60 µM) and a rank order of haloperidol < diazepam < chlorpromazine < d-tubocurarine < pentazocine < pentobarbital . They did, however, vary in efficacy with maximum inhibitions ranging from < 20% of control binding (chlorpromazine and pentobarbital) to approximately 50% of control (haloperidol, diazepam and pentazocine). By contrast, DPH had minimal effects (< 10% of control) at its saturation limits (10^{-4} M). These results are similar to those reported for *Torpedo* nAChR as well as for $GABA_A$ receptors (Schwartz RD Biochem Pharmacol **37**:3369-3375, 1988) and support the hypothesis of functional as well as structural homology between nicotinic and $GABA_A$ receptor-gated ion channels. (Supported by Univ. Ca. TRDRP Grant 1RT-352)

384.14

EFFECTS OF A NOVEL NITROMETHYLENE INSECTICIDE ON COCKROACH NICOTINIC SINGLE CHANNELS.
[1,2]H. Cheung*, [2]E.W Harris and [1]D.J. Beadle*.
[1]School Biol & Mol Sci, Oxford Polytechnic, Oxford UK & [2]Biology Dept., Fisons Pharmaceut., Rochester, NY USA

The suggested site of action of the nitromethylene heterocycle (NMH) insecticides is at the nicotinic acetylcholine receptor. A potent new NMH insecticide, Compound I produced prolonged inward currents under voltage-clamped conditions. Both acetylcholine and Compound I produced transient inward currents, but the rate of current inactivation for acetylcholine was much faster than for Compound I. Single-channel studies were therefore performed to reveal the mechanism of action of Compound I.

Under cell-attached patch, Compound I induced unitary currents similar to those induced by acetylcholine at both resting and hyperpolarizing potentials. At resting potential, two categories of unitary currents were observed for both acetylcholine (1.66±0.34 & 2.83±0.37 pA) and Compound I (1.89±0.4 & 3.42±0.4 pA). With a 60mV hyperpolarization, the mean amplitudes and mean open-times increased for both acetylcholine and Compound I. The probability of channel opening was higher for Compound I (0.096±0.004 vs. 0.042±0.007). Compound I also induced unitary currents with a longer mean open-time than did acetylcholine.

These results suggest that NMH insecticides may act by prolonging nicotinic channel-opening. The longer mean open-time observed in the presence of Compound I may explain the slow inactivation observed under whole-cell voltage-clamp studies.

(This work generously supported by SERC & Shell Research Centre, Sittingbourne, Kent UK.)

384.15

THE EFFECTS OF DITHIOTHREITOL (DTT), A REDUCING AGENT, ON THE INHIBITORY NICOTINIC RECEPTOR ON RAT DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS. E.M. Sorenson and J.P. Gallagher. Dept of Pharmacol. and Toxicol., Univ. of Texas Med. Br., Galveston, TX 77550.

This laboratory has previously demonstrated that nicotinic agonists produce a direct inhibitory response at rat DLSN neurons (Wong and Gallagher, Nature 341:439, 1989 and J. Physiol. (Lond.) 436:325, 1991). To determine whether the agonist binding site on this novel inhibitory nicotinic receptor has structural similarities to that on excitatory nicotinic receptors, we have examined the effects of DTT on the inhibitory response. DTT abolishes excitatory nicotinic responses by reducing a disulfide bond at cysteines 192 and 193 in the alpha subunits of excitatory receptors. This disulfide bond is essential for agonist activation of the receptors (Kao et al., J. Bio. Chem. 259:11662, 1984; Loring et al., J. Neurosci. 9:2423, 1989).

Standard intracellular recording techniques were used to record from DLSN neurons in the *in vitro* brain slice. A control response to 1,1-methyl-4-phenylpiperazinium (DMPP), a nicotinic agonist, was obtained. The slice was then superfused with 1mM DTT at pH 8.0 for 20 min and the response to DMPP was retested. DTT did not abolish the response to DMPP (n=5). The effects of DTT on the response of each neuron to serotonin (5-HT) or N-methyl-D-aspartate (NMDA) were also tested. 5-HT_{1A} responses were unaffected but the NMDA responses were potentiated, as has been reported by others (Aizenman et al., Neuron 2:1257, 1989). The DTT treatment was also effective in abolishing an excitatory nicotinic response found in rat vestibular neurons.

We conclude that, unlike excitatory nicotinic receptors, the inhibitory nicotinic receptor on DLSN neurons does not require a disulfide bond at the agonist binding site for receptor activation.

(Supported by The Council for Tobacco Research-USA, Inc.)

384.16

EFFECTS OF REPLACEMENT OF BUFFER Na WITH N-METHYL-D-GLUCAMINE (NMDG) ON NICOTINE-INDUCED CHANGES IN SYNAPTOSOMAL ACCUMULATION OF [^{3}H]TETRAPHENYL-PHOSPHONIUM (^{3}H-TPP). C.J. Hillard and J.J. Pounds*. Dept. of Pharmacology, Medical College of WI, Milwaukee, WI 53226.

^{3}H-TPP is a membrane permeant, lipophilic cation which accumulates in synaptosomes and can be used to estimate membrane potential difference. We have demonstrated that nicotine and four other nicotinic agonists decrease the accumulation of ^{3}H-TPP in synaptosomes from rat cerebral cortex, suggesting that these agonists depolarize the synaptic plasma membrane. This effect of nicotine is blocked by the ganglionic blockers mecamylamine and hexamethonium but not by atropine. If the mechanism of action of nicotine in the CNS is the same as at peripheral nicotinic cholinergic receptors (nAChR), the effect of nicotine on ^{3}H-TPP accumulation ought to be sodium dependent. In this series of experiments, we have replaced sodium in the incubation buffer with NMDG. Removal of buffer sodium resulted in an increase in the synaptic membrane potential difference calculated using the concentration gradient of ^{3}H-TPP from -58 mV to -76 mV. Veratridine (60 µM), which produced a 40% decrease in ^{3}H-TPP accumulation in the presence of sodium, produced a 15% decrease in NMDG buffer. The depolarizing effect of increasing extrasynaptosomal potassium concentration was identical in both buffers. The decrease in ^{3}H-TPP accumulation produced by nicotine (100 µM) was enhanced in the NMDG buffer (42 fmol/0.4 mg protein compared to 18 fmol/0.4 mg protein in sodium buffer). If buffer KCl concentration was increased to 12 mM to reduce the initial membrane potential to -64 mV, nicotine decreased ^{3}H-TPP accumulation by 15 fmol/0.4 mg protein. Therefore, replacement of buffer sodium with NMDG did not diminish the effect of nicotine on membrane depolarization. However, since the effect of nicotine was found to be dependent on initial membrane potential, these studies raise the possibility NMDG may itself enter and depolarize the synaptosome through the nAChR ionophore. Supported by USPHS grant R29-DA04800.

384.17

ENRICHMENT OF PRESYNAPTIC CHOLINERGIC RECEPTOR BINDING SITES ON PERCOLL GRADIENTS S. Wonnacott & G. Wilkie*. Dept. Biochem., Univ. Bath, Bath BA2 7AY, UK.

Subcellular fractionation of brain tissue on Percoll discontinuous density gradients resolves intact nerve terminals (synaptosomes) from membrane fragments of pre- and postsynaptic origin[1]. This contrasts with isopycnic procedures, in which membranes and synaptosomes are recovered in the same fraction.

We have characterised Percoll gradient fractions from rat cortex with respect to marker enzymes for plasma membranes (AChE), synaptosomes (occluded lactate dehydrogenase) and mitochondria (succinate dehydrogenase). The fractions were assayed for muscarinic and nicotinic cholinergic ligand binding sites. Whereas muscarinic receptors labelled with [^{3}H]QNB and [^{3}H]pirenzepine are enriched in the membrane fractions at the top of the gradient, specific binding of the nicotinic ligands [^{3}H]nicotine and [^{3}H]cytisine has a biphasic distribution, with enrichment also in the nerve terminal fractions. This is consistent with a predominantly presynaptic localisation of nicotinic receptors in the CNS, in accordance with their role in mediating transmitter release. The muscarinic agonist [^{3}H]oxotremorine-M displayed a similar biphasic profile, indicating that this fractionation technique may be useful in discriminating presynaptic binding sites.

[1] Thorne et al., 1991, J. Neurochem. 56, 479-484.

384.18

NICOTINIC RECEPTORS IN RAT CEREBRAL CORTEX ARE ASSOCIATED WITH THALAMOCORTICAL AFFERENTS. P.B.S. Clarke. Dept. of Pharmacology, McGill Univ., Montreal, Canada H3G 1Y6.

Nicotinic receptors labelled with ^{3}H-nicotine are prominent in most thalamic nuclei and in layers III/IV of cerebral cortex, which receive a major thalamic input (Clarke et al 1984). To test whether cortical nicotinic receptors are located on afferent terminals from thalamus, male Wistar rats were infused unilaterally with excitotoxic doses of N-methyl-D-aspartate (NMDA) under anaesthesia into one of four sites: anterior thalamus, ventral thalamus, medial geniculate nucleus or dorsal lateral geniculate nucleus. Control subjects received phosphate buffer. After one week survival, brain sections were processed for ^{3}H-nicotine receptor autoradiography. Within thalamic nuclei infused with NMDA, ^{3}H-nicotine binding appeared little altered or unchanged, despite a marked loss of neurons. In contrast, there was a substantial reduction in ^{3}H-nicotine binding in ipsilateral cerebral cortex, localized to the projection zone of the thalamic nuclei that had been lesioned. Thus, in several cortical areas, nicotinic receptors that are labelled with high affinity by ^{3}H-nicotine appear to reside on thalamocortical inputs. Supported by MRC (Canada).

384.19

MECHANISM OF THE HYPERTENSIVE RESPONSE TO CENTRAL INJECTION OF NICOTINE. J.J. Buccafusco. Dept. Pharmacology & Toxicology, Medical College of Georgia & the Department of Veterans Affairs Medical Center, Augusta, GA 30912.

The purpose of this study was to examine the effects of nicotine administered directly into the CNS on mean arterial pressure (MAP) and heart rate (HR) to avoid the direct peripheral action of the drug. Also, since nicotine has been reported to enhance the release of endogenous brain acetylcholine, we sought to determine the role of this mechanism in mediating the cardiovascular response. Rats were previously implanted with intracerebroventricular (icv) cannula guides and an arterial line (iliac artery). One week later each animal received a series of increasing doses of nicotine (or saline vehicle) from 2-100 μg (in a 10 μl volume) with each dose separated by at least one day. MAP increased immediately following all doses of nicotine, however, the maximal response was obtained following the 50 μg dose. In general, the response began immediately after injection and peaked within 2-3 min and returned to baseline within about 15 min. HR changes were often not dramatic and highly variable. In order to examine the dependence of the pressor response to nicotine on brain acetylcholine, rats were pretreated with 20 μg (icv) of hemicholinium-3 (HC-3) 1 hr prior to nicotine to deplete endogenous acetylcholine. HC-3 pretreatment resulted in a significant reduction in the magnitude and duration of the pressor response to nicotine. Likewise, pretreatment with atropine inhibited the pressor response to subsequent injection of nicotine. These data support the concept that icv injection of nicotine induces a pressor response through the release of endogenous acetylcholine possibly acting on central muscarinic receptors. Supported by HL30046 and the Dep. Vet. Affairs Med. Ctr.

PEPTIDES: ANATOMICAL LOCALIZATION I

385.1

THREE-DIMENSIONAL RECONSTRUCTION OF THE NEUROPEPTIDE NETWORK IN THE HUMAN CAUDATE NUCLEUS. M.S. Manley, S.J. Young, M.E. Martone, and P.M. Groves *Depts. Psychiatry & Neurosciences, Univ. Calif. San Diego, La Jolla, CA 92093-0603.*

Studies of rat and cat striatum have established the existence of a patch, or striosomal compartment, embedded in a surrounding matrix region. We have examined the distribution of neuropeptide markers for leucine enkephalin (LENK), Substance P (SP) and calbindin D (CaBD) in alternate 70μ sections of formalin-fixed postmortem tissue from the head of the human caudate nucleus, using computer-assisted three-dimensional reconstruction. Reconstructions were derived from camera lucida drawings outlining striatal regions of high or low immunoreactivity. Consistent with previous studies, LENK immunoreactivity in the human dorsal striatum appeared annular in contrast to the more homogenously labeled regions seen in the cat. Similar to the cat, reconstructions revealed the existence of considerable long-range order. Patches appeared aligned over distances of several millimeters to form long, horizontally oriented tubular structures in the dorsolateral caudate nucleus, with occasional orthogonal interconnecting crossbridges between the tubular structures. LENK-immunoreactive elements tended to fill the matrix in the ventromedial caudate and nucleus accumbens. In this area, LENK-poor areas are in register with CaBD-poor areas, which are known to correspond to striosomes, and can be followed over successive serial sections to similarly reveal tubular structures. As seen in rat and cat, CaBD-poor patches were more distinctly demarcated ventrally and generally smaller than LENK-patches. Reconstructions derived from this marker revealed a network similar to the LENK network in the dorsal striatum. The distribution of SP staining appeared complex. For example, in the mid-ventral caudate, SP-poor zones were in register with enkephalin-rich patches, while in the dorsal caudate some SP-rich zones were in register with LENK patches. Thus, the pattern of SP staining corresponds in a complicated way to the LENK network. This network is strikingly similar across individuals and may relate to the segregation and/or integration of parallel striatal circuits. *(Supported by NIDA 02854 & K0500079; ONR N00014-89-J-1254; and PHS 5T32 MH18398)*

385.2

RELATIONSHIPS OF PEPTIDE-CONTAINING NERVE FIBRES WITH NORADRENERGIC AND PUTATIVE CHOLINERGIC PELVIC NEURONS. J.R. Keast, Dept. Physiol. & Pharmacol., Univ. Queensland, Qld. 4072, Australia.

The major pelvic ganglion (MPG) of the male rat contains cholinergic and noradrenergic postganglionic neurons which supply the reproductive, lower urinary and digestive organs. Many peptides are also found in somata and nerve fibres of the MPG. These neurons receive inputs from either sympathetic or parasympathetic preganglionic neurons, and possibly also collaterals from visceral primary afferent axons. In the present study multiple staining immunohistochemical analyses of fixed MPG sections were carried out to determine the pattern of distribution of these nerve fibres with somata of different neurochemical types. Four main groups of neurons could be identified: noradrenergic neurons containing neuropeptide Y (NPY), and three groups of non-noradrenergic (putative cholinergic) neurons, those with NPY, those with vasoactive intestinal peptide (VIP) and a smaller group of enkephalin neurons (ENK); the latter contained various other combinations of additional peptides (galanin (GAL), substance P (SP), NPY and VIP). The peptides studied in varicose nerve fibres were ENK, GAL, SP, somatostatin (SOM), cholecystokinin (CCK) and bombesin (BOM). Each group of these peptide-containing fibres (except some ENK and fewer GAL) were closely associated almost exclusively with putative cholinergic neurons - very few noradrenergic neurons were surrounded by peptide containing fibres. Further specific associations were demonstrated within the non-noradrenergic neurons, with the majority of SOM and CCK fibres associated with non-noradrenergic NPY neurons and most SP fibres associated with VIP somata.

These results suggest that preganglionic inputs to noradrenergic neurons either contain no peptide or a peptide not included in this study. Furthermore, peptide markers for different subsets of preganglionic inputs to putative cholinergic neurons have been demonstrated, and may represent different functional pathways. Sensory fibres (represented by SP distribution) are also likely to communicate with a select group of MPG neurons. This information has provided further insights into the organization of neural pathways to the pelvic organs.

385.3

Catecholamine (CA) input to stress responsive, c-fos containing paraventricular (PVN) neurons. S.Pretel and D.T.Piekut, Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642

Peptide (e.g. enkephalin, CRF) containing neurons of the PVN are known to receive CA input. CA release, in particular that of noradrenaline (NA), is important for the modulation of stress response behavior. We have examined the CA input to PVN neurons of the hypothalamus that are specifically responsive to nociceptive stress.

Under ether anaesthesia, nociceptive primary afferents of the rat hindfoot were activated through application of mustard oil. Two-3hrs later, the animals were transcardially perfused with phosphate buffer followed by 4% paraformaldehyde. Vibratome cut 40μm tissue sections were processed for double-label immunocytochemistry using the Vectastain ABC Kit. C-fos like immunoreactivity (antibodies kindly provided by Drs.Slammon and Curran) was localized first, followed by dopamine-β-hydroxylase (DBH) or phenylethanolamine-N-methyltransferase (PNMT; both, Eugene, Inc.) using the regular or nickel enhanced 3,3' diaminobenzidine procedure. Some double-labeled sections were subsequently embedded in plastic (Immunobed, Inc.) and processed for semi-thin (1-2μm) sections.

The results showed c-fos labeled neurons predominantly in the parvocellular nuclei of the PVN and numerous DBH labeled fibers in the PVN, particularly in the dorsal and medial nuclei. PNMT labeled fibers were less numerous. However, the location of fibers labeled for either enzyme overlapped that of c-fos labeled neurons, as visible in 40μm sections. Analysis of semi-thin sections showed that numerous DBH as well as PNMT immunoreactive fibers were located in close anatomical proximity to c-fos positive neurons. This suggests that CA are able to potentially modulate the activity of stress responsive, i.e.c-fos containing PVN neurons. NS18626

385.4

PROJECTIONS OF THE COMMISSURAL NUCLEUS OF THE SOLITARY TRACT DEMONSTRATED BY PHA-L. L.J. Sim and S.A. Joseph, The Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642.

The commissural nucleus of the solitary tract (cNTS) participates in central autonomic control *via* catecholamine and opioid mechanisms. A small opiocortin neuronal pool has been identified in the cNTS and projects locally to brainstem nuclei. This is in contrast to the large arcuate opiocortin pool, from which we have previously identified extensive projections to the telencephalon, diencephalon, periaqueductal gray (PAG) and midline brainstem nuclei. This study was performed to expand upon our previous studies of the NTS opiocortin system by elucidating cNTS efferents and determining the relationship to specific neurons in terminal fields. PHA-L was iontophoresed into cNTS and immunocytochemically localized with subsequent dual immunostaining for peptides and transmitters. PHA-L-immunoreactive (-ir) fibers with terminals were identified in the bed nucleus of the stria terminalis, periventricular thalamus, hypothalamus and amygdala. In the brainstem, PHA-L-ir fibers and terminals were identified the PAG, lateral parabrachial nucleus, locus coeruleus/Barrington's nucleus and NTS, as well as lateral pontine and medullary nuclei important in cardiovascular function. Dual immunostaining revealed a correlation between NTS terminal fields and catecholaminergic cell groups. PHA-L-ir terminals were also identified in putative contact with peptidergic neurons in the brainstem. We provide neuroanatomical evidence that the cNTS can influence autonomic function. (Supported by USPHS DA07232, NS 21323 & AHA 87 1011)

385.5

EFFERENT PROJECTIONS FROM THE PARABRACHIAL NUCLEAR COMPLEX: CONNECTIVITY WITH NEUROPEPTIDERGIC FOREBRAIN STRUCTURES. S.A. Joseph and L.J. Sim. Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642.

Electrophysiological studies have shown that either directly or as a relay site the parabrachial nuclear complex communicates with important forebrain structures which modulate autonomic information. The parabrachial nucleus and its subdivisions have been singled out as an important pivot by which visceral afferent information is relayed to forebrain centers.

For this study we have utilized a sensitive anterograde tracer to elucidate connectivity from this complex and by dual labeling we have identified neurochemical substances which possibly mediate the connectivity. *Phaseolus vulgaris* leucoagglutinin (PHA-L), an anterograde tracing lectin, was iontophoresed into discrete regions of the parabrachial nuclear organization for subsequent analysis of the efferent terminations from the nuclear complex and their putative contact with neuropeptidergic neuronal elements. This analysis was performed using single and dual immunocytochemistry.

The study demonstrates parabrachial projections to periaqueductal grey, locus coeruleus, amygdala, bed nucleus of the stria terminalis and the paraventricular nucleus. With dual immunochemistry, some of these efferent terminals were seen in close proximity to CRF and enkephalin containing neuronal elements in the forebrain. These studies substantiate the supposition that the parabrachial autonomic center communicates with centers in the forebrain known to be important in regulating certain autonomic mechanisms. (Supported by American Heart Association #871011 and NIH NS21323 grants.)

385.6

SEXUAL DIFFERENCES IN THE PEPTIDERGIC INNERVATION OF THE QUAIL SEPTAL REGION UNDER VARIOUS HORMONAL CONDITIONS. C.Viglietti-Panzica, N. Aste, A. Fasolo, J. Balthazart and G.C.Panzica. Dept. Human Anatomy & Physiology, and Dept. Animal Biology, I-10126 Torino, Italy, Lab. Biochemistry, Univ. Liège, B-4020 Liège, Belgium.

A large population of LH-RH immunoreactive neurons was identified within the septal region of the Japanese quail (Cell Tissue Res., 253, 327-335, 1988), suggesting a direct involvement of this area in the control of avian reproduction. We have now analyzed the immunocytochemical distribution of four peptidergic systems [vasotocin (VT), corticotropin-releasing factor, substance P, and neuropeptide Y] within the quail septal region. The immunoreactivity showed a peculiar distribution in several nuclei, and some of them [lateral septum (SL), nucleus of the pallial commissure, and nucleus of the stria terminalis (nST)] contained more than two of the considered peptides. The VT innervation of SL and nST is sexually dimorphic: a dense network of immunoreactive fibres was seen in adult sexually stimulated males but not in females. The VT immunoreactivity was virtually absent when male quail were put in a short day photoperiod or were castrated and it was restored to the original level in castrated males by silastic implants of testosterone (T). No immunoreactivity was observed in adult sexually stimulated females, or in the other female groups (exposed to short-day, ovariectomized, or treated with T). In conclusion, present results suggest a direct role of peptidergic systems in regulating septal circuitries, and the possibility of their dependency by the levels of circulating sex hormones. *Supported by EEC (SC1-0230C/TT), CNR (89.03043.04, 90.02456.04), MURST 40%, and FRFC (9.4601.90 and 2.9003.91).*

385.7

ATRIAL NATRIURETIC PEPTIDE (ANP)-POSITIVE NEURONS OF THE CANINE ISLANDS OF CALLEJA COMPLEX (ICC) ARE COMPONENTS OF THE VENTRAL PALLIDUM. J. Kennedy*, L. Compton*, B.H. Turner and J.C. McKenzie*. Dept of Anatomy, Coll. of Medicine, Howard University, Washington, DC, 20059.

ANP has been localized in the ICC, distinct structures in the olfactory tubercle with unknown function. The purpose of the present study was to define the type and distribution of ANP-positive neurons in the canine ICC and to characterize these neurons by identification of their afferents. Dogs (6) were deeply anesthetized with sodium pentobarbital and perfused with saline followed by 4% formaldehyde. Brains were removed, blocked, immersed in sucrose and sectioned at 50 μm on a freezing microtome. Sections through the olfactory tubercle were incubated in ANP antibody (1:10,000) for 72 h at 4° C and processed by the ABC technique. Serial sections were immunostained for ANP, Substance P (SP, 1:2,000) and met-enkephalin (mENK, 1:10,000). Some sections were double stained for ANP and either tyrosine hydroxylase (TH), SP, mENK or neuropeptide Y (NPY) using the ABC and glucose oxidase (GO) techniques. ANP was localized in large neurons of the core, cap and arched bridge regions of the ICC. These neurons were similar in morphology to large neurons of the pallidum which also stained for ANP. Immunoreactivity for TH did not overlap with ANP-positive neurons. ANP-positive neurons were enmeshed within networks of SP- and mENK-positive fibers. It is concluded that ANP-positive neurons of the ICC are similar to large pallidal neurons in morphology, immunoreactivity and innervation, and may represent the most ventral portion of the pallidum. Supported by NIH grant HL45241 and a grant from Howard University.

385.8

THE EFFECT OF BILATERAL ELECTROLYTIC LESIONS OF THE CENTRAL AMYGDALOID NUCLEUS ON THE CONCENTRATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) IN MICRODISSECTED BRAIN REGIONS. S.M. Koegler, C.D. Kilts, M.J. Owens, G.N. Ervin, G. Bissette, and C.B. Nemeroff. Depts. Pharmacol. and Psychiat., Duke University Medical Center, Durham, NC 27710.

The projection fields of CRF-containing perikarya in the rat central nucleus of the amygdala (Ce) were studied using a combination of electrolytic lesioning methods, microdissection techniques and radioimmunoassay. After bilateral electrolytic lesions of the Ce, CRF concentrations were measured by a sensitive and specific radioimmunoassay (RIA) in the locus coeruleus, entorhinal cortex, prefrontal cortex, paraventricular nucleus, dorsal and ventral bed nucleus of the stria terminalis, arcuate nucleus and median eminence, lateral septal nucleus, lateral and ventromedial hypothalamic nuclei, dorsal and ventral parabrachial nuclei, and the nucleus of the solitary tract. Ce lesions significantly decreased the CRF concentration in the locus coeruleus (LC) and was the only brain region altered. This suggests the existence of a CRF pathway from the Ce to the LC which may mediate stress responsiveness. Plasma corticosterone concentrations of the lesioned rats were significantly increased when compared to the control animals. Subsequent studies will determine whether the marked increase in CRF concentrations in the LC after stress can be attenuated in rats with Ce lesions. (Supported by NIMH MH-42088).

385.9

COMPARATIVE FETAL DEVELOPMENT OF TACHYKININS IN THE BED NUCLEUS OF STRIA TERMINALIS AND AMYGDALA OF THE CAT. M.K. Boylan and R.S. Fisher. MRRC and Dept. of Anatomy, UCLA, Los Angeles, CA 90024.

The bed nucleus of the stria terminalis (BNST) and the central and medial nuclei of the amygdala are considered to be related developmentally. Similarities in their cytoarchitecture, connections and neuropeptide content have been characterized in the adult. Developmental studies indicate similar neuronal morphogenesis in these regions. However, neurochemical development in these structures has not been detailed. In order to test the hypothesis that the BNST and amygdala are developmentally related, we compared the ontogeny of tachykinin immunoreactivity (TK-I) within these regions in 18 fetal (F) kittens from F30-F60 (gestation in cat is 65 days). The anterior BNST (antBNST) is known to receive TK axons from the central amygdaloid nucleus (CNA), while the posterior BNST (postBNST) receives TK input mainly from the medial amygdaloid nucleus (MNA). The results of this study indicate that developmental TK expression in the antBNST parallels that in the CNA, and the postBNST parallels TK expression in the MNA. At F45, the MNA undergoes a significant increase in TK neuronal and fiber frequency, correlated with increased fiber density laterally in the postBNST, with more frequent cells medially and ventrally. Likewise, as CNA neurons increase substantially by F50, fibers become abundant in the lateral antBNST and cells more numerous in the middle and ventral regions. In addition, TK growth cones and fibers are seen entering the amygdala from the postBNST and visa versa, as early as F30, constituting a known reciprocal TK pathway. Thus, the development of TKs in the amygdala and BNST are closely related, and may influence the early ontogeny of the amygdala/BNST system. Supported by USPHS Grants NS 24596 and HD 05958.

385.10

DISTRIBUTION OF NEUROKININ B IN THE CENTRAL NERVOUS SYSTEM OF THE MALE SYRIAN HAMSTER. C. B. Newton and J.M. Swann. Dept. of Biol. Sci., Rutgers University, Newark, NJ 07102.

The three mammalian tachykinins are encoded by two genes. PPT-A encodes for substance P and substance K, while PPT-B encodes for neurokinin B (NKB). While the distribution of substance P in cells and fibers of the CNS of the rat and the hamster has been extensively described, the distribution of NKB in the hamster has not been reported. Studies utilizing in situ hybridization suggest that mRNA for PPT-A and PPT-B are expressed in overlapping areas. However, it has not been conclusively demonstrated whether or not substance P and NKB co-occur within the same neurons. In the present study we used immunohistochemistry and double label immunofluorescence to determine the location of neurokinin B and the extent of its co-occurrence with substance P in the cells and fibers of the male hamster CNS.

Colchicine treated tissue from adult male Syrian hamsters was either immunolabelled for NKB with polyclonal antisera to peptide 2, a fragment of the NKB precursor protein, (generously donated by Dr. J. Krause, Washington University) or immunolabelled for substance P using a monoclonal antiserum (Acurate Scientific)

Heaviest cell and fiber labelling obtained with the peptide 2 antisera was located in the medial habenular. NKB fibers exiting this nucleus formed a heavily labelled projection to the interpeduncular nucleus via the fasiculus retroflexus. Light to moderate cell and fiber labelling was found in the cingulate and frontal cortex, ventral and external bed nucleus of the stria terminalis, lateral preoptic area, lateral hypothalamic area, anterior hypothalamic area, ventral tegmental area, central grey, paraventricular and arcuate nuclei. Fiber labelling was also found in the infra limbic and dorsal peduncular cortex, tenia tecta, shell of the accumbens, basolateral, central and intercalated nuclei of the amygdala and the substantia nigra pars reticulata. Substance P containing cells are located in the medial and lateral septum, medial and lateral subdivisions of the BNST, the medial preoptic area, the medial, cortical and central nuclei of the amygdala, and the ventromedial hypothalamic nucleus. Colchicine treated tissue that was double labelled for substance P and NKB failed to reveal colocalization of these peptides in neurons or fibers of the hamster CNS. Thus, PPT-A and PPT-B are expressed in the cells and fibers of the hamster CNS but do not appear to be colocalized within these structures. Supported by MPRC-B 3 S06 GMO8223-06S2.

385.11

SUBSTANCE P IMMUNOREACTIVITY IN THE MYENTERIC AND SUBMUCOSAL PLEXUS FOLLOWING APPLICATION OF BENZYLDIMETHYLTETRADECYLAMMONIUM CHLORIDE DIHYDRATE TO THE DUODENAL SEROSA. E.H. South and P.D. Huff*. Dept. of Food Sci. & Toxicol., Univ. of Idaho, Moscow, ID 83843.

Serosal application of benzyldimethyltetradecylammonium chloride dihydrate (BAC) to the serosa of discrete regions of rat intestine ablates the myenteric plexus without an apparent reduction in neuron number in the submucosal plexus (Herman and Bass, 1989). The neuropeptide, substance P, is found in both the submucosal and myenteric plexus as well as extrinsic nerves in the gut wall and, thus, may serve as a peptide marker for selective damage to the myenteric plexus. We found that bathing the duodenal serosa in a 2mM solution of BAC ablated substance P-like immunoreactivity (SPLI) along the entire extent of the duodenal myenteric plexus. SPLI in the submucosal plexus was not diminished in the duodenum or in the adjacent or distal myenteric and submucosal plexus of the jejunum. The rats tolerated the BAC treatment well, recovered to presurgery body weight within 10 days of BAC treatment and were equivalent in weight to control rats twelve wks after BAC treatment when whole mounts were prepared for assessment of SPLI. The BAC denervated duodenum may provide a model for use in acute or chronic examinations of the anatomical or functional contribution of the myenteric plexus to intestinal function.

385.12

SUBSTANCE P DELINEATES CYTOARCHITECTONIC SUBDIVISIONS OF THE SOLITARY NUCLEAR COMPLEX AND THE INTERMEDIATE RETICULAR ZONE OF THE MEDULLA OBLONGATA IN THE HUMAN. D.A. McRitchie*, I. Törk, X.-F. Huang* and G. Paxinos, Schools of Anatomy and Psychology, University of New South Wales, Kensington, NSW 2033, Sydney, Australia.

The solitary nuclear complex (Sol) and the intermediate reticular zone (IRt) contain high levels of substance P-like immunoreactivity (SP-LI). We investigated the distribution of SP-LI in four brainstems from adult patients with no known neurological disorders. SP-LI was found in both small diameter varicose fibers as well as in medium and large diameter non-varicose fibers, and in small to medium sized cells. Of the ten subnuclei comprising Sol, the most dense SP-LI was found in the intermediate, medial, dorsal, and subregions of the dorsolateral nuclei. Moderate SP-LI was observed in the paracommissural and gelatinosus nuclei and the remainder of the dorsolateral nucleus. Sparse SP-LI was recorded in the commissural, ventral and ventrolateral nuclei, while the interstitial nucleus and the solitary tract itself displayed very sparse immunoreactivity. All subnuclei, with the exception of the paracommissural and ventrolateral nuclei, contained medium sized, spindle shaped, bipolar and bitufted SP-LI neurons. Only the nucleus gelatinosus contained a high density of SP-LI cells. The IRt displayed two parallel layers, with the external (lateral) lamina displaying high SP-LI. SP-LI positive neurons were mostly found in the part of the IRt between the ambiguus nucleus and the solitary/vagal nuclear complex. The above findings demonstrate that the pattern of SP-LI in these two autonomic control regions of the medulla closely follows their cytoarchitectonic boundaries and accentuates the internal subdivisions of these regions. Supported by the National Heart Foundation and the National Health and Medical Research Council of Australia.

385.13

POSSIBLE SOURCES OF SP REINNERVATION IN THE DEAFFERENTED TRIGEMINAL SPINAL NUCLEUS. **J.D. Stover*, C.A. Schwab*, K.D. Hoffmann* and M.A. Matthews (LSU School of Dentistry, New Orleans, LA, USA).**

To study the likely role of neurons exhibiting SP-like immunoreactivity (SPLIR) in deafferentation pain, the contribution of cervical and trigeminal primary afferents to recovery of SPLIR in trigeminal subnucleus caudalis (Vc) was assessed in 13 cats. Animals underwent either retrogasserian rhizotomy, cervical tractotomy or a combination trigeminal and cervical tractotomies. One cat served as a control. After perfusion and fixation at 11 and 45 days postoperatively, serial trigeminal brainstem sections were incubated in monoclonal SP antiserum and then reacted in avidin-biotin ABC solution for demonstration of SPLIR.

Loss of SPLIR was moderate to severe in middle and rostral Vc in rhizotomized cats and in middle and caudal Vc in cats subjected to cervical tractotomy, with predominant loss in the lateral Vc. Cats receiving two tractotomies showed moderate loss of SPLIR in middle Vc, with significant dorsomedial retention. In all cases SPLIR was retained in the trigeminal interstitial nuclear complex. Large, punctate boutons consistent with previously described glomerular terminals were much reduced at 11 days, however recovered at 45 days. Heavily labeled cell bodies were seen throughout Vc after tractotomies.

We conclude that an increased metabolic activity of intrinsic SP-containing neurons in Vc may correlate with expansion of terminal projections within the subnucleus, thus potentiating deafferentation pain. **This study was supported by NIH Grants, DE00199, DE08052 and BRSG Grant RR 05704.**

385.14

RELATIONSHIP BETWEEN LEU-ENKEPHALIN AFFERENTS AND A10 DOPAMINERGIC NEURONS: LIGHT AND ELECTRON MICROSCOPIC EXAMINATION. C.-L. Liang* and D.C. German. Dept. of Psychiat., UT Southwestern Medical Cntr., Dallas, TX.

Previous studies have indicated that opioid peptides are located within the midbrain regions containing the ventral tegmental area (nucleus A10) dopaminergic (DA) neurons. The present experiment sought to determine the synaptic relationship between leu-enkephalin (LE)-containing terminals and A10 DA neurons in the rat. Midbrain sections were double stained for LE (1:1000) and tyrosine hydroxylase (TH; 1:4000) using the peroxidase antiperoxidase and silver-intensified colloidal gold reactions, respectively. At the light microscope level, dense LE fiber and terminal immunoreactivity was observed within all of the A10 subnuclei (interfascicular n., paranigralis n., central linear n., ventral tegmental area, parabrachialis pigmentosus n.). At the EM level, there were direct synaptic contacts between LE axon terminals and DA dendrites. Two types of LE boutons, symmetric and asymmetric, could be identified. LE terminals also contacted DA somata and unlabeled dendrites. These findings indicate that LE, or related opioid peptides, can directly influence A10 DA neurons. Supported by DA-05314.

385.15

ULTRASTRUCTURAL CHARACTERIZATION OF THE DENSITY AND DISTRIBUTION OF BETA-ENDORPHINERGIC NEURONAL ELEMENTS IN THE ROSTRAL FOREBRAIN OF THE FEMALE C57BL/6J MOUSE. M.M.Miller, O. Zingg,* and L. Zhu.* Departments of Obstetrics and Gynecology, Experimental Medicine and Anatomy, McGill University, Montreal, Quebec,.

Beta-endorphin is synthesized in a subpopulation of neurons in the arcuate nucleus of the hypothalamus which send projections to the rostral forebrain. The present ultrastructural study was designed to determine the density and distribution of beta-endorphinergic (B-EP) neuronal elements in the preoptic area (POA) and diagonal band of Broca (DBB), regions known to be important to reproductive function. Sections from five normally cycling female C57BL/6J mice (2 mo old) were evaluated at proestrous using a pre-embedding immunogold labeling technique. Vibratome sections from regions including the rostral DBB through the caudal POA were incubated with rabbit anti B-EP followed by protein-A gold and processed for immunocytochemical labeling. Ultrathin tissue sections were analysed for density and distribution of labeled neuronal elements. All neuronal elements from all photomicrographs were classified according to category and counted. Categories included neurons, dendrites, axons, and glia. Immunolabeled neuronal elements were also counted and classified. Both density and distribution of gold-containing structures were similar in the POA and DBB. The total number of B-EP-containing neuronal elements was 2.1% of all identified structures. Among axons, 1.8% were labeled, while 1.5% of all terminals examined contained B-EP. Immunoreactive terminals only rarely impinged upon neuronal perikarya. Gold label was found in 3.6% of all dendrites and 1.9% of all neurons within examined regions. In no case were myelinated axons or glia labeled. The present study indicates that the rostral forebrain of the C57BL/6J mouse demonstrates a B-EP-containing network which is separate from that already identified in the arcuate nucleus in this strain of mouse. Supported by NIH R01 AG7795 (MMM).

385.16

DISTRIBUTION OF [LEU]ENKEPHALIN AND [MET]ENKEPHALIN IN BRAIN REGIONS OF THE TWO-DAY-OLD CHICK. P.J. Colombo, G. Schulteis, E.L. Bennett, J.L. Martinez, Jr., & M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA 94720

Chick brains were dissected into five regions and examined independently for [leu]enkephalin (LE) and [met]enkephalin (ME) content by radioimmunoassay. The brain regions examined were these: cerebellum (CER), optic tectum (OT), brain stem (BS), dorsal forebrain (DF) and ventral forebrain (VF). DF contained the neostriatum and ectostriatum. VF contained the mammalian basal ganglia homologues lobus parolfactorius and paleostriatum augmentatum. Values for both LE and ME were significantly different in each brain region examined except BS and VF and followed this descending rank order: BS = VF > OT > DF > CER. Values for ME were significantly higher than for LE in all brain regions except CER which was near the lower limit for detection. These findings suggest that the regional differences in enkephalin concentrations in chick brain are similar to those reported for mammalian brain.

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385.17

OPIOID IMMUNOREACTIVITY IN THE NERVOUS SYSTEM OF C. ELEGANS
Z.Aguilar*, E.Piros*[1], N.Maidment[1], J.R.Mancillas and C.J.Evans.[1]
Brain Research Institute and Departments of Psychiatry[1], Anatomy and Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024

The vertebrate opioid system has evolved a complex array of ligands and receptors. Three known opioid precursors, namely POMC, proenkephalin and prodynorphin have been extremely well characterized from amphibians to man. Characterizing the opioids in invertebrates is of interest from a molecular evolutionary standpoint and may identify primary behavioral goals of the endogenous opioid system. We have developed an immunoassay which identifies all vertebrate opioid peptides and used this assay to probe acid/acetone extracts of C. elegans. Immunoreactive material co-chromatographing on Sephadex G50 with enkephalins was detected and is presently being further characterized.

To localize opioids in C. elegans, we used immunocytochemistry with antibodies against enkephalin. Leu-enkephalin (-ir) was observed in a) two bilaterally symmetrical cells in a position consistent with that of the IL2DL and IL2DR (inner labial sensilla neurons presumed to be chemoreceptors) and b) two bilaterally symmetrical neurons in a slightly dorsal midbody position, which could be the ALM mechanosensory receptors, or the CAN neurons, believed to regulate the excretory canal.

The non-selective opiate etorphine was tested for its effects on locomotion, swimming, feeding and egg-laying behaviors. No significant effects were observed. We are currently evaluating opiate effects on chemoreception, chemotaxis and osmoregulation.

Supported by NIDA and a grant from the Keck Foundation.

385.18

DISTRIBUTION AND SEASONAL VARIATIONS OF THE IMMUNOREACTIVITY TO LEU- AND MET- ENKEPHALINS IN THE PERIOESOPHAGEAL GANGLIA OF THE SNAIL Helix aspersa.
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División de Inv. en Neurociencias, Instituto Mexicano de Psiquiatría, AP 14370, México, D.F.

The snail Helix aspersa has been subjected to intense neurobiological research, thus it was of interest to investigate the presence and the distribution of enkephalins in this organism. Given the distribution, we studied changes in the intensity of the immonoreactivity (IR) to enkephalins along the year. Thirty-five snail ganglia were used in a two year interval. The ganglia were extracted, fixed and cut into 10 µm thick slices. The indirect immunofluorescence method was used. Leu-enkephalin IR was found in neurons and fibers of the parietal, pleural and cerebral ganglia; fibers were also present in pedal ganglia. Met-enkephalin IR was present in cells and fibers of all ganglia, and in fibers of cerebro-pedal and cerebro-pleural connectives. Our data show a seasonal variation in the IR to enkephalins, having its lowest intensity in winter. This work and other studies carried out in less evolved invertebrates show that enkephalins are present in these organisms as neuro-modulators.

This work was financially supported by Dr. F. Pellicer project Conacyt P228CCOX891549 and Dr. M. Condés-Lara project Conacyt P228CCOX891536.

385.19

EXPRESSION OF GASTRIN-RELEASING PEPTIDE IN THE RAT BRAIN: IMMUNOHISTOCHEMICAL ANALYSIS WITH AN ANTISERUM AGAINST C-TERMINAL EXTENSION PEPTIDE.
P. Panula and J.F. Battey. Department of Anatomy, Univ. Helsinki and Laboratory of Neurochemistry, NINCDS, Bethesda, Maryland, USA.

A specific antiserum against the C-terminal extension peptide of GRP (GRP-CEP) was used to elucidate its distribution in the rat brain. In colchicine-treated rats, a prominent group of neurons in the paraventricular nucleus displayed strong GRP-CEP-ir. Faintly immunoreactive neurons were seen in the suprachiasmatic nucleus. Immunoreactive neurons were also found in the medial preoptic area, lateral hypothalamus, amygdala, central grey and dorsolateral tegmental nucleus. A few neurons were seen in the cerebral cortex and anterior olfactory nucleus. Immunoreactive nerve fibers were dense in the hypothalamus and some medullary nuclei. Occasional fibers were seen in the zona compacta but not in the zona reticulata of the substantia nigra. The distribution of GRP-CEP was not entirely identical to the distribution of GRP mRNA in normal rat brain or immunohistochemical distribution of GRP/bombesin-like immunoreactivity. Intraventricular injection of colchicine may affect expression of GRP in the brain.

385.20

THE COLLOIDAL GOLD TECHNIQUE FOR THE CELLULAR LOCALIZATION OF GSH RECEPTORS.
N. Guo and C. Shaw, Dept. of Ophthalmol., Univ.of British Columbia, Vancouver, B.C., Canada V6T 1Z3

Previous studies have shown that biotinyl-glutathione (GSH) binding sites are present in the white matter of the brain and thus suggested that GSH receptors might be on glial cells. Radioligand receptor assay performed on cultured astrocytes revealed the characteristics for a receptor, such as saturable and reversable binding, ligand specificity, and high affinity (Guo et al, 1991). Although these results sggested that there might be GSH receptors on astrocytes, they did not allow a direct cellular resolution.

To confirm that GSH receptors exist on astrocytes, appropriate cytochemical techniques are used. In the present study, the colloidal gold method was performed on the primary cultures of astrocytes to visualize the GSH receptors at the cellular level. Streptavidin-gold was used to bind to biotinyl-GSH molecules bound by their receptors on the cells. On addition of silver enhancer, precipitation of metallic silver occured, which enlarged the colloidal gold label normally visible only at the electron microscopy level, yielding high-contrast signals visible by light microscopy. The colloidal gold decoration of biotin-GSH-GSH receptor complex revealed that these receptors were on both astrocytic cell bodies and their processes. Double staining with GFAP-Rhodamine showed that all cells which were stained by colloidal gold-silver were immunoactive to GFAP, i.e. these cells which possess GSH receptors were astrocytes. Many GFAP positive cells, which look smaller and have not differentiated their processes, are colloidal gold negative, implying that these cells do not have GSH receptors at that ontogenic stage.

PEPTIDES: ANATOMICAL LOCALIZATION II

386.1

IMMUNOHISTOCHEMICAL DEMONSTRATION OF PRO-NEUROTENSIN/NEUROMEDIN N PROCESSING INTERMEDIATES IN RAT BRAIN.
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The neurotensin (NT)/neuromedin N (NN) precursor molecule possesses 4 lys-arg dibasic residues which represent potential sites of cleavage by maturation enzymes. Two of these sites flank a non-NT/NN-containing segment located between amino acids 87 and 140 within the precursor's primary sequence. Region-specific antisera generated against the exposed N-(KLPLVL;K6L) and C-terminal (EKEEVI;E6I) sequences of this segment were employed to immunohistochemically examine its topographic distribution relative to that of endogenous NT in serial adjacent sections through the rat brain. E6I and K6L immunoreactivity (IR) was detected in both nerve cell bodies and axon terminals. Both antigens were confined to areas displaying NT-IR suggesting a cellular co-distribution of E6I, K6L and NT. The intensity of E6I-IR in axon terminals was comparable to that of NT in all regions examined. However, there were far fewer E6I-IR cell bodies. K6L-IR in both axon terminals and cell bodies was consistently weak relative to that of E6I and NT. The differential intensity of E6I-IR in axon terminals versus perikarya suggests that the maturation cleavage exposing the E6I sequence occurs further distal to the cell body than that giving rise to NT. The relatively weak K6L-IR suggests that cleavage of the dibasic site adjacent to the K6L sequence may occur differentially in a restricted sub-population of NT-synthesizing neurons. The ability to detect both the N- and C-terminal extremities of the 87-140 precursor fragment in axon terminals suggests that it may represent an independent, biologically-active peptide.

386.2

ULTRASTRUCTURAL LOCALIZATION OF NEUROTENSIN IMMUNOREACTIVITY IN THE CAT NUCLEUS SOLITARIUS.
B. E. MALEY, Dept. of Anatomy and Neurobiology, Univ. of Kentucky Med. Ctr., Lexington, KY 40536-0084.

Neurotensin immunoreactivity has been reported in neurons and axons in the cat nucleus solitarius at the light microscopic level. The nucleus solitarius is known to be involved in the central regulation of cardiovascular function. Neurotensin plays a role in this regulation, although the morphological basis for its action is not understood. The present study was intended to define the specific synaptic circuits involving neurotensin in the nucleus solitarius. All animals used in the present study were perfused with 4% paraformaldehyde and 0.5% glutaraldehyde in phosphate buffer, pH 7.4. The nucleus solitarius was isolated, cut on a vibrating microtome and immunostained with neurotensin antiserum using the PAP method.

Neurotensin was localized to synaptic terminals and axons throughout the various regions of the nucleus solitarius. The reaction product was typically associated with synaptic vesicles in terminals that contacted various size dendrites, spines, cell bodies and other axons. The majority of labelled terminals contacted spines and distal regions of the dendritic tree. Very few of the synaptic contacts involved proximal dendrites or the neuron's cell body. Results of this study indicate that neurotensin is present in presynaptic terminals in the cat nucleus solitarius, suggesting a possible role for it in the synaptic circuitry of this neural region. The morphological characteristics of neurotensin labelled terminals is very similar to that of other peptidergic terminals in the cat's nucleus solitarius. However, its pattern of termination is distinctive, suggesting that the pattern of synaptic contacts is a critical component of the circuitry in the nucleus solitarius.

386.3

NEUROTENSIN IMMUNOREACTIVITY IN THE POSTERIOR CINGULATE AND RETROSPLENIAL REGIONS OF THE MONKEY. K. Satoh, H. Matsumura* and M. Narita*, Dept. of Psychiatry, Shiga Univ. of Medical Science, Otsu 520-21, Japan.

The tridecapeptide, neurotensin, has been demonstrated in the forebrain limbic structures of many mammalian species, including primates. In the present study, we examined the anatomical organization of neurotensin-containing fiber networks in the posterior cingulate and retrosplenial regions of the *Macaca fuscata* by immunoperoxidase histochemistry. In the caudal half of the cingulate cortex, a zone of dense deposit of granular, neurotensin-containing puncta was observed in a subdivision of area 29l (Insausti). This neurotensin-rich field occupied the lateral part of its layer IV with a wedge-like appearance in the coronal sections. This area was clearly demarcated and the density of fibers appeared to be much higher than that observed in the nucleus accumbens. This field extended caudally to the level of the retrosplenial cortex, and made a curve around the caudal portion of the corpus callosum. After a ventro-rostral swift, the dense neurotensin-containing fiber network occupied a large area in the medial temporal region, and appeared to emerge with another neurotensin-rich field in the presubiculum. Such characteristic, regional distribution was not observed in other cortical systems that contained either substance P, somatostatin or tyrosine hydroxylase. The present study revealed that the cortical neurotensin system is distributed specifically to certain regions of the primate limbic structures. (Supported by grants from the ministry of education, science and culture.)

386.4

NEURON-GLIA INTERACTIONS IN THE REGULATION OF OXYTOCIN AND VASOPRESSIN RELEASE FROM THE RAT NEURAL LOBE. C.J.C.Boersma, M.A.F.Sonnemans* and F.W.van Leeuwen. Neth. Inst. for Brain Res., Amsterdam, the Netherlands.

The release of oxytocin (OT) and vasopressin (VP) from the neural lobe (NL) is modulated by both opioid peptides and OT/VP itself. Apart from these peptides, relatively high levels of F8Famide-immunoreactive (F8Fa-ir) peptides are present, which become undetectable after osmotic stress. We tried to find morphological evidence for VP and F8Fa-ir peptide involvement in this regulation mechanism. For staining of F8Fa-ir nerve terminals we used a polyclonal antiserum (kindly provided by H-Y.T.Yang), raised to synthetic F8Fa. VP and F8Fa-ir nerve terminals were shown to make synaptoid contact with pituicytes. With a combination of preembedding peroxidase staining for F8Fa and a postembedding immunogold labeling for the C-terminal part of VP-Neurophysin (VP-NP), it was shown that F8Fa-ir peptides were colocalized with VP-NP in the same granules. The present results suggest that VP and F8Fa-ir peptides can be co-released and that both might modulate OT and VP release indirectly, with the pituicyte as an intermediate link.

386.5

DISTINCT PATTERNS OF CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY (CGRP-IR) IN THE MAMMALIAN PANCREAS. R. De Giorgio, C. Sternini, K. Anderson, P.C. Watt*, F.C. Brunicardi*, A. L. Widdison*, H.A. Reber*, and V.L.W. Go*. CURE, Depts. of Med. & Surg., UCLA & VAMC at Wadsworth & Sepulveda, Los Angeles, CA 90073.

In rodent pancreas, CGRP-IR has been reported in nerves and islet cells. The aim of this study was to evaluate whether neuronal and endocrine CGRP-IR structures are a common feature of mammalian pancreas. CGRP-IR is present in nerve fibers innervating the parenchyma and vasculature of different species with varying densities (rat, mouse, and rabbit >> pig, guinea pig, cat >> dog > human). CGRP-IR is not detected in intrapancreatic ganglion cells, indicating an extrinsic origin of CGRP innervation. In human pancreas, CGRP-IR fibers are mainly restricted to intrapancreatic ganglia. In the pig, islets are devoid of CGRP innervation. Only the rat pancreas contains CGRP-IR islet D cells. No immunoreaction is detectable in islet cells of pig or rabbit pancreas. In other species, islet B cells are labeled with rabbit CGRP antibodies. However, this staining is abolished by incubating the tissue at 37°C (which does not affect D cell and neuronal labeling) and is not reproducible with guinea pig or mouse CGRP antibodies (which strongly stain nerve fibers in each species and D cells in rat). Whereas the presence of CGRP-IR nerves is a common feature of mammalian pancreas, CGRP-IR endocrine cells appear to be restricted to the rat pancreas. Supported by NIH grant DK38752.

386.6

ONTOGENY OF CALCITONIN GENE-RELATED PEPTIDE-LIKE IMMUNO REACTIVITY IN THE RAT PANCREAS. K.C. Hunt and C. Sternini. Departments of Anatomy & Cell Biology and Medicine and CURE/DDC, UCLA School of Medicine and VAMC - Wadsworth, Los Angeles, CA 90024.

Calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) has previously been observed in the adult rat in endocrine cells at the periphery of the islets of Langerhans and in afferent fibers innervating the endocrine and exocrine pancreas and blood vessels. The purpose of this study was to trace the development of this pattern. Sprague-Dawley rats at embryonic (E) days 12-21 (the day of discovery of the vaginal plug = day 0) and postnatal days 0-4, 8, 12, and 16 were processed for CGRP immunoreactivity using the avidin-biotin technique. Specificity controls included normal serum and preabsorption of CGRP antibody to CGRP, gastrin, and other pancreatic peptides. CGRP-LI was observed in scattered cells within the pancreas at E12. Nerve fibers adjacent to blood vessels in connective tissue associated with the pancreas stained for CGRP-LI at E16. CGRP-LI could be found in fiber bundles in the exocrine pancreas at E17 and in thin fibers in the endocrine pancreas at E18. By E20, the islets of Langerhans had assumed their adult form and CGRP-LI was observed in cells at their periphery. Thus, the adult pattern of CGRP-LI is completed prior to the birth of the rat.

Supported by NIH grant DK37852.

386.7

ASSOCIATIONS BETWEEN CGRP-IMMUNOREACTIVE FIBERS AND NPY-IMMUNOREACTIVE NEURONS THAT PROJECT FROM THE PELVIC PARACERVICAL GANGLION TO THE CERVIX AND BLADDER. R.E. Papka, D.L. McNeill and D. Quiver*. Dept. Anatomical Sciences, Univ. of Oklahoma, Oklahoma City, OK 73190.

We are attempting to determine if neurons in the paracervical ganglia (PG) which contain the same neurotransmitter code, but project to different end organs, receive similar or different patterns of input by identifiable nerves. Our preliminary work compared neurons that project to uterine cervix (cervical neurons) with those that project to urinary bladder (bladder neurons); specifically we focused on perikarya containing neuropeptide Y (NPY)-immunoreactivity (I) which are associated with calcitonin gene-related peptide (CGRP)-I fibers. The retrograde axonal tracers fluorogold and fast blue were used to identify cervical- versus bladder-neurons. After 7-14 days the PG were removed and sectioned on a cryostat. Sections containing tracer-labeled cells were double immunostained for NPY and CGRP. Counts of the following cells were made:(1) tracer-labeled cervical-neurons, (2) NPY-I cervical-neurons, (3) cervical-neurons associated with CGRP-I fibers and (4) cervical-NPY-I-neurons associated with CGRP-I fibers. Similar counts were made for bladder NPY-I neurons. Preliminary data indicate: (1) of the tracer-labeled-cervical-neurons about 50% were NPY-I, about 44% were associated with CGRP-I fibers and about 32% were both NPY-I and associated with CGRP-I fibers and (2) of the tracer-labeled-bladder neurons about 67% were NPY-I, about 51% were associted with CGRP-I fibers and about 37% were both NPY-I and associated with CGRP-I fibers. (Supported by NIH Grant NS22526 and the Presbyterian Health Foundation).

386.8

CALCITONIN GENE-RELATED PEPTIDE AND GALANIN IMMUNOREACTIVITY IN NERVES OF THE RAT UTERUS: LOCALIZATION, CO-LOCALIZATION AND EFFECTS ON UTERINE CONTRACTION. R.L. Shew and R.E. Papka. Department of Anatomical Sciences, University of Oklahoma, Health Sciences Center, Oklahoma City, OK 73190.

Immunoreactivity to the neuropeptides galanin (GAL) and calcitonin gene-related peptide (CGRP) were examined in nerves in the rat uterus as a prelude to studying their effects on uterine contractility. GAL-immunoreactivity (GAL-I) and CGRP-I were localized in myometrial nerves throughout the uterine horns. CGRP-I nerves were the most prevalent. Double labeling revealed GAL- and CGRP-I co-exist in a subpopulation of uterine nerve fibers; e.g. GAL-I was not present in all CGRP-I nerves. Effects of these neuropeptides on uterine contractility was examined in vitro on uterine horns from diethylstilbestrol-treated rats (50 µg/rat, i.p., 14-16 hours prior to the study). GAL (10^{-6} to 10^{-8} M) stimulated uterine contraction in a dose-related manner, while CGRP had no effect on baseline uterine tension. However, CGRP (10^{-7} M) reduced GAL-stimulated (10^{-7} M) uterine contraction by 92.5%. These results demonstrate that GAL- and CGRP-I are present in, and co-exist in some, uterine nerves, presumably afferent nerves. Both GAL and CGRP could be released from the afferent fibers in an "efferent" fashion and affect uterine contractility; GAL having a contractile effect and CGRP having a relaxing effect. (Supported in part by NIH grant NS 22526 and Presbyterian Health Foundation).

386.9

SPROUTING OF CALCITONIN GENE-RELATED PEPTIDE-LIKE IMMUNOREACTIVE (CGRP-LI) NERVES IN RAT CORNEAS FOLLOWING NEONATAL ADMINISTRATION OF CAPSAICIN (CAP). C. F. Marfurt and M. A. Jones*. Northwest Center for Medical Education, Indiana University School of Medicine, Gary, IN 46408.

Trigeminal sensory nerve fibers exert important trophic effects on the corneal epithelium, perhaps by releasing axonally transported neuropeptides. In the present study, we investigated the initial loss, and subsequent return, of corneal CGRP-LI nerve fibers following neonatal CAP administration, and the relationship between these changes and the development of neuroparalytic keratitis. Seventy-five newborn rats were given CAP (50mg/kg, i.p.) on each of the first 3 days of life. CGRP-LI corneal nerves began to degenerate within 1 hour of CAP administration and disappeared totally by 36 hours. Over the next 3-4 weeks, a dramatic reinnervation of the cornea took place and by 6-8 weeks the CGRP-LI innervation density approached, and occasionally exceeded, that of age-matched control corneas. However, the morphology and pattern of CGRP-LI innervation was abnormal, and included numerous myelinated axons, a robust subepithelial plexus of filamentous, randomly oriented fibers, and occasional "hyperinnervation" of the epithelium by unusually large numbers of densely packed basal epithelial leashes. Retrograde transport of WGA-HRP from the central cornea in control and CAP-treated adult animals labeled 121 and 35 cells, respectively in the trigeminal ganglion, representing a cell loss of approximately 70% in the CAP animals. Chronic keratitis, often with extensive neovascularization, developed in all of the CAP treated animals by approximately 3 weeks, reached a maximum between 4-6 weeks, and showed gradual improvement thereafter. However, the keratitis never completely disappeared, even after 15 months. In conclusion, these data show that CGRP-LI, CAP-resistant corneal nerve fibers undergo extensive sprouting following subtotal corneal sensory denervation. However, the resultant reinnervation is morphologically altered and, for reasons unknown, functionally incapable of preventing or totally reversing the keratitis. This research was supported by NIH grant EY05717 to C.F.M.

386.10

CALCITONIN GENE-RELATED PEPTIDE DEPLETION IN SENSORY NERVE AND GANGLIA OF DIABETIC BB-RATS. **L.C. Russell[1,2], X.M. Dong*[2], R.P. Lee[1], M. Byers[3] and K.J. Burchiel[4]. [1]Veterans Affairs Med. Ctr. and [2]Dept. of Neurological Surgery, [3]Dept. of Anesthesiology and Biological Structure, University of Washington, Seattle, WA 98195, and [4]Oregon Health Sciences Univ., Portland OR.**

Immunocytochemical studies of skin biopsies from diabetic patients have revealed calcitonin gene-related peptide (CGRP) depletion in peripheral C-fibers (Levy, D.M. et al., 1989). Therefore we studied the distribution of CGRP immunoreactivity (CGRP-I) in sensory peripheral nerve and dorsal root ganglia in the spontaneously diabetic BB Wistar rat. Saphenous nerve and L5 and L6 dorsal root ganglia were obtained from diabetic BB rats which had been maintained hyperglycemic for 9 or12 months, or nondiabetic littermates. Samples were then prepared for routine immunocytochemistry. CGRP-I was detected in saphenous nerve from control rats but not in nerve from rats which had been diabetic either 9 or 12 months. CGRP-I was also detected in approximately 50% of the DRG cells from both control and 9 month diabetic rats, but was not found in DRG from rats which had been hyperglycemic for 12 months. These findings indicate that axonal CGRP depletion precedes this peptide's depletion from sensory ganglia in the sequelae of diabetic polyneuropathy.

386.11

CO-LOCALIZATION OF CGRP AND GALANIN IN THE TROCHLEAR NUCLEUS OF THE CAT. Wang, X.H.,[1] Iannuzzelli, P.,[1] Baker, R.[2] and Murphy, E.H.[1] [1]Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA, 19129, and [2]Department of Physiology and Biophysics, NYU Medical Center, New York, 10016.

The neuropeptides, Calcitonin Gene Related Peptide (CGRP) and Galanin (GAL), are expressed in spinal and brainstem motoneurons. In hypoglossal and facial motoneurons, CGRP and GAL are expressed in separate populations of neurons (Moore 1989). In order to determine the generality of this observation for all brainstem motoneurons, we have examined the distribution of CGRP and GAL in trochlear motor neurons (TMNs) in the adult cat. In order to intensify the visualization of the peptides we pretreated the cats with colchicine. CGRP and GAL were visualized by using immunocytochemical methods on adjacent sections. The percentage of CGRP+ and GAL+ neurons was determined by counting the number of CGRP+ or GAL+ neurons and comparing this number to the total number of neurons counted in adjacent Nissl stained sections. Approximately 90% of TMNs showed CGRP immunoreactivity and 30% of TMNs showed GAL immunoreactivity. Comparing the distributions of these two neuropeptides, we conclude that there is a small population of motor neurons in the cat trochlear nucleus in which CGRP and GAL are co-localized. The immunoreactivity of both CGRP+ and GAL+ motoneurons is increased following axotomy (Wang et 1990, Moore 1989). Although the function of these peptides is not fully understood, they may play a role in modulating the action of both normal and axotomized cholinergic motoneurons. Supported by NS24707 to EHM and EY20007 to RGB.

386.12

EFFECT OF SELECTIVE DEAFFERENTATION OF CONVERGENT INPUTS ON TRIGEMINAL CGRP DISTRIBUTION. **C.A. Schwab*, J.D. Stover*, K.D. Hoffmann* and M.A. Matthews. Depts. of Anatomy and Oral and Maxillofacial Surgery, LSU Medical Center, New Orleans, LA 70119.**

Combinations of lesions designed to interrupt trigeminal and/or cervical inputs to the cat's spinal trigeminal nucleus reveal extensive overlapping fiber degeneration in the spinal tract together with distinctive changes in the immunocytochemical labeling pattern of calcitonin gene-related peptide at 11, 30, 45 and 60 days following surgery.

CGRP-containing cervical inputs to the subnucleus caudalis were shown by cervical tractotomy to be most prominent in the dorsomedial and ventrolateral portion of the caudal and middle 1/3 of the subnucleus whereas lesions of trigeminal inputs revealed that CGRP-containing trigeminal inputs predominate in the dorsolateral and lateral portions of the rostral and middle 2/3 of the subnucleus. Combined trigeminal and cervical tractotomies showed that CGRP inputs to the dorsomedial portion of the middle 1/3 of the subnucleus are least disturbed by the lesions used in this study. This is an area in which concentrations of convergent nociceptive inputs have been demonstrated previously using electrophysiological methods.

It is concluded that the anatomical divergence of cervical primary afferents from their point of entry into the CNS with an overlap onto trigeminal nuclei may provide a substrate for pathological alterations in receptive field size and spontaneous hyperactivity seen with clinical pain syndromes.

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386.13

CHANGES IN BRAINSTEM CGRP AFTER SEVENTH AND EIGHTH NERVE LESIONS. K.W. Brewer[1], A.M. Thompson[1,2], K.R. Moore[1], J.M. Byers[1], C.D. Ross[1], and G.C. Thompson[1,2]. Depts. of [1]Otorhinolaryngology and [2]Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Cranial nerve motor nuclei contain moderate amounts of the neuropeptide, calcitonin gene-related peptide (CGRP). Previous studies suggest that CGRP may serve as a neuromodulator of CNS events following peripheral nerve transection. The present study investigated the effect of seventh and eighth cranial nerve lesions on the presence of CGRP in brainstem. To examine this relationship, guinea pigs were anesthetized and subjected to unilateral cochlear removal, vestibular end organ ablation, and seventh nerve transection. They were then allowed to survive 1-5 days before intracardial perfusion. Formalin-fixed cryostat sections were collected through the brainstem and incubated in rabbit anti-CGRP (Amersham) with the Vectastain ABC kit and protocol.

Cranial nuclei including vagus (X), glossopharyngeal (IX), facial (VII), abducens (VI), trigeminal (V), trochlear (IV), and oculomotor (III) and olivocochlear (auditory VIII) and vestibular (vestibular VIII) efferents were analyzed for side to side differences. Motor nuclei III, VI, and VII were significantly different with increased CGRP staining on the side ipsilateral to the lesion in VI and VII and contralateral to the lesion in III.

Since no peripheral damage occurred to any structure other than those related to the VII and VIII nerves, the changes observed in motor nuclei III and VI may be related to the underlying processes causing eye nystagmus secondary to VIIIth nerve ablation. Greater staining motor nuclei in VI ipsilateral and III contralateral to the lesion is consistent with an eye movement having a slow phase velocity toward the side of the lesion.

[Supported by NIH grant DC00381]

386.14

GALANIN IMMUNOREACTIVITY IN THE MONKEY AND HUMAN BRAIN H.K. Le*, C. Anderson, E.J. Mufson, and J. H. Kordower, Department of Anatomy & Cell Biology, Univ. Illinois School of Medicine., Department of Neurological Sciences., Rush Presbyterian Medical Center., Chicago Ill. 60612 USA

Galanin-immunoreactivity (GAL-ir) was examined throughout the brain and spinal cord of Cebus monkeys, baboons and humans. In nonhuman primates, GAL-ir perikarya were observed within the basal forebrain. A dense continuum of neurons and processes were found extending from the bed nucleus of stria terminalis, through the substantia innominata, to the ventral portion of the bed nucleus. Small GAL-ir neurons were also seen within the caudate and putamen. In the diencephalon, GAL-ir neurons were also seen within the suprachiasmatic, periventricular, paraventricular, and arcuate nuclei as well as the lateral hypothalamic area. In the brain stem, GAL-ir cell bodies were seen within the mesencephalic nucleus of the trigeminal nerve (V), nucleus of the solitary tract and hypoglossal nucleus. Fibers were observed within the nucleus of the descending tract of V extending caudally to include its spinal cord analog within the substantia gelatinosa. GAL-ir were also seen within spinal cord anterior horn cells. GAL-ir differs between monkeys and humans within the basal forebrain (Kordower and Mufson, *J. Comp. Neurol.* 294: 281-292, 1990). Another dramatic difference in GAL-ir between these species is the observation that numerous GAL-ir neurons are seen in the human supraoptic nucleus while monkeys only display a dense fiber plexus in this region. We are presently investigating further the scope of GAL-ir species differences between monkeys and humans.

386.15

DISTRIBUTION OF GALANIN-LIKE IMMUNOREACTIVITY IN THE ADULT AND DEVELOPING BRAZILIAN OPOSSUM BRAIN. J. K. Elmquist, C. A. Fox, and C.D. Jacobson. Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

We have studied the anatomical distribution of galanin immunoreactive (GAL IR) cell bodies and fibers in the brain of the adult and developing Brazilian short-tailed opossum, *Monodelphis domestica. Monodelphis* is a marsupial whose young are born in a very immature, accessible state making it an ideal model to study development of the brain. Galanin polyclonal antibodies obtained from CRB and Peninsula were utilized in an indirect immunohistochemical technique to identify GAL IR structures in intact animals. Somata containing GAL IR were seen in the hypothalamus and brainstem while fibers were seen in the hypothalamus, thalamus, midbrain, and brainstem in the adult brain. Specifically, GAL IR cell bodies were consistently seen in the bed nucleus of the stria terminalis, medial preoptic area, and arcuate, dorsomedial, paraventricular, periventricular, and supraoptic hypothalamic nuclei. In the brainstem somata were seen in the nucleus of the solitary tract. Dense collections of GAL IR fibers were seen in the median eminence and the above areas. In the developing brain, GAL IR somata and fibers were seen as early as day 1 postnatal (PN). By days 5 and 10 PN there was a robust expression of GAL IR fibers and cell bodies in specific regions of the brain. These results indicate that galanin is present and may play a role during development of the brain.

386.16

GALANIN-LIKE IMMUNOREACTIVITY OCCURS IN RAT VAGAL SENSORY NEURONS. N. Calingasan and S. Ritter. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Galanin (GAL), a 29 amino acid peptide originally isolated from the porcine upper small intestine, is widely distributed in the rat central nervous system, including the nucleus of the solitary tract (NTS). Although vagal sensory neurons terminate in the NTS, it is not known whether these neurons contain GAL in the rat. Therefore, we attempted to determine the presence of GAL in the sensory neurons of the vagus nerve. We employed avidin-biotin-peroxidase immunocytochemistry using an antiserum raised in rabbits against synthetic GAL and preadsorbed controls to study the occurrence and distribution of GAL in colchicine-treated nodose ganglia of adult rats. GAL-like immunoreactive cell bodies were observed in nodose ganglia, possibly comprising about 10 - 15% of the total ganglion cell population. They were unevenly scattered, but were concentrated mostly in the rostral pole. Nodose ganglionectomy reduced GAL immunostaining in certain regions of the ipsilateral NTS but this observation was not conclusive. These findings demonstrate that GAL may serve as a neurotransmitter in rat vagal sensory neurons.

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386.17

PREFERENTIAL LOCALIZATIN OF A GALANIN-LIKE PEPTIDE IN AUTONOMIC CELL BODIES OF FROG CNS. S.M. Henry, D. Peruzzi and C.J. Forehand. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Galanin is a biologically active neuropeptide with a widespread distribution in the nervous system of a variety of species. In amphibians, galanin-like immunoreactivity (gal-IR) is prominent in peripheral autonomic neurons (McKeon et al., Cell Tiss. Res. 262:461, '90; Morris et al., Neurosci. Lett. 102:142, '89); however, its localization in amphibian CNS is unknown. We have examined the distribution of galanin in frog CNS by flourescence immunohistochemical staining.

Cell body staining was noted in several autonomic regions of the CNS. Labeled somata were observed in the hypothalamus in the preoptic nucleus, paraventricular nucleus and the tuberal region. Cells containing a galanin-like peptide were also observed in an area medial to the solitary tract and in a subpopulation of sympathetic preganglionic neurons. Galanin positive fiber systems were abundant throughout the tegmentum of the brain stem, but were not seen in the cerebellum and were sparsely present in the tectum.

All somata that demonstrated gal-IR were localized in regions involved in control of autonomic function. Thus, galanin may be a neuromodulator in this system in frogs. Co-localization studies are underway to further characterize the role of this neuropeptide in frog CNS.

386.18

COLOCALIZATION OF GALANIN AND VASOPRESSIN mRNA IN THE MEDIAL AMYGDALA OF THE RAT. M.A. Miller, P.E. Kolb*, M.A. Raskind. Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195.

Galanin (GAL) and vasopressin (VP) have previously been colocalized in magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei by both immunocytochemical and in situ hybridization techniques. Here we provide the first evidence for the colocalization of GAL and VP mRNAs in the steroid sensitive VP cells of the medial amygdala (AMe). Coronal brain sections (20 μm) through the AMe were double labeled for VP and GAL mRNAs using RNA probes labeled with digoxigenin-UTP (VP) and ^{35}S-UTP (GAL). Radioactive grains were detected over VP cells in the AMe indicating the presence of GAL gene expression. To avoid artifacts induced in darkfield by the digoxigenin color reagent, grains were visualized using reflected-light polarization. Double-labeling of AMe cells was determined to be specific since 1) radio-labeled cells were detected in the same region of the AMe in slides assayed for GAL only 2) digoxigenin labeled VP cells in other brain regions (SCN and parts of the PVN) did not appear to contain grains and 3) distributions of mRNAs for GAL and VP were the same as has been reported previously. The observation that VP and GAL are coexpressed in the AMe permits studies on the coregulation of these peptides under various physiological conditions.

386.19

TRIGEMINAL TRACTOTOMY DOES NOT ALTER CCK LEVELS, BUT DOES ALTER CCK, GASTRIN, CGRP AND SP IMMUNO-STAINING, IN NORMAL AND NEONATALLY DEAFFERENTED ADULT MEDULLARY DORSAL HORN. M.C. Beinfeld, D.S. Zahm, N.L. Chiaia, & M.F. Jacquin. Pharm. & Physio., Anat. & Neurobiol., St. Louis Univ., MO 63104; Anatomy, Med. Coll. Ohio, Toledo, Ohio 43699.

Recent in situ hybridization studies have questioned the primary afferent origin of dorsal horn CCK. CCK8-specific radioimmunoassay (RIA) and immunohistochemical (IHC) methods were used 4-10 days following left trigeminal tractotomy at the obex to test the hypothesis that medullary dorsal horn CCK arises from higher-order neurons. Caudal to the lesion, RIA of punches from superficial laminae in 9 rats revealed a 15% decrease in CCK (L vs R: 1.04 ± 0.73 vs 1.22 ± 1.14 ng/mg; $p > .05$); IHC revealed modest reductions in CCK, Gastrin, and SP terminals (N=5). CGRP terminal staining was virtually eliminated. CCK labeling in inner lamina II was unaffected. Only Gastrin (courtesy of Dr. J. Walsh) labeled dorsal horn cells (inner lamina II) and their numbers were reduced. Rostral to the lesion, IHC staining did not reveal axotomy-induced sprouting. In 16 other adults with left infraorbital nerve cut at birth, similar lesion and RIA methods revealed a 20% increase in CCK caudal to the tractotomy (L vs R: 2.74 ± 1.93 vs 2.29 ± 1.62 ng/mg; $p > .05$); in 4 others processed for IHC, peptide staining was reduced as in the tractotomy-alone cases. These data suggest that 1) most CCK in medullary dorsal horn is of higher-order origin, 2) neonatal deafferentation-induced increases in medullary CCK (Beinfeld et al., Neurosci. Abstr. 16, '90) reflect reorganization of higher-order inputs, and 3) higher-order SP inputs to medullary dorsal horn are robust. Support: NIH DE07734, DE07662, NS18667, NS23805, DE08971.

386.20

ORIGIN OF THE CHOLECYSTOKININ (CCK) IMMUNOREACTIVE (ir) TERMINALS IN THE RAT THALAMIC RETICULAR NUCLEUS (RTN). G. Battaglia, S. De Biasi♯, A. Amadeo* and C. Frassoni*. Neurological Institute "C. Besta", Milano, Italy; ♯Dept. of Physiology and Biochemistry, Univ. of Milano, Italy.

Recent immunocytochemical studies reveal that the rat RTN is densely innervated by CCKir fibers. The origin of these afferents has been investigated in the present study by means of light and EM immunocytochemistry, combined with the retrograde transport of WGAapoHRP-gold. Small CCKir terminals contact both distal and proximal dendrites, and also cell bodies, throughout the entire rostrocaudal extent of RTN. CCKir neurons are present in different brain areas known to project to the thalamus. Some pyramidal or multipolar neurons are CCKir in layer V and VI of the cerebral cortex. CCKir neurons are also present in the cholinergic mesopontine tegmental nuclei (PPTg and LDTg), and in the amygdaloid complex, particularly in the dorsal division of the medial amygdaloid nucleus (MePD). Tracing experiments reveal the presence of double-labeled CCKir neurons in PPTg and MePD, after WGAapoHRP-gold injection in RTN. The present experiments suggest that different brain areas give origin to the CCKir terminals in RTN. Further experiments are in progress to evaluate the contribution by each of these areas to the CCK innervation of RTN.

386.21

ONTOGENY OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY IN THE BRAZILIAN SHORT-TAILED OPOSSUM BRAIN. C. A. Fox, M. M. Jeyapalan* and C. D. Jacobson. Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

We have studied the anatomical distribution of cholecystokinin immunoreactive (CCK IR) somata and fibers in the brain of the adult and developing Brazilian short-tailed opossum, *Monodelphis domestica*. A nickel enhanced, avidin-biotin, indirect immunohistochemical technique was used to identify CCK IR structures. Cholecystokinin immunoreactive cell bodies were located throughout the cerebral cortex and hippocampus of adult opossums. Somata containing CCK immunoreactivity were also observed in the hypothalamus, thalamus, midbrain, and brainstem. Cholecystokinin immunoreactive fibers had a wide distribution in the adult *Monodelphis* brain. The earliest expression of CCK immunoreactivity was found in fibers in the dorsal brainstem of 5 day old opossum pups. Cholecystokinin immunoreactive somata were observed in the brainstem on day 10 of postnatal life (10PN). Most regions of the hypothalamus did not contain CCK IR until 35PN. An exception to this was the medial preoptic area (MPA). The male MPA had CCK IR cells and fibers on 25PN. However, the female MPA did not contain significant CCK IR elements until the 60PN time point. A broad spectrum of patterns of onset of CCK expression was observed in the opossum brain. The early occurrence, wide distribution, and varied ontogenesis of CCK IR structures indicates CCK may be involved in several different functions in the adult and developing opossum brain.

PEPTIDES: ANATOMICAL LOCALIZATION III

387.1

NEUROPEPTIDE-Y IMMUNOREACTIVITY IN THE BRAIN OF THE FROG RANA PIPIENS. C.J. Tyler, K.V. Fite, and G.J. DeVries. Neuroscience and Behavior Program, Univ. Of Massachusetts, Amherst, MA 01003

Immunocytochemistry was used to characterize the presence of neuropeptide Y (NPY) in the frog brain, placing emphasis on the assessment of NPY in primary retinal terminal fields and thalamic nuclear groups postsynaptic to primary retinal terminal zones.

Preliminary observations revealed the presence of NPY-like immunoreactive perikarya in tectal layer 4, ventral portion of tectal layer 6, anterior portions of posterocentral and posterolateral nuclei, suprachiasmatic nucleus (SCN), and anterior portion of the ventromedial thalamic nucleus. In addition, labelled perikarya were located in the glossopharyngeal nerve motor nucleus, nucleus cerebelli, nucleus of the medial longitudinal fasiculus, the ventral, dorsal, and lateral hypothalamic nuclei, and the medial, dorsal, and lateral pallium. The hypothalamic nuclei contained the largest population of labelled cells while the SCN contained cells with the most intense perikaryal labelling.

Although most areas of the brain contained light, homogeneous immunoreactive (IR) puncta accompanied by scattered IR fibers, certain areas were markedly different in NPY fiber and puncta labelling. While both divisions of the optic tract were relatively devoid of immunoreactivity, tectal layers 3 and 5, the ventral third and dorsal margin of tectal layer 6, and the dorsal portion of tectal layer 9 displayed dense IR puncta. Both immuno-negative nucleus isthmi and rostral visual nucleus were offset by their dense puncta-labelled surrounds. The SCN and the anterior thalamic nucleus contained a denser concentration of puncta and fibers. While both the nucleus and neuropil of Bellonci appeared to be relatively immuno-negative, the Corpus geniculatum contained IR puncta only within its central core. Intense fiber and puncta labelling in the medial and lateral amygdala, accessory olfactory tract, and lateral forebrain bundle, separated these structures from other forebrain areas. (Supported by NSF Grant BNS 8819870 to K.V.F.)

387.2

DISTRIBUTION OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVE PERIKARYA IN THE FELINE HYPOTHALAMUS AND FOREBRAIN. V.J. Massari, Y. Tizabi, Z.Z. Song*, H. M. Rhee#, & P. J. Gatti. Dept. of Pharmacology, Howard University, College of Medicine, Washington, D.C. 20059, and #Food & Drug Administration, Rockville, MD. 27457

Neuropeptide Y (NPY) is considered to be a neurotransmitter candidate in the central nervous system. Hypothalamic NPY may be involved in the regulation of the hypothalamo-pituitary-adrenal axis through regulation of the secretion of ACTH. NPY is also believed to influence other hypothalamic functions such as reproduction, feeding, and control of fluid homeostasis. We have therefore examined the distribution of NPY-like immunoreactivity (NPY-LI) in the cat hypothalamus and forebrain. Four adult male mongrel cats were anesthetized with 50mg/kg pentobarbital and perfused with 2 liters of phosphate buffered saline (PBS) and 3 liters of 4% paraformaldehyde in PBS. Frozen sections (50u) were cut in a cryostat and processed using an avidin-biotin based immunoperoxidase method. The largest number of NPY-LI cells in the hypothalamus were observed in the arcuate nucleus. NPY-LI cells were also seen in the lateral hypothalamic area, paraventricular nucleus, suprachiasmatic nucleus, and anterior hypothalamic nucleus. NPY-LI cells were also observed in the cerebral cortex, caudate nucleus, putamen, claustrum, olfactory tubercle, bed nucleus of the stria terminalis, nucleus of the anterior commissure, nucleus accumbens, amygdala, dentate gyrus, and several thalamic nuclei. These data indicate that NPY-LI cells have a wider distribution in the feline hypothalamus and forebrain than was previously suggested. Supported by The American Heart Association

387.3

NEUROPEPTIDE Y IMMUNOCYTOCHEMICAL EXPRESSION IN HUMAN NEOCORTEX. W.E. Kaufmann and N.A. Cuello*. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Neuropeptide Y is the most abundant neuropeptide in human cortex and is expressed by intrinsic (nonpyramidal) neurons. Its distribution in some cortical areas and its changes in degenerative diseases have become a recent focus of interest. Studies of monkeys suggest differences in distribution between primary sensory and association neocortices. Therefore, we studied the neuropeptide Y cell body and axonal immunoreactivity in six normative subjects (control individuals for Alzheimer's disease), comparing different association cortical areas with primary somatosensory, visual, and motor regions. The relative distribution of elements was analyzed in each individual. Associational areas showed a more profuse and randomly oriented fiber pattern, particularly prefrontal cortex, as well as neurons with a complex dendritic tree. In contrast, primary regions exhibited a rather laminar axonal arrangement and simplified cells. It seems that cortical areas involved in cognitive functions have a richer neuropeptide Y network than those processing sensory information.

387.4

SOMATOSTATIN AND NEUROPEPTIDE Y ARE COLOCALIZED BUT NOT COEXTENSIVE WITHIN THE RAT CAUDATE-PUTAMEN. C.C.G. Naus, W.J. Rushlow and B.A. Flumerfelt. Department of Anatomy, University of Western Ontario, London, Canada N6A 5C1.

It has been reported previously that somatostatin (SS) and neuropeptide Y (NPY) are colocalized in the rat striatum within neurons of the medium-sized aspiny category. Experiments involving the selective lesioning of glutamatergic and dopaminergic inputs, however, yield conflicting results with respect to both mRNA and protein expression. Furthermore, topographical data for NPY and SS neurons conflict with repect to the medial to lateral gradient described for SS. This suggests that although both neuroactive substances may be colocalized they are not coextensive. In order to investigate this possibility, the striatum of five adult female Wistar rats (260-280g) were sectioned at 40 μm on the freezing microtome. NPY (Incstar) was then localized immunocytochemically and visualized by the ABC-peroxidase technique using 3,3'diaminobenzidine (DAB) as the chromogen. In situ hybridization was then carried out on the immunostained sections using an [S^{35}]-labelled anti-sense riboprobe for somatostatin. The hybridization signal was detected using autoradiography. Examination of the sections revealed that a large proportion of the neurons labelled immunocytochemically for NPY were also positive for SS mRNA. However, a significant proportion of neurons containing SS mRNA did not display detectable NPY immunoreactivity. The mismatch between in situ signal and immunocytochemically labelled neurons could be seen throughout the striatum but was most concentrated in the ventral and central caudate-putamen. These results suggest that although SS and NPY are frequently colocalized within the neostriatum they are not coextensive.

Supported by NSERC (C.C.G.N.) and MRC (B.A.F.).

387.5

ALL SOMATOSTATIN INTERNEURONS IN THE RAT CAUDATE-PUTAMEN CONTAIN SOMATOSTATIN-28 BUT NOT ALL CONTAIN SOMATOSTATIN-14. W.J.Rushlow, C.C.G. Naus and B.A. Flumerfelt. Department of Anatomy, University of Western Ontario, London, Canada N6A 5C1.

In the rat striatum, both somatostatin-28 (SS28) and somatostatin-14 (SS14) are found within neurons of the medium-sized aspiny category. SS28 is likely produced within these cells by the cleavage of prosomatostatin at an Arg site while SS14 is obtained by cleavage of SS28 at an Arg-Lys dibasic site (Benoit R., Metabolism 39(9) suppl.2: 30-32, 1990). The difference in the type of cleavage sites between the two forms of somatostatin raises the possibility that two different subpopulations of these neurons may exist. In order to investigate this possibility, the striatum of five adult female Wistar rats (260-280g) were sectioned at 40μm on the freezing microtome. SS14 and SS28 were then localized immunocytochemically on alternate sections using antisera S310 or S309 respectively (kindly provided by Dr. R. Benoit) and visualized by the ABC-peroxidase technique using 3,3'diaminobenzidine tetrahydrochloride as the chromogen. In situ hybridization was carried out on the immunostained sections using an [S^{35}]-labelled anti-sense riboprobe for somatostatin. The hybridization signal was detected using autoradiography. For the SS28 immunostained sections, the positive grain densities were all localized to immunostained neurons. In the SS14 immunostained sections, however, a significant proportion of the grain clusters were found overlying neurons where no reaction product was present. Further investigation revealed that the SS14 deficient region is most pronounced in a wide medial to lateral band in the dorsal caudate-putamen (just beneath the corpus callosum) and the medial aspect of the striatum (dorsal to ventral adjacent to lateral ventricle). These results suggest that two subpopulations of somatostatin neurons exist within the striatum; one containing only SS28, and one containing both SS14 and SS28 (the precursor of SS14). Supported by NSERC (C.C.G.N.) and MRC (B.A.F.).

387.6

SOMATOSTATIN-IMMUNOREACTIVITY IN THE HIPPOCAMPUS OF MOUSE, RAT, GUINEA PIG, AND RABBIT. P.S. Buckmaster[1], D.D. Kunkel[2], M.E. Gross*[2] and P.A. Schwartzkroin[1,2]. Depts. of [1]Physiology & Biophysics, and [2]Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

We have examined the somatostatin-immunoreactivity of hippocampi of animals commonly used for electrophysiological studies to determine if there are regional or species-specific variations. Animals (adult female) were deeply anesthetized (100 mg/kg pentobarbital IP) and perfused with fixative. After cryoprotection, hippocampi were removed, slightly flattened, and sectioned at 40 μ perpendicular to the septo-temporal axis. Sections from 5 evenly spaced regions along the septo-temporal axis were processed with an antibody against part of the precursor molecule of somatostatin (SS320, kindly provided by Dr. R. Benoit) and the ABC method. Sections from all regions and from all species were processed together to eliminate variations in immunostaining that occur between runs. The pattern of somatostatin-immunoreactivity is generally quite similar across the four species examined: 1] There are more somatostatin-positive somata and a denser fiber plexus in the ventral hippocampus; 2] The majority of the somatostatin-positive somata occur in the hilus of the dentate gyrus and in stratum oriens and alveus of CA1 and CA3; 3] There is a dense somatostatin-positive plexus in stratum lacunosum-moleculare of CA1. However, there are some interesting species variations: 1] Mice and rats have proportionally more somatostatin-immunoreactive neurons in stratum lucidum of CA3 than rabbit and guinea pig; 2] Rats, guinea pigs, and rabbits have a dense somatostatin-positive fiber plexus in the outer molecular layer of the dentate gyrus, but mice do not. The functional significance of these differences is unknown.

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387.7

VASOPRESSIN AND CRF IMMUNOSTAINING FOLLOWING ADRENALECTOMY IN SYRIAN HAMSTER HYPOTHALAMUS. S.N. Del Paine and R.L. Meisel. Dept. of Psychological Sciences, Purdue University, West Lafayette, IN 47907.

In a continuing effort to determine why compensatory growth following unilateral adrenalectomy is lacking in hamsters, we examined the roles of vasopressin (VP) and CRF in pituitary-adrenal regulation. Immunocytochemistry for VP and CRF was conducted in alternate 50 um sections through the PVN using primary antibodies to arg-VP and oCRF and a peroxidase-based Vector ABC kit. Immunostaining was qualitatively and quantitatively assessed in female Syrian hamsters that were either intact, bilaterally (5 days), or unilaterally (5 days) adrenalectomized. Preliminary results indicated a wide distribution of intense VP immunostaining regardless of treatment. The most salient VP fiber and cell body staining was observed throughout all levels of the PVN and supraoptic nucleus as well as in the suprachiasmatic, retrochiasmatic, nucleus circularis, lateral and anterior hypothalamic areas. In addition, both bilateral and unilateral adrenalectomy appeared to increased the number of VP immunostaining PVN neurons as compared to intact controls. Furthermore, bilateral adrenalectomy appeared to change the distribution of the VP-immunostained neurons in the PVN, possibly by increasing the number of parvocellular VP-stained neurons. Virtually no staining of CRF neurons was observed in intact and unilateral adrenalectomized hamsters, whereas CRF was observed in PVN neurons following bilateral adrenalectomy. Bilateral, but not unilateral, adrenalectomy appears to be a potent trigger for ACTH secretagogues in the Syrian hamster hypothalamus.

387.8

ABSENCE OF ADRENALECTOMY INDUCED ALTERATIONS IN CRF/VASOPRESSIN NEUROSECRETORY VESICLE MORPHOLOGY IN HOMOZYGOUS BRATTLEBORO RATS. J.Z. Kiss and L.T. Bertini*. Dpt. of Morphology, Univ. of Geneva, Sch. of Med., Geneva Switzerland.

Dense core vesicle /DCV/ provide a mechanism whereby neurohormones are packaged and secreted in discrete quanta. The regulation of the amount of neurohormones released may in part be controlled by vesicle size which in turn determines the number of molecules per quanta. We previously reported that hypohysiotrophic corticotropin-releasing factor /CRF/ neurons are capable to alter the size of DCV after removal of glucocorticoid negative feedback by adrenalectomy /ADX/. ADX results in a progressive increase in the mean volume of DCV to about three times normal. Changes in size are accompanied by a substantial increase in immunoreactive sites for vasopressin /VP/ co-packaged with CRF in the same DCV compartment.

To determine as to whether changes in size of the DCV is simply due to elevated intravesicular concentrations of VP, we conducted a quantitative electron microscopic study to estimate the size distribution of CRF positive vesicles in adrenalectomized homozygous Brattleboro rats that lacks vasopressin. CRF immmunoreactivity was present within a 95nm vesicle population in both homozygous and heterozygous /control/ rats. In heterozygous rats CRF was co-packaged with VP in the same DCV, and ADX induced a significant increase in the mean vesicle diameter. However, in homozygous rats the size distribution of CRF positive vesicles was not affected by ADX. These results are consistent with the hypothesis that the biosynthesis of neurosecretory material itself determines the quantal size of DCV.

387.9

VASOACTIVE INTESTINAL PEPTIDE (VIP)- AND NEUROPEPTIDE Y (NPY)-LIKE IMMUNOREACTIVITY (IR) IN NORMAL AND SYMPATHETICALLY DENERVATED RAT PINEAL GLAND. S.J. Piszczkiewicz, C. Baldwin and R.E. Zigmond. Dept. of Neurosciences, Case Western Reserve Univ., School of Medicine, Cleveland, OH 44106.

The rat pineal gland is innervated primarily by the superior cervical ganglia (SCG). This innervation drives the diurnal rhythm of pineal melatonin production by regulating serotonin N-acetyltransferase (NAT) activity. Either bilateral transection of the post-ganglionic axons in the internal carotid nerve (ICNX) or removal of the ganglia (SCGX) abolishes this rhythm. While norepinephrine (NE) release appears to be mainly responsible for the increase in NAT activity, other transmitters, such as VIP, may also be involved. VIP-IR, found in nerve fibers in the pineal, has been proposed to originate, at least in part, from VIPergic neurons in the SCG (Soc. Neuro. Abst. 15:836). VIP stimulates NAT activity *in vivo* in gerbil pineals and in cultured rat pinealocytes. Thus, VIP originating in the SCG may be released in the pineal and act synergistically with NE to increase NAT activity.

To examine the origin of the VIPergic input to the gland, pineals were removed and assayed for VIP- and NPY-IR by radioimmunoassay 48 hr post-ICNX. NPY is present in nerve fibers in the pineal and is known to disappear from the pineal following SCGX. The NPY-IR was reduced in the ICNX groups to 11% of the control or sham-operated groups. No significant differences in VIP-IR were found among any of the groups, although there was a large variability (about 25%) within each group. The identity of the 2 peptides in the pineal extracts were authenticated by HPLC. Additionally, bilateral SCGX were performed and the pineals removed 1-6 weeks later and examined immunohistochemically. The density of VIP-IR fibers was highly variable with no apparent difference in pineals from both control and SCGX animals. These data suggest that VIP innervation to the rat pineal gland is variable and does not originate to a significant extent from the SCG. Therefore, the origin of VIP fibers in the pineal, the control of its release *in vivo*, and verification of its role in the *in vivo* regulation of NAT remain to be elucidated. (GM07250-15, NS17512 and MH00162)

387.10

VASOACTIVE INTESTINAL PEPTIDE IN PROLACTIN-DEFICIENT DWARF MOUSE PITUITARY. C.J. Phelps, D.L. Hurley, M.Y. Vaccarella* and A.J. Carrillo, Depts. of Anatomy, Tulane Univ. Sch. Med., New Orleans, LA 70112, and NEOUCOM, Rootstown, OH 44272.

Ames (df/df) dwarf mice manifest a spontaneous homozygous mutation in which prolactin (PRL) is not expressed. Since recent evidence supports a role for VIP as a PRL-stimulating factor, and a pituitary source for PRL-regulating VIP, it was of interest to examine whether pituitary VIP was altered in dwarfs. Immunoreactive VIP in df/df and normal (DF/?) littermate mouse pituitaries was investigated using ICC and RIA techniques, using the same VIP antiserum (Carrillo *et al.*, ENDO 128:131, 1991) in both assays. For ICC, paraffin-embedded sections of adult glands (both sexes) were incubated in a-VIP at 1:2000, developed using the ABC method, and visualized using either DAB with $NiSO_4$ or Texas red-labeled avidin. For additional PRL ICC, sections were incubated in a-mPRL (1:5000, A.F. Parlow) and visualized with DAB or fluorescein-labeled avidin, respectively. VIP immunostaining in both DF/? and df/df anterior pituitaries was diffuse, of low intensity, and widespread. VIP and PRL were in distinct cells in normal mice, and VIP was apparent in dwarf gland where PRL cells are absent. In posterior pituitary, staining suggestive of neural fibres was present and was particularly intense in dwarfs. For RIA measurement, neurointermediate (NIL) and anterior lobes were dissected and assayed separately, in supernatants of HCl-treated, boiled extracts. Anterior pituitaries averaged 391.3±86.2 pg VIP for normals and 6.8±1.6 pg VIP for dwarfs. NIL averaged 6.9±1.0 pg in normals and 7.6±3.83 pg in dwarfs. Taken together, the results suggest that VIP in the tiny dwarf mouse anterior pituitary is diminished relative to size, and that VIP localization and content in dwarf posterior pituitary is comparable to that of normal mice.

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387.11

VASOACTIVE INTESTINAL PEPTIDE IN PROLACTIN-DEFICIENT DWARF MOUSE BRAIN: IMMUNOCYTOCHEMICAL AND RIA ASSESSMENT.

H. Dalcik, D.L. Hurley, A.J. Carrillo, and C.J. Phelps, Depts. of Anatomy, Tulane Univ. Sch. Med., New Orleans, LA 70112, and NEOUCOM, Rootstown, OH 44272.

Ames dwarf mice contain a recessive mutation which, in the homozygous condition (df/df), results in absence of pituitary prolactin (PRL). Previous studies have indicated that factors which inhibit GH or PRL secretion are absent in the brains of dwarf mice. Recent reports have documented the role of VIP as a PRL-stimulating factor. Thus, it was of interest to examine immunoreactive VIP in PRL-deficient df/df compared with normal (DF/?) littermate brain. For ICC, some mice were treated with ICV colchicine (30mg/ml) prior to sacrifice. Thirty µm brain sections from perfused adults (both sexes) were processed using anti-VIP serum (1:3000, Incstar and Carrillo *et al.*, ENDO 128:131, 1991) and ABC technique (Vectastain), and visualized using DAB. For RIA measurement, blocks of neocortex, and of dorsal (including paraventricular nucleus, PVN) and basal (including median eminence) hypothalamus were dissected from 2 mm-thick coronal sections of fresh frozen brain, and were assayed in supernatants of HCl-treated, boiled extracts; protein levels were measured in precipitates. VIP-immunoreactive cell bodies were found primarily in neocortex, limbic system, pyriform and entorhinal cortex, and suprachiasmatic nuclei (SCN). Dense fiber patterns were observed in amygdala, bed nucleus of stria terminalis, and SCN. Upon colchicine treatment, additional VIP cell bodies were visualized, particularly in PVN and periventricular areas. Small numbers of fibers were present in hypothalamic internal median eminence, and vertical profiles of VIP immunoreactivity, suggestive of "tanycytic" cells, extended through the median eminence. Immunostaining with both antisera, in both sexes, and in df/df and DF/? brains was comparable. VIP levels averaged (pg/µg protein): cortex 11.2±1.3 (DF/?), 14.4±1.0 (df/df); dorsal hypothalamus 1.5±0.2 (DF/?), 1.6±0.3 (df/df); ventral hypothalamus 2.6±0.3 (DF/?), 4.0±1.0 (df/df). Taken together, the results indicate that VIP measured in the ventral hypothalamus appears not to be perikaryal, and that brain VIP localization and content is unaltered in PRL-deficient Ames dwarf mouse.

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387.12

A LIGHT- (LM) AND ELECTRON-MICROSCOPIC (EM) STUDY OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) NEURONS IN A CIRCUMVENTRICULAR ORGAN OF THE CHICK.

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The lateral septal organ (LSO) of the chick is composed of lateral and medial components. The lateral component is marked by a vascularized ependyma that is conspicuous with nissl staining. The ependyma of the medial component located adjacent to the lateral septal area includes approximately 1000 cerebrospinal-fluid (CSF) contacting neurons of about 12 µm in diameter that immunostain for VIP. Both LM and EM analyses show that the VIP immunoreactive (VIPir) perikarya are situated directly beneath the ependymal cells. A typical CSF-contacting, VIPir-neuron extends a long, club-like neurite through the ependymal layer into the supraependymal region of the lateral ventricle. The club-like process is surrounded by cilia extending from ependymal cells. These neurons appear to project to the septal area where a dense VIPir neuropil is found. A third cell type with a distinctive shape and nuclear appearance is located in the LSOm along the ependymal wall where VIPir cells are present. The function of these neurons is unknown. They may be encephalic photoreceptors (Silver et al., Cell Tissue Res.(1988)253:189). Supported by USDA Grant #90-37240-5506.

387.13

IMAGE ANALYSIS OF VASOACTIVE INTESTINAL POLYPETIDE IMMUNOREACTIVE (VIP-IR) NEURONS IN THE MOUSE BARRELFIELD CORTEX

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The radial orientation of VIP-IR dendrites in the rat visual cortex suggests, that VIP neurons may play a regulatory role in columnar cortical units (Magistretti and Morrison, *Neuroscience* 24: 367, 1988). However, Hajós et al. (*Anat. Embryol.* 178:197, 1988) demonstrated a distribution of VIP-IR boutons with no signs of a columnar organization. The present observation tests the hypothesis of a cortical-column associated function of VIP by immunohistochemistry in the barrelfield cortex of the mouse, a cortical area with an anatomically demonstrable columnar organization. Tangential sections were stained with a VIP-antiserum using avidin-biotin and DAB for visualization. A barrel-like distribution of the VIP-IR structures was found. In order to analyze quantitatively the distribution of VIP-IR boutons within single barrels, we have used a previously published image analyzing technique (Hajós et al., *Anat. Embryol.* 176:207, 1988) for automatic measurement. The results demonstrate for the first time the columnar distribution of VIP-IR boutons. The highest density of boutons is found in the septum region of a barrel with a lower density in the periphery of the barrel core and the lowest density in the center of the core region. Since VIP-IR boutons are rarely found, where the highest density of synapses with the primary afferent fibers occurs (core), but most densely packed where the blood vessel density is maximal (septum), our results are in accordance with a presumed metabolic regulatory function of VIP in cortical columns. Supported by the DFG (Zi 192/6-3) and the Hung. Acad. Sci.

387.14

MAMMALIAN FMRF-NH_2-LIKE PEPTIDE, FLFQPQRF-NH_2, IN PERIPHERAL SYSTEM.

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FLFQPQRF-NH_2, neuropeptide FF (NPFF) detected initially by the FMRF-NH_2 antiserum, was isolated from bovine brain. NPFF is widley distributed in CNS and accumulating evidence suggests that NPFF may function as an antiopiate in the CNS. In the peripheral system, NPFF can increase blood pressure and inhibit insulin secretion from pancreas, however, whether there is NPFF in peripheral tissue is unknown. In this study, presence of NPFF in rat peripheral tissues including superior cervical ganglia, plasma, vas deferens, adrenal and pancreas was examined by HPLC coupled with NPFF RIA . NPFF was detected in plasma and superior cervical ganlia (0.10 ± 0.011 pmol/mg prot.) but not in other tissues. Analysis of pancreatic extract revealed two major forms of NPFF immunoreactive peptides which were also reactive to FMRF-NH_2 antiserum but were not identical to NPFF or FMRF-NH_2. Other investigators have found that FMRF-NH_2 (µM) and NPFF (nM) can inhibit pancreatic insulin secretion. Whether pancreatic FMRF-NH_2-like peptides have a similar inhibitory effect on insulin secretion remains to be determined.

387.15

A STUDY OF GnRH NEURONS THAT PROJECT TO THE MEDIAL BASAL HYPOTHALAMUS IN THE MALE DJUNGARIAN HAMSTER.

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In the Djungarian hamster at the onset of puberty (15 to 20d of age), increased gonadotropin secretion suggests well established GnRH projections to the medial basal hypothalamus (MBH). As part of a developmental study to determine the distribution and morphology of GnRH neurons which terminate in the MBH, 15d males in long days (16L:8D; n = 4) were intracardially perfused with 4% paraformaldehyde. Brains were removed and crystals of DiI, a fluorescent tract tracer, were implanted directly into the median eminence. After 8 weeks in phosphate buffer in the dark, brains were sectioned (60µm) and immunocytochemistry performed for GnRH (0.02% saponin for 0.5-1h prior to incubation with LR1 antisera) using the fluorescent marker avidin-bodipy. The majority of GnRH cells were found in the rostral forebrain, i.e., the medial preoptic area, diagonal band of Broca, and septal regions. Of these GnRH somata, 68% contained DiI indicating a projection to the MBH. Regions closer to the DiI implant site, the lateral preoptic area and rostral hypothalamus, had fewer GnRH cells labelled with DiI (26%). Morphologically, GnRH somata were bipolar or unipolar and present in a 2:1 ratio, respectively. Both subtypes were labelled with DiI in proportion to this ratio. Later in development, the brains from postpubertal 40d males (n = 4) were processed as described above. Again, bipolar and unipolar GnRH cells were DiI labelled in a 2:1 ratio. However, compared to 15d males, fewer GnRH perikarya were labelled with DiI in all areas, e.g. rostral forebrain (34.1%), especially the medial preoptic area (40d: 13% vs 15d: 48%). These data suggest that the MBH is innervated primarily by rostral forebrain GnRH cell bodies, both bipolar and unipolar. Findings raise the possibility that GnRH neuronal projections involved in the control of gonadotropin secretion change with maturation. (NIH HD22479)

387.16

NOVEL SITES OF MELANIN CONCENTRATING HORMONE mRNA AND PEPTIDE EXPRESSION IN LACTATING RATS.

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The deduced structure of the rat melanin-concentrating hormone (MCH) precursor (Endocrinology 125:2056, 1989) predicted the existence of at least two peptides that may be processed from it, one similar to teleost MCH and a second novel neuropeptide, NEI. Cellular localization studies confirmed that prepro-MCH (ppMCH) mRNA and the MCH and NEI peptides are expressed predominantly in cells in the dorsolateral hypothalamic area with minor contingents seen in the olfactory tubercle and pons. A moderate MCH- and NEI-IR axonal projection to the median eminence and, particularly, to oxytocin-rich regions of the posterior pituitary suggested some anatomical heterogeneity of ppMCH-expressing neurons in the hypothalamus, and an involvement in neuroendocrine function. In the present study, immuno- and hybridization histochemical methods were used to follow MCH gene and peptide expression as a function of reproductive status in female rats. Nursing dams sacrificed after 6-21 days of lactation displayed ppMCH mRNA and MCH- and NEI-IR in rostral, oxytocin-rich aspects of the paraventricular nucleus of the hypothalamus, and in a discrete, ventromedial, aspect of the medial part of the medial preoptic nucleus. This apparent induction was not apparent in animals sacrificed 7 days after weaning, during late pregnancy, or at any point in the estrous cycle. No frank alterations in ppMCH mRNA were evident in the dorsolateral hypothalamus as a function of reproductive status. The loci and apparent state-dependency of the induction of MCH mRNA and peptide expression suggests a role for these gene products in the control of lactation.

387.17

IMMUNOHISTOCHEMICAL LOCALIZATION OF PANCREASTATIN IN RAT PANCREATIC ISLETS

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Pancreastatin is a pancreatic peptide isolated from the porcine pancreas (K. Tatemoto et al., Nature 324: 476, 1986). It has been reported that pancreastatin in vitro inhibits glucose-induced insulin release. Immunohistochemical studies have shown that pancreastatin was located in porcine endocrine cells: pancreas, gut and pituitary. In the present study, using a specific antiserum against rat pancreastatin, we investigated the localization of pancreastatin in rat pancreatic islets by immunofluorescence techniques. The rats were perfused with 4% paraformaldehyde. The pancreas was sectioned at 10μm or 5μm (serial sections) on a cryostat. The tissue sections were processed by an indirect immunofluorescence method. Pancreastatin-like immunoreactivity was observed in ß-cells of the rat pancreatic islets. Serial sections of the pancreas showed that pancreastatin and insulin co-existed in ß-cells of the rat islets. Our results suggest that pancreastatin may play important roles in the regulation of ß-cell function and carbohydrate metabolism.

387.18

NEURONS IN THE SUBSTANTIA NIGRA OF RAT, MONKEY, AND HUMAN CONTAIN ACIDIC FIBROBLAST GROWTH FACTOR.

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Acidic fibroblast growth factor (aFGF) is one of a family of growth factors which have been characterized based on their mitogenic potential. In contrast to other FGF's, aFGF has been isolated from a limited number of tissues. We have used in situ hybridization histochemistry as well as Northern and Western blotting techniques to study the localization of aFGF mRNA and protein in rat, monkey, and human brain.

aFGF mRNA positive neurons were found principally in motor nuclei from the brainstem to the spinal cord in rat and monkey. In addition, cells containing aFGF mRNA were observed in the substantia nigra. aFGF mRNA was found in a subpopulation of nigral cells in both rat and monkey brain, although in the monkey not many nigral cells contained detectable aFGF mRNA. Unilateral 6-OHDA pretreatment of the rat substantia nigra (8ug/4ul, 2 weeks prior to sacrifice) produced a loss of both TH mRNA and aFGF mRNA on the lesioned side. Nigral localization of aFGF (mRNA and protein) was also observed in the human substantia nigra. These data suggest that some mesencephalic dopamine-containing neurons contain aFGF.

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387.19

ALTERATION IN CALBINDIN EXPRESSION IN THE CAUDATE PUTAMEN AND N. ACCUMBENS OF GENETICALLY EPILEPTIC PRONE RATS (GEPR 3 AND 9)

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It has been acknowledged that genetic factors may significantly contribute to the generation of seizure activity. In view of the role of Ca^{++} in neuronal excitability it is feasible that some of the genetically determined alterations may involve calcium dependent mechanisms. Calbindin (CB) and calretinin (CR) are neuronal peptides with high affinity for Ca^{++} and it has been suggested that they function as intraneuronal Ca^{++} buffering systems.In situ hybridization histochemistry has been used to selectively label the neurons expressing either of the two peptides. The regional and cellular distribution of the CB and CR mRNAs for the most part do not overlap. The level of expression of the mRNA coding for the two peptides was quantified in control rat versus Genetically Epileptic Prone Rats : GEPR-3 and GEPR-9 . These two strains of rats had been genetically selected to have enhanced suceptibility to audiogenic seizure (GEPR9 > GEPR3).CB mRNA is highly expressed in the ventral part of the caudate putamen and n.accumbens. A quantitative analysis in this region was performed since a visual variation of the labelling was apparent. Based on densitometric measurements of autoradiograms and grain counting of emulsion dipped slides we estimated the alteration in the basal level of expression of CB mRNA in the GEPR rats to be as follows: GEPR-3 (N=3, mean= -30.2 % +/- 4.3 ($p < 0.05$) ; GEPR-9 (N=3, mean = -59.8 % +/- 3.4 ($p < 0.001$).The corresponding levels of expression of CB was accessed using western blot semi-quantitative method. No apparent difference was observed between the 2 strains of GEPR rats; however a significant reduction in calbindin peptide imunodetection was observed in these strains compared to control rats . The results suggest that prior to the induction of seizure, the basal level of expression of CB mRNA in the ventral caudate putamen / n.accumbens is decreased and correlates with the severity of the seizure succeptibility.

387.20

PARVALBUMIN-IMMUNOREACTIVE STRUCTURES IN THE ENTORHINAL CORTEX OF THE HUMAN ADULT.

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Frontal sections of the adult human entorhinal cortex were incubated with anti-parvalbumin. Parvalbumin-immunoreactive neurons occur in all layers. The neurons in the external principal layers (pre-α, pre-β, pre-γ) outnumber those within the inner ones (pri-α pri-β, pri-γ). Small, medium-sized and large somata can be distinguished. They are either multipolar or bipolar in shape. Axon initial segments of several pyramidal neurons in layers pre-β, pri-α and pri-γ are enwrapped by immunoreactive fibres. The density of immunoreactive fibres is already high within the cellular islands of the upper cellular layer (layer pre-α) and increases further to a dense plexus in layer pre-γ. Towards the lamina dissecans the fiber density decreases abruptly and remains to be low in the inner layers. All observed immunoreactive cells belong to the class of non-pyramidal neurons, some of them are chandelier cells.

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387.21

ISOLATION OF A PORCINE cDNA CLONE FOR THE TRYPSIN INHIBITOR PEPTIDE LIKE PEC-60: WIDE SPREAD EXPRESSION AND PRESENCE OF THE PEC-60 PEPTIDE ALSO IN THE IMMUNE SYSTEM.

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A porcine cDNA clone of the trypsin-inhibitor like peptide PEC-60 which may serve as a neuropeptide signaling molecule (Fuxe et al. 1990, Soc Neurosci Abstr 16, 800) was isolated. The deduced amino acid sequence revealed a 87 amino acid long precursor protein that includes a 27 amino acid signal sequence. RNA blot analysis showed that the porcine PEC-60 gene is abundantly expressed in the pig as a 0.7 Kb mRNA in duodenum, bone marrow and peripheral blood cells. Lower levels were found in spleen. Immunohistochemical studies in the pig using a rabbit antiserum against PEC-60 (No. 2510) in combination with an immunofluorescence or an immunoperoxidase protocol demonstrated very strong cytoplasmatic specific PEC-60 like immunoreactivity (IR) in the goblet cells of the columnar epithelium of the duodenum and in probable epithelioid cells of the marrow of the thymus. A moderate degree of specific PEC-60 like IR was observed in the cytoplasm of islands of adrenal medullary gland cells present mainly in the marginal part close to the adrenal cortex as well as in the cytoplasm of sympathetic ganglion cells of the superior cervical gangion, in which also scattered strongly PEC-60 like IR cells were present, probably representing SIF cells. After absorption with PEC-60 no PEC-60 like IR could be demonstrated in the various tissues analyzed. The results indicate a role of PEC-60 like peptides not only in the neuronal, gastrointestinal and endocrine systems but also in the immune system. The results open up the possibility that PEC-60 like peptides could act to produce coordinated responses in the major regulatory systems of the body besides having a local role.

388.1

CHOLECYSTOKININ (CCK) ANTAGONISTS DECREASE THE SUSCEPTIBILITY OF RAT CAUDATE TO KCl-INDUCED SLOW WAVE DEPOLARIZATION. K. Kedzie*, J. Wilkerson*, R. Kasser and K. Renner. Dept. of Biomedical Sciences, Southwest Missouri State Univ., Springfield, MO 65804.

The effect of sulfated CCK octapeptide (CCK-8S) on the generation of slow wave depolarization (SWD) in the rat caudate (CPu) was studied using in vivo voltammetry. Pressure-ejection of 50 μM CCK-8S into the CPu induced signals recorded with Nafion-coated carbon fiber electrodes (CFM). Based on in vitro selectivity properties of the CFMs, these signals were predominantly due to increases in dopamine (DA). The similar propagation rates of signals induced by CCK-8S (2.2 ± 0.3 mm/min) and 100 mM KCl (1.8 ± 0.2 mm/min) suggests that the CCK-8S-induced signals represent a SWD. The CPu failed to respond to a second CCK-8S stimulus. Dose-dependent decreases in KCl-induced DA signals were recorded following i.v. proglumide (4-64 mg/kg) and lorglumide (Rotta Labs., 20-640 μg/kg). KCl-induced signals returned to 69% of pre- lorglumide signals after 130 min. These results suggest that the susceptibility of the CPu to KCl-induced SWD is enhanced by CCK-8S. Studies testing this possibility are in progress. (Supported by MH-44893)

388.2

GASTRIC SALINE LOADS AND CELIAC ARTERY CHOLECYSTOKININ INFUSIONS ACT SYNERGISTICALLY TO STIMULATE GASTRIC VAGAL AFFERENT ACTIVITY IN RATS. G.J. Schwartz, P.R. McHugh & T.H. Moran, Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We have previously demonstrated that doses of cholecystokinin (CCK) ineffective in inhibiting food intake when given alone become effective when paired with a non-nutrient gastric load. To determine whether the gastric vagal afferent response to CCK is also modified by the presence of gastric loads, we examined the neurophysiological responses of single cervical vagal afferent fibers with gastric receptive fields that responded to both gastric loads and CCK (N=6). Anesthetized rats received 1) a range of intra-celiac artery infusions of CCK alone (10-1000 pmol), 2) various intragastric physiological saline loads alone (2-7 ml), and 3) combinations of CCK and gastric loads. Doses of CCK that were subthreshold when given alone always increased vagal afferent discharge rates when given in combination with gastric loads. Doses of CCK that were effective alone elicited greater discharge rates when combined with a load, whether or not the load alone elicited an increase in discharge rate. Thus, the presence of a gastric load 1) lowered the threshold CCK dose required to elicit an increase in vagal afferent discharge rate, and 2) always magnified the discharge rate elicited by suprathreshold doses of CCK. These results demonstrate a peripheral integration of CCK- and load- elicited signals at the level of the afferent vagus, and may provide a mechanism for the synergistic actions of gastric loads and CCK in the inhibition of food intake. (Supported by DK19302).

388.3

EFFECTS OF THE NEUROPEPTIDE CHOLECYSTOKININ ON THE GOLDFISH MAUTHNER CELL. D. P. Yox, M. J. Titmus, and D. S. Faber. *Neurobiology Laboratory, Dept. of Physiology, State University of New York at Buffalo, Buffalo, NY 14214.*

Current evidence suggests that neuroactive peptides may act to modulate the effects of nonpeptide neurotransmitters. In the medulla of the goldfish, antibodies against the neuropeptide cholecystokinin (CCK) were used to identify the presence of CCK-like immunoreactivity in proximity to the Mauthner (M-) cell. CCK-like immunoreactive varicosities were observed in close proximity to the M-cell body and the lateral dendrite. To examine the physiological effects of locally applied CCK, we pressure ejected solutions of CCK (30-60 μM) near the M-cell soma or lateral dendrite while recording intracellularly from the same respective areas. Local application of CCK near the M-cell body (N=3) was accompanied by an enhanced input resistance, as evidenced by an increase (23.7±5.0%) in antidromic spike height and by single electrode voltage clamp. In contrast, local application of CCK to the lateral dendrite caused a decrease in input resistance (N=3), as evidenced by a decrease in antidromic spike height (-22.7±9.4%) and a similar reduction in excitatory and inhibitory synaptic responses. In both cases, the resting membrane potential was either unchanged or slightly hyperpolarized. Local application of the CCK vehicle had no effects on input resistance or resting membrane potential. These data support the hypothesis that CCK is a neuromodulator at the M-cell membrane. While the effects of CCK alone may be difficult to interpret, the role of CCK at this level may be to modulate the effects of other transmitters such as dopamine or GABA. (Supported by DHHS: NS08819 and NS15335.)

388.4

IN VITRO EXCITATION OF RAT NODOSE GANGLION CELLS BY CHOLECYSTOKININ OCTAPEPTIDE. N. J. Dun and S. Y. Wu* Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

Cholecystokinin (CCK) has been implicated as one of the putative transmitters involved in the production of satiety by acting on vagal afferents. Current/voltage clamp recordings from isolated rat nodose ganglion cells show that pressure applications of CCK octapeptide (CCK-8) evoke in the large majority of ganglion cells a rapid depolarization or a fast inward current and in a few cells a slow depolarization or a slow inward current. The fast response is associated with a fall of input resistance and is increased by membrane hyperpolarization. The extrapolated reversal potential is about -10 mV. This response is reduced in a Na^+-free, Tris-Cl solution and by d-tubocurarine but is not reduced in a low Cl^- solution. The slow response is associated with an increase of membrane resistance and is decreased by hyperpolarization; the extrapolated reversal potential is around -90 mV. The results suggest that CCK-8 produces two types of depolarizing response in rat nodose ganglion cells, probably by acting on two distinct types of receptors. The fast response appears to be caused by an increase of cation conductance, whereas the slow response is likely to be due to a reduction of membrane K^+ conductance. (Supported by NS18710 & NS24226).

388.5

BOMBESIN INHIBITORY EFFECT ON GASTRIC VAGAL EFFERENT DISCHARGE (GVED). E. Yoshida-Yoneda, J.Y. Wei, and Y. Taché. CURE/VA Medical Center, Dept. of Medicine and Brain Res. Inst., UCLA, CA 90073.

Surgical and pharmacological studies indicate that central and peripheral bombesin actions on gastric function and food intake involve the autonomic nervous system. In the present study we use electrophysiologic approach to investigate whether central and peripheral injection of bombesin will influence GVED.

Male SD rats were anesthetized with urethane (1.25g/kg im). A microdissection technique was used to isolate efferent fibers from the ventral gastric branch of the vagus. Strands were cut distally to record efferent multi-unit activity. Three minute peak changes from basal GVED were calculated. Bombesin and saline were injected intravenously (iv) or intracisternally (ic) through a catheter into the cisterna magna.

Bombesin ic (6.2, 62, 620, 6200 pmol, n=5) induced a dose-dependent inhibitory effect on GVED (100.2 ± 11.4%, 77.5 ± 9.5%, 50.3 ± 3.5%, 42.5 ± 3.1%, respectively). Bombesin iv (6.2 x 10^{-6}, 6.2 x 10^{-2} and 62 pmol n=4) also suppressed GVED (68.0 ± 3.9%, 76.5 ± 7.2%, 38.8 ± 9.5%, respectively). Bombesin iv produced stronger inhibition compared with ic. Continuously iv infusion of bombesin monoclonal antibody 2A11 did not reduce the inhibitory effect (61.3 ± 14.1%, n=5) of ic bombesin (620 pmol) compared with control (41.5 ± 14.1%, n=5), whereas the antibody blocked the inhibition of iv bombesin in the same animal. Bombesin (62 pmol, iv) reduced VED recorded from cervical fibers to 24.8±6.1% ; a second bombesin injection following bilateral cervical vagotomy below the site of recording reduced by 56% the inhibitory effect of bombesin (n=4). In control group (n=4), two successive injections of bombesin had similar inhibitory effect.

These data indicate that (1) bombesin acts in the brain to decrease GVED, (2) bombesin iv exerts a potent inhibitory effect on GVED which is probably mediated in part through an action on vagal afferent.

388.6

BOMBESIN-INDUCED HYPOTHERMIA IN HYPOGLYCEMIC RATS: A DOSE-RESPONSE STUDY. AM Babcock[1*], P. Lomax[2], and TW Moody[3]. [1]Dept. of Psych., Univ. of S. AL, Mobile, AL 36688; [2]Dept. of Pharm., UCLA Sch of Med, Los Angeles, CA 90024; [3]Dept. of Biochem, George Washington Univ Med Center, Washington D.C. 20037.

Infusion of bombesin (BOM) at a dose of 50 ng or more into the POA produces hypothermia in rats made hypoglycemic with insulin (Babcock et al.,'89 Pharm, Biochem, & Behav 34, 539). The present Exp examined the dose-response relationship of this hypothermic action. Using a randomized block design, 5 insulin-pretreated rats (10U/kg; i.m.) received POA microinfusions of BOM (0, 5, 12, 25, 50 ng/ .25 μl). Rectal temperatures (T_b) were measured prior to insulin and at 60 mins following BOM. Treatments were separated by at least 2 days. BOM produced a significant reduction in T_b ($F(4,16)=3.817$ $p<.02$) that was dose-related ($r=.58$ $p<.002$). Our findings support the notion that the POA is a sensitive site for BOM-induced hypothermia under hypoglycemic conditions. We are presently examining the ability of (Psi[13,14], Leu[14])BOM to antagonize the response (data to be presented).

388.7

EFFECT OF CHOLECYSTOKININ ON GENE EXPRESSION IN THE RAT BRAIN. R. Miyoshi, S. Kito, T. Nomoto*, M. Weiser and T.H. Joh. Dept. of Pharmacol., Tokyo Women's Med. Coll., Tokyo 162, Division of Health Sciences, Univ. of the Air, Chiba 260, Japan and Lab. of Molecular Neurobiology, Cornell Univ. Med. Coll., New York 10605, U.S.A.

Excitation of cells by means of growth factors and others causes changes of gene expression which result in long-term response of cells. The response is due to sequential induction of mRNAs, involving the proto--oncogenes. Clinically, it has been observed that a single dosis of caerulein, an analog of cholecystokinin -8, causes improvement of involuntary movements lasting for much longer period than being expected from its short half-life time in circulating blood. We investigated effects of caerulein on changes of gene expression in the rat brain through in situ hybridization. Intraperitoneal injection of caerulein did not affect basal levels of c-fos and c-jun mRNAs. It is known that administration of convulsants such as pentylenetetrazole (PTZ) causes rapid increases of proto-oncogenes in the rat brain. In our experiments, increases of c-fos and c-jun mRNAs were observed in the hippocampus and cerebral cortex one hour after PTZ injection. Administration of caerulein one hour prior to PTZ decreased the effect of PTZ on the expression of the mRNAs. It is considered that clinically-experienced long-term effects of caerulein are the results of such changes of gene expression.

388.8

ALTERATION OF JEJUNAL ABSORPTION OF AMINO ACIDS BY INTRACEREBRAL INJECTIONS OF VASOACTIVE INTESTINAL PEPTIDE (VIP). C.F. Nassar*, S. Itani*, N.E. Saadé and S.J. Jabbur Fac. of Med., American University of Beirut, Lebanon.

High concentrations of VIP were found in the gut and in areas of the central nervous system involved in processing visceral information. Intravenous injection of 11.2 ng/Kg /min produced an immediate and maintained decrease in the absorption of amino acids in the jejunum similar to that produced by ulcerogenic agents (Nassar, C.F. et al., FASEB J. 2:734, 1988) but without an apparent ulceration.

To investigate whether this effect is neurally mediated, 11.2 ng/Kg of VIP were stereotaxically microinjected either in the lateral ventricle (n=5 rats) or in the dorsal motor nucleus of the vagus (n=5 rats). Amino acid absorption in a jejunal segment was determined, for 2-3 hrs following injections using the single-pass perfusion technique and labelled alanine as the permanent probe (Nassar, C.F. et al., Comp. Biochem. Physiol. 89:61, 1988). The results show a significant decrease in alanine absorption ($P<0.05$) from 0.2 µmole/20 min.cm to 0.12-0.11 µmole /20 min.cm in both injected groups. Water movement across the jejunal segment did not show a significant change resulting from these injections.

These results demonstrate that alteration of jejunal absorption by VIP is neurally mediated and may act, at least partially, through the parasympathetic system. (Supported by grants from LNRC and DTSabbagh Fund).

388.9

TRH-INDUCED GASTRIC MOTILITY INCREASES ARE MEDIATED BY A CHOLINERGIC MECHANISM IN YOUNG RATS. M.M. Heitkemper*, E.F. Bond*, K. Gruver*, & A. Horita. Depts. of Physiological Nursing & Pharmacology, University of Washington, Seattle, WA 98195

Centrally administered thyrotropin releasing hormone (TRH) increases gastric motility and small intestine transit in rats as young as 14 days (D). Prior treatment with serotonin (5-HT) blocking agents decreases TRH-induced intestinal transit increases in adult rats. This study examined effects of 5-HT blocking agents xylamidine 10mg/kg, ketanserin 5mg/kg, cyproheptadine 1.5mg/kg, and the anticholinergic atropine 2mg/kg on TRH-induced gastric contractile activity in rats 7,10,14, & 21D. Rats treated with p-chlorophenylalanine (PCPA) 48 hrs prior were also studied. Rats were anesthetized (urethane, 1.25mg/kg). A tension transducer was implanted on the anterior gastric corpus. After 1hr baseline recording, test rats were injected with blocker IP. Control and PCPA treated rats received no pretreatment. After 30min TRH in saline (5 or 10µg in 0.6µl) or saline was injected intracisternally and motility recorded. Data were expressed relative to baseline. In 10,14, and 21D rats TRH produced a significant increase in gastric contractile activity. In all age groups, cyproheptadine inhibited TRH-induced increases in gastric motility. However, inhibition was not observed with xylamidine, ketanserin or PCPA. Atropine blocked TRH-induced gastric motility indicating TRH stimulation of gastric motility is via a cholinergic mechanism in young rats (≤21D). (Supported by N00007, NCNR, NIH).

388.10

THYROTROPIN RELEASING HORMONE (TRH) EXCITES THE CHOLINERGIC SYSTEM ORIGINATING IN THE NUCLEUS BASALIS OF MEYNERT.

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It has been reported that cerebral blood flow (CBF) increases following either an intravenous or a central administration of thyrotropin releasing hormone (TRH) in anesthetized animals. The study in our laboratory has recently suggested that the cholinergic vasodilative system contributes to the TRH-induced increase in the CBF in the cortex[1]. The present experiment aimed to clarify whether TRH actually excites cholinergic neurons originating in the nucleus basalis of Meynert (NBM) and projecting to the cortex and eventually increases exracellular concentration of acetylcholine (ACh) in the cortex using anesthetized rats. Single neurons in the NBM were identified by antidromic stimulation of their axons in the cortex. ACh was measured using a microdialysis method. Intravenous administrations of 300 µg/kg and 3,000 µg/kg of TRH increased both the nerve activity and the concentration of ACh dose-dependently. 30 µg/kg of TRH was ineffective to the both parameters. The results suggest that the TRH-induced activation of the cholinergic fibers originating in the NBM is involved in the TRH-induced increases in the cortical CBF.

1) Inanami, O. *et al.* (1988) Neurosci. Lett. 88:184-188.

388.11

PREPROTRH 53-74 STIMULATES PRIMARY CULTURES OF RAT ANTERIOR PITUITARY TO ENTER EARLY S-PHASE. R. Toni, M. Vitale*, S. Mosca*, L. Zamai*, A. M. Martelli*, L. Cocco*. Insts. Human Anatomy and Ort. Rizzoli, University of Bologna, Italy.

The N-terminal fragment of the thyrotropin-releasing hormone precursor, preproTRH 53-74 (pYT22) has been identified in axon terminals in the rat median eminence but its physiological role is still unknown. To determine whether pYT22 may influence the growth of anterior pituitary cells we studied its effect on the incorporation of the synthetic analogue of thymidine, bromodeoxyuridine (BrdU) in the nuclei of cells from primary cultures of rat anterior pituitary. Primary adenohypophyseal cell cultures were grown 72 hs, then the cells incubated in serum-free medium with 100 nM pYT22 up to 18 hs plus 100 um BrdU in the last 12 hs. The percentage of DNA replicating cells was identified by flow cytometry, using directly FITC-conjugated antibodies to BrdU and a bivariate DNA/BrdU analysis focused on early S-phase vs G0/G1. Primary cultures treated with pYT22 showed 58.9% (control 44.9%) and 46.0% (control 34.5%) of cells in early S-phase after 12 and 18 hs of treatment, respectively. These results show that pYT22 increases the number of cultured adenohypophyseal cells that enter early S-phase and suggest that it may play a role on growth regulation of anterior pituitary.

388.12

SOMATOSTATIN (SST) INHIBITS NICOTINIC CHOLINOCEPTOR MEDIATED OESOPHAGOMOTOR ACTIVITY IN VIVO. Y.T. Wang, R.S. Neuman and D. Bieger, Fac. of Med., Memorial Univ. of Nfld. St. John's, NF Canada A1B 3V6

Work from this lab has revealed the presence of excitatory nicotinic cholinoceptors on oesophagomotor neurons of the ambigual compact formation (AMB_c) of the rat. In vitro, AMB_c neurons respond to acetylcholine (ACh) with a fast depolarisation that is reversibly inhibited by SST, unlike the response to S-glutamate (GLU). Here we report oesophagomotor responses evoked by application of these substances at deglutitive sites in the solitarius and ambiguus complex of the intact urethane-anaesthetized rat (adult male Sprague-Dawley). ACh (0.5 M), S-glutamate (0.2 M) and SST (0.001 M) were dissolved in artificial cerebrospinal fluid and injected in volumes of 20-100 pl from three-barreled glass micropipettes (tip dia < 10 µm). Oesophageal responses to ACh and GLU ejection in AMB_c usually consisted of short-latency, single, nonpropulsive pressure waves and showed little evidence of desensitisation when evoked at 2-3 min intervals. SST (0.1-0.3 pmol) produced a feeble, inconsistent oesophageal pressure wave following which the ACh response was reversibly inhibited, whereas that to GLU was slightly enhanced or unchanged. The SST-ACh antagonism showed dose-dependence and surmountability. Interestingly, SST did not eliminate facilitation by Ach of GLU responses. In contrast, SST and ACh displayed no inhibitory interaction at solitarial oesophagomotor sites, where ACh responses are mediated by muscarinic cholinoceptors. In conclusion, SST produces a selective inhibition of nicotinic cholinoceptor activity at the level of the AMB_c. As this peptide has been demonstrated in a subpopulation of solitarial premotor neurons projecting to the AMB_c, our observations strengthen the hypothesis that SST plays a role in the generation of oesophageal peristalsis. Supported by MRC (Canada).

389.1

NPY INHIBITS FEEDFORWARD IPSP'S IN HIPPOCAMPUS BY REDUCING EXCITATORY INPUT TO INTERNEURONS. G.J. Klapstein and W.F. Colmers. Dept of Pharmacology, Univ. of Alberta, Edmonton, T6G 2H7.

Neuropeptide Y (NPY) has been shown to selectively inhibit excitatory synaptic transmission in the *in vitro* hippocampus by a presynaptic mechanism. However, orthodromically-evoked IPSP's are also reduced by NPY in area CA1. To examine whether NPY's action was due to a direct effect on the interneurons mediating the GABAergic (A and B receptor mediated) IPSP's, we examined focally-evoked monosynaptic IPSP's in principal neurons in hippocampus under pharmacological blockade of excitatory amino acid neurotransmission.

Intracellular recordings were made from pyramidal neurons in areas CA1 and CA3 of transverse (450 μm) slices of hippocampus, maintained submerged in oxygenated buffer at 34°C. Synaptic potentials were evoked with a pair of sharpened tungsten electrodes mounted on independent micromanipulators, permitting optimal placement. All components of the feedforward EPSP-IPSP (E/I) complex evoked by stimulation of stratum radiatum in CA1 were inhibited equally by NPY (1 μM). However, when IPSP's (having both $GABA_A$ and $GABA_B$ components) were isolated by the presence of CNQX (10 μM) and APV (50 μM), NPY had no effects. Isolated IPSP's evoked in CA3 pyramidal neurons were similarly unaffected by application of the peptide. As previously reported, NPY caused no detectable changes in the properties of the postsynaptic neurons.

The simplest explanation for the observed results is that NPY presynaptically inhibits excitatory inputs to interneurons mediating the feedforward IPSP in area CA1. NPY does not appear to act on the interneurons themselves. The results help clarify the role of NPY in the hippocampus by helping define the synaptic connections subject to its influence.

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389.2

NEUROPEPTIDE Y (NPY) DOES NOT ALTER POSTSYNAPTIC NMDA CURRENTS IN PYRAMIDAL NEURONS OF AREA CA3 OF THE RAT HIPPOCAMPAL SLICE A.R. McQuiston and W.F. Colmers. Dept of Pharmacology, Univ. of Alberta, Edmonton, T6G 2H7.

NPY affects synaptic transmission in hippocampus by a presynaptic mechanism. Recently authors have postulated that NPY may also act at the sigma and PCP receptors. Binding studies have shown inconsistent results, some investigators finding specific high affinity binding of NPY to the sigma and PCP receptors while others did not. Iontophoretically- applied NPY increases the rate of firing of rat hippocampal CA3 pyramidal neurons evoked by iontophoresis of NMDA in vivo, consistent with the idea that NPY acts at the PCP receptor. However, these studies are in direct contrast to studies from our laboratory showing that NPY has no postsynaptic actions in area CA1, including no effect on the response to iontophoretically-applied glutamate.

To test whether NPY affected NMDA conductances, we used the tight-seal, whole-cell technique to record responses of pyramidal neurons in area CA3 of submerged hippocampal slices (450 μm, 34°C) to iontophoretically applied NMDA. The intracellular solution contained (in mM) Cs-gluconate, 135; HEPES, 10; EGTA, 5.5; $MgCl_2$, 2; pH 7.2-7.3. TTX (500 nM) was applied in the perfusate to block Na^+ currents. NMDA (50 mM, pH 8.0) was applied by brief (<10 msec) pulses of negative current (50-120 nA) to proximal dendrites of CA3 cells. Under these conditions bath application of NPY (1 uM) did not alter the response to NMDA. However, in other experiments, bath application of NPY (1μM, in the presence of 50 μM picrotoxin) inhibited EPSC's evoked by extracellular stimulation of the mossy fibers, consistent with earlier work.

The results show that NPY does not alter NMDA currents and is thus unlikely to be a ligand at the PCP receptor. The role for NPY in hippocampus thus appears to be only its inhibition of synaptic transmission.

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389.3

THE SITE AND MECHANISM OF ACTION OF NEUROPEPTIDE Y IN CULTURED RAT HIPPOCAMPAL NEURONS **D. Bleakman, N. L. Harrison†, W.F. Colmers‡ and R.J. Miller**. Dept. of Pharmacol. and Physiol. Sci., and †Dept. of Anesthesia and Critical Care, Univ. of Chicago, Chicago, IL 60637 and ‡Dept. of Pharmacol., Univ. of Alberta, Edmonton, Canada.

We have examined the effects of neuropeptide Y (NPY) on synaptic transmission and $[Ca^{2+}]_i$ signals in rat hippocampal neurons (E17) grown in cell culture. Using combined whole cell patch clamp/Fura-2 based microfluorimetry we were able to observe frequent fluctuations in $[Ca^{2+}]_i$ which corresponded to spontaneous synaptic activity. These fluctuations were inhibited by tetrodotoxin (1μM), and combinations of CNQX (10μM) and APV (50μM) indicating that they were the result of glutamatergic transmission occurring between neurons. $[Ca^{2+}]_i$ fluctuations and spontaneous activity were also abolished by Ni^{2+}(200μM) and reduced by baclofen (10μM), NPY (100nM) and fragments of NPY implicating the involvement of the Y2 receptor. Following treatment of cells with pertussis toxin, the effects of baclofen, NPY and NPY fragments were reduced. NPY had no effect on somal Ba^{2+} or Ca^{2+} currents measured in hippocampal neurons under whole cell voltage clamp even in the presence of GTP-γ-S. Furthermore, NPY (100nM) had no effect on the cell soma $[Ca^{2+}]_i$ signal accompanying the Ca^{2+} current in these cells even though Ba^{2+} currents were inhibited by both Cd^{2+} (200μM) and baclofen (10μM). Current clamp recordings from neurons demonstrated the occurence of spontaneous E.P.S.P.'s and action potential firing which was accompanied by increases in $[Ca^{2+}]_i$. This activity and the accompanying $[Ca^{2+}]_i$ signal was reduced by NPY (100nM), NPY fragments and baclofen (10μM). However, when increases in $[Ca^{2+}]_i$ were induced by trains of evoked action potentials in the presence of CNQX and APV, NPY (100nM) had no effect on these cell soma $[Ca^{2+}]_i$ signals. Hence, NPY is able to inhibit excitatory neurotransmission in these neurons through a pertussis toxin sensitive mechanism although no effect of NPY on Ca^{2+} influx into the cell soma of these neurons was discernable.

389.4

NPY AND SIGMA LIGANDS ACT IN THE PARAVENTRICULAR NUCLEUS TO BLOCK CRF-INHIBITED GASTRIC ACID SECRETION IN URETHANE ANESTHETIZED RATS. M. Gué*, J.L. Junien*§, M. Yoneda*, H. Mönnikes*, L. Diop§and Y. Taché, CURE/VA Wadsworth Medical Center, Department of Medicine and Brain Research Institute UCLA, Los Angeles, CA 90073 / §Institut de Recherche Jouveinal, 1 Rue de Moissons, Fresnes, France

CRF has been shown to inhibit gastric acid secretion after injection into the cerebral spinal fluid, with a site of action in the PVN. Since NPY and sigma ligand act through a common receptor to antagonize stress and CRF-induced colonic hyperactivity, the aim of this study was to evaluate whether NPY and JO 1784 a specific sigma ligand can alter the CRF-inhibited gastric secretion by acting in the PVN.

Gastric output was recorded with a double-lumen cannula every 10 min. by flushing the gastric lumen. Intracisternal (IC) injection of NPY or JO 1784 (0.01 to 0.5μg in 5μl) was performed 10 min before IC injection of CRF. In a second series of experiments unilateral microinjection of NPY or JO 1784 (30 ng in 50 nl) into the PVN was done 10 min before microinjection of CRF (3 μg) into the PVN. Immediately after IC or microinjections, pentagastrin (10 μg/kg/hr) was infused through the femoral vein for 2 hours. Acid output was determined by titration of the flushed perfusate with 0.1 NaOH to pH 7.0

Intracisternal injection of CRF resulted in an inhibition of 71.6% of the gastric output in the rat when acid secretion was stimulated by IV infusion of pentagastrin. The inhibitory effect of CRF was dose dependently blocked by concomitant IC injection of JO 1784 or NPY (0.01 to 0.1 μg) the complete reversal was obtained at a dosage of 0.1 μg. CRF microfused into the PVN (3 μg) also reduced by 88.3% the gastric output acid stimulated by pentagastrin. Microinjection into the PVN of NPY (30 ng) or JO 1784 (30 ng) had no effect on acid secretion but abolished the inhibitory effect of CRF.

These results indicate that central administration of NPY and JO 1784 abolished CRF-induced inhibition of gastric acid secretion in urethane anesthetized rats and that they act directly on PVN structures to exert their inhibitory effect on CRF.

389.5

THE DEPRESSOR EFFECT OF INTRATHECAL NEUROPEPTIDE Y DEPENDS ON SPINAL CATECHOLAMINES. X. Chen and T.C. Westfall Dept. Pharmacol. & Physiol. Science, Saint Louis Univ. Sch. of Med., St. Louis, MO 63104

We have shown previously that the intrathecal (int) injection of NPY C-terminal fragments produces a depressor effect similar to NPY. In the present study, we examined the ability of the fragments to block the response to NPY and evaluated the role of spinal catecholamines (CA) in mediating the depressor effect of NPY. For int injections, the atlanto-occipital membrane was exposed by a midline incision, a PE-10 catheter was inserted 6 cm down the thoracic spinal subarachnoid space (T10). Drugs were dissolved in saline and slowly injected at a volume of 5 µl followed by 5 µl of vehicle to wash the catheter. Co-administration of NPY^{14-36} (1.0 nmol) and NPY (0.1 nmol) did not affect the depressor effect of int NPY. NPY^{18-36}, also behaved in a similar manner as NPY^{14-36} suggesting that these fragments do not possess antagonistic or partial agonistic activity. [Leu^{31},Pro^{34}]-NPY has been shown to be a specific Y_1 receptor ligand. Int injection of the Y_1 ligand (0.1 and 1.0 nmol) did not significantly change arterial pressure (AP) suggesting that the depressor effect of NPY is not mediated by spinal Y_1 receptors and further supports the hypothesis that Y_2 receptors are involved. Some rats were pretreated with an int injection of 6-OHDA (20 μg) and the effect of NPY tested after 7 days. At the end of each experiment, rats were decapitated and a spinal thoracic-lumbar segment (about T_6-L_3), brain and heart were removed and prepared for monoamine measurement. Int 6-OHDA pretreatment significantly depleted norepinephrine (NE) (81%) and epinephrine (EPI) (46.5%) in the spinal cord but not in the brain and heart. Such pretreatment completely blocked the depressor effect of int NPY suggesting that NE and EPI play an important role in mediating the depressor effect of NPY. (Supported by HL 26319 and HL 35202).

389.6

ANTAGONISM OF THE ACTIONS OF [11-36]NPY AND [$LEU^{31}PRO^{34}$]NPY (LP) at Y2 AND Y1 RECEPTORS IN THE RAT MESENTERIC ARTERIAL BED. M.A. McAuley*, A. Howlett and T.C. Westfall, Dept. of Pharmacol. and Physiol. Sci., Saint Louis Univ. Sch. of Medicine, St. Louis MO 63104.

Earlier investigation of the vascular actions of Neuropeptide Y (NPY), led us to propose that distinct NPY receptors mediated the pre- and postsynaptic actions of this peptide in the isolated and perfused rat mesenteric arterial bed. In this model of the vascular neuroeffector synapse, a presynaptic NPY receptor was previously designated Y2 based on the ability of NPY C-terminal fragments to mimic the attenuation of the periarterial nerve-stimulated (PNS) release of norepinephrine. At high concentrations the intact peptide but not C-terminal fragments could potentiate the nerve stimulated increase in perfusion pressure and thus in accordance with other studies, the postsynaptic receptors were classified as Y1 receptors. In the present study further examination of the involvement of these putative receptor subtypes in the actions of NPY has revealed a more complicated organization of these receptor subtypes in this preparation. Perfusion of [11-36]NPY (10^{-10}-10^{-8}M) produced a concentration-related potentiation of the increase in perfusion pressure elicited by subsequent administration of norepinephrine (NE) alone, suggesting the existence of a postsynaptic Y2 receptor. This potentiation had a latency of onset of 8 mins, reached a maximum at 30 mins and was abolished by Phentolamine(Phen), attenuated by Benextramine(Benxt) but not significantly altered by the novel NPY antagonist PYX-1. The reputedly Y1 selective NPY analogue LP(10^{-10}-10^{-7}M) attenuated the PNS release of NE indicating that the Y1 subtype may also be located presynaptically. Moreover LP produced an immediate potentiation of the increase in perfusion pressure elicited by exogenous NE substantiating the presence of a postsynaptic Y1 receptor. This action was also differentially effected by Phen., Benxt. and PYX-1. Supported in part by HL 26319 and HL 35202.

389.7

NPY(13-36) AND [LEU-PRO]NPY CONSTRICT RAT FEMORAL ARTERY RINGS, AND PROTECT NPY FROM BENEXTRAMINE BLOCKADE, BY ACTING AT Y1 AND Y2 RECEPTORS. R.E. Tessel, D. Xu*, D.W. Miller*, and M.B. Doughty*. Depts. of Pharmacol. & Toxicol. and Med. Chem, Univ. of Kansas, Lawrence, KS 66045-2505.

Rings were attached to transducers (Time 0) and equilibrated in warmed physiological buffer prior to being maximally contracted with 10 uM norepinephrine both 30 and 60 min after Time 0. 70 min after Time 0, tissues were exposed to 1.0 uM of NPY, NPY(13-36), [Leu^{31}-Pro^{36}]NPY (LP-NPY) or vehicle 5 min prior to a 10-min exposure to benextramine (BXT; 10 uM) or saline. A cumulative concentration-effect curve for one of the above three peptides (0.001-1.0 uM) was then constructed beginning 95 min after Time 0. Except during drug or peptide exposure, buffer was changed every 10 min. All three NPY congeners induced concentration-dependent constriction and with similar apparent EC_{50}'s (60 +/- 19, 38 +/- 12 and 44 +/- 9 nM for NPY, NPY (13-36) and LP-NPY, respectively). BXT essentially abolished these effects, probably irreversibly. All three peptides protected NPY from BXT blockade, and desensitized NPY-induced constriction. In contrast, NPY and LP-NPY desensitized LP-NPY-induced responses and protected them from BXT-induced blockade but NPY(13-36) did neither. These data suggest that: 1) postsynaptic vascular Y2 receptors do exist; 2) rat femoral arteries contain both Y1 and Y2 constriction-mediating, BXT-sensitive receptors; 3) NPY is an agonist at both receptors; and 4) NPY(13-36) and LP-NPY are selective Y2 and Y1 agonists, respectively, in the rat vasculature. (Supported by grants from the American Heart Association, National and Kansas Affiliates.)

389.8

EFFECT OF CALCITONIN GENE-RELATED PEPTIDE ON HYPOXIA IN THE RAT HIPPOCAMPUS IN VITRO. **F.Z. Wang and A.S. Ding, Dept. of Neurobiology, Institute of Basic Medical Sciences, Beijing 100850, China**

The effects of calcitonin gene-related peptide on hypoxia were examined in the rat hippocampal slice. When hippocampal slice was immersed in the superfusing medium with 95%O_2 + 5%CO_2(normoxic), the field population spike (FPS) was recorded in CA_1 pyramidal cells by stimulating Schaffer collateral pathways. The FPS was gradually abolished following the hypoxia produced by superfusing hypoxia medium with 95%N_2 + 5%CO_2 mixture. At 150ml/min flow of hypoxia mixture the average time when the FPS was initially attenuated and completely abolished were 3.60±1.01 min and 4.90± 1.21min respectively (N=27), when the flow was increased to 200 ml/min, the average time were 2.46±0.80min and 4.33±1.54min respectively. While the FPS vanished, its latency extended and its duration increased. In addition, the presynaptic responses disappeared subsequently. When oxegen was recharged the FPS in the most slices (80%) failed to recover after 20 min of hypoxic superfusion. When 4nM β-CGRP- containing medium was superfused to the slice before hypoxia, the time at which FPS began to attenuate and completely abolished were post-poned. They were 6.70±3.40 min and 15.96±2.64min respectively. 100% of slices appeared FPS again when they reoxgenated after 20 min of hypoxic and the percentage was 70% when hypoxic exposure lasted 40 min (N=10). Superfusion of CGRP during the period of hypoxia also increased the recovery of FPS.

The results show that the time of FPS's attenuating and vanishing varied according to the degree of hypoxic exposure, therefore, it may be used as a comparable parameter of hypoxia. Also, neuropeptide -CGRP has appearently protective effect when hippocampus was exposed to hypoxia in vitro.

389.9

AMYLIN INCREASES TRANSPORT OF TYROSINE AND TRYPTOPHAN INTO BRAIN. A.Balasubramaniam*, F.S.Zhang*, I.Thomas* and W.T. Chance. Dept. Surgery, Univ. of Cincinnati Med. Ctr. and VA Med. Ctr., Cincinnati, OH.

Amylin (AMY) is a 37 amino acid peptide of the CGRP family isolated from pancreatic amyloid deposits of type II diabetic patients. We reported anorexia, elevated tryptophan (TRP) and increased metabolism of monoamines following intrahypothalamic (iht) injection of AMY (Brain Res. 539:352,1991). In the present study, we specifically investigated whether iht AMY would further increase brain concentrations of TRP and tyrosine (TYR) following their systemic injection. Following the implantation of hypothalamic cannulae, groups (n=5-7/gp) of adult, male, SD rats were treated (ip) with either 50 mg/kg each of TRP and TYR ethyl ester or saline (SAL) followed (15 min) by either iht AMY (2 ug) or CSF (2 ul). Sixty min later all rats were decapitated and the levels of monoamines, precursors and metabolites were determined by HPLC-EC. Striatal concentrations of TYR were elevated ($p<0.05$) by 39%, while TRP levels were increased 69% in AMY-treated TYR-TRP rats as compared to the SAL-TYR-TRP group. Levels of DOPAC and HVA were also elevated significantly by 21% and 44% in these rats. Similar alterations in TYR and TRP were observed in the hypothalamus of AMY-TYR-TRP rats. These results suggest that blood-brain-barrier transport of TYR and TRP may be increased by iht AMY. Supported by Dept. of Veterans Affairs and PHS #CA 48057.

389.10

A PEPTIDE ANTAGONIST BLOCKS THE SPINAL EFFECT OF INTRATHECAL GALANIN IN RATS

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The neuropeptide galanin (GAL) applied intrathecally (i.t.) has a complex effect on spinal flexor reflex excitability. At low doses (3 and 30 pmol) i.t. GAL purely facilitates the reflex, whereas at higher doses (300 pmol and 3 nmol), facilitation, facilitation followed by depression or pure depression occurs (Brain Res., 486, 1989, 205-213). We have presented evidence that GAL, which is present in primary afferents and spinal cord interneurons, acts as a functional antagonist of the excitatory neuropeptides substance P and calcitonin gene-related peptide and blocks the C-afferent conditioning stimulus (CS) induced central facilitation of reflex excitability (Eur. J. Neurosci., 2, 1990, 733-743).

We now report that a peptide antagonist to GAL has been developed. This GAL antagonist applied i.t. at 30 pmol - 3 nmol reduces the facilitatory effect of 30 pmol i.t. GAL on the flexor reflex in a dose-dependent fashion. The inhibitory effect of this dose of GAL on the C-afferent CS-induced central facilitation is also reversed by this GAL antagonist.

Since GAL has a variety of functions in the CNS and other systems, an antagonist of GAL may prove to be experimentally and clinically useful.

389.11

GALANIN CAUSES A HYPERPOLARIZATION OF LOCUS COERULEUS NEURONS IN THE *IN VITRO* BRAIN SLICE . Vincent A. Pieribone[1], Ulo Langel[2], Tamas Bartfai[2] and Tomas Hökfelt[1] . [1]Dept. of Histology and Neurobiology, Karolinska Institutet & [2]Dept. of Biochem., Arrhenius Lab. Stockholm, Sweden.

Both anatomic and physiologic studies indicate that LC neurons form axo- and dendro-dendritic synapses with surrounding LC neurons. This has led to the proposal that LC neurons modulate their own discharge through the feedback release of noradrenaline (NA) onto surrounding LC neurons. Since virtually all NA containing neurons of the LC also contain and may release the neuropeptide galanin (GAL), the present study sought to determine what, if any effects GAL has on the membrane properties of LC neurons recorded in the *in vitro* brain slice preparation. Horizontal slices (300 μm thick) containing the LC were cut on a Vibratome and immediately transferred to a slice chamber. Slices were maintained at 37° C in a Ringer's solution containing (in mM): 124 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.24 NaH_2PO_4, 2.4 $CaCl_2$, 25 $NaHCO_3$ and 10 glucose and saturated with 95% O_2 / 5% CO_2. Neurons of the LC were impaled with microelectrodes containing K^+acetate (DC resistances of 90-110 megohms). Precise quantities (100-1000 nl) of a GAL solution (10^{-5} M) were applied from a calibrated micropipette positioned adjacent (100 μm) to the recording electrode. The resting membrane potential and input impedance of cells were monitored during GAL application. GAL caused a dose dependent hyperpolarization and decrease in membrane impedance in all LC neurons tested. Since the hyperpolarization could be reversed in neurons in which the resting potential was held at about -95 mV, it appears that GAL increases K^+ conductance in these neurons. Further characterization of GAL effects on LC neurons will be reported. In addition, a putative peptide antagonist of 20 amino acids with nM affinity for CNS GAL receptors reversed the GAL induces hyperpolarization. VAP was supported by an NSF (INT-8908720) and a Fogarty (1 F20 TWO 1586-01) post-doctoral fellowship.

389.12

ENDOGENOUS GALANIN (GAL) MODULATES THE GONADOTROPIN AND PROLACTIN (PRL) PROESTROUS SURGES IN THE RAT. F.J. López, E.H. Meade, A. Daniels and A. Negro-Vilar. Reproductive Neuroendocrinology Section, LMIN, NIEHS, NIH. Research Triangle Park, NC 27709.

Studies from our laboratory have clearly demonstrated that GAL is secreted to the hypophyseal portal circulation and that plays an important role in controlling gonadotropin secretion by both stimulating LHRH release from nerve terminals of the median eminence and acting directly at the level of the pituitary gland stimulating and enhancing LHRH-induced LH secretion. We have utilized passive immunoneutralization using an anti rat GAL sera raised in sheep in order to evaluate the role of endogenous GAL in the events leading to the preovulatory surges of gonadotropins and PRL. Female rats receiving either 1 ml normal sheep serum (NSS) or GAL antiserum (GAL-AS) i.v. were bled through an indwelling intraatrial cannula at hourly intervals from 1400 to 2300 h on the day of proestrus. GAL-AS administration significantly reduced by 40% the preovulatory LH surge as measured by the area under the curve. Analysis of the time points revealed statistically significant lower LH levels at 1700, 1900 and 2000 h in GAL-AS-treated animals. The FSH surge was not altered (area under the curve and maximum FSH levels) by GAL-AS treatment, although GAL-AS-treated animals presented lower FSH levels at 1700 h. In contrast, the PRL proestrous surge was dramatically reduced by passive immunization against GAL. Statistically significant differences were observed in the area under the curve and at 1700, 1800, 1900, 2000, 2100 and 2200 h in the GAL-AS-treated group. Our results indicate that endogenous GAL participates in mechanisms leading to a full expression of the LH and PRL preovulatory surges.

389.13

INHIBITION OF ENDOGENOUS GLUTAMATE AND ASPARTATE RELEASE IN RAT HIPPOCAMPUS BY GALANIN

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Galanin, a 29 amino-acid peptide, has potent inhibitory effects in the central nervous system, notably in the hippocampus where it modulates the release of acetylcholine via presynaptic receptors (Fisone, et al., 1987). Since excitatory amino-acids (EAA) play an important role in the synaptic transmission of the hippocampus, we have examined the effect of galanin on endogenous EAA release.

Superfused rat hippocampal slices were used to investigate the effect of galanin in the K^+ -evoked release of endogenous glutamate and aspartate. Preincubation of slices during 15 min with 1 μM of the (1-16) biologically active galanin fragment reduced the basal efflux of EAA of 20%. The stimulation of the slices by 50 mM K^+ during 6 min induced an increase of the EAA release by 250-300%. In the presence of galanin (1 μM) K^+ -evoked release of glutamate and aspartate were reduced by 70% and 50% respectively (n = 5), with a half-maximal effective concentration in the nM range.

These results suggest a modulation of EAA release by galanin in the hippocampus. This peptide could constitute a metabolic stress regulator which protects against excessive neurotransmitter release, notably during ischemic situations.

Fisone, G., et al., Proc. Natl. Acad. Sci., 1987, 84, 7339-7343.

389.14

EFFECTS OF NEONATAL TREATMENT WITH TYR-MIF-1, MORPHICEPTIN, AND MORPHINE ON DEVELOPMENT, TAIL-FLICK AND BLOOD-BRAIN BARRIER TRANSPORT.

W.A. Banks*, J.E. Zadina, L.M. Harrison, M.A. Oleshansky[1], J.W. Holaday[1], and A.J. Kastin. VAMC and Department of Medicine and Neuroscience Training Prog, Tulane U. Sch. of Med, New Orleans, LA 70146 and [1]Walter Reed Army Medical Center.

Morphine (M) and endogenous peptides are known to alter developmental processes, inducing changes that can endure into adulthood. Morphiceptin (Mcp) binds to mu opiate receptors and to non-opiate sites labeled by Tyr-MIF-1 (TM), a brain peptide known to modulate opiate effects. M,Mcp,TM, M+TM, and Mcp+TM (50μg, sc) were given to rats during the first week of life. Animals given M alone or in combination with TM had significantly lower body weights for the first 3 weeks of life and reduced eye opening on day 16. Rats given M+TM had hypersensitive tail flick responses on day 3. Locomotor, passive avoidance, and rotorod behaviors were not altered by the neonatal treatments. Brain catecholamines, their metabolites, and Tyr-MIF-1 concentrations were also not altered at day 23. On day 23, transport of ^{125}I-Tyr-MIF-1 out of the brain was significantly increased by neonatal M, an effect significantly potentiated by neonatal TM. These results indicate that neonatal administration of peptides and opiates can affect later peptide transport as well as selected developmental landmarks.

389.15

THE EFFECT OF ALTERING ADENYLATE CYCLASE ACTIVITY ON THE RESPONSE OF ANTRAL CIRCULAR MUSCLE TO SUBSTANCE P (SP).

H.-S.Feng* and A.Ouyang. Department of Medicine/G.I. Section, University of Pennsylvania, Philadelphia, PA 19104.

SP causes a contractile response in the antral circular muscle in cats. The mechanism of this action is unknown. In this study, the **aims** were to determine 1) the site of action of SP in the circular muscle and 2) the involvement of the adenylate cyclase system in the action of SP.

Methods: Circular muscle strips from the antrum in cats were mounted in individual muscle bath chambers and attached to force transducers after removal of the mucosa. Contractile responses of the strips to SP and drugs were examined at isometric maximal tension expressed as Grams Force x min/gram tissue±SEM. **Results**: SP10^{-7}M and 10^{-6}M caused a contractile response. Neither atropine (10^{-6}M) nor tetrodotoxin (TTX 10^{-6}M) blocked this response. There was no significant difference between the response to SP 10^{-7}M and to SP after atropine(3.57±0.52 and 4.02±0.71, n=6), nor between the responses to SP 10^{-6}M with or without atropine(9.37±1.48 and 10.29±0.73, n=6). Similar responses were seen with TTX. Exposure of muscle strips to forskolin 10^{-5}M significantly increased the response to SP 10^{-7}M from 2.72±0.83 to 10.52±1.44 (n=8, p=0.001). Theophylline10^{-5}M also increased the response to SP 10^{-7}M from 3.04±1.18 to 14.54±2.91 (n=8, p=0.002). In contrast, 2',3'-dideoxyadenosine10^{-4}M decreased but did not abolish the response to SP 10^{-7}M from 9.90±1.62 to 2.72±0.83 (n=7, p=0.013). **Conclusions**: SP acts at the antral circular muscle via smooth muscle receptors. In view of the known inhibitory effects of c-AMP on smooth muscle contraction, we postulate that the enhancing effect of increasing c-AMP is mediated via an interacting neural pathway.

389.16

RELEASE Of FLFQPQRF-NH_2, A MAMAMLIAN FMRF-NH_2-LIKE PEPTIDE, FROM RAT SPINAl CORDS BY SUBSTANCE P.

J.M. Zhu and H.-Y.T. Yang. Lab. of Biochem. Genetics, NIMH Neuroscience Center at St. Elizabeths, Washington D.C. 20032.

The mammalian FMRF-NH_2-like peptide, FLFQPQRF-NH_2 (NPFF) initially isolated from bovine brain, was found to reduce rat tail flick latencies and modulate opiate mediated analgesia. NPFF immunoreactive nerve terminals and NPFF receptors are highly localized in superficial laminae of dorsal spinal cords. Previously we have found that NPFF release from rat spinal cords can be evoked by 56 mM KCl. In this study, effects of various transmitters on secretions of NPFF were investigated by in vitro superfusions of rat spinal cords. Substance P significantly increased the efflux of NPFF-immunoreactivity from the spinal cord in a dose dependent manner while substance K, noradrenaline, serotonin and morphine failed to show the effct. HPLC analysis of the superfusate after the substance P revealed two NPFF immunoreactive peaks and one of them was eluted in the position of NPFF. These results together with the biological activity and anatomical location of NPFF suggest that NPFF in the spinal cord may have a role in senssory transmission.

389.17

HISTAMINE RELEASE INDUCED BY SUBSTANCE P AND CGRP 8-37 FROM RAT PERITONEAL MAST CELLS

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The effect of substance P (SP) and calcitonin gene-related peptide fragment 8-37 (CGRP 8-37) on histamine (HA) release from rat peritoneal mast cells was studied and compared to those of compound 48/80 (C48/80). Rat peritoneal mast cells were incubated 10 min with SP, CGRP 8-37 or C48/80 in normal Tyrode's solution (Na^+ 137 mM) or Na^+-substituted solutions in the presence (1.5 mM) or absence (+1 mM EGTA) of extracellular calcium ($[Ca^{2+}]_o$). The effects of different Ca^{2+} blockers were studied. HA concentration was assayed enzymatically.

The results demonstrated that 10 μM SP and CGRP 8-37 in the concentration of 1 μM induce HA release which is $[Na^+]_o$-dependent and inhibited by $[Ca^{2+}]_o$, whereas a higher CGRP 8-37 concentration (10 μM) and C48/80 (5 μg/ml) induced HA release is dependent on $[Ca^{2+}]_o$ and is not influenced by changes in $[Na^+]_o$.

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390.1

INTERRUPTION OF THE MEDIAL FOREBRAIN BUNDLE (MFB) REDUCES ANGIOTENSIN II RECEPTOR BINDING IN THE RAT FOREBRAIN. R.C. Speth, K.L. Grove, K.N. Stephenson*, W.F. Ganong and M.K. Steele. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520 and Dept. of Physiology, University of California, San Francisco, San Francisco, CA 94143.

Angiotensin II (AII) regulates luteinizing hormone (LH) release in the female rat via its actions on AII receptors (AIIR) in or near the ventral portion of the bed nucleus of the stria terminalis (BSTV) of the brain (Steele, *Neuroendo.* 46:401; Grove et al., *Neuroendo.* 53:339). To determine the cellular structures that contain the AIIR binding sites in the rat forebrain, inputs from the brainstem via the MFB were unilaterally cut with a knife. Two weeks later rats were sacrificed and AIIR binding was determined by *in vitro* receptor autoradiography using ^{125}I-Sar^{1},Ile^{8} AII in the piriform cortex (PC), BSTV, medial preoptic n. (MPO), lateral septum (LS), paraventricular n. of the hypothalamus (PVN) and ventrolateral hypothalamus (VLH).

Nucleus	Ipsilat. Side	Contralat. Side.	% Decrease
PC	318±179	423±224	26±13*
BSTV	196±80	288±103	30±17*
MPO	381±218	394±217	3±15
LS	241±52	242±54	3±7
PVN	775±403	795±317	6±14
VLH	322±205	371±173	19±19

* $p < 0.01$ paired t test. Values are mean±SD fmol/g, n = 9

These data suggest that some forebrain AIIR, including those in brain areas that regulate LH release, are in part located presynaptically on the terminals of axons that ascend in the MFB.

390.2

ANGIOTENSIN RECEPTOR SUBTYPES, WATER INTAKE AND LH AND PROLACTIN SECRETION IN FEMALE RATS. M.K.Steele, Physiology Department, University of California, San Francisco, 94143.

These experiments were done to determine which of the recently identified Ang II receptor subtypes (AT-1 or AT-2) mediates the stimulation of LH secretion, the inhibition of prolactin release and the dipsogenesis induced by third cerebroventricular (icv) administration of Ang II in female rats.

Icv cannulae were implanted at least one week prior to use in experiments. Estradiol benzoate and progesterone were administered 72 h, and a jugular cannula implanted 48 h, prior to the experiment. The animals were non-stressed, conscious and had access to food and water during blood withdrawal. Either artificial cerebrospinal fluid or 10, 50, 100, 500 or 1000 ng of DUP 753 (AT-1 antagonist, Dupont) or of PD 123177 (AT-2 antagonist, Parke Davis) was administered icv, followed 10 minutes later by Ang II (100 ng). Multiple blood samples were taken for determination of plasma LH and prolactin levels. Water intake was also measured.

Ang II-induced water intake was attenuated (65%) by 1000 ng DUP 753; lower doses of DUP and all of the doses of PD had no effect.

Ang II-induced inhibition of prolactin was prevented by 1000 ng of both DUP and PD. The prolactin-reducing effects of Ang II were unaffected by lower doses of either drug.

Ang II-induced stimulation of LH was attenuated in a dose-dependent manner by both DUP and PD: 1000 ng totally abolished the stimulation while 100 ng prevented the rise by about 50%; 10 ng had no effect.

These data demonstrate that water intake induced by Ang II is mediated by the AT-1 receptor subtype. Both AT-1 and AT-2 receptor subtypes mediate the effects of Ang II on LH and prolactin. Ang II-induced LH release shows greater sensitivity than water intake to blockade of the AT-1 receptor.

390.3

DIFFERENTIAL DRINKING RESPONSES TO CHRONIC INFUSION OF ANGIOTENSIN III IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS. Julie Y.H. Chan, Jing-Shiou Yang* and Samuel H.H. Chan. Inst. of Pharmacology, National Yang-Ming Medical College, and Dept. of Medical Research, Veterans General Hospital-Taipei, Taipei, Taiwan, R.O.C.

We evaluated the chronic effect of angiotensin III (AIII) in the elicitation of drinking behavior in spontaneously hypertensive (SH) and normotensive Wistar-Kyoto (WKY) rats, using conscious, freely moving male animals that have been instrumented with an intracerebroventricular (i.c.v.) cannula connected to an osmotic minipump for 7-day application. Chronic infusion of AIII (5 or 10 pmol/min) elicited robust, dose-dependent and Ile^{7}-AIII (100 pmol/min, as specific antagonist)-reversible dipsogenesis in both SH and WKY rats. However, the drinking response in the SHRs exhibited a sharp decline after 3 days of AIII infusion, during which acute AIII (80 pmol, i.c.v.) challenges also failed to induce dipsogenesis. Chronic i.c.v. infusion of bestatin (150 pmol/min), an aminopeptidase inhibitor, did not by itself discernibly affect basal drinking. When combined with AIII (5 or 10 pmol/min), however, bestatin respectively augmented and suppressed the dipsogenic response of WKY and SH rats to the heptapeptide. These results suggest that chronic administration of AIII did not produce sustained drinking behavior in SHRs because of the development of early desensitization of the AIII receptors.

390.4

DRINKING RESPONSE IN RATS TO THE INTRAVENTRICULAR INJECTION OF VARIOUS ANALOGS OF ANGIOTENSIN. **D. G. Changaris,* L. T. Harrison,* and R. S. Levy. Departments of Neurology and Biochemistry and The Laboratory of Biological Psychiatry, University of Louisville School of Medicine, Louisville, Kentucky 40292.**

Rats will drink when angiotensin II (AII) is injected into the brain's third ventricle. For the endogenous generation of AII to initiate drinking, the prevailing view is that the hydrolysis of a dipeptide from the decapeptide angiotensin I (AI) by the brain's angiotensin converting enzyme is required. However, we have found that other angiotensin analogs initiate drinking when injected intraventricularly into male Sprague Dawley rats. Des-leu angiotensin I (des-leu AI) initiates a drinking response, but the D-his substituted nonapeptide is not nearly as potent a dipsogen. Similarly, AI initiates a drinking response, but the D-leu substituted decapeptide is not nearly as potent a dipsogen. Since both the AI and des-leu AI responses are blocked by captopril, and since we have found that des-leu AI is not a substrate for converting enzyme, this would indicate the existence of an alternate pathway for the synthesis of AII that requires a two-step hydrolysis of leucine and histidine from AI. Changes in the conformation of the N-terminus by injecting the D-asp substituted des-leu AI does not affect the drinking response. **(Supported by NIH-CIDA 5 KO8 SO1164; VA DOD 003; and the Humana Centers of Excellence - Humana Heart Institute International.)**

390.5

INVOLVEMENT OF NUCLEUS TRACTUS SOLITARIUS IN ANGIOTENSIN III-INDUCED SUPPRESSION OF BARORECEPTOR REFLEX RESPONSE IN THE RAT. Samuel H.H. Chan, K.S. Lin* and Julie Y.H. Chan. Institute of Pharmacology, National Yang-Ming Medical College, and Department of Medical Research, Veterans General Hospital-Taipei, Taipei, Taiwan, R.O.C.

Previous work from our laboratory suggests that the endogenous angiotensin III (AIII) exerts a tonic inhibitory action on the baroreceptor reflex (BRR) response. The present study further examined the role of the nucleus tractus solitarius (NTS), the terminal site of the baroreceptor afferents, in this process. Adult, male Sprague-Dawley rats anesthetized with pentobarbital sodium were used. Bilateral microinjection of AIII (40 pmol) into the NTS significantly inhibited the BRR response. On the other hand, local application of the specific AIII antagonist, Ile^{7}-AIII (1.6 nmol), appreciably augmented the same reflex. In addition, intracerebroventricular administration of AIII (100 pmol) and Ile^{7}-AIII (100 nmol) respectively suppressed and enhanced the responsiveness of the arterial pressure-related neurons in the NTS to transient hypertension induced by phenylephrine (5 µg/kg, i.v.). Similar results were obtained when AIII or Ile^{7}-AIII was given microiontophoretically. Based on these findings, it is possible that neurons which contain AIII may decrease the BRR response by tonically reducing the sensitivity of baroreceptive neurons in the NTS to arterial pressure perturbations.

390.6

ANGIOTENSIN CONVERTING ENZYME (ACE) AND ANGIOTENSIN II (AT) RECEPTORS ARE REGULATED BY ESTROGEN IN THE ANTERIOR PITUITARY OF THE FEMALE RAT. A.M. Seltzer, F.M.A. Correa, M. Steele and J.M.Saavedra. Section on Pharmacology, Laboratory of Clinical Science, NIMH, Bethesda, MD 20892.

We have studied anterior pituitary ACE and AT receptors during the estrous cycle, in ovariectomized (OVX) rats, and after estrogen replacement (17-β-estradiol, 0.05 mg pellets, s.c., for 1 week). ACE binding was determined by quantitative autoradiography after incubation of pituitary sections with the ACE inhibitor [^{125}I]351A. ACE activity was measured by a radioenzymatic method using [^{14}C]hippuryl-L-histidyl-L-leucine. AT receptors were quantified by autoradiography after incubation of pituitary sections with the AT agonist [^{125}I]Sar^{1}-AT. There were no differences in ACE binding or activity during the estrous cycle. Both the binding to and the activity of ACE were increased in OVX rats when compared to normally cycling animals, and they were both returned to normal after estrogen replacement. Marked differences in AT receptor expression occurred during the estrous cycle, with concentrations highest in the metestrous phase (metestrous>diestrous>estrous>proestrous). OVX produced a large increase in the anterior pituitary AT receptor number, and the AT receptor concentration was markedly reduced after estrogen replacement. Our results indicate a dual regulation of the anterior pituitary AT system by estrogen, both at the level of the peptide synthesis and AT receptor number.

390.7

REGIONAL *C-FOS* EXPRESSION IN THE AV3V, HYPOTHALAMUS AND AMYGDALA FOLLOWING INTRAVENTRICULAR ANGIOTENSIN II AND ITS MODULATION BY DRINKING. J. Herbert, S.R.Howes* and P.M Stacey*, Department of Anatomy, University of Cambridge, CB2 3DY, UK

The release of angiotensin II (AII) both centrally and peripherally has been implicated in the behavioural and physiological response to hypovolaemic stress. A number of sites of action, including the pre-optic area and the so-called 'anterior region of the third ventricle' (AV3V) have been defined by either lesion or local application of AII. We have studied the expression of *c-fos* protein immunohistochemically in the limbic system following intraventricular (icv) infusion of AII to determine the pattern of activation and whether this is altered by allowing the animals to drink. In the first experiment, either 1000 or 250 pmols AII were infused and animals examined 60 minutes later; water was removed during this interval. Both doses induced high levels of *c-fos* expression in a band of neurons extending from the OVLT ventrally through the medial septum to the sub-fornical organ dorsally; that is, corresponding to AV3V. There were also high levels of *c-fos* in the hypothalamic paraventricular nucleus (PVN), principally in the lateral magnocellular group of cells (though some parvicellular areas showed activity) in the supraoptic nucleus (SON), and in the central nucleus of the amygdala. *C-fos* also increased in the ventral BNST. Allowing animals access to water during the 60 minutes following AII infusion modified the pattern of *c-fos* expression. *C-fos* was reduced dramatically in the SON and the magnocellular PVN, but not in the AV3V and the other areas examined. If water was withheld for 15 minutes, and then allowed, *c-fos* expression was not altered. Much greater amounts of 0.9% saline than water were ingested following icv AII , but had no effect on *c-fos* in the PVN or SON. These experiments show that this peptide induces highly localised expression of *c-fos* in areas known to be concerned with its dipsogenic and endocrine actions, and that this pattern is selectively altered by allowing the animal to drink either hypo- or isotonic solutions.

390.8

CELLULAR TARGETS FOR PEPTIDERGIC TRANSMISSION IN THE HIPPOCAMPUS. T. Smock, Albeck, D., Cooper, R.*, Garritano, J.*, Marrs, J. K.*, Minerbo, G.* and Alvarado, M. Behav. Neurosci. Prog., Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

A peptide similar in structure to vasopressin or oxytocin acts as a transmitter in the rat hippocampus (**Br. Res.**, 1990, 511: 7 and 15, **Peptides**, 1991, 12: 47 and 53). The peptidergic fibers arise in the amygdala and project mostly to the ventral hippocampus. In this study we sampled CA_1 pyramidal cells in both the ventral and the dorsal hippocampus and granule cells in the dentate gyrus to determine the anatomical specificity of responsiveness to the peptide.

With acute single-unit recording techniques and ratemeter analysis we studied six pyramidal cells in ventral CA_1 (AP -6.0, ML 5.8, 6.0 deep) and ten cells in dorsal CA_1 (AP -4.4, ML 2.9, 2.0 deep). Three of the six ventral pyramidal cells were inhibited following electrical activation of the peptidergic pathway, and seven of the ten dorsal pyramidal cells were inhibited. We made use of the fact that the influence of peptidergic transmission is invariably repeatable within experiments to test for antagonist blockade. The signal in all three ventral units was blocked by a specific anti-oxytocic antagonist and two of three dorsal units tested showed antagonist blockade. To ascertain the responsiveness of granule cells we performed field potential recording in the dentate gyrus, activated the peptidergic pathway, moved to field potential recordings in CA_1, and activated it again. In each of three such experiments, peptidergic inhibition was apparent in CA_1 but not in the dentate gyrus.

These results indicate that the neural targets of the peptide transmitter include pyramidal cells throughout the hippocampus (and, by inference, interneurons) but do not include granule cells. As for peptidergic transmission in other systems (e.g., ELH in *Aplysia*, LHRH in frog autonomic ganglia), the peptide targets in our system include cells that are close to the sites of release as well as targets that are remote from the sites of release.

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390.9

Ca^{2+} HOMEOSTASIS IN CEREBRAL CORTICAL NEURONS OF EMBRYONIC RAT BRAIN: LACK OF PEPTIDERGIC EFFECT. M.L. Koenig, M.A. DeCoster, and M.A. Oleshansky. Medical Neurosciences Branch, Div. Neuropsych., Walter Reed Army Inst. Research, Wash. DC 20307-5100.

Peptides have been found to be the primary neurotransmitter in some types of neurons and are co-released with "classical" neurotransmitters in many others. Since many peptides have been shown to have neuromodulatory activity, we have investigated the possibility that at least part of the peptidergic effect may be attributable to alterations in intraneuronal Ca^{2+} homeostasis. Cortical neurons were isolated from fetal rat brains, loaded with a membrane-permeable form of the Ca^{2+} indicator fluo-3, and peptide-induced changes in intraneuronal Ca^{2+} were monitored using the ACAS 570 interactive laser cytometer (Meridian Instr., Okemos, MI). Sequential image scans of fields containing 5-10 neurons were used to construct kinetic profiles of peptidergic effects. Identification and verification of the viability of individual neurons was made by exposing the cells to a depolarizing (15 mM) solution of KCl. None of 10 peptides, shown in other studies to have neuromodulatory properties, had any effect on basal or depolarization-dependent levels of free Ca^{2+} over time periods of 5-10 min. The peptides studied (10^{-7} to 10^{-6} M) were arginine vasopressin, cholecystokinin, corticotropin releasing hormone, neuropeptide Y, neurotensin, somatostatin, thyrotropin releasing hormone, vasoactive intestinal peptide, endothelin, and parathyroid hormone. The possibility that some or all of the peptides may have effects measurable only over longer time periods and/or on receptor-gated Ca^{2+} fluxes is currently being investigated.

390.10

INSULIN, GLUCAGON AND SOMATOSTATIN IN THE CONTROL OF ENERGY METABOLISM. J. Menéndez and D. Atrens*. Dept. of Psychology, University of Sydney, NSW 2006, Australia.

Although there is a great deal of information on the effects of insulin, glucagon and somatostatin on carbohydrate, fat and protein metabolism, there is little information on how these peptides modulate thermogenesis and substrate utilisation. The present study analyses the effects on these parameters of systemic injections of insulin (4-8 U/kg), glucagon (25-100 ug/kg) and somatostatin (0.5-10 ug/kg). Energy expenditure (EE) and respiratory quotient (RQ) were respectively used as indices of thermogenesis and energy substrate utilisation. They were calculated in an open-circuit calorimeter from oxygen consumption, carbon dioxide production and locomotor activity. An increase in RQ indicates the preferential combustion of carbohydrates, and even the synthesis of fat from carbohydrate when values are above 1.00. A decrease in RQ indicates the preferential combustion of fat. 4 U/kg insulin had no effect, while 8 U/kg insulin increased both EE and RQ. Glucagon also increased EE and RQ, though in a more clear dose-related manner. Somatostatin decreased RQ and EE. Neither peptide affected locomotor activity which indicates that the effects on EE and RQ represent primary metabolic effects on thermogenesis and substrate utilisation. The conclusions are: 1- Insulin, glucagon and somatostatin are key regulators of thermogenesis and energy substrate utilisation; 2- Insulin and glucagon have similar metabolic roles in terms of thermogenesis and fat deposition (despite well-known opposite effects on carbohydrate metabolism and blood glucose levels); 3- Somatostatin is antithermogenic and fat depleting; these effects add to other reported somatostatin's anti-insulin and anti-glucagon effects. The present data support, therefore, the view of a more complex role for insulin and glucagon. They can no longer be simply considered as anabolic or catabolic in nature. This assertion is further supported by new data showing that the metabolic effects of insulin (Menéndez and Atrens, Brain Research, in press) and glucagon (Menéndez and Atrens, in preparation) injected into the paraventricular nucleus of the hypothalamus are opposite to their systemic metabolic effects. Supported by an A.R.C. grant to D.M.A.

390.11

COMPARISON OF THE EFFECTS OF SOUND STRESS (SS) WITH THOSE OF INTRACRANIAL CORTICOTROPIN RELEASING FACTOR (CRF) ON RAT BRAIN TRYPTOPHAN HYDROXYLASE (TrpH). K.C. Corley, T-H. Phan* and M.C. Boadle-Biber. Dept. Physiol., Va Commonwealth University, Richmond, VA 23298.

In the present study, we tested whether repeated intracranial CRF mimics the effect of repeated SS on TrpH activity. An acute, 1-h exposure to SS produces a rapidly reversible increase in cortical and midbrain TrpH acvitity which can be abolished in vitro by incubation with alkaline phosphatase[1]. This effect can be mimicked by a single intracranial injection of CRF into the amygdala[2]. In contrast, repeated SS results in increased enzyme activity which is stable for at least 24 h and is unaffected by incubation with alkaline phosphatase, in vitro[1]. A more detailed examination of the time course of this more stable increase in enzyme activity now reveals that the 85% increase at 24 h was down to 70% at 28 h, 50% at 32 h, 31% at 36 h and had disappeared after 48 h.

The effects of repeated CRF were assessed by the injection on 3 successive days of CRF (0.5 ug/2ul per side) into the amygdaloid central nucleus via bilateral guide cannulae implanted 3 days earlier under surgical anesthesia. The increase in TrpH activity in these animals, sacrificed immediately after the last injection, was alkaline reversible and no different than that obtained from animals after a single CRF injection. Thus the long-lasting increase in TrpH after repeated SS was not observed after repeated injections of CRF, a finding that suggests that this peptide does not play a role in this effect of SS on enzyme activity.

[1] Boadle-Biber et al. Brain Res., 482: 306-316, 1989.

[2] Singh et al. Soc. Neurosci. Abstr., 15: Abstr. # 91.18, p 225, 1989.

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390.12

IMMUNOCYTOCHEMICAL ANALYSIS OF NEUROPEPTIDE CHANGES IN RAT CEREBROVASCULAR AND DURAL NERVES AFTER EXPERIMENTAL SAH. A.G. ARAND*, L.L. LANKER, M. ZUCCARELLO*, AND J.T. KELLER. Depts. Neurosurg. U. Cincinnati, The Christ Hosp., J.N.G. Inst. Med. Res and Mayfield Neurol Inst. Cinti., OH 45219.

Previous studies have demonstrated the presence of a robust cerebral and dural perivascular neural network containing multiple neurotransmitters/neuropeptides, believed to be involved in the regulation of cerebral blood flow. Changes in the concentration of various neuropeptides after experimental subarachnoid hemorrhage (SAH), due to as yet undefined mechanism(s), has been documented previously in cerebral vessels and recently in the dura mater. The purpose of this study was to simultaneously examine changes in cerebrovascular and dural neuropeptide concentrations after cisternal injection of 0.3 ml. of autologous arterial blood (experimental) or 0.3 ml. buffered lactated ringers (sham) in male Sprague-Dawley rats. Changes in calcitonin gene related peptide (CGRP), substance P(SP), and neuropeptide Y (NPY) staining of cerebral and dural vessels were examined and evaluated by independent observers at 6,24. and 48 hours after SAH. CGRP was reduced in cerebral vessels at 6 hours and returned to normal at 24 hours. In the dura CGRP staining remained unchanged in all time periods. A marked decrease in SP and NPY immunostaining was noted at 6 hrs in cerebral vessels and dura of all animals. SP immunostaining in both tissues returned to control (normal) levels at 48 hours, while NPY staining had not yet reached control levels. The similar changes in experimental and sham SP and NPY animals suggests a general physiologic response eg. increased intracranial pressure to SAH. Furthermore differences of CGRP immunostaining in cerebral versus dural vessels suggests that changes in dural CGRP immunostaining may in part be mediated by a central neural mechanism.

*This study was supported in part by NIH grant NS22969 (JTK)

390.13

THE EFFECT OF CENTRALLY ADMINISTERED ATRIAL NATURIURETIC POLYPEPTIDE (ANP) ON ANTERIOR PITUITARY HORMONE RELEASE IN RATS. L.A. De Luca Jr., L.Y. Ma*, J.Y. Liu*, Z.M. Qi*, B.Z. Ho*, M.L. Zhang*, S.Q. Xi*, Z.S. Wang*, with advice of E. Stellar and A.N. Epstein. Tianjin Medical College, P.R. China, and University of Pennsylvania.

ANP immunoreactivity is present in many areas of the central nervous system, including anterior pituitary. The possibility of a role for ANP in the control of anterior pituitary prompted us to investigate the effect of ANP on the hormonal release of this gland. Female Sprague-Dawley rats were injected intracerebroventricularly with ANP 2 μg or 4 μg/4μl or vehicle. Twenty minutes after the injections, the animals were decapitated, blood was collected and the pituitaries were rapidly removed. Administration of ANP resulted in a significant dose-related increase in plasma growth hormone (GH) level, and, in contrast, the GH content in the anterior pituitary was significantly reduced by injection of 4 μg ANP. Injection of 2 μg of ANP caused a highly significant plasma increase, and anterior pituitary decrease in prolactin levels. ANP had no effect on serum or anterior pituitary levels of thyroid stimulating hormone , nor did it affect the level of follicle stimulating hormone in the anterior pituitary. These results suggest a role for ANP in the control of anterior pituitary hormone secretion. Further studies are necessary to elucidate the mechanisms of action of ANP on the anterior pituitary and its significance.

390.14

SIGMA LIGAND (JO 1784) REDUCES CRF-INDUCED INHIBITION OF GASTRIC ACID SECRETION AND GASTRIC EMPTYING IN CONSCIOUS RATS. M. Yoneda, M. Gué, H. Yang and Y. Taché. CURE/VA Medical Center, Dept. of Medicine and Brain Res. Inst., UCLA, Los Angeles, CA 90073.

Sigma binding sites have been characterized most extensively in brain though their presence has also been recognized in several peripheral tissues, including the digestive tract. Sigma ligands recently have been reported to block central CRF-induced colonic motor activation in conscious rats. The aim of this study is to investigate whether a specific interaction between JO 1784, a specific sigma ligand, and CRF can be demonstrated in relation to central CRF-induced alterations of gastric acid secretion and emptying.

Under ether anesthesia rats were injected intracisternally (ic) or intravenously (iv) either with saline or JO 1784 (0.05-5 μg ic, 50 μg iv) immediately followed by another ic or iv injection of saline or CRF (5 μg ic, 20 μg iv). The pylorus was ligated and rats were sacrificed 2 h after for measurement of gastric acid secretion. In another experiment, rats were injected ic with saline or JO 1784 (0.5-50 μg) followed by ic saline or CRF (0.6 μg), and then 1.5 ml of a non-caloric solution was given orally and 20 min after rats were sacrificed for measurement of gastric emptying.

JO 1784 (5 μg ic or 50 μg iv) had no effect on basal acid secretion in conscious, pylorus-ligated rats. CRF (5 μg ic or 20 μg iv) inhibited gastric acid secretion by 95 and 90%, respectively. JO 1784 (0.05-0.5 μg ic) reversed dose-dependently ic CRF-induced gastric acid inhibition. The maximum reversal effect of ic JO 1784 (0.5 μg) against ic CRF (5 μg) was 28%. JO 1784 (50 μg iv) against ic CRF (5 μg) and ic JO 1784 (5 μg) against iv CRF (20 μg) had no effect on gastric acid secretion. JO 1784 (0.5-5 μg ic) reversed dose-dependently ic CRF (0.6 μg)-induced gastric emptying inhibition. The maximum reversal effect of ic JO 1784 (5 μg) against ic CRF (0.6 μg) on gastric emptying was 44%. These results suggest that the sigma ligand, JO 1784, acts in the brain to partially abolish the inhibitory effect of central CRF on both gastric acid secretion and gastric emptying in conscious rats.

390.15

NEUROPEPTIDE FF DOES NOT CLEARLY ANTAGONIZE THE EFFECTS OF MORPHINE ON CEREBRAL MONOAMINES. M. Attila, U. Vuorela*, L. Kivipelto*, P. Panula and L. Ahtee. Div. of Pharmacology and Toxicology, Dept. of Pharmacy, and Dept. of Anatomy, Univ. of Helsinki, SF-00170 Helsinki, Finland.

Neuropeptide FF (NFF) isolated from bovine brain attenuates morphine-induced tail-flick latency (Yang *et al.* Proc.Natl.Acad.Sci.USA 1985:**82**:7757). Because morphine alters cerebral monoamine metabolism (Ahtee *et al.* J.Pharmacol. Exp.Ther. 1989:**249**:303) we have studied the interaction of NFF with morphine on cerebral monoamine metabolism. Male Wistar rats were given 10 mg/kg of morphine or saline (s.c.; 30 min before decapitation) followed by three injections of saline or NFF (10 μg in 20 μl saline) i.c.v. at 30, 20 and 10 min before decapitation. The NFF tended to potentiate the morphine-induced decrease in hypothalamic noradrenaline (NA) as well as the increase of the NA metabolite, sulphated MOPEG. In the caudate putamen NFF attenuated the morphine-induced elevation of the acidic dopamine (DA) metabolites, DOPAC and HVA as well as potentiated the morphine-induced elevation of 5-hydroxytryptamine (5-HT) metabolite 5-HIAA. These results suggest that NFF might attenuate morphine-induced increase in striatal DA turnover, but tends to enhance morphine's effects on NA and 5-HT turnover.

390.16

NALOXONE INDUCED MODIFICATION OF OXYTOCIN RELEASE FROM SPINAL CORD SYNAPTOSOMAL PREPARATION BY HIGH KCl STIMULUS. M. M. Daddona* and Jaya Haldar. Dept. of Biology, St. John's Univ., Jamaica, NY 11439.

Previous experimentation carried out by our laboratory demonstrated for the first time that high KCl can effectively release oxytocin (OT) from spinal cord (SC) synaptosomal preparations. We have also established that the release pattern varied: 1) between the two pellets which were isolated at different centrifugation speed, 2) among the different SC regions and posterior pituitary of the individual pellets.

Recent experimentation was designed to determine if the secretory process resulting in the various OT release patterns: a) was calcium dependent, b) regulated by endogenous SC opioids. Rat SC was separated into cervical, thoracic and lumbosacral regions. Synaptosomes were prepared in 270 mM buffered sucrose. Samples were collected at 3,100g (pellet 2) and 12,000g (pellet 3). The pellets were incubated for 15 min. in artificial CSF for controls and with 56 mM KCl for stimulus. a) The role of opioid peptides in KCl-induced OT release from synaptosomal preparations was investigated by the addition of naloxone (2 μM) to both pellets 1 minute prior to the incubation with stimulus solution. b) In studying calcium dependency EGTA was added to all solutions used. OT concentration was determined by radioimmunoassay. Current data demonstrates that the patterns of OT release from SC synaptosomes are increased by the addition of 2μM naloxone to the pellets one minute prior to the application of the high KCl stimulus. Furthermore, it shows that the KCl-induced OT release is calcium dependent. Such findings further support a role for oxytocin as a neurotransmitter. They also suggest the involvement of endogenous opioids in the release of OT from nerve terminals within the spinal cord. (Supported by NIH grant DK-40160)

CATECHOLAMINES: BIOSYNTHESIS II

391.1

EXPRESSION OF DIFFERENT FORMS OF TYROSINE HYDROXYLASE IN MONKEY AND HUMAN BRAIN. D.S. Melchitzky, M.J. Scolieri*, J.W. Haycock and D.A. Lewis. Depts. of Behav. Neurosci. and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15213 and Dept. of Biochem. and Mol. Biol., Louisiana State Univ., New Orleans, LA 70119.

A single gene for tyrosine hydroxylase (TH) gives rise to a single form of TH mRNA in most species. However, in humans alternative splicing produces four distinct TH mRNAs. Translation of these mRNAs into different isoforms of TH has recently been demonstrated in human adrenal medulla (J.W. Haycock, *J Neurochem* 56:2139, 1991). In this study, polyclonal antisera directed against the unique amino acid sequences of the four types of human TH, and antibodies that recognize sequences common to all forms of TH, were used in immunohistochemical and Western analyses to determine the cell specific expression of TH isoforms in human and monkey (*M. mulatta* and *M. fascicularis*) brain and adrenal medulla. The specificity of each antiserum for its target TH isoform was demonstrated in competition experiments in all species. All four isoforms of TH were present in human and monkey adrenal medulla, but only types 1 and 2 were detected in brain. Both TH isoforms were present in most catecholaminergic neurons and processes. However, some structures of the ventral mesencephalon may contain only one TH isoform; dual labeling studies are in progress. These findings demonstrate that at least two isoforms of TH are expressed in human and monkey brain and suggest that additional mechanisms for the regulation of catecholamine biosynthesis may exist in primate brain.

391.2

EFFECT OF ACUTE AND CHRONIC ADMINISTRATION OF NICOTINE ON TYROSINE HYDROYXLASE ACTIVITY IN RAT ADRENAL MEDULLA. A.W. Tank, L.H. Fossom and C. Sterling*. Univ. of Rochester Med. Ctr., Rochester, NY 14642.

A single injection of 2.3 mg/kg nicotine is associated with a 2–3 fold activation of rat adrenal tyrosine hydroxylase (TH). This activation is not blocked by prior administration of 15 mg/kg hexamethonium. A single injection of 2.3 mg/kg nicotine also activates TH in denervated adrenal glands; however, hexamethonium completely blocks this activation in the denervated gland. When rats are repeatedly injected with nicotine (1.0 or 2.3 mg/kg) once every 30 min for 3 hr, adrenal TH activity remains persistently elevated 3–4 fold over controls; however, TH is not activated in denervated adrenal glands 3 hr after repeated injections of nicotine. These results suggest that the systemic administration of nicotine activates adrenal TH by at least two mechanisms: (1) direct stimulation of nicotinic receptors on adrenal chromaffin cells; and (2) stimulation of non-nicotinic receptors on chromaffin cells by neurotransmitters released from the splanchnic nerve. Interestingly, after 3 days of chronic infusion with 48 mg/kg/day nicotine via Alzet osmotic pumps, complex changes occur in the adrenal medulla, such that TH enzyme levels are induced approximately 2-fold and this elevated level of enzyme is activated when the rat is administered a challenge injection of 2.3 mg/kg nicotine. (Supported by DA05014, DA07232 and AHA grant 89-050G).

392.1

NMDA-EVOKED INCREASES IN $[Ca^{2+}]_i$IN RAT SPINAL CORD NEURONS ARE ENHANCED BY TACHYKININS. K.I.Rusin*, D.Bleakman#, P.S.Chard#, R.J. Miller#, and M.Randić. Dept.of Vet.Physiol.Pharmac.,Iowa State Univ.,Ames,IA 50011 and Dept.of Pharmac. Physiol.Sci., Univ.of Chicago, Chicago,IL 60637#.

Tachykinins enhance the responses of rat dorsal horn (DH) neurons to N-methyl-D-aspartate (NMDA) (Neurosci. Lett., 117:74-80, 1990) but the mechanism underlying this effect remains unclear. In order to address this question we have monitored the effects of tachykinins and NMDA on intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$)in acutely isolated rat DH neurons using Fura-2 based microfluorimetry. The $[Ca^{2+}]_i$ was monitored in single DH neurons perfused with a physiological solution containing no added Mg^{2+} and 0.5 μM TTX. Drugs were applied rapidly with a large Y-tube to the entire neuron. In confirmation of previous findings NMDA, glutamate (50-100 μM) and substance P (SP) (2-10nM) by themselves induced increases in the peak $[Ca^{2+}]_i$ in a proportion of DH neurons, whereas neurokinin A (NKA) (2nM) was without effect. The peak of the NMDA-evoked increase in $[Ca^{2+}]_i$ was potentiated up to 285% (in 7 of 8 cells) by 2nM SP applied for periods of 2-4 min, and this effect lasted up to 90 min. Subsequent applications of SP failed to elicit similar increases in the NMDA response. SP had no effect on either 50mM K^+- or kainate (20μM) -induced increases in $[Ca^{2+}]_i$. Glutamate-evoked increases in $[Ca^{2+}]_i$ were also enhanced by SP. To determine the subclass of tachykinin receptors involved in the SP effect, we compared the effect of SP, which acts primarily at the NK1 class of tachykinin receptor, with that of NKA which has higher affinity than SP for NK2 and NK3 receptors. We found that in the same DH cells that responded to SP, NKA (2nM) also enhanced the NMDA-evoked increases in $[Ca^{2+}]_i$ suggesting that the responses to the tachykinins are mediated by the activation of both NK1 and NK2 receptors. These results provide direct evidence that tachykinins enhance NMDA evoked increases in $[Ca^{2+}]_i$ in spinal cord neurons. (Supported by NIH and NSF).

392.2

MODULATION OF NMDA-INDUCED CURRENTS BY SUBSTANCE P: EFFECTS OF SPANTIDE II AND GLYCINE. M. Randic, K.I. Rusin* and P.D. Ryu. Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA 50011.

In acutely isolated spinal dorsal horn neurons of the rat, SP enhances N-methyl-D-aspartate (NMDA)-induced current responses recorded under whole-cell voltage-clamp conditions (Neurosci. Lett., 117:74-80 1990). Molecular mechanisms underlying the SP effect have yet to be elucidated. We have used a novel tachykinin antagonist spantide II (D-NicLys[1], 3-Pal[3], D-Cl^{2}Phe[5], Asn[6], D-Trp[7,9], Nle[11]-SP), glycine and 7-chlorokynurenic acid to examine the possibility that the modulation of the NMDA-induced current by SP may involve interaction of the tachykinin receptors with the strychnine-insensitive glycine binding site located at the NMDA receptor-ion channel complex. Glycine is thought to act either as a co-agonist or a regulator of the rate of desensitization of NMDA receptors. Spantide II (10nM) co-administered with NMDA (100μM) slightly depressed the NMDA-induced current (86.3 ± 2.9%, n=11), but effectively blocked the SP (2nM) -induced potentiation of the responses of dorsal horn neurons to NMDA (n=6). In the presence of glycine (0.1μM), the SP-evoked increase of the NMDA-induced current was prevented (n=18). 7-Chlorokynurenic acid (2μM), a competitive antagonist at the glycine modulatory site of the NMDA receptor, led to a re-establishment of the SP effect. These results are consistent with a possibility that tachykinins might directly modulate the NMDA receptor-ion channel complex, either by interacting with the regulatory site(s) or by acting through a distinct cellular site. (Supported by NS 26352 and BNS 841 8042).

392.3

NMDA-EVOKED RELEASE OF ENDOGENOUS ADENOSINE FROM THE RAT HIPPOCAMPUS IN VIVO Y. Chen* and T.W. Stone, Dept. of Pharmacology, Glasgow University, Glasgow, U.K. G12 8QQ (Spon. Brain Research Association, U.K.)

Adenosine (Ado), as an inhibitory neuromodulator in the CNS, exhibits a role in protecting neurons from excitotoxicity and ischaemia. To assess the endogenous release of Ado during abnormal neuronal excitation, in vivo microdialysis was employed, using a probe implanted in the ventral hippocampus of an anaesthetized rat. Ado was analyzed on HPLC with UV detection. Perfusion of NMDA (0.1-25mM), K^+(100mM) and veratridine (100μM) for 5min evoked a 2-15 fold increase of the purines from the basal level. Incorporation of verapamil (100μM), Cd^{++}(300μM) did not alter the basal release, although they attenuated the evoked release, suggesting a Ca^{++}-independent releasable pool of Ado. The NMDA evoked release was dose-dependent, and was completely blocked by APV. In addition, much of the K^+ evoked response was also prevented by APV. The results demonstrate the endogenous release of Ado from the hippocampus by neuroexcitants, and also indicate a large contribution of NMDA receptors to the release.

(*Supported by Scottish Home and Health Dept.)

392.4

EFFECT OF LUTEINIZING HORMONE-RELEASING HORMONE ON IN VIVO RELEASE OF NEUROTRANSMITTERS FROM THE RAT HIPPOCAMPUS. M. Shimoyama, R. Miyoshi and S. Kito. University of the Air, Chiba 260, Japan and Department of Pharmacology, Tokyo Women's Medical College, Tokyo 162, Japan.

The function of luteinizing hormone-releasing hormone (LHRH) of the hypothalamus is well documented and the mechanism of its action is fully understood. However, the role of LHRH being played in the central nervous system is yet unclear. It has been reported that the hippocampus contains the highest density of LHRH receptors of the brain, while LHRH immunoreactivities are by no means rich in this brain area. To elucidate the role of LHRH in the hippocampus, we investigated the effect of LHRH on neurotransmitter release from the rat hippocampus using an intracerebral dialysis technique in combination with HPLC and an electrochemical detector.

LHRH was dissolved in perfusion medium and administered into the rat hippocampus via a dialysis tube. LHRH had no effect on the levels of acetylcholine in hippocampal perfusates. In contrast, LHRH(10^{-5}, 10^{-6} M) caused a significant decrease of levels of glutamate(-37, -34%, respectively. $p<0.05$).

It was ssumed that LHRH was playing a role in the hippocampal function by interacting with the glutamatergic system.

392.5

MECHANISM OF CRF DEPRESSION OF QUISQUALTE RESPONSE IN CULTURED CEREBELLAR PURKINJE NEURONS. E.A. Fox and D.L. Gruol, Res. Inst. of the Scripps Clinic, La Jolla, CA, 92037.

We previously showed that corticotrophin-releasing-factor (CRF) depressed quisqualate (Quis)-induced biphasic responses (increased firing rate followed by decreased firing rate and then recovery) in rat cerebellar Purkinje neurons (PNs; Neurosci. Abst. 16:521, 1990). In the present study intracellular recordings were used to determine whether CRF depressed the Quis-induced increase in firing rate by preventing the underlying membrane depolarization. CRF effects on PN membrane properties were also examined.

The major findings were that CRF did not decrease the Quis-induced membrane depolarization and that CRF prevented the repetitive spiking induced by depolarizing current steps. CRF also decreased the amplitude of the off response evoked at the termination of a hyperpolarizing current pulse and the afterhyperpolarization evoked at the termination of a depolarizing pulse. CRF antagonists (CRF (9-41) and CRF (12-41)) applied in combination with CRF did not block these CRF effects and applied alone acted as partial agonists.

These results suggest that CRF depresses the PN response to Quis by altering a conductance that contributes to spike generation. We are currently studying this with voltage clamp recording and calcium imaging. The significance of CRF suppression of excitatory-amino-acid responses in PNs may be that it contributes to the long term depression observed at the parallel fiber-PN synapse after repeated pairing of parallel and climbing fiber activation. Grant #NS21777 and #AA0756.

392.6

NOREPINEPHRINE ALTERS MEMBRANE RESPONSES OF LAYER V CORTICAL NEURONS TO EXCITATORY SYNAPTIC INPUTS. R.D. Mouradian, F.M. Sessler, B.J. Gwag, J.E. Springer and B.D. Waterhouse, Depts. of Physiol. & Biophys. and Neurology, Hahnemann Univ., Phila., PA. 19102-1192

Previous studies from our laboratory indicate that under in vivo conditions subliminal synaptic inputs to sensory cortical neurons can be "gated" by norepinephrine (NE). Under in vitro conditions, locally applied NE can reveal robust excitatory responses of somatosensory cortical neurons to otherwise subthreshold iontophoretic doses of glutamate or NMDA. The present study was conducted to examine the effects of NE on synaptically-evoked membrane responses of layer V somatosensory cortical neurons. Intracellular recordings were made from a submerged brain slice preparation. Excitatory post-synaptic potentials (EPSPs) were evoked via a bipolar stimulating electrode (0.02-0.5 mA, 0.05-0.2 ms) placed in the cortical white matter. Experiments were performed under current clamp conditions to control for small depolarizations (1-4 mV) that sometimes accompany bath application of NE. The amplitude of EPSPs evoked by stimuli which were subthreshold for spike generation were increased during bath application of NE (10 uM). In many instances the probability of spiking in response to otherwise subthreshold stimuli was markedly enhanced during NE at 10 uM. By contrast, a higher concentration of NE (100 uM) reversibly suppressed both EPSP amplitude and spiking evoked by synaptic stimulation. In several experiments stimulus-evoked spiking and EPSPs could be partially antagonized by bath application of the NMDA receptor blocker, 2-amino 5- phosphonovaleric acid (APV, 50 uM), suggesting that the pathway activated was glutamatergic. These results provide further support for the possibility that noradrenergically activated mechanisms can regulate the threshold for spike generation in neocortical neurons. Since, NE-induced enhancement of membrane responsiveness to synaptic input was observed under current clamp conditions and at levels of stimulation subthreshold for evoking spikes, the mechanism(s) underlying this facilitation seem unlikely to be dependent upon membrane depolarization or blockade of accommodation. A possibility that is under investigation is that these effects involve direct actions on spike generating mechanisms and/or presynaptic release. Overall, these results suggest that noradrenergic enhancement of cortical neuronal excitability may be expressed as a shift in the ability of the membrane to respond to excitatory synaptic stimuli. (Supported by AFOSR-87-0138, NINCDS 18081 and Klingenstein Foundation award to B.D.W.)

391.9

TEMPORAL CHANGES IN TYROSINE HYDROXYLASE (TH) mRNA LEVELS FOLLOWING ELECTRICAL STIMULATION (ES) OF A1-NORADRENERGIC NEURONS. **J-J. Liaw, J-R. He and C.A.Barraclough.** Dept. Physiology, Sch. Med., Univ. Maryland, Baltimore, MD 21201.

TH is the rate limiting enzyme for synthesis of norepinephrine (NE) and stimuli which release NE also may increase TH mRNA. To establish, further, whether the increased release of hypothalamic NE is accompanied by an increase in TH mRNA levels, we electrically activated A1 neurons using stimuli which increase NE release. The following groups were studied: unstimulated or sham-stimulated controls and ES rats. All sham or ES male rats were anesthetized with chloral hydrate, and a coaxial electrode was stereotaxically inserted into the right medullary A1 region In the sham group, no stimulation was performed, whereas, other rats received 20 min of ES in the right A1 area. TH mRNA levels were measured in untreated controls and 1, 6 and 12 h after sham or ES. In situ hybridization was performed and message levels were analyzed by quantitative image analysis. TH mRNA levels in untreated and sham-stimulated controls did not differ at anytime time period nor did mRNA levels in right versus left A1 regions. One hour after ES, TH mRNA levels had increase in right but not left A1 cells. By 6 h, TH mRNA levels were significantly higher in right A1 neurons compared to 0 or 1 h values and a small increase in message had occurred in left A1 cells. Twelve hours after ES, TH mRNA levels remained high but did not differ from 6 h values on either right or left side of brain. Thus, stimuli which activate NE release also increase transcription of TH mRNA. HD-02138.

391.10

DIFFERENTIAL REGULATION OF LOCUS COERULEUS (LC) LEVELS OF TYROSINE HYDROXYLASE (TH) BY CHRONIC STRESS AND ANTIDEPRESSANT TREATMENT IN TWO DIFFERENT STRAINS OF RATS. K.R. Melia and R.S. Duman. Laboratory of Molecular Psychiatry, Dept. of Psychiatry, Yale Univ. School of Med., New Haven CT 06508.

The tendency for stress to precipitate and/or exacerbate major depression is widely accepted. However, the literature supporting this relationship is inconsistent. Such inconsistencies might result from genetically based individual differences in vulnerability to the deleterious effects of stress.

Previous studies have identified LC TH as a common site of regulation by chronic stress and antidepressant treatments. In the present experiments we investigated the effects of chronic cold stress or chronic imipramine (a tricyclic antidepressant) on LC levels of TH in two different strains of outbred rats: Sprague Dawley and Wistar (Camm). While chronic stress produced a significant increase of 34% ($p < .03$) in LC TH of Sprague Dawleys, chronic stress had no effect on LC TH in Wistar rats. Chronic imipramine treatment produced a significant 44% ($p < .005$) decrease in LC levels of TH in Sprague Dawleys, but had no effect on LC TH in Wistar rats. Consistent with these biochemical data, behavioral studies using the Porsolt swim test revealed differences in basal rates of immobility between the two rat strains. More importantly, imipramine produced an expected 66% decrease in immobility in Sprague Dawley rats ($p < .05$), but had no effect on immobility in Wistar rats.

The present experiments demonstrate a differential effect of chronic stress and antidepressant treatment on the neurochemistry and behavior of two different strains of rats. These findings, together with clinical studies implicating the noradrenergic system in responses to stress and major depression, raise the possibility that variation in the responsivity of this system to perturbations may underly individual differences in vulnerability to stress-related psychiatric disorders.

391.11

THE EFFECT OF CHRONIC HALOPERIDOL ADMINISTRATION ON TYROSINE HYDROXYLASE (TH) ACTIVITY, mRNA, AND PROTEIN LEVELS. H.M. Hallak, A.J. Azzaro, K.E. Vrana. Depts. Biochemistry and Neurology, West Virginia University HSC, Morgantown, WV 26506.

The nigrostriatal dopamine (DA) pathway has an important function in regulation of normal motor control. Administration of DA receptor antagonists is known to alter DA synthesis presumably through the rate-limiting TH-catalyzed step. This study was designed to determine the effect of haloperidol (a DA receptor antagonist) on TH activity, TH protein and TH mRNA levels in the substantia nigra (SN) and TH activity and protein in the corpus striatum (CS) of rats. After 21 days of haloperidol administration (1mg/kg/day s.c.), TH mRNA in the SN increased five fold in comparison to control. TH activity and TH protein was not changed significantly from control in the SN or CS. Experiments performed with polysomes showed that this increase in TH mRNA was not reflected in an increase in mRNA bound to ribosomes. This suggests that a translational-block may be an additional mechanism for the regulation of DA synthesis in the CNS. (Supported by NIH #GM-38931).

391.12

TYROSINE HYDROXYLASE (TH) mRNA IS TRANSIENTLY DECREASED FOLLOWING CHRONIC SELEGILINE (DEPRENYL) ADMINISTRATION IN RAT BRAIN. S.L. Vrana, A.J. Azzaro and K.E. Vrana. Depts. of Behavioral Medicine/Psychiatry, Neurology and Biochemistry, West Virginia Univ., Morgantown, WV 26506.

Selegiline, a selective monoamine oxidase type B (MAO-B) inhibitor, decreases TH activity in the rat nigrostriatal (NS) pathway. Selegiline administration (0.1 mg/kg s.c.) for 3, 7, 14 or 21 days decreased TH activity in the terminal fields (corpus striatum; CS) of the dopamine-containing NS pathway, which recovered by 14 days. TH activity was decreased in the cell bodies (substantia nigra; SN) of the NS pathway throughout the entire treatment period. We tested whether the decrease in TH activity was mediated by a decrease in TH mRNA. Northern blot and RNA dot blot analyses (using a TH-specific cDNA probe) of SN homogenates revealed a significant decrease in TH mRNA at 3, 7 and 14 days of selegeline treatment as compared with controls. Conversely, after 21 days of selegiline, TH mRNA levels were significantly higher (3-fold) than controls which was not reflected in SN TH activity. It is possible that the recovery in TH activity in CS is a result of increased TH protein transport from the SN to the CS, or that TH exists in a more stabilized state.

391.13

CULTURE OF E14 MESENCEPHALIC DOPAMINERGIC CELLS: A HIGH YIELD OF TYROSINE HYDROXYLASE POSITIVE CELLS. K. Shimoda and J.W. Commissiong. Lab. Biochem. Gen., Center for Neurosci., St.Eliz. Hosp. Washington, D.C. 20032. and NIH-NINDS-CNB, Bldg.10/5N214, Bethesda, MD. 20892.

In the E14 rat brain, a <2.0 mm^3, region of the ventral mesencephalon, containing > 90% of the DA, was dissected, pooled in oxygenated culture medium (DMEM/F12 1:1, 10% FCS, 4 mM glutamine), and mechanically dispersed. Cell viability, was 65-75%. 5.0 x 10^5 Cells/well, were cultured in 8-well chamber slides (Lab-Tek) coated with poly-D-lysine. At 1, 3, 5, 7, 10 and 14 days after plating, the cells were immunostained with a mouse primary tyrosine hydroxylase (TH) monoclonal antibody (Boehringer-Mannheim), and an FITC-conjugated secondary antibody, raised against mouse IgG. The % of TH +ve cells at the days listed above was 4.1, 5.0, 4.8, 8.0, 9.1 and 6.9 respectively. Treatment of the cells with a rat striatal extract, caused a significant ($p<0.01$) increase in the % of TH +ve cells at 3 and 5 days, but not at 7 and 10 days. This procedure can be used to make cultures with >5% TH +ve dopaminergic cells, at 5 to 10 days after plating, for a variety of studies.

392.1

NMDA-EVOKED INCREASES IN $[Ca^{2+}]_i$IN RAT SPINAL CORD NEURONS ARE ENHANCED BY TACHYKININS. K.I.Rusin*, D.Bleakman#, P.S.Chard#, R.J. Miller#, and M.Randić. Dept.of Vet.Physiol.Pharmac.,Iowa State Univ.,Ames,IA 50011 and Dept.of Pharmac. Physiol.Sci., Univ.of Chicago, Chicago,IL 60637#.

Tachykinins enhance the responses of rat dorsal horn (DH) neurons to N-methyl-D-aspartate (NMDA) (Neurosci. Lett., 117:74-80, 1990) but the mechanism underlying this effect remains unclear. In order to address this question we have monitored the effects of tachykinins and NMDA on intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$)in acutely isolated rat DH neurons using Fura-2 based microfluorimetry. The $[Ca^{2+}]_i$ was monitored in single DH neurons perfused with a physiological solution containing no added Mg^{2+} and 0.5 μM TTX. Drugs were applied rapidly with a large Y-tube to the entire neuron. In confirmation of previous findings NMDA, glutamate (50-100 μM) and substance P (SP) (2-10nM) by themselves induced increases in the peak $[Ca^{2+}]_i$ in a proportion of DH neurons, whereas neurokinin A (NKA) (2nM) was without effect. The peak of the NMDA-evoked increase in $[Ca^{2+}]_i$ was potentiated up to 285% (in 7 of 8 cells) by 2nM SP applied for periods of 2-4 min, and this effect lasted up to 90 min. Subsequent applications of SP failed to elicit similar increases in the NMDA response. SP had no effect on either 50mM K^+- or kainate (20μM) -induced increases in $[Ca^{2+}]_i$. Glutamate-evoked increases in $[Ca^{2+}]_i$ were also enhanced by SP. To determine the subclass of tachykinin receptors involved in the SP effect, we compared the effect of SP, which acts primarily at the NK1 class of tachykinin receptor, with that of NKA which has higher affinity than SP for NK2 and NK3 receptors. We found that in the same DH cells that responded to SP, NKA (2nM) also enhanced the NMDA-evoked increases in $[Ca^{2+}]_i$ suggesting that the responses to the tachykinins are mediated by the activation of both NK1 and NK2 receptors. These results provide direct evidence that tachykinins enhance NMDA evoked increases in $[Ca^{2+}]_i$ in spinal cord neurons. (Supported by NIH and NSF).

392.2

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392.3

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392.4

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392.5

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The major findings were that CRF did not decrease the Quis-induced membrane depolarization and that CRF prevented the repetitive spiking induced by depolarizing current steps. CRF also decreased the amplitude of the off response evoked at the termination of a hyperpolarizing current pulse and the afterhyperpolarization evoked at the termination of a depolarizing pulse. CRF antagonists (CRF (9-41) and CRF (12-41)) applied in combination with CRF did not block these CRF effects and applied alone acted as partial agonists.

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392.6

NOREPINEPHRINE ALTERS MEMBRANE RESPONSES OF LAYER V CORTICAL NEURONS TO EXCITATORY SYNAPTIC INPUTS. R.D. Mouradian, F.M. Sessler, B.J. Gwag, J.E. Springer and B.D. Waterhouse, Depts. of Physiol. & Biophys. and Neurology, Hahnemann Univ., Phila., PA. 19102-1192

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392.7

ACTIONS OF SEROTONIN ON GLUTAMATERGIC SYNAPTIC TRANSMISSION IN LAYERS III AND IV OF RAT BARRELFIELD CORTEX. W. Liu, F.M. Sessler, C-S. Lin and B.D. Waterhouse, Dept. Physiol and Biophys. Hahnemann Univ., Phila., PA 19102

Serotonin (5-HT) containing neurons in the dorsal raphe nucleus are the source of an extensive network of fibers that are found in all regions and all layers of the neocortex. Despite the well documented anatomy of this cortical serotonergic innervation, the potential impact of 5-HT release on heterogeneous populations of neurons in functionally distinct regions of the cortex is unclear. In the present investigation we have examined the effects of 5-HT on amino acid neurotransmitter- induced and synaptically-evoked responses of cortical neurons in the "barrelfield" region of rat somatosensory cortex. Both intra- and extracellular recordings were made from cortical neurons in layers III and IV using a submerged in vitro brain slice preparation. Excitatory postsynaptic potentials (EPSPs) were evoked in cells by stimulating the cortical white matter (0.04-0.5mA, 0.1-0.2ms). In all cases, EPSPs displayed conventional voltage-dependence, being increased with membrane hyperpolarization and decreased with membrane depolarization. Bath application of 5-HT (30uM) reversibly suppressed the amplitude and duration of these EPSPs and abolished the action potentials induced by EPSPs which were otherwise suprathreshold for spike generation. 5-HT also reduced the membrane depolarizing effect of locally applied glutamate. In each of these experimental situations, resting membrane potential and rheobase were unchanged during 5-HT perfusion. Under extracellular recording conditions, microiontophoretic application of 5-HT at ejection currents (5-20nA), which caused little or no suppression of background firing, produced marked suppression of glutamate-evoked excitatory discharges. Similar suppressant effects were observed when 5-HT was interacted with excitatory neuronal responses to NMDA, kainate or quisqualate. Our results indicate that 5-HT can suppress glutamate-evoked excitatory responses of somatosensory cortical neurons without directly changing membrane excitability. These data are consistent with the hypothesis that serotonergic afferents to the neocortex may play a role in regulating the flow of sensory information through local sensory circuits by way of an action on glutamatergic synaptic transmission. (Supported by AFOSR 870138 and an award from the Klingenstein Foundation to BDW)

392.8

INTERACTIONS BETWEEN CENTRAL GLUTAMATERGIC, CATECHOLAMINERGIC AND CHOLINERGIC SYSTEMS WITH REGARD TO PSYCHOMOTOR FUNCTIONS. M. Carlsson, A. Carlsson* and A. Svensson*. Dept. of Pharmacology, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden.

The purpose of the present investigation was to study the effects of simultaneous manipulations of central cholinergic, adrenergic and glutamatergic systems on locomotion in an animal model of Parkinson's disease.

Mice were deprived of their monoamine stores by pretreatment with the monoamine depleter reserpine and the catecholamine synthesis inhibitor α-methyl-p-tyrosine, given 18 h and 60 min., respectively, before the acute experiment. Traditionally, only dopaminergic agonists have been shown to reverse the akinesia thus produced. However, in the present study it is demonstrated that if a muscarine receptor antagonist (atropine or biperiden) is *combined* with an α-adrenergic agonist/α-adrenergic agonist precursor (clonidine or L-α-methyl-DOPA), a marked locomotor stimulation can be achieved, although either agent given alone is ineffective. Adding an NMDA antagonist (MK-801, ketamine or SDZ EAA494) to the combination biperiden+clonidine resulted in further potentiation of the locomotor stimulatory effects.

From our findings it can be inferred that (1) DA is not indispensable for initiation and execution of locomotion, (2) central glutamatergic and catecholaminergic systems are functionally opposed with regard to locomotion - possibly this antagonistic interaction takes place within the striatum, in analogy to the presumed cholinergic/dopaminergic antagonism within this structure.

392.9

EPINEPHRINE FACILITATES GLUTAMATE-INDUCED RESPONSES IN ACUTELY DISSOCIATED FROG MOTONEURONS. S. Adachi*, J-I. Oka, T. Nagao* and H. Fukuda*. Dept. of Toxicology and Pharmacology, Fac. of Pharmaceutical Sciences, Univ. of Tokyo, Tokyo 113, JAPAN.

Epinephrine (Epi) functions as the major catecholaminergic neurotransmitter in frogs, and has been reported to augment spinal reflexes. We previously reported that Epi causes slow depolarization with an increase and a decrease in input resistance by suppression of K^+-conductances via α_1-adrenergic receptor and induction of Na^+-dependent inward current via β-adrenergic receptor, respectively, in a single motoneurons dissociated from frog. The purpose of this study was to elucidate the modulating effects of Epi on the responsiveness of motoneurons to glutamate (Glu). Whole-cell patch clamp technique was applied to the motoneurons acutely dissociated from adult bullfrogs (*Rana catesbeiana*) with trypsin and collagenase. Motoneurons were identified by retrograde labelling with Evance blue. Epi (10^{-6}M) facilitated Glu ($3x10^{-4}$M)-, quisqualate (Qui, $3x10^{-5}$M)- or kainate (Kai, 10^{-5}M)-induced depolarization. At the holding potential of -70 mV, Epi augmented Glu-induced inward currents, but did not affect Qui- or Kai-induced inward currents. These results suggest that Epi facilitates the responses of motoneurons to Glu through modulation of passive membrane electrical properties of motoneurons and through direct enhancement of Glu-, presumably NMDA-, induced currents.

392.10

GLUTAMATE RELEASES HYPOTHALAMIC NOREPINEPHRINE: I MODULATION BY GLYCINE. D. Johnson*, J. Goldfarb, J. Walcott* and P. Blandina, Department of Pharmacology, Mount Sinai School of Medicine, City University of New York, N.Y.,N.Y. 10029

Hypothalamic slices (400 μ) from male Sprague-Dawley rats (200g) were perfused with a Mg^{2+}-free medium containing nomifensine(10μM) and tyrosine(50μM). Spontaneous release of endogenous norepinephrine(NE), measured by HPLC-ECD after alumina extraction, averaged 0.1 pmol/3min/mg protein. Both 1 and 3 mM glutamate (GLU) more than doubled NE release. GLU effect was totally Ca^{2+}-dependant, and abolished by 0.1 μM tetrodotoxin. Preincubation of slices with 1.2 mM Mg^{2+}, 30 μM 2-amino-5- phosphonopentanoate, or 30 nM MK-801 completely blocked GLU effects, suggesting mediation by an NMDA receptor. Preincubation with 100 μM kynurenic acid (KYN) reduced GLU-evoked NE release, suggesting the presence of endogenous glycine (GLY) modulation. However, up to 100 μM, GLY did not reduce the inhibitory effect of 100 μM KYN. Glycine alone produced no effect, yet simultaneous perfusion with GLY(10-100μM) and GLU(1mM) greatly reduced GLU-evoked NE release. This effect was not blocked by 100 μM strychnine (STR), thus suggesting that GLY inhibits NMDA-mediated release of NE through a STR-insensitive mechanism.

Supported by grants (DA 07135 and DA 04507) from N.I.D.A.

392.11

GLUTAMATE RELEASES HYPOTHALAMIC NOREPINEPHRINE: II MODULATION BY 5-HT. J. Goldfarb, P. Blandina, D. Johnson*, J. Walcott* and J. P. Green, Department of Pharmacology, Mount Sinai School of Medicine, City University of New York, N. Y., N. Y. 10029

Hypothalamic slices (400 μ) from male Sprague-Dawley rats (200 g) were perfused with a Mg^{2+}-free medium containing nomifensine(10 μM) and tyrosine(50 μM). Spontaneous release of endogenous norepinephrine (NE), measured by HPLC-ECD after alumina extraction, averaged 0.1 pmol/3min/mg protein. Glutamate(GLU) (1 mM) more than doubled the rate of NE release, presumably by activation of NMDA receptors (see previous abstract). Preincubation with 5-HT (1 and 10 μM) produced no change in spontaneous NE release but reduced the GLU-induced NE release by about 60-70 %. This effect was mimicked by 2-CH_3-5-HT (10μM), and blocked by ICS 205-930 (2nM) but not by ritanserin (1μM), thus suggesting that activation of 5-HT_3 receptors reduces GLU-evoked NE release. Whereas ritanserin (or another 5-HT_{1C}/5-HT_2 antagonist) must be present to observe 5-HT-induced inhibition of K^+-evoked NE release (Blandina et. al., J. Pharmacol. Exp. Ther. 256: 341-347, 1991), there is no such requirement for 5-HT inhibition of GLU-evoked NE release.

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392.12

THE EFFECT OF MK-801 ON DOPAMINE METABOLISM OF RAT MEDIAL FRONTAL CORTEX.

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Div. Mental Disorder Res., Natl. Inst. Neurosci. NCNP, Tokyo, 187 and Psychiatric Res. Inst. of Tokyo, Tokyo, 156, Japan.

An acute intraperitoneal injection of MK-801 caused a tetrodotoxin-reversible increase in extracellular release of dopamine(DA), 3,4-dihydroxyphenylacetic acid(DOPAC) and homovanillic acid(HVA) in the dialysates from rat medial frontal cortex. Intra-frontal cortex infusion of the drug via dialysis tubing also augmented the cortical DA release. Moreover, there was an increase in the tissue content of DOPAC and HVA with acceleration of DA utilization in the cortical area after systemic administration of MK-801. These results suggest that MK-801 facilitates DA metabolism in the medial frontal cortex by increasing impulse flow in the DA neurons projecting to the prefrontal region. Since MK-801 is a selective non-competitive antagonist of NMDA receptor, the present study supports the view that the NMDA receptor may be involved in a tonic inhibitory control of the DA neurons in the medial frontal cortex.

392.13

THE PHARMACOLOGICAL PROFILE OF GLUTAMATE-EVOKED ASCORBIC ACID EFFLUX MEASURED BY IN VIVO ELECTROCHEMISTRY. J. Cammack, B. Ghasemzadeh, and R.N. Adams. Departments of Pharmacology and Chemistry, University of Kansas, Lawrence KS 66045.

A recently described in vivo voltammetric electrode selectively records rapid changes in extracellular fluid (ECF) levels of ascorbic acid. Using this detector, the nature of glutamate induced efflux of ascorbate into ECF was investigated using pharmacological tools. Ascorbate signals were shown to be directly related to amounts of microinjected glutamate. Blockers of glutamate reuptake homocysteic acid and d,l-threo-B-hydroxy-aspartic acid virtually eliminate the ascorbate signal. A more specific reuptake blocker (the stilbene isothiocyano derivative, SITS, which blocks glial transport of glutamate) does not completely inhibit ascorbate efflux though, suggesting that ascorbic acid exchange during glutamate transport is both neuronal and glial in nature. Other pharmacological experiments indicate that excitatory amino acid receptors are not involved in the glutamate elicited ascorbate efflux. The possible role(s) of brain ascorbate in the general functioning of the pervasive glutamate neurotransmitter systems are discussed.

392.14

A MICRODIALYSIS STUDY ON AGE-RELATED CHANGES IN STRIATAL GLUTAMATE RELEASE. B. A. Donzanti, J. F. Hite*, B. K. Yamamoto. Pharmacology Dept., Battelle Memorial Institute, Columbus, Ohio 43201 and Dept. of Psychiatry, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106.

We have previously shown that D-2 receptor agonists can inhibit K^+-evoked glutamate release in vivo from the striatum. Thus, a decline in D-2 receptors, which occurs during the course of natural aging, may lead to the enhanced release of glutamate, a known excitotoxin. In an attempt to examine this potential mechanism of age-related cell death, the present study will determine: 1) if in vivo glutamate release is altered with age and 2) whether a D-2 agonist (LY163502) and antagonist (sulpiride) will differentially alter glutamate release in an age-dependent manner. Male Fischer 344 rats (4, 12, 18, and 26 months old) were anesthetized with urethane and placed in a stereotaxic frame. Microdialysis probes were placed in either the lateral or medial striatum and perfused at a rate of 1.5 μl/min with artificial CSF. Basal, drug-induced, and K^+-evoked glutamate release was examined at 20 min intervals using HPLC-EC analysis. It was observed that: 1) lateral, but not medial, basal glutamate release increases with age; 2) KCl (80 mM)-evoked glutamate release in the lateral striatum was not influenced by age; and 3) the direct infusion of sulpiride (500 μm) and LY163502 (1 μm) did not alter basal glutamate release. We are currently determining whether these drugs will alter K^+-evoked glutamate release. Thus, the data suggest that elevated glutamate release could be a plausible explanation for age-related striatal cell loss; however, a role for D-2 receptors in this process remains unclear. (Supported by AFAR grant).

392.15

SIGMA RECEPTOR AGONISTS INHIBIT N-METHYL-D-ASPARTATE (NMDA)-STIMULATED [^{3}H]DOPAMINE ([^{3}H]DA) RELEASE FROM RAT STRIATAL SLICES. G.M. Gonzalez, D. Sachdeva*, and L.L. Werling. Dept. Pharmacology, The George Washington University Medical Ctr., Washington, D.C. 20037.

We have previously reported the stimulation of preloaded [^{3}H]DA release from rat striatum by NMDA receptor/channel activators in a static incubation system. We now have confirmed the properties of NMDA receptor regulation of [^{3}H]DA release from this tissue using a superfusion system. Striata were dissected, chopped, and washed in Mg^+-free modified Krebs-HEPES buffer, then incubated with 15 nM [^{3}H]DA for 30 min. Tissue was washed and resuspended in buffer containing nomifensine (10 μM) and domperidone (1μM) to prevent reuptake of and feedback inhibition by released [^{3}H]DA. Slices were loaded into superfusion chambers and superfused with buffer to establish a low, stable baseline release. Tissue was stimulated to release [^{3}H]DA by 2 min exposures to 25 μM NMDA.

The sigma agonists (+)-pentazocine and (+)-SKF10,047 inhibited NMDA-stimulated [^{3}H]DA release from rat striatal slices in a concentration dependent manner. Although (+)-SKF10,047 has significant affinity for the phencyclidine (PCP) receptor located within the NMDA-gated cation channel, (+)-pentazocine has negligible affinity for the PCP receptor. Naloxone (1μM) did not reverse the inhibition by 10 μM (+)-pentazocine, obviating any contribution by opioid receptors. In contrast, the sigma antagonists ditolylguanidine (DTG), haloperidol and BMY14,802 reversed inhibition by (+)-pentazocine. These data suggest a potential involvement of sigma receptors in regulation of DA release from striatum. (Supported by an NIH BRSG to LLW.)

392.16

COMBINED ANTAGONISM OF MUSCARINIC AND NMDA SYSTEMS IMPAIRS MAZE LEARNING IN RATS. P. Garofalo, E.L. Spangler, K. Yamagami, D.K. Ingram. Gerontol. Res. Ctr., NIA, NIH, Baltimore, MD 21224

The cholinergic muscarinic antagonist scopolamine (SCOP) impairs learning in a variety of tasks. Similarly the glutamatergic antagonist dizocilpine (DIZO), which is specific to the N-methyl-D-aspartate (NMDA) ion channel, impairs learning in several complex tasks. Thus, both the cholinergic and glutamatergic systems appear involved in memory processes. The present study assessed the interaction between the two systems in acquisition of a 14-unit T-maze that has provided robust evidence of age-related memory impairment (Ingram, *Neurobiol. Aging,* 1 9:475, 1988). Young (3 mo) male F-344 rats were pretrained in 1-way active avoidance to a criterion (13 avoidances/15 trials). About 24 hr later each rat received an injection of either saline, SCOP (125 μg/kg i.p.), DIZO (12.5 μg/kg s.c.) or a combination of the drugs at these doses, 30 and 20 min, respectively, before they began training (15 trials) in the T-maze. The rat had to run through 5 maze segments each within 10 sec to avoid footshock (0.8 mA). Measures of maze performance included errors, alternation errors, runtime, shock duration and frequency. The individual doses of either SCOP or DIZO had no effects on any maze variable; whereas, the combination treatment at these doses produced marked increases in maze errors without effects on other noncognitive performance variables (run time, shock frequency and shock duration). Our results clearly support the hypothesis of an interaction between the two systems. Although our data do not address the nature of this interaction, results indicate that the cumulative impact of blockade of muscarinic and NMDA receptors is significantly greater than blockade of each one alone. Thus, it is possible that the decline in memory that occurs in aging might be due to relatively minor deficiencies in both systems and could suggest novel therapeutic strategies targeting both systems.

392.17

Increase of Free Radicals (OH°), glutamate and 5-HT in artery and vein of dog after ischemia/reperfusion/insult. B. Delbarre, G. Delbarre and F. Calinon*. Faculté de Médecine, 37032, Tours, France.

In addition to their effect on parenchymal tissue, OH° impair vascular function. To determine the effect of ischemia reperfusion insults (IRI) on the vessels, we have used Beagle anesthetized (Nembutal = 40 mg.kg-1). After 5 min occlusion and 5 min reperfusion, carotid, femoral artery and femoral vein were dissected. We have determined the levels of glutamate (Xu, X., J. Liquid Chromatog., 9, 2253, 1986), 5-HT (Rips, R., Progress in HPLC, Vol.2 : 375-94, 1986) and OH° (Floyd R.A., J. Free Radic. Biol. & Med., 2 : 13, 1986). After IRI, glutamate, 5-HT and OH° were significantly increased in arteries and vein. The vascular effect of OH° may be mediated by inhibition of synthesis of 5-HT by and oxydative mechanism and of glutamate by an action at the level of glutamate system (Oliver, C.N., Proc. Natl. Acad. Sci.,87 : 5144-7, 1986).

In conclusion, after IR, in artery and vein of dog, simultaneous increase of OH°, glutamate and 5-HT may be explained by the direct action of OH°.

Percentage of increase versus control dog (n = 5)

	Femoral vein	Femoral artery	Carotid
OH°	299.36 *	548.70 *	169.54 *
Glutamate	415,25 *	381.21 **	194.08**
5-HT	195.58 **	291.95 **	511.54**

Unpaired Student t test, $p < 0.05$*, $p < 0.01$**, $p < 0.001$**

392.18

SIMULTANEOUS MEASUREMENT OF THE MONOAMINE AND AMINO ACIDERGIC PATHWAYS IN BRAIN TISSUE P. H. Gamache*[1], M. J. During[2], C. N. Svendsen[1], and I. N. Acworth[1]. [1]ESA Inc., Bedford, MA 01730. [2]Section Neurosurgery, Yale Univ., Sch. of Med., New Haven, CT 06510.

Normal brain function results from the interplay of several distinct neurotransmitter systems. Although many techniques are available for the measurement of a specific class of neurochemicals, analysis of several classes involves separate sample processing protocols and multiple analytical systems. Practical and temporal constraints may thus prevent an accurate assessment of possible underlying neurochemical relationships. The objective of this study is to gain maximum information about the amino acidergic and monoaminergic pathways from a single sample in one analysis. We have developed a technique which is based on HPLC with coulometric array detection. Automated sample delivery, derivatization of amino acids with OPA/ßME, and column switching sequences present the analytes for separation and detection. Multiple series coulometric detectors, set at different oxidative potentials, increase the resolution provided by reverse phase chromatography. Using external standards, comparison of the electrochemical "signature" across the detector array is used in conjunction with retention time for each sample peak to aid in identification and peak purity assessment. We have investigated tissue levels of neurochemicals from 5 brain regions of the adult male Sprague-Dawley rat. Representative data from 100 µl of a hippocampal tissue extract is shown below:

	n mole/g Tissue	Ratio Accur.%		n mole/g Tissue	Ratio Accur.%
Aspartate	232	98	HVA	0.03	-
Dopamine	0.26	96	5HIAA	1.84	96
Dopac	0.09	-	Serotonin	2.55	89
GABA	600	91	Norepinephrine	2.08	98
Glutamate	1232	99	Taurine	1385	99
Glycine	135	100			

Comparable ratio accuracy data has been obtained from the brain stem, cerebellum, corpus striatum, and cortex. This data suggests that unique insight into the relationship between amino acidergic and monoaminergic neurotransmitter pathways can be obtained.

392.19

FURTHER INVESTIGATIONS FOR RAPIDLY DETERMINING MULTIPLE NEUROCHEMICAL SPECIES USING A NEUROBIOLOGICAL ANALYZER.

DOUGLAS J. TURK and C. LEROY BLANK, Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK, USA, 73019.

The separation and determination of a variety of catecholamine, indoleamine and acetylcholine related neurochemicals and metabolites has previously been demonstrated using a multiple column, multiple electrode Neurobiological Analyzer, NEUBA (see related abstract in Society for Neuroscience, November, 1990). The Neurobiological Analyzer provides a three dimensional separation of potential, current and time for the analysis of electroactive species during a single thirty minute chromatogram.

Improvements with this instrument have included the ability to deconvolute overlapping peaks resulting in a truly three dimensional separation for any given analysis along with the development of an electrochemical reference library of close to two hundred compounds on each of three systems. Computer controlled potential, current offset and autoranging capabilities and overall improved operating conditions have resulted in a detection limit of approximately 10 femtomoles (at a S/N ratio of 2) for most neurochemical species.

A practical application with the Neurobiological Analyzer has been the qualitative analysis and quantitative assay of a variety of neurochemical species of urine samples from normal and hypertensive children (diagnosed either by WISCR or STARTLE criteria). The three dimensional nature of NEUBA greatly simplifies the analysis and data handling for these samples and facilitates the determination of a possible chemical categorization of ADHD subgroups.

INTERACTIONS BETWEEN NEUROTRANSMITTERS III

393.1

DOPAMINE/NEUROTENSIN INTERACTIONS IN THE RAT STRIATUM. Mary Ann Chapman and Ronald E. See. Dept. of Psychology, Washington State University, Pullman, WA, 99164-4820

Several lines of research indicate that neurotensin (NT) acts as a neuromodulator of dopamine function in the striatum. This study employed an intracranial microdialysis technique in order to more precisely characterize these interactions. Female, Sprague-Dawley rats were anesthetized with Equithesin and bilateral guide cannulae implanted (A +0.2, L +3.1, V -5.0). Following one week of recovery, microdialysis probes with 3 mm of exposed dialysis membrane were inserted into the guide cannulae and perfused with Ringer's solution. Samples were collected at 20 min intervals throughout the experiments. One group of rats was treated with 3 incremental concentrations of NT (0.1, 1.0, 10.0 uM). Each concentration of NT was flowed out the dialysis membrane for one hour and was followed by 80 min. of perfusion with Ringer's solution alone. Another group of rats was given an IP injection of quinpirole (0.03 mg/kg) during infusion of NT (0.1 uM) into the striatum. This dose has previously been shown to decrease DA release by 50%. Samples were injected onto an HPLC column and analyzed for DA, DOPAC, and HVA using electrochemical detection. Neurotensin (10 uM) produced an increase in DA levels. The low concentration of NT blocked the quinpirole-induced decrease in DA but not DOPAC and HVA. Further interactions between neurotensin and dopamine will be discussed.

393.2

HIGH AFFINITY REUPTAKE OF DOPAMINE IN RAT NUCLEUS ACCUMBENS SLICES IS MODULATED BY THE PEPTIDE DES-ENKEPHALIN-γ-ENDORPHIN

E. Ronken, and V.M. Wiegant. Rudolf Magnus Institute, Dept. of Pharmacology, University of Utrecht, The Netherlands

γ-Endorphin (γE) and N^{α}-acetyl-γE are endogenous neuropeptides in rat brain, and express non-opioid biological activity upon central or peripheral administration. These properties are shared by N-terminally truncated, opioid-inactive congeners like des-Tyr^1-γE (DTγE;βE(2-17)), and des-enkephalin-γE (DEγE;βE(6-17)), and are thought to act through modulation of the mesolimbic dopamine system. Non-opioid γ-type endorphins do not display affinity for dopamine receptors, and no conclusive evidence has been found for modulation of dopamine release. Recently, specific binding sites for non-opioid γ-type endorphins have been characterized, preferentially located mesocorticolimbic brain structures.

In slice preparations of rat nucleus accumbens (20μmx20μm) active, GBR12909-sensitive [^{3}H]dopamine uptake kinetics were studied and uptake was found to be concentration-dependently inhibited by DEγE (EC_{50}=20nM) to a maximum of 52% at saturation concentrations of dopamine. In contrast, the structurally related peptide DEαE (βE(6-16); 1μM) was found to be inactive. DEγE both affected maximal uptake rate and observed k_m, suggesting the presence in the rat nucleus accumbens several distinct dopamine carrier systems, one of which is sensitive to γ-type endorphins. Interestingly, DEγE inhibited the [^{3}H]noradrenaline uptake in both accumbens and striatal slices, whereas no effect of DEγE was observed on dopamine uptake in striatal slices. At present, the molecular mechanism with which DEγE interferes with uptake of catecholamines in brain tissue is unknown, modulation of reuptake systems-particularly in meso(cortico)limbic dopaminergic projection areas-may underlie the biological actions of γ-type endorphins.

Support by the Dutch Society for Scientific Research (N.W.O. Grant 900-543-063).

393.3

NEUROTENSIN MODULATION OF DOPAMINE INHIBITION IN THE RAT BRAIN: AN IONTOPHORETIC ANALYSIS. M. Beauregard, A. Ferron and L. Descarries. Centre de recherche en sciences neurologiques, Dép. physiologie, Univ. de Montréal, Montréal (Québec), CANADA H3C 3J7.

Biochemical and behavioral data have suggested functional interactions between dopamine (DA) and neurotensin (NT) in regions of the brain receiving a separate and/or coexistent innervation by these two transmitters. To further elucidate these interactions, the effects of iontophoretically applied DA and NT were measured and compared in the prefrontal (PF) and anterior cingulate (ACg) cortex (coexistent innervation), and in the neostriatum (NS) and nucleus accumbens (Acb) (distinct innervation) of urethane-anesthetized rats. Under similar conditions of application (50 nA during 50 s), most spontaneously firing neurons were inhibited by DA (decrease of 40% or more) in all four regions, whereas NT had no apparent effect on the same cells, except for a few units in Acb (3/21), which were also depressed by the peptide. When DA and NT were concomitantly administered, the percentage of maximal inhibition induced by DA was modified in a majority of the units tested (66/93). A 30% or more decrease in DA responsiveness was observed in 100% of ACg (24/24), 74% of PF (20/27) and 48% (10/21) of Acb units. In NS, DA responsiveness was increased by NT in 60% of the units (12/20). In every region, all remaining neurons showed less than 30% changes in DA responsiveness and were therefore considered as unaffected by NT. Inhibitions induced by the DA agonists SKF 38393 (D1) and LY 171555 (D2) were also decreased during simultaneous application of NT in ACg (30/30 and 4/8 units, respectively). These results support the likelyhood of DA and NT interactions in every brain region innervated by these two transmitters. Why DA and NT interactions might have opposite effects in NS versus PF, ACg and Acb remains to be determined. (Supported by Fonds internes de la Faculté de médecine and MRC grant MT-3544).

393.4

SPECIFICITY OF THE INCREASE IN NEUROTENSIN CONCENTRATIONS AFTER CHRONIC ANTIPSYCHOTIC DRUG TREATMENT. B. Myers, B. Levant, G. Bissette and C.B. Nemeroff. Duke Univ. Med. Center, Durham, NC 27710

Neurotensin (NT) is an endogenous brain and gut peptide that shares many CNS effects with antipsychotic drugs. This study examined the specificity of the antipsychotic-drug induced increase in NT concentrations in the rat nucleus accumbens and caudate nucleus. After sub-chronic (once daily for three weeks) or acute (once) intraperitoneal injection of either the antipsychotic drug, haloperidol (1mg/kg), the benzodiazepine anxiolytic, chlordiazepoxide (25mg/kg), the tricyclic antidepressant, desipramine (10mg/kg), or the antihistamine, diphenhydramine (acute treatment only with three injections of 20mg/kg, i.p. every six hours), six discrete brain regions were dissected and assayed: nucleus accumbens, anterior and posterior caudate nucleus, frontal cortex, amygdala and substantia nigra/ventral tegmental area. Regional mean NT concentrations were expressed as pg NT/mg protein.

As previously reported, sub-chronic and acute treatment with haloperidol significantly increased NT concentrations in the nucleus accumbens and the caudate nucleus. Neither chronic nor acute treatment with desipramine, chlordiazepoxide or acute treatment with diphenhydramine affected NT concentrations in any of the brain regions examined. Regionally specific alterations in NT concentration are not seen with drugs that possess effects similar to the side effects often observed with antipsychotic drug treatment. Thus these NT concentration alterations may mediate some of the therapeutic effects of antipsychotic drug administration. (Supported by NIMH MH-39415).

393.5

ANGIOTENSIN II INCREASES DOPAMINE RELEASE FROM RAT STRIATAL SLICES IN VITRO. A.L. Jewell, S.T. Buxton, L.L. Leibee, L.A. Cassis* and L.P. Dwoskin. College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082.

Components of the renin-angiotensin system including angiotensinogen, angiotensin converting enzyme, angiotensin II (AII) immunoreactivity and AII receptors have been localized in striatum. Recently, we reported (Dwoskin, Naunyn-Schmiedeberg's Arch. Pharmacol., in press, 1991) that in vitro superfusion with DuP 753(0.1 - 10 nM), a specific nonpeptide AII-1 receptor antagonist, decreased endogenous dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) overflow from rat striatal slices. These concentrations of DuP 753 did not alter either DA uptake or MAO activity, suggesting a direct effect of DuP 753 to decrease DA release. Futhermore, these results suggest that endogenous AII may modulate DA neurotransmission in striatum. Therefore, the present study determined if exogenous AII modulates DA release in superfused rat striatal slices. In the absence of BSA, high concentrations (1 and 10 uM) of AII robustly increased (49-fold) the outflow of endogenous DOPAC in striatal superfusate. In the presence of BSA, much lower concentrations (0.1 nM) of AII increased (1.5-fold) the outflow of tritium in striatal slices preloaded with [3H]DA. Therefore, these results suggest that AII modulates DA release in striatum. (Supported by NIH LBI R29-HL-41954)

393.6

EFFECT OF APOMORPHINE ON EXTRACELLULAR GABA LEVELS IN THE VENTRAL PALLIDUM OF RATS WITH 6OHDA LESIONS OF THE NUCLEUS ACCUMBENS. A.J. Bourdelais and P.W. Kalivas. Dept. of VCAPP, Washington State University, Pullman, Washington 99164.

Apomorphine (APO) stimulated locomotor activity in the rat is greatly enhanced following lesions of the nucleus accumbens (NA) dopamine (DA) terminals using 6OHDA. This increased behavioral response is thought to be due to the action of APO on supersensitive DA receptors, resulting from the reduction of extracellular DA in the NA. Further experimental evidence has shown that DA terminals in the NA synapse on GABA-containing cell bodies located in the NA, and that these GABAergic neurons project to the ventral pallidum (VP). It has also been demonstrated that these GABA neurons are involved in psychostimulant induced locomotor activity, and are inhibited by DA neurotransmission in the NA. The purpose of this study was to determine if DA in the NA regulates GABA release in the VP. To accomplish this, we utilized the DA supersensitivity model described by Swerdlow and Koob, 1984. Bilateral DA leions of the NA were made with 6OHDA. Alterations of the extracellular GABA levels in the VP were measured using microdialysis in conscious freely moving rats following the systemic administration of APO. A significant (88%) depleation of DA in the NA was found following 6OHDA injection into the NA. There was a slight but not significant increase in basal GABA levels in the VP of 6OHDA-lesioned rats. APO induced a significant decrease in basal GABA levels in the VP of both 6OHDA- and sham-lesioned rats, this decrease was augmented in the 6OHDA-lesioned animals. Behavioral augmentation folllowing APO administration was seen in only the 6OHDA-lesioned rats.

393.7

MODULATION OF STRIATAL ACETYLCHOLINE RELEASE BY DOPAMINE AFTER 6-HYDROXYDOPAMINE. D. Jackson, B.A. Vogta*, and M.J. Zigmond, Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA. 15260

Striatal acetylcholine (ACh) interneurons are in close proximity to [inhibitory] nigral dopamine (DA) afferents. In the present study, we have examined the capacity of DA released from residual terminals to modulate the overflow of ACh formed from labelled choline. Striatal slices (350 μ) were prepared from rats given the neurotoxin, 6-hydroxydopamine (6-OHDA, 8 μg), along the nigrostriatal pathway. ACh efflux in response to electrical field depolarization was enhanced by the D2 antagonist sulpiride (1 μM SULP,94+5%) and inhibited by the DA uptake blocker, nomifensine (10 μM NOMI,-51+4%) in slices from untreated rats. The effects of these drugs were reduced in slices from rats lesioned with 6-OHDA 3-6 days before sacrifice (1 μM SULP, 22+7%, NOMI,-24+2%). ACh efflux in slices from rats lesioned 1-2 months earlier with 6-OHDA was similar to efflux in slices from intact rats (1 μM SULP,60+9%, NOMI,-36+1%). Inhibition of ACh efflux by the DA agonist, apomorphine (APO, 10^{-9}-10^{-5} M), was greater in slices from rats lesioned 1-2 months earlier with 6-OHDA than in slices from intact rats or acute 6-OHDA rats. These data suggest that destruction of nigrostriatal DA neurons reduces effectiveness of endogenous DA to act on striatal targets but that over time adaptations involving residual terminals and upregulation of D2 receptors occur leading to a restoration of at least some dopaminergic functions.

393.8

EFFECT OF ENDOGENOUS EXCITATORY AMINO ACIDS ON THE OUTFLOW OF DOPAMINE IN THE NUCLEUS ACCUMBENS. *D. Daly*, K.D. Youngren, and B. Moghaddam, Department of Psychiatry and Neuroscience Program, Yale University School of Medicine, New Haven, CT 06510, and West Haven VA Medical Center, West Haven, CT 06516.*

Intracerebral microdialysis was utilized to assess the effect of endogenous excitatory amino acids - glutamate (GLU) and aspartate (ASP) - on the extracellular level of dopamine in the rat nucleus accumbens. Addition of 5 and 10 mM GLU through the microdialysis probe increased the outflow of dopamine by nearly 200%. The increase was not significantly different between the two doses. The stimulatory effect of GLU at both 5 and 10 mM was partially blocked by the non-NMDA antagonist CNQX (100 uM through the dialysis probe), but not by the NMDA antagonist APV (up to 1 mM). ASP, in contrast to GLU, displayed a dose dependent effect on dopamine release at the doses tested (1 - 10 mM). ASP-stimulated dopamine release was partially blocked by both the NMDA and non-NMDA antagonists, APV and CNQX, respectively. Perfusion of TTX concomitantly with either GLU or ASP completely inhibited the excitatory effect of these amino acids on dopamine release. These results indicate that in the nucleus accumbens, ASP and GLU enhance dopamine outflow through distinct trans-synaptic mechanisms.
Supported in part by VA Centers for Schizophrenia & Alcoholism.

393.9

INCREASE IN GASTRIN-RELEASING PEPTIDE(GRP) IN RAT BRAIN AFTER INTERRUPTION OF DOPAMINE PATHWAYS PRODUCED BY D1/2 ANTAGONISTS AND 6-HYDROXYDOPAMINE. A.Masui, M.Sadamatsu*, K.Tsunashima*, N.Yanaihara* and N.Kato. Dept. of Psychiatry, Shiga Univ. of Med. Science, Otsu 520-21, Japan.

Various neuropeptides such as bombesin(BN), substance P and somatostatin are known to induce excessive grooming in which BN was most potent (this Meeting, 1989). Since haloperidol (HAL; a D1/2 antagonist) suppresses this behavior, the involvement of dopaminergic(DA) transmission has been suggested. We examined the effect of DA interruption induced by HAL and 6-hydroxydopamine(6-OH-DA) on the brain immunoreactive GRP(IR-GRP), mammalian BN, in rats. Chronic HAL treatment via osmotic pump induced the increase of IR-GRP in the striatum and mesolimbic area. The elevation of IR-GRP in the mesolimbic area was also noted following 6-OH-DA icv. treatment. Between SCH23390 (D1 antagonist) and sulpiride (D2), SCH23390 appeared to more preferentially affect brain IR-GRP. This finding is coincident with the behavioral study in which D1 antagonist selectively inhibits the excessive grooming induced by several peptides. It will be of interest that somatostatin was increased after 6-OH-DA treatment in the same study.

393.10

POSSIBLE ROLE FOR ADENOSINE AS A NEUROMODULATOR OF METHAMPHETAMINE EFFECTS K.T. DelleDonne, R.E. Heikkila, D.E. Riordan' and P.K. Sonsalla. Department of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

Adenosine is an inhibitory modulator of neuronal activity whose action at its receptors is blocked by the methylxanthine caffeine. Substantial evidence indicates that adenosine is a neuroprotectant against glutamate-mediated neurodegeneration. Repeated high doses of methamphetamine (METH) to mice cause damage to neostriatal dopaminergic neurons and there is evidence that both dopamine and glutamate play a role in the production of METH toxicity. The purpose of these studies was to assess if treatment with chronic or acute caffeine alters METH-induced neurotoxicity. Pretreatment of mice with caffeine prior to each injection of METH resulted in a dose-related potentiation of METH-induced decrements in neostriatal dopamine content and tyrosine hydroxylase activity. In contrast, chronic pretreatment of mice for four weeks with caffeine followed by drug wash-out caused a significant attenuation of METH-induced toxicity. Chronic caffeine treatment did not alter neostriatal levels of METH but did up-regulate the number of adenosine A_1 receptors. These results suggest that adenosine may be able to modulate METH-induced dopaminergic neurotoxicity in the mouse neostriatum. Effects of adenosine on either dopaminergic or glutamatergic neurotransmission may underlie its protectant properties.

393.11

EFFECT OF INTRAPALLIDAL MORPHINE INJECTIONS ON EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS (NAC).
C. Spyraki and Y.E. Anagnostakis. Lab. of Pharmacology, Medical School, University of Crete, 71409 Heraklion, GR.

The Ventral Pallidum (VP) has direct and indirect projections to the region of the ventral mesencephalon containing dopamine perikarya and to certain terminal fields, including the NAC. The present study was undertaken in order to examine the functional properties of some of these projections and to associate locomotor stimulant response seen in earlier studies following intrapallidal morphine, with possible alterations in the activity of the mesolimbic dopamine (DA) system. Microdialysis probes (2.3 mm) were implanted unilaterally in the nucleus accumbens (AP: +1.6 L: -1.5, V: -8.2 from bregma) and in the ipsilateral ventral pallidum (AP: -0.3 L: -2.5 V: -8.5 from bregma) of Sprague-Dawley male rats (N=10). The day following the probe implantation, dopamine (DA) and metabolites (DOPAC, HVA) were determined in NAC dialysates using HPLC-E.C. (ESA) coupled. Samples were collected at 45 min intervals (flow rate: 0.75 µl/min) over 3 and 6 hrs before and after challenging the rats with morphine (10 µl of 2.6, 4.0 and 26 mM) through the pallidal dialysis probe, respectively. Basal levels were for DA 0.1 pmol, for DOPAC 29 pmol, and for HVA 6.5 pmol/20µl. Increased KCl 50 mM for 10 min) in the perfusate medium (artificial CSF) induced a 500% increase of DA. Basal and K^+ evoked release of DA was reduced in the absence of Ca^{++} in the perfusate medium. We have found that injections of morphine at concentrations higher than 2.6 mM into the ventral pallidum increased levels of DA, DOPAC and HVA in the NAC (20 - 100%) in a dose-dependent manner. The results show that stimulation of VP with morphine modulates the mesolimbic DA system. These findings suggest that the increased locomotion seen in earlier studies following intrapallidal morphine is associated with enhancement of DA overflow in the NAC.

393.12

DARPP-32 is phosphorylated by GABA and dopamine in rat substantia nigra. Gretchen L. Snyder, Shelley Halpain, and Paul Greengard, Lab. of Molec. and Cell. Neurosci., The Rockefeller Univ., New York, NY 10021. DARPP-32 (dopamine- and cAMP-regulated phosphoprotein, Mr=32,000) is enriched in dopaminoceptive cells such as the cell bodies (striatum) and terminals (substantia nigra) of striatonigral neurons and is phosphorylated by cAMP-dependent protein kinase. We used an antibody selective for phosphorylated DARPP-32 (mab 23) to study the regulation of phospho-DARPP-32 by DA and GABA in rat substantia nigra. Minces of nigra were incubated in warm, oxygenated RPMI-1640 medium 30 min prior to addition of drugs for 2-5 min. Tissue was frozen, then sonicated in hot 1% SDS for 10 min. Proteins were separated by SDS-PAGE, transferred to nitrocellulose, immunoblotted with mab 23, and phospho-DARPP-32 detected with ^{125}I-protein A. DARPP-32 was identified in SDS homogenates as a doublet of Mr≅32 kD. GABA (100 uM, 2 min) increased phospho-DARPP-32 in the upper band of the doublet but not in the lower band. This effect was reduced by 5 min pretreatment with the GABA-A antagonist, picrotoxin (100 uM), but not by phaclofen, a GABA-B antagonist (100 uM). L-DOPA (1-100 uM) and the dopamine D-1 agonist, SKF 38393 (1-100 uM), each increased phospho-DARPP-32 in both bands of the doublet. These data suggest that GABA and DA release in the nigra may influence activity of striatonigral terminals through differential effects on the phosphorylation of DARPP-32.

393.13

NICOTINE EFFECT ON LOCOMOTOR ACTIVITY IS REDUCED BY DOPAMINE RECEPTOR BLOCKADE. E. J. Cline and C. Ksir. Department of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY 82071.

We previously reported (Soc. Neurosci Abstr., 14, 1137, 1988) that rats tested for ambulatory activity did not show a diminished response to 0.2 mg/kg nicotine after pretreatment with α-flupenthixol (FPT), a dopamine receptor antagonist. This was puzzling in light of evidence indicating that the mesolimbic dopamine system is important for the locomotor stimulant effects of nicotine (e.g. Clarke et al, JPET., 246, 701-708, 1988).

The current experiment used a different measure of locomotor activity and a different sequence of drug testing. Rats were maintained on daily saline or nicotine injections, and FPT effects were assessed in twice-weekly test sessions. Saline or FPT (0.05, 0.1, 0.2, and 0.4 mg/kg) was followed 90 min later by a test dose of 0.2 mg/kg nicotine or saline, and activity was monitored for one hour. The same animals were then tested for their response to 1.0 mg/kg d-amphetamine after saline, 0.05. and 0.1 mg/kg FPT.

FPT produced significant diminution of the locomotor response to both nicotine and amphetamine, even at the lowest dose tested. These results are consistent with the notion that dopaminergic mechanisms are involved in the expression of increased locomotor activity following nicotine injections.

393.14

DEPLETION OF BRAIN SEROTONIN BY PCPA AFFECTS DOPAMINE AND SEROTONIN SYSTEMS DIFFERENTIALLY AS REFLECTED BY BEHAVIOR AND NEUROCHEMISTRY IN PIGEONS L. Zhang and J. E. Barrett. Dept. of Psychiatry, USUHS., Bethesda, MD 20814.

Serotonin (5-HT)-depleted pigeons were subjected to behavioral and biochemical studies. In the behavioral procedure, a multiple fixed interval (FI 3 min) fixed ratio (FR 30) schedule was employed. The 5-HT_{1A} agonist 8-OH-DPAT (0.3 mg/kg) and the dopamine (DA) agonist piribedil (3.0 mg/kg) were tested before, during and after 5-HT depletion produced by systemic injection of pCPA (150 mg/kg/day for 3 days). Response rate increases produced initially by 8-OH-DPAT were not changed during pCPA treatment. Fourteen days later, 8-OH-DPAT produced increases in FI rates even higher than those before pCPA. Response rate increases produced by piribedil initially were attenuated during pCPA but 14 days later the piribedil effects were recovered. This suggests that 5-HT depletion results in less sensitivity to DA agonists and during 5-HT recovery induces supersensitivity to 5-HT_{1A} agonists. PCPA decreased the 5-HT metabolite 5-HIAA and the DA metabolite DOPAC. These findings suggest that 5-HT depletion either directly or indirectly affects DA system as well. Supported by DA 02873.

393.15

IN-VIVO FACILITATION OF DOPAMINE (DA) RELEASE BY SEROTONIN (5-HT): STUDIES ON CALCIUM DEPENDENCE AND RECEPTOR SELECTIVITY.
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Electrophysiological and behavioral evidence indicates that 5-HT can both facilitate and inhibit DA activity. Previously, we reported that striatal infusion of 5-HT_1 agonists increased DA release as measured by in-vivo microdialysis. This report describes the calcium dependence and receptor selectivity of this facilitation. Agents were dissolved in artificial CSF and perfused through microdialysis probes located in the anterior lateral striata of chloral hydrate anesthetized rats. Drug-induced changes in levels of DA and metabolites were monitored in 20 min. fractions by HPLC-EC and compared with control responses from the contralateral striatum. Perfusion of the calcium channel blocker cadmium (0.1 mM for 1 h) attenuated by 40% the 5-HT (10 µM) induced facilitation of DA release ($p < .01$, $n = 5$). Perfusion of barium (2 mM), cesium (3 mM), or nifedipine (0.1 mM) did not block the elevation of DA levels ($n = 3 - 5$ each). Treatment with antagonists selective for 5-HT receptor subtypes indicated selectivity for the 5-HT_1 subtype. Perfusion of the 5-HT_2 antagonist ritanserin (100 µM) did not attenuate DA facilitation by 5-HT ($n = 4$). While the 5-HT_3 antagonist MDL 72222 (100 µM) reduced 5-HT induced potentiation of DA release by 50% ($p < .05$, $n = 5$), the 5-HT_3 antagonist ICS 205930 (100 µM) did not block enhanced DA release caused by the 5-HT_3 agonist 2-methyl-5HT (50 µM, $n = 5$). In contrast, the 5-HT_1 antagonist pindolol (100 µM) reversed by 50% the facilitation of DA release by both 5-HT (10 µM, $p < .05$, $n = 5$) and the 5-HT_1 agonist RU 24969 (50 µM, $n = 3$). (Supported by DA-04120, and the State of Michigan Dept. of Mental Health).

393.16

THE EFFECT OF NEUROTENSIN AND HALOPERIDOL ON BODY TEMPERATURE, OXYGEN CONSUMPTION AND HEAT EXCHANGE IN THE RAT.
C. M. Handler, E. B. Geller and M. W. Adler.* Dept. of Pharmacology, Temple University School of Medicine, Philadelphia, PA. 19140.

The neuropeptide neurotensin (NT; pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) was injected ICV into the right lateral ventricle of unrestrained, male, S-D rats. At an ambient temperature of 20 ± 1°C, brain surface temperature (Tb), oxygen consumption (VO_2), and heat exchange (Q) were measured for three hours post-injection in a gradient-layer calorimeter. A dose of 0.025 µg neurotensin (0.0149 nmol) did not cause any significant alterations in Tb, VO_2, or Q. Both 0.125 µg (0.0747 nmol) and 0.625 µg (0.373 nmol) of neurotensin significantly reduced Tb during the post-injection period when compared to baseline control levels. VO_2 and Q were also reduced during the period of hypothermia. Haloperidol (H) is a dopamine receptor (D2 > D1) antagonist (and a sigma receptor ligand). A dose of 2 mg/kg (IP) produced slight reductions in the levels of Tb, VO_2, and Q. Haloperidol (30 min pre-treatment), in combination with neurotensin, resulted in a decrease in VO_2 prior to the hypothermic episodes and an increase just before recovery. Q was depressed during the entire post-injection period. At the doses used, the combination of haloperidol and neurotensin does not appear to be other than additive with respect to the magnitude of the hypothermia while the duration of the hypothermic period is shortened.

drug/dose	n	onset of hypothermia	duration of hypothermia	max ΔT ± SE
H 2.0 mg/kg	4	30 min	135 min	-0.36 ± 0.20°C
0.125µg NT	3	30 min	150 min	-1.04 ± 0.46°C
H+0.125µgNT	2	15 min	75 min	-1.42 ± 0.15°C
0.625µg NT	5	0 min	>180 min	-0.67 ± 0.60°C
H+0.625 µg NT	3	0 min	135 min	-1.48 ± 1.00°C

This attenuation of the duration of neurotensin-induced hypothermia results from a smaller decrease in levels of VO_2 and a continuous reduction in Q, resulting in the return of Tb to pre-injection baseline levels. The data support the hypothesis that neurotensin, acting in conjunction with dopamine receptors, has a significant effect on thermoregulation. (Supported by Grant DA 00376 from the National Institute of Drug Abuse)

394.1

SEROTONIN UPTAKE INHIBITION INCREASES PREPROTACHYKININ mRNA AND DECREASES TRYPTOPHAN HYDROXYLASE ACTIVITY IN EMBRYONIC MEDULLARY RAPHE EXPLANTS. L.A. Riley, R.P. Hart and G.M. Jonakait, Dept. of Biol. Sci., Rutgers Univ., Newark, NJ 07102.

Almost all serotonin-containing neurons in the medullary raphe also contain Substance P, but its role and regulation there is not well understood. We have sought to determine whether changes in 5-HT levels affect the biosynthesis of colocalized SP. In previous studies, we have shown that pharmacological manipulations of 5-HT levels in vivo change the steady state levels of the mRNA coding for the prohormone precursor of SP, preprotachykinin (PPT; Walker et al., Mol. Brain Res. 8:113 1990; Riley et al., Mol. Cell. Neurosci., in press).

Changes effected by altered 5-HT levels may be due secondarily to changes in serotonergic neurotransmission. In order to test this hypothesis directly, we have begun to use a culture system in which medullary raphe neurons are grown as organotypic explants where complicating afferent input to the raphe can be kept to a minimum. Raphe are discretely dissected from rat medullae at embryonic day 16 and grown in Maximow-slide depression chambers as lying-drop preparations. In these explants, levels of SP, PPT mRNA and tryptophan hydroxylase (TPH; first enzyme in 5-HT biosynthesis) activity are stable over the first 10 days in culture. Growth for 5 days in the presence of the specific 5-HT uptake inhibitor norzimelidine increased levels of PPT mRNA 48±9% ($p<0.01$) as measured by a sensitive nuclease protection assay. TPH activity decreased 66±6% ($p<0.05$) while levels of SP-like immunoreactivity were unchanged. Since raphe neurons are the only SP-containing neurons in the explant, these data suggest that 5-HT, while regulating its own biosynthesis, simultaneously regulates the biosynthesis of its colocalized peptide partner. Supported by MH43365 and TSA.

394.2

DOSE-DEPENDENT EFFECTS OF THE SEROTONIN$_2$ RECEPTOR ANTAGONIST, RITANSERIN, ON TISSUE AND DIALYSATE LEVELS OF DOPAMINE AND SEROTONIN IN THE RAT BRAIN. L. L. DEVAUD, DIV. OF PHARMACOLOGY, WELLCOME RESEARCH LABS, RESEARCH TRIANGLE PARK, N C 27709

Ritanserin (RIT) was used to study the serotonin (5-HT) component of neurotransmitter modulation in relation to atypical antipsychotics, which have both 5-HT$_2$ and dopamine$_2$ (DA$_2$) receptor antagonist activities. Systemic RIT potently decreased tissue levels of DA and 5-HT with a maximal effect of 21.4 or 22.2% reduction, respectively. It had no consistent effects on DOPAC, HVA or 5-HIAA levels. ED$_{50}$ values were 0.27 mg/kg (0.21-0.35)mg/kg for DA and 0.39(0.15-1.0)mg/kg for 5-HT in the nucleus accumbens. Similar potencies were noted for RIT in the frontal cortex. No dose response was seen in the striatum. In general, these findings correspond to RIT-induced increases in DA levels in dialysate from the nucleus accumbens and the frontal cortex collected from freely moving animals. At 60 min after a dose of 0.3mg/kg, RIT increased DA 147±22 above baseline while 0.63mg/kg RIT increased DA 213±58. RIT treatment also increased DA and 5-HT release in the frontal cortex. No effects of RIT were noted on dialyzed DA or 5-HT levels collected from the striatum. These results show that selective 5-HT$_2$ receptor antagonism modulates DA and 5-HT neurotransmission in a dose-dependent and site-selective manner.

394.3

***IN VIVO* PRESYNAPTIC MODULATION OF SEROTONERGIC TRANSMISSION BY α_2-ADRENOCEPTORS IN THE RAT HIPPOCAMPUS.** R. Mongeau, P. Blier and C. de Montigny. Neurobiological Psychiatry Unit, Dept. of Psychiatry, McGill University, Montréal, Québec, Canada H3A 1A1

In vitro studies have demonstrated the presence of inhibitory noradrenergic (NE) α_2-heteroreceptors on 5-HT terminals modulating the release of [^{3}H]5-HT in rat brain slices. This modulation, nevertheless, remained to be documented *in vivo*. The effect of the activation of these NE heteroreceptors was thus studied by comparing the effectiveness of the electrical stimulation (200 pulses: 0.5 msec, 300 µA, 1 Hz) of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus pyramidal neurons prior to, and following, the intravenous administration of NE agents. Desipramine (2 mg/kg), a selective NE reuptake blocker, reduced the efficacy of the stimulation; this effect was completely reversed by the α_2-adrenergic antagonist yohimbine (500 µg/kg), a preferential ligand for the phenylethylamine site, but was only partially reversed by idazoxan (500 µg/kg), a preferential ligand for the imidazoline site. Small doses of the α_2-adrenergic agonist clonidine (2-10 µg/kg) enhanced the efficacy of the stimulation, while high doses (100-400 µg/kg) reduced it. These incremental and the decremental effects of clonidine were reversed by 0.1 and 1 mg/kg of yohimbine, respectively. These results suggest that low doses of clonidine preferentially activate α_2-autoreceptors on NE neurons resulting in a reduction of the tonic inhibitory effect of endogenous NE on 5-HT transmission, while higher doses of clonidine would decrease 5-HT transmission through the activation of α_2-heteroreceptors on 5-HT terminals. This interpretation is consistent with the report of Maura et al. (*Eur. J. Pharmacol.* 116: 335, 1985) showing that the affinity of clonidine is 10 times higher for α_2-autoreceptors on NE terminals than for α_2-heteroreceptors on 5-HT terminals.

394.4

THE "5HT/NE LINK" BEYOND THE BETA ADRENOCEPTOR. A. Eiring*, D.H. Manier* and F. Sulser. Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

C_6 rat glioma cells cultured in Ham's F-10 medium were used as a model system to study the interaction of aminergic signals beyond the beta-adrenoceptor. In confirmation of the results obtained by Yoshikawa and Sabol (Mol. Brain Res. 1, 75-83, 1986), the beta-adrenoceptor mediated expression of preproenkephalin A (ppEK) mRNA in C_6 rat glioma cells is markedly enhanced by glucocorticoids. Isoproterenol caused a long lasting increase in the formation of cyclic AMP and a 3,6 and 9 fold enhancement in the ppEK mRNA steady state levels at 2,4 and 8 hours of incubation respectively. Serotonin, (5HT) while exerting no effect on beta-adrenoceptor density or K_D values and only slightly attenuating the isoproterenol enhanced formation of cyclic AMP, had no effect on the steady state level of ppEK mRNA but markedly reduced the beta agonist induced increase. The 5HT receptor subtype mediating the antagonistic response to the beta signal remains to be determined. The results demonstrate the convergence of aminergic signals beyond the beta-adrenoceptor and its importance in the regulation of gene expression (Supported by USPHS grant MH-29228).

394.5

IPSPs IN PYRAMINDAL CELLS IN PIRIFORM CORTEX EVOKED BY MONOAMINE EXCITATION OF INTERNEURONS DEMONSTRATE A CONVERGENCE OF INPUTS. R.L. Gellman and G. K. Aghajanian. Depts. of Psychiatry and Pharmacology; Yale University, New Haven, CT 06510.

Previous work demonstrated a subpopulation of interneurons on the border of layers II/III in piriform cortex that are excited by serotonin (5-HT) via 5-HT$_2$ receptors. Excitation of these interneurons induces IPSPs (inhibitory post-synaptic potentials) in pyramidal cells (layer II). The present study looked at the effects of three different monoamines on the layer II/III interneuron-pyramidal cell network. In brain slices, brief bath applications of 100 µM 5-HT, dopamine (DA) or norepinephrine (NE) produced an increase in firing rate in 30-40% of extracellularly recorded interneurons, a decrease in 10% and no effect in the remainder. Of the interneurons that did respond, 52% responded to all three of the monoamines, 13% responded to two and 35% responded only to a single transmitter. These data suggest that responsivity to applied monoamines can define subpopulations of interneurons.

Intracellular recordings from layer II pyramidal cells (n=29) using KCl filled electrodes demonstrated spontaneous, bicucculline-sensitive reverse IPSPs. 5-HT, NE or DA increased IPSPs in 65%, 45% or 24% of cells respectively. The number of IPSPs increased in response to all three monoamines in 39% of the cells, to two in 28% and to one in 33%. The pattern of IPSPs evoked by the monoamines was unique for each cell. Typically, within a given pyramidal cell, the array of IPSPs evoked by one monoamine contained amplitudes common to that evoked by the other monoamines as well as unique amplitudes, suggesting that multiple interneurons had been activated.

We propose that the specific pattern of IPSPs seen in a pyramidal cell responding to more than one monoamine demonstrates a convergence of inputs from different populations of interneurons onto that pyramidal cell.

394.6

INTERACTIONS BETWEEN RESPONSES MEDIATED BY SIMULTANEOUS ACTIVATION OF ADENOSINE A2 AND ALPHA1 ADRENERGIC RECEPTORS (AAR). H.L.Wiener, G.P. Thalody* and S.Maayani. Dept. Pharm. Sci. (HLW & GPT), St. John's Univ., Jamaica, NY 11439 and Depts. Anesthesiol. (HLW & SM), and Pharmacol. (SM), Mt. Sinai Sch. Med., CUNY, New York, NY, 10029.

Blood vessel tone is regulated by the action of different substances, such as neurotransmitters, blood-borne compounds, and agents released from the vascular endothelium. Some characteristics of the interactions between responses mediated by two of these substances are reported in this communication. Two membrane receptors, the adenosine (AD) A_2 and the AAR, were chosen as a model to study functional antagonism, measured as operational partial agonism, in the adventitia- and endothelium-denuded isolated rabbit thoracic aorta. AD agonists (AD, cyclohexyladenosine (CHA), methyladenosine (MeAD), 5'-N-ethylcarboxamidoadenosine (NECA), and R(-)-N6-(2-phenylisopropyl)adenosine (R-PIA)) did not affect basal tissue tone; they did, however, relax aortic rings pre-contracted with the AAR agonist phenylephrine (PE). The AD receptor mediating this functional antagonism was tentatively classified as the AD A_2 receptor based on the rank order of potency: NECA > AD = CHA = R-PIA > MeAD. The fractional relaxation (relaxation/contraction; R/C) mediated by each drug was inversely proportional to PE concentration in naive tissues, and was greater in tissues following partial AAR alkylation with dibenamine, indicating that the magnitude of functional antagonism measured as operational partial agonism, is inversely dependent on the contractile stimulus. The concentration dependence and saturability of R/C elicited by AD A_2 agonists indicates the involvement of a drug-receptor complex in the generation of the relaxation response. We propose that in a given tissue the efficacy of distinct endogenous substances is mutually and continuously altered when they activate their respective receptors. These interactions are the underlying mechanisms of the resultant physiological responses. (GM-34852).

394.7

EFFECTS OF REPEATED ADMINISTRATION OF AZAPIRONES ON CNS MONOAMINES IN RATS.

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Using HPLC-EC techniques, the effects of 1-28 days of treatment with the azapirone 5-HT_{1A} agonists, buspirone or gepirone (1.0 mg/kg; ip) on monoamine (MA) levels in cortex, brainstem and spinal cord were studied. In the cortex and brainstem, buspirone produced peak MA changes at days 1 and 7. In these loci, MA changes returned to basal levels by 28 days of treatment. In the spinal cord, no effects were detected prior to the occurrence of peak changes at 14 and 28 treatment days. Gepirone produced peak MA changes in the cortex and brainstem at 1 and 7 days, with a returned to baseline levels by days14 and 28. Gepirone-induced MA changes in the spinal cord occured at 14 days, and persisted to 28 days of treatment.

394.8

ROLE OF CALCIUM IN THE MODULATION OF NE-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS BY cAMP AND ADENOSINE.

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In DDT_1 smooth muscle cells the adenosine A_1-selective agonist cyclopentyladenosine (CPA) induced a twofold potentiation of norepinephrine-stimulated (NE-stimulated) phoshatidylinositol (PI) turnover, whereas the activation of adenosine A_1 receptors in the absence of NE had no significant effect upon PI turnover. This behavior occurred in the absence of extracellular calcium and was not blocked by calcium channel blockers. However, pretreatment with the membrane-permeant calcium chelator, Quin-2 AM, blocked the potentiation, suggestive of a role for intracellular calcium in the potentiation phenomenon. In the presence of a calcium ionophore, CPA stimulated PI hydrolysis in a calcium dependent manner, without the addition of NE.

Both 8-bromo cAMP and forskolin were able to selectively inhibit the potentiation of NE-stimulated PI hydrolysis, due to CPA, without significantly affecting the stimulation by NE alone. Measurement of the intracellular calcium transient response to NE with Fura-2 revealed that forskolin reduced both the magnitude and the duration of the rise in intracellular calcium.

The data are supportive of a model for the activation of PI hydrolysis via two different pathways: 1) a CPA-stimulated pathway which requires increased intracellular calcium,and 2) a NE-stimulated pathway, active at basal calcium. The level of intracellular calcium, and thus the magnitude of CPA-stimulated PI hydrolysis, can be regulated via [cAMP]. (NS-26934 and GM-31155)

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION I

395.1

Fornix-lesions do not affect diurnal rhythms, stress responsiveness or ACTH sensitivity to corticosterone (B) feedback in rats with fixed B replacement.

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While B shapes both circadian rhythms and stress-induced ACTH responses, the sites in the CNS at which B acts to control ACTH are not known. Although some reports implicate a hippocampal site [through hippocampectomy or fornix-lesions (FL)],others do not find effects of such surgery on ACTH or B (for review, see Jacobson & Sapolsky, 1991). First, we damaged either the dorsal fornix (df) (n=5 rats), the df and 1/2 to 3/4 of the ventral hippocampal commisure (VHC) (n=9), the df and the columns of the posterior fornix (n=4) or the overlying cortex (CL) (n=4). In the morning 4 days after the lesion, there were no differences in plasma B among the groups (B≤1.4 µg/dl in all but 2 rats). Next, rats were provided with chronic catheters; in 9, the df and the VHC were completely lesioned (FL). 7 rats were CL and 3 were only cannulated. The diurnal rhythm of B was the same among the groups days 1-6 after surgery. In both exps, thymus and adrenal weights were the same in all groups, providing no evidence for prolonged stimulation of B. Finally, we asked whether FL rats would be as sensitive to fixed negative feedback as CL rats. 2 days after cannulation and FL or CL, rats were ADX and given either 20% B pellets (n: FL= 5, CL=7) or 25% pellets (n: FL=3, CL=5). The diurnal rhythm of ACTH was not different among the groups 4-7 days after ADX. On day 8, rats were exposed to hypoxia (10% O_2) for ~55 min and then to room air for 45 min. The rising phase of ACTH, the peak ACTH response and the decrease of ACTH after hypoxia in FL and CL rats were the same. Initial B in FL and CL rats was the same within pellet doses. From these data, we conclude that the fornix lesions we made did not disrupt a major B-mediated feedback pathway during the times examined.

395.2

MORPHOLOGICAL EVIDENCE FOR HIPPOCAMPAL INTERACTION WITH THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS

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Recent data have implicated the hippocampal formation in the negative feedback inhibition of the hypothalamic-pituitary-adrenal (HPA) axis, suggesting inhibitory influences on the neurons of the medioparvocelluar division of the hypothalamic paraventricular nucleus (PVN). However, hippocampal efferents apparently do not reach the PVN, and are generally thought to be excitatory, suggesting that the effects are mediated by one or more intervening neurons. A possible site of such an interaction is the bed nucleus of the stria terminalis (BST), which is known to receive hippocampal input, project to the PVN, and to contain a rich population of GABA neurons. This possibility was examined in the present experiments. Iontophoretic injections of the retrograde tracer Fluoro- gold were made in the PVN in male rats. Injections of the anterograde tracer Phaseolus vulgaris-leucoagglutinin (PHA-L) were delivered to the ipsilateral ventral subiculum. Light microscopic PHA-L/Fluoro-gold double-labeling studies suggested hippocampal input to PVN-projecting neurons in the BST. To test the possibility that PVN-projecting neurons in the BST contain the neurotransmitter GABA, sections from cases containing Fluoro-gold injections in the PVN were processed for *in situ* hybridization for glutamic acid decarboxylase, the GABA synthesizing enzyme. Double-labeled neurons were detected in the BST, rostromedial portion of the zona incerta, as well as in several other regions of the medial basal hypothalamus. The findings are consistent with the notion that hippocampal influence on the HPA axis may be mediated in part by the BST. Supported by DA02265 and MH422251.

395.3

THE EFFECTS OF NEONATAL HANDLING ON SEROTONIN TURNOVER AND RECEPTOR BINDING.

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Neonatal rats handled during the first week postnatally exhibit increased glucocorticoid (GC) receptor densities in hippocampus (HPC) and frontal cortex. Cultured fetal HPC cells also possess higher GC receptor densities following exposure to serotonin (5HT), an effect mediated via $5HT_2$ receptors. Handled (H) rats manifest increased 5HT turnover in HPC, leading us to ask if this effect is selective for the HPC and whether it involves changes in $5HT_2$ receptor densities. Brain tissue obtained from H or non-handled (NH) rats was assayed by HPLC for 5HT and 5HIAA content, and $5HT_2$ binding was assessed using 3H-ketanserine (3H-KET) as ligand. The 5HT/5HIAA ratio was higher in H than in NH rats in frontal cortex and HPC, but not in hypothalamus or amygdala, regions unaffected by handling. 3H-KET binding in the frontal cortex did not differ between groups, since neither Kd's nor Bmax's varied. Thus handling could alter GC receptor densities by affecting 5HT turnover in specific brain regions, but not by altering $5HT_2$ receptor densities.

395.4

INHIBITORY EFFECT OF CENTRALLY ADMINISTERED SEROTONIN-1_A AGONISTS UPON ADRENOCORTICAL SECRETION.

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Serotonin (5-HT) has generally been considered to serve a facilitatory role in the CNS regulation of adrenocortical secretion. Numerous studies have shown that administration of 5-HT_{1A} receptor agonists increase plasma corticosterone (CS) concentrations in rats, however the mechanism by which this response is elicited has not been demonstrated to date. Rats were prepared with a cannula implanted above the lateral cerebral ventricle, or bilateral cannulae above the hypothalamic paraventricular nuclei (PVN), the site of corticotropin-releasing factor (CRF)-secreting neurons regulating adrenocortical secretion. The 5-HT_{1A} agonists 8-OH-DPAT and ipsapirone caused significant decreases in plasma CS levels at all doses tested (5-20 nmol), as compared with saline-treated control rats, under sodium pentobarbital anesthesia. In contrast, intraperitoneal injection of 8-OH-DPAT (2 µmol/kg) increased plasma CS concentrations, and this effect was not prevented by prior central administration of the 5-HT_{1A} antagonists, NAN-190 or spiroxatrine (20 nmol). Pretreatment of animals with 6-hydroxydopamine, injected via the PVN cannulae, caused a large depletion in local norepinephrine content, without affecting 5-HT content, and abolished the CS responses following peripheral 8-OH-DPAT administration. In view of the adrenocortical activating effects of hypotensive stimuli, this suggests that the well-documented hemodynamic responses following 5-HT_{1A} receptor stimulation were probably responsible for the adrenocortical responses observed. Our data indicate that 5-HT_{1A} receptor stimulation within the CNS decreases adrenocortical secretion, perhaps via a direct effect in the PVN involving CRF-secreting neurons therein.